UNIVERSIDADE DA BEIRA INTERIOR
Ciências da Saúde

Study of the etiopathogenesis of Chronic Rhinosinusitis with Nasal Polyps: focus on the host-environment interaction

Rafaela da Cruz Vieira Veloso Teles

Thesis submitted for the degree of Doctor of Philosophy in

Medicine

Primary Supervisor: Rosa Roque Farinha, MD, PhD
Associate Supervisor: Christian von Buchwald, MD, DMSc

Covilhã, Janeiro de 2020

UNIVERSIDADE DA BEIRA INTERIOR
Ciências da Saúde

Estudo da etiopatogenia da Rinossinusite Crónica com Pólipos Nasais: foco na interação ambiente-hospedeiro

Rafaela da Cruz Vieira Veloso Teles

Tese para obtenção do Grau de Doutor em
Medicina
(3º ciclo de estudos)

Orientador: Professora Doutora Rosa Roque Farinha
Co-orientador: Professor Doutor Christian von Buchwald

Covilhã, Janeiro de 2020

Dedication

To my Grandparents, that have always inspired me and taught me how to guide by intuition.

To my Parents, that always believed in me and to all the effort they made to turn everything possible.

To Rui, that showed me how it is delightful to share the wins but also to have a comfort during defeats. Thank you for being the mirror of my soul.

To Miguel, for giving another meaning to my life.

To all the family and friends, for the stimulus they gave me.

To Professor Rosa Roque Farinha, for accepting the challenge of being my supervisor, and for her optimism and driving force concerning this investigation.

To Professor Christian von Buchwald, I will be always grateful for his sharing of expertise in Rhinology, for the invaluable advices and for the wonderful time that I spent in his department.

To the memory of Professor Sousa Martins, that inspired different generations of medical students, for his devotion as a doctor and to his merits in divulging throughout Europe the unique therapeutic properties of the Serra da Estrela air in diseases of the respiratory tract.

To all the people that helped me in this journey.

Publications

The investigation done for this Thesis lead to the publication of the following papers in indexed and peer-reviewed journals:

Veloso-Teles R, Cerejeira R. Endoscopic sinus surgery for chronic rhinosinusitis with nasal polyps: clinical outcome and predictive factors of recurrence*. Am J Rhinol Allergy. 2017 Jan 1; 31(1):56-62. (Appendix 1)*

Veloso-Teles R, Cerejeira R, Roque-Farinha R, von Buchwald C. Higher prevalence of nasal polyposis among textile workers: an endoscopic based and controlled study. *Rhinology. 2018 Jan 1; 56(2):99-105. (Appendix 2)*

Veloso-Teles R, Cerejeira R, Rodrigues D, Roque-Farinha R, von Buchwald C. Food specific IgE and IgG antibodies in patients with chronic rhinosinusitis with nasal polyps: a case-control study. *Ear Nose Throat J. 2019 Sep 23:145561319867668. doi: 10.1177/0145561319867668. [Epub ahead of print] (Appendix 3)*

Veloso-Teles R, Cerejeira R, Roque-Farinha R, von Buchwald C. Systemic immune profile in patients with chronic rhinosinusitis with nasal polyps. *Ear Nose Throat J. 2019 Dec 4:145561319893163. doi: 10.1177/0145561319893163. [Epub ahead of print] (Appendix 4)*

Presentations

The investigation done for this Thesis lead to the following Conference made at scientific events:

Veloso-Teles R, Cerejeira R. Epidemiologia da RSCcPN em Portugal: estudos baseados na endoscopia. (Epidemiology of CRSwNP in Portugal: endoscopic based studies). *65th Annual Meeting of the Portuguese Society of Otorhinolaryngology–Head and Neck Surgery.* Aveiro, 2018 *(Appendix 5)*

The investigation done for this Thesis lead to the following oral presentation made at scientific events:

Veloso-Teles R**,** Cerejeira R, Rodrigues D, Roque-Farinha R, von Buchwald C. Food specific IgE and IgG-antibodies levels in patients with chronic rhinosinusitis with nasal polyps: a case-control study. *5th Congress of the Confederation of European Otorhinolaryngology–Head and Neck Surgery (CEORL-HNS).* Brussels, 2019 *(Appendix 6)*

The investigation done for this Thesis lead to the following poster presentation made at scientific events:

Veloso-Teles R, Cerejeira R, Roque-Farinha R, von Buchwald C. Systemic immune profile in patients with chronic rhinosinusitis with nasal polyps: a case-control study. *5th Congress of the Confederation of European Otorhinolaryngology–Head and Neck Surgery (CEORL-HNS)*. Brussels, 2019 *(Appendix 7)*

Prizes

The investigation done for this Thesis received the following prize:

"Clinical Research Prize ERS 2018" for the best clinical investigation with the manuscript entitled "Higher prevalence of nasal polyposis among textile workers: an endoscopic based and controlled study", assigned by the *European Rhinologic Society*, 2018 *(Appendix 8)*

Resumo alargado

Estudo da etiopatogenia da Rinossinusite Crónica com Pólipos Nasais: foco na interação ambiente-hospedeiro

Introdução

A rinossinusite crónica (RSC) é uma doença inflamatória crónica do nariz e seios perinasais, que engloba dois fenótipos clínicos: a Rinossinusite Crónica sem Pólipos Nasais (RSCsPN) e a Rinossinusite Crónica com Pólipos Nasais (RSCcPN). Esta última destaca-se pela presença de formações polipoides, hiperplásicas, pedunculadas e edematosas nas cavidades nasais e seios perinasais, geralmente de forma bilateral. A RSCcPN é uma entidade clínica comum, com elevada morbilidade e cronicidade, já descrita no tempo do Antigo Egipto (2000 a.C.) e sobre a qual a investigação científica tem incidido de forma intensa nas últimas duas décadas. No entanto, apesar de toda a pesquisa realizada, a RSCcPN continua a ser um enigma na história da Medicina, permanecendo como uma doença idiopática de prevalência desconhecida, cuja fisiopatologia é em grande parte oculta. Estas incertezas refletem-se na eficácia limitada dos tratamentos disponíveis e explicarão, em parte, a elevada refratariedade da doença ao tratamento médico e cirúrgico. As principais limitações no estudo desta patologia têm sido: a sua sintomatologia inespecífica que dificulta o diagnóstico diferencial com outras patologias nasossinusais; os estudos epidemiológicos pouco fiáveis que estimam a prevalência da doença e avaliam os seus fatores de risco baseando-se em questionários sobre sintomas; a frequente ausência de diferenciação entre os tipos de RSC (RSCsPN e RSCcPN) em diferentes estudos e a inclusão de subtipos de doença (ex. RSCcPN no contexto de Fibrose Quística, Discinésias Ciliares primárias, Vasculites), que sendo casos raros e com mecanismos fisiopatológicos particulares, deverão ser alvo de estudos individualizados. Tendo em conta o consenso atual de que a RSC resultará de uma interação disfuncional entre hospedeiro-ambiente, pretende-se estudar fatores de risco exógenos e endógenos que possam estar na origem e perpetuação da inflamação da mucosa nasal que caracteriza a RSCcPN.

Este trabalho tem como objetivos:

1) Avaliar a eficácia da cirurgia endoscópica nasossinusal (CENS) no tratamento da RSCcPN e estabelecer fatores prognósticos de recidiva da doença;
2) Comparar a prevalência da polipose nasal (PN) num grupo de trabalhadores com e sem exposição ocupacional a poeiras;
3) Caracterizar e comparar alterações imunológicas sistémicas dos doentes com RSCcPN *versus* grupo controlo;

4) Clarificar o papel da alergia alimentar na RSCcPN, comparando os níveis séricos de anticorpos IgE e IgG específicos contra antigénios alimentares em casos e controlos.

Material e Métodos

1) Estudo observacional retrospetivo de 85 doentes submetidos a CENS e com um follow-up mínimo de 9 meses. Os dados demográficos, a exposição ocupacional, as comorbilidades, a história cirúrgica prévia, os sintomas pré e pós-operatórios, os dados do exame ORL, resultados da TC e a informação sobre o tratamento médico e cirúrgico foram obtidos através da revisão dos processos clínicos. A análise estatística foi efetuada com recurso ao SPSS v.23. A estatística descritiva foi utilizada na caracterização da amostra. Utilizou-se o teste de McNemar na comparação dos sintomas pré e pós-operatórios. Os doentes com e sem recidiva de RSCcPN foram divididos em dois grupos independentes e foram comparados para múltiplos fatores: na avaliação da associação entre a recidiva e variáveis categóricas utilizou-se o teste de Qui-Quadrado (ou o teste exato de Fisher quando não se verificavam as assunções necessárias à execução do teste anterior); no estudo de associação entre a recidiva e variáveis quantitativas utilizou-se o teste de Mann-Whitney. Realizou-se uma regressão logística multivariada para avaliar a existência de fatores preditivos independentes na recidiva da polipose nasal. O teste de razão de verossimilhança, o teste de Hosmer e Lemeshow, a área sob a curva ROC foram realizados/calculados para avaliação do modelo criado, e procedeu-se à determinação do coeficiente de Nagelkerke's. O teste de Wald's e o teste de score foram obtidos para cada variável independente, assim como o Odds Ratio e seu intervalo de confiança a 95%.
2) Estudo epidemiológico transversal numa amostra randomizada de trabalhadores têxteis (n=215) e de trabalhadores de venda a retalho (n=101). Realizou-se uma entrevista clínica sistematizada, que incluiu os questionários RhinoQOL-pv e CAT™, e uma avaliação endoscópica com ótica rígida 0º, com determinação do *score* endoscópico de Lund-Kennedy em cada participante. A análise estatística foi efetuada com recurso ao SPSS v.23. Utilizou-se a estatística descritiva na caracterização dos dois grupos de trabalhadores e procedeu-se à sua comparação. Na comparação de variáveis categóricas entre os grupos, utilizou-se o teste de Qui-Quadrado (ou teste exato de Fisher, quando adequado) e na comparação de variáveis quantitativas utilizou-se o teste de Mann-Whitney. O teste Binomial foi utilizado na comparação da prevalência de PN no grupo de trabalhadores têxteis com a prevalência de outros estudos publicados na literatura.
3) Estudo caso-controlo de 37 doentes com RSCcPN e 34 controlos sem RSC. Os dados clínicos foram obtidos por entrevista clínica e exame ORL. Realizaram-se TC do nariz e seios perinasais, teste cutâneo de Prick, espirometria; determinação dos parâmetros imunológicos no plasma (contagem diferencial de leucócitos, classes e

subclasses de imunoglobulinas) e também dos níveis de 25-hidroxivitamina D (25-HOD), alfa-1-antitripsina (A1AT) e proteína C reativa (PCR). A análise estatística foi efetuada com recurso ao SPSS v.23. Utilizou-se a estatística descritiva na caracterização do grupo de casos e controlos. O teste de Mann-Whitney foi utilizado na comparação de variáveis contínuas entre os dois grupos e o teste de Qui-Quadrado ou o teste exato de Fisher na comparação de variáveis categóricas. Realizou-se uma subanálise com o teste de Kruskal-Wallis na comparação dos parâmetros analíticos entre 3 grupos (grupo controlo sem doenças respiratórias crónicas inferiores (DRCI), grupo RSCcPN sem DRCI e grupo RSCcPN com DRCI), seguido de comparações múltiplas interpares com o teste *post-hoc* de Dunn.

4) Estudo caso-controlo de 33 doentes com RSCcPN e 31 controlos sem RSC. Os dados clínicos foram obtidos por entrevista clínica, incluindo a aplicação do Questionário de Frequência Alimentar (QFA). Realizou-se o teste de ELISA com o kit OmegaDiagnostics® com 40 antigénios alimentares para determinação de anticorpos IgG específicos e procedeu-se ao teste de imunoensaio usando o ImmunoCAP™ na avaliação de anticorpos IgE específicos contra 11 antigénios alimentares. Procedeu-se à análise estatística dos dados obtidos, com recurso ao SPSS v.23. Utilizou-se a estatística descritiva na caracterização dos dois grupos. O teste de Mann-Whitney foi utilizado na comparação de variáveis quantitativas entre os dois grupos, enquanto o teste de Qui-Quadrado ou o teste exato de Fisher foi utilizado na comparação de variáveis categóricas. O teste não-paramétrico de Spearman foi utilizado na avaliação de correlação entre variáveis quantitativas.

Resultados

1) Houve uma melhoria significativa de todos os sintomas rinológicos após a CENS. As prevalências de complicações major e minor foram de 1,2 e 15,3%, respetivamente. A proporção de doentes com RSCcPN com recidiva da patologia após CENS foi de 31%, com 7% a necessitar de reintervenção cirúrgica. 60% dos doentes com RSCcPN reportaram exposição ocupacional a poeiras, das quais 90,4% correspondiam a poeiras de baixo peso molecular (BPM) (<5KDa). Os doentes com exposição ocupacional a poeiras apresentaram uma recorrência da doença significativamente superior ao grupo não exposto (48% vs 3%, $p=5,5x10^{-6}$). A análise de regressão logística multivariada identificou a exposição ocupacional a poeiras (p=0,001, OR=38,02, IC95%: [4,18; 345,69]) e a asma não-atópica (p=0,012, OR=8,65, IC95%: [1,62; 46,16]) como fatores preditivos independentes de recidiva da RSCcPN ao contrário das outras variáveis analisadas: idade, sexo, asma atópica, rinite alérgica, hábitos tabágicos, classificação endoscópica da polipose nasal, score de Lund-Mackay e uso pós-operatório de corticoide tópico. O modelo logístico apresentou área sob a curva ROC de 0,82 (p<0,001; IC95%: [0,73; 0,91]). O teste de razão de verossimilhança do

modelo criado obteve um $p=1,2x10^{-7}$, o teste de Hosmer e Lemeshow demonstrou um p=0,503 e o coeficiente de Nagelkerke's foi de 0,44.

2) A PN foi diagnosticada em 19 participantes do grupo dos trabalhadores têxteis (8,8%) e em nenhum do grupo controlo (p=0,001). A prevalência da PN aumentou conforme o estrato etário (p=0,03) e dependendo do número de anos de exposição às poeiras (p=0,017). A degenerescência polipoide do corneto médio foi mais prevalente no grupo exposto (p=0,001), que também obteve um score de Lund-Kennedy mais elevado (p<0,001). No RhinoQOL-pv e no CAT™ obtiveram-se scores significativamente mais elevados entre os trabalhadores têxteis. A prevalência de PN nos trabalhadores têxteis (8.8%) foi significativamente superior às prevalências reportadas em estudos endoscópicos prévios (2,7% e 5,5%; p<0,001 e p=0,029, respetivamente).
3) No grupo dos doentes com RSCcPN a prevalência de doenças respiratórias crónicas inferiores (DRCI) foi significativamente superior ao grupo controlo (p<0,001), ao contrário da patologia atópica que não diferiu. Nos doentes com RSCcPN obteve-se uma contagem relativa de eosinófilos (p<0,001) e de basófilos (p=0,022) no plasma significativamente mais elevada do que no grupo controlo, ao contrário dos neutrófilos cuja contagem foi significativamente menor (p=0,013). Os doentes com RSCcPN apresentaram níveis mais elevados de IgG1 (p=0,022), mas mais reduzidos de IgG2 (p=0,014) e IgG3 (p=0,018) comparativamente aos controlos. Essas diferenças observadas foram mais evidentes nos doentes com RSCcPN e DRCI concomitante. Os níveis de IgG4, IgG total, IgA, IgM e IgE não diferiram entre os grupos, assim como a prevalência das deficiências de classes e subclasses de imunoglobulinas; os níveis de 25-HOD, A1AT e PCR também não diferiram de forma significativa.
4) No grupo com RSCcPN verificou-se uma concentração total de anticorpos alimentares do tipo IgG significativamente menor do que a do grupo controlo (p=0,012); esta diferença foi também observada para diferentes anticorpos IgG específicos (milho, soja, leguminosas, maçã e pera, frutos vermelhos, citrinos). No grupo controlo verificou-se uma correlação positiva entre os níveis de IgG1 séricos e a soma da concentração dos anticorpos IgG alimentares (p=0,049). Pelo contrário, no grupo com RSCcPN observou-se uma correlação negativa entra essas variáveis (p=0,048). Os níveis de IgG1 encontravam-se significativamente elevados no grupo com RSCcPN (p=0,041). Os níveis séricos de IgE específicas contra os diferentes alergénios alimentares avaliados, bem como a concentração total de IgE específicas alimentares, não diferiram de forma estatisticamente significativa entre os grupos.

Conclusões

Apesar da cirurgia endoscópica ser um tratamento eficaz na RSCcPN, com benefícios óbvios na resolução de sintomas no pós-operatório, a recorrência da doença é considerável. O

primeiro estudo deste trabalho demonstrou que a exposição ocupacional a poeiras e a asma não-atópica são fatores preditivos independentes de recidiva da doença. A identificação da exposição a partículas de BPM como principal exposição ocupacional reportada é também relevante. Estas partículas de BPM têm sido associadas ao risco de desenvolver asma ocupacional (não-atópica) e ao contrário das partículas de alto peso molecular (APM) que atuam por mecanismos IgE-mediados, não têm os seus mecanismos de ação bem estabelecidos. A distinção realizada entre asma atópica e não-atópica permitiu clarificar o impacto da asma nos resultados pós-operatórios, que era até então controverso.

O estudo epidemiológico realizado, baseado em endoscopia, foi pioneiro na avaliação do impacto da exposição ocupacional a poeiras na prevalência da PN e aponta para uma importante associação entre ambas, ao demonstrar uma prevalência da doença significativamente elevada nos trabalhadores têxteis. Os resultados deste trabalho alertam para um relevante problema de Saúde Pública, reforçando a necessidade de medidas de proteção dos trabalhadores expostos a poeiras (ex. uso de máscara com filtros apropriados) e a necessidade de controlo no funcionamento dos sistemas de exaustão de partículas e filtros de ar que garantam a qualidade do ar. Depreende-se assim que os doentes com RSCcPN devem sempre que possível trabalhar em ambientes livres de poeiras, de forma a reduzir o risco de recidiva e melhorar o seu prognóstico. Outros estudos epidemiológicos serão necessários na avaliação de outro tipo de exposições ocupacionais, tentando se possível comparar o impacto de exposição a poeiras de BPM e APM na prevalência de RSCcPN.

O estudo prospetivo, clínico-laboratorial, demonstrou ainda que os doentes com RSCcPN apresentam um perfil imune sistémico distinto dos controlos, com variações na contagem diferencial de leucócitos e um desvio IgG1 a nível das subclasses de IgG. Estas diferenças estão de acordo com o que tem sido reportado a nível local, nos pólipos nasais. De notar, que essas diferenças eram mais marcadas nos doentes com DRCI, o que reforça o conceito de "*one airway, one disease*".

Relativamente ao estudo sobre o impacto da alergia alimentar na RSCcPN, esta não parece ter um papel relevante na sua etiopatogenia, seja esta resposta imune IgG ou IgE mediada. Além do mais, observou-se uma supressão de anticorpos IgG específicos contra antigénios alimentares nos doentes com RSCcPN, uma correlação negativa da sua soma com os níveis séricos de IgG1 e valores de IgG1 significativamente elevados nestes doentes. Esta supressão poderá estar relacionada com um desvio da resposta imune IgG-mediada contra outros agentes (p.ex. partículas inalantes) na RSCcPN e deverá ser investigada no futuro.

Palavras-chave

Pólipos nasais, rinossinusite, exposição ocupacional, sistema imune, alergia alimentar

Abstract

Study of the etiopathogenesis of Chronic Rhinosinusitis with Nasal Polyps: focus on host-environment interaction

Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a common disease, with high morbidity and chronicity, but its exact etiology is still unclear and remains a difficult to treat condition. Considering the emerging consensus that chronic rhinosinusitis (CRS) results from a dysfunctional host-environment interaction, this study pretends to clarify exogenous and endogenous factors, which can contribute to the occurrence and perpetuation of sinonasal mucosa inflammation observed in CRSwNP.

The aims of this study are:

1) To evaluate endoscopic sinus surgery (ESS) efficacy in CRSwNP treatment and to establish prognostic factors for disease recurrence;
2) To compare the prevalence of nasal polyps (NP) in a group of workers with occupational dust exposure and in a control group;
3) To characterize systemic immunological alterations that occur in patients with CRSwNP compared to controls;
4) To clarify the role of food allergy in CRSwNP disease, comparing serum levels of food specific IgE and IgG antibodies in cases and controls.

Material and Methods

1) Retrospective observational study in 85 patients with CRSwNP submitted to ESS and a minimum follow-up of 9 months. Patients' demographics, occupational exposure, comorbidities, previous nasal surgeries, pre and postoperative symptoms and ENT examination findings, CT results, medical and surgical treatment information were collected from medical records.
2) Cross-sectional study with a random sample of textile (n=215) and retail store employees (n=101). Clinical data was gathered through a systematic interview, which included RhinoQOL-pv and CAT™ questionnaires. A systematic endoscopic nasal examination was performed using a 0º rigid endoscope and Lund-Kennedy endoscopic score was determined for each participant.
3) Case-control study with 37 CRSwNP patients and 34 controls without CRS. Clinical data was gathered through a systematic interview. CT scan, skin prick test, spirometry, immunological parameters (leukocyte differential count, immunoglobulin

classes and IgG subclasses) and 25-hydroxyvitamin D (25-HOD), alpha1-antitrypsin (A1AT) and C-reactive protein (CRP) dosage in serum specimens were obtained.

4) Case-control study with 33 patients with CRSwNP and 31 controls without CRS. Clinical data was gathered through a systematic interview (including the application of Food Frequency Questionnaire (QFA)). ELISA tests using OmegaDiagnostics® kit with 40 food allergens for detection of specific IgG antibodies were performed and food specific IgE antibodies were determined by immunoassay using ImmunoCAP™ against 11 food antigens.

Statistical analysis was performed using SPSS v.23.

Results

1) All rhinologic symptoms improved after ESS. The major and minor complications prevalences were 1.2% and 15.3%, respectively. Disease recurrence occurred in 31% of cases, but only 7% required surgical reintervention. Multivariate logistic regression analysis identified occupational dust exposure (p=0.001) and non-atopic asthma (p=0.012) as independent predictive variables in CRSwNP recurrence, unlike the other tested variables: age, sex, atopic asthma, allergic rhinitis, smoking habits, nasal polyps endoscopic grade, Lund-Mackay score and postoperative topical corticoid use. The adjusted logistic model had a ROC area under curve of 0.82 (p<0.001; CI95%: [0.73; 0.91]).
2) 316 participants were included in the study, i.e. 215 textile workers and 101 retail store workers. NP were found in 19 subjects (8.8%) among textile workers and none in the control group (p=0.001). The prevalence of NP increased by age strata (p=0.03) and by years of dust exposition (p=0.017). Polypoid degeneration of the middle turbinate was more prevalent in the exposed group (p=0.001) with Lund-Kennedy scoring also higher (p<0.001). RhinoQOL-pv and CAT™ questionnaires had both significantly higher scores among textile employees.
3) A significantly higher eosinophil (p<0.001) and basophil relative count (p=0.022) and a lower relative neutrophil count (p=0.013) were found among CRSwNP group. Patients with CRSwNP had higher IgG1 (p=0.022), but lower IgG2 (p=0.014) and IgG3 (p=0.018) serum levels compared to controls. IgG4, total IgG, IgA, IgM and IgE serum levels did not differ between groups, as well as the prevalence of immunoglobulin classes or IgG subclasses deficiency; 25-HOD, A1AT and CRP dosage had also no significant difference.
4) The overall sum of food IgG antibodies was significantly lower in CRSwNP compared to control group (p=0.012), and this difference was also observed for different specific IgG antibodies (corn, soya, grain legumes, pear and apple, berries, citric fruit). In controls a positive correlation between IgG1 and the sum of food IgG antibodies was

seen (p=0.049) but in CRSwNP group a negative correlation was found (p=0.048). Significant higher level of IgG1 was found among CRSwNP patients (p=0.041). Levels of serum specific IgE antibodies against the different studied food allergens, as well as the sum of food IgE antibodies, did not differ significantly between the groups.

Conclusions

Endoscopic sinus surgery proved to be an effective treatment in CRSwNP, but with a considerable disease recurrence. The first study of this investigation demonstrated that occupational dust exposure and non-atopic asthma are independent predictive factors of disease recurrence risk.

The epidemiologic study performed, based on endoscopy, was pioneer in the evaluation of occupational exposure to dust impact on NP prevalence and the results pointed to an important association between them by demonstrating a significantly higher prevalence of the disease among textile workers.

This investigation also showed a distinct systemic immunologic profile in CRSwNP patients compared to controls, and the variation observed in peripheral relative leukocyte count and the systemic IgG1 subclass shift are similar to what is known to happen in nasal polyp tissue.

Concerning food allergy, it does not seem to have an important role in CRSwNP etiopathogenesis, whether if it is IgG or IgE-mediated. Moreover, the observed suppression of specific IgG antibodies against food allergens, its negative correlation with IgG1 and the raised IgG1 serum levels in CRSwNP, can be related to deviated IgG responses against other targets (e.g. airborne particles) and warrants future investigation.

Keywords

Nasal polyps, rhinosinusitis, occupational exposure, immune system, food allergy

Index

Acronym List

A1AT	Alpha-1-antitripsin
AAOHNS	American Academy of Otolaryngology, Head and Neck Surgery
ACOS	Asthma-COPD overlap syndrome
AERD	Aspirin-exacerbated respiratory disease
AFRS	Allergic fungal rhinosinusitis
ARS	Acute rhinosinusitis
BCE	Before Common Era
ANCA	Anti-neutrophil cytoplasmic antibodies
CAT	COPD Assessment Test
CEBM	Centre for Evidence Based Medicine
CFTR	Cystic fibrosis transmembrane conductance regulator
CLRD	Chronic lower respiratory diseases
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
COPD	Chronic obstructive pulmonary diseases
CRP	C-reactive protein
CRS	Chronic rhinosinusitis
CRSsNP	Chronic rhinosinusitis without nasal polyps
CRSwNP	Chronic rhinosinusitis with nasal polyps
CT	Computed Tomography
DBPCFC	Double-blind placebo-controlled food challenge
EAACI	European Academy of Allergy and Clinical Immunology
EAST	Enzyme allergosorbent test
EGPA	Eosinophilic granulomatous polyangiitis
ELISA	Enzyme-linked immunosorbent assay
ENT	Ear, nose and throat / otorhinolaryngology
EPOS	European position paper on rhinosinusitis and nasal polyps
ESS	Endoscopic sinus surgery
HMW	High molecular weight

ICAR:RS	International Consensus Statement on Allergy and Rhinology: Rhinosinusitis
Ig(s)	Imunoglobulin(s)
IgA(s)	Imunoglobulin(s) A
IgE(s)	Imunoglobulin(s) E
IgG(s)	Imunoglobulin(s) G
IgM(s)	Imunoglobulin(s) M
IL	Interleukine
ILC2s	Type 2 innate lymphoid cells
LGS	Leaky gut syndrome
LMW	Low molecular weight
MDI	Methylene diphenyl diisocyanate
mRNA	Messenger ribonucleic acid
NP	Nasal polyposis
NSAID	Nonsteroidal anti-inflammatory drugs
OSAS	Obstructive sleep apnea syndrome
PGE2	Prostaglandin E2
QFA	Questionário de Frequência Alimentar (Food Frequency Questionnaire)
RAST	Radioallergosorbent test
RhinoQOL-pv	Rhinosinusitis Quality of Life Survey Instrument, portuguese version
ROC curve	Receiver operating characteristic curve
SDB	Sleep-disordered breathing
SERPINA1	Serpin family A member 1 gene
SPSS®	Statistical Package of the Social Sciences
SPT	Skin prick test
Th cell	T helper cell
VDR	Vitamin D receptor
25-HOD	25-hydroxyvitamin D

Index of Tables and Graphics

TABLES

Chapter 3. Endoscopic Sinus Surgery for CRSwNP: clinical outcome and predictive factors of recurrence

Chapter 4. Higher prevalence of nasal polyposis among textile workers: an endoscopic based and controlled study

Chapter 5. Systemic immune profile in patients with CRSwNP

Chapter 6. Food specific IgE and IgG antibodies in patients with CRSwNP

GRAPHICS

Chapter 3. Endoscopic Sinus Surgery for CRSwNP: clinical outcome and predictive factors of recurrence

Chapter 5. Systemic immune profile in patients with CRSwNP

Chapter 6. Food specific IgE and IgG antibodies in patients with CRSwNP

Chapter 1.
INTRODUCTION

1. Introduction

1.1 Chronic Rhinosinusitis with Nasal Polyps (CRSwNP): the scope of the problem

Chronic Rhinosinusitis (CRS) is an inflammatory disease of nasal and paranasal sinus mucosa, which includes two different clinical phenotypes: Chronic Rhinosinusitis without Nasal Polyps (CRSsNP) and Chronic Rhinosinusitis with Nasal Polyps (CRSwNP). CRSwNP distinguishes itself by the presence of polypoid, pedunculated, edematous and hyperplastic tissue masses in nasal cavity and paranasal sinus, most often bilaterally and generally arising from the middle meatus.[1–3] Nasal polyposis (NP) have been a medically recognised condition since the time of ancient Egypt, with literature records about it of approximately 2000 years BCE. Egypt civilization was known for its familiarity and dexterity in the nasal cavity because of their routinely removal of intracranial contents throughout the nose during the mummification process.[4] Later, Hippocrates (460-370 BCE), during the apex of Greek civilization, observed and described different otorhinolaryngology diseases, referring to the "nasal growths" as "polypus" due to their resemblance to the sea-polyp.[4] The word "polúpous" from the ancient Greek drives from the conjugation of "polús" (many) and "poús" (feet), which was later in the origin of the word "polypus" in Latin. Hippocrates also described a treatment modality for this disease with removal of nasal polyps with a snare (polypectomy), a method which persisted well into the second half of the 20th century.[4]

In the last two decades, a lot of investigation has been made on CRS, about its epidemiology, modifying factors, associated comorbidities, phenotypes and endotypes, disease biomarkers, prognosis and treatment. In 2005, the first *European Position Paper on Rhinosinusitis and Nasal Polyps (EP^{3}OS)*[5] was published, then it was actualized in 2007 (EPOS2007)[6], with the ultimate edition released in 2012 (EPOS2012).[1] This last consensus document of the European Rhinologic Society (ERS) was particularly exhaustive, trying to actualize the acquired knowledge about Rhinosinusitis and Nasal Polyps, to establish uniform definitions and diagnosis criteria and using CEBM (*Centre for Evidence Based Medicine* 1998) levels of evidence when making recommendations about diagnosis, treatment and disease approach. In 2007, the *American Academy of Otolaryngology, Head and Neck Surgery* (AAOHNS) also published a guideline for clinical practice concerning adult rhinosinusitis[7], that was recently actualized and reedited in 2015.[8] The rapid growth in number of publications in the last years led rhinologic experts from around the world, in an effort to both consolidate and critically appraise that information, to produce the International Consensus Statement on Allergy and Rhinology: Rhinosinusitis (ICAR:RS), published in 2016.[9]

In spite of all the research that has been done, CRSwNP is still an enigma in Medicine. CRSwNP remains as an idiopathic disease, its true prevalence is unknown, its pathophysiology is unclear, it has an important impact in patient quality of life with high morbidity, and despite the advances in surgical and medical treatment, it has a considerable rate of refractoriness and recurrence. There are different factors that have hampered the investigation about this clinical entity. First of all, symptoms of CRSwNP are nonspecific, making it difficult to distinguish it from other sinonasal diseases. Unfortunately, most of epidemiological studies about CRS have based their diagnosis in symptoms questionnaires, turning the prevalences they found merely speculative. The second problem is that many of the investigation that has been carried out does not separate CRS in its two main phenotypic types (CRSwNP and CRSsNP), or even its subtypes (e.g. CRSwNP associated with Cystic Fibrosis, Vasculitis, Primary Ciliary Dyskinesias, Major Immunodeficiencies, etc). These subtypes of CRSwNP, comprise uncommon cases, that must be evaluated in specific studies, since it is expected the involvement of different physiopathological mechanisms. Moreover, the etiopathogenesis of CRSwNP can have origin in different individual and environmental factors that are difficult to define, creating controversy and uncertainty about it.

Many researches have been carried out around CRS endotyping, trying to reach a classification of CRS based on the cellular and molecular physiopathological mechanisms (e.g. eosinophil-based endotype, Th2-based endotype, IgE-based endotype, cysteinyl-based endotype).[10] Until now there is no single and precise classification for CRSwNP endotyping and endotype-based treatments are still not approved for routine clinical practice, being reserved only for clinical trial settings, in which results are awaited. The difficulties around endotyping have to do with the largely unknown pathophysiology behind CRSwNP.

In western countries, 85% of the CRSwNP disease reveals a type 2 inflammatory pattern. In the last 15 years, several randomized double-blind studies on monoclonal antibodies in CRSwNP were performed, namely anti-IL-5 (reslizumab, mepolizumab), anti-IL4 receptor alpha (dupilumab), and anti-IgE (omalizumab).[11] These biological agents target specific immune pathways, such as IL-5 orchestrating the survival of eosinophils, or IL-4 and IL-13 regulating the formation of IgE and the chemotaxis of eosinophils, among other effects. It was observed for the first time that biologics targeting type 2 immune reactions might be successful in nasal polyps but those studies were characterized by limited number of patients and heterogeneous populations.[11] Until now, however, no biomarkers have been identified to predict response to a specific biologic or to monitor treatment success. Phase-3 studies on monoclonal antibodies with larger populations are being conducted and only with their results it will be feasible to define treatment indications, patient selection and possible side effects. In June 2019, the Food and Drug Administration from United States of America has approved the first monoclonal antibody (dupilumab) for inadequately controlled CRSwNP.

Type-2-cell-mediated immunity, rich in eosinophils, basophils, mast cells, CD4(+) Th2 cells, ILC2s, and type 2 subset of natural killer T cells and macrophages play a role in chronic eosinophilic diseases, such as Western CRSwNP, asthma, and atopic dermatitis.[12] However, in Asian subjects a primarily neutrophilic process occurs in adult bilateral polyps. Studies done in Asian countries like China, Thailand, Singapore and Malaysia found that neutrophil-predominant polyps occur in a large percentage of patients (approximately 49%).[13] The etiology of nasal polyposis in Caucasians and Asians may be different and probably need to be managed differently.

To overcome the previously mentioned limitations on CRS research and taking advance of all the acquired knowledge in the field, future studies must use a rigorous methodology, defining the types and subtypes of the disease that they intend to evaluate. Only then, we can make studies comparable, trying to uncover the subjacent physiopathological mechanisms and finally allow the development of specific treatment options for different CRS subgroups.

1.1.1. Definition

1.1.1.1 Clinical definition of chronic rhinosinusitis

The European consensus document EPOS2012[1], clinically defines CRS (with or without nasal polyps) in the adult as an inflammation of the nose and the paranasal sinuses characterised by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip), with or without other symptoms of facial pain/pressure, and reduction or loss of smell. To establish the diagnosis of CRS there must be either endoscopic signs of nasal polyps and/or mucopurulent discharge primarily from middle meatus and/or edema/mucosal obstruction primarily in middle meatus; and/or Computed Tomography (CT) signs of mucosal changes within the ostiomeatal complex and/or sinuses. To be considered as chronic, the symptoms must persist for 12 or more weeks. Questions on allergic symptoms (i.e. sneezing, watery rhinorrhea, nasal itching, and itchy watery eyes) should be included.[1] Chronic rhinosinusitis with nasal polyps (CRSwNP) is diagnosed if chronic rhinosinusitis as defined above is present and bilateral polyps are endoscopically detected in the middle meatus.[1]

1.1.1.2 Definition for use in General Practice and epidemiology studies

About the definition to use in General Practice or for epidemiological studies, EPOS2012[1] states that it should be based on symptomatology without the need of otorhinolaryngological or imagiological examination.

1.1.1.3 Definition for research

For research purposes, EPOS2012[1] considers that CRS must be defined as per the clinical definition. For the purpose of a study, the differentiation between CRSsNP and CRSwNP must be based on endoscopy.

1.1.2 Epidemiology

CRSwNP is a common clinical entity, but the epidemiological studies about it are scarce, especially in Europe.[1] Another important issue is that most of the epidemiological studies are based on symptoms questionnaires[14–16] and do not allow the distinction between CRSwNP and CRSsNP. The use of a special definition for epidemiology studies as recommended by EPOS2012, based only in sinonasal symptoms, make it impossible to distinguish the main types of CRS, or even from other sinonasal diseases, which can origin an overestimation of CRS. Moreover, many of the studies have used email questionnaires and telephone interviews that reduces, even more, the acuity of diagnosis.

A previous study in Sweden[17] has already showed that data obtained with questionnaires about CRSwNP prevalence can be unreliable, since not all patients that claim to have NP have polyps on nasal endoscopy and asymptomatic polyps will be missing, meaning that cases of subclinical disease will be dropped out.

Despite not having yet changed its epidemiological definition for CRS, EPOS2012[1] alerts that nasal endoscopy is a prerequisite for an accurate estimate of the prevalence of NP and that in the light of epidemiologic research, a distinction needs to be made between clinically silent NP or preclinical cases, and symptomatic NP.

Having all these facts in consideration, we can assume that the ideal methodology to use for CRS epidemiological studies includes standardized and validated symptom questionnaires for the disease, associated with nasal endoscopy.

In Europe, only two endoscopic-based studies have evaluated NP prevalence: an *in-vivo* study done in Sweden[17] that found a prevalence of 2.7% and a cadaver study done in Portugal[18] that showed a prevalence of 5.5%.

1.1.3 Predisposing factors and associated comorbidities

1.1.3.1 Demography

According to published data, CRSwNP prevalence seems to change according to demographical parameters. It is a disease that occurs in all ethnicities, becomes more common with age and appears to have a preference for male gender.[1] The average age of onset is 42 years and is rare under the age of 20.[1]

In the population-based study done in Sweden[17], Johansson et al. verified through a multivariate regression logistic analysis that male gender (OR: 2.7 [95% CI: 1.33–5.5]) and seniors (>60 years; 5%) were more commonly affected by the disease.

Despite being a universal disease with similar clinical manifestations, the subjacent physiopathological mechanisms seem to diverge between Caucasian and Asiatic patients. Comparing nasal polyps tissue of Caucasian and Asiatic origin, the last ones have a minor eosinophilic inflammation.[19]

1.1.3.2 Atopic status

In the past, it was tempting to speculate that atopy could predispose to CRSwNP occurrence, and this was one of the first theories about CRSwNP etiopathogeny. However, many studies that tried to correlate allergic rhinitis (IgE-mediated) with CRSwNP did not find any raise of atopic prevalence in this patients.[1] Furthermore, it was also demonstrated that patients with allergic rhinitis had prevalences of CRSwNP between 0.5-4.5%, which are similar to the prevalences found in general population.[1] More recently, a prospective study that included 210 CRSwNP patients found that atopic status did not correlate with disease severity or with disease recurrence in postoperative follow-up.[20]

Moreover, Settipane et al. and Grigoreas et al. conducted two large epidemiological studies and both showed higher prevalences of CRSwNP in patients with non-allergic rhinitis (4.7% and 8.9%) than in patients with allergic rhinitis (1.5% and 1.7%), with statistically significant differences.[21,22]

1.1.3.3 Asthma and other Chronic Lower Respiratory Diseases (CLRD)

The extent of the interrelationship between CRSwNP and chronic lower respiratory diseases (CLRD) and the subjacent physiopathological mechanism behind it still have to be clarified, deserving a close collaboration between Otorhinolaryngologists, Pneumologists/ Respiratory Physicians and Immunoallergologists. Once more, the epidemiological studies that exist about this topic are mostly based on symptom questionnaires[23] and have only evaluated CRS in general.[24] Nevertheless, there is convincing evidence that these diseases are correlated, reinforcing the concept of the unified airway (“*one airway, one disease*”[25]). In a study conducted by Settipane et al.[21] that included 2228 patients with asthma, it was demonstrated that patients with non-atopic asthma (non-IgE-mediated asthma) had a

significantly higher prevalence of NP when compared to patients with atopic asthma (IgE-mediated asthma), with prevalences of 12.5% vs 5.0%, respectively ($p<0.01$). These findings were also corroborated by Grigoreas et al., that also found a prevalence of NP in patients with non-atopic asthma (13%) much superior than in patients with atopic asthma (2.4%), in a sample of 1877 asthmatic patients.[22] A Danish study, that evaluated 40 patients with CRSwNP referred for surgery, clinically and objectively through peak expiratory flow, spirometry and bronchodilation tests, found a very high prevalence of asthma (65%), that was frequently previously undiagnosed (25%). This value diverged in a statistical significant manner from the control group.[26] More recently, that investigation team also reported a prevalence of asthma in CRSwNP patients among the primary care setting of 44%.[27] Even though the prevalence of asthma was still high, it was significantly less prevalent than the one found in patients undergoing endoscopic sinus surgery (ESS). This suggests that patients with more severe upper airway disease have more frequently associated lower airway disease.[26]

The impact of asthma in ESS postoperative results for CRSwNP has generated a lot of controversy among researchers, with ones finding a negative impact of this comorbidity[28,29] and others not finding any influence at all.[30,31] An important methodological limitation of those studies is the absence of differentiation between atopic and non-atopic asthma, beside the non-differentiation of CRS types, that can definitely contribute to the divergence of the results that have been found.

Concerning chronic obstructive pulmonary disease (COPD), its relationship with CRS is even more poorly defined. A study made in Norway in 2015, with patients with COPD, asthma and controls submitted to magnetic resonance (MR), showed that the probability of paranasal sinus opacification was 6 times higher in COPD and two fold higher in asthmatic patients compared to the control group.[32] In addition, there are clinical research studies showing a high prevalence of sinonasal symptoms in COPD patients (75%) [33], and an inverse correlation between nasal patency evaluated by rhinomanometry and pulmonary airflow obstruction (FEV1%), and therefore to COPD disease severity.[34] It would be of value to determine with an endoscopic based study the prevalence of CRSsNP and CRSwNP in a COPD group of patients.

As discussed above, CRSwNP has a higher prevalence among elderly and it is important to be aware that in this age strata it is sometimes difficult to distinguish asthma from COPD, a fact that has inclusively introduced a new but still not well defined clinical entity, the asthma-COPD overlap syndrome (ACOS).[35] More investigation is needed to study the association of CRSwNP and these chronic lower respiratory diseases (CLRD).

1.1.3.4 Aspirin exacerbated respiratory disease (AERD)

Aspirin-exacerbated respiratory disease (AERD) is a condition characterized by CRSwNP, asthma and sensitivity to aspirin and other nonsteroidal anti-inflammatory drugs (NSAID), a triad called as Samter's triad, in behalf of Samter and Beers[36] that made it well known in scientific community. In these patients, aspirin and other NSAID that inhibit cyclooxygenase-1 (COX-1) induce unique non–IgE-mediated reactions that include a spectrum of respiratory reactions, including rhinitis, flushing, congestion, laryngospasm, and asthma exacerbations.[37] The prevalence of nasal polyposis in aspirin-sensitive asthmatics may be as high as 60-70%, as compared to less than 10% in the population of aspirin-tolerant asthmatics.[1] CRS patients with AERD tend to suffer from more extensive sinus disease and they benefit from ESS to a lesser extent than patients without AERD, with more predisposition for disease recurrence.[1] Among patients with CRSwNP, AERD represents 9.7% of the patients.[38] Patients with aspirin sensitivity, asthma and NP are usually non-atopic and the prevalence increases over the age of 40 years. It has a predominance for male gender and also seems to have an hereditary predisposition.[1,37]

1.1.3.5 Ciliary dyskinesia

Primary ciliary dyskinesia (PCD) is an autosomal recessive genetic disease, characterized by ciliary mobility disfunction with improper airway mucociliary clearance. There are currently 33 known genes associated with PCD, with mutation at *DNAI1* and *DNAH5*, which encode for components of the outer dynein arm complex in cilia, being the two most common genes associated with PCD.[39] About half of these patients presents with *situs inversus*.[39] In 1933, Kartagener recognized the combination of *situs inversus*, bronchiectasis and chronic rhinosinusitis, that was designated as Kartagener Syndrome.[40] In 1977, Eliasson et al. first coined the term "immotile cilia syndrome" for Kartagener Syndrome to associate infertility with chronic sinopulmonary infections.[40] In the literature, it has been published different cases of patients with Kartagener Syndrome presenting the CRSwNP phenotype.[40–42].

CRS affects $\geq$ 70% of patients with PCD and nasal polyps are prevalent in 15% to 30% of these patients, a much higher value compared to 3-4% in the general population.[43] Alanin MC et al. studied 24 PCD patients and found a significant improvement in CRS-related symptoms 12 months after ESS and adjuvant therapy (saline irrigation, topical nasal steroids and 2 weeks of systemic antibiotics plus instigations of local colistin if *Pseudomonas aeruginosa* was cultured at ESS). Moreover, a trend toward better lung function was observed after ESS. In this cohort nasal polyps were observed in 42% of the patients, including polyps in 2 children. Sinus hypoplasia was also a common trait in more than 50% with PCD.[43]

Secondary ciliary dyskinesia (SCD) can occur after epithelium injuries (e.g. viral airway infections, exposition to pollutants) and is commonly temporary. Mucostasis, hypoxia, microbial products, and mediators and toxic proteins generated during chronic inflammation probably all contribute to diminished mucociliary function. These factors decrease

mucociliary function by direct toxic effects on cilia, ciliary loss, other ultrastructural alterations in the epithelium and changes in the viscoelastic properties of the mucus.[44] In the majority of CRS patients, dysfunction in mucociliary function seems to be more a secondary than primary event, and is probably reversible, although restoration takes some time.[1]

1.1.3.6 Cystic Fibrosis

Cystic Fibrosis (CF) is caused by a defect in the protein responsible for chloride and bicarbonate transport (cystic fibrosis transmembrane conductance regulator protein, CFTR).[10] In patients with CF, the inability of the cilia to transport the viscous mucus cause mucociliary malfunction and consequently CRS. NP are present in about 40% of patients with CF and are generally more neutrophilic.[1] Nasal Polyps are rare in children and it has been reported that the majority of children with CRSwNP has CF[45], making it mandatory to exclude CF in every child presenting with nasal polyposis.

Annaes K et al. followed a cohort of 106 CF patients submitted to ESS plus adjuvant antibiotic treatment (two weeks of intravenous and 6 months of topical antibiotics) founding that the frequency of pulmonary samples with CF pathogens was reduced one-year after surgery, and the symptoms of CRS and quality of life significantly improved.[46] In CF patients a marked association exists between upper and lower airway cultures, with paranasal sinuses often being colonised by CF-lung-pathogenic Gram-negative bacteria. The combined treatment of CRS (ESS plus systemic and topical antibiotic treatment) seems to be of benefit in upper and lower airway disease in CF patients.[46]

1.1.3.7 Vasculitis

Eosinophil granulomatosis with polyangiitis (EGPA), formerly known as Churg-Strauss syndrome, is a rare systemic disease characterized by necrotising, granulomatous inflammation involving small to medium vessels, associated to severe asthma, peripheral and tissue eosinophilia and CRS. CRSwNP is present in about 60% of patients with EGPA[47], and there are many case reports about this association.[48–50] Following the criteria of the American College of Rheumatology, the diagnosis can be confirmed with high specificity and sensitivity if 4 of 6 of the following manifestations are present: bronchial asthma; eosinophilia >10%; sinusitis (acute or chronic; alternatively also opacity in imaging); (if applicable transient) pulmonary infiltrates; histologically confirmed vasculitis with detection of extravascular eosinophils; mononeuritis multiplex or polyneuropathy.[51] EGPA is considered as a ANCA (anti-neutrophil cytoplasmic antibodies) associated vasculitis, but less than 50% of patients have ANCA positivity.[50] When present they correspond to IgG class antibodies with a predominant perinuclear pattern (p-ANCA).(37, 40)

1.1.3.8 Immunodeficiency

Congenital immunodeficiencies typically manifest during infancy and childhood as frequent, chronic or opportunistic infections, with symptoms appearing early in life, commonly during infancy and childhood.[1] However, depending on the deficiency and dysfunction of the immune system, it can manifest later in life and present with CRS.[1] Therefore, EPOS2012 considers that immunological testing should be an integral part of the diagnostic pathway of patients with CRS.[1]

In 2015, a systematic literature review and meta-analysis has alerted that humoral immunodeficiency could be a frequent condition in patients with CRS.(46) This study revealed a immunodeficiency of total IgG, IgA and/or IgM in 13% of patients with recurrent CRS (not controlled by conservative management) and in 23% of patients with difficult-to-treat CRS (not controlled despite successful ESS and appropriate conservative treatment for at least one year).(46) The prevalence of IgG subclasses and specific antibody deficiency in CRS patients was 5 to 50% and 8 to 34%, respectively.(46) This study concluded that immunoglobulin deficiency is a frequent condition in CRS patients.(46) However, this study presented limitations that are inherent to the analysed studies: most of them were not controlled, had incomplete information about possible secondary causes of hypogammaglobulinemia (such as systemic corticoid use), did not separate CRS with and without nasal polyps, and used different methodologies and study designs.

Concerning immunological testing specifically in CRSwNP, the published literature is very scarce. In 2014, a non-controlled study in 161 patients with CRSwNP found a prevalence of IgG subclass deficiency of 13.7%, and they found no correlation between the presence of humoral deficiency and either symptom evolution after medical and surgical treatment or the dose of corticosteroids needed to control disease, after a follow-up of 5 years.[53] The authors concluded that a link between IgG subclass deficiency and nasal polyposis seemed unlikely. The lack of reference data on general population prevalence of IgG subclass deficiency and the absence of a control group, made it impossible to find any association between IgG subclass deficiency and NP.[53]

1.1.3.9 Environmental factors

The role of environmental factors in the development of CRSwNP remains undervalued and only a few studies have payed attention to it. In 1995, a Portuguese clinical-pathological study in 92 cases of CRSwNP, demonstrated with appropriate histochemical techniques that 69 out of 92 nasal polyps (75%) had inclusions of an exogenous material in the interior of the polyps (namely, particles of iron, wood, cement, cork, paper, glass, tobacco, textile fibres and chalk), that could be either localized inside the macrophages or free and sparse in the polyp tissue.[54] In that study, it was also interesting to observe that in 36 patients that abandoned their occupational exposure, only two (5.5%) demonstrated disease recurrence after a follow-up of 12 years.[54] In 2002, Kim J and Hanley J, suggested in a case control study with 55 CRSwNP patients and 55 controls, that the use of woodstove as a

principal source of heating or the occupational exposure to noxious inhalant compounds (other than tobacco smoke) constitute risk factors for NP development.[55] In 2012, Hox et al. also found that occupational exposures can be a risk factor for the occurrence of CRS and for its recurrence or persistence.[56] In 467 patients submitted to ESS for CRS, 25% had a relevant occupational exposure (reported on questionnaires sent by mail) and the prevalence of those exposures increased linearly and significantly with the number of ESS procedures.[56] Lastly, a multicentric study based on self-administered questionnaires conducted in China, that included 10,633 subjects, found a prevalence of CRS of 8.0% and found that some occupational and environmental exposures were strongly associated with CRS (namely, having a clearance-related job, occupational exposure to dust, occupational exposure to poisonous gas, a pet at home or carpet at home or at the workplace).[16] As discussed above, on epidemiology subchapter (see 1.1.2), studies as this last one based on self-administered questionnaires, are not truly reliable and do not allow to distinguish between CRSwNP and CRSsNP.

About cigarette smoking, there is controversial evidence about its role on CRSwNP. There are studies demonstrating that the prevalence of CRSwNP is reduced in smokers compared to the general population[57,58], while others reported smoking as a risk factor for CRSwNP recurrence.[59,60]

1.1.3.10 Anatomic Variations

Some authors have mentioned certain anatomic variations such as concha bullosa, deviated nasal septum or Haller's cells (infraorbital ethmoid cells), as potential risk factors for CRS development.[61,62] However, the studies that have made these assertations often establish CRS diagnosis by mucosal thickening on CT scan, when it has already been demonstrated that incidental mucosal thickening can occur in almost a third of asymptomatic population.[63] The majority of these studies are old, do not take in consideration the clinical definition of the disease and do not differentiate between acute rhinosinusitis (ARS), CRSsNP or CRSwNP.[9] Moreover, there are already published studies showing that the prevalence of anatomic variations in CRS patients is not superior to the ones found in the general population.[63] The International Consensus Statement on Allergy and Rhinology: Rhinosinusitis published in 2016 (ICAR:RS 2016) considers that CRSwNP patient populations have rarely been independently studied to determine the influence of anatomic variation on this disease and the few studies that independently evaluated this group of patients suggested a minimal influence on pathophysiology and instead favoured a systemic inflammatory process leading to sinonasal disease.[9] Later, a study in CRSwNP patients and controls found a higher prevalence of some anatomic variations in CRSwNP patients (i.e. deviated septum, *concha bullosa, agger nasi* cells, frontal hypoplasia and accessory ostium in maxillary sinus).[64] In this study, the sample selection for control group is not well explicit and only some kind of septum deviations were considered. As already reported on PCD

subheading, sinus hypoplasia seems to be a common trait in these patients that are also prone to develop nasal polyposis.[43]

Further research is needed about this topic. The role of anatomic variations on CRSwNP pathogenesis remains controversial, meanwhile it is valuable to recognize them for a good surgical planning of these patients.

1.1.3.11 Other factors

Concerning alpha-1-antitrypsin (A1AT), there are two published articles about SERPINA1 gene polymorphisms in CRSwNP patients. One of these case-control studies was realized in Canada and demonstrated an association between single nucleotide polymorphisms (SNPs) of the SERPINA1 gene (rs1243168 e rs4900229) with clinically severe CRSwNP.[65] The other study took place in Germany and reported a significantly higher prevalence of genetic A1AT polymorphisms (PI-MS and MZ) among CRSwNP patients compared to controls.[66] It is a topic with scarce data about it, but the existing results seem to be consistent and deserve to be replicated in other populations.

The role of vitamin D in CRSwNP etiopathogeny has been ambiguous. Vitamin D has been shown to be a potent immunomodulatory steroid hormone involved in the regulation of epithelial cells, dendritic cells, monocytes, macrophages and T-cells functions.[9] In 2016, a systematic review suggested a correlation between low vitamin D and polypoid CRS phenotypes.[67] However, this review included only seven articles, three of them with retrospective character, the majority without a control group, and with heterogeneous methods for vitamin D dosage, for reporting the outcomes and for analysis of the confounding variables. More studies with rigorous methodology must be done to clarify this possible association.

1.1.4 Historical overview on CRSwNP etiopathogeny

Historically, CRSwNP used to be seen as the end stage of severe atopic status.[1] The limitations of this theory were obvious for many authors but the lack of consistent alternative hypothesis hampered its withdrawal.

One of the first attempts to explain CRS etiopathogeny was called "fungal hypothesis", that resulted from Ponikau JU et al. research in 1999 [68], which attributed all CRS to an excessive host response to Alternaria fungi. The role of fungi in CRS has generated many debates in scientific community, while new and more sensitive techniques have shown that the presence of intranasal fungi is ubiquitous, in CRS and controls.[69] Almost one decade after, this theory was rejected due to the absence of convincing immunologic data or clinical evidence of benefit with antifungal treatment. However, fungi are still believed by many to play a role as a disease modifier in some subtypes of CRSwNP, e.g. Allergic fungal rhinosinusitis (AFRS).[1]

Meanwhile, another mechanism has been proposed for CRSwNP etiopathogeny, the "leukotriene hypothesis", which considers the existence of defects in arachidonic acid metabolization (the eicosanoid pathway), leading to increased synthesis of pro-inflammatory leukotrienes and decreased synthesis of prostaglandin E2 (PGE2), that has an important anti-inflammatory function on respiratory tract.[1] This theory is most closely associated to patients with aspirin intolerance but have also been proposed as a potential cause of CRSwNP in general.[1] One of the studies about this topic, found that the level of cysteine-leukotrienes were significantly elevated in polyp tissue of patients with CRSwNP and aspirin sensitivity, followed by patients with CRSwNP and aspirin tolerance, and by patients with CRSsNP (in a decrescent way) when compared to levels of nasal mucosa of control subjects.[70] The reduction in PGE2 synthesis and mRNA expression of cyclooxygenase-2 (COX-2) were specifically observed in CRSwNP group, and although more evident in patients with aspirin sensitivity were not restricted to that subgroup.[70,71] While some evidence supports this theory in CRSwNP, enthusiasm is muted by the limited clinical efficacy of leukotriene pathway inhibitors.[1,9]

Another proposed theory was the "staphylococcal superantigen hypothesis" based on the findings that the most common bacterial species found in the nasal mucus is Staphylococcus aureus, and that specific IgE antibodies against staphylococcal enterotoxin A (SEA) and B (SEB) have been detected in nasal polyps[72], which were associated with eosinophilic inflammation and raise in total IgE detected in CRSwNP.[1] Superantigens are capable of stimulating T cells in a nonspecific way, causing a polyclonal activation of T cells with a massive cytokine release. The net effect is a Th2 skewing, accentuated eosinophil and mast cell activity and heightened tissue damage and remodelling.[1] However, the superantigens effect was only demonstrated in approximately half of CRSwNP patients, being regarded by many as disease modifiers, rather than discrete aetiologic agents. The

superantigen effect primarily addresses Caucasian patients with CRSwNP as it was rarely observed in Asiatic patients.[73]

Other formulated hypothesis was called the "immune barrier hypothesis" that defended that defects in mechanical barrier and/or innate immunity response of the sinonasal epithelium could manifest as CRS, leading to increased microbial colonization and accentuated barrier damage, causing stimulation of the compensatory adaptative immune response. This theory does not specifically explain the differential Th2 skewing and B-cell infiltrate seen in Western CRSwNP.[1,73]

Lastly, the "biofilm hypothesis" suggested that biofilms, in particular staphylococcal biofilms, could serve as etiologic agents in CRS.(66) It can be speculated that a defect in immune barrier might facilitate formation on biofilms. The mechanism of biofilms formation and worsening of CRS remains unclear[1], but it has been suggested that staphylococci sequestered in biofilms can lead to superantigens production that trigger Th2 skewing and eosinophilic polyposis.[73]

To sum up, CRS is still described as a multifactorial disease with obscure etiopathogeny. There is however, an emerging consensus that the persistent inflammation that defines CRS results from a dysfunctional host-environment interaction involving exogenous agents and changes in sinonasal mucosa. This overall concept is in agreement with the current understanding of the etiology and pathogenesis of chronic mucosal inflammatory disorders in general, which describe multiple balance interactions between host, commensal flora, potential pathogens and exogenous stresses.[1]

Chapter 2.
AIMS OF THE THESIS

2. Aims of the thesis

CRSwNP still has an unknown etiopathogeny and there is controversial evidence about the risk factors for disease occurrence and recurrence. Considering the emerging consensus that chronic rhinosinusitis (CRS) results from a dysfunctional host-environment interaction, this study pretended to clarify which exogenous and endogenous factors contribute to the occurrence and perpetuation of sinonasal mucosa inflammation observed in CRSwNP.

The objectives of this study were:

1) To evaluate endoscopic sinus surgery (ESS) efficacy in CRSwNP treatment and to establish prognostic factors for disease recurrence (Chapter 3);

2) To compare the prevalence of nasal polyposis (NP) in a group of workers with occupational dust exposure and in a control group (Chapter 4);

3) To characterize systemic immunological alterations that occur in patients with CRSwNP compared to controls (Chapter 5);

4) To clarify the role of food allergy in CRSwNP disease, comparing serum levels of food specific IgE and IgG antibodies in cases and controls (Chapter 6).

Chapter 3.

ENDOSCOPIC SINUS SURGERY FOR CRSwNP:

CLINICAL OUTCOME AND PREDICTIVE FACTORS OF RECURRENCE

3. Endoscopic Sinus Surgery for CRSwNP: clinical outcome and predictive factors of recurrence

3.1 Introduction

Endoscopic sinus surgery (ESS) for nasal polyposis has been generally reported to be a safe and effective procedure and is usually done in CRSwNP that is refractory to medical treatment.[1] In 2006, a systematic review about safety and effectiveness of ESS in CRSwNP treatment was published, reporting recurrence rates between 4-60% and a need for reintervention of 3-42%.[74]

Concerning prognostic factors that can help to identify patients that are more prone to disease recurrence or in need for a reintervention, there are important differences among the published studies. This variability can, in part, be due to the used methodology, including in sample selection (which in general do not distinguish between CRSwNP and CRSsNP) and in the statistical analysis performed (commonly using bivariate analysis without control on the interaction and confounding effect between variables).

One of the factors that has created controversy, is the asthma impact on postoperative results of ESS in CRS, with some studies showing a negative impact[28,29] and others not displaying any influence at all.[30,31] These published studies held important limitations that can contribute to this divergence, namely the absence in distinction between atopic (IgE-mediated) and non-atopic (non-IgE-mediated) asthma, plus the absence in CRS types differentiation.

Our hypothesis is that ESS is an effective treatment for medically recalcitrant CRSwNP and that some independent variables may influence surgical outcomes in these patients, namely: sex, age, occupational dust exposure, atopic and non-atopic asthma, allergic rhinitis, smoking habits, nasal polyps endoscopic grade, Lund-Mackay score or postoperative topical steroid compliance. Our main goals were to evaluate the efficacy of ESS in patients with CRSwNP, including symptoms relief, complications and recurrence rate, and to try to identify independent predictors of recurrence in a multivariate regression analysis.

The identification of predictive factors of recurrence can help to establish a more accurate prognosis concerning surgical results and, when possible, it can help to prevent or reduce that risk after modifying those factors.

3.2 Materials and Methods

The present study was performed according to established ethical guidelines in accordance to Declaration of Helsinki Principles. Retrospective analysis of patients submitted to endoscopic sinus surgery (ESS) due to CRSwNP, in a district hospital center (Alto Ave Hospital Center), from January 2004 to December 2013.

Diagnosis of CRSwNP was established using the definition of EPOS2012.[1] Patients proposed for ESS had refractory disease to topical long-term and systemic short-term steroids. A total of 85 patients were selected from the hospital database after applying the following exclusion criteria: patients with concomitant benign or malignant sinonasal tumors; no available preoperative computer tomography (CT) scan on hospital computer network and a follow-up period lasting less than 9 months. Postoperative appointments were scheduled quarterly with discharge at 2 years of follow-up if no symptoms or signs of recurrence were present.

Clinical records were reviewed to collect information on demographics, occupational history (including types of dust exposure), smoking and alcoholic habits, comorbidities, previous nasal surgeries, pre and postoperative symptoms and ENT examination findings, CT results, medical and surgical treatment, perioperative complications and histopathological findings. Whenever the occupational information was missing from clinical records, data was collected by phone interview. Patients were considered to have occupational dust exposure if it occurred during the performance of their job duties, almost daily and in a repetitive way. To evaluate for disease recurrence and postoperative symptoms it was considered the last ENT outpatient clinic visit of the patient, in order to have the longest possible follow-up period. The diagnosis of IgE-mediated and non-IgE-mediated asthma and allergic and non-allergic rhinitis was established by a respiratory physician or an immunoallergologist. Nasal polyps were classified endoscopically in grade I, II or III, according to Lund criteria.[2] Anatomical variations and compatible CRSwNP findings in CT scan were also registered, as well as Lund-Mackay score.[75]

Statistical Analysis

Statistical analysis was performed with *Statistical Package for Social Sciences* (IBM® SPSS® Statistics for Windows, Version 23.0).

Descriptive statistics was used in sample characterization. An evaluation of surgical efficacy for each symptom, comparing the pre and postoperative group, was done using McNemar test (for two related samples), except for nasal obstruction were a Binomial test had to be used since a 100% of preoperative group had the symptom. Patients with and without recurrence of nasal polyposis were then divided in two independent groups and compared for multiple factors. For categorical variables, Chi-Square test (or Fisher exact test when assumptions needed for the previous test were not verified) was used to test for variable association. For continuous quantitative variables, Mann-Whitney test was applied.

A logistic regression analysis was carried out to evaluate the association of multiple different variables with nasal polyposis recurrence. Likelihood ratio test and Hosmer and Lemeshow test were performed in order to evaluate model significance and its goodness of fit. Nagelkerke's coefficient determination (pseudo-R^2) was obtained to determine the proportion of dependent variable variance explained by the logistic regression model. Wald's and score tests were done to evaluate the impact of each independent variable on the recurrence of the disease. For each significant independent variable, Odds Ratio (OR) and 95% confidence interval (CI95%) were calculated. Analysis of residuals, control variables and multicollinearity were performed. Finally, the area under the ROC Curve was calculated to establish the discriminatory power of the model.

Statistical significance was accepted to correspond to a p-value of less than 0.05.

3.3 Results

A total of 85 patients were included, 55 males and 30 females, with a mean age of 47 ±13 years. Thirty-eight percent of patients suffered from concomitant asthma (from these, 56% had IgE-mediated asthma and 44% non-IgE-mediated asthma); 31% had allergic rhinitis and 17% presented chronic obstructive pulmonary disease (COPD). Samter's triad prevalence was 6%. There were no patients with cystic fibrosis on this sample. Seventy-seven percent of patients were non-smokers. When asked about occupational inhalant exposure, 60% of patients reported exposure to dust (85% to organic and 15% to inorganic dust). Using the European Academy of Allergy and Clinical Immunology (EAACI) task force[76] classification for inhalant agents, 9.6% of patients were exposed to high molecular weight (HMW) (>5 kDa) substances, while 90.4% were exposed to low molecular weight (LMW) (<5 kDa) particles. The prevalence of atopy (IgE-mediated allergy) among patients exposed to occupational dust exposure was 27%. Sample demographics, comorbidities, smoking habits and occupational history to dust inhalation are summarized in Table 1.

Table 1- Sample demographics, comorbidities, smoking habits and occupational history. (N=85).

- Demographics		
Age	47 ± 13 Years	[15 ; 73]
Gender	**n**	**%**
Male	55	65
Female	30	35
- Comorbidities		
Asthma	32	38
IgE-mediated (atopic)	18	21
Non-IgE-mediated (non-atopic)	14	17
Samter's Triad	5	6
Allergic Rhinitis	26	31
COPD	14	17
- **Smoking Habits**		
Non-smoker	65	77
Ex-smoker	14	17
Smoker	6	7
- Occupational history		
Inhalant dust exposure	52	60
Organic agents		
Cotton, Flax, Sisal	23	27
Fuel gas, Paints, Glues	15	18
Flours	5	6
Wood dust	1	1
Inorganic agents		
Metallic compounds	2	2
Cement	2	2
Pesticides	2	2
Bleach, Chlorine dioxide	2	2
No dust exposure	33	40

COPD- chronic obstructive pulmonary disease

Twenty-five percent of patients had previous history of nasal surgery. Of those, 12 patients had already been submitted to ESS, seven to septoplasty and two to both of these surgeries. The majority of cases (53%) were staged as grade III polyposis, 34% as grade II and the remaining 13% as grade I. Anatomical variations detected in preoperative CT scan are depicted in Table 2. Mean bilateral Lund-Mackay score was 16.6±4.6 (range 6 to 24).

Table 2- Anatomical variations on CT scan and their absolute and relative frequencies.

Anatomical Variations	n	%
Deviated Septum	46	54
Kuhn Cells	24	28
Bullous Concha	25	29
Frontal Sinus Hypoplasia	15	18
Haller Cell	7	8
Frontal Sinus Agenesis	5	6
Paradoxal Turbinate	5	6
Maxillary Sinus Hypoplasia	5	6
Overpneumatized Ethmoid Bulla	1	1

Endoscopic sinus surgery was performed bilaterally in every case and included always the anterior ethmoidectomy, followed in frequency by maxillary sinus antrostomy and posterior ethmoidectomy, associated with other procedures as needed (Table 3). In the majority of patients in whom frontal sinusotomy was necessary, a type I drainage (Draf I) was established by anterior ethmoidectomy, identifying frontal recess, without touching its mucosa. In 30% of patients, with more severe disease and in revision cases, a Draf IIa was performed with extended drainage achieved after ethmoidectomy by resecting the floor of the frontal sinus between the lamina papyracea and the middle turbinate (Table 3).

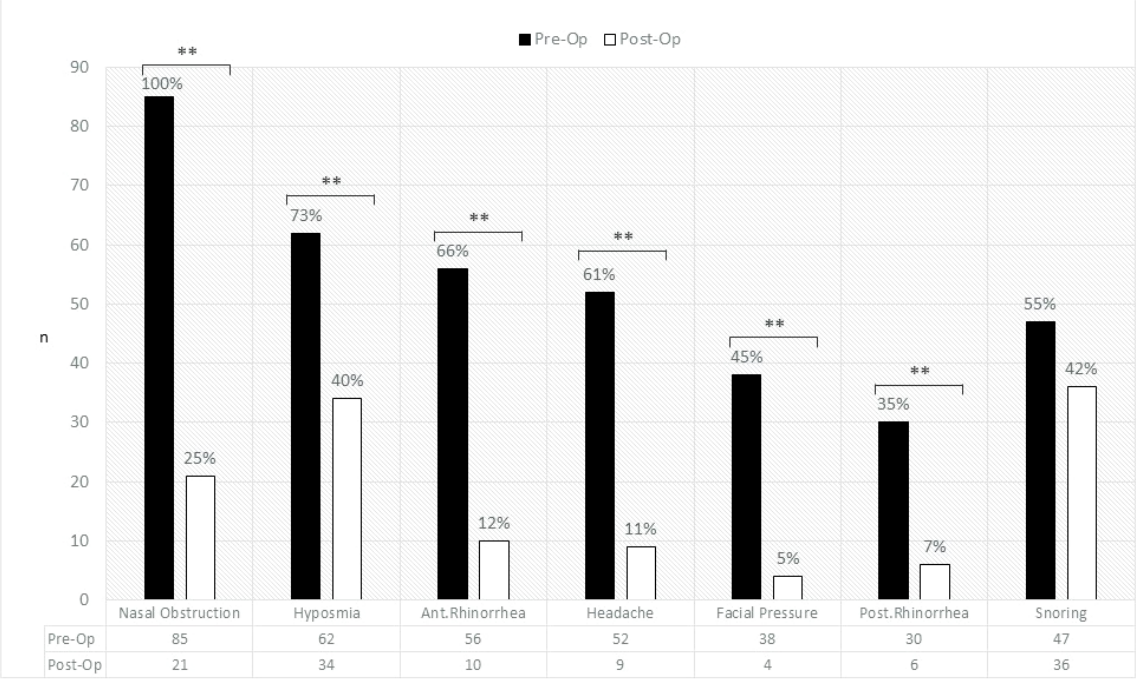
One case (1.2%) of cerebrospinal fluid (CSF) fistula occurred, which was repaired with a medium turbinate flap and fibrin glue. Other minor complications were observed in 13 cases (15.3%): periorbital cellulitis (1 case), discreet periorbital ecchymosis (9 cases) and epistaxis (3 cases).

The routine histopathological evaluation with hematoxylin and eosin staining confirmed CRSwNP diagnosis and revealed tissue eosinophilia (>10 eosinophils/hpf [77]) in all cases. Topical steroids were prescribed to all patients postoperatively, with a medication compliance rate of 47%. Non-compliers included 29% of occasional steroids users and 24% of no compliance at all. The prescribed steroids were fluticasone propionate or mometasone intranasal spray, applied twice-a-day.

Table 3- Surgical procedures and their absolute and relative frequencies.

Surgical Procedures	n	%
Anterior Ethmoidectomy	85	100
Maxillary Antrostomy	79	93
Posterior Ethmoidectomy	69	81
Frontal Sinusotomy ▪ **Draf Type I** ▪ **Draf Type IIa**	68 42 26	80 49 31
Sphenoidotomy	13	15
Inferior Turbinoplasty	28	33
Middle Turbinoplasty	15	18
Septoplasty	31	36

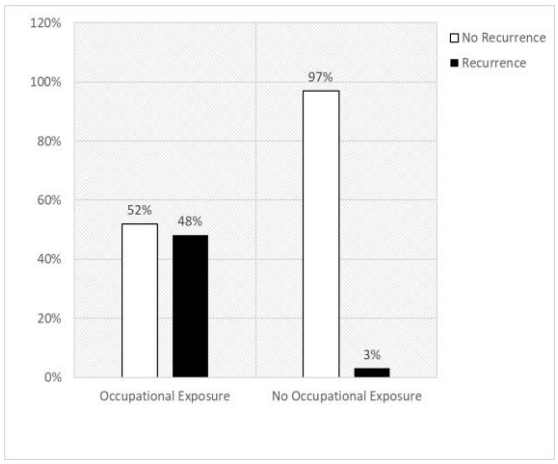
The main preoperative reported complaints were nasal obstruction (100%), hyposmia (73%), anterior rhinorrhea (66%) and headache (61%). All rhinologic symptoms had a statistically significant reduction after endoscopic surgery, in contrast with snoring which did not improved significantly (Graphic 1).



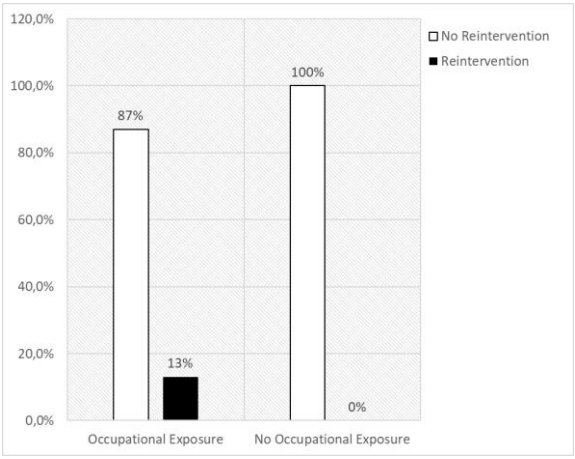
Graphic 1- Relative frequencies of the preoperative and postoperative symptoms. Absolute frequencies are presented in the table under the graphic bars. Abbreviation Legend: Pre-op= preoperative; Post-op= postoperative; Ant. Rhinorrhea= anterior rhinorrhea; Post. Rhinorrhea= posterior rhinorrhea, **p<0.01.

Postoperative recurrence was diagnosed when endoscopic nasal polyps were detected. The main recurrence site was the ethmoid region. The mean follow-up time was 27 months (range 9-108), the recurrence rate was 31%, but with only 7% requiring surgical reintervention. The need for new surgery was determined according to patient´s complaints and ENT examination. The rate of follow-up loss was 5.9%.

Patients with occupational dust exposure had a statistically significant higher recurrence of polyps (48%), compared to the recurrence of patients with no dust exposure (3%), $\chi^2(1)=19.25$, $p=5.5x10^{-6}$ (Graphic 2). Thirteen percent of patients exposed to dust needed surgical reintervention, in contrast with patients not exposed, who did not need revision surgery; this difference was significant using Fisher exact test (p=0.04) (Graphic 3). Patients with Samter's triad did not present a statistically significant higher recurrence rate compared to aspirin tolerant patients (Fisher exact test, p=0.165).



Graphic 2- Relative frequencies of CRSwNP recurrence in the group with and without occupational dust exposure.



Graphic 3- Relative frequencies of surgical reintervention for CRSwNP in the group with and without occupational dust exposure.

Multivariate analysis was performed with logistic regression analysis using the Forward LR method. Likelihood ratio test demonstrated that the created adjusted model was significantly better than the null model ($p=1.2x10^{-7}$). Hosmer and Lemeshow test had a p-value of 0.503, which means that the adjusted logistic model fits well to the data. Nagelkerke's coefficient determination (pseudo-R^2) was 0.44, suggesting that 44% of disease recurrence variability is explained by the adjusted logistic model.

Wald's test and score test identified occupational dust exposure and non-IgE-mediated asthma as independent predictive factors for CRSwNP recurrence (p=0.001 and p=0.012, respectively), unlike the other studied variables that were found to be non-significant: sex, age, IgE-mediated asthma, allergic rhinitis, smoking habits, Lund-Mackay score, nasal polyps endoscopic grade, and postoperative topical corticoid use (Table 4).

Patients with occupational exposure to dust had 38 times more chance of recurrence than the non-exposure group, CI95%: [4; 345]. Patients with non-IgE-mediated asthma were more likely to develop recurrence, with 8.7 times more chance than non-asthmatic patients, CI95%: [2; 46]. The potential confounding effects of sex, age, smoking habits, Samter's triad, Lund-Mackay score, nasal polyps endoscopic grade, postoperative topical corticoid use, have been statistically controlled. No problems of multicollinearity were identified when analyzing all the variables in study (condition indices for all 11 dimensions below 30). Two outliers were identified and evaluated, but we did not find a reason to exclude them. The final model allowed to correctly identify the recurrence disease in 79% of the cases and presented a good discriminatory capacity (ROC Area under curve=0.822; p<0.001; CI95%: [0.73;0.91]).

Table 4- Independent predictive factors of recurrence in logistic regression analysis.

Variables	B	DF	Wald	p-value	Exp(B)	CI95%
Occupational Dust Exposure	3.638	1	10.433	**0.001** *	38.015	4.18 - 345.69
Non-IgE-mediated Asthma	2.158	1	6.378	**0.012** *	8.650	1.62 - 46.16
Constant	-4.072	1	13.254	**0.000** *	0.017	

* p<0.05; DF= Degrees of freedom; Exp(B)= exponentiation of the B coefficient, odds ratio; CI= Confidence Interval

3.4 Discussion

In accordance to previously published studies that found a male predominance in CRSwNP, an average age of disease onset of 42 years and rare cases under 20 years[1], we also observed a higher frequency of nasal polyps in men (male to female ratio of 1.8:1), a mean age of 47 years and only 3 cases under 20 years.

Asthma prevalence in our study (38%) is similar to the one found by the questionnaire-based study of Klossek et al.[78], which found wheezing and respiratory discomfort to be present in 31% and 42% of patients with CRSwNP, respectively. Another recently published study, which performed peak expiratory flow, spirometry and bronchodilation tests in every patient with CRSwNP undergoing ESS (N=40), found an asthma prevalence of 65%, with 25% of previously undiagnosed and unrecognized asthma.[26] This call for a closer collaboration between otorhinolaryngology and respiratory medicine, in research as well as in clinical practice.[26]

A systematic review about symptom-specific outcomes of endoscopic sinus surgery in CRS concluded that all studied symptoms improved in a similar way for major CRS symptoms with the exception of nasal obstruction, which improved more than headache or hyposmia.[79] In accordance to it, our study demonstrated a statistically significant improvement in all rhinologic symptoms and found hyposmia to be the most difficult one to treat, remaining the first reported postoperative complain, as already stated in previous studies.[80] The outcomes of endoscopic sinus surgery on olfaction are challenging to predict and previous studies have documented a wide range (17-75%) of improvement in olfactory function.[81] Our 33% improvement on impaired sense of smell is in accordance to these data. On the other hand, no statistically significant reduction in snoring was observed; this fact is not surprising as sleep-disordered breathing (SDB) is often due to a multilevel collapse of the superior airways. A direct correlation between the degree of nasal obstruction and the severity of SDB has not been found and, certainly, nasal obstruction does not appear to be the main contributing factor in the majority of patients with moderate to severe obstructive sleep apnea syndrome (OSAS).[82] A recent study, with 139 patients with CRS, found a prevalence of OSAS of 64.7%; despite the high prevalence found, OSAS and rhinosinusitis severity were not correlated and nasal polyps did not worsen sleep problems in CRS patients.[83]

As previously described, a 2006 systematic review about ESS effectiveness in the treatment of CRSwNP found a recurrence rate between 4-60% with a median value of 20% across all evaluated studies and a need for revision surgery in the range of 3–42% with a median of 6%.[74] The recurrence found in our study of 31% and the need of re-intervention of 7% are in accordance with these values. The wide dispersion of the recurrence rates found in the literature can be explained not only by the used technique and surgeon expertise, but also due to geographic differences. Recurrence can be higher in areas in which primary and secondary work sectors predominate, where employees have occupational dust exposure. Our

prevalences of major and minor complications (1.2% and 15.3%) are also comparable to the ones reported in the 2006 systematic review [74], which found prevalences of major and minor complications, of 0-1.5% and 1.1-20.8%, respectively.

It is accepted that there is a difference in the pathology of nasal polyps between Western and Asian populations. While approximately 80% of polyps in Western patients are eosinophilic, <50% of polyps in Asian patients show tissue eosinophilia above that seen in control tissues. A recent work done in Japan has shown that mucosal eosinophilia was significantly correlated with recurrence after ESS.[84] Since in our case, all patients presented eosinophilic polyps this topic could not be addressed.

Samter's triad is considered as a subgroup of CRS characterized by a higher burden of disease and a challenging disease entity to manage, with surgery aimed toward symptom control rather than cure.[85] The fact that we did not find a statistically significant difference in recurrence rate in these patients may be due to the small representation of these patients in our sample (5 cases, 6% of patients).

A great point of controversy has been the impact of asthma in surgical outcomes of CRS. There are some studies pointing to a negative impact[28,29], while others found no influence at all.[30,31] A great limitation of these published studies is that most of them do not separate atopic (IgE-mediated) and non-atopic (non-IgE-mediated) asthma. In order to comply with EAACI recommendations, and because the mechanisms initiating non-IgE-mediated allergic asthma and non-allergic asthma are not well defined, we classified asthma as IgE-mediated or non-IgE-mediated asthma.[86] We found a significant negative impact of non-IgE-mediated asthma in surgical outcomes of CRSwNP, raising the chance of recurrence about nine times ($p=0.012$, OR=8.7, CI95%: [2; 46]), in contrast to IgE-mediated asthma with no significant impact ($p=0.274$) on disease recurrence. A recent systematic review about management of CRSwNP and coexisting asthma stated that the effect of ESS on pulmonary outcomes has been a question of great debate and it was found a low strength of evidence to support a positive effect of ESS on asthma.[87] It would be interesting to analyze and compare the surgical impact of ESS on pulmonary function of patients with IgE-mediated and non-IgE-mediated asthma.

In 2012, Hox et al. suggested that occupational exposures can be a risk factor for the occurrence of CRS and for its recurrence or persistence.[56] Patients submitted to ESS for CRS were inquired and a relevant occupational exposure was reported in 25% of all responding patients (n=467). The prevalence of occupational exposures increased linearly with the number of ESS procedures, from 21% in those who had one ESS to 44% in those who had four or more ESS ($p<0.001$).[56] This study presented some limitations, namely the fact that it was based on a self-administered questionnaire sent by mail and the evaluation of CRS in general. Our work was pioneering in studying the impact of occupational exposure specifically on CRSwNP. Our prevalence of reported exposure to organic and inorganic dust was much higher (60%) comparing to the results of Hox et al. (25%), pointing to a meaningful effect of this factor in CRSwNP, which is supposed to be higher than in CRSsNP, according to these results.

In accordance, our study reveals that occupational exposure to dust has a negative outcome on CRSwNP treatment, with exposed patients having 38 times more chance of recurrence compared to the non-exposed group (p=0.001, OR= 38, CI95%: [4; 395]). We can speculate that similar to what has already been described for occupational rhinitis, occupational dust exposure can be involved in the pathophysiology of CRSwNP by different mechanisms: immunologically mediated hypersensitivity reactions (antibody or cell-mediated), designated as allergic reactions or through irritant, non-immunological mechanisms.[76] While HMW agents are biological substances from vegetable or animal origin (e.g. flour, latex, laboratory animals) known to cause IgE-mediated immune responses, the mechanisms linked to LMW agents have not been fully characterized. LMW agents induce non-IgE-mediated mechanisms as airway sensitizers (e.g. wood and metal dust, resins), with a latency period between exposure to symptoms of weeks to years, or as airway irritants (ex. chlorine, ammonia) with symptoms of acute onset.[88] Hox et al. reported an exposure to HMW agents in 5% and to LMW agents in 95% of the cases with CRS and relevant occupational exposures[56], with very similar results to what we found on the subgroup of CRSwNP (9.4% and 90.6% for HMW and LMW, respectively). Since LMW substances are also known to cause more severe occupational asthma than HMW and that they act mostly through non-IgE related mechanisms[89], this can explain our finding that non-IgE-mediated asthma was a poor prognostic factor for CRSwNP recurrence and it may be a marker of disease severity. The fact that the prevalence of atopy (IgE-mediated allergy) among patients exposed to occupational dust exposure was 27%, and that this value is in the range of the reported prevalence of atopy in general population (20-30%)[90], also supports the hypothesis of important non-IgE related mechanisms in occupational airway inflammation, including immune and non-immune ones.

As this represents a problem of Public Health, these findings show the importance of employee's protective measures, such as mask use during work, and reinforces the need for legislation and control to guarantee the functioning of air dust filters and exhausting systems. Patients with CRSwNP should work in a free dust environment, whenever possible.

There are wide differences among the published literature about prognostic factors that can identify the most susceptible patients to CRSwNP recurrence and predict the need for reintervention. This fact can be explained by different methodologies and types of studies used, including sample selection (which often includes patients with CRSwNP and CRSsNP, mixed together), and type of statistical analysis performed. In many cases, the correlations of dependent and independent variables are done individually, but in this kind of study, logistic regression analysis with multiple variables is the best way to identify the influence of the different variables, controlling at the same time the problems of multicollinearity and confounding effects. This study has some limitations: retrospective character of the study, subjective evaluation of occupational exposure by the patients and a moderate sample size.

By the time the patients were observed in consultation there was not still available a validate quality of life survey in European Portuguese, which would have added value to this

work, if applied. In our center, CRSwNP postoperative protocol treatment includes only topical corticoid spray. Recently, postoperative corticoid nasal irrigation was introduced as a therapeutic aid in CRS, with safety and effectiveness studies that are making this procedure becoming accepted by many physicians, especially in most difficult to treat cases.[91,92] This issue should be addressed in a future multivariate logistic regression analysis, to see if the corticoid delivery method has independent prognostic impact on CRSwNP recurrence.

The fact that the study was conducted in a single hospital unit, limits the variability in therapeutic options, operative techniques and postoperative care, making the groups, with and without nasal polyposis recurrence, more comparable.

More clinical investigations are needed to clarify these associations, namely prospective studies that can evaluate if there is a cause-effect relation between occupational dust exposure and CRSwNP, and if concomitant non-IgE-mediated asthma represents a mark of disease severity (following the concept of "one airway, one disease"[25]).

Conclusion

These results suggest an important role of occupational inhalants exposure in the physiopathology of chronic rhinosinusitis with nasal polyps. Avoidance of dust can be an important measure to prevent disease progression and recurrence after treatment, and non-IgE-mediated asthma may represent a mark of disease severity.

Chapter 4.

HIGHER PREVALENCE OF NASAL POLYPOSIS AMONG TEXTILE WORKERS: AN ENDOSCOPIC BASED AND CONTROLLED STUDY

4. Higher prevalence of nasal polyposis among textile workers: an endoscopic based and controlled study

4.1 Introduction

There is a deficit of epidemiologic studies exploring the prevalence of chronic rhinosinusitis with nasal polyps (CRSwNP), especially in European countries.[1] When reviewing the current literature, it becomes clear that giving an accurate estimate of CRSwNP prevalence remains speculative, mainly due to the diagnostic imprecision often used in publications, which are mostly based on symptoms questionnaires. The data obtained with such approach can be unreliable as not all patients that claim to have the disease have nasal polyps on endoscopy and asymptomatic polyps will be unaccounted for. Therefore, EPOS2012 expert panel considers endoscopy as a prerequisite for accurate estimate of the prevalence of nasal polyposis (NP) and alerts to the need to distinguish between clinically silent NP or preclinical cases, and symptomatic NP.[1] In Europe, there are only two endoscopic based studies published, an in-vivo study done in Sweden which found a prevalence of 2.7%[17] and a cadaver study done in Portugal, which found a prevalence of 5.5%.[18]

Recent data suggests that occupational dust exposure may be involved in chronic rhinosinusitis (CRS) physiopathology. In 2012, Hox et al. stated that occupational exposures can be a risk factor for the occurrence of CRS and for its recurrence or persistence.[56] Patients submitted to endoscopic sinus surgery (ESS) for CRS were inquired and a relevant occupational exposure was reported in 25% of all responding patients (N=467). The prevalence of occupational exposures increased linearly with the number of ESS procedures needed by each patient ($p<0.001$).[56] In 2016, a multicenter cross-sectional study done in China was published (N=10,633) suggesting that some occupational and environmental exposures (specifically, having a clearance-related job, occupational exposure to dust, occupational exposure to poisonous gas, a pet at home or carpet at home or at the workplace) were risk factors for CRS.[16] In that study, CRS had a prevalence of 8% according to EPOS2012 criteria for epidemiology studies.[16] Nevertheless, that study was based on self-administered questionnaires for CRS diagnosis and occupational and environmental history; and no separation between patients with CRSwNP or patients with CRSsNP (chronic rhinosinusitis without nasal polyps) was made.

As presented in the previous chapter (Chapter 3), our investigational work also demonstrated in a multivariate logistic regression analysis, that occupational exposure to dust had a negative impact on CRSwNP postoperative outcome (N=85), with exposed patients (60%

of the sample) having 38 times more chance of recurrence compared to the non-exposed group (p=0.001).

In light of the above, we performed an epidemiological study comparing the prevalence of NP in workers with and without occupational exposure to dust, using nasal endoscopy for NP screening. We decided to make this screening among textile workers as we found in our previous retrospective study that 60% of CRSwNP patients were exposed to dust during their job duties, most of it being textile particles (Table 1, Chapter 3).

The aim of this research was to establish the prevalence of NP in a group of workers with occupational exposure to dust (textile workers) and to compare it with its frequency among a non-exposed group (retail store workers).

4.2 Materials and Methods

The study was performed according to established ethical guidelines and approval of Ethics Committee at the Health Sciences Faculty, Beira Interior University. A signed informed consent was obtained from each participant in the study.

A descriptive cross-sectional study was carried out to determine NP prevalence among two groups: textile workers (exposed group) and retail store workers (controls). This study took place in Castelo Branco District, within the Interior Centre Region of Portugal. This area is internationally known for its textile industry, mainly wool manufacturing. For the purpose of the study, a total of 357 workers were recruited, 254 textile workers and 103 controls. The sample was randomly selected using employee's numbers at the personnel database of the Factory/Retail Store. Textile workers were recruited from an industrial unit with a total workforce of 509 employees, comprising subjects from every working sector (spinning, warping, weaving, dyeing, finishing, quality control, storing and packing, informatics and marketing, administration, designing, woodwork). There were only included those with a minimum of one year's work. This Factory manufactures mainly pure wool fabrics and wool rich or polyester/wool mixtures and, in lesser extent, cotton, linen and lycra products. The individuals for the control group were recruited from two retail stores from the same geographic area of the factory. In the control group, individuals who referred previous jobs in textile industry at any time or other jobs/hobbies with relevant dust exposures (such as construction workers, woodworkers, bakers) in the last 10 years were also excluded.

Clinical data was gathered through a systematic interview to collect information on demographics, occupational history (including working sector, types of dust exposure, years of exposure, mask use), domestic or hobby dust exposures, smoking and alcoholic habits, comorbidities, previous nasal surgeries, nasal symptoms and their duration. Atopy (based on positive skin prick test or IgE antibodies in serum), chronic lower airway disease and obstructive sleep apnea syndrome (OSAS) history were only considered positive when previously diagnosed by a specialist physician in each area. Subjective assessment of upper and lower airway symptoms and quality of life was obtained applying two Portuguese validated questionnaires: Rhinosinusitis Quality of Life Survey – portuguese version (*RhinoQOL-pv*)[93] and *COPD Assessment Test™* (CAT)[94]. RhinoQOL-pv scores for the symptom frequency and impact scales ranged from 1 (''never'') to 5 (''always'') in each question, while for the bothersomeness scale, scores ranged from 0 to 10, in accordance with the question's possible answers. We choose the CAT™ test because it consists of nonspecific questions about lung disease impacts and has already been studied not only for chronic obstructive pulmonary diseases (COPD), but also asthma and asthma–COPD overlap syndrome (ACOS).[95]

A systematic endoscopic examination of both nasal cavities was performed by an otolaryngologist, using a 0º, 2.7 mm rigid endoscope from Karl-Storz®; decongestion with

vasoconstrictor was used on an as-needed basis, especially to make middle meatus inspection easier. Nasal polyps were classified endoscopically in grade I, II or III, according to Lund criteria.[2] Lund-Kennedy endoscopic score for CRS[96] was also determined for each participant. For the study purpose, cases of antrochoanal polyps, polypoid lesions with features suggestive of benign neoplasia (e.g. sinonasal papillomas) or with malignancy suspicion were excluded. All employees were observed in the doctor's office located at the company during their working shifts.

Terminology Usage Notes

CRSwNP according to EPOS2012 is defined as a symptomatic clinical entity, not contemplating asymptomatic polyposis. So, it was decided to use "Nasal Polyposis" terminology, to include symptomatic CRSwNP and subclinical disease.

Statistical Analysis

Statistical analysis was performed with *Statistical Package for Social Sciences* (IBM® SPSS® Statistics for Windows, Version 23.0).

Textile workers and retail store workers were grouped in two independent samples and compared for multiple factors. Descriptive statistics was used in those samples' characterization. For categorical variables, Chi-Square test (or Fisher's exact test/ Likelihood ratio test when assumptions needed for the previous test were not verified) was used to test for variable association. For continuous quantitative variables, Mann-Whitney test was applied.

Binomial test was used to compare our prevalence of NP with previous data published on the literature.

Statistical significance was accepted to correspond to a p-value of less than 0.05.

4.3 Results

A total of 316 individuals were included in the study: 215 textile workers (exposed group) and 101 retail store workers (control group). A total of 41 subjects were excluded: two retail store workers with a past working history in the textile industry; 15 textile workers with less than one year of work experience; 22 employees that were temporarily absent from work (ex. maternity leave, medical reason) and other two that refused to participate.

Sample demographics, comorbidities, smoking and alcoholic habits are summarized in Table 5.

Table 5- Demographics, comorbidities, smoking and alcoholic habits in the exposed and control group and their comparison. (N=316).

	Exposed Group		**Control Group**		***p* value**
- Demographics					
Age (mean ± SD; [range])	50 ± 11; [21; 67]		41 ± 10; [20; 65]		**< 0.001**
	n	**%**	**n**	**%**	
Gender: Male	92	42.3	35	34.7	0.120
Race					
Caucasian	214	99.5	101	100	0.680
African	1	0.5	0	0	
- Comorbidities (n, %)					
Allergic Rhinitis	43	20.0	13	12.9	0.080
Asthma	17	7.9	11	10.9	0.252
COPD	1	0.5	0	0	0.680
Sinonasal Tumor	0	0	1	1.0	0.320
OSAS	11	5.1	0	0	**0.013**
Atopic Dermatitis	26	12.1	14	12.1	0.392
Salicylates Intolerance	3	1.4	1	1.0	0.617
- **Smoking Habits**					
Non-smoker	124	57.7	65	64.4	0.832
Ex-smoker	41	19.1	8	7.9	
Smoker	50	23.3	28	27.7	
- **Alcoholic Habits**					
Non-alcoholic habits	107	49.8	60	59.4	0.057
Light-to-moderate	97	45.1	40	39.6	
Heavy drinking	11	5.1	1	1.0	

SD- standard deviation; COPD- chronic obstructive pulmonary disease; OSAS- obstructive sleep apnea syndrome

When asked about exposure to domestic fumes (ex. use of firewood/ coal) no difference was observed between groups, with 12.2% of retail store employees and 13.1% of textile workers answering positively (Chi-Square test, p=0.854). Regarding to domestic animals, the non-exposed group (retail store workers) had more pets in a statistically significant way (Chi-Square test, p=0.004).

The previous history of nasal surgery did not differ among the study groups (Table 6).

Table 6- History of nasal surgical procedures with absolute and relative frequencies.

Surgery	Exposed Group		Control Group		*p* value
	n	%	n	%	
Septoplasty	3	1.4	2	2.0	0.656
ESS	2	1.8	1	1.0	1.00
Polypectomy	0	0	0	0	-

ESS – endoscopic sinus surgery

When asked about sinonasal symptoms with at least three months duration, the textile group reported significantly higher prevalence of hyposmia (16% vs 5%, p=0.003), headache (38% vs 22%, p=0.003), facial pressure (38% vs 24%, p=0.008), sneezing (62% vs 38%, p<0.001) and nasal pruritus (61% vs 37%, p<0.001). The proportion of patients with snoring was also significantly higher among the exposed group (54% vs 38%, p=0.005). Despite having all higher prevalences in the exposed group, other symptoms such as nasal congestion/blockage/obstruction, anterior and posterior rhinorrhea, and epistaxis did not differ statistically.

RhinoQOL-pv (total and by scales) and CAT™ mean scores are specified by group on Table 7.

Table 7- RhinoQOL-pv and CAT™ mean scores in the exposed and control groups.

Questionnaire	Exposed Group	Control Group	
	mean± SD; [range]	mean± SD; [range]	
RhinoQOL-pv	26.31 ± 15.4; [14; 116]	21.45 ± 10.1; [14; 50]	**0.005**
• **Frequency Scale**	8.25 ± 5.9; [5; 81]	7.12 ± 2.8; [5; 16]	**0.011**
• **Impact Scale**	11.92 ± 5.3; [9; 37]	10.12 ± 2.6; [9; 25]	**0.013**
• **Bothersomeness Scale**	6.14 ± 7.0; [0; 30]	4.21 ± 6.0; [0; 24]	**0.009**
CAT	2.67 ± 5.36; [0; 29]	1.37 ± 3.12; [0; 17]	**0.023**

The endoscopic findings as well as the Lund-Kennedy endoscopic score for both groups are summarized in Table 8. In nine cases (4.2%) from the exposed group and one case (1.0%)

from the control group, it was impossible to adequately inspect the middle meatus bilaterally due to severe septal deviation of the nasal cavity. In those cases, the Lund-Kennedy score and the polypoid status of the mucosa was inferred from the contralateral side.

The prevalence of septal deviation did not differ between the exposed and control groups (Table 8); also, total RhinoQOL-pv scores did not differ significantly between patients with and without nasal septum deviation (Mann-Whitney test, p=0.505).

One case of unilateral polyp was found in the control group. This patient had an history of a previous unilateral endoscopic surgery for an antrochoanal polyp (in the same side), and so it was assumed to correspond to disease recurrence.

Table 8- Results of endoscopic evaluation, including Lund-Kennedy score, by group.

Endoscopic Evaluation	**Exposed Group**		**Control Group**		***p* value**
	n	%	n	%	
Nasal Polyposis	19	8.8	0	0	**0.001**
Antrochoanal Polyp	0	0	1	1.0	0.320
Polypoid degeneration of the middle turbinate	24	11.2	1	1.0	**0.001**
Septal Deviation	71	33.0	40	39.6	0.258
Lund-Kennedy score (mean± SD; [range])	3.43 ± 2.43; [0; 12]		1.76 ± 1.48; [0; 6]		**<0.001**

NP was found in 19 subjects (8.8%) among the textile workers group (12 men and 7 women, ratio 1.7:1; mean age of 55 years) and none in the control group. From the 19 individuals found to have NP, only two (11%) were previously aware of the diagnosis and had been previously submitted to ESS. All cases of NP presented bilateral disease. Characterization of nasal polyps according to Lund criteria[2] is displayed on table 9.

Table 9- Nasal polyps' classification according to Lund criteria by group.

Grade	**Exposed Group**		**Control Group**	
	n	%	n	%
0 (no polyps)	196	91.2	101	100
I	9	4.2	0	0
II	9	4.2	0	0
III	1	0.5	0	0
Total	**215**	**100**	**101**	**100**

When performing a statistical analysis stratified for age, we verified that in the subgroup of subjects with less than 45 years (N=120) the prevalence of NP was higher among the exposed ones (p=0.035, Likelihood ratio test), with 3 cases among them (4.8%) and none in the control group. In the subgroup of individuals with 45 or more years (N=196), the prevalence of NP was also higher in the exposed group with 16 cases among textile workers (11.3%) and no cases found in the control group (p=0.024, Fisher's exact test).

The prevalence of NP by age and years of dust exposition strata among textile workers is presented in Table 10. The mean time of years of textile dust exposure was 26±15 years, with a minimum of one year and a maximum of 54.

Table 10- Absolute prevalence of nasal polyposis by age and years of dust exposition strata among textile workers.

Age Strata	**Exposed Group**		**Years of Dust Exposition**	**Exposed Group**	
	Nasal Polyposis	No Nasal Polyposis		Nasal Polyposis	No Nasal Polyposis
< 25 years	0	3	**< 15 years**	3	50
[25; 50[years	3	81	**[15; 35[years**	5	92
≥ 50 years	16	112	**≥ 35 years**	11	54
Fisher's exact test (p-value)	**0.030**		**Fisher's exact test (p-value)**	**0.017**	

Distribution of individuals with and without NP across working textile sectors is presented in Table 11. We found individuals with NP across almost every sector, apart from informatic/marketing and administration teams. Only 21 (9.8%) of textile workers referred to use occasional mask-protection during their job duties.

Concerning medication habits, 4.7% of textile workers referred regular use of nasal steroids and 9.3% of anti-histamines. Only two subjects with nasal polyps (10.5%) were using nasal steroids and were the ones with history of previous ESS.

The percentage of clinically silent NP or preclinical cases was 21% (4 cases) versus 79% (15 cases) of symptomatic NP. From the seven textile workers (3.3%) who claimed to have a previous medical diagnosis of CRSwNP, the diagnosis was confirmed in only two (being both workers with symptomatic NP). In the control group, two patients claimed also to have CRSwNP but the diagnosis was not confirmed in both of them. In total, from the 9 subjects claiming to have CRSwNP, seven (78%) had no signs of NP on endoscopic nasal evaluation, neither previous history of nasal surgery. Of the 19 individuals found to have NP, only two (11%) were previously aware of the diagnosis.

Table 11- Distribution of individuals with and without NP across working sectors in textile industry.

Work Sector	Exposed Group			
	Nasal Polyposis		No Nasal Polyposis	
	n	%	n	%
Spinning	2	10.5	40	20.4
Warping	1	5.3	15	7.7
Weaving	5	26.3	30	15.3
Dyeing	2	10.5	18	9.2
Finishing	4	21.1	54	27.6
Quality Control	1	5.3	7	3.6
Storing, Packing	2	10.5	14	7.1
Informatics, Marketing	0	0	6	3.1
Administration	0	0	8	4.1
Designing	1	5.3	3	1.5
Woodwork	1	5.3	1	0.5
TOTAL	19	100	196	100

Chronic lower respiratory diseases (27 cases with asthma, i.e. 21 atopic asthma and 6 non-atopic asthma) have been previously diagnosed and treated in 9% of the total 297 individuals without polyps (total sample, exposed and not exposed) and in 10.5% of NP subjects (1 case of non-atopic asthma and 1 case of COPD) (p=0.689, Fisher's exact test). Among patients with clinically silent NP there were no cases of previously diagnosed CLRD. When comparing the exposed and control group for CLRD there was also no statistically significant difference (Table 5).

Concerning atopy in general, five individuals from the 19 subjects with NP (26%) reported a previous diagnosed of atopy by an immunoallergologist versus 51 in 297 individuals without NP (17%) (p=0.349, Fisher's exact test).

Comparing with the two published European endoscopic based studies[17,18], our prevalence of NP among textile workers was significantly higher: 8.8% versus 5.5% found in the Portuguese cadaver-study (p=0.029, Binomial test) and 8.8% versus 2.7% found in the Swedish in-vivo study (p<0.001, Binomial test).

4.4 Discussion

Despite all investigation on CRSwNP, there is still a deficit of information about its prevalence and risk factors. The remarkable proportion of clinically silent disease that we found (21%) and the fact that the diagnosis was not confirmed in seven out of nine patients (78%) who claimed to have a previous medical diagnosis of CRSwNP, confirms the need of endoscopic based studies over the non-reliable questionnaire-based ones.

Our epidemiologic study is to our knowledge the first endoscopic based one to address whether occupational dust exposure has influence on CRSwNP prevalence. All employees were observed in the doctor's office located at the company during their working shifts, allowing a truly workplace assessment of sinonasal diseases. This fact, along with the use of a control group without dust exposure represent some of the main study's strengths. Moreover, all participants were observed by the same otorhinolaryngology specialist which reduces inter-observational biases.

Our prevalence results in the exposed group are significantly higher than the two European population-based studies, in Portugal and Sweden[17,18], which highlights the importance of occupational dust exposure as a risk factor for the occurrence of CRSwNP. This is also accentuated by the significantly higher NP prevalence among textile workers comparing to the control group.

The control of potential confounding variables, such as domestic fumes exposures or the presence of pets at home, was also important to this work. Interestingly, no difference on domestic dust exposures was observed among exposed and non-exposed group, but more retail workers owned animals at their homes in a statistically significant way. This fact reinforces the role of occupational dust exposure in sinonasal inflammatory pathology detected in the textile population.

One of this study limitations is the difference in age between the exposed and the control group. The textile industry in central Portugal is led by an ageing population and it was not possible to equalize the groups concerning this factor. However, we analyzed the groups by age strata and the difference in NP prevalence was still evident and significant. There was also a female predominance in the control group, despite not being significantly different from the exposed group. These two factors, in addition to a relatively small sample size might have contributed to the absence of nasal polyposis among the controls. These limitations are exceeded when comparing results of the exposed group with the prior cadaver endoscopic study done in Portugal, which had an even older population (mean age of 77 years, N=200) and a male predominance (58.5%). The prevalence results among these textile workers are significantly higher than the prevalence found in the cadaver study.[18]

Another limitation is the fact that in a few cases (3.2%) it was impossible to adequately inspect the middle meatus bilaterally due to marked septal deviation. Small polyps may have also been missed in a slight percentage of the sample since rigid endoscopy

must be done gently and no topical anesthesia was used. These factors in combination can lead to an underestimation of NP prevalence.

In accordance to previously published studies that suggested an increase of NP prevalence with age[17,22,97], and a male predominance[1,17], we also found an higher prevalence among older strata and a male-female ratio of 1.7:1.

The prevalence of NP also rose by strata according to the number of years of textile dust exposition, suggesting that a longer occupational dust exposition increases the risk of CRSwNP occurrence. The fact that we found NP across every textile sector apart from informatic/marketing and administration teams is not surprising, since these two sectors are the least exposed ones to textile dust.

Comparing the comorbidities between volunteers from the exposed and control group (Table 5), we notice that OSAS prevalence is higher among the exposed ones. This fact can be attributed to the older age of this group since we know that the frequency of this pathology also increases with age, but also due to higher prevalence of sinonasal disease, as we can infer by RhinoQOL-pv results and Lund-Kennedy scores (significantly higher on the exposed group). OSAS and rhinosinusitis severity seem not to be correlated but the prevalence of OSAS in patients with chronic rhinosinusitis is considered to be high (with a recent study finding a prevalence of 65%).[83]

The fact that all rhinologic symptoms were higher among textile workers compared with the control group, and especially the statistically significant rhinosinusitis characteristic symptoms like hyposmia, headache and facial pressure, support the hypothesis previously formulated in Chapter 3 discussion, that occupational/ work-exacerbated rhinitis may progress towards occupational/work-exacerbated rhinosinusitis and contribute to CRSwNP occurrence by different mechanisms: immunologically mediated hypersensitivity reactions (antibody or cell-mediated), designated as allergic reactions or through irritant, non-immunological mechanisms.

Moreover, RhinoQOL-pv, which was designed for rhinosinusitis, scored higher in a statistically significant way between the exposed and control group.

Another interesting issue is that patients reporting atopic diseases previously diagnosed by an immunoallergologist did not differ significantly between the exposed and control groups or even between CRSwNP patients and subjects without the disease. Moreover, the prevalence of atopy was 20% in the exposed group and 26% among CRSwNP patients and these values are in the range of the reported prevalence of atopy in the general population (20–30%)[90]. These findings corroborate our previous work (Chapter 3) and other published studies[22], supporting the hypothesis of important non–IgE-related mechanisms on CRSwNP etiopathogenesis, including immune and non-immune ones.

We found not only a significant difference among NP prevalence between the groups ($p=0.001$) but also among polypoid degeneration of the middle turbinate ($p=0.001$) and overall inflammatory state of the nose as shown by higher Lund-Kennedy score among textile workers

($p<0.001$). By these findings, we can state that occupational exposure to dust is associated to general inflammatory changes of the nose and paranasal sinus.

No statistically significant difference was found on the frequency of previously diagnosed CLRD in subjects with and without NP, contrary to what was expected by recent studies on CRSwNP with prevalences as high as 72.5%[26] and even by our retrospective study in which a prevalence of 54% was reported (Chapter 3). The relatively small number of cases with NP in our sample (only 19 cases) and the proportion of patients with subclinical and early NP stages (mainly grade I/II) may contribute to these figures.

The prevalence of previously diagnosed CLRD did not differ also between exposed and control groups. However, many epidemiologic studies assessing lower respiratory symptoms and spirometry results among textile workers and controls have been alerting to higher frequency of lower respiratory symptoms and deterioration in spirometric parameters in the first group.[98–100] Moreover, it is clear by CAT^{TM} score that lower respiratory symptoms are more frequent among exposed individuals, with a statistically significant difference, pointing up to a probable underdiagnosis of lower respiratory diseases among textile workers. The fact that in this study it was only considered the diagnosis of CLRD previously diagnosed by a respiratory physician can partially explain this finding, as many employees are treated by general practitioner or workplace doctor.

As this represents a problem of Public Health, these findings show the importance of employee's protective measures, such as mask use during work, and reinforces the need for legislation and control to guarantee the functioning of air dust filters and exhausting systems.

More epidemiologic investigations are needed, namely to establish the NP prevalence in other types of occupational dust exposure. The concomitant involvement of otolaryngologists and a pneumologists on this type of studies can be helpful to clarify the association between CRSwNP and lower respiratory diseases.

Conclusion

This investigation was the first endoscopic based epidemiological study to evaluate the impact of occupational dust exposure on NP prevalence. Our results revealed a higher prevalence of NP among textile workers compared to our control group, but also compared to previous in-vivo and cadaver endoscopic based studies done in Europe. These results point to an important correlation between occupational dust exposure and NP occurrence, justifying more research in this area. Meanwhile, Public Health policies like employee's protective measures must be reinforced.

Chapter 5.

SYSTEMIC IMMUNE PROFILE IN PATIENTS WITH CRSwNP

5. Systemic immune profile in patients with CRSwNP

5.1 Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a common clinical entity, but despite the high prevalence, morbidity and chronicity, its etiopathogeny remains obscure.[1] Systemic immunological changes associated with CRSwNP may provide important clues to a better knowledge of the involved immune pathways.

Local immune modifications in nasal mucosa have been intensely investigated in CRSwNP. It is known that Western patients with CRSwNP show local tissue immune effects, such as skewing of the inflammatory response in a T helper cell type 2 (Th2) direction, generation of local polyclonal Immunoglobulin E (IgE) antibodies, promotion of eosinophil survival and mast cell degranulation.[1] Van Zele et al. demonstrated significantly higher values of Immunoglobulin A (IgA), Immunoglobulin G (IgG) and IgE in polyps homogenates, as well as a higher percentage of Immunoglobulin G class 1 (IgG1), when compared to nasal tissue samples from controls.[101] Concerning Immunoglobulin M (IgM), no difference was observed between the two groups.[101] It has also been recently demonstrated that local IgG1 can act as a "superantibody" against staphylococcus enterotoxins.[102]

However, data about systemic modification of immune system in CRSwNP is scarce, namely about humoral immunity. To our knowledge, the only controlled study to address IgG subclasses serum modifications in CRSwNP included 15 patients and 10 controls, not finding any significant difference.[101]

Despite all the efforts in trying to correlate atopy (IgE-mediated allergy) with CRSwNP, many reports failed to show a higher prevalence of atopy in these patients.[1] The study published by Hox et al.[56] in CRS patients submitted to ESS and our retrospective study about predictive factors of recurrence in CRSwNP patients submitted to ESS (Chapter 3) highlighted the importance of occupational exposure to dust in disease recurrence, namely the inhalation of lower molecular weight (LMW) particles (<5 kDa). Contrary to high molecular weight (HMW) particles that induce a well-known IgE-mediated immune response, LMW particles induce airway inflammation through mechanisms that are far less known. It can include the classical "irritant response" plus LMW sensitization of the adaptive immune system by acting as haptens (attached to large carriers such as a proteins).[56,88] An investigation about sensitization to methylene diphenyl diisocyanate (MDI), an LMW particle, in a car upholstery factory found that the prevalence of MDI-induced occupational asthma/eosinophilic bronchitis was strongly associated with the presence serum-specific IgG antibodies to an MDI-human serum albumin conjugate.[103]

CRSwNP and asthma are frequently associated in the same patients, but their inter-relationship is poorly understood. As expected by the previous consideration about atopy, CRSwNP seems to be more associated with non-atopic asthma than with atopic asthma[1], and this fact was also corroborated by our investigation (Chapter 3) that found non-atopic asthma to be an independent predictive factor of recurrence in CRSwNP after surgery. The clarification of systemic immunopathogenesis can be a clue factor to better understand the association between CRSwNP and chronic lower respiratory diseases (CLRD).

Conflict evidence has been published about D vitamin levels influence on CRSwNP.[67] On the other hand, there is scarce but important evidence for a possible role for mutations in *SERPINA1*, the gene that codes for alpha-1-antitrypsin (A1AT), in CRSwNP physiopathology.[65]

Our main goal was to characterize systemic immunological alterations that occur in Western patients with CRSwNP compared to controls, namely: leukocyte differential count and evaluation of humoral immune profile, based on immunoglobulin classes and subclasses dosage. Our secondary objectives were to compare serum levels of 25-hydroxyvitamin D (25-HOD), A1AT and C-reactive protein (CRP) between the two groups.

5.2 Materials and Methods

A observational case-control study was performed according to established ethical guidelines and approval of Ethics Committee at the Cova da Beira Hospital Centre (deliberation number 82/2015). A signed informed consent was obtained from each participant in the study.

2.1- *Sample*

All the cases were about to undergo endoscopic sinus surgery (ESS) for CRSwNP refractory to medical treatment (topical long-term and systemic short-term steroids), in a district hospital center, from January 2016 to October 2018. Diagnosis of CRSwNP was established using the definition of EPOS2012.[1] The cases were selected consecutively from the waiting list and had their disease confirmed endoscopically, by computed tomography (CT) and histological examination of the subsequent surgical specimen. Patients were selected after applying the following exclusion criteria: concomitant benign or malignant sinonasal tumors; chronic rhinosinusitis without nasal polyps; antrochoanal polyps; polyps associated with fungal rhinosinusitis; primary ciliary dysfunction; cystic fibrosis; acquired immunodeficiency (i.e. human immunodeficiency virus; immunosuppressive drugs), autoimmune diseases (e.g. systemic vasculitis) or history of or under allergen-specific immunotherapy. No course of systemic corticosteroids was given to CRSwNP patients at least three months before serum specimens collection.

Controls were selected from patients in the waiting list for septoplasty, after excluding subjects with symptoms and endoscopic or CT signs of CRS, with acquired immunodeficiency, autoimmune diseases or with history of or under allergen-specific immunotherapy. All the cases and controls were adults (> than 18 years old).

2.2- *Data Collection*

Clinical data was gathered through a systematic interview to collect information on demographics, occupational history and comorbidities. Subjective assessment of upper and lower respiratory disease was obtained through Rhinosinusitis Quality of Life Survey – portuguese version (*RhinoQOL-pv*)[93] and *COPD Assessment Test™* (CAT) [94]. Nasal polyps were classified endoscopically according to Lund criteria and Lund-Mackay imagiological score was obtained for each participant.

All patients and controls were submitted to skin prick test (SPT) and spirometry, while specific serum IgE antibodies for inhalants were requested as needed. CLRD diagnosis were established by a respiratory physician and classified according to ICD-10™ *(International Classification of Diseases, version 10*).

Blood sample was collected to obtain a leukogram, to determine immunoglobulin classes and IgG subclasses levels, CRP, A1AT and 25-HOD levels. Immunoglobulin classes, CRP and A1AT were obtained by immunoturbidity assay, and 25-HOD was determined by electrochemiluminescence immunoassay, all using Cobas 6000 analyzer (Roche Diagnostics®,

Mannheim, Germany). Immunoglobulin subclasses levels were obtained through the Optilite turbidimetric analyzer (The Binding Site®, Birmingham, United Kingdom). The reference range used for total IgG (700-1600 mg/dL), IgA (70-400 mg/dL), IgM (40-230) was the one suggested by the International Federation of Clinical Chemistry.[104] Immunoglobulin subclasses levels were considered to be low if IgG1 < 405 mg/dL, IgG2 < 169 mg/dL, IgG3< 11 mg/dL or IgG4 < 3 mg/dL according to the laboratory reference values.

2.4- *Statistical Analysis*

Statistical analysis was performed with *Statistical Package for Social Sciences* (IBM® SPSS® Statistics for Windows, Version 23.0). Descriptive statistics was used in sample characterization. Mann-Whitney test was used to compare continuous variables between CRSwNP and controls. A subanalysis with Kruskal-Wallis test was performed to compare continuous variables between three groups (control group without CLRD, CRSwNP without CLRD and CRSwNP with CLRD) and Dunn's post hoc test was carried out for multiple pairwise comparisons. Chi-square test (or Fisher's exact test/ Likelihood ratio test when needed) was used to test association between categorical data. A *p*-value <0.05 was considered as statistically significant.

5.3 Results

Seventy-one individuals completed the study: 37 patients with CRSwNP and 34 controls. In total, one individual refused to participate in the study, four decided to be operated elsewhere, one case was considered unfit for anesthesia procedure and other was excluded due to primary ciliary dysfunction, and one control was excluded due to psoriatic arthritis.

Demographics, comorbidities and occupational dust exposure of CRSwNP and controls are presented and compared in Table 12. All participants were Caucasians.

Table 12- Demographics, comorbidities and occupational dust exposure in CRSwNP and control groups and their comparison. (N=71).

	CRSwNP Group (n=37)		**Control Group (n=34)**		***p* value**
- Demographics					
Age (mean ± SD; [range])	58± 2; [30; 82]		54± 2; [30; 79]		0.060
	n	**%**	**n**	**%**	
Gender: Male	18	48.6	20	58.8	0.477
- Comorbidities					
Allergic Rhinitis	12	32.4	5	14.7	0.100
Asthma					
- **Atopic**	8	21.6	2	5.9	0.087
- **Non-Atopic**	9	29.7	0	0	**0.002**
COPD	8	21.6	1	2.9	**0.019**
AERD	7	18.9	0	0	**0.008**
- **Occupational Dust Exposure**	31	83.8	19	55.9	**0.010**

COPD- chronic obstructive pulmonary disease; AERD- Aspirin-exacerbated respiratory disease

RhinoQOL-pv and CAT™ total mean scores were significantly higher among the CRSwNP group (60.4±3.5 and 15.9±1.7, respectively) versus the mean values for the control group (49.3±2.7 and 7.3±1.2) [p=0.010 for RhinoQOL-pv and $p=1x10^{-4}$ for CAT™, Mann-Whitney test].

Twenty-five patients with CRSwNP (67.6%) presented concomitant CLRD (Table 12) which, compared with the control group (three individuals, 8.8%), means a higher prevalence of pulmonary diseases ($p=0.17x10^{-7}$, Fisher's exact test). Aspirin exacerbated respiratory disease (AERD) was more prevalent among CRSwNP group (Table 1), with 16.2% of CRSwNP presenting the Samter's triad (six cases). In CRSwNP group, 21 out of 37 (56.7%) were under

treatment with inhaled steroids with ß2-agonists; whereas in the control group only one patient was being treated with inhaled steroids.

Twelve individuals from the 37 subjects with CRSwNP (32.4%) and five individuals from the control group (14.7%) had atopic disease, without a statistically significant difference (p=0.100, Fisher's exact test).

Nasal polyps were classified endoscopically as grade I in five cases (13.5%), grade II in eleven cases (29.7%) and grade III in 21 cases (56.8%), according to Lund criteria and the mean Lund-Mackay imagiological score was 15.22±0.78. All nasal specimens of CRSwNP patients showed tissue eosinophilia (>10 /hpf).

The analyzed systemic immunological parameters are presented and compared in Table 13. In respect to leukogram, CRSwNP patients had significant lower levels of relative neutrophil count but higher values of relative eosinophil and basophil counts. Concerning humoral immunity, CRSwNP showed an IgG1 subclass switching, with reduced levels of IgG2 and IgG3. No significant differences were observed for total IgG, IgA, IgM and IgE serum levels.

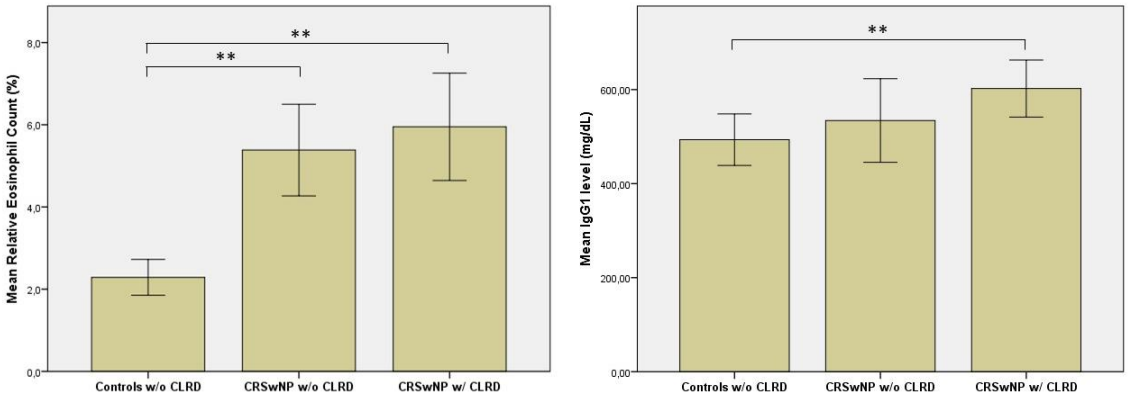
Table 13- Comparison of systemic immunological parameters between groups. (N=71).

Parameters	CRSwNP Group (n=37)	Control Group (n=34)	*p* value
WBC (10^3/uL)	7.24 ± 0.31	7.31 ± 0.39	0.717
Neutrophils (%)	51.26 ± 1.44	56.00 ± 1.41	**0.013**
Lymphocytes (%)	33.44 ± 1.58	32.92 ± 1.30	0.872
Monocytes (%)	7.96 ± 0.30	7.71 ± 0.27	0.378
Eosinophils (%)	5.67 ± 0.47	2.29 ± 0.20	**$3.56x10^{-9}$**
Basophils (%)	0.06 ± 0.009	0.04 ± 0.009	**0.022**
IgE (kU/L)	284.97 ± 92.8	173.12 ± 53.6	0.079
IgG (mg/dL)	995.05 ± 38.27	931 ± 32.97	0.451
IgG1	578.35 ± 25.32	502.20 ± 26.12	**0.022**
IgG2	349.40 ± 18.55	403.88 ± 20.9	**0.014**
IgG3	63.28 ± 8.13	68.97 ± 4.00	**0.018**
IgG4	65.89 ± 10.56	44.70 ± 5.74	0.272
IgM (mg/dL)	99.75 ± 9.54	97.58 ± 8.63	0.608
IgA (mg/dL)	254.72 ± 17.90	218.15 ± 16.43	0.105
25-HOD (ng/mL)	19.7 ± 1.26	23.9 ± 1.82	0.195
A1AT (mg/dL)	130.7 ± 4.27	123.81 ± 5.33	0.153
CRP (mg/dL)	0.30 ± 0.05	0.28 ± 0.06	0.489

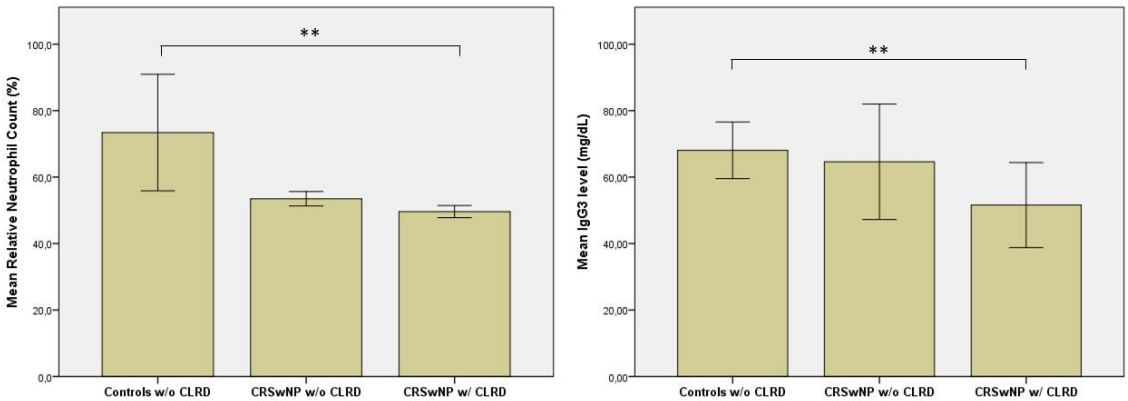
WBC- white blood cells; Ig- Immunoglobulin; 25-HOD- 25-hydroxyvitamin D; A1AT- alpha-1-antitrypsin; CRP- C-reactive protein. Comparison using Mann-Whitney test.

In addition, we tried to find out if AERD patients diverge from the remaining patients in the CRSwNP group but no significant differences about these immunological variables were detected.

A Kruskal-Wallis test (N=68) was also run for these continuous variables considering three different groups: control group without CLRD, CRSwNP without CLRD and CRSwNP with CLRD. Patients from the control group with CLRD (n=3) were not included in this sub-analysis because of their limited number. Neutrophil (p=0.031) and eosinophil relative count ($p=1.5x10^{-7}$), and IgG1 (p=0.027), IgG2 (p=0.015) and IgG3 (p=0.028) differed significantly between the groups. For relative eosinophil count and IgG1 concentration levels there was a crescendo trend in their mean values between control group without CLRD, CRSwNP without CLRD and CRSwNP with CLRD, and an inverse pattern (decrescendo) was observed for relative neutrophil count and IgG2 and IgG3. (Graphics 4 and 5).



Graphic 4- Mean relative eosinophil count and serum concentrations of IgG1 in controls without chronic lower respiratory diseases (Controls w/o CLRD), in CRSwNP patients without CLRD (CRSwNP w/o CLRD) and CRSwNP with CLRD (CRSwNP w/ CLRD). N=68. Significance levels are marked as * if p<0.05 and ** if p<0.01. Kruskal-Wallis test (p<0.001 and p=0.037, respectively) was performed and pairwise comparisons were made using Dunn's post hoc test.



Graphic 5- Mean relative neutrophil count and serum concentration levels of IgG3 in controls without chronic lower respiratory diseases (Controls w/o CLRD), in CRSwNP patients without CLRD (CRSwNP w/o CLRD) and CRSwNP with CLRD (CRSwNP w/ CLRD). N=68. Significance levels are marked as * if p<0.05

and ** if p<0.01. Kruskal-Wallis test was performed (p=0.031 and p=0.018, respectively) and pairwise comparisons were made using Dunn's post hoc test.

Relating to humoral immunodeficiency (Table 14), we did not find a higher prevalence of immunoglobulin classes (five cases, 13.5%) or subclasses deficiency (seven cases, 18.9%) compared to our control group [three cases (8.8%) and seven cases (20.6%), respectively]. No cases of common variable immunodeficiency (CVID) were diagnosed in the entire sample. One case (2.9%) among CRSwNP and two cases among controls (5.4%) had an IgAGMD plus IgG1-3 deficiency (combination of selective classes (IgA, IgG or IgM) plus IgG subclasses deficiency).

Table 14- Prevalence of immunoglobulin classes and subclasses deficiency in CRSwNP and control groups. (N=71).

	CRSwNP Group (n=37)	**Control Group** (n=34)	***p* value**
Igs classes deficiency	**n, (%)**	**n, (%)**	
- **IgG**	2, (5.4)	2, (5.9)	1.000
- **IgA**	1, (2.7)	0	1.000
- **IgM**	3, (8.1)	2, (5.9)	1.000
IgG subclasses deficiency			
- **IgG1**	6, (16.2)	7, (20.6)	0.762
- **IgG2**	1, (2.7)	2, (5.9)	0.604
- **IgG3**	1, (2.7)	0	1.000
- **IgG4**	1, (2.7)	0	1.000
Multiple Igs deficiency	2, (5.4)	1, (2.9)	1.000
Total patients with Igs deficiency (classes or subclasses)	11, (29.7)	9, (26.5)	0.797

CRSwNP- Chronic Rhinosinusitis with Nasal Polyps; Igs- Immunoglobulins;
Comparison using Chi-square test (or Fisher's exact test).

5.4 Discussion

Our case and control groups had no significant differences concerning baseline demographic features, such as race, gender or age.

Comparing comorbidities (Table 12), the prevalence of allergic rhinitis and atopic asthma did not differ significantly between cases and controls. In addition, the prevalence of atopy was 32% in the CRSwNP group, which is close to the range values of the reported prevalence of atopy in the general population (20–30%).[90] These results support the involvement of non–IgE-related mechanisms on CRSwNP etiopathogenesis.

On the contrary, there was a statistically significant higher prevalence of non-atopic asthma and COPD (both classified as CLRD) among CRSwNP patients compared with controls. In total, 67.6% of CRSwNP patients had a concomitant diagnose of CLRD, and this value is in accordance with a recent study which reported a CLRD prevalence (asthma plus COPD) of 72.5%.[26] However, the COPD prevalence reported on that study (7.5%) was much lower than the 21.6% found in our study. The association between CRSwNP and asthma is well established, but the association of CRS and COPD despite being suggested, has been much less investigated.[105] In 2011, a study in ninety COPD patients demonstrated that 53% had concomitant symptoms of rhinosinusitis and 64% had signs of CRS in CT scan.[105] Our study took place in Castelo Branco District, within the Interior Centre Region of Portugal, an area internationally known for its textile industry, mainly wool manufacturing, potentially explaining the high prevalence of COPD in our CRSwNP sample. Another important point, is that Asthma and COPD may be difficult to distinguish, especially in patients with history of tobacco consumption or exposure to noxious particles or gases, and in the elderly, who often have overlapping clinical features of both diseases.[35]

The higher frequency of occupational dust exposure among CRSwNP patients compared to controls corroborates the important role of this factor in the etiopathogenesis of CRSwNP, as already suggested by our previous retrospective (Chapter 3) and epidemiologic studies (Chapter 4). Occupational exposure to dust has already demonstrated to trigger CLRD in different studies [98,100], and can be a key factor in understanding the relation between CRSwNP and diseases of the lower airways.

In Western patients, it has been suggested that CRSsNP is more distinctly a neutrophilic process, while CRSwNP is more eosinophilic, based on the relative degree of nasal tissue infiltration.[1] It was interesting to observe that in CRSwNP the peripheral relative counts of eosinophils and neutrophils had the same behavior to what happens locally. There was a significant reduction in the relative neutrophil count among CRSwNP, with a decrescendo tendency if comorbid CLRD was present and the opposite occurred with the eosinophil count.

Similarly to other published study[101], serum IgG, IgA, IgM and IgE concentration values did not show a significant difference between CRSwNP and controls. But contrary to

that study, where no significant difference about serum IgG subclasses concentration values was observed [101], in our study we found higher IgG1, but lower IgG2 and IgG3 serum levels in CRSwNP compared to controls, and this variations were more pronounced if CLRD were present.

In asthma patients, it has already been studied that IgG subclasses in bronchoalveolar lavage (BAL) and epithelial lining fluid are significantly higher than in controls with IgG1 quotient between BAL fluid and serum concentrations having the highest value and IgG3 the lowest.[106] Similar studies should be done in CRSwNP, in nasal lavage fluid and tissue homogenates, to shed light in this subject. Meanwhile, an immunofluorescent study in nasal polyps from 100 patients has already shown a positive labelling for IgG in all specimens, for C3b complement fraction in 80% and negative immunofluorescency for IgM or IgA.[107] Van Zele et al. demonstrated that nasal tissue homogenates showed significantly higher concentrations by immunonephelometry of IgG, IgE and IgA in CRSwNP and also found by ELISA that the percentage of IgG1 subclass was significantly higher among tissue homogenates in CRSwNP compared to controls.[101] A recent study in patients with CRSwNP and AERS also pointed to the importance of IgG1 against staphylococcus enterotoxins (SEE), showing that this antibody can enhance the activity of anti-SEE IgEs as conventional antibodies or as "superantibodies" through complementary determining regions (CDRs) and framework regions to SEEs in SEE-anti-SEE IgE-FcεRI complexes.[102]

We hypothesize that systemic IgG1 subclass switching is involved in CRSwNP pathogenesis and may be an important link to lower airway diseases, as there is a progression toward increasing mean plasmatic values in those patients. This is interesting, since each IgG subclass has a unique profile, for example, soluble protein antigens and membrane proteins primarily induce IgG1 switching.[108] This subclass is also important in antibody response to allergens, has the longest serum half-life, is capable of complement activation by C1q binding and is the main IgG subclass to cross placenta and mucosal barrier.[108]

Moreover, there are previous studies showing that IgG1 has a strong binding affinity to the neonatal Fc receptor (FcRn) compared to the other subclasses[109,110], which is a receptor that has been recently identified in human nasal epithelium.[111] This receptor was originally identified in suckling rats as the receptor involved in IgG transport across the intestinal epithelium into the bloodstream, but has now been demonstrated to be expressed in many adult tissues and cell types[112], with predominant expression in respiratory system[113]. Apart from regulating and extending the serum half-life of IgG, FcRn orchestrates IgG-based immune responses at mucosal sites, contributing to immunosurveillance at host-environment interfaces within the adult organism.[114]

In addition, in vitro studies with serum from asthmatic patients showed that antigen-specific IgG1 and IgG3 antibodies can induce eosinophil degranulation, and that IgG-depleted serum but not IgE-depleted serum abolished this degranulation.[115] A possible link between IgG1 and tissue and peripheral eosinophilia has to be addressed in future investigations, since

inflammation seen in CRSwNP can be reliant on IgG-dependent eosinophil-mediated cytotoxicity.

All these recent findings seem relevant to understand the systemic immune profile results that we found. It will be necessary future studies to compare FcRn expression among CRSwNP and controls and its relationship with IgG1 subclass switching in this disease. Following the concept of "one airway, one disease" this IgG1-mediated immune response in CRSwNP patients may be a key piece in understanding its interrelation with CLRD, namely non-atopic asthma and COPD and to clarify the role of occupational dust exposure, specifically LMW particles, in their etiopathogenesis.

Our prevalence results of IgG subclasses (18.9%) and immunoglobulin classes (13.5%) deficiency in CRSwNP are similar to the previous reported values in the literature for CRS (5% to 50% for IgG subclasses deficiency and 13% for IgG, IgA or IgM antibody deficiency).[116] However, since no significant difference was detected between our CRSwNP and control groups (20.6% for IgG subclasses and 8.8% for Ig classes deficiency in controls), it seems unlikely that immunoglobulin class and subclass deficiency are important factors in CRSwNP etiopathogenesis. Larger prospective and controlled studies about this topic are needed.

In 2016, a systematic review suggested a correlation between low vitamin D and polypoid CRS phenotypes.[67] However, this review included only seven articles, three of them with retrospective character, and with heterogeneous methods for vitamin D dosage, for reporting the outcomes and for analysis of the confounding variables (e.g. ethnicity, corticoid use, concomitant chronic diseases, seasonal vitamin variation).[67] A Turkish study published later, did not find any significant difference between systemic levels of vitamin D between cases and controls, but found levels of vitamin D receptor (VDR) significantly higher in nasal tissue sample of CRSwNP patients.[117] In our Caucasian sample, no significant difference was found between serum vitamin D levels in CRSwNP and controls, which adds to the necessity of prospective and randomized trials, with standardized methods to clarify if there is a relationship between vitamin D and CRSwNP.

In our study, serum level of A1AT did not differ significantly between CRSwNP patients and controls, despite a higher mean enzyme level in patients with nasal polyps. It has already been published a case-control study which found an association between single nucleotide polymorphisms (SNPs) of the *SERPINA1* gene with clinically severe CRSwNP.[65] Interestingly, some of the patients who were homozygous for those SNP were submitted to measurement of serum levels of A1AT and were all in the normal reference range for the enzyme.[65] This alerts to the fact that this serum marker can be artificially elevated in uncontrolled sinus disease, as A1AT is an acute phase reactant and/or the possibility of a less functional enzyme with more difficult to reach the mucosal surface and incorporation into secretions. A German study published in 1995, also found a higher prevalence of genetic A1AT polymorphisms (PI-MS and MZ) among CRSwNP patients than in controls.[66] All of these facts highlight the useless of serum level determination for A1AT in CRSwNP patients and the

importance to study genetic A1AT polymorphisms in future investigations, as this could be a target for specific therapy.

There are few studies about the hypothesis of CRP rising in CRSwNP. In 2011, a Turkish study in 100 participants equally divided in four groups (CRSwNP, CRSsNP, Allergic Rhinitis and Control group) did not find any significant difference in CRP serum dosage between those groups.[118] In the same manner, we also did not find any difference in CRP values between cases and controls.

Our study has some limitations: a limited sample size from a single institution, the use of patients waiting for septoplasty as controls and a high percentage of CRSwNP under inhalant steroids. Nonetheless, this is to our knowledge the largest controlled study to analyze IgG subclasses dosage in CRSwNP. Patients under inhalant steroids were taking it in combination with long-acting B2-agonists that allows asthma control at lower corticosteroids doses, with negligible systemic side effects.[119] Moreover, immunological differences found between CRSwNP and patients submitted to septoplasty would be probably even bigger if we used a randomly selected group of non-CRS subjects from the general population, as it is known that patients undergoing septoplasty also suffer from relevant nasal symptoms, in association with some degree of nasal inflammation. This investigation deserves replication in larger samples and with patients from other institutions.

Conclusion

A distinct systemic immunologic profile in CRSwNP patients compared to controls, concerning leukogram and humoral immunity was observed. The observed changes in peripheral leukocyte count and the systemic IgG1 subclass shift are similar to what is described to happen in nasal polyp tissue of Caucasian CRSwNP patients, and these differences were even more marked if CLRD were present. These variations may be involved in CRSwNP pathogenesis and a possible role for IgG1-mediated response must be investigated.

Chapter 6.

FOOD SPECIFIC IgG AND IgE ANTIBODIES IN PATIENTS WITH CRSwNP

6. Food specific IgG and IgE antibodies in patients with CRSwNP

6.1 Introduction

EPOS2012 states that further research is needed to investigate a possible role for food allergy in the initiation and perpetuation of CRSwNP.[1] A possible role of food allergy in CRSwNP physiopathology has been raised in two case-control studies[120,121] that found a significant higher prevalence of intradermal food test positivity in CRSwNP patients compared to controls (70% and 81% in CRSwNP vs 34% and 11% in the control group, respectively). The authors argued that the fact that intradermal food test correlated poorly with serum specific immunoglobulin E (IgE) levels pointed to the involvement of other non-IgE-mediated food hypersensitivities.[121]

Food allergy is an adverse food reaction that involves abnormal immune responses to food allergens, which can involve immunoglobulin E (IgE)-mediated (immediate food responses) or non-IgE-mediated reactions (delayed food responses).[122] Food allergy prevalence is known to be higher in patients with allergic rhinitis[123] and cross-reactivity is known to occur between aeroallergens and food allergens.[124] Moreover, food allergy is known to be a risk factor for asthma development and lower respiratory symptoms may be seen in food-induced allergic reactions in asthmatic patients.[122] In addition, it is known that inhalation of food allergens can induce respiratory symptoms, as for example in occupational asthma in workers handling food products and derivates.[125]

Controversial evidence exists about IgE food sensitization in patients with CRSwNP, with authors reporting prevalences between 22-74% depending on the used technique (e.g. SPT - skin prick test, RAST - radioallergosorbent test).[126,127] In 2016, a comparative study between patients with CRSwNP and CRSsNP using enzyme allergosorbent test (EAST), found no significant difference between food IgE sensitization prevalence, type and severity, concluding that food atopy is unlikely to be a major factor in nasal polyposis etiopathogenesis.[128] The prevalence of food allergy is difficult to estimate mainly due to diagnosis issues. For example, SPT positivity does not necessarily prove that the food is causal as the positive predictive value is less than 50% but if negative essentially confirms the absence of IgE-mediated allergic reactivity, since negative predictive value is greater than 95%.[122] In vitro assays are also useful modalities in food allergy study. Initially, RAST test were used, but more recently quantitative measurement of food-specific IgE antibodies have been utilized (such as ImmunoCAP™) and studies have found them to be more predictive of symptomatic IgE-mediated food allergy.[122] Moreover, ImmunoCAP™ has the advantage of the lack of interference from allergen-specific IgG antibodies.[129] However, undetectable

serum food-specific IgE levels may be associated with clinical reactions as well and the double-blind placebo-controlled food challenge (DBPCFC) is still considered the gold standard for food allergy diagnosis, but because of its methodological difficulties it is rarely conducted in clinical practice.

Recently, immunoglobulin G (IgG) antibodies against food antigens have been suggested to cause low grade inflammation in irritable bowel syndrome with symptoms improvement after dietary elimination based on specific IgG profile of the patient.[130] This theory, called the "leaky gut syndrome" (LGS), proposes that it is the increased permeability of the gut wall to macromolecules that leads to activation of the immune system, which may initiate production of specific IgG antibodies against food, resulting in chronic inflammation sustained by repeated intake of allergenic foods.[131,132] The LGS and the IgG-mediated food allergy have been investigated in different chronic inflammatory pathologies such as obesity[133], type 1 and type 2 diabetes[134,135], chronic liver disease[136], chronic kidney disease[137], chronic heart failure[138], depression[132,139] and asthma[140]. The role of IgG antibodies against food remains controversial, namely because they can be detected in healthy individuals, there are no standardized cut off values and because the methods for allergen extraction and purification used to be diverse and to give poorly reproductive results. Actually, IgG food detection is still only recommended for investigational purpose and not for individual use.[141] The development of commercial kits with standardized food antigen extraction and purification, using an optimized and validated ELISA method, have allowed to overcome matters of reproducibility and variability and will make it possible to compare results between research centers.

Our main goal was to compare specific IgG and IgE antibodies dosage against food allergens in patients with CRSwNP and controls, trying to clarify if food allergy can be associated with CRSwNP.

6.2 Materials and Methods

A case-control observational study was performed according to established ethical guidelines and approval of Ethics Committee at the Cova da Beira Hospital Centre (deliberation number 82/2015). A signed informed consent was obtained from each participant in the study.

2.1- *Sample*

All the cases were about to undergo endoscopic sinus surgery (ESS) for CRSwNP refractory to medical treatment (topical long-term and systemic short-term steroids), in a district hospital center, from January 2016 to October 2018. Diagnosis of CRSwNP was established using the definition of EPOS2012.[1] The cases were selected consecutively from the waiting list and had their disease confirmed endoscopically, by CT scan and histological examination of the subsequent surgical specimen. Patients were selected after applying the following exclusion criteria: concomitant benign or malignant sinonasal tumors; chronic rhinosinusitis without nasal polyps; antrochoanal polyps; polyps associated with fungal rhinosinusitis; primary ciliary dysfunction; cystic fibrosis; innate or acquired immunodeficiency (e.g. human immunodeficiency virus; immunosuppressive drugs), autoimmune diseases (e.g. systemic vasculitis; inflammatory bowel disease) or patients with history of or under allergen-specific immunotherapy. No course of oral corticosteroids was given to CRSwNP patients at least three months before serum specimens collection.

Controls were selected from patients in the waiting list for septoplasty, after excluding subjects with symptoms and endoscopic or imagiological signs (in CT scan) of CRS; controls with innate or acquired immunodeficiency, autoimmune diseases or patients with history of or under allergen-specific immunotherapy were also excluded.

2.2- *Data Collection*

Clinical data was gathered through a systematic interview. Dietary habits were collected using the Portuguese validated Food Frequency Questionnaire (QFA) [142]. Every questioned food item was scored between an intake of 0 (“Never or less than 1 per month”) to 8 (“Six or more per month”).

Chronic lower respiratory diseases (CLRD) diagnosis was established by a respiratory physician.

Blood sample was collected to determine immunoglobulin classes, IgG subclasses levels and specific IgG and IgE against food allergens. Immunoglobulin classes were determined by electrochemiluminescence immunoassay using Cobas 6000 analyzer (Roche Diagnostics®, Mannheim, Germany) and IgG subclasses through the Optilite turbidimetric analyzer (The Binding Site®, Birmingham, United Kingdom).

2.3- *Enzyme Linked Immunosorbent Assay* (ELISA)

ELISA tests for semi-quantitative analysis of serum IgG antibodies to 40 food allergens was performed using OmegaDiagnostics® (Cambridgeshire, UK) detection kit. A microplate reader with 450 nm filter was used to read the final absorbance of each well. The given concentration of the two standards provided on the kit allowed to infer about antibody IgG concentration (Arbitrary Units/ml) on each well. Some food antigens are grouped in the same well as food mixtures, confering in total 21 wells [Corn; Oat; Rice; Rye; Wheat; Cow's Milk; Egg White; Egg Yolk; White Fish Misture (Cod, Haddock, Plaice); Shellfish Misture (Crab, Lobster, Prawn); Soya; Legume Bean Misture (Haricot, Kidney, Pea); Mustard Mixture (Cabbage, Broccoli, Cauliflower); Gluten, Apple and Pear; Berries Misture (Rasperry, Strawberry, Blackberry); Citrus Mixture (Orange, Lemon, Grapefruit); Nut Mixture (Almond, Cashew, Hazelnut, Peanut); Yeast (Bakers and Brewer's); Chicken and Turkey; Pork and Beef]. A positive response was considered if concentration value was ≥ 8 AU/ml, and the response of the reaction was classified as Grade 1+ if ≥ 8 and < 12.5, Grade 2+ if ≥ 12.5 and < 25 and Grade 3+ if ≥ 25 AU/ml, according to suggested ranges of the manufacturer.

2.4- ImmunoCAP™ Food Allergen Test

Specific IgE antibodies for food antigens were determined by immunoassay using ImmunoCAP™ Food Allergen tests by Thermo Fisher Scientific® for the following food allergens: fx5- Egg white, Milk, Fish, Wheat, Peanut, Soybean; f24- Shrimp; f44- Strawberry; f33- Orange; f5- Rye and f75- Egg yolk. Positive results were considered if serum concentration was above 0.35 KUA/L.

2.4- *Statistical Analysis*

Statistical analysis was performed with *Statistical Package for Social Sciences* (IBM® SPSS® Statistics for Windows, Version 23.0). Descriptive statistics was used in sample characterization. Mann-Whitney test for independent samples was used to compare continuous variables between the two groups. Pearson's chi-square test (or Fisher's exact test when appropriate) was used to test association between categorical data. A nonparametric Spearman correlation was used to examine the relation between quantitative variables.

A p-value <0.05 was considered as statistically significant.

6.3 Results

Sixty-four individuals were included: i.e. 33 patients with CRSwNP and 31 controls.

Demographics, body mass index (BMI) and QFA scores of patients with CRSwNP and controls are presented and compared in Table 15. There were no significant differences between CRSwNP and control groups based on age, gender distribution and BMI. According to QFA total score and sub-scores, no significant difference in the food consumption pattern was observed between groups.

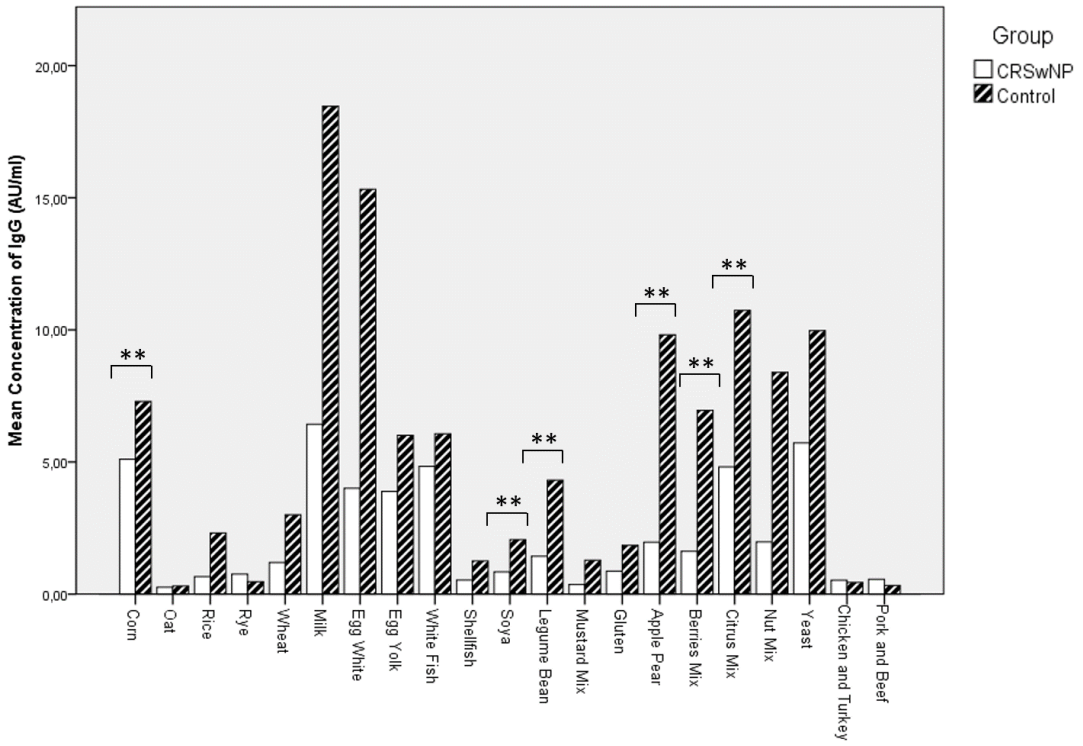
Table 15- Comparison of sample demographics, BMI, presence of CLRD, QFA score and subscores between patients with CRSwNP and controls. (N=64).

	CRSwNP Group (n=33)	**Control Group (n= 31)**	***p* value**
- Demographics			
Age	58.4 ± 2.4; [30; 82]	52.9 ± 2.2; [32; 75]	0.073
Gender: Male/Female, n	17/16	19/12	0.461
- BMI	27.4 ± 0.6; [19; 37]	26.17 ± 0.9; [17; 34]	0.282
- CLRD (n, %) *	21 (63.6)	2 (6.5)	**<0.001**
Atopic Asthma	6 (18.2)	2 (6.5)	0.149
Non-atopic Asthma	9 (27.3)	0 (0)	**0.001**
COPD	9 (27.3)	0 (0)	**0.033**
- **QFA**	155.27 ± 8.9; [79; 301]	153.19 ± 5.6; [91; 219]	0.481
Dairy Products	12.55 ± 5.50	9.94 ± 5.45	0.070
Eggs	2.21 ± 1.08	2.61 ± 1.05	0.102
Cereals	16.45 ± 1.26	16.03 ± 1.22	0.783
Rice	2.76 ± 1.00	2.94 ± 0.57	0.153
White Fish	7.06 ± 2.64	5.87 ± 0.47	0.110
Shellfish	0.73 ± 0.94	1.19 ± 0.21	0.083
Legumes	3.52 ± 2.42	3.13 ± 1.86	0.984
Vegetables	8.73 ± 5.08	9.77 ± 5.08	0.319
Apple and Pear	3.76 ± 1.64	3.61 ± 1.38	0.618
Berries	1.82 ± 1.78	2.48 ± 1.61	0.069
Citrus Fruits	4.45 ± 2.45	3.42 ± 2.22	0.092
Chicken and Turkey	3.48 ± 1.71	4.19 ± 1.78	0.124
Pork and Beef	6.48 ± 3.75	6.90 ± 2.86	0.398

CRSwNP- Chronic Rhinosinusitis with Nasal Polyps; BMI- Body Mass Index; CLRD- chronic lower respiratory diseases; COPD- Chronic Obstructive Pulmonary Disease; QFA - Food Frequency Questionnaire. Data are expressed as mean± SEM (standard error of mean). Comparison was made using Mann-Whitney test for continuous variables and Pearson's chi-square test for categorical data. [*] 3 out of 21 patients with CLRD presented clinical features associated with both asthma and COPD

CLRD were more prevalent in CRSwNP patients (21 patients - 63.6%) than in the control group (two participants - 6.5%), in a statistically significant way (p<0.001, Fisher's exact test) – Table 15. In CRSwNP group, 16 participants (48.4%) were under treatment with inhaled steroids with β2-agonists; whereas in the control group only two (6.4%) were being treated with inhaled steroids.

Using the ELISA tests for serum IgG antibodies to 40 food allergens analysis, we found the overall sum of IgG levels to be significantly lower in CRSwNP patients compared to the control group (p=0.013) and this difference was also observed for specific IgG antibodies against corn (p=0.009), soya (p=0.002), grain legumes (p=0.004), pear and apple (p=0.0025), berries (p=0.0005) and citric fruit (p=0.007) (Graphic 6). Moreover, the prevalence of patients with positive food IgG antibodies was significantly lower in CRSwNP (48.5%) compared to the control group (74.2%) (Table 16). The overall sum of specific food IgG antibodies did not correlate significantly with BMI (p=0.719) or age (p=0.228).



Graphic 6- Mean concentration values of specific IgG antibodies for different food allergens in patients with Chronic Rhinosinusitis with Nasal Polyps (CRSwNP) and controls. (N=64).

** p<0.01 using Mann-Whitney test.

No correlation between specific food IgG antibodies and the frequency of consumption that particular food was observed: i.e. IgG antibodies against cow's milk and dairy products consumption frequency (p=0.710); IgG antibodies against egg white and egg consumption frequency (p=0.505); IgG antibodies against wheat and cereals consumption frequency (p=0.676), among others. In global, considering the all sample, the specific IgG antibodies against food with higher mean concentration values in our sample were cow's milk

(12.25 AU/ml), egg white (9.49 AU/ml), citrus fruits (7.68 AU/ml), bakers and brewer's yeast (7.78 AU/ml) and corn (6.17 AU/ml).

Table 16- Comparison of food specific IgG antibodies levels and prevalence of test positivity between patients with chronic rhinosinusitis with nasal polyps (CRSwNP) and controls. (N=64).

	CRSwNP Group (n=33)	**Control Group (n= 31)**	***p* value**
Sum [Food IgG] (AU/ml)	48.24 ± 3.9; [18; 89]	115.5 ± 32.1; [22; 940]	**0.013**
Positive Food IgG (n, %)	16, 48.5%	23, 74.2%	**0.032**
Multiple Positive Food IgG (n, %)	9, 27.3%	16, 51.6%	**0.041**

Positive food IgG was considered if at least one food specific IgG concentration was ≥ 8 AU/ml and multiple positive if two or more food specific IgG antibodies were ≥ 8 AU/ml. Data are expressed as mean± SEM (standard error of mean). Comparison was made using Mann-Whitney test for continuous variables and Pearson's chi-square test for categorical data.

No correlation between total serum IgG level and sum of specific IgG against food was observed (p=0.748). However, considering subclasses and the two sample groups, we found a negative correlation between IgG1 serum levels and sum of food specific IgG concentration values in CRSwNP patients and a positive correlation between IgG1 serum levels and sum of food specific IgG concentration values in controls (Table 17).

Table 17- Correlation between the sum of food specific IgG concentration and total IgG and IgG subclasses in the serum among patients with CRSwNP and controls. (N=64).

	CRSwNP Group (n=33)		**Control Group (n= 31)**	
	R	**p value**	**R**	**p value**
Correlation between Sum of [Food IgG] and Total IgG	-0.014	0.937	0.197	0.289
Correlation between Sum of [Food IgG] and IgG1	-0.295	**0.048**	0.302	**0.049**
Correlation between Sum of [Food IgG] and IgG2	0.242	0.174	-0.036	0.845
Correlation between Sum of [Food IgG] and IgG3	0.103	0.568	-0.225	0.223
Correlation between Sum of [Food IgG] and IgG4	0.094	0.603	0.076	0.686

Correlation performed with Spearman test.

Moreover, in CRSwNP a statistically significant higher level of IgG1 subclass in the serum was detected compared to controls (p=0.041), and lower levels of IgG2 (p=0.048) and IgG3 (p=0.014). Concerning total IgG and IgG4 subclass levels, no difference was identified.

No significant difference in IgE food sensitization was observed in relation to the overall sum of specific food IgE concentrations or the prevalence of positive IgE values between CRSwNP and control group (Table 18). Concerning specific food IgE dosage, no significant differences were observed for fx5- Egg white, Milk, Fish, Wheat, Peanut, Soybean (p=0.271); Shrimp (p=0.629); Strawberry (p=0.207); Orange (p=0.509); Rye (p=0.585) or Egg yolk (p=0.066).

Table 18- Comparison between the sum of food specific IgE antibodies levels and the prevalence of test positivity between patients with CRSwNP and controls. (N=64).

	CRSwNP Group (n=33)	**Control Group (n= 31)**	***p* value**
Sum [Food IgE] (AU/ml)	0.49 ± 0.21; [0.01; 5.91]	0.40 ± 0.23; [0.01; 7.03]	0.234
Positive Food IgE (n, %)	5, 15.2%	4, 12.9%	1.000
Multiple Positive Food IgE (n, %)	3, 9.1%	2, 6.5%	1.000

Data are expressed as mean± SEM (standard error of mean). Comparison was made using Mann-Whitney test for continuous variables and Pearson's chi-square test for categorical data.

A positive correlation between total serum IgE levels and total specific IgE against food was observed ($p=5.12x10^{-9}$). Total IgE serum levels were significantly elevated in CRSwNP compared to controls (p=0.03).

No correlation between the overall sum of specific IgG and IgE antibodies against food was observed (p=0.936).

6.4 Discussion

Our results do not support the existence of an important role for food allergy in CRSwNP pathogenesis, independently of being an IgE- or IgG-mediated immune response.

As already stated, the possible role of food allergy in CRSwNP has been raised in two case-control studies[120,121] that found 70% and 81% positivity for intradermal food tests in CRSwNP patients and only 34% and 11% in the control group, respectively, with statistically significant differences. Meanwhile, some authors have criticized the value of intradermal food tests namely for their increased risk of systemic reactions comparatively with skin prick testing, non-correlation to specific IgE levels and high frequency of false-positive results.[143] Moreover, these two case-control studies have been carried out in the same investigational center and until now no replication of their results have been published elsewhere.

Regarding IgE-mediated food sensitization, we did not find any significant difference in ImmunoCAP™ results considering IgE food serology test positivity or even in the sum of specific IgE antibodies against food between CRSwNP and controls, in accordance to a previous publication that used EAST technique.[128] In that study, published in 2016, a positive correlation between total serum IgE level and sum of specific IgE antibodies against food was observed[128], as we also found in this investigation. We report here prevalences of IgE food sensitization in patients with CRSwNP (15%) and in controls (13%) which are similar to the values described by Lill C et al.[127] (22% in CRSwNP and 14% in controls). Lill C et al. reported that patients with CRSwNP had higher frequency of IgE positivity for milk compared to controls using RAST. However this result was not replicated in other studies so far and the fact that they did not find any control with IgE positivity for milk must be seen with caution, since milk has been reported as one of the most frequently food allergens in IgE dosage screenings in general population.[144] We did not find significant difference comparing fx5-multifood allergen panel (that includes milk) or in single specific IgE antibody dosage for milk in case of fx5 positivity. The discrepancy in the prevalence of IgE-mediated food allergy among CRSwNP between published studies might be due to different methodologies used, such as food allergy skin prick test, RAST or EAST. The ImmunoCAP™ is considered to have higher sensitivity and better diagnostic capacity compared to RAST[145], and this study was to our knowledge the first to use this technique in CRSwNP.

Concerning IgG-mediated food sensitization, our results did not show higher antibodies dosage in CRSwNP, but otherwise revealed an immune suppression of IgG response against food allergens in CRSwNP. The overall sum of food specific IgG antibodies concentration was significantly reduced in CRSwNP comparing to controls and was not correlated with BMI or age. Moreover, a statistically significant reduction in IgG concentration against particular foods such as corn, soya, legume beans, pear and apple, berries and citric fruits, was observed in the CRSwNP group, irrespective of food type consumption. We

hypothesize that this suppression of IgG-mediated immune response against food allergens could be the result of deviated IgG responses to other agents (e.g. airborne particles) in CRSwNP, which must be clarified in future investigations. In accordance with this hypothesis it is interesting to note that in the CRSwNP group a significant inverted correlation was observed between the sum of food specific IgG antibodies and IgG1 subclass level in serum, as opposed to the control group in which a positive correlation was found. Moreover, we observed that CRSwNP patients showed a subclass switching towards IgG1, with significant higher values of this IgG subclass and lower levels of IgG2 and IgG3, compared to controls.

As already discussed in Chapter 3 and Chapter 4, there is growing evidence that occupational exposure to dust can be related to the occurrence and persistence of chronic rhinosinusitis (CRS) and specifically to CRSwNP phenotype. The existing evidence point to a risk factor for CRS that is mainly due to the inhalation of lower molecular weight (LMW) particles (<5 kDa) which, contrary to high molecular weight (HMW) particles that induce a well-known IgE-mediated immune response, induce airway inflammation through mechanisms that are far less known. It can include the classical "irritant response" plus LMW sensitization of the adaptive immune system by acting as haptens.[49,81] HMW agents are generally proteins from animal and vegetal origin while LMW agents include a wide variety of organic and inorganic compounds. In contrast to protein allergens, LMW agents are incomplete antigens (i.e. haptens) that must bind to carrier macromolecules to become immunogenic. For example, LMW agents causing occupational asthma are typically highly reactive electrophilic compounds that are capable of combining with hydroxyl, amino and thiol functionalities on airway proteins.[146] The innate chemical reactivity of most LMW agents has largely hampered the investigation of immunological mechanisms, owing to uncertainty about the antigens that elicit immunological responses.[146] The understanding of the interaction between LMW particles and respiratory proteins, can lead to the identification of antigenic determinants involved in CRSwNP and non-IgE-mediated asthma and to the development of hapten-specific monoclonal antibodies to clarify the subjacent immunologic mechanisms.

As previously discussed in Chapter 5, IgG1 which was significantly elevated in the CRSwNP group, is an important IgG subclass in antibody response to soluble protein antigens, membrane proteins and allergens, has the longest serum half-life, is capable of complement activation by C1q binding, is the main IgG subclass to cross placental and mucosal barriers[108] and has the strongest affinity to FcRn.[109,110] The FcRn receptor has been shown in an animal model to have a predominant expression in the airways compared to other organs[113] and has already been demonstrated to be expressed in nasal mucosa.[111] In both human asthmatics and animal models of allergy, it has been showed that allergen-specific IgG mediated response can contribute to Th2-mediated inflammation[147], the predominant type of inflammation seen in Western CRSwNP patients.[1] IgG1 ratio between bronchoalveolar fluid and serum concentrations has been showed to be elevated in asthmatic patients compared to the ratios of other IgG subclasses[106] and the relative concentration of IgG1 in

nasal tissue homogenates in CRSwNP has been demonstrated to be significantly elevated compared to controls.[101] Based on this facts and integrating the results of our previous studies, we hypothesized that an augmented IgG1 response mounted in airways could make the immune system more tolerant to food allergens in the intestinal mucosa, mainly to protein food antigens.

Since food IgG sensitization seem to be reduced in CRSwNP comparing to controls, it seems unlikely to be an important factor in nasal polyposis etiopathogenesis. These results need to be replicated in other populations and investigation on the subjacent immune mechanisms of this phenomenon are needed. The use of a randomly selected group of non-CRS subjects from the general population as a control group would be of benefit in future investigations.

To our knowledge, this is the first study to investigate the hypothesis of IgG-mediated food allergy in CRSwNP pathogenesis and to use ImmunoCAP™ technology to evaluate IgE food sensitization in this disease. Our study has some limitations, namely a relatively small sample size from a single institution, the use of patients waiting for septoplasty as controls and a high percentage of CRSwNP under inhalant steroids. Nonetheless, patients were taking a combination of inhaled steroids with long-acting B2-agonists that allows asthma control at lower corticosteroids doses, with negligible systemic side effects[119].

Conclusion

In conclusion, our findings suggest that food allergy does not have an important role in CRSwNP etiopathogenesis, neither through IgE-mediated mechanisms nor through IgG-mediated hypersensitivity. Moreover, we observed a suppression in development of IgG sensitization against food allergens in CRSwNP patients which may be related to deviated IgG responses against other important targets (e.g. airborne particles). Supporting this theory, is the fact that an IgG1 subclass switching was observed in CRSwNP patients and its values were negatively correlated to the sum of food specific IgG concentration values.

Chapter 7.

FINAL DISCUSSION AND CONCLUSION

7. Final Discussion

In spite of all the investigation carried out about CRSwNP, its risk factors and the characterization of the underlying physiopathological mechanisms are still unclear. Even though new medical treatments and surgical techniques have been developed, bringing some benefits on patient's quality of life, CRSwNP has remained as a difficult to treat condition.

The research conducted for this thesis comprehended an investigation on CRSwNP etiopathogeny focusing on host-environment interaction and comprising a clinical, epidemiological and clinical-laboratory integrated approach. It pretended to evaluate specifically CRSwNP, with meticulous differential diagnosis to other clinical entities, but not including its special subsets which deserve a separate and special research attention as defined in EPOS2012[1] (e.g. Cystic Fibrosis, Vasculitis, Primary Ciliary Dyskinesia, Allergic fungal rhinosinusitis).

Our first study was a clinical retrospective study about ESS results on CRSwNP treatment outcomes and identification of recurrence prognostic factors, which confirmed an obvious benefit on postoperative symptom resolution, but with still high recurrence rate, which represents a source of concern. This study also demonstrated that occupational dust exposure and the concomitant presence of non-atopic asthma were independent predictive factors of recurrence of CRSwNP after surgery. The option for a multivariate logistic regression analysis was a main strength of this research compared to previous studies of this kind, that often used only bivariate analysis.

Concerning the importance of occupational dust exposure, Hox et al.[56] in 2012 had already alerted for a possible role of it in CRS recurrence and persistence. Compared to that study, our research pointed to an even more pronounced effect of occupational dust exposure on CRSwNP. Other data that is important to emphasize, was the identification of low molecular weight particles (LMW) as the main occupational risk factor for disease recurrence, also similar to what was reported by Hox et al.[56] It is known that, contrary to high molecular weight particles (HMW) that act by IgE-mediated immune responses, LMW do not have their mechanisms of action well established. It is important to notice that LMW particles are also known to be associated with severe occupational asthma (non-atopic asthma).[89] The distinction made in our study between atopic and non-atopic asthma, permitted to highlight the role of non-atopic asthma as a risk factor for disease recurrence and to clarify asthma impact in endoscopic sinus surgery results.

The results found on this clinical retrospective study led us to design an epidemiological study, which aimed to determine Nasal Polyposis prevalence in workers with and without occupational exposure to dust, using nasal endoscopic for NP screening. We decided to make this screening among textile workers as we found in the previous study that

60% of CRSwNP patients were exposed to dust during their job routine, the majority of them being textile particles. This investigation was pioneering in demonstrating a significantly higher prevalence of NP among textile workers compared to the control group, but also significantly higher than the prevalence found in the two European population-based studies, in Portugal and Sweden[17,18], which highlighted the importance of occupational dust exposure as a risk factor for the occurrence of CRSwNP. A longer occupational dust exposition also seemed to increase the risk of CRSwNP occurrence. Rhinologic symptoms were more prevalent among textile workers compared to controls, with rhinosinusitis symptoms having all a statistically significant higher prevalence. We hypothesize, then, that occupational/work-exacerbated rhinitis may progress toward occupational/work-exacerbated rhinosinusitis, which in turn may contribute to CRSwNP development. The absence of a significant difference in the prevalence of reported atopic diseases between exposed and control groups, supports that IgE-mediated allergy do not seem to be important on CRSwNP etiopathogenesis. The non-despicable proportion of clinically silent disease and of patients that claimed to have CRSwNP that was not confirmed on the endoscopic screening, confirmed the importance of endoscopic based studies over the non-reliable questionnaire-based ones.

The results of this work alert for a problem of Public Health, showing the importance of employee's protective measures, such as mask use during work, and reinforces the need for legislation and control on the functioning of air dust filters and exhausting systems to guarantee air quality in this type of industry. Whenever possible patients with CRSwNP should work in free dust environments.

More epidemiologic endoscopic based investigations are needed to establish the NP prevalence in other types of occupational dust exposure, and it would be desirable to differentiate the effect of LMW and HMW particles. The concomitant involvement of otolaryngologists and pneumologists on this type of studies would be helpful to clarify the association between CRSwNP and lower respiratory diseases (namely, asthma and COPD) and to try to define the subjacent physiopathology mechanisms of these airway diseases that can have common triggers.

The clinical laboratory study that we performed for this investigation supported once more the importance of occupational exposure to dust on CRSwNP, and the association of this clinical entity with CLRD (namely, non-atopic asthma and COPD). A distinct systemic immunologic profile in CRSwNP patients compared to controls, concerning leukogram and humoral immunity was observed. The changes that were found in peripheral leukocyte count (higher eosinophil relative count and lower neutrophil relative count) and the systemic IgG1 subclass switch are similar to what is described to happen in nasal polyp tissue of Caucasian CRSwNP patients, and these differences were even more marked if CLRD were present. Following the concept of "one airway, one disease" this IgG1-mediated immune response in CRSwNP patients may be a key piece in understanding its interrelation with CLRD, namely non-atopic asthma and COPD and to clarify the role of occupational dust exposure,

specifically LMW particles, in their etiopathogenesis. The FcRn receptor, already identified in nasal mucosa[111], known for its regulation of IgG serum half-life and to orchestrate IgG-based immune responses at mucosal sites, needs to be studied in CRSwNP patients, to see if it can be associated to IgG1 subclass switching in this disease.

The last work of this investigation, included as part of the clinical laboratory study, intended to evaluate the role of food allergy in CRSwNP. To our knowledge, this was the first study to investigate the hypothesis of IgG-mediated food allergy in CRSwNP pathogenesis and to use ImmunoCAP™ technology to evaluate IgE food sensitization in this disease. Our findings suggest that food allergy does not have an important role in CRSwNP etiopathogenesis, neither through IgE-mediated mechanisms nor through IgG-mediated hypersensitivity. Moreover, we observed a suppression in the development of IgG sensitization against food allergens in CRSwNP patients. We hypothesize that this suppression of IgG-mediated immune response against food allergens could be the result of deviated IgG responses to other agents (e.g. airborne particles) in CRSwNP. Supporting this theory, is the fact that an IgG1 subclass switching was observed in CRSwNP patients and its values were negatively correlated to the sum of food specific IgG concentration values. These results need to be replicated in other populations and investigation on the subjacent immune mechanisms of this phenomenon are needed.

Understanding CRSwNP etiopathogenesis and its relationship with CLRD, is a critical step towards airway disease management and control. Otolaryngologists, Pneumologists and Immunoallergologists must unify their research efforts, share their knowledge in this area, in order to find crucial clues about CRSwNP physiopathology. It is also important to continue the investigation that has been made in LMW agents causing occupational asthma[89,103] and use it as model to study the impact of them on CRSwNP. The pathogenesis of occupational asthma caused by LMW agents remains unclear, since the innate chemical reactivity of these agents has hampered the investigation of their immunological mechanisms. This is due to uncertainty about the antigens that elicit the immune response and the same must be happening with CRSwNP. Recently, important findings have been made about this type of particles. We currently know that LMW agents causing occupational asthma are typically highly reactive electrophilic compounds that are capable of combining with hydroxyl, amino and thiol functionalities on airway proteins, acting as haptens.[146] Some of their structural and physicochemical properties have been found to be important to determine their potential for inducing respiratory sensitisation (e.g. capacity to form at least two bonds with human proteins and the agent's potential for chemical binding, as reflected by Hansen polarity and hydrogen-bond acceptance).[146] The advances in the characterization of the molecular interactions between LMW particles and airway proteins should lead to a re-exploration of the nature of the involved immunological mechanisms and to the definition of the biologically

relevant antigenic determinants associated to CRSwNP and non-atopic asthma. The development of hapten-specific monoclonal antibodies would greatly enhance the understanding of the complex interactions between LMW agents and respiratory proteins and could bring new treatment strategies.

In future research it is essential to use careful methodologies, defining the CRS types and CRSwNP subtypes that are intended to study, because only in this manner it will be possible to define individual and environmental predisposing and modifying disease factors for those subgroups, and subsequently their best treatment and preventive options.

In conclusion, with this investigation we add new data about the importance of occupational exposure to dust on CRSwNP etiopathogenesis, which deserves to be further investigated. The exposition to LMW agents can be a key piece in CRSwNP and non-atopic asthma development, reflecting their interrelationship and common subjacent immunological mechanisms. The modifications that we found on the systemic immune profile of CRSwNP, highlights that this disease cannot be viewed as a local nasal disease and also opens a new perspective as a possible role for IgG1-mediated immune response in its physiopathology. We also discarded an ultimate role for food allergy in CRSwNP etiopathogenesis, either through IgE-mediated mechanisms or through IgG-mediated hypersensitivity, with an interesting suppression of this latest in CRSwNP patients. All of these results may have a critical contribution in the future of CRSwNP research and help in disease prevention and treatment.

8. REFERENCES

References

[1] Fokkens WJ, Lund VJ, Mullol J, Bachert C, Alobid I, Baroody F et al. European position paper on rhinosinusitis and nasal polyps 2012. Rhinology 2012;50:1–329.

[2] Lund VJ. Diagnosis and treatment of nasal polyps. BMJ 1995;311:1411–4.

[3] Hulse KE, Stevens WW, Tan BK, Schleimer RP. Pathogenesis of nasal polyposis. Clin Exp Allergy 2015;45:328–46. doi:10.1111/cea.12472.

[4] Önerci T, Ferguson B. Nasal Polyposis: Pathogenesis, Medical and Surgical Treatment. Springer-Verlag Berlin Hedelberg 2010.

[5] European Academy of Allergology and Clinical Immunology. European position paper on rhinosinusitis and nasal polyps. Rhinol Suppl 2005;18:1–87.

[6] Fokkens WJ, Lund V, Mullol J. European position paper on rhinosinusitis and nasal polyps 2007. Rhinology 2007;45:1–136.

[7] Rosenfeld RM. Clinical Practice Guideline on Adult Sinusitis. Otolaryngol Neck Surg 2007;137:365–77. doi:10.1016/j.otohns.2007.07.021.

[8] Rosenfeld RM, Piccirillo JF, Chandrasekhar SS, Brook I, Ashok Kumar K, Kramper M, et al. Clinical Practice Guideline (Update): Adult Sinusitis. Otolaryngol Neck Surg 2015;152:S1–39. doi:10.1177/0194599815572097.

[9] Orlandi RR, Kingdom TT, Hwang PH. International Consensus Statement on Allergy and Rhinology: Rhinosinusitis Executive Summary. Int Forum Allergy Rhinol 2016;6:S3–21. doi:10.1002/alr.21694.

[10] Koennecke M, Klimek L, Mullol J, Gevaert P, Wollenberg B. Subtyping of polyposis nasi: phenotypes, endotypes and comorbidities. Allergo J Int 2018;27:56–65. doi:10.1007/s40629-017-0048-5.

[11] Ren L, Zhang N, Zhang L, Bachert C. Biologics for the treatment of chronic rhinosinusitis with nasal polyps - state of the art. World Allergy Organ J 2019;12:100050. doi:10.1016/j.waojou.2019.100050.

[12] Bachert C, Akdis CA. Phenotypes and Emerging Endotypes of Chronic Rhinosinusitis. J Allergy Clin Immunol Pract 2016;4:621–8. doi:10.1016/j.jaip.2016.05.004.

[13] Tikaram A, Prepageran N. Asian nasal polyps: a separate entity? Med J Malaysia 2013;68:445–7.

[14] Hastan D, Fokkens WJ, Bachert C, Newson RB, Bislimovska J, Bockelbrink A, et al. Chronic rhinosinusitis in Europe - an underestimated disease. A GA2LEN study. Allergy 2011;66:1216–23. doi:10.1111/j.1398-9995.2011.02646.x.

[15] Shi JB, Fu QL, Zhang H, Cheng L, Wang YJ, Zhu DD, et al. Epidemiology of chronic

rhinosinusitis: results from a cross-sectional survey in seven Chinese cities. Allergy 2015;70:533–9. doi:10.1111/all.12577.

[16] Gao WX, Ou CQ, Fang SB, Sun YQ, Zhang H, Cheng L, et al. Occupational and environmental risk factors for chronic rhinosinusitis in China: a multicentre cross-sectional study. Respir Res 2016;17:54. doi:10.1186/s12931-016-0366-z.

[17] Johansson L, Akerlund A, Holmberg K, Melen I, Bende M. Prevalence of nasal polyps in adults: the Skovde population-based study. Ann Otol Rhinol Laryngol 2003;112:625–9.

[18] Cerejeira R, Veloso-Teles R, Lousan N MC. Prevalence of nasal polyps in Northern Portugal: a cadaver endoscopic study. Rhinology 2014;52:386–9. doi:10.4193/Rhin.

[19] Zhang N, Holtappels G, Claeys C, Huang G, van Cauwenberge P, Bachert C. Pattern of inflammation and impact of Staphylococcus aureus enterotoxins in nasal polyps from southern China. Am J Rhinol n.d.;20:445–50.

[20] Li Q, Cheng K, Wang F, Zhou S. Role of atopy in chronic rhinosinusitis with nasal polyps: does an atopic condition affect the severity and recurrence of disease? J Laryngol Otol 2016;130:640–4. doi:10.1017/S0022215116008112.

[21] Settipane GA, Chafee FH. Nasal polyps in asthma and rhinitis. A review of 6,037 patients. J Allergy Clin Immunol 1977;59:17–21.

[22] Grigoreas C, Vourdas D, Petalas K, Simeonidis G, Demeroutis I, Tsioulos T. Nasal Polyps in Patients with Rhinitis and Asthma. Allergy Asthma Proc 2002;23:169–74.

[23] Habib A-RR, Javer AR, Buxton JA. A population-based study investigating chronic rhinosinusitis and the incidence of asthma. Laryngoscope 2015. doi:10.1002/lary.25831.

[24] Ragab A, Clement P. Objective assessment of lower airway involvement in chronic rhinosinusitis. Am J Rhinol 2004:15–21.

[25] Grossman J. One airway, one disease. Chest 1997;111:11S-16S.

[26] Håkansson K, Thomsen SF, Konge L, Mortensen J, Backer V, von Buchwald C. A comparative and descriptive study of asthma in chronic rhinosinusitis with nasal polyps. Am J Rhinol Allergy 2014;28:383–7. doi:10.2500/ajra.2014.28.4076.

[27] Frendø M, Håkansson K, Schwer S, Rix I, Ravn AT, Backer V, et al. Asthma in ear, nose, and throat primary care patients with chronic rhinosinusitis with nasal polyps. Am J Rhinol Allergy 2016;30:67–71. doi:10.2500/ajra.2016.30.4304.

[28] Dinis PB, Gomes A. Sinusitis and asthma: how do they interrelate in sinus surgery? Am J Rhinol 1997;11:421–8.

[29] Dejima K, Hama T, Miyazaki M, Yasuda S, Fukushima K, Oshima A, et al. A clinical study of endoscopic sinus surgery for sinusitis in patients with bronchial asthma. Int Arch Allergy Immunol 2005;138:97–104. doi:10.1159/000088430.

[30] Mehanna H, Mills J, Kelly B, McGarry GW. Benefit from endoscopic sinus surgery. Clin Otolaryngol Allied Sci 2002;27:464–71.

[31] Kountakis SE, Bradley DT. Effect of asthma on sinus computed tomography grade and symptom scores in patients undergoing revision functional endoscopic sinus surgery. Am J Rhinol 2003;17:215–9.

[32] Hansen AG, Helvik AS, Thorstensen WM, Nordgård S, Langhammer A, Bugten V, et al. Paranasal sinus opacification at MRI in lower airway disease (the HUNT study-MRI). Eur Arch Oto-Rhino-Laryngology 2015:1761–8. doi:10.1007/s00405-015-3790-7.

[33] Roberts NJ, Lloyd-Owen SJ, Rapado F, Patel IS, Wilkinson TMA, Donaldson GC, et al. Relationship between chronic nasal and respiratory symptoms in patients with COPD. Respir Med 2003;97:909–14. doi:10.1016/S0954-6111(03)00114-8.

[34] Hurst JR, Kuchai R, Michael P, Perera WR, Wilkinson TM WJ. Nasal symptoms, airway obstruction and disease severity in chronic obstructive pulmonary disease. Clin Physiol Funct Imaging 2006;26:251–6.

[35] van den Berge M, Aalbers R. The asthma-COPD overlap syndrome: how is it defined and what are its clinical implications? J Asthma Allergy 2016;9:27–35. doi:10.2147/JAA.S78900.

[36] Samter M, Beers RF. Concerning the nature of intolerance to aspirin. J Allergy 1967;40:281–93.

[37] Kennedy JL, Stoner AN, Borish L. Aspirin-exacerbated respiratory disease: Prevalence, diagnosis, treatment, and considerations for the future. Am J Rhinol Allergy 2016;30:407–13. doi:10.2500/ajra.2016.30.4370.

[38] Rajan JP, Wineinger NE, Stevenson DD, White AA. Prevalence of aspirin-exacerbated respiratory disease among asthmatic patients: A meta-analysis of the literature. J Allergy Clin Immunol 2015;135:676-81.e1. doi:10.1016/j.jaci.2014.08.020.

[39] Stern BM, Sharma G. Ciliary Dysfunction (Kartagener Syndrome, Primary Ciliary Dyskinesia). StatPearls Publishing; 2019.

[40] Tadesse A, Alemu H, Silamsaw M, Gebrewold Y. Kartagener's syndrome: a case report. J Med Case Rep 2018;12:5. doi:10.1186/s13256-017-1538-2.

[41] Zhou B, Guan Y, Liu S, Sun J, Zhang L, Han D. Observation of nasal mucosal cilia ultrastructure of Kartagener's syndrome. Zhonghua Er Bi Yan Hou Ke Za Zhi 2001;36:323–5.

[42] Nowakowska-Trzcińska J, Janicki K. A case of Kartagener's syndrome. Otolaryngol Pol = Polish Otolaryngol 2002;56:741–4.

[43] Alanin MC, Aanaes K, Høiby N, Pressler T, Skov M, Nielsen KG, et al. Sinus surgery can improve quality of life, lung infections, and lung function in patients with primary

ciliary dyskinesia. Int Forum Allergy Rhinol 2017;7:240–7. doi:10.1002/alr.21873.

[44] Baroody FM. Mucociliary transport in chronic rhinosinusitis. Clin Allergy Immunol 2007;20:103–19.

[45] Cornet ME, Georgalas C, Reinartz SM, Fokkens WJ. Long-term results of functional endoscopic sinus surgery in children with chronic rhinosinusitis with nasal polyps. Rhinology 2013;51:328–34. doi:10.4193/Rhin13.079.

[46] Aanaes K, Johansen HK, Skov M, Buchvald FF, Hjuler T, Pressler T, et al. Clinical effects of sinus surgery and adjuvant therapy in cystic fibrosis patients - can chronic lung infections be postponed? Rhinology 2013;51:222–30. doi:10.4193/Rhino12.207.

[47] Beule A. Epidemiology of chronic rhinosinusitis, selected risk factors, comorbidities, and economic burden. GMS Curr Top Otorhinolaryngol Head Neck Surg 2015;14:Doc11. doi:10.3205/cto000126.

[48] Yılmaz İ, Çelik G, Aydın Ö, Özdemir SK, Soyyiğit Ş, Sözener Z, et al. Churg-Strauss syndrome: a new endotype of severe asthma? Results of 14 Turkish patients. Clin Respir J 2015;9:350–8. doi:10.1111/crj.12154.

[49] Olsen KD, Neel HB, Deremee RA, Weiland LH. Nasal manifestations of allergic granulomatosis and angiitis (Churg-Strauss syndrome). Otolaryngol Head Neck Surg 1979;88:85–9.

[50] Jerrentrup A, Müller T, Mariss J, Dylla S. Eosinophile Granulomatose mit Polyangiitis (Churg-Strauss-Syndrom). Internist 2017:1–6. doi:10.1007/s00108-017-0303-3.

[51] Masi AT, Hunder GG, Lie JT, Michel BA, Bloch DA, Arend WP, et al. The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). Arthritis Rheum 1990;33:1094–100.

[52] Basu N, Erwig L-P. Pathophysiological importance of antineutrophil antibodies in vasculitis. Curr Opin Hematol 2011;18:25–9. doi:10.1097/MOH.0b013e328340dc2a.

[53] Tran Khai Hoan N, Karmochkine M, Laccourreye O, Bonfils P. Nasal polyposis and immunoglobulin-G subclass deficiency. Eur Ann Otorhinolaryngol Head Neck Dis 2014;131:171–5. doi:10.1016/j.anorl.2013.11.002.

[54] Pimentel JC. [92 cases of allergic-type nasal polyp. A methodology for its etiological characterization]. Acta Med Port 1995;8:379–84.

[55] Kim J, Hanley JA. The role of woodstoves in the etiology of nasal polyposis. Arch Otolaryngol Head Neck Surg 2002;128:682–6.

[56] Hox V, Delrue S, Scheers H, Adams E, Keirsbilck S, Jorissen M, et al. Negative impact of occupational exposure on surgical outcome in patients with rhinosinusitis. Allergy 2012;67:560–5. doi:10.1111/j.1398-9995.2011.02779.x.

[57] Erbek SS, Erbek S, Budakoğlu I, Cakmak O. The relationship between smoking and nasal

polyposis. Kulak Burun Bogaz Ihtis Derg 2008;18:216–20.

[58] Rugina M, Serrano E, Klossek JM, Crampette L, Stoll D, Bebear JP, et al. Epidemiological and clinical aspects of nasal polyposis in France; the ORLI group experience. Rhinology 2002;40:75–9.

[59] Wu AW, Ting JY, Platt MP, Tierney HT, Metson R. Factors affecting time to revision sinus surgery for nasal polyps: A 25-year experience. Laryngoscope 2014;124:29–33. doi:10.1002/lary.24213.

[60] Görgülü O, Özdemir S, Canbolat EP, Sayar Ç, Olgun MK, Akbaş Y. Analysis of the Roles of Smoking and Allergy in Nasal Polyposis. Ann Otol Rhinol Laryngol 2012;121:615–9. doi:10.1177/000348941212100909.

[61] Zinreich SJ, Mattox DE, Kennedy DW, Chisholm HL, Diffley DM, Rosenbaum AE. Concha bullosa: CT evaluation. J Comput Assist Tomogr 1988;12:778–84.

[62] Caughey RJ, Jameson MJ, Gross CW, Han JK. Anatomic risk factors for sinus disease: fact or fiction? Am J Rhinol 2005;19:334–9.

[63] Jones NS. CT of the paranasal sinuses: a review of the correlation with clinical, surgical and histopathological findings. Clin Otolaryngol Allied Sci 2002;27:11–7.

[64] Bilge T, Akpinar M, Mahmutoğlu AS, Uçak I, Uslu Coşkun B. Anatomic Variations in Paranasal Sinuses of Patients With Sinonasal Polyposis. J Craniofac Surg 2016;27:1336–9. doi:10.1097/SCS.0000000000002731.

[65] Kilty SJ, Bossé Y, Cormier C, Endam LM, Desrosiers MY. Polymorphisms in the SERPINA1 (Alpha-1-Antitrypsin) gene are associated with severe chronic rhinosinusitis unresponsive to medical therapy. Am J Rhinol Allergy 2010;24:e4-9. doi:10.2500/ajra.2010.24.3429.

[66] Maune S, Rath NF, Görögh T, Steinert R. Genetic disposition to chronic polypoid sinusitis and alpha 1-proteinase inhibitor deficiency types. HNO 1995;43:537–9.

[67] Stokes PJ, Rimmer J. The relationship between serum Vitamin D and chronic rhinosinusitis: A systematic review. Am J Rhinol Allergy 2016;30:23–8. doi:10.2500/ajra.2016.30.4267.

[68] Ponikau JU, Sherris DA, Kern EB, Homburger HA, Frigas E, Gaffey TA, et al. The Diagnosis and Incidence of Allergic Fungal Sinusitis. Mayo Clin Proc 1999;74:877–84. doi:10.4065/74.9.877.

[69] Braun H, Buzina W, Freudenschuss K, Beham A, Stammberger H. ???Eosinophilic Fungal Rhinosinusitis???: A Common Disorder in Europe? Laryngoscope 2003;113:264–9. doi:10.1097/00005537-200302000-00013.

[70] Pérez-Novo CA, Watelet JB, Claeys C, Van Cauwenberge P, Bachert C. Prostaglandin, leukotriene, and lipoxin balance in chronic rhinosinusitis with and without nasal

polyposis. J Allergy Clin Immunol 2005;115:1189–96. doi:10.1016/j.jaci.2005.02.029.

[71] Pujols L, Mullol J, Alobid I, Roca-Ferrer J, Xaubet A, Picado C. Dynamics of COX-2 in nasal mucosa and nasal polyps from aspirin-tolerant and aspirin-intolerant patients with asthma. J Allergy Clin Immunol 2004;114:814–9. doi:10.1016/j.jaci.2004.07.050.

[72] Bernstein JM, Ballow M, Schlievert PM, Rich G, Allen C, Dryja D. A superantigen hypothesis for the pathogenesis of chronic hyperplastic sinusitis with massive nasal polyposis. Am J Rhinol 2003;17:321–6.

[73] Flint PW, Haughey BH, Lund VJ, Niparko JK, Robbins KT, Thomas JR, et al. Cummings otolaryngology : head and neck surgery. 6th edition. Saunders 2014.

[74] Dalziel K, Stein K, Round A, Garside R, Royle P. Endoscopic sinus surgery for the excision of nasal polyps: A systematic review of safety and effectiveness. Am J Rhinol 2006;20:506–19.

[75] Lund VJ, Mackay IS. Staging in rhinosinusitus. Rhinology 1993;31:183–4.

[76] Moscato G, Vandenplas O, Gerth Van Wijk R, Malo JL, Quirce S, Walusiak J, et al. Occupational rhinitis. Allergy 2008;63:969–80. doi:10.1111/j.1398-9995.2008.01801.x.

[77] Soler ZM, Sauer D, Mace J, Smith TL. Impact of mucosal eosinophilia and nasal polyposis on quality-of-life outcomes after sinus surgery. Otolaryngol Head Neck Surg 2010;142:64–71. doi:10.1016/j.otohns.2009.10.005.

[78] Klossek JM, Neukirch F, Pribil C, Jankowski R, Serrano E, Chanal I, et al. Prevalence of nasal polyposis in France: a cross-sectional, case-control study. Allergy 2005;60:233–7. doi:10.1111/j.1398-9995.2005.00688.x.

[79] Chester AC, Antisdel JL, Sindwani R. Symptom-specific outcomes of endoscopic sinus surgery: a systematic review. Otolaryngol Head Neck Surg 2009;140:633–9. doi:10.1016/j.otohns.2008.12.048.

[80] Dufour X, Bedier A, Ferrie J-C, Gohler C, Klossek J-M. Diffuse nasal polyposis and endonasal endoscopic surgery: long-term results, a 65-case study. Laryngoscope 2004;114:1982–7. doi:10.1097/01.mlg.0000147933.14014.12.

[81] Kim BG, Kang JM, Shin JH, Choi HN, Jung YH, Park SY. Do sinus computed tomography findings predict olfactory dysfunction and its postoperative recovery in chronic rhinosinusitis patients? Am J Rhinol Allergy 2015;29:69–76. doi:10.2500/ajra.2015.29.4120.

[82] Georgalas C. The role of the nose in snoring and obstructive sleep apnoea: an update. Eur Arch Otorhinolaryngol 2011;268:1365–73. doi:10.1007/s00405-010-1469-7.

[83] Jiang RS, Liang KL, Hsin CH, Su MC. The impact of chronic rhinosinusitis on sleep-disordered breathing. Rhinology 2015;54:75–9. doi:10.4193/Rhin15.204.

[84] Tokunaga T, Sakashita M, Haruna T, Asaka D, Takeno S, Ikeda H, et al. Novel scoring

system and algorithm for classifying chronic rhinosinusitis: the JESREC Study. Allergy 2015;70:995–1003. doi:10.1111/all.12644.

[85] Yip J, Yao CM, Lee JM. State of the art: A systematic review of the surgical management of aspirin exacerbated respiratory disease. Am J Rhinol Allergy 2014;28:493–501. doi:10.2500/ajra.2014.28.4103.

[86] Johansson SGO, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol 2004;113:832–6. doi:10.1016/j.jaci.2003.12.591.

[87] Rix I, Håkansson K, Larsen CG, Frendø M, von Buchwald C. Management of chronic rhinosinusitis with nasal polyps and coexisting asthma: A systematic review. Am J Rhinol Allergy 2015;29:193–201. doi:10.2500/ajra.2015.29.4178.

[88] Hox V, Steelant B, Fokkens W, Nemery B, Hellings PW. Occupational upper airway disease: How work affects the nose. Allergy Eur J Allergy Clin Immunol 2014;69:282–91. doi:10.1111/all.12347.

[89] Meca O, Cruz M-J, Sánchez-Ortiz M, González-Barcala F-J, Ojanguren I, Munoz X. Do Low Molecular Weight Agents Cause More Severe Asthma than High Molecular Weight Agents? PLoS One 2016;11:e0156141. doi:10.1371/journal.pone.0156141.

[90] Pawankar R, Canonica GW, Holgate ST, Lockey RF. White Book on Allergy. World Allergy Organization (WAO); 2011.

[91] Wook T, Jae K, Chung H, Hyun S, Seung C, Lee H, et al. The Effectiveness of Budesonide Nasal Irrigation After Endoscopic Sinus Surgery in Chronic Rhinosinusitis With Asthma. Clin Exp Otorhinolaryngol 2016:[Epub ahead of print].

[92] Kosugi EM, Moussalem GF, Simões JC, Souza R de P e SF de, Chen VG, Saraceni Neto P, et al. Topical therapy with high-volume budesonide nasal irrigations in difficult-to-treat chronic rhinosinusitis. Braz J Otorhinolaryngol 2016;82:191–7. doi:10.1016/j.bjorl.2015.03.014.

[93] Cerejeira R, Veloso-Teles R, Lousan N, Moura CP. The Portuguese version of the RhinoQOL Questionnaire: validation and clinical application. Braz J Otorhinolaryngol 2015;81:630–5. doi:10.1016/j.bjorl.2015.08.015.

[94] CAT Development Steering Group. Healthcare Professional User Guide - CAT 2012:5–12.

[95] Kurashima K, Takaku Y, Ohta C, Takayanagi N, Yanagisawa T, Sugita Y. COPD assessment test and severity of airflow limitation in patients with asthma, COPD, and asthma–COPD overlap syndrome. Int J COPD 2016;11:479–87. doi:10.2147/COPD.S97343.

[96] Lund VJ, Kennedy DW. Staging for rhinosinusitis. Otolaryngol Head Neck Surg

1997;117:S35-40. doi:10.1016/S0194-59989770005-6.

[97] Settipane GA. Epidemiology of Nasal Polyps. Allergy and Asthma Proc 1996;17:231–6.

[98] Nagoda M, Okpapi JU, Babashani M. Assessment of respiratory symptoms and lung function among textile workers at Kano Textile Mills, Kano, Nigeria. Niger J Clin Pract 2012;15:373–9. doi:10.4103/1119-3077.104505.

[99] Hinson AV, Lokossou VK, Schlünssen V, Agodokpessi G, Sigsgaard T, Fayomi B. Cotton Dust Exposure and Respiratory Disorders among Textile Workers at a Textile Company in the Southern Part of Benin. Int J Environ Res Public Health 2016;13(6):895 doi:10.3390/ijerph13090895.

[100] Mansouri F, Pili JP, Abbasi A, Soltani M, Izadi N. Respiratory problems among cotton textile workers. Lung India 2016;33(2):163–6. doi:10.4103/0970-2113.177444.

[101] Van Zele T, Gevaert P, Holtappels G, Van Cauwenberge P, Bachert C. Local immunoglobulin production in nasal polyposis is modulated by superantigens. Clin Exp Allergy 2007;37:1840–7. doi:10.1111/j.1365-2222.2007.02838.x.

[102] Chen JB, James LK, Davies AM, Wu YCB, Rimmer J, Lund VJ, et al. Antibodies and superantibodies in patients with chronic rhinosinusitis with nasal polyps. J Allergy Clin Immunol 2017;139:1195-1204.e11. doi:10.1016/j.jaci.2016.06.066.

[103] Hur GY, Koh DH, Choi GS, Park HJ, Choi SJ, Ye YM, et al. Clinical and immunologic findings of methylene diphenyl diisocyanate-induced occupational asthma in a car upholstery factory. Clin Exp Allergy 2008;38:586–93. doi:10.1111/j.1365-2222.2008.02935.x.

[104] Dati F, Schumann G, Thomas L, Aguzzi F, Baudner S, Bienvenu J, et al. Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP Reference Material (CRM 470). International Federation of Clinical Chemistry. Community Bureau of Reference of the Commission of the European Communities. College of American Pathologists. Eur J Clin Chem Clin Biochem 1996;34:517–20.

[105] Kelemence A, Abadoglu O, Gumus C, Berk S, Epozturk K, Akkurt I. The frequency of chronic rhinosinusitis/nasal polyp in COPD and its effect on the severity of COPD. COPD 2011;8:8–12. doi:10.3109/15412555.2010.540272.

[106] Out TA, van de Graaf EA, van den Berg NJ, Jansen HM. IgG subclasses in bronchoalveolar lavage fluid from patients with asthma. Scand J Immunol 1991;33:719–27.

[107] Zivic M, Antic M. Immunofluorescent study of polyps in nose and paranasal cavities. Bratislava Med J 2009;110:181–4.

[108] Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: From structure to

effector functions. Front Immunol 2014;5:1–17. doi:10.3389/fimmu.2014.00520.

[109] Zhao C, Gao Y, Yu N, Li T, Zhang Y, Zhang H, et al. Unidirectional transport of IgG by neonatal Fc receptor in human thyrocytes varies across different IgG subclasses. Mol Cell Endocrinol 2018;477:103–11. doi:10.1016/j.mce.2018.06.006.

[110] Stapleton NM, Andersen JT, Stemerding AM, Bjarnarson SP, Verheul RC, Gerritsen J, et al. Competition for FcRn-mediated transport gives rise to short half-life of human IgG3 and offers therapeutic potential. Nat Commun 2011;2:599. doi:10.1038/ncomms1608.

[111] Heidl S, Ellinger I, Niederberger V, Waltl EE, Fuchs R. Localization of the human neonatal Fc receptor (FcRn) in human nasal epithelium. Protoplasma 2016;253:1557–64. doi:10.1007/s00709-015-0918-y.

[112] Yoshida M, Masuda A, Kuo TT, Kobayashi K, Claypool SM, Takagawa T, et al. IgG transport across mucosal barriers by neonatal Fc receptor for IgG and mucosal immunity. Springer Semin Immunopathol 2006;28:397–403. doi:10.1007/s00281-006-0054-z.

[113] Tian Z, Sutton BJ, Zhang X. Distribution of rat neonatal Fc receptor in the principal organs of neonatal and pubertal rats. J Recept Signal Transduct 2014;34:137–42. doi:10.3109/10799893.2013.865745.

[114] Rath T, Baker K, Pyzik M, Blumberg RS. Regulation of immune responses by the neonatal Fc receptor and its therapeutic implications. Front Immunol 2014;5:22–9. doi:10.3389/fimmu.2014.00664.

[115] Kaneko M, Swanson MC, Gleich GJ, Kita H. Allergen-specific IgG1 and IgG3 through FcγRII induce eosinophil degranulation. J Clin Invest 1995;95:2813–21. doi:10.1172/JCI117986.

[116] Schwitzguébel AJ-P, Jandus P, Lacroix J-S, Seebach JD, Harr T. Immunoglobulin deficiency in patients with chronic rhinosinusitis: Systematic review of the literature and meta-analysis. J Allergy Clin Immunol 2015:1–9. doi:10.1016/j.jaci.2015.07.016.

[117] Erdag O, Turan M, Ucler R, Berkoz M, Garca MF, Bozan N, et al. Is Nasal Polyposis Related to Levels of Serum Vitamin D and Vitamin D Receptor Gene Expression? Med Sci Monit 2016;22:4636–43. doi:10.12659/MSM.898410.

[118] Yıldırım YS, Apuhan T, Koçoğlu E, Simşek T, Kazaz H. High sensitivity C-reactive protein levels in chronic rhinosinusitis and allergic rhinitis. Turkish J Ear Nose Throat 2011;21:266–9. doi:10.5606/kbbihtisas.2011.039.

[119] Barnes PJ. Inhaled Corticosteroids. Pharmaceuticals 2010;3:514–40. doi:10.3390/ph3030514.

[120] Collins MM, Loughran S, Davidson P, Wilson JA. Nasal polyposis: prevalence of positive food and inhalant skin tests. Otolaryngol Head Neck Surg 2006;135:680–3.

doi:10.1016/j.otohns.2006.07.005.

[121] Pang YT, Eskici O, Wilson JA. Nasal polyposis: role of subclinical delayed food hypersensitivity. Otolaryngol Head Neck Surg 2000;122:298–301.

[122] Kewalramani A, Bollinger ME. The impact of food allergy on asthma. J Asthma Allergy 2010;3:65–74.

[123] Pang KA, Pang KP, Pang EB, Tan YN, Chan YH, Siow JK. Food allergy and allergic rhinitis in 435 asian patients - A descriptive review. Med J Malaysia 2017;72:215–20.

[124] Popescu F-D. Cross-reactivity between aeroallergens and food allergens. World J Methodol 2015;5:31–50. doi:10.5662/wjm.v5.i2.31.

[125] Cartier A. The Role of Inhalant Food Allergens in Occupational Asthma. Curr Allergy Asthma Rep 2010;10:349–56. doi:10.1007/s11882-010-0130-2.

[126] Doğru H, Tüz M, Uygur K, Akkaya A, Yasan H. Asymptomatic IgE mediated food hypersensitivity in patients with nasal polyps. Asian Pacific J Allergy Immunol 2003;21:79–82.

[127] Lill C, Loader B, Seemann R, Zumtobel M, Brunner M, Heiduschka G, et al. Milk Allergy is Frequent in Patients with Chronic Sinusitis and Nasal Polyposis. Am J Rhinol Allergy 2011;25:e221–4. doi:10.2500/ajra.2011.25.3686.

[128] Al-Qudah M. Food Sensitization in Medically Resistant Chronic Rhinosinusitis with or without Nasal Polyposis. Int Arch Allergy Immunol 2016;169:40–4. doi:10.1159/000443737.

[129] van Hage M, Hamsten C, Valenta R. ImmunoCAP assays: Pros and cons in allergology. J Allergy Clin Immunol 2017;140:974–7. doi:10.1016/j.jaci.2017.05.008.

[130] Atkinson W, Sheldon TA, Shaath N, Whorwell PJ. Food elimination based on IgG antibodies in irritable bowel syndrome: a randomised controlled trial. Gut 2004;53:1459–64. doi:10.1136/gut.2003.037697.

[131] Littman DR, Pamer EG. Role of the commensal microbiota in normal and pathogenic host immune responses. Cell Host Microbe 2011;10:311–23. doi:10.1016/j.chom.2011.10.004.

[132] Karakuła-Juchnowicz H, Szachta P, Opolska A, Morylowska-Topolska J, Gałęcka M, Juchnowicz D, et al. The role of IgG hypersensitivity in the pathogenesis and therapy of depressive disorders. Nutr Neurosci 2014. doi:10.1179/1476830514Y.0000000158.

[133] Wilders-Truschnig M, Mangge H, Lieners C, Gruber HJ, Mayer C, Mrz W. IgG antibodies against food antigens are correlated with inflammation and intima media thickness in obese juveniles. Exp Clin Endocrinol Diabetes 2008;116:241–5. doi:10.1055/s-2007-993165.

[134] Li X, Atkinson MA. The role for gut permeability in the pathogenesis of type 1 diabetes

- a solid or leaky concept? Pediatr Diabetes 2015;16:485–92. doi:10.1111/pedi.12305.

[135] Panwar H, Rashmi HM, Batish VK, Grover S. Probiotics as potential biotherapeutics in the management of type 2 diabetes - prospects and perspectives. Diabetes Metab Res Rev 2013;29:103–12. doi:10.1002/dmrr.2376.

[136] Seo YS, Shah VH. The role of gut-liver axis in the pathogenesis of liver cirrhosis and portal hypertension. Clin Mol Hepatol 2012;18:337. doi:10.3350/cmh.2012.18.4.337.

[137] Anders H-J, Andersen K, Stecher B. The intestinal microbiota, a leaky gut, and abnormal immunity in kidney disease. Kidney Int 2013;83:1010–6. doi:10.1038/ki.2012.440.

[138] Ebner N, Földes G, Schomburg L, Renko K, Springer J, Jankowska EA, et al. Lipopolysaccharide responsiveness is an independent predictor of death in patients with chronic heart failure. J Mol Cell Cardiol 2015;87:48–53. doi:10.1016/j.yjmcc.2015.07.029.

[139] Maes M, Kubera M, Leunis J-C, Berk M. Increased IgA and IgM responses against gut commensals in chronic depression: Further evidence for increased bacterial translocation or leaky gut. J Affect Disord 2012;141:55–62. doi:10.1016/j.jad.2012.02.023.

[140] Walker J, Dieleman L, Mah D, Park K, Meddings J, Vethanayagam D. High prevalence of abnormal gastrointestinal permeability in moderate-severe asthma. Clin Invest Med 2014;37:E53-7.

[141] Stapel SO, Asero R, Ballmer-Weber BK, Knol EF, Strobel S, Vieths S, et al. Testing for IgG4 against foods is not recommended as a diagnostic tool: EAACI Task Force Report. Allergy 2008;63:793–6. doi:10.1111/j.1398-9995.2008.01705.x.

[142] Lopes C. Reprodutibilidade e validação do questionário semi-quantitativo de frequência alimentar. Aliment e Enfarte Agudo Do Miocárdio Um Estud Caso-Controlo Base Comunitária [PhD] 2000.

[143] Nesselroad TD, Hrabak TM. Concerns for food allergen intradermal skin testing in nasal polyposis patients. Otolaryngol Head Neck Surg 2007;137:692–3; author reply 693. doi:10.1016/j.otohns.2007.05.015.

[144] Benninger MS, Daly T, Graffmiller K. Positivity rates of in vitro inhalant/respiratory and food allergy tests in the northern midwestern United States. Ear, Nose Throat J 2018;97:296–322. doi:10.1177/014556131809700919.

[145] Alonso R, Botey J, Pena JM, Eseverri JL, Marín A, Ras RM. Specific IgE determination using the CAP system: comparative evaluation with RAST. J Investig Allergol Clin Immunol 1995;5:156–60.

[146] Sastre J, Vandenplas O, Park H, de Alergia S, Jiménez Díaz F, Jiménez F. Pathogenesis

of occupational asthma. Eur Respir J 2003;22:364–73. doi:10.1183/09031936.03.00045103.

[147] Williams JW, Tjota MY, Sperling AI. The contribution of allergen-specific IgG to the development of th2-mediated airway inflammation. J Allergy 2012;2012:236075. doi:10.1155/2012/236075.

9. APPENDIX

APPENDIX 1

Endoscopic sinus surgery for chronic rhinosinusitis with nasal polyps: Clinical outcome and predictive factors of recurrence

Rafaela Veloso-Teles, M.D.,[1,2] and Rui Cerejeira, M.D., Ph.D.[1,2]

ABSTRACT

Background: *Chronic rhinosinusitis with nasal polyps (CRSwNP) is a highly prevalent disease of the nasal cavity and paranasal sinus, but its exact etiology is still unclear and remains a difficult-to-treat condition.*

Hypothesis: *Endoscopic sinus surgery (ESS) is an effective treatment for medically recalcitrant CRSwNP. There are independent variables that can predict surgical outcomes in patients with CRSwNP.*

Objectives: *To evaluate ESS efficacy in CRSwNP treatment and to establish prognostic factors for disease recurrence.*

Methods: *Eighty-five patients with CRSwNP submitted to ESS, and a minimum follow-up of 9 months was selected. Patient demographics, occupational organic exposure (e.g., cotton, fuel gas, wood dust) and inorganic dust exposure (e.g., bleach, metals, cement), comorbidities, previous nasal surgeries, pre- and postoperative symptoms, ear, nose and throat examination findings, computed tomography results, and medical and surgical treatment information were collected from medical records. Statistical analysis was performed.*

Results: *All rhinologic symptoms improved after surgery, in a statistically significant way, with the best recovery rate for nasal obstruction and the worst for hyposmia. The major and minor complications rates were 1.2 and 15.3%, respectively. Disease recurrence occurred in 31% of the patient, but only 7% required surgical reintervention. Multivariate logistic regression analysis identified occupational dust exposure ($p = 0.001$) and non–immunoglobulin E (IgE) mediated asthma ($p = 0.012$) as independent predictive variables in CRSwNP recurrence, unlike the other tested variables: age, sex, IgE-mediated asthma, allergic rhinitis, smoking habits, nasal polyps endoscopic grade, Lund-Mackay score, and postoperative topical corticoid use. The adjusted logistic model presented a good discriminatory capacity with a receiver operating characteristic area under the curve of 0.82 (95% confidence interval, 0.73–0.91; $p < 0.001$).*

Conclusion: *ESS proved to be an effective treatment in CRSwNP but with a considerable rate of recurrence. These results indicated an important correlation of occupational dust exposure and non–IgE-mediated asthma with disease recurrence.*

(Am J Rhinol Allergy 31, 56–62, 2017; doi: 10.2500/ajra.2017.31.4402)

Chronic rhinosinusitis (CRS) has been defined in European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2012 as an inflammation of the nose and paranasal sinus that lasts for >12 weeks, characterized by specific nasal symptoms, and endoscopic signs and/or computed tomography (CT) changes.[1] CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP) are distinguished for research purposes by endoscopic visualization of bilateral polyps in the middle meatus.[1] There is still a deficit of epidemiologic studies that used nasal endoscopy to explore the prevalence and incidence of CRSwNP.[1] A population-based study done in Sweden found a prevalence of 2.7%,[2] and a recently published cadaver-based study in Portugal found a prevalence of 5.5%.[3]

Despite the relatively high prevalence of this condition, its exact etiology is still unclear and remains a difficult-to-treat condition.[4] Endoscopic sinus surgery (ESS) for nasal polyposis has generally been reported to be a safe and effective procedure and is usually performed for CRSwNP that is refractory to medical treatment.[1] However, massive nasal polyposis can have a recurrence rate after ESS as high as 50%.[4] Our hypothesis is that ESS is an effective treatment for medically recalcitrant CRSwNP and that some independent variables may influence surgical outcomes in these patients, *viz.*, sex, age, occupational dust exposure, atopic and nonatopic concomitant respiratory diseases, smoking habits, nasal polyps endoscopic grade, Lund-Mackay score, or postoperative topical steroid compliance. Our main goals were to evaluate the efficacy of ESS in patients with CRSwNP, including symptom relief, complications, and recurrence rate, and to try to identify independent predictors of recurrence.

From the [1]Department of Otorhinolaryngology, Cova da Beira Hospital Center, Covilhã, Portugal, and [2]Otorhinolaringology Division, Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal

No external funding sources reported

The authors have no conflicts of interest to declare pertaining to this article

Presented at the 25th Congress of the European Rhinology Society in conjunction with 32nd International Symposium of Infection and Allergy, June 22–26, 2014, Amsterdam, the Netherlands

Address correspondence to Rafaela Veloso Teles, M.D., Cova da Beira Hospital Center, Quinta do Alvito, 6200–251 Covilhã, Portugal

E-mail address: rafaelateles84@gmail.com

METHODS

The present study was performed according to established ethical guidelines and approval of the ethics for health comittee of Alto Ave Hospital Center. This study was performed according the principles of the Declaration of Helsinki. This was a retrospective analysis of patients who presented for ESS due to CRSwNP in a district hospital center from January 2004 to December 2013. The diagnosis of CRSwNP was established by using the definition of European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2012.[1] Patients proposed for ESS had refractory disease to topical long-term and systemic short-term steroids. A total of 85 patients were selected from the hospital data base after applying the following exclusion criteria: patients with concomitant benign or malignant sinonasal tumors; no available preoperative CT in the hospital computer network, and a follow-up period of <9 months. Postoperative appointments were scheduled quarterly, with discharge at 2 years of follow-up if no symptoms or signs of recurrence were present. Authorship contribution included the following: R. Veloso-Teles, study design, data collection, statistical analysis, discussion, and revision; and R. Cerejeira, statistical analysis, discussion, and revision.

Clinical records were reviewed to collect information on demographics, occupational history (including types of dust exposure), smoking and alcoholic habits, comorbidities, previous nasal surgeries, pre- and postoperative symptoms, ear, nose and throat (ENT) examination findings, CT results, medical and surgical treatment, perioperative complications, and histopathologic findings. Whenever the occupational information was missing from the clinical records, data were collected by a telephone interview. Patients were considered to

Table 1 **Sample demographics, comorbidities, smoking habits, and occupational history (when considering almost daily dust exposure during job duties) (N = 85)**

Demographics	
Age, mean ± SD (range), y	47 ± 13 (15–73)
Sex, no. (%)	
Male	55 (65)
Female	30 (35)
Comorbidities, no. (%)	
Asthma	32 (38)
IgE mediated	18 (21)
Non–IgE mediated	14 (17)
Samter's triad	5 (6)
Allergic rhinitis	26 (31)
COPD	14 (17)
Smoking habits, no. (%)	
Non-smoker	65 (77)
Ex-smoker	14 (17)
Smoker	6 (7)
Occupation, no. (%)	
Inhalant dust exposure	
Organic dust	52 (60)
Cotton, flax, sisal	23 (27)
Fuel gas, paints, glues	15 (18)
Flours	5 (6)
Wood dust	1 (1)
Inorganic dust	
Pesticides	2 (2)
Bleach, chlorine dioxide	2 (2)
Metallic compounds	2 (2)
Cement	2 (2)
No dust exposure	33 (40)

SD = Standard deviation; IgE = immunoglobulin E; COPD = chronic obstructive pulmonary disease.

Table 2 **Anatomic variations on computed tomography, absolute and relative frequencies**

Anatomic Variations	No. (%)
Deviated septum	46 (54)
Kuhn cells	24 (28)
Bullous concha	25 (29)
Frontal sinus hypoplasia	15 (18)
Paradoxical turbinate	5 (6)
Overpneumatized ethmoid bulla	1 (1)
Maxillary sinus hypoplasia	5 (6)
Haller cell	7 (8)
Frontal sinus agenesis	5 (6)
Total	68 (80)

Table 3 **Surgical procedures with absolute and relative frequencies**

Surgical Procedure	No. (%)
Anterior ethmoidectomy	85 (100)
Maxillary antrostomy	79 (93)
Posterior ethmoidectomy	69 (81)
Frontal sinusotomy	68 (80)
Draf type I	42 (49)
Draf type IIa	26 (31)
Sphenoidotomy	13 (15)
Inferior turbinoplasty	28 (33)
Middle turbinoplasty	15 (18)
Septoplasty	31 (36)

have occupational dust exposure if it occurred during the performance of their job duties, almost daily, and in a repetitive way. To evaluate for disease recurrence and postoperative symptoms, it was considered the last ENT outpatient clinic visit of the patient so to have the longest possible follow-up period. The diagnosis of IgE-mediated and non–IgE-mediated asthma and of allergic and nonallergic rhinitis were established by a respiratory physician or an allergologist. Nasal polyps were classified endoscopically as grade I, II, or III, according to the Lund criteria.[5] Anatomic variations and compatible CRSwNP findings on the CT were also registered as well as the Lund-Mackay score.[6]

Statistical Analysis

Statistical analysis was performed with Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 23.0, IBM Corporation, Chicago, IL). Descriptive statistics were used in the sample characterization. An evaluation of surgical efficacy for each symptom by comparing the pre- and postoperative groups, was done by using the McNemar test (for two related samples), except for nasal obstruction, for which a binomial test had to be used because 100% of the preoperative group had the symptom. Patients with and those without recurrence of nasal polyps were then divided in two independent groups and compared for multiple factors. For categorical variables, χ^2 test (or the Fisher exact test when assumptions needed for the previous test were not verified) was used to test for variable association. For continuous quantitative variables, the Mann-Whitney test was used.

A logistic regression analysis was carried out to evaluate the association of multiple different variables with nasal polyposis recurrence. The likelihood ratio test and the Hosmer and Lemeshow test were performed to evaluate model significance and its goodness of fit. The Nagelkerke coefficient determination (pseudo-R^2) was used to determine the proportion of the dependent variable variance explained by the logistic regression model. The Wald test and the score test were used to test the impact of each independent variable on the recurrence of the disease. For each significant independent variable, the odds ratio and 95% confidence interval (CI) were calculated. Analyses of residuals, control variables, and multicollinearity were performed. Also, the area under the receiver operating characteristic curve was calculated to establish the discriminatory power of the model. Statistical significance was accepted to correspond to a p value of <0.05.

RESULTS

A total of 85 patients were included, 55 male and 30 female patients. The mean (standard deviation) age was 47 ± 13 years (range, 15–73 years). Thirty-eight percent of the patients had concomitant asthma (of these, 56% had IgE-mediated asthma and 44% had non–IgE-mediated asthma), 31% had allergic rhinitis, and 17% presented chronic obstructive pulmonary disease. Samter's triad prevalence was 6%. There were no patients with cystic fibrosis in this sample. Seventy-seven percent of patients were non-smokers. When asked about occupational inhalant exposure, 60% of the patients reported exposure to dust (85% to organic dust and 15% to inorganic dust). By using the European Academy of Allergy and Clinical Immunology (EAACI) task force[7] classification for dust particles, 9.6% of the exposed patients inhaled high-molecular-weight (HMW) (>5 kDa) substances, whereas 90.4% of the patients were exposed to low-molecular-weight (LMW) (<5 kDa) particles. The prevalence of atopy (IgE-mediated allergy) among the patients exposed to occupational dust exposure was 27%. Sample demographics, comorbidities, smoking habits, and occupational history to dust inhalation are summarized in Table 1. Twenty-five percent of the patients had a history of nasal surgery. Of these, 12 patients had already had ESS, 7 had septoplasty, and

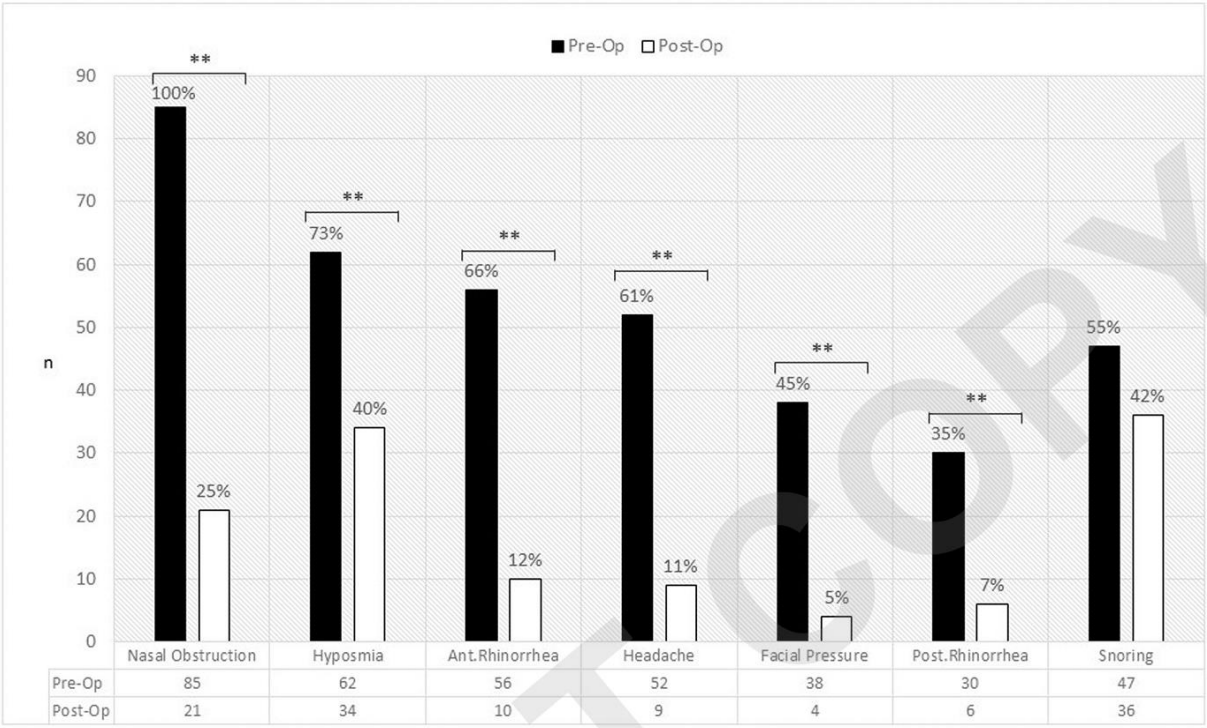


Figure 1. *Relative frequencies of the pre- and postoperative symptoms. Absolute frequencies are presented in the table under the graphic bars. Pre-op = Preoperative; post-op = postoperative; Ant. Rhinorrhea = anterior rhinorrhea; Post. Rhinorrhea = posterior rhinorrhea. **$p < 0.01$.*

2 had both of these surgeries. The majority of the patients (53%) were staged as having grade III polyposis, 34% as having grade II, and the remaining 13% as having grade I. Anatomic variations detected in a preoperative CT are depicted in Table 2. The mean bilateral Lund-Mackay score was 16.6 ± 4.6 (range, 6–24).

ESS was performed bilaterally in every case and always included the anterior ethmoidectomy, followed in frequency by maxillary sinus antrostomy and posterior ethmoidectomy associated with other procedures as needed (Table 3). In the majority of the patients in whom frontal sinusotomy was necessary, a type I drainage (Draf I) was established by anterior ethmoidectomy, which identified the frontal recess, without touching its mucosa. In 30% of the patients with more-severe disease and in revision cases, a Draf IIa was performed with extended drainage achieved after ethmoidectomy by resecting the floor of the frontal sinus between the lamina papyracea and the middle turbinate (Table 3). One case of cerebrospinal fluid fistula (1.2%) occurred, which was repaired with a medium turbinate flap and fibrin glue. Other minor complications were observed in 13 patients (15.3%): periorbital cellulitis (1 patient), discreet periorbital ecchymosis (9), and epistaxis (3). Routine histopathologic evaluation with hematoxylin and eosin staining confirmed the CRSwNP diagnosis and revealed tissue eosinophilia (>10 eosinophils/hpf[8]) in all the patients. Topical steroids were prescribed to all the patients after surgery, with a medication compliance rate of 47%. Patients who were noncompliant included 29% of occasional steroids users and 24% of those who were not compliant at all. The prescribed steroids were fluticasone propionate or mometasone intranasal spray applied twice daily.

The main preoperative reported concerns were nasal obstruction (100%), hyposmia (75%), anterior rhinorrhea (56%), and headache (61%). All rhinologic symptoms had a statistically significant reduction after endoscopic surgery, in contrast with snoring, which did not improve significantly (Fig. 1). Postoperative recurrence was diagnosed when endoscopic nasal polyps were detected. The main recurrence site was the ethmoid region. The mean follow-up time was 27 months (range, 9–108 months), the recurrence rate was 31% but with only 7% of patients who required surgical reintervention. The need for revision surgery was determined according to patient's concerns and ENT examination. The rate of follow-up loss was 5.9%.

The patients with occupational dust exposure had statistically significant higher rates of recurrence of polyps (48%) compared with the recurrence rate of the patient groups with no exposure (3%), $\chi^2(1) = 19.25$, $p < 0.001$ (Fig. 2). Thirteen percent of the patients exposed to dust needed surgical reintervention, in contrast to patients not exposed, who did not need revision surgery; this difference was significant when using the Fisher's exact test ($p = 0.04$) (Fig. 3). Patients with Samter's triad did not present a statistically significant higher rate of recurrence compared with patients with aspirin tolerance ($p = 0.165$, Fisher exact test). Multivariate analysis was performed with logistic regression analysis by using the forward likelihood ratio method. The likelihood ratio test demonstrated that the created adjusted model was significantly better than the null model ($p < 0.001$). The Hosmer and Lemeshow test had a p value of 0.503, which meant that the adjusted logistic model fit the data. The Nagelkerke coefficient determination (pseudo-R^2) was 0.44, which indicted that 44% of disease recurrence variability was explained by the adjusted logistic model.

The Wald test and the score test identified occupational dust exposure and non–IgE-mediated asthma as independent predictive factors for CRSwNP recurrence ($p = 0.001$ and $p = 0.012$, respectively), unlike the other studied variables that were found to be nonsignificant: sex, age, IgE-mediated asthma, allergic rhinitis, smoking habits, the Lund-Mackay score, nasal polyps endoscopic grade, and postoperative topical corticoid use (Table 4). The logistic model algorithm can be described as $\text{logit}(\pi) = -4.702 + 3.64$ occupational exposure $+ 2.16$ non–IgE-mediated asthma. Patients with occupational exposure to

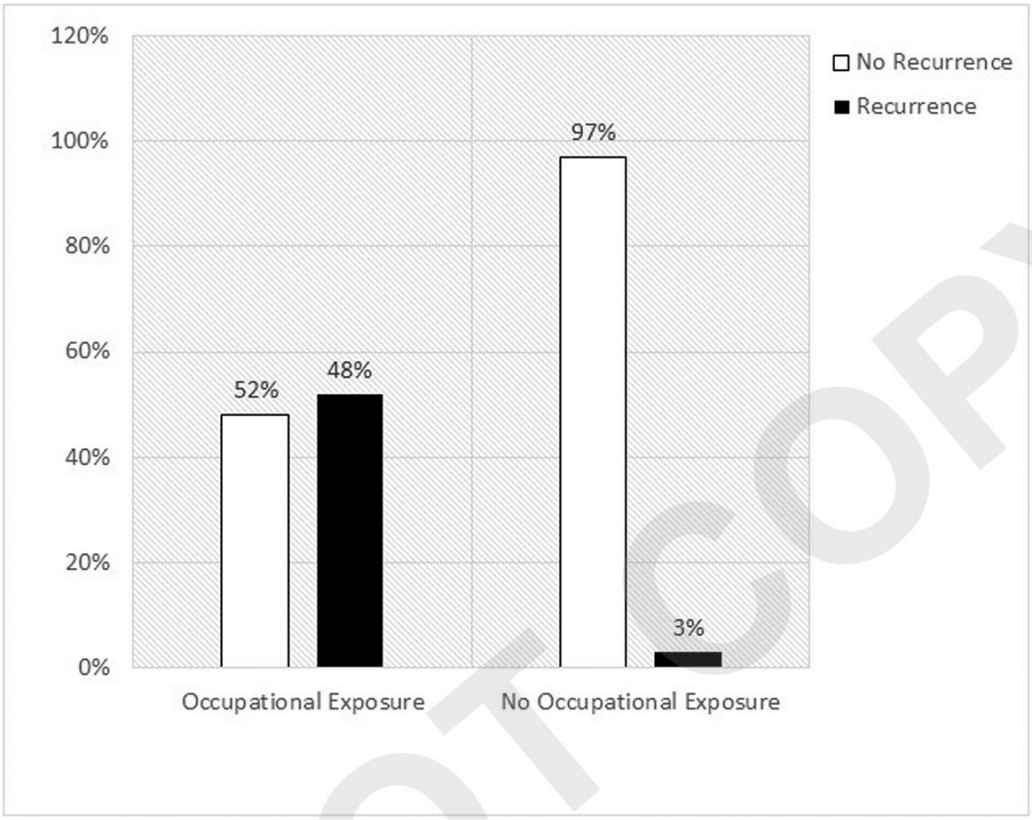


Figure 2. *Relative frequencies of recurrence of chronic rhinosinusitis with nasal polyps (CRSwNP) in the group with and in the group without occupational exposure to inhalants.*

dust had a 38 times greater chance of recurrence than the nonexposure group (95% CI, 4–345). The patients with nonallergic asthma were more likely to develop recurrence, with a 8.7 times greater chance (95% CI, 2–46). The potential confounding effects of sex, age, smoking habits, Samter's triad, Lund-Mackay score, nasal polyps endoscopic grade, and postoperative topical corticoid use were statistically controlled. No problems of multicollinearity were identified when analyzing all the variables in this study (condition indices for all 11 dimensions were <30). Two outliers were identified and evaluated, but we did not find a reason to exclude them. The final model allowed correct identification of disease recurrence in 79% of the patients and presented a good discriminatory capacity (receiver operating characteristic area under the curve 0.822 [95% CI, 0.73–0.91]; $p < 0.001$).

DISCUSSION

In accordance with previously published studies that found a male predominance in CRSwNP, an average age of disease onset of 42 years and rare cases of patients < 20 years old,[1] we also observed a higher frequency of nasal polyps in men (male-to-female ratio, 1.8:1), a mean age of 47 years, and only three patients who were <20 years old. Asthma prevalence in our study (38%) was similar to that found by the questionnaire-based study of Klossek *et al.*[9] which found wheezing and respiratory discomfort to be present in 31 and 42% of patients with CRSwNP, respectively. Another recently published study, which performed peak expiratory flow, spirometry, and bronchodilation tests in every patient with CRSwNP ($N = 40$), found an asthma prevalence of 65%, with 25% of previously undiagnosed and unrecognized asthma,[10] which calls for a closer collaboration between otorhinolaryngology and respiratory medicine specialists in research as well as in clinical practice.[10]

A systematic review about symptom-specific outcomes of ESS in CRS concluded that all studied symptoms improved in a similar way for major CRS symptoms, with the exception of nasal obstruction, which improved more than headache or hyposmia.[11] In accordance with this, our study demonstrated a statistically significant improvement in all rhinologic symptoms and found hyposmia to be the most difficult symptom to treat, which remained the first reported postoperative symptom, as already stated in previous studies.[12] The outcomes of ESS on olfaction are challenging to predict, and previous studies documented a wide range (17–75%) of improvement in olfactory function.[13] Our 33% improvement on impaired sense of smell was in accordance with these data.

To overcome this problem and to help patient counseling about the likelihood of postoperative olfactory recovery, Kim *et al.*[13] recently purposed the combined use of the butanol threshold test and sinonasal CT findings before surgery because they showed that patients with anosmia in the butanol threshold test and partly opacified anterior ethmoid on the CT benefited the most from surgery with respect to olfaction. However, no statistically significant reduction in snoring was observed; this fact is not surprising because sleep-disordered breathing is often due to a multilevel collapse of the superior airways. A direct correlation between the degree of nasal obstruction and the severity of sleep-disordered breathing has not been found and, certainly, nasal obstruction does not seem to be the main contributing factor in the majority of patients with moderate-to-severe obstructive sleep apnea syndrome.[14] A recent study, with 139 patients with CRS, found a prevalence of obstructive sleep apnea syndrome of

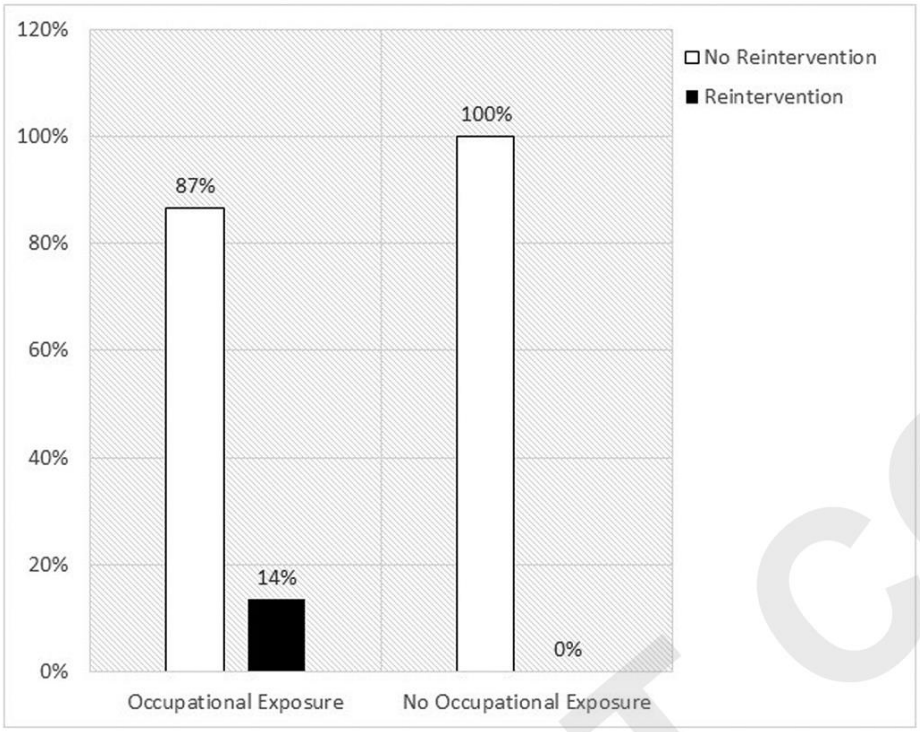


Figure 3. *Relative frequencies of the need for surgical reintervention for chronic rhinosinusitis with nasal polyps (CRSwNP) in the group with and the group without occupational exposure to inhalants.*

Table 4 **Variables in equation in logistic regression analysis**

	B	DF	Wald	*p* Value	Exp (B)	95% CI
Variable						
Occupational dust exposure	3.638	1	10.433	0.001*	38.015	4.18–345.69
Non–IgE-mediated asthma	2.158	1	6.378	0.012*	8.650	1.62–46.16
Constant	−4.072	1	13.254	0.000*	0.017	

DF = Degrees of freedom; Exp (B) = exponentiation of the B coefficient, odds ratio; CI = confidence interval; IgE = immunoglobulin E.
**$p < 0.05$.*

64.7%; despite the high prevalence found, obstructive sleep apnea syndrome and rhinosinusitis severity were not correlated, and nasal polyps did not worsen sleep problems in patients with CRS.[15]

A 2006 systematic review about ESS effectiveness in the treatment of CRSwNP found a recurrence rate between 4 and 60%, with a median value of 20% across all evaluated studies and a need for revision surgery in the range of 3–42%, with a median of 6%.[16] The recurrence rate in our study of 31% and the need of reintervention of 7% is in accordance with these values. The wide dispersion of the recurrence rates found in the literature can be explained not only by the technique used and surgeon expertise but also the geographic differences. Recurrence rates can be higher in areas in which primary and secondary work sectors predominate, where employees have occupational dust exposure. Our rates of major and minor complications (1.2% for major complications and 15.3% for minor complications) are also comparable with the complication rates reported in the 2006 systematic review,[16] which found a rate of major and minor complications, of 0–1.5% and 1.1–20.8%, respectively.

It is accepted that there is a difference in the pathology of nasal polyps between Western and Asian populations. Although ~80% of polyps in Western patients are eosinophilic, <50% of polyps in Asian patients show tissue eosinophilia above that seen in control tissues. Recent work done in Japan showed that mucosal eosinophilia was significantly correlated with recurrence after ESS.[17] Because, in our case, all the patients presented with eosinophilic polyps, this topic could not be addressed. Samter's triad is considered a subgroup of CRS characterized by a higher burden of disease and a challenging disease entity to manage, with surgery aimed toward symptom control rather than cure.[18] That we did not find a statistically significant difference in recurrence rates in these patients may be due to the small representation of these patients in our sample (five cases, 6% of patients).

A point of controversy has been the impact of asthma in surgical outcomes of CRS. There are some studies that point to a negative impact,[19,20] whereas other studies found no influence.[21,22] A limitation of these published studies is that most of them do not separate atopic (IgE mediated) and nonatopic (non-IgE mediated) asthma. To comply with the European Academy of Allergy and Clinical Immunology (EAACI) recommendations, and, because the mechanisms that initiate non–IgE-mediated allergic asthma and nonallergic asthma are not well defined, we classified asthma as IgE-mediated or non–IgE-mediated asthma.[23] We found a significant negative impact of non–IgE-mediated asthma in surgical outcomes of CRSwNP, which raised the chance of recurrence by approximately nine times (odds ratio 8.7 [95% CI, 2–46]; $p = 0.012$), in contrast to IgE-mediated asthma, with no significant impact ($p = 0.274$) on disease recurrence. A recent systematic review about the management of CRSwNP and coexisting asthma stated that the effect of ESS on pulmonary outcomes has been a question of debate, with low strength of evidence supporting a pos-

itive effect of ESS on asthma.[24] It would be interesting to analyze and compare the surgical impact of ESS on the pulmonary function of patients with IgE-mediated asthma and patients with non–IgE-mediated asthma.

In 2012, Hox *et al.*[25] indicated that occupational exposures can be a risk factor for the occurrence of CRS and for its recurrence or persistence. Patients had ESS for CRS were questioned, and a relevant occupational exposure was reported in 25% of all the responding patients ($n = 467$). The prevalence of occupational exposures increased linearly with the number of ESS procedures, from 21% in those who had one ESS to 44% in those who had four or more ESS procedures ($p < 0.001$).[25] This work presented some limitations, *viz.*, the fact that it was based on a self-administered questionnaire sent by mail and no separation between patients with CRSwNP or patients with CRSsNP was made. Our work was pioneering in studying the impact of occupational exposure specifically on CRSwNP. Our rate of reported exposure to organic and to inorganic dust was much higher (60%) when compared with the results of Hox *et al.*[25] (25%), which points to a meaningful effect of this factor in CRSwNP, which is supposed to be higher than in CRSsNP according to these results. In accordance, our study revealed that occupational exposure to dust had a negative outcome on CRSwNP treatment, with patients who were exposed having a 38 times greater chance of recurrence compared with the nonexposed group (odds ratio 38 [95% CI, 4–395]; $p = 0.001$). We can speculate that, similar to what has already been described for occupational rhinitis, occupational dust exposure can be involved in the pathophysiology of CRSwNP by different mechanisms: immunologically mediated hypersensitivity reactions (antibody or cell mediated), designated as allergic reactions or through irritant, nonimmunologic mechanisms.[7]

Although HMW agents are biologic substances from vegetable or animal origin (*e.g.,* flour, latex, laboratory animals) known to cause IgE-mediated immune responses, the mechanisms linked to LMW agents have not been fully characterized. LMW agents induce non–IgE-mediated mechanisms as airway sensitizers (*e.g.,* woods, metals, resins), with a latency period between exposure to symptoms of weeks to years, or as airway irritants (*e.g.,* chlorine, ammonia), with symptoms of acute onset.[26] Hox *et al.*[25] reported an exposure to HMW agents in 5% and to LMW agents in 95% of the patients with CRS and relevant occupational exposures, with very similar results to what we found in the subgroup of CRSwNP (9.4 and 90.6% for HMW and LMW, respectively). Because LMW substances are also known to cause more-severe occupational asthma than HMW and that they act mostly through non–IgE-related mechanisms,[27] this can explain our finding that non–IgE-mediated asthma was a poor prognostic factor for CRSwNP recurrence and that it may be a marker of disease severity. That the prevalence of atopy (IgE-mediated allergy) among patients exposed to occupational dust exposure was 27% and that this value was in the range of the reported prevalence of atopy in the general population (20–30%),[28] also supported the hypothesis of important non–IgE-related mechanisms in occupational airway inflammation, including immune and nonimmune ones. Because this represents a problem of public health, these findings showed the importance of protective measures for employees, such as the use of a mask during work, and reinforces the need for legislation and control to guarantee the functioning of air-dust filters and exhausting systems. It is advisable that patients with CRSwNP should work in a dust-free environment whenever possible.

There are wide differences in the published literature about prognostic factors that can identify the most susceptible patients to CRSwNP recurrence and predict the need for reintervention. This fact can be explained by different methodologies and types of studies used, including sample selection (which often includes patients with CRSwNP and CRSsNP mixed together) and the type of statistical analysis performed. In many cases, the correlation of dependent and independent variables are done individually, but, in this kind of study, logistic regression analysis with multiple variables is the best way to identify the influence of the different variables by controlling, at the same time, the problems of multicollinearity and confounding effects. This study had some limitations: the retrospective character of the study, subjective evaluation of occupational exposure by the patients, and a moderate sample size.

By the time the patients were observed in consultation, there no longer was available a validate quality of life survey in European Portuguese, which would have added value to this work if applied. In our center, CRSwNP postoperative protocol treatment includes only topical corticoid spray. Recently, postoperative corticoid nasal irrigation was introduced as a therapeutic aid in CRS, with safety and effectiveness studies that make this procedure acceptable to many physicians, especially in the most difficult-to-treat cases.[29,30] This issue should be addressed in a future multivariate logistic regression analysis to see if the corticoid delivery method has an independent prognostic impact on CRSwNP recurrence. That the study was conducted in a single hospital unit limited the variability in therapeutic options, operative techniques, and postoperative care, which made the groups, with and without nasal polyps recurrence, more comparable. More clinical investigations are needed to clarify these associations, *viz.*, prospective studies that can evaluate if there is a cause-effect relationship between occupational dust exposure and CRSwNP, and if concomitant non–IgE-mediated asthma represents a mark of disease severity (when following the concept of "one airway, one disease"[31]).

CONCLUSION

These results indicated an important role of occupational inhalant exposure in the physiopathology of CRSwNP. Avoidance of dust can be an important measure to prevent disease progression and recurrence after treatment, and non–IgE-mediated asthma may represent a mark of disease severity.

ACKNOWLEDGMENTS

We thank the Otorhinolaryngology Department, Alto Ave Hospital Center, where the study was conducted, especially Fausto Fernandes, M.D., and Francisco Moreira da Silva, M.D., for their support.

REFERENCES

1. Fokkens WJ, Lund VJ, Mullol J, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. Rhinol Suppl (23):3 p. preceding table of contents, 1–329, 2012.
2. Johansson L, Akerlund A, Holmberg K, et al. Prevalence of nasal polyps in adults: The Skovde population-based study. Ann Otol Rhinol Laryngol 112:625–629, 2003.
3. Cerejeira R, Veloso-Teles R, and Lousan N, and Moura CP. Prevalence of nasal polyps in northern Portugal: A cadaver endoscopic study. Rhinology 52:386–389, 2014.
4. Hoseini SM, Saedi B, and Aghazadeh K. Meticulous endoscopic sinus surgery to prevent recurrence of massive nasal polyposis. J Laryngol Otol 126:789–794, 2012.
5. Lund VJ. Diagnosis and treatment of nasal polyps. BMJ 311:1411–1414, 1995.
6. Lund VJ, and Mackay IS. Staging in rhinosinusitis. Rhinology 31:183–184, 1993.
7. EAACI Task Force on Occupational Rhinitis, Moscato G, Vandenplas O, et al. Occupational rhinitis. Allergy 63:969–980, 2008.
8. Soler ZM, Sauer D, Mace J, and Smith TL. Impact of mucosal eosinophilia and nasal polyposis on quality-of-life outcomes after sinus surgery. Otolaryngol Head Neck Surg 142:64–71, 2010.
9. Klossek JM, Neukirch F, Pribil C, et al. Prevalence of nasal polyposis in France: A cross-sectional, case-control study. Allergy 60:233–237, 2005.
10. Håkansson K, Thomsen SF, Konge L, et al. A comparative and descriptive study of asthma in chronic rhinosinusitis with nasal polyps. Am J Rhinol Allergy 28:383–387, 2014.
11. Chester AC, Antisdel JL, and Sindwani R. Symptom-specific outcomes of endoscopic sinus surgery: A systematic review. Otolaryngol Head Neck Surg 140:633–639, 2009.

12. Dufour X, Bedier A, Ferrie JC, Gohler C, and Klossek JM. Diffuse nasal polyposis and endonasal endoscopic surgery: Long-term results, a 65-case study. Laryngoscope 114:1982–1987, 2004.
13. Kim BG, Kang JM, Shin JH, et al. Do sinus computed tomography findings predict olfactory dysfunction and its postoperative recovery in chronic rhinosinusitis patients? Am J Rhinol Allergy 29:69–76, 2015.
14. Georgalas C. The role of the nose in snoring and obstructive sleep apnoea: An update. Eur Arch Otorhinolaryngol 268:1365–1373, 2011.
15. Jiang RS, Liang KL, Hsin CH, and Su MC. The impact of chronic rhinosinusitis on sleep-disordered breathing. Rhinology 54:75–79, 2016.
16. Dalziel K, Stein K, Round A, et al. Endoscopic sinus surgery for the excision of nasal polyps: A systematic review of safety and effectiveness. Am J Rhinol 20:506–519, 2006.
17. Tokunaga T, Sakashita M, Haruna T, et al. Novel scoring system and algorithm for classifying chronic rhinosinusitis: The JESREC Study. Allergy 70:995–1003, 2015.
18. Yip J, Yao CM, and Lee JM. State of the art: A systematic review of the surgical management of aspirin exacerbated respiratory disease. Am J Rhinol Allergy 28:493–501, 2014.
19. Dinis PB, and Gomes A. Sinusitis and asthma: How do they interrelate in sinus surgery? Am J Rhinol 11:421–428, 1997.
20. Dejima K, Hama T, Miyazaki M, et al. A clinical study of endoscopic sinus surgery for sinusitis in patients with bronchial asthma. Int Arch Allergy Immunol 138:97–104, 2005.
21. Mehanna H, Mills J, Kelly B, and McGarry GW. Benefit from endoscopic sinus surgery. Clin Otolaryngol Allied Sci 27:464–471, 2002.
22. Kountakis SE, and Bradley DT. Effect of asthma on sinus computed tomography grade and symptom scores in patients undergoing revision functional endoscopic sinus surgery. Am J Rhinol 17:215–219, 2003.
23. Johansson SG, Bieber T, Dahl R, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol 113:832–836, 2004.
24. Rix I, Håkansson K, Larsen CG, et al. Management of chronic rhinosinusitis with nasal polyps and coexisting asthma: A systematic review. Am J Rhinol Allergy 29:193–201, 2015.
25. Hox V, Delrue S, Scheers H, et al. Negative impact of occupational exposure on surgical outcome in patients with rhinosinusitis. Allergy 67:560–565, 2012.
26. Hox V, Steelant B, Fokkens W, et al. Occupational upper airway disease: How work affects the nose. Allergy 69:282–291, 2014.
27. Meca O, Cruz MJ, Sánchez-Ortiz M, et al. Do Low molecular weight agents cause more severe asthma than high molecular weight agents? PLoS One 11:e0156141, 2016.
28. Pawankar R, Canonica GW, Holgate ST, and Lockey RF. White Book on Allergy. World Allergy Organization, United States of America, 2011.
29. Kang TW, Chung JH, Cho SH, et al. The effectiveness of budesonide nasal irrigation after endoscopic sinus surgery in chronic rhinosinusitis with asthma. Clin Exp Otorhinolaryngol doi: 10.21053/ceo.2016.00220. Epub ahead of print July 21, 2016.
30. Kosugi EM, Moussalem GF, Simões JC, et al. Topical therapy with high-volume budesonide nasal irrigations in difficult-to-treat chronic rhinosinusitis. Braz J Otorhinolaryngol 82:191–197, 2016
31. Grossman J. One airway, one disease. Chest 111(suppl.):11S–16S, 1997. □

APPENDIX 2

ORIGINAL CONTRIBUTION

Higher prevalence of nasal polyposis among textile workers: an endoscopic based and controlled study*

Rafaela Veloso-Teles[1,2], Rui Cerejeira[1,2], Rosa Roque-Farinha[2,3], Christian von Buchwald[4]

[1] Department of Otorhinolaryngology, Cova da Beira Hospital Centre (CHCB), Covilhã, Portugal
[2] Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal
[3] Department of Otorhinolaryngology, Lisboa Norte Hospital Centre, Lisbon, Portugal
[4] Department of Otorhinolaryngology, Head and Neck Surgery and Audiology, Rigshospitalet-Copenhagen University Hospital, Copenhagen, Denmark

Rhinology 56; 2: 99-105, 2018
https://doi.org/10.4193/Rhin17.228

***Received for publication:**
November 8, 2017
Accepted: January 2, 2018

Background: There is a deficit of reliable epidemiologic studies exploring the prevalence of Chronic Rhinosinusitis with Nasal Polyps (CRSwNP). Recent data suggests that occupational dust exposure may be involved in its physiopathology.

Objectives: To compare the prevalence of nasal polyposis (NP) in a group of workers with occupational dust exposure (textile workers) and in a control group (retail store workers).

Methods: Cross-sectional study with a random sample of textile and retail store employees. Clinical data was gathered through a systematic interview, which included RhinoQOL and CAT™ questionnaires. A systematic endoscopic nasal examination was performed using a 0º rigid endoscope. Lund-Kennedy endoscopic score was determined for each participant. Statistical analysis was performed with SPSS.

Results: 316 participants were included in the study, i.e. 215 textile workers and 101 retail store workers. NP was found in 19 subjects among textile workers and none in the control group. The prevalence of NP increased by age strata and by years of dust exposition. Polypoid degeneration of the middle turbinate was more prevalent in the exposed group with Lund-Kennedy scoring also higher. RhinoQOL and CAT™ questionnaires had both significantly higher scores among textile employees. Previous medical diagnosis of atopic diseases or chronic lower airway diseases did not differ between exposed and control groups or even between subjects with and without NP.

Conclusions: These results point to an important correlation between occupational dust exposure and NP occurrence.

Key words: nasal polyps, paranasal sinus diseases, sinusitis, occupational exposure

Introduction

There is a deficit of epidemiologic studies exploring the prevalence of Chronic Rhinosinusitis with Nasal Polyps (CRSwNP), especially in European countries[(1)]. When reviewing the current literature, it becomes clear that giving an accurate estimate of CRSwNP prevalence remains speculative, mainly due to the diagnostic imprecision often used in publications, which are mostly based on symptoms questionnaires. The data obtained with such approach can be unreliable as not all patients that claim to have the disease have nasal polyps on endoscopy and asymptomatic polyps will be unaccounted for. Therefore, EPOS 2012 expert panel considers endoscopy as a prerequisite for accurate estimate of the prevalence of nasal polyposis (NP) and alerts to the need to distinguish between clinically silent NP or preclinical cases, and symptomatic NP[(1)]. In Europe, there are only two endoscopic based studies published, an in-vivo study done in Sweden which found a prevalence of 2.7%[(2)] and a cadaver study done in Portugal, by our investigation group, which found a prevalence of 5.5%[(3)].

Recent data suggests that occupational dust exposure may be involved in Chronic Rhinosinusitis (CRS) physiopathology. In 2012, Hox et al. stated that occupational exposures can be a risk

factor for the occurrence of CRS and for its recurrence or persistence[4]. Patients submitted to endoscopic sinus surgery (ESS) for CRS were inquired and a relevant occupational exposure was reported in 25% of all responding patients (N= 467). The prevalence of occupational exposures increased linearly with the number of ESS procedures needed by each patient ($p<0.001$)[4]. Our investigation group have also demonstrated, in a multivariate regression analysis, that occupational exposure to dust has a negative impact on CRSwNP postoperative outcome (N=85), with exposed patients (60% of the sample) having 38 times more chance of recurrence compared to the non-exposed group ($p=0.001$)[5]. However, both studies were retrospective analysis with their inherent limitations. In 2016, a multicenter cross-sectional study done in China was published (N=10,633) suggesting that some occupational and environmental exposures are strongly associated with CRS[6]. Nevertheless, that epidemiologic study was based on self-administered questionnaires for CRS diagnosis and occupational and environmental history; and no separation between patients with CRSwNP or patients with CRSsNP (Chronic Rhinosinusitis without Nasal Polyps) was made. Until present, there are no epidemiologic data comparing the prevalence of NP in workers with and without occupational exposure to dust.

The objective of this research was to establish the prevalence of NP in a group of workers with occupational exposure to dust (textile workers) and to compare it with its frequency among a non-exposed group (retail store workers).

Materials and methods

The study was performed according to established ethical guidelines and approval of Ethics Committee at the Health Sciences Faculty, Beira Interior University. A signed informed consent was obtained from each participant in the study.

A descriptive cross-sectional study was carried out to determine NP prevalence among two groups: textile workers (exposed group) and retail store workers (controls). This study took place in Castelo Branco District, within the Interior Centre Region of Portugal. This area is internationally known for its textile industry, mainly wool manufacturing. For the purpose of the study, a total of 357 workers were recruited, 254 textile workers and 103 controls. The sample was randomly selected using employee's numbers at the personnel database of the Factory/Retail Store. Textile workers were recruited from an industrial unit with a total workforce of 509 employees, comprising subjects from every working sector (spinning, wrapping, weaving, dyeing, finishing, quality control, storing and packing, informatics and marketing, administration, designing, woodwork). There were only included those with a minimum of one year's work. This factory manufactures mainly pure wool fabrics and wool rich or polyester/wool mixtures and, in lesser extent, cotton, linen and lycra products. The individuals for the control group were recruited from two retail stores from the same geographic area of the factory. In the control group, individuals who referred previous jobs in textile industry at any time or other jobs/hobbies with relevant dust exposures (such as construction workers, wood workers, bakers) in the last 10 years were also excluded.

Clinical data was gathered through a systematic interview to collect information on demographics, occupational history (including working sector, types of dust exposure, years of exposure, mask use), domestic or hobby dust exposures, smoking and alcoholic habits, comorbidities, previous nasal surgeries, nasal symptoms and their duration. Atopy (based on positive skin prick test or IgE antibodies in serum), lower airway disease and obstructive sleep apnea syndrome (OSAS) history were only considered positive when diagnosed by a specialist physician in each area. Subjective assessment of upper and lower airway symptoms and quality of life was obtained applying two Portuguese validated questionnaires: Rhinosinusitis Quality of life survey – Portuguese version (RhinoQOL-pv)[7] and COPD Assessment Test™ (CAT)[8]. RhinoQOL-pv scores for the symptom frequency and impact scales ranged from 1 ("never") to 5 ("always") in each question, while for the bothersomeness scale, scores ranged from 0 to 10, in accordance with the questions possible answers. We chose the CAT™ test because it consists of nonspecific questions about lung disease impacts and has already been studied not only for Chronic Obstructive Pulmonary Disease (COPD), but also Asthma and Asthma–COPD overlap syndrome (ACOS)[9].

A systematic endoscopic examination of both nasal cavities was performed by an otolaryngologist, using a 0º, 2.7 mm rigid endoscope from Karl-Storz®; decongestion with vasoconstrictor was used on an as-needed basis, especially to make middle meatus inspection easier. Nasal polyps were classified endoscopically in grade I, II or III, according to Lund criteria(10); Lund-Kennedy endoscopic score for CRS(11) was also determined for each participant. For the study purpose, cases of antrochoanal polyps, polypoid lesions with features suggestive of benign neoplasia (e.g. sinonasal papillomas) or with malignancy suspicion were excluded. All employees were observed in the doctor's office located at the company during their working shifts.

Terminology Usage Notes

CRSwNP according to EPOS 2012 is defined as a symptomatic clinical entity, not contemplating asymptomatic polyposis. So, it was decided to use "Nasal Polyposis" terminology, to include symptomatic CRSwNP and subclinical disease.

Statistical analysis

Statistical analysis was performed with Statistical Package for Social Sciences (IBM® SPSS® Statistics for Windows, Version 23.0). Textile workers and retail store workers were grouped in two independent samples and compared for multiple factors. Descrip-

Table 1. Sample demographics, comorbidities, smoking habits and occupational history (N=315).

	Exposed Group		Control Group		p value
Demographics					
Age (years ± SD; [range])	50 ± 11; [21; 67]		41 ± 10; [20; 65]		< 0.001
	n	%	n	%	
Gender: Male	92	42.3	35	34.7	0.120
Race					0.680
Caucasian	214	99.5	101	100	
African	1	0.5	0	0	
Comorbidities (n, %)					
Allergic Rhinitis	43	20.0	13	12.9	0.080
Asthma	17	7.9	11	10.9	0.252
COPD	1	0.5	0	0	0.680
Sinonasal Tumour	0	0	1	1.0	0.320
OSAS	11	5.1	0	0	0.013
Atopic Dermatitis	26	12.1	14	12.1	0.392
Salicylates Intolerance	3	1.4	1	1.0	0.617
Smoking Habits					0.832
Non-smoker	124	57.7	65	64.4	
Ex-smoker	41	19.1	8	7.9	
Smoker	50	23.3	28	27.7	
Alcoholic Habits					0.057
Non-alcoholic habits	107	49.8	60	59.4	
Light-to-moderate	97	45.1	40	39.6	
Heavy drinking	11	5.1	1	1.0	

tive statistics was used in those samples characterization. For categorical variables, Chi-Square Test (or Fisher's Exact Test/ Likelihood Ratio Test when assumptions needed for the previous test were not verified) was used to test for variable association. For continuous quantitative variables, Mann-Whitney Test was applied. Binomial test was used to compare our prevalence of NP with previous data published on the literature.
Statistical significance was accepted to correspond to a p-value of less than 0.05.

Results

A total of 316 individuals were included in the study: 215 textile workers (exposed group) and 101 retail store workers (control group). A total of 41 subjects were excluded: two retail store workers with a past working history in the textile industry; 15 textile workers with less than one year of work experience; 22 employees that were temporarily absent from work (ex. maternity leave, medical reason) and other two that refused to participate. Sample demographics, comorbidities, smoking and alcoholic habits are summarized in Table 1.
When asked about exposure to domestic fumes (ex. use of firewood/ coal) no difference was observed between groups with 12.2% of retail store employees and 13.1% of textile workers answering positively (Chi-Square Test, p=0.854). With regard to domestic animals, the non-exposed group (retail store workers) had more pets in a statistically significant way (Chi-Square Test, p=0.004).
The previous history of nasal surgery did not differ among the study groups (Table 2).
When asked about sinonasal symptoms with at least three months duration, the textile group reported significantly higher rates of hyposmia (16% vs 5%, p=0.003), headache (38% vs 22%, p=0.003), facial pressure (38% vs 24%, p=0.008), sneezing (62% vs 38%, p<0.001) and nasal pruritus (61% vs 37%, p<0.001). Snoring had also a significantly higher rate among the exposed group (54% vs 38%, p=0.005). Despite having all higher rates in the exposed group, other symptoms as nasal congestion/ blockage/obstruction, anterior and posterior rhinorrhea, and epistaxis did not differed statistically.
RhinoQOL-pv (total and by scales) and CAT™ mean scores are specified by group in Table 3.
The endoscopic findings as well as the Lund-Kennedy endoscopic score for both groups are summarized in Table 4. In nine cases (4.2%) from the exposed group and one case (1.0%) from

Table 2. History of nasal surgical procedures with absolute and relative frequencies. ESS – endoscopic sinus surgery.

Surgery	Exposed Group		Control Group		p value
	n	%	n	%	
Septoplasty	3	1.4	2	2.0	0.656
ESS	2	1.8	1	1.0	1.00
Polipectomy	0	0	0	0	-

Table 3. RhinoQOL-pv and CAT™ mean scores by group.

Questionnaire	Exposed Group	Control Group	p value
	mean± SD; [range]	mean± SD; [range]	
RhinoQOL-pv	26.31 ± 15.4 [14; 116]	21.45 ± 10.1 [14; 50]	0.005
Frequency Scale	8.25 ± 5.9 [5; 81]	7.12 ± 2.8 [5; 16]	0.011
Impact Scale	11.92 ± 5.3 [9; 37]	10.12 ± 2.6 [9; 25]	0.013
Bothersomeness Scale	6.14 ± 7.0 [0; 30]	4.21 ± 6.0 [0; 24]	0.009
CAT	2.67 ± 5.36 [0; 29]	1.37 ± 3.12 [0; 17]	0.023

Table 4. Results of endoscopic evaluation, including Lund-Kennedy score, by group.

Endoscopic Evaluation	Exposed Group		Control Group		p value
	n	%	n	%	
Nasal Polyposis	19	8.8	0	0	0.001
Antrochoanal Polyp	0	0	1	1.0	0.320
Polypoid degeneration of the middle turbinate	24	11.2	1	1.0	0.001
Septal Deviation	71	33.0	40	39.6	0.258
Lund-Kennedy score (mean± SD; [range])	3.43 ± 2.43; [0; 12]		1.76 ± 1.48; [0; 6]		<0.001

Table 5. Nasal polyps classification according to Lund criteria by group.

Grade	Exposed Group		Control Group	
	n	%	n	%
0 (no polyps)	196	91.2	101	100
I	9	4.2	0	0
II	9	2.2	0	0
III	1	0.5	0	0
Total	215	100	101	100

the control group, it was impossible to adequately inspect the middle meatus bilaterally due to severe septal deviation of the nasal cavity. In those cases, the Lund-Kennedy score and the polypoid status of the mucosa was inferred from the contralateral side.

The prevalence of septal deviation did not differ between the exposed and control groups (Table 4); also, total RhinoQOL-pv scores did not differ significantly between patients with and without nasal septum deviation (Man-Whitney Test, p=0.505).

One case of unilateral polyp was found in the control group. This patient had an history of a previous unilateral endoscopic surgery for an antrochoanal polyp (in the same side), and so it was assumed to correspond to disease recurrence.

NP was found in 19 subjects (8.8%) among the textile workers group (12 men and 7 women, ratio 1.7:1; mean age of 55 years) and none in the control group. From the 19 individuals found to have NP, only two (11%) were previously aware of the diagnosis and had been previously submitted to ESS. All cases of NP presented bilateral disease. Characterization of nasal polyps according to Lund criteria[10] is displayed on table 5.

When performing a statistical analysis stratified for age, we verified that in the subgroup of subjects with less than 45 years (N=120, 62 from exposed group and 55 from control group) the prevalence of NP was higher among the exposed ones (p=0.035, Likelihood Ratio Test), with 3 cases among them (4.8%) and none in the control group. In the subgroup of individuals with 45 or more years (N=196, 141 from exposed group and 39 from control group), the prevalence of NP was also higher in the exposed group with 16 cases among textile workers (11.3%) and no cases found in the control group (p=0.024, Fisher's Exact Test).

The prevalence of NP by age and years of dust exposition strata among textile workers is presented in Table 6. The mean time of years of textile dust exposure was 26±15 years, with a minimum of one year and a maximum of 54. Distribution of individuals with and without NP across working textile sectors is presented in Table 7. We found individuals with NP across almost every sector, apart from informatic/marketing and administration teams. Only 21 (9.8%) of textile workers referred to use occasional mask-protection during their job duties.

Concerning medication habits, 4.7% of textile workers referred regular use of nasal steroids and 9.3% of anti-histamines. Only two subjects with nasal polyps (11.7%) were using nasal steroids and were the ones with history of previous ESS.

The percentage of clinically silent NP or preclinical cases was 21% (4 cases) versus 79% (14 cases) of symptomatic NP. From the seven textile workers (3.3%) which claimed to have a previous medical diagnosis of CRSwNP, the diagnosis was confirmed in only two (being both workers with symptomatic NP). In the control group, two patients claimed also to have CRSwNP but

exposed and the control group. The textile industry in central Portugal is led by an ageing population and it was not possible to equalize the groups concerning this factor. However, we analyzed the groups by age strata and the difference in NP prevalence is still evident and significant. There was also a stronger female predominance in the control group, despite not being significantly different from the exposed group. These two factors, in addition to a relative small sample size might have contributed to the absence of nasal polyposis among the controls. These limitations are exceeded when comparing results of the exposed group with the prior cadaver endoscopic study done in Portugal, which had an even older population (mean age of 77 years, N=200) and a male predominance (58.5%). The prevalence results among these textile workers are significantly higher than the prevalence found in the cadaver study[3].

Another limitation is the fact that in a few cases (3.2%) it was impossible to adequately inspect the middle meatus bilaterally due to marked septal deviation. Small polyps may have also been missed in a small percentage of the sample since rigid endoscopy must be done gently and no topical anesthesia was used. These factors in combination can lead to an underestimation of NP prevalence.

In accordance to previously published studies that suggested an increase of NP prevalence with age[2,12,13], and a male predominance[1,2], we also found an higher prevalence among older strata and a male-female ratio of 1.7:1.

The prevalence of NP also rose by strata according to the number of years of textile dust exposition, suggesting that a longer occupational dust exposition increases the risk of CRSwNP occurrence. The fact that we found NP across every textile sector apart from informatic/marketing and administration teams is not surprising, since these two sectors are the least exposed ones to textile dust.

Comparing the comorbidities between volunteers from the exposed and control group (Table 1), we notice that OSAS prevalence is higher among the exposed ones. This fact can be attributed to the older age of this group since we know that the frequency of this pathology also increases with age, but also due to higher rates of sinonasal disease, as we can infer by RhinoQOL-pv results and Lund-Kennedy scores (significantly higher on the exposed group). OSAS and rhinosinusitis severity seem not to be correlated but the prevalence of OSAS in patients with chronic rhinosinusitis is high (with a recent study finding a prevalence of 65%)[14].

The fact that all rhinologic symptoms were higher among textile workers compared with the control group, and especially the statistically significant rhinosinusitis characteristic symptons like hyposmia, headache and facial pressure, are in accordance with our previously published hypothesis that occupational/work-exacerbated rhinitis may progress toward occupational/work-exacerbated rhinosinusitis and may contribute to CRSwNP development[5]. Moreover, RhinoQOL-pv, which was designed for rhinosinusitis, scored higher in a statistically significant way between the exposed and control group.

Another interesting issue is that patients reporting atopic diseases previously diagnosed by an immunoallergologist did not differ significantly between the exposed and control groups or even between CRSwNP patients and subjects without the disease. Moreover, the prevalence of atopy was 20% in the exposed group and 26% among CRSwNP patients and these values are in the range of the reported prevalence of atopy in the general population (20–30%)[15]. These findings corroborate our previous work[5] and other published studies[13], supporting the hypothesis of important non–IgE-related mechanisms on CRSwNP etiopathogenesis, including immune and non-immune ones.

We found not only a significant difference among NP prevalence between the groups (p=0.001) but also among polypoid degeneration of the middle turbinate (p=0.001) and overall inflammatory state of the nose as shown by higher Lund-Kennedy score among textile workers (p<0.001). By these findings, we can state that occupational exposure to dust is associated to general inflammatory changes of the nose and paranasal sinus.

No statistically significant difference was found on the frequency of previously diagnosed chronic lower respiratory diseases in subjects with and without NP, contrary to what was expected by recent studies on CRSwNP which found prevalences as high as 55%[5] or 72.5%[16]. The relative small number of cases with NP in our sample (only 19 cases) and the rate of subclinical and early NP stages (mainly grade I/II) may contribute to these figures.

The prevalence of previously diagnosed chronic lower respiratory diseases did not differ also between exposed and control groups. However, many epidemiologic studies assessing lower respiratory symptoms and spirometry results among textile workers and controls have been alerting to higher rates of respiratory symptoms and deterioration in spirometric parameters in the first group[17–19]. Moreover, it is clear by CAT™ score that lower respiratory symptoms are more frequent among exposed individuals, with a statistically significant difference, pointing up to a probable underdiagnosis of lower respiratory diseases among textile workers. The fact that in this study it was only considered the diagnosis of chronic lower respiratory diseases previously diagnosed by a respiratory physician can partially explain this finding, as many employees are treated by general practitioner or workplace doctor.

As this represents a problem of Public Health, these findings show the importance of employee's protective measures, such as mask use during work, and reinforces the need for legislation and control to guarantee the functioning of air dust filters and exhausting systems.

More epidemiologic investigations are needed, namely to establish the NP prevalence in other types of occupational dust

exposure. The concomitant involvement of otolaryngologists and a pneumologists on this type of studies can be helpful to clarify the association between CRSwNP and lower respiratory diseases.

Conclusion

This investigation was the first endoscopic based epidemiological study to evaluate the impact of occupational dust exposure on NP prevalence. Our results revealed a higher prevalence of NP among textile workers compared to our control group, but also comparing with previous in-vivo and cadaver endoscopic based studies done in Europe. These results point to an important correlation between occupational dust exposure and NP occurrence, justifying more research in this area. Meanwhile, Public Health policies like employee's protective measures must be reinforced.

Acknowledgements

We thank to António Santos Silva, MD, for his support concerning research authorizations in the involved Industrial and Retail Store Enterprises.

Authorship contribution

RVT: Study design, data collection, statistical analysis, discussion, revision. RC: Study design, discussion, revision. RF: Study design, discussion, revision. CvB: Study design, discussion, revision.

Conflict of interest

No conflicts of interest or financial disclosure to declare.

References

1. Fokkens WJ, Lund VJ MJ, Mullol J, Bachert C, Alobid I, Baroody F et al. European position paper on rhinosinusitis and nasal polyps 2012. Rhinology. 2012; 50(Supp 23): 1–329.
2. Johansson L, Akerlund A, Holmberg K, Melen I, Bende M. Prevalence of nasal polyps in adults: the Skovde population-based study. Ann Otol Rhinol Laryngol. 2003; 112(7): 625–9.
3. Cerejeira R, Veloso-Teles R, Lousan N, Moura CP. Prevalence of nasal polyps in Northern Portugal: a cadaver endoscopic study. Rhinology. 2014; 52(4): 386–9.
4. Hox V, Delrue S, Scheers H, Adams E, Keirsbilck S, Jorissen M, et al. Negative impact of occupational exposure on surgical outcome in patients with rhinosinusitis. Allergy. 2012; 67(4):560–5.
5. Veloso-Teles R, Cerejeira R. Endoscopic sinus surgery for chronic rhinosinusitis with nasal polyps: Clinical outcome and predictive factors of recurrence. Am J Rhinol Allergy. 2017; 31(1):56–62.
6. Gao WX, Ou CQ, Fang SB, Sun YQ, Zhang H, Cheng L, et al. Occupational and environmental risk factors for chronic rhinosinusitis in China: a multicentre cross-sectional study. Respir Res. 2016; 17(1): 54.
7. Cerejeira R, Veloso-Teles R, Lousan N, Moura CP. The Portuguese version of the RhinoQOL Questionnaire: validation and clinical application. Braz J Otorhinolaryngol. 2015 Jan; 81(6): 630–5.
8. CAT Development Steering Group. Healthcare Professional User Guide - CAT. 2012;(3):5–12.
9. Kurashima K, Takaku Y, Ohta C, Takayanagi N, Yanagisawa T, Sugita Y. COPD assessment test and severity of airflow limitation in patients with asthma, COPD, and asthma–COPD overlap syndrome. Int J COPD. 2016;11(1):479–87.
10. Lund VJ. Diagnosis and treatment of nasal polyps. BMJ. 1995; 311(7017): 1411–4.
11. Lund VJ, Kennedy DW. Staging for rhinosinusitis. Otolaryngol Head Neck Surg. 1997; 117(3 Pt 2):S35-40.
12. Settipane GA. Epidemiology of Nasal Polyps. Allergy Asthma Proc. 1996; 17(5): 231-6. Review.
13. Grigoreas C, Vourdas D, Petalas K, Simeonidis G, Demeroutis I, Tsioulos T. Nasal Polyps in Patients with Rhinitis and Asthma. Allergy Asthma Proc. 2002; 23(3): 169-74.
14. Jiang RS, Liang KL, Hsin CH, Su MC. The impact of chronic rhinosinusitis on sleep-disordered breathing. Rhinology. 2015; 54(1):75–9.
15. Pawankar R, Canonica GW, Holgate ST, Lockey RF. White Book on Allergy. (WAO). WAO, editor. World Allergy Organization (WAO). 2011; 1-220 p.
16. Håkansson K, Thomsen SF, Konge L, Mortensen J, Backer V, von Buchwald C. A comparative and descriptive study of asthma in chronic rhinosinusitis with nasal polyps. Am J Rhinol Allergy. 2014; 28(5):383–7.
17. Nagoda M, Okpapi JU, Babashani M. Assessment of respiratory symptoms and lung function among textile workers at Kano Textile Mills, Kano, Nigeria. Niger J Clin Pract. 2012; 15(4): 373-9.
18. Hinson AV, Lokossou VK, Schlünssen V, Agodokpessi G, Sigsgaard T, Fayomi B. Cotton Dust Exposure and Respiratory Disorders among Textile Workers at a Textile Company in the Southern Part of Benin. Int J Environ Res Public Health. 2016; 13(9): pii: E895.
19. Mansouri F, Pili JP, Abbasi A, Soltani M, Izadi N. Respiratory problems among cotton textile workers. Lung India. 2016; 33(2): 163-6.

Rafaela Veloso-Teles
Cova da Beira Hospital Centre
Quinta do Alvito
6200-251 Covilhã
Portugal

Mobile: +351-96-565 0303
E-mail: rafaelateles84@gmail.com

APPENDIX 3

Clinical Study

Ear, Nose & Throat Journal
1–8

DOI: 10.1177/0145561319867668
journals.sagepub.com/home/ear
SAGE

Food-Specific IgE and IgG Antibodies in Patients With Chronic Rhinosinusitis With Nasal Polyps: A Case–Control Study

Rafaela Veloso-Teles, MD[1,2,3], Rui Cerejeira, MD, PhD[1,2,3], Débora Rodrigues, MBiolSc[4], Rosa Roque-Farinha, MD, PhD[1], and Christian von Buchwald, MD, DMSci[5]

Abstract

EPOS 2012 states that investigation is needed to study a possible role for food allergy in the initiation and perpetuation of chronic rhinosinusitis with nasal polyps (CRSwNP). Our main goal was to compare serum levels of food-specific immunoglobulin G (IgG) and IgE antibodies in patients with CRSwNP and controls. A prospective case–control study with 33 patients with CRSwNP and 31 controls without CRS was carried out. Clinical data were gathered through a systematic interview and blood sample was collected. Enzyme-linked immunosorbent assay tests using OmegaDiagnostics kit with 40 food allergens for detection of specific IgG antibodies were performed and food-specific IgE antibodies were determined by immunoassay using ImmunoCAP. Immunoglobulin classes and IgG subclasses levels were also evaluated. Statistical analysis was performed using SPSS v.23. The overall sum of food IgG antibodies was significantly lower in CRSwNP compared to control group, and this difference was also observed for different specific IgG antibodies (corn, soya, grain legumes, pear and apple, berries, citric fruit). In controls, a positive correlation between IgG1 and the sum of food IgG antibodies was seen, but in CRSwNP group a negative correlation was found. In addition, a significant higher level of IgG1 and lower IgG2 and IgG3 was found among patients with CRSwNP. Levels of serum-specific IgE antibodies against multiallergen food mix (fx5) and against shrimp, strawberry, orange, rye, or egg yolk, as well as the sum of food IgE antibodies, did not differ significantly between the groups. These findings suggest that food allergy does not have an important role in CRSwNP etiopathogenesis, whether it is IgG or IgE mediated. Moreover, the observed suppression of specific IgG antibodies against food allergens, its negative correlation with IgG1 and the IgG1 switching in CRSwNP, can be related to deviated IgG responses against other targets (eg, airborne particles) and warrants future investigation.

Keywords

nasal polyps, paranasal sinus disease, sinusitis, food allergy

Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a common chronic inflammatory disease with high morbidity that results in a significant decrease in patient quality of life. The etiopathogeny of CRSwNP remains obscure, and it is still considered a difficult-to-treat pathology. There is, however, a movement away from the pathogen-driven hypotheses, and an emerging consensus that the persistent inflammation that defines chronic rhinosinusitis (CRS) results from a dysfunctional host–environment interaction, involving various exogenous agents and changes in the sinonasal mucosa.[1] This overall concept is in agreement with the current understanding of the etiopathogenesis of chronic mucosal inflammatory disorders in general, driven by an imbalance between the host, commensal

[1] Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal
[2] Department of Otorhinolaryngology, Cova da Beira Hospital Centre (CHCB), Covilhã, Portugal
[3] Otorhinolaryngology Unit of Beira Interior (UOBI), Covilhã, Portugal
[4] Department of Clinical Pathology, Cova da Beira Hospital Centre (CHCB), Covilhã, Portugal
[5] Department of Otorhinolaryngology, Head and Neck Surgery and Audiology, Rigshospitalet-Copenhagen University Hospital, Copenhagen, Denmark

Received: June 08, 2019; accepted: July 11, 2019

Corresponding Author:
Rafaela Veloso-Teles, MD, Departamento de Ciências Médicas, Faculdade de Ciências da Saúde, Universidade da Beira Interior, Av Infante D. Henrique, Covilhã 6200-506, Portugal.
Email: rafaelateles84@gmail.com

flora, potential pathogens, and exogenous stresses.[1,2] Chronic rhinosinusitis with nasal polyps and asthma (mainly non-immunoglobulin E [IgE]-mediated asthma) frequently coexist in the same patients,[1] and although their etiopathogenesis may be linked, their interrelationship and subjacent mechanisms are unclear.

Food allergy is an adverse food reaction that involves abnormal immune responses to food allergens, which can involve IgE-mediated (immediate food responses) or non-IgE-mediated reactions (delayed food responses).[3] The prevalence of food allergy is known to be higher in patients with allergic rhinitis,[4] and cross-reactivity is known to occur between aeroallergens and food allergens.[5] Moreover, food allergy is known to be a risk factor for asthma development and lower respiratory symptoms may be seen in food-induced allergic reactions in asthmatic patients.[3] In addition, it is known that inhalation of food allergens can induce respiratory symptoms, for example, in occupational asthma in workers handling food products and derivates.[6]

A possible role of food allergy in CRSwNP has been raised in 2 case–control studies[7,8] that found 70% and 81% positivity for intradermal food tests in patients with CRSwNP and only 34% and 11% in the control group, respectively. The authors argued that the fact that intradermal food test correlated poorly with serum-specific IgE levels pointed to the involvement of other non-IgE-mediated food hypersensitivities.[8] Meanwhile, *EPOS 2012* states that further research is needed to investigate a possible role for food allergy in the initiation and perpetuation of CRSwNP.[1]

Controversial evidence exists about IgE food sensitization in patients with CRSwNP, with authors reporting prevalence between 22% and 74% depending on used technique (skin prick test [SPT] vs radioallergosorbent test [RAST]).[9,10] In 2016, a comparative study between patients with CRSwNP and chronic rhinosinusitis without nasal polyps (CRSsNP) using enzyme allergosorbent test (EAST) found no significant difference between food IgE sensitization prevalence, type, and severity, concluding that food atopy is unlikely to be a major factor in nasal polyposis etiopathogenesis.[11] The prevalence of food allergy is difficult to estimate mainly due to diagnosis issues. For example, SPT positivity does not necessarily prove that the food is causal as the positive predictive value is less than 50% but if negative essentially confirms the absence of IgE-mediated allergic reactivity since negative predictive value is greater than 95%.[3] In vitro assays are also useful modalities in food allergy study. Initially, RAST were used, but more recently quantitative measurement of food-specific IgE antibodies such as ImmunoCAP have been utilized and studies have found them to be more predictive of symptomatic IgE-mediated food allergy.[3] Moreover, ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) has the advantage of the lack of interference from allergen-specific immunoglobulin G (IgG) antibodies.[12] However, undetectable serum food-specific IgE levels may be associated with clinical reactions as well and the double-blind placebo controlled food challenge is still considered the gold standard for food allergy diagnosis, but because of its methodological difficulties, it is rarely conducted in clinical practice.

Recently, IgG antibodies against food antigens have been suggested to cause low-grade inflammation in irritable bowel syndrome, with symptoms improvement after dietary elimination based on specific IgG profile of the patient.[13] This theory, called the "leaky gut syndrome" (LGS), proposes that it is the increased permeability of the gut wall to macromolecules that leads to activation of the immune system, which may initiate production of specific IgG antibodies against food, resulting in chronic inflammation sustained by repeated intake of allergenic foods.[2,14] The LGS and the IgG-mediated food allergy have been investigated in different chronic inflammatory pathologies, such as obesity,[15] type 1 and type 2 diabetes,[16,17] chronic liver disease,[18] chronic kidney disease,[19] chronic heart failure,[20] depression,[14,21] and asthma.[22] The role of IgG antibodies against food remains controversial, namely because they can be detected in healthy individuals and there are no standardized cutoff values. In fact, IgG food detection is still only recommended for investigational purpose and not for individual use.[23] The development of commercial kits with standardized food antigen extraction and purification, using an optimized and validated enzyme-linked immunosorbent assay (ELISA) method, permits to overcome matters of reproducibility and variability.

Our main goal was to compare specific IgG and IgE antibodies dosage against food allergens in patients with CRSwNP and controls, trying to clarify if food allergy can be associated with CRSwNP.

To our knowledge, this is the first study to investigate the hypothesis of IgG-mediated food allergy in CRSwNP pathogenesis and to use ImmunoCAP technology to evaluate IgE food sensitization in CRSwNP.

Patients and Methods

A case–control observational study was performed according to established ethical guidelines and approval of ethics committee at the Cova da Beira Hospital Centre (deliberation number 82/2015). A signed informed consent was obtained from each participant in the study.

Sample

All the cases were about to undergo endoscopic sinus surgery for CRSwNP refractory to medical treatment (topical long-term and systemic short-term steroids), in a district hospital center, from January 2016 to October 2018. Diagnosis of CRSwNP was established using the definition of *EPOS 2012*.[1] The cases were selected consecutively from the waiting list and had their disease confirmed endoscopically by computed tomography (CT) scan and histological examination of the subsequent surgical specimen. Patients were selected after applying the following exclusion criteria: concomitant benign or malignant sinonasal tumors, CRSsNP, antrochoanal polyps, polyps associated with fungal rhinosinusitis, primary ciliary dysfunction, cystic fibrosis, innate or acquired immunodeficiency (eg, human immunodeficiency virus; immunosuppressive drugs), autoimmune diseases (eg, systemic vasculitis,

inflammatory bowel disease), or patients with history of or under allergen-specific immunotherapy. No course of oral corticosteroids was given to patients with CRSwNP at least 3 months before serum specimen collection.

Controls were selected from patients in the waiting list for septoplasty, after excluding patients with symptoms and endoscopic or imagiological signs (in CT scan) of CRS; controls with innate or acquired immunodeficiency, autoimmune diseases, or patients with history of or under allergen-specific immunotherapy were also excluded.

Data Collection

Clinical data were gathered through a systematic interview. Dietary habits were collected using the Portuguese-validated Food Frequency Questionnaire (QFA).[24] Every questioned food item was scored between an intake of 0 (never or less than 1 per month) and 8 (6 or more per month).

Chronic lower respiratory diseases diagnosis was established by a respiratory physician. Blood sample was collected to determine immunoglobulin classes, IgG subclasses levels and specific IgG and IgE against food allergens. Immunoglobulin classes were determined by electrochemiluminescence immunoassay using Cobas 6000 analyzer (Roche Diagnostics, The Binding Site Group, Birmingham, United Kingdom) and IgG subclasses through the Optilite turbidimetric analyzer (Binding Site).

Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assay tests for semiquantitative analysis of serum IgG antibodies to 40 food allergens were performed using OmegaDiagnostics (Littleport, Cambridgeshire, United Kingdom) detection kit. A microplate reader with 450-nm filter was used to read the final absorbance of each well. The given concentration of the 2 standards provided on the kit allowed to infer about antibody IgG concentration (arbitrary units/mL [AU/mL]) on each well. Some food antigens are grouped in the same well as food mixtures, confering in total 21 wells (corn; oat; rice; rye; wheat; cow's milk; egg white; egg yolk; white fish misture [cod, haddock, plaice]; shellfish misture [crab, lobster, prawn]; soya; legume bean misture [haricot, kidney, pea]; mustard mixture [cabbage, broccoli, cauliflower]; gluten, apple, and pear; berries misture [rasperry, strawberry, blackberry]; citrus mixture [orange, lemon, grapefruit]; nut mixture [almond, cashew, hazelnut, peanut]; yeast [bakers and brewer's]; chicken and turkey; and pork and beef). A positive response was considered if concentration value was $\geq$8 AU/mL, and the response of the reaction was classified as grade 1+ if $\geq$8 and <12.5, grade 2+ if $\geq$12.5 and <25, and grade 3+ if $\geq$25 AU/mL, according to suggested ranges of the manufacturer.

ImmunoCAP Food Allergen Test

Specific IgE antibodies for food antigens were determined by immunoassay using ImmunoCAP Food Allergen tests by Thermo Fisher Scientific (Uppsala, Sweden) for the following food allergens: fx5—egg white, milk, fish, wheat, peanut, and soybean; f24—shrimp; f44—strawberry; f33—orange; f5—rye; and f75—egg yolk. Positive results were considered if serum concentration was above 0.35 KUA/L.

Statistical Analysis

Statistical analysis was performed with Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 23.0). Descriptive statistics was used in sample characterization. Mann-Whitney *U* test for independent samples was used to compare continuous variables between the 2 groups. Pearson χ^2 test (or Fisher exact test when appropriate) was used to test association between categorical data. A nonparametric Spearman correlation was used to examine the relation between quantitative variables.

A P value <.05 was considered statistically significant.

Results

Sixty-four individuals were included: 33 patients with CRSwNP and 31 controls. Demographics, body mass index (BMI), and QFA scores of patients with CRSwNP and controls are presented and compared in Table 1. There were no significant differences between CRSwNP and control groups based on age, gender distribution, and BMI. According to QFA total score and subscores, no significant difference in the food consumption pattern was observed between groups.

Chronic lower respiratory diseases were more prevalent in patients with CRSwNP (21 patients, 63.6%) than that in the control group (2 participants, 6.5%), in a statistically significant way (P < .001, Fisher exact test; Table 1). In CRSwNP group, 16 (48.4%) participants were under treatment with inhaled steroids with β2-agonists, whereas in the control group, only 2 (6.4%) were being treated with inhaled steroids.

Using the ELISA tests for serum IgG antibodies to 40 food allergens analysis, we found the overall sum of IgG levels to be significantly lower in patients with CRSwNP compared to the control group (P = .013) and this difference was also observed for specific IgG antibodies against corn (P = .009), soya (P = .002), grain legumes (P = .004), pear and apple (P = .0025), berries (P = .0005), and citric fruit (P = .007; Figure 1). Moreover, the prevalence of patients with positive food IgG antibodies was significantly lower in patients with CRSwNP (48.5%) compared to the control group (74.2%; Table 2). The overall sum of specific food IgG antibodies did not correlate significantly with BMI (P = .719) or age (P = .228).

No correlation between specific food IgG antibodies and the frequency of consumption that particular food was observed: IgG antibodies against cow's milk and dairy products consumption frequency (P = .710); IgG antibodies against egg white and egg consumption frequency (P = .505); and IgG antibodies against wheat and cereals consumption frequency (P = .676), among others. In global, considering the all sample, the specific

Table 1. Comparison of Sample Demographics, Body Mass Index (BMI), Presence of CLRDs (Chronic Lower Respiratory Diseases), Food Frequency Questionnaire (QFA) Score, and Subscores Between Patients With CRSwNP and Controls (N = 64).[a]

	CRSwNP Group, n = 33	Control Group, n = 31	*P* Value
- Demographics			
Age	58.4 ± 2.4; [30; 82]	52.9 ± 2.2; [32; 75]	.073
Gender: male/female, n	17/16	19/12	.461
- BMI	27.4 ± 0.6; [19; 37]	26.17 ± 0.9; [17; 34]	.282
- CLRDs, n (%)	21 (63.6)	2 (6.5)	<.001
Atopic asthma	6 (18.2)	2 (6.5)	.149
Nonatopic asthma	9 (27.3)	0 (0)	.001
COPD	9 (27.3)	0 (0)	.033
- QFA	155.27 ± 8.9; [79; 301]	153.19 ± 5.6; [91; 219]	.481
Dairy products	12.55 ± 5.50	9.94 ± 5.45	.070
Eggs	2.21 ± 1.08	2.61 ± 1.05	.102
Cereals	16.45 ± 1.26	16.03 ± 1.22	.783
Rice	2.76 ± 1.00	2.94 ± 0.57	.153
White fish	7.06 ± 2.64	5.87 ± 0.47	.110
Shellfish	0.73 ± 0.94	1.19 ± 0.21	.083
Legumes	3.52 ± 2.42	3.13 ± 1.86	.984
Vegetables	8.73 ± 5.08	9.77 ± 5.08	.319
Apple and pear	3.76 ± 1.64	3.61 ± 1.38	.618
Berries	1.82 ± 1.78	2.48 ± 1.61	.069
Citrus fruits	4.45 ± 2.45	3.42 ± 2.22	.092
Chicken and turkey	3.48 ± 1.71	4.19 ± 1.78	.124
Pork and beef	6.48 ± 3.75	6.90 ± 2.86	.398

Abbreviations: COPD, chronic obstructive pulmonary disease; CRSwNP, chronic rhinosinusitis with nasal polyps.
[a]Data are expressed as mean ± standard error of mean. Comparison was made using Mann-Whitney *U* test for continuous variables and Pearson χ^2 test for categorical data.

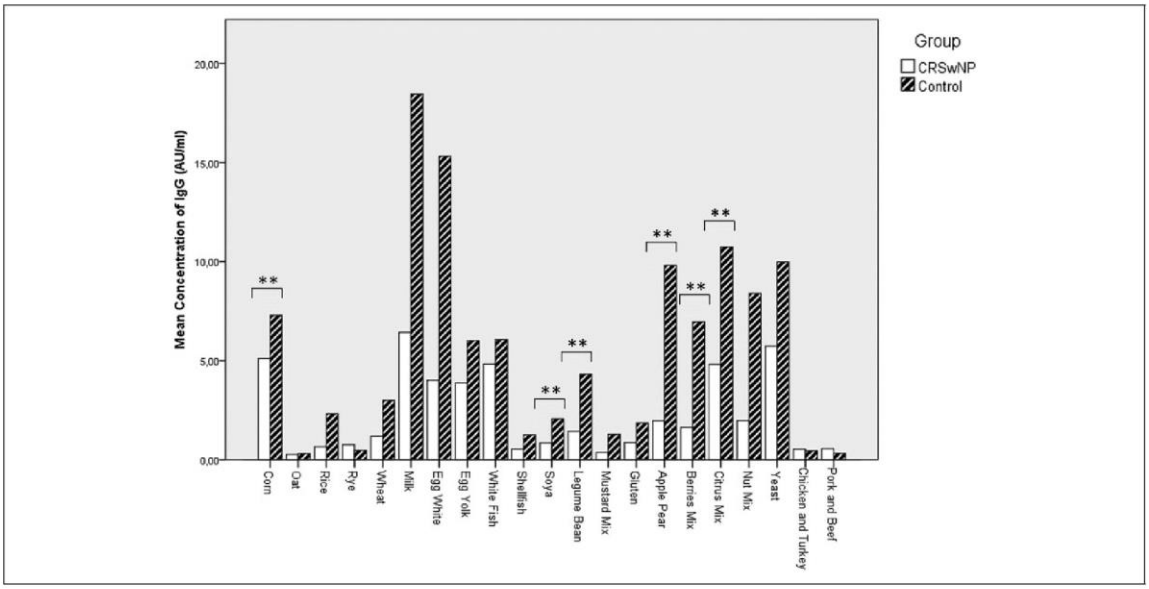


Figure 1. Comparison of the mean concentration values of specific IgG antibodies for different food allergens in patients with CRSwNP and controls, obtained in ELISA test. N = 64. **$P < .01$ using Mann-Whitney *U* test. CRSwNP indicates chronic rhinosinusitis with nasal polyps; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G.

Table 2. Comparison Between Serum Levels of Specific Food IgG Antibodies and Prevalence of Test Positivity Between Patients With CRSwNP and Controls (N = 64).[a]

	CRSwNP Group, n = 33	Control Group, n = 31	P Value
Sum [food IgG], AU/mL	48.24 ± 3.9; [18; 89]	115.5 ± 32.1; [22; 940]	.013
Positive food IgG, n (%)	16, 48.5	23, 74.2	.032
Multiple positive food IgG, n (%)	9, 27.3	16, 51.6	.041

Abbreviations: CRSwNP, chronic rhinosinusitis with nasal polyps; IgG, immunoglobulin G.
[a]Positive food IgG was considered if at least 1 food-specific IgG concentration was ≥8 AU/mL and multiple positive if 2 or more food-specific IgG antibodies were ≥8 AU/mL. Data are expressed as mean ± standard error of mean. Comparison was made using Mann-Whitney *U* test for continuous variables and Pearson χ^2 test for categorical data.

Table 3. Correlation Between the Overall Sum of Food Specific IgG Concentration and Total IgG and IgG Subclasses Levels in the Serum Among Patients With CRSwNP and Controls (N = 64).[a]

	CRSwNP Group, n = 33		Control Group, n = 31	
	R	*P* Value	*R*	*P* Value
Correlation between sum of [Food IgG] and total IgG	−0.014	.937	0.197	.289
Correlation between sum of [Food IgG] and IgG1	−0.295	.048	0.302	.049
Correlation between sum of [Food IgG] and IgG2	0.242	.174	−0.036	.845
Correlation between sum of [Food IgG] and IgG3	0.103	.568	−0.225	.223
Correlation between sum of [Food IgG] and IgG4	0.094	.603	0.076	.686

Abbreviations: CRSwNP, chronic rhinosinusitis with nasal polyps; IgG, immunoglobulin G.
[a]Correlation was performed with Spearman test.

IgG antibodies against food with higher mean concentration values in our sample were cow's milk (12.25 AU/mL), egg white (9.49 AU/mL), citrus fruits (7.68 AU/mL), bakers and brewer's yeast (7.78 AU/mL), and corn (6.17 AU/mL).

No correlation between total serum IgG level and sum of specific IgG against food was observed ($P = .748$). However, considering subclasses and the 2 sample groups, we found a negative correlation between IgG1 serum levels and sum of food-specific IgG concentration values in patients with CRSwNP and a positive correlation between IgG1 serum levels and sum of food-specific IgG concentration values in controls (Table 3).

Table 4. Comparison Between the Overall Sum of Serum Levels of Specific Food IgE Antibodies and the Prevalence of Test Positivity Between Patients With CRSwNP and Controls (N = 64).[a]

	CRSwNP Group, n = 33	Control Group, n = 31	P Value
Sum [Food IgE], AU/mL	0.49 ± 0.21; [0.01; 5.91]	0.40 ± 0.23; [0.01; 7.03]	.234
Positive food IgE, n (%)	5, 15.2	4, 12.9	1.000
Multiple positive food IgE, n (%)	3, 9.1	2, 6.5	1.000

Abbreviations: CRSwNP, chronic rhinosinusitis with nasal polyps; IgE, immunoglobulin E.
[a]Data are expressed as mean ± standard error of mean. Comparison was made using Mann-Whitney *U* test for continuous variables and Pearson χ^2 test for categorical data.

Moreover, in CRSwNP, a statistically significant higher level of IgG1 subclass in the serum was detected compared to controls ($P = .041$) and lower levels of IgG2 ($P = .048$) and IgG3 ($P = .014$). Concerning total IgG and IgG4 subclass levels, no difference was identified.

No significant difference in IgE food sensitization was observed in relation to the overall sum of specific food IgE concentrations or the prevalence of positive IgE values between CRSwNP and control group (Table 4). Concerning specific food IgE dosage, no significant differences were observed for fx5: egg white, milk, fish, wheat, peanut, and soybean ($P = .271$); shrimp ($P = .629$); strawberry ($P = .207$); orange ($P = .509$); rye ($P = .585$); or egg yolk ($P = .066$).

A positive correlation between total serum IgE levels and total specific IgE against food was observed ($P = 5.12 \times 10^{-9}$). Total IgE serum levels were significantly elevated in patients with CRSwNP compared to controls ($P = .03$). No correlation between the overall sum of specific IgG and IgE antibodies against food was observed ($P = .936$).

Discussion

Our results do not support the existence of an important role for food allergy in CRSwNP pathogenesis, independently of being an IgE- or IgG-mediated immune response. As already stated, the possible role of food allergy in CRSwNP has been raised in 2 case–control studies[7,8] that found 70% and 81% positivity for intradermal food tests in patients with CRSwNP and only 34% and 11% in the control group, respectively, with statistically significant differences. Meanwhile, some authors have criticized the value of intradermal food tests, namely for their increased risk of systemic reactions comparatively with SPT, noncorrelation to specific IgE levels, and high frequency of false-positive results.[25] Moreover, these 2 case–control studies have been carried out in the same investigational center and until now no replication of their results has been published elsewhere.

Regarding IgE-mediated food hypersensitivity, we did not find any significant difference in ImmunoCAP results considering IgE food serology test positivity or even in the sum of specific IgE class against food between CRSwNP and controls, in accordance to a previous publication that used EAST technique.[11] In that study, published in 2016, a positive correlation between total serum IgE level and sum of specific IgE antibodies against food was observed,[11] as we also found in this investigation. We report here prevalence of IgE food sensitization in patients with CRSwNP (15%) and in controls (13%), which are similar to the values described by Lill et al[10] (22% in CRSwNP and 14% in controls). Lill et al reported that patients with CRSwNP had higher rates of IgE positivity for milk compared to controls using RAST. However, this result was not replicated in other studies so far and the fact that they did not find any control with IgE positivity for milk must be seen with caution since milk has been reported as one of the most frequently food allergens in IgE dosage screenings in general population.[26] We did not find significant difference comparing fx5-multifood allergen panel (that includes milk) or in single specific IgE antibody dosage for milk in case of fx5 positivity. The discrepancy in the prevalence of IgE-mediated food allergy among CRSwNP between published studies might be due to different methodologies used, such as food allergy SPT, intradermal food allergy test, RAST, or EAST. The ImmunoCAP is considered to have higher sensitivity and better diagnostic capacity compared to RAST,[27] and this study was to our knowledge the first to use this technique in CRSwNP.

Concerning IgG-mediated food hypersensitivity, our results did not show higher antibodies dosage in CRSwNP, otherwise revealing an immune suppression of IgG response against food allergens in CRSwNP. The overall sum of food-specific IgG antibodies concentration was significantly reduced in CRSwNP comparing to controls and was not correlated with BMI or age. Moreover, a statistically significant reduction in IgG concentration against particular foods, such as corn, soya, legume beans, pear and apple, berries, and citric fruits, was observed in the CRSwNP group, irrespective of food-type consumption. We hypothesize that this suppression of IgG-mediated immune response against food allergens could be the result of deviated IgG responses to other agents (eg, airborne particles) in CRSwNP, which must be clarified in future investigations. In accordance with this hypothesis, it is interesting to note that in the CRSwNP group, a significant inverted correlation was observed between the sum of food-specific IgG antibodies and IgG1 subclass level in serum, as opposed to the control group in which a positive correlation was found. Moreover, we observed that patients with CRSwNP showed a subclass switching toward IgG1, with significant higher values of this IgG subclass and lower levels of IgG2 and IgG3, compared to controls.

There is recent evidence suggesting that occupational exposure to dust can be related to the occurrence and persistence of CRS[28,29] and specifically to CRSwNP phenotype.[30,31] These studies point to a risk factor for CRS that is mainly due to the inhalation of lower-molecular-weight (LMW) particles (<5 kDa) which, contrary to high-molecular-weight particles that induce a well-known IgE-mediated immune response, induce airway inflammation through mechanisms that are far less known. It can include the classical "irritant response" plus LMW sensitization of the adaptive immune system by acting as haptens.[28,31,32] High-molecular weight agents are generally proteins from animal and vegetal origin while LMW agents include a wide variety of organic and inorganic compounds. In contrast to protein allergens, LMW agents are incomplete antigens (ie, haptens) that must bind to carrier macromolecules to become immunogenic. For example, LMW agents causing occupational asthma are typically highly reactive electrophilic compounds that are capable of combining with hydroxyl, amino, and thiol functionalities on airway proteins.[33] The innate chemical reactivity of most LMW agents has largely hampered the investigation of immunological mechanisms, owing to uncertainty about the antigens that elicit immunological responses.[33] The understanding of the interaction between LMW particles and respiratory proteins can lead to the identification of antigenic determinants involved in CRSwNP and non-IgE-mediated asthma and to the development of hapten-specific monoclonal antibodies to clarify the subjacent immunologic mechanisms.

Immunoglobulin G1, which was significantly elevated in the CRSwNP group, is an important subclass in antibody response to soluble protein antigens, membrane proteins, and allergens; has the longest serum half-life; is capable of complement activation by C1q binding; and is the main IgG subclass to cross placental and mucosal barriers.[34] The neonatal Fc receptor (FcRn), the receptor involved in IgG transport across mucosa barriers, has been demonstrated to be expressed in many adult tissues and cell types, including nasal mucosa,[35] has a strong binding affinity of IgG1 to FcRn compared to other subclasses,[36,37] and in animal models, it has been demonstrated a predominant expression in the airways compared to the intestine, skin, liver, spleen, and skeletal muscle.[38] In both human asthmatics and animal models of allergy, it has been showed that allergen-specific IgG-mediated response can contribute to Th2-mediated inflammation,[39] the predominant type of inflammation seen in Western patients with CRSwNP.[1] One study in patients with asthma has showed that IgG1 ratio between bronchoalveolar fluid and serum concentrations had the highest value among IgG subclasses.[40] Another study in patients with CRSwNP demonstrated that the relative concentration of IgG1 subclass was significantly higher among nasal tissue homogenates in CRSwNP compared to controls.[41] It is possible that an augmented IgG1 response mounted in airways could make the immune system more tolerant to food allergens in the intestinal mucosa, mainly to protein food antigens.

To our knowledge, this is the first investigation concerning food-specific IgG antibody levels in CRSwNP. Since food IgG sensitization seems to be reduced compared to controls, it seems unlikely to be an important factor in nasal polyposis pathogenesis. Our results showed an association between CRSwNP and an immune suppression of IgG response against food allergens, and additional studies are needed to confirm and explain this phenomenon. The use of a randomly selected

group of patients with non-CRS from the general population as a control group would be of benefit in future investigations.

Our study has some limitations, namely a relatively small sample size from a single institution, the use of patients waiting for septoplasty as controls, and a high percentage of CRSwNP under inhalant steroids, which can bring together some bias to interpretation of immunological parameters. Nonetheless, patients were taking a combination of inhaled steroids with long-acting β_2-agonists that allows asthma control at lower corticosteroids doses, with negligible systemic side effects.[42]

In conclusion, our findings suggest that food allergy does not have an important role in CRSwNP etiopathogenesis, neither through IgE-mediated mechanisms nor through IgG-mediated hypersensitivity. Moreover, we observed a suppression in development of IgG sensitization against food allergens in patients with CRSwNP, which may be related to deviated IgG responses against other important targets (eg, airborne particles). Supporting this theory is the fact that an IgG1 subclass switching was observed in patients with CRSwNP and its values were negatively correlated to the sum of food-specific IgG concentration values.

Authors' Note

Rafaela Veloso-Teles contributed to study design, data collection, statistical analysis, discussion, and revision. Rui Cerejeira, Rosa Farinhaand, and Christian von Buchwald contributed to study design, discussion, and revision. Débora Rodrigues did data collection and revision. This study was performed according the principles of the Declaration of Helsinki. The work was performed at Cova da Beira Hospital Centre.

Acknowledgments

The authors thank Conceição Faria, MD, director of clinical pathology department of Cova da Beira Hospital Centre, for laboratory availability for this investigation.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Rafaela Veloso-Teles https://orcid.org/0000-0003-4319-6085

References

1. Fokkens WJ, Lund VJ, Mullol J, et al. European position paper on rhinosinusitis and nasal polyps 2012. *Rhinology*. 2012;50(supp 23):1-329.
2. Littman DR, Pamer EG. Role of the commensal microbiota in normal and pathogenic host immune responses. *Cell Host Microbe*. 2011;10(4):311-323.
3. Kewalramani A, Bollinger ME. The impact of food allergy on asthma. *J Asthma Allergy*. 2010;3:65-74.
4. Pang KA, Pang KP, Pang EB, Tan YN, Chan YH, Siow JK. Food allergy and allergic rhinitis in 435 asian patients – a descriptive review. *Med J Malaysia*. 2017;72(4):215-220.
5. Popescu F-D. Cross-reactivity between aeroallergens and food allergens. *World J Methodol*. 2015;5(2):31-50.
6. Cartier A. The role of inhalant food allergens in occupational asthma. *Curr Allergy Asthma Rep*. 2010;10(5):349-356.
7. Collins MM, Loughran S, Davidson P, Wilson JA. Nasal polyposis: prevalence of positive food and inhalant skin tests. *Otolaryngol Head Neck Surg*. 2006;135(5):680-683.
8. Pang YT, Eskici O, Wilson JA. Nasal polyposis: role of subclinical delayed food hypersensitivity. *Otolaryngol Head Neck Surg*. 2000;122(2):298-301.
9. Doğru H, Tüz M, Uygur K, Akkaya A, Yasan H. Asymptomatic IgE mediated food hypersensitivity in patients with nasal polyps. *Asian Pacific J allergy Immunol*. 2003;21(2):79-82.
10. Lill C, Loader B, Seemann R, et al. Milk allergy is frequent in patients with chronic sinusitis and nasal polyposis. *Am J Rhinol Allergy*. 2011;25(6):e221-e224.
11. Al-Qudah M. Food sensitization in medically resistant chronic rhinosinusitis with or without nasal polyposis. *Int Arch Allergy Immunol*. 2016;169(1):40-44.
12. van Hage M, Hamsten C, Valenta R. ImmunoCAP assays: pros and cons in allergology. *J Allergy Clin Immunol*. 2017;140(4):974-977.
13. Atkinson W, Sheldon TA, Shaath N, Whorwell PJ. Food elimination based on IgG antibodies in irritable bowel syndrome: a randomised controlled trial. *Gut*. 2004;53(10):1459-1464.
14. Karakuła-Juchnowicz H, Szachta P, Opolska A, et al. The role of IgG hypersensitivity in the pathogenesis and therapy of depressive disorders. *Nutr Neurosci*. 2017;20(2):110-118.
15. Wilders-Truschnig M, Mangge H, Lieners C, Gruber HJ, Mayer C, März W. IgG antibodies against food antigens are correlated with inflammation and intima media thickness in obese juveniles. *Exp Clin Endocrinol Diabetes*. 2008;116(4):241-245.
16. Li X, Atkinson MA. The role for gut permeability in the pathogenesis of type 1 diabetes – a solid or leaky concept? *Pediatr Diabetes*. 2015;16(7):485-492.
17. Panwar H, Rashmi HM, Batish VK, Grover S. Probiotics as potential biotherapeutics in the management of type 2 diabetes – prospects and perspectives. *Diabetes Metab Res Rev*. 2013;29(2):103-112.
18. Seo YS, Shah VH. The role of gut–liver axis in the pathogenesis of liver cirrhosis and portal hypertension. *Clin Mol Hepatol*. 2012;18(4):337.
19. Anders H-J, Andersen K, Stecher B. The intestinal microbiota, a leaky gut, and abnormal immunity in kidney disease. *Kidney Int*. 2013;83(6):1010-1016.
20. Ebner N, Földes G, Schomburg L, et al. Lipopolysaccharide responsiveness is an independent predictor of death in patients with chronic heart failure. *J Mol Cell Cardiol*. 2015;87:48-53.
21. Maes M, Kubera M, Leunis J-C, Berk M. Increased IgA and IgM responses against gut commensals in chronic depression: further evidence for increased bacterial translocation or leaky gut. *J Affect Disord*. 2012;141(1):55-62.

22. Walker J, Dieleman L, Mah D, Park K, Meddings J, Vethanayagam D. High prevalence of abnormal gastrointestinal permeability in moderate-severe asthma. *Clin Invest Med*. 2014;37(2):E53-E57.
23. Stapel SO, Asero R, Ballmer-Weber BK, et al. Testing for IgG4 against foods is not recommended as a diagnostic tool: EAACI task force report. *Allergy*. 2008;63(7):793-796.
24. Lopes C. Reprodutibilidade e validação do questionário semi-quantitativo de frequência alimentar. *Alimentação e enfarte agudo do miocárdio um Estudo caso-controlo de base comunitária [PhD]*. 2000.
25. Nesselroad TD, Hrabak TM. Concerns for food allergen intradermal skin testing in nasal polyposis patients. *Otolaryngol Head Neck Surg*. 2007;137(4):692-693.
26. Benninger MS, Daly T, Graffmiller K. Positivity rates of in vitro inhalant/respiratory and food allergy tests in the northern midwestern United States. *Ear, Nose Throat J*. 2018;97(9):296-322.
27. Alonso R, Botey J, Pena JM, Eseverri JL, Marín A, Ras RM. Specific IgE determination using the CAP system: comparative evaluation with RAST. *J Investig Allergol Clin Immunol*. 1995; 5(3):156-160.
28. Hox V, Delrue S, Scheers H, et al. Negative impact of occupational exposure on surgical outcome in patients with rhinosinusitis. *Allergy*. 2012;67(4):560-565.
29. Gao WX, Ou CQ, Fang SB, et al. Occupational and environmental risk factors for chronic rhinosinusitis in China: a multicentre cross-sectional study. *Respir Res*. 2016;17(1):54.
30. Veloso-teles R, Cerejeira R, Roque-farinha R, Von Buchwald C. Higher prevalence of nasal polyposis among textile workers: an endoscopic based and controlled study*. *Rhinology*. 2018;56(2): 99-105.
31. Veloso-Teles R, Cerejeira R. Endoscopic sinus surgery for chronic rhinosinusitis with nasal polyps: clinical outcome and predictive factors of recurrence. *Am J Rhinol Allergy*. 2017;31(1):56-62.
32. Hox V, Steelant B, Fokkens W, Nemery B, Hellings PW. Occupational upper airway disease: how work affects the nose. *Allergy Eur J Allergy Clin Immunol*. 2014;69(3):282-291.
33. Sastre J, Vandenplas O, Park H. Pathogenesis of occupational asthma. *Eur Respir J*. 2003;22(2):364-373.
34. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. *Front Immunol*. 2014;5:1-17.
35. Heidl S, Ellinger I, Niederberger V, Waltl EE, Fuchs R. Localization of the human neonatal Fc receptor (FcRn) in human nasal epithelium. *Protoplasma*. 2016;253(6):1557-1564.
36. Zhao C, Gao Y, Yu N, et al. Unidirectional transport of IgG by neonatal Fc receptor in human thyrocytes varies across different IgG subclasses. *Mol Cell Endocrinol*. 2018;477:103-111.
37. Stapleton NM, Andersen JT, Stemerding AM, et al. Competition for FcRn-mediated transport gives rise to short half-life of human IgG3 and offers therapeutic potential. *Nat Commun*. 2011;2(1):599.
38. Tian Z, Sutton BJ, Zhang X. Distribution of rat neonatal Fc receptor in the principal organs of neonatal and pubertal rats. *J Recept Signal Transduct*. 2014;34(2):137-142.
39. Williams JW, Tjota MY, Sperling AI. The contribution of allergen-specific IgG to the development of Th2-mediated airway inflammation. *J Allergy*. 2012. doi:org/10.1155/2012/236075.
40. Out TA, van de Graaf EA, van den Berg NJ, Jansen HM. IgG subclasses in bronchoalveolar lavage fluid from patients with asthma. *Scand J Immunol*. 1991;33(6):719-727.
41. Van Zele T, Gevaert P, Holtappels G, Van Cauwenberge P, Bachert C. Local immunoglobulin production in nasal polyposis is modulated by superantigens. *Clin Exp Allergy*. 2007;37(12): 1840-1847.
42. Barnes PJ. Inhaled corticosteroids. *Pharmaceuticals*. 2010;3(3): 514-540.

APPENDIX 4

Clinical Study

Ear, Nose & Throat Journal
1–8

DOI: 10.1177/0145561319893163
journals.sagepub.com/home/ear
SAGE

Systemic Immune Profile in Patients With CRSwNP

Rafaela Veloso-Teles, MD[1,2,3], Rui Cerejeira, MD, PhD[1,2,3], Rosa Roque-Farinha, MD, PhD[1], and Christian von Buchwald, MD, DMSci[4]

Abstract

The immune pathogenesis of chronic rhinosinusitis with nasal polyps (CRSwNP) remains obscure. Our aim was to compare humoral immunity and white blood cell counts in patients with CRSwNP and controls. A prospective case–control study was carried out in 37 patients with CRSwNP and 34 controls without CRS. Clinical data were gathered through a systematic interview. Computed tomography scan, skin prick test, spirometry, and immunological parameters (leukocyte differential count, immunoglobulin classes, and immunoglobulin [Ig] G subclasses) in serum specimens were obtained. Statistical analysis was performed using SPSS v.23. The prevalence of chronic lower respiratory diseases was greater in the CRSwNP group ($P < .001$), but atopic disease had no significant difference. A significantly higher eosinophil ($P < .001$) and basophil relative count ($P = .022$) and a lower relative neutrophil count ($P = .013$) were found among CRSwNP group. Patients with CRSwNP had higher IgG1 ($P = .022$), but lower IgG2 ($P = .014$) and IgG3 ($P = .018$) serum levels compared to controls; IgG4, total IgG, IgA, IgM, and IgE serum levels did not differ between groups, as well as the prevalence of immunoglobulin classes or IgG subclasses deficiency. The variation observed in peripheral relative leukocyte count and the systemic IgG1 subclass shift are similar to what is known to happen in nasal polyp tissue. A unique systemic immune profile seems to be present in patients with CRSwNP.

Keywords

nasal polyps, paranasal sinus diseases, sinusitis, immune system

Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a common clinical entity, but despite the high prevalence, morbidity, and chronicity, its etiopathogeny remains obscure.[1] Systemic immunological changes associated with CRSwNP may provide important clues to a better knowledge of the involved immune pathways.

Local immune modifications in nasal mucosa have been intensely investigated in CRSwNP. It is known that Western patients with CRSwNP show local tissue immune effects, such as skewing of the inflammatory response in a T helper cell type 2 direction, generation of local polyclonal immunoglobulin E (IgE) antibodies, promotion of eosinophil survival, and mast cell degranulation.[1] In polyps homogenates, it has already been demonstrated significantly higher values of immunoglobulin A (IgA), immunoglobulin G (IgG) and IgE, as well as a higher percentage of IgG class 1 (IgG1), when compared to nasal tissue samples from controls.[2] An immunofluorescent study in nasal polyps from 100 patients has shown a positive labeling for IgG in all specimens, for C3b complement fraction in 80% but negative immunofluorescency for immunoglobulin M (IgM) or IgA.[3]

However, data about systemic modification of immune system in CRSwNP are scarce, namely about immunoglobulin classes and subclasses. To our knowledge, the only controlled study to address IgG subclasses serum modifications in

[1] Faculty of Health Sciences, University of Beira Interior, Covilhã, Portugal
[2] Department of Otorhinolaryngology, Cova da Beira Hospital Centre (CHCB), Covilhã, Portugal
[3] Otorhinolaryngology Unit of Beira Interior (UOBI), Covilhã, Portugal
[4] Department of Otorhinolaryngology, Head and Neck Surgery and Audiology, Rigshospitalet-Copenhagen University Hospital, Copenhagen, Denmark

Received: July 19, 2019; revised: November 13, 2019; accepted: November 15, 2019

Corresponding Author:
Rafaela Veloso-Teles, MD, Departamento de Ciências Médicas, Faculdade de Ciências da Saúde, Universidade da Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal.
Email: rafaelateles84@gmail.com

CRSwNP included 15 patients and 10 controls, not finding any significant difference.[2]

Our main goal was to characterize systemic immunological alterations that occur in patients with CRSwNP compared to controls, namely in leukocyte differential count and humoral immune profile, based on immunoglobulin classes and subclasses dosage.

Materials and Methods

A prospective and observational case–control study was performed according to the established ethical guidelines and approval of Ethics Committee at the Cova da Beira Hospital Centre (deliberation number 82/2015). A signed informed consent was obtained from each participant in the study.

Sample

All the cases were about to undergo endoscopic sinus surgery for CRSwNP refractory to medical treatment (topical long-term and systemic short-term steroids), in a district hospital center, from January 2016 to October 2018. Diagnosis of CRSwNP was established using the definition of *EPOS 2012*.[1] The cases were selected consecutively from the waiting list and had their disease confirmed endoscopically, by computed tomography (CT) and histological examination of the subsequent surgical specimen. Patients were selected after applying the following exclusion criteria: concomitant benign or malignant sinonasal tumors, chronic rhinosinusitis without nasal polyps (CRSsNP), antrochoanal polyps, polyps associated with fungal rhinosinusitis, primary ciliary dysfunction, cystic fibrosis, acquired immunodeficiency (ie, human immunodeficiency virus; immunosuppressive drugs), autoimmune diseases (eg, systemic vasculitis), or history of or under allergen-specific immunotherapy. No course of systemic corticosteroids was given to patients with CRSwNP at least 3 months before serum specimens collection, and inhalant steroids were only prescribed if needed for asthma control and in combination with long-acting B2-agonists.

Controls were selected from patients in the waiting list for septoplasty, after excluding patients with symptoms and endoscopic or CT signs of chronic rhinosinusitis (CRS), with acquired immunodeficiency, autoimmune diseases, or with history of or under allergen-specific immunotherapy. All the cases and controls were adults (older than 18 years).

Data Collection

Clinical data were gathered through a systematic interview to collect information on demographics, occupational history, and comorbidities. Subjective assessment of upper and lower respiratory disease was obtained through Rhinosinusitis Quality of life survey—Portuguese version (RhinoQOL-pv)[4] and chronic obstructive pulmonary disease (*COPD*) *assessment test* (CAT).[5] The CAT test was chosen because it consists of nonspecific questions about lung disease impacts and has already been studied not only for COPD, but also for Asthma.[6] Nasal polyps were classified endoscopically according to Lund criteria, and Lund-Mackay imagiological score was obtained for each participant. These clinical, endoscopic and imagiological evaluations were done 3 months before surgery.

All patients and controls were submitted to skin prick test, while specific serum IgE antibodies for inhalants were requested as needed. Participants also performed a spirometry test, which is considered an essential assessment of patients with suspected chronic lower respiratory diseases (CLRD).[7] In case of abnormal test result or if patients presented lower respiratory symptoms, the participant was further evaluated by a respiratory physician and subsequent examinations were demanded as needed in order to establish a final diagnosis (eg, bronchial challenge, day-to-day peak expiratory flow variation, imagiological examination). Chronic lower respiratory diseases were classified according to *International Classification of Diseases*, *10th Revision*.[8] Patients with CRSwNP were also subdivided into CRSwNP with CLRD and CRSwNP without CLRD for data analysis. It was decided to classify according to the presence of CLRD and not only asthma, since there is growing evidence for the coexistence of CRS and CLRD other than asthma (eg, COPD).[9-12]

Blood sample was collected to obtain a leukogram, to determine the levels of immunoglobulin classes and IgG subclasses. Laboratory testing was performed 2 weeks before surgery at the same time of preoperative workout. Total white blood cell (WBC) count and 5-part leukocyte differentiation were determined automatically using DxH 800 (Beckman Coulter). The results of differential counts were presented and compared in relative numbers as traditionally done and currently performed in the literature (evidence exists that relative numbers are more accurate than absolute leukocyte differential counts[13]). Immunoglobulin classes were obtained by immunoturbidity assay using Cobas 6000 analyzer (RocheDiagnostics®, Mannheim, Germany) and levels of immunoglobulin subclasses were obtained through the Optilite turbidimetric analyser (The Binding Site®, Birmingham, United Kingdom). The reference range used for total IgG (700-1600 mg/dL), IgA (70-400 mg/dL), and IgM (40-230) was the one suggested by the International Federation of Clinical Chemistry.[14] Levels of immunoglobulin subclasses were considered to be low if IgG1 <405 mg/dL, IgG2 <169 mg/dL, IgG3 <11 mg/dL, or IgG4 <3 mg/dL according to the laboratory reference values.

Statistical Analysis

Statistical analysis was performed with *Statistical Package for Social Sciences* (IBM SPSS Statistics for Windows, Version 23.0). Descriptive statistics was used in sample characterization. Nonparametric tests were used for continuous variables since our data did not meet the assumptions needed for parametric tests, namely normal distribution of the dependent variable in each group and homogeneity of variances. Mann-Whitney test was used to compare continuous variables between CRSwNP and controls. A subanalysis with Kruskal-

Table 1. Characterization of Sample Demographics, Comorbidities, and Occupational Dust Exposure.[a]

	CRSwNP Group (n = 37)		Control Group (n = 34)		*P* Value
Demographics					
Age, years ± SD (range)	58 ± 2 (30-82)		54 ± 2 (30-79)		.060
	n	%	n	%	
Gender: male	18	48.6	20	58.8	.477
Comorbidities					
Allergic rhinitis	12	32.4	5	14.7	.100
Asthma					
– Atopic	8	21.6	2	5.9	.087
– Nonatopic	9	29.7	0	0	.002
COPD	8	21.6	1	2.9	.019
AERD	7	18.9	0	0	.008
Occupational dust exposure	31	83.8	19	55.9	.010

Abbreviations: AERD, aspirin exacerbated respiratory disease; COPD, chronic obstructive pulmonary disease; CRSwNP, chronic rhinosinusitis with nasal polyps; SD, standard deviation.
[a]N = 71.

Wallis test was performed to compare continuous variables between 3 groups (control group without CLRD, CRSwNP without CLRD, and CRSwNP with CLRD), and Dunn post hoc test was carried out for multiple pairwise comparisons. Chi-square test (or Fisher exact test/likelihood ratio test when needed) was used to test association between categorical data. A P value <.05 was considered as statistically significant.

Results

Seventy-one individuals completed the study: 37 patients with CRSwNP and 34 controls. Demographics, comorbidities, and occupational dust exposure of CRSwNP and controls are presented and compared in Table 1. All participants were Caucasians.

RhinoQOL-pv and CAT total mean scores were significantly higher among the CRSwNP group (60.4 ± 3.5 and 15.9 ± 1.7, respectively) versus the mean values for the control group (49.3 ± 2.7 and 7.3 ± 1.2; $P = .010$ for RhinoQOL-pv and $P = 1 \times 10^{-4}$ for CAT, Mann-Whitney test).

Twenty-five (67.6%) patients with CRSwNP presented concomitant CLRD (Table 1) which, compared with the control group (3 individuals, 8.8%), means a higher prevalence of pulmonary diseases ($P = 0.17 \times 10^{-7}$, Fisher exact test). Aspirin exacerbated respiratory disease (AERD) was more prevalent among CRSwNP group (Table 1), with 16.2% of CRSwNP presenting the Samter's triad (6 cases). In CRSwNP group, 21 (56.7%) of 37 were under treatment with inhaled steroids with β2-agonists; whereas in the control group, only 1 patient was being treated with inhaled steroids.

Twelve (32.4%) individuals from the 37 patients with CRSwNP and 5 (14.7%) individuals from the control group had atopic disease, without a statistically significant difference ($P = .100$, Fisher exact test).

Nasal polyps were classified endoscopically as grade I in 5 (13.5%) cases, grade II in 11 (29.7%) cases, and grade III in 21 (56.8%) cases, according to Lund criteria, and the mean Lund-Mackay imagiological score was 15.22 ± 0.78. All nasal specimens of patients with CRSwNP showed tissue eosinophilia (>10/hpf).

The analyzed systemic immunological parameters are presented and compared in Table 2. In respect to leukogram, patients with CRSwNP had significant lower levels of relative neutrophil count but higher values of relative eosinophil and basophil counts. Concerning humoral immunity, CRSwNP showed an IgG1 subclass switching, with reduced levels of IgG2 and IgG3. No significant differences were observed for total IgG, IgA, IgM, and IgE serum levels.

In addition, we tried to find out if patients with AERD diverge from the remaining patients in the CRSwNP group but no significant differences about these immunological variables were detected. When we exclude these 6 patients with AERD from the CRSwNP group, we still found a significant difference concerning higher eosinophil and basophil relative count ($P = 5.41 \times 10^{-9}$ and $P = .020$), higher IgG1, and lower IgG3 concentration values ($P = .048$ and $P = .044$), between CRSwNP without AERD and control groups.

A Kruskal-Wallis test (N = 68) was also run for these continuous variables considering 3 different groups: control group without CLRD, CRSwNP without CLRD, and CRSwNP with CLRD. Patients from the control group with CLRD (n = 3) were not included in this subanalysis because of their limited number. Neutrophil ($P = .031$) and eosinophil relative count ($P = 1.5 \times 10^{-7}$), and IgG1 ($P = .027$), IgG2 ($P = .015$), and IgG3 ($P = .028$) differed significantly between the groups. For relative eosinophil count and IgG1 concentration levels, there was a crescendo trend in their mean values between control group without CLRD, CRSwNP without CLRD, and CRSwNP with CLRD; and an inverse pattern (decrescendo) was observed for relative neutrophil count and IgG2 and IgG3 (Figures 1 and 2).

Relating to humoral immunodeficiency (Table 3), we did not find a higher prevalence of immunoglobulin classes (5 cases, 13.5%) or subclasses deficiency (7 cases, 18.9%) compared to our control group (3 [8.8%] and 7 [20.6%] cases, respectively). No cases of common variable immunodeficiency were diagnosed in the entire sample. One (2.9%) case among CRSwNP and 2 (5.4%) cases among controls had an IgAGMD plus IgG1-3 deficiency (combination of selective classes [IgA, IgG, or IgM] plus IgG subclasses deficiency).

Discussion

Our case and control groups had no significant differences concerning baseline demographic features, such as race, gender, or age.

Comparing comorbidities (Table 1), the prevalence of allergic rhinitis and atopic asthma did not differ significantly

Table 2. Comparison of Systemic Immunological Parameters.[a]

Parameters	CRSwNP Group (n = 37)	Control Group (n = 34)	*P* value
WBC ($10^3/\mu L$)	7.24 ± 0.31	7.31 ± 0.39	.717
Neutrophils (%)	51.26 ± 1.44	56.00 ± 1.41	.013
Absolute count ($10^3/\mu L$)	3.71 ± 0.19	4.18 ± 0.12	
Lymphocytes (%)	33.44 ± 1.58	32.92 ± 1.30	.872
Absolute count ($10^3/\mu L$)	2.47 ± 0.14	2.37 ± 0.12	
Monocytes (%)	7.96 ± 0.30	7.71 ± 0.27	.378
Absolute count ($10^3/\mu L$)	0.57 ± 0.03	0.55 ± 0.03	
Eosinophils (%)	5.67 ± 0.47	2.29 ± 0.20	3.56×10^{-9}
Absolute count ($10^3/\mu L$)	0.40 ± 0.04	0.17 ± 0.02	
Basophils (%)	0.81 ± 0.05	0.65 ± 0.05	.022
Absolute count ($10^3/\mu L$)	0.06 ± 0.01	0.04 ± 0.01	
IgE (kU/L)	284.97 ± 92.8	173.12 ± 53.6	.079
IgG (mg/dL)	995.05 ± 38.27	931 ± 32.97	.451
IgG1	578.35 ± 25.32	502.20 ± 26.12	.022
IgG2	349.40 ± 18.55	403.88 ± 20.9	.014
IgG3	63.28 ± 8.13	68.97 ± 4.00	.018
IgG4	65.89 ± 10.56	44.70 ± 5.74	.272
IgM (mg/dL)	99.75 ± 9.54	97.58 ± 8.63	.608
IgA (mg/dL)	254.72 ± 17.90	218.15 ± 16.43	.105

Abbreviations: CRSwNP, chronic rhinosinusitis with nasal polyps; Ig, immunoglobulin; SEM, standard error of mean; WBC, white blood cell.
[a]N = 71. Data expressed as mean ± SEM. Comparison using Mann-Whitney test.

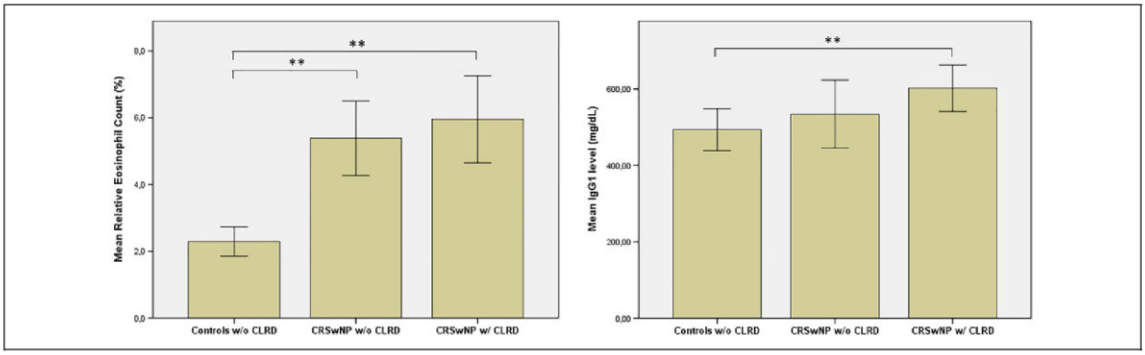


Figure 1. Mean relative eosinophil count (%) and serum concentration levels of IgG1 in controls without chronic lower respiratory diseases (controls w/o CLRD), in patients with CRSwNP without CLRD (CRSwNP w/o CLRD), and in patients with CRSwNP with CLRD (CRSwNP w/ CLRD). N = 68. Significance levels are marked as "*" if $P < .05$ and "**" if $P < .01$. Kruskal-Wallis test ($P < .001$ and $P = .037$, respectively) was performed and pairwise comparisons were made using Dunn post hoc test. CLRD indicates chronic lower respiratory diseases; CRSwNP, chronic rhinosinusitis with nasal polyps; IgG1, immunoglobulin G class 1; w/, with; w/o, without.

between cases and controls. In addition, the prevalence of atopy was 32% in the CRSwNP group, which is close to the range values of the reported prevalence of atopy in the general population (20%-30%).[15] These results corroborate previous studies[16-18] that support the involvement of non–IgE-related mechanisms on CRSwNP etiopathogenesis.

On the contrary, there was a statistically significant higher prevalence of nonatopic asthma and COPD (both classified as CLRD) among patients with CRSwNP compared with controls. In total, 67.6% of patients with CRSwNP had a concomitant diagnose of CLRD, and this value is in accordance with a recent study which reported a CLRD prevalence (asthma plus COPD) of 72.5%.[19] However, the COPD prevalence reported on that study (7.5%) was lower than the 21.6% found in our study. The association between CRSwNP and asthma is well established, but the association of CRS and COPD despite being suggested has been much less investigated.[11] In 2011, a study in 90 patients with COPD demonstrated that 53% had concomitant symptoms of rhinosinusitis and 64% had signs of CRS in CT scan.[20] A study made in Norway in 2015 (the HUNT study-MRI: The Nord-Trøndelag Health Study with magnetic resonance imaging), with patients with COPD, asthma, and controls submitted to MRI, showed that the probability of paranasal sinus opacification was 6 times higher in

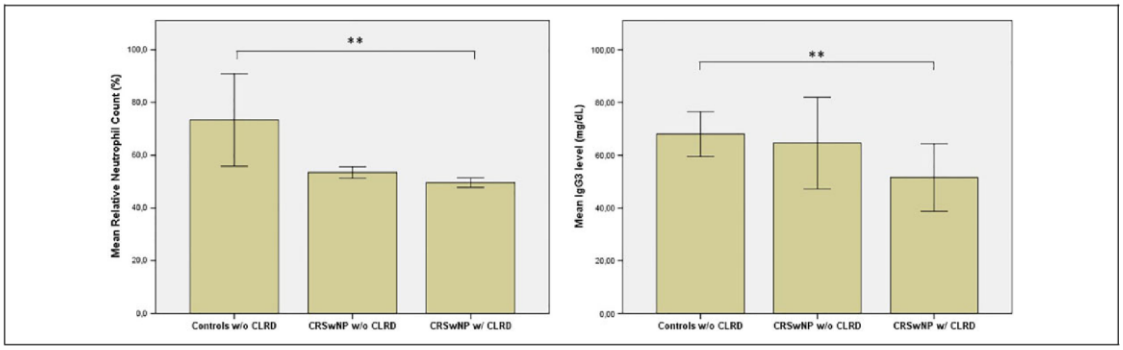


Figure 2. Mean relative neutrophil count (%) and serum concentration levels of IgG3 in controls without chronic lower respiratory diseases (controls w/o CLRD) and in patients with CRSwNP without CLRD (CRSwNP w/o CLRD). N = 68. Significance levels are marked as "*" if $P < .05$ and "**" if $P < .01$. Kruskal-Wallis test was performed ($P = .031$ and $P = .018$, respectively) and pairwise comparisons were made using Dunn post hoc test. CLRD indicates chronic lower respiratory diseases; CRSwNP, chronic rhinosinusitis with nasal polyps; IgG3, immunoglobulin G class 3; w/o, without.

Table 3. Prevalence of Immunoglobulin Classes and Subclasses Deficiency.[a]

	CRSwNP Group (n = 37)	Control Group (n = 34)	*P* Value
Igs classes deficiency	n (%)	n (%)	
IgG	2 (5.4)	2 (5.9)	1.000
IgA	1 (2.7)	0	1.000
IgM	3 (8.1)	2 (5.9)	1.000
IgG subclasses deficiency			
IgG1	6 (16.2)	7 (20.6)	.762
IgG2	1 (2.7)	2 (5.9)	.604
IgG3	1 (2.7)	0	1.000
IgG4	1 (2.7)	0	1.000
Multiple Igs deficiency	2 (5.4)	1, (2.9)	1.000
Total patients with Igs deficiency (classes or subclasses)	11 (29.7)	9, (26.5)	.797

Abbreviations: CRSwNP, chronic rhinosinusitis with nasal polyps; Igs, immunoglobulins; IgA, immunoglobulin A; IgG, immunoglobulin G.
[a]N = 71. Comparison using chi-square test (or Fisher exact test).

COPD and 2-fold higher in patients with asthma compared to the control group.[21] In addition, there are clinical research studies showing a high prevalence of sinonasal symptoms in patients with COPD (75%)[22] and an inverse correlation between nasal patency evaluated by rhinomanometry and pulmonary airflow obstruction (FEV1%), and therefore to COPD disease severity.[9] The true prevalence of CRSwNP among patients with COPD needs future investigation using endoscopic-based approaches. Our study took place in Castelo Branco District, within the Interior Centre Region of Portugal, an area internationally known for its textile industry, mainly wool manufacturing, potentially explaining the high prevalence of COPD in our CRSwNP sample. Another important point is that asthma and COPD may be difficult to distinguish, especially in patients with history of tobacco consumption or exposure to noxius particles or gases, and in the elderly individuals, who often have overlapping clinical features of both diseases.[23]

=In Western patients, it has been suggested that CRSsNP is more distinctly a neutrophilic process, while CRSwNP is more eosinophilic, based on the relative degree of nasal tissue infiltration.[1] It was interesting to observe that in CRSwNP the peripheral relative counts of eosinophils and neutrophils had the same behavior to what happens locally. There was a significant reduction in the relative neutrophil count among CRSwNP, with a decrescendo tendency if comorbid CLRD was present and the opposite occurred with the eosinophil count.

Similarly to other published study,[2] concentration values of serum IgG, IgA, IgM, and IgE did not show a significant difference between CRSwNP and controls. But contrary to that study, where no significant difference about serum IgG subclasses concentration values was observed,[2] in our study we found higher IgG1, but lower IgG2 and IgG3 serum levels in CRSwNP compared to controls, and these variations were more pronounced if CLRD were present. In patients with asthma, it has already been studied that IgG subclasses in bronchoalveolar lavage (BAL) and epithelial lining fluid are significantly higher than in controls, mainly due to increased leakage from blood, with IgG1 quotient between BAL fluid and serum concentrations having the highest value and IgG3 the lowest.[24] Similar studies should be done in CRSwNP, in nasal lavage fluid and tissue homogenates, to shed light in this subject. Meanwhile, an immunofluorescent study in nasal polyps from 100 patients has already shown a positive labelling for IgG in all specimens, for C3b complement fraction in 80% and negative immunofluorescency for IgM or IgA3. Van Zele et al demonstrated that nasal tissue homogenates showed significantly higher concentrations by immunonephelometry of IgG, IgE, and IgA in CRSwNP and also found by enzyme-linked immunosorbent assay that the percentage of IgG1 subclass was significantly higher among tissue homogenates in CRSwNP compared to controls.[2] A recent study in patients with

CRSwNP and AERS also pointed to the importance of local IgG1 against staphylococcus enterotoxins (SEE), showing that this antibody can enhance the activity of anti-SEE IgEs as conventional antibodies or as "superantibodies" through complementary determining regions and framework regions to SEEs in SEE-anti-SEE IgE-FcεRI complexes.[25]

We hypothesize that systemic IgG1 subclass switching is involved in CRSwNP pathogenesis and may be an important link to lower airway diseases, as there is a progression toward increasing mean plasmatic values in those patients. This is interesting, since each IgG subclass has a unique profile, for example, soluble protein antigens and membrane proteins primarily induce IgG1 switching.[26] This subclass is also important in antibody response to allergens, has the longest serum half-life, is capable of complement activation by C1q binding, and is the main IgG subclass to cross placenta and mucosal barrier.[26]

Moreover, there are previous studies showing that IgG1 has a strong binding affinity to the neonatal Fc receptor (FcRn) compared to the other subclasses.[27,28] This receptor was originally identified in suckling rats as the receptor involved in IgG transport across the intestinal epithelium into the bloodstream but has now been demonstrated to be expressed in many adult tissues and cell types,[29] with predominant expression in respiratory system.[30] Recently, this receptor has also been identified in human nasal epithelium.[31] Apart from regulating and extending the serum half-life of IgG, FcRn orchestrates IgG-based immune responses at mucosal sites, contributing to immunosurveillance at host-environment interfaces within the adult organism.[32]

In addition, in vitro studies with serum from patients with asthma showed that antigen-specific IgG1 and IgG3 antibodies can induce eosinophil degranulation, and that IgG-depleted serum but not IgE-depleted serum abolished this degranulation.[33] A possible link between IgG1 and tissue and peripheral eosinophilia has to be addressed in future investigations, since inflammation seen in CRSwNP can be reliant on IgG-dependent eosinophil-mediated cytotoxicity.

The higher rate of occupational dust exposure among patients with CRSwNP compared to controls corroborates the important role of this factor in the etiopathogenesis of CRSwNP and CLRD, as already suggested by previous studies.[17,34] Evidence points to a risk factor for CRS that is mainly due to the inhalation of lower molecular weight (LMW) particles (<5 kDa) which, contrary to high-molecular weight) particles that induce a well-known IgE-mediated immune response, induce airway inflammation through mechanisms that are far less known.[16,17] It can include the classical "irritant response" plus LMW sensitization of the adaptive immune system by acting as haptens (attached to large carriers such as airway proteins).[16,35,36] An investigation about sensitization to methylene diphenyl diisocyanate (MDI), an LMW particle, in a car upholstery factory found that the prevalence of MDI-induced occupational asthma/eosinophilic bronchitis was strongly associated with the presence of serum-specific IgG antibodies to an MDI–human serum albumin conjugate.[37]

All these recent findings seem relevant to understand the systemic immune profile results that we found. It will be necessary future studies to compare FcRn expression among CRSwNP and controls and its relationship with IgG1 subclass switching in this disease. Following the concept of "one airway, one disease," this IgG1-mediated immune response in patients with CRSwNP may be a key piece in understanding its interrelation with CLRD, namely nonatopic asthma and COPD and to clarify the role of occupational dust exposure, specifically LMW particles, in their etiopathogenesis.

Our prevalence results of IgG subclasses (18.9%) and immunoglobulin classes (13.5%) deficiency in CRSwNP are similar to the previous reported values in the literature for CRS (5% to 50% for IgG subclasses deficiency and 13% for IgG, IgA, or IgM antibody deficiency).[38] However, since no significant difference was detected between our CRSwNP and control groups (8.8% and 20.6%, respectively), it seems unlikely that immunoglobulin class and subclass deficiencies are important factors in CRSwNP etiopathogenesis. Larger prospective and controlled studies about this topic are needed.

Our study has some limitations: a limited sample size from a single institution, the use of patients waiting for septoplasty as controls, and a high percentage of CRSwNP under inhalant steroids. Nonetheless, this is to our knowledge the largest controlled study to analyze IgG subclasses. To surpass the potential bias effect caused by corticosteroids influence on WBC count, systemic steroids were not prescribed within 3 months before serum specimen's collection. However, patients who needed steroids plus long-acting β2-agonists for their asthma control were permitted to keep their long-term medication. These inhalant associations are considered to have negligible systemic side effects.[39] Although, if we consider a potential effect of steroids intake on our hematological findings, it would be in the opposite way of our observed results (higher total leukocyte count,[40] higher relative neutrophil count,[41] lower relative eosinophil[42] and lymphocyte count, lower levels of total IgG/IgA/IgE and IgG1 and IgG2 subclass deficiencies[43]). This means that our observed differences in immunological profile might have been even more pronounced if patients with CRSwNP were not taking inhalant corticosteroids. Moreover, immunological differences found between CRSwNP and patients submitted to septoplasty would be probably even bigger if we used a randomly selected group of non-CRS individuals from the general population, as it is known that patients undergoing septoplasty also have significant nasal symptoms, in association with some degree of nasal inflammation. This investigation deserves further replication in larger samples and with patients from other institutions. In a bigger sample, a multivariate regression analysis would be of added value to identify the variables that have an independent correlation with the disease, allowing at the same time to control the effect of multicollinearity and outliers.

Conclusion

A distinct systemic immunologic profile in patients with CRSwNP concerning leukogram and humoral immunity was observed. Changes in peripheral leukocyte count and the

systemic IgG1 subclass shift are similar to what is described to happen in nasal polyp tissue. The observed differences were more marked if CLRD were present. These variations may be involved in CRSwNP pathogenesis and a possible role for IgG1-mediated response must be investigated.

Authors' Note

Rafaela Veloso-Teles contributed to study design, data collection, statistical analysis, discussion, and revision. Rui Cerejeira, Rosa Roque-Farinha, and Christian von Buchwald contributed to study design, discussion, and revision. This study was performed according to the principles of the Declaration of Helsinki Institution at which the work was performed: Cova da Beira Hospital Centre.

Acknowledgments

The authors thank Professor Søren Jacobsen, M.D., D.M.Sci, from Department of Rheumatology at Rigshospitalet (Copenhagen, Denmark) for is valuable comments and suggestions.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

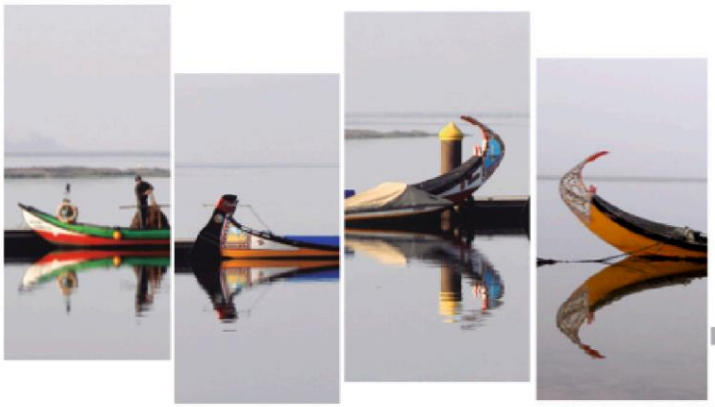
Rafaela Veloso-Teles https://orcid.org/0000-0003-4319-6085

References

1. Fokkens WJ, Lund VJ, Mullol J, et al. European position paper on rhinosinusitis and nasal polyps 2012. *Rhinology*. 2012;50(suppl 23):1-329.
2. Van Zele T, Gevaert P, Holtappels G, Van Cauwenberge P, Bachert C. Local immunoglobulin production in nasal polyposis is modulated by superantigens. *Clin Exp Allergy*. 2007;37(12): 1840-1847. doi: 10.1111/j.1365-2222.2007.02838.x.
3. Zivic M, Antic M. Immunofluorescent study of polyps in nose and paranasal cavities. *Bratislava Med J*. 2009;110(3):181-184.
4. Cerejeira R, Veloso-Teles R, Lousan N, Moura CP. The Portuguese version of the RhinoQOL Questionnaire: validation and clinical application. *Braz J Otorhinolaryngol*. 2015;81(6): 630-635. doi: 10.1016/j.bjorl.2015.08.015.
5. CAT Development Steering Group. https://www.catestonline.org/patient-site-portuguese_portugal.html. Accessed November 2, 2015.
6. Kurashima K, Takaku Y, Ohta C, Takayanagi N, Yanagisawa T, Sugita Y. COPD assessment test and severity of airflow limitation in patients with asthma, COPD, and asthma–COPD overlap syndrome. *Int J COPD*. 2016;11(1):479-487. doi: 10.2147/COPD.S97343.
7. Global Initiative for Asthma. *Glob Strateg Asthma Manag Prev*. 2016. Available from: www.ginasthma.org. Accessed May 1, 2016.
8. WHO (World Health Organization). *ICD-10 Version:2016*. https://icd.who.int/browse10/2016/en. Published 2016. Accessed October 1, 2017.
9. Hurst JR, Kuchai R, Michael P, Perera WR, Wilkinson TMA, Wedzicha JA. Nasal symptoms, airway obstruction and disease severity in chronic obstructive pulmonary disease. *Clin Physiol Funct Imaging*. 2006;26(4):251-256.
10. Hurst JR.Upper airway. 3: sinonasal involvement in chronic obstructive pulmonary disease. *Thorax*. 2010;65(1):85-90. doi: 10.1136/thx.2008.112888.
11. Kelemence A, Abadoglu O, Gumus C, Berk S, Epozturk K, Akkurt I. The frequency of chronic rhinosinusitis/nasal polyp in COPD and its effect on the severity of COPD. *COPD*. 2011;8(1): 8-12. doi: 10.3109/15412555.2010.540272.
12. Kim HY, So YK, Dhong HJ, et al. Prevalence of lower airway diseases in patients with chronic rhinosinusitis. *Acta Otolaryngol Suppl*. 2007;(558):110-114. doi: 10.1080/0365523070 1624988.
13. Arneth B.Complete blood count: absolute or relative values? *J Hematol*. 2016;5(2):49-53. doi: 10.14740/jh265w.
14. Dati F, Schumann G, Thomas L, et al. Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP Reference Material (CRM 470). *Eur J Clin Chem Clin Biochem*. 1996; 34(6):517-520.
15. Pawankar R, Canonica GW, Holgate ST, Lockey RF. *White Book on Allergy*. Milwaukee, WI: World Allergy Organization (WAO); 2011.
16. Veloso-Teles R, Cerejeira R. Endoscopic sinus surgery for chronic rhinosinusitis with nasal polyps: clinical outcome and predictive factors of recurrence. *Am J Rhinol Allergy*. 2017; 31(1):56-62. doi: 10.2500/ajra.2017.31.4402.
17. Veloso-teles R, Cerejeira R, Roque-farinha R, Buchwald CV. Higher prevalence of nasal polyposis among textile workers: an endoscopic based and controlled study*. *Rhinology*. 2018;56(2): 99-105.
18. Grigoreas C, Vourdas D, Petalas K, Simeonidis G, Demeroutis I, Tsioulos T. Nasal polyps in patients with Rhinitis and Asthma. *Allergy Asthma Proc*. 2002;23(3):169-174.
19. Håkansson K, Thomsen SF, Konge L, Mortensen J, Backer V, von Buchwald C. A comparative and descriptive study of asthma in chronic rhinosinusitis with nasal polyps. *Am J Rhinol Allergy*. 2014;28(5):383-387. doi: 10.2500/ajra.2014.28.4076.
20. Hedman J, Kaprio J, Poussa T, Nieminen MM. Prevalence of asthma, aspirin intolerance, nasal polyposis and chronic obstructive pulmonary disease in a population-based study. *Int J Epidemiol*. 1999;28(4):717-722.
21. Hansen AG, Helvik AS, Thorstensen WM, et al. Paranasal sinus opacification at MRI in lower airway disease (the HUNT study-MRI). *Eur Arch Otorhinolaryngol*. 2016;273(7): 1761-1768. doi: 10.1007/s00405-015-3790-7.
22. Kim JS, Rubin BK. Nasal and sinus inflammation in chronic obstructive pulmonary disease. *COPD*. 2007;4(2):163-166. doi: 10.1080/15412550701341228.

23. van den Berge M, Aalbers R. The asthma-COPD overlap syndrome: how is it defined and what are its clinical implications? *J Asthma Allergy*. 2016;9:27-35. doi: 10.2147/JAA.S78900.
24. Out TA, van de Graaf EA, van den Berg NJ, Jansen HM. IgG subclasses in bronchoalveolar lavage fluid from patients with asthma. *Scand J Immunol*. 1991;33:719-727.
25. Chen JB, James LK, Davies AM, et al. Antibodies and superantibodies in patients with chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol*. 2017;139(4):1195-1204.e11. doi: 10.1016/j.jaci.2016.06.066.
26. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. *Front Immunol*. 2014; 5(OCT):1-17.
27. Zhao C, Gao Y, Yu N, et al. Unidirectional transport of IgG by neonatal Fc receptor in human thyrocytes varies across different IgG subclasses. *Mol Cell Endocrinol*. 2018;477:103-111. doi: 10.1016/j.mce.2018.06.006.
28. Stapleton NM, Andersen JT, Stemerding AM, et al. Competition for FcRn-mediated transport gives rise to short half-life of human IgG3 and offers therapeutic potential. *Nat Commun*. 2011;2(1): 599. doi: 10.1038/ncomms1608.
29. Yoshida M, Masuda A, Kuo TT, et al. IgG transport across mucosal barriers by neonatal Fc receptor for IgG and mucosal immunity. *Springer Semin Immunopathol*. 2006;28(4):397-403. doi: 10.1007/s00281-006-0054-z.
30. Tian Z, Sutton BJ, Zhang X. Distribution of rat neonatal Fc receptor in the principal organs of neonatal and pubertal rats. *J Recept Signal Transduct*. 2014;34(2):137-142. doi: 10.3109/10799893.2013.865745.
31. Heidl S, Ellinger I, Niederberger V, Waltl EE, Fuchs R. Localization of the human neonatal Fc receptor (FcRn) in human nasal epithelium. *Protoplasma*. 2016;253(6):1557-1564. doi: 10.1007/s00709-015-0918-y.
32. Rath T, Baker K, Pyzik M, Blumberg RS. Regulation of immune responses by the neonatal Fc receptor and its therapeutic implications. *Front Immunol*. 2014;5(DEC):22-29. doi: 10.3389/fimmu.2014.00664.
33. Kaneko M, Swanson MC, Gleich GJ, Kita H. Allergen-specific IgG1 and IgG3 through FcγRII induce eosinophil degranulation. *J Clin Invest*. 1995;95(6):2813-2821. doi: 10.1172/JCI117986.
34. Nagoda M, Okpapi JU, Babashani M. Assessment of respiratory symptoms and lung function among textile workers at Kano Textile Mills, Kano, Nigeria. *Nigerian J of Clinical Practice*. 2012; 15(4):373-379.
35. Hox V, Steelant B, Fokkens W, Nemery B, Hellings PW. Occupational upper airwaydisease: how work affects the nose. *Allergy Eur J Allergy Clin Immunol*. 2014;69(3):282-291. doi: 10.1111/all.12347.
36. Hox V, Delrue S, Scheers H, et al. Negative impact of occupational exposure on surgical outcome in patients with rhinosinusitis. *Allergy*. 2012;67(4):560-565. doi: 10.1111/j.1398-9995.2011.02779.x.
37. Hur GY, Koh DH, Choi GS, et al. Clinical and immunologic findings of methylene diphenyl diisocyanate-induced occupational asthma in a car upholstery factory. *Clin Exp Allergy*. 2008;38(4):586-593. doi: 10.1111/j.1365-2222.2008.02935.x.
38. Schwitzguébel AJP, Jandus P, Lacroix J-S, Seebach JD, Harr T. Immunoglobulin deficiency in patients with chronic rhinosinusitis: Systematic review of the literature and meta-analysis. *J Allergy Clin Immunol*. 2015;136(6):1523-1531. doi: 10.1016/j.jaci.2015.07.016.
39. Barnes PJ. Inhaled Corticosteroids. *Pharmaceuticals*. 2010;3(3): 514-540. doi: 10.3390/ph3030514.
40. Fay ME, Myers DR, Kumar A, et al. Cellular softening mediates leukocyte demargination and trafficking, thereby increasing clinical blood counts. *Proc Natl Acad Sci U S A*. 2016;113(8): 1987-1992. doi: 10.1073/pnas.1508920113.
41. Busti J. *A General Review of the Mechanisms for Steroid or Glucocorticoid Induced Increases in the White Blood Cell (WBC) Count*. https://www.ebmconsult.com/articles/steroids-glucocorticoids-wbc-neutrophiles-increase. Published 2015.
42. Ortega H, Llanos JP, Lafeuille MH, et al. Effects of systemic corticosteroids on blood eosinophil counts in asthma: real-world data. *J Asthma*. 2019;56(8):808-815. doi: 10.1080/02770903.2018.1502301.
43. Klaustermeyer WB, Gianos ME, Kurohara ML, Dao HT, Heiner DC. IgG subclass deficiency associated with corticosteroids in obstructive lung disease. *Chest*. 1992;102(4):1137-1142. doi: 10.1378/chest.102.4.1137.

APPENDIX 5



65º CONGRESSO
da Sociedade Portuguesa
de Otorrinolaringologia
e Cirurgia Cérvico-Facial

4-6
maio 2018
CENTRO DE CONGRESSOS DE AVEIRO

CERTIFICADO

Certifica-se que, **Rafaela da Cruz Vieira Veloso Teles**
participou no 65º Congresso Nacional da Sociedade Portuguesa de Otorrinolaringologia e Cirurgia Cérvico-Facial, que se realizou de 4 a 6 de Maio de 2018 no Centro de Congressos de Aveiro, como Palestrante na Conferência "***Epidemiologia da RSCcPN em Portugal: Estudos baseados na Endoscopia*** ".

SPORL
Sociedade Portuguesa de Otorrinolaringologia e Cirurgia Cérvico-Facial

Ezequiel Barros
Presidente da Sociedade Portuguesa de Otorrinolaringologia e Cirurgia Cérvico-Facial

APPENDIX 6

5TH CONGRESS OF EUROPEAN ORL-HNS
BRUSSELS, JUNE 29 – JULY 3, 2019
www.ceorlhns2019.org

CERTIFICATE OF ORAL PRESENTATION

This is to certify that

Rafaela Veloso-Teles

Presented the following Oral abstract

Food specific IgE and IgG-antibodies levels in patients with chronic rhinosinusitis with nasal polyps: a case-control study

at

5th Congress of European ORL-HNS 2019

from June 29 – July 3, 2019 in Brussels, Belgium

Marc Remacle
Congress President

CONFEDERATION OF EUROPEAN ORL-HNS

Elisabeth Sjogren
Scientific Chair

APPENDIX 7

5TH CONGRESS OF EUROPEAN ORL-HNS
BRUSSELS, JUNE 29 – JULY 3, 2019
www.ceorlhns2019.org

CERTIFICATE OF E-POSTER PRESENTATION

This is to certify that

Rafaela Veloso-Teles

Presented the following E-Poster abstract

Systemic immune profile in patients with chronic rhinosinusitis with nasal polyps: a case-control study

at

5th Congress of European ORL-HNS 2019

from June 29 – July 3, 2019 in Brussels, Belgium

Marc Remacle
Congress President

CONFEDERATION OF EUROPEAN ORL-HNS

Elisabeth Sjogren
Scientific Chair

APPENDIX 8

European Rhinologic Society

THE EUROPEAN RHINOLOGIC SOCIETY
awards the
Clinical Research Prize
to

Rafaela Veloso-Teles

Higher prevalence of Nasal Polyposis among textile workers:
an endoscopic based and controlled study

ERS2018, London, United Kingdom

Par Stjarne
President

Wytske Fokkens
General Secretary

Sean Carry
President-Elect

APPENDIX 9

RHINOQOL (RHINOSINUSITIS QUALITY OF LIFE SURVEY) – VERSÃO PORTUGUESA

Responda às seguintes questões marcando uma cruz (x) na resposta mais adequada para cada pergunta.

	Nunca	Pouco tempo	Algum tempo	Muito tempo	Sempre
1. Nos últimos 7 dias, durante quanto tempo sentiu **dor de cabeça**, **dor na face** ou **pressão na face**?					
1A. Usando uma escala de 0 a 10, em que 0 significa "nada incomodado" e 10 significa "muito incomodado", que valor atribuiria ao incómodo causado pelas dores de cabeça, dor na face ou pressão na face?	☐ 0	☐ 1 ☐ 2 ☐ 3	☐ 4 ☐ 5 ☐ 6	☐ 7 ☐ 8 ☐ 9	☐ 10
2. Nos últimos 7 dias, durante quanto tempo sentiu ter o **nariz tapado ou congestionado**?					
2A. Usando uma escala de 0 a 10, em que 0 significa "nada incomodado" e 10 significa "muito incomodado", que valor atribuiria ao incómodo causado por ter o nariz tapado ou congestionado?	☐ 0	☐ 1 ☐ 2 ☐ 3	☐ 4 ☐ 5 ☐ 6	☐ 7 ☐ 8 ☐ 9	☐ 10
3. Nos últimos 7 dias, durante quanto tempo sentiu **escorrência por detrás do nariz**?					
3A. Usando uma escala de 0 a 10, em que 0 significa "nada incomodado" e 10 significa "muito incomodado", que valor atribuiria ao incómodo causado pela escorrência por detrás do nariz?	☐ 0	☐ 1 ☐ 2 ☐ 3	☐ 4 ☐ 5 ☐ 6	☐ 7 ☐ 8 ☐ 9	☐ 10
4. Nos últimos 7 dias, durante quanto tempo teve saída de **secreções nasais espessas**?					
5. Nos últimos 7 dias, durante quanto tempo sentiu o **nariz a pingar**?					
Para responder às próximas perguntas, por favor considere todos os sintomas nasais que tem sentido recentemente.					
6. Nos últimos 7 dias, durante quanto tempo se sentiu **cansado(a)** *por causa dos seus sintomas nasais*?					
7. Nos últimos 7 dias, durante quanto tempo sentiu **dificuldade em dormir** *por causa dos seus sintomas nasais*?					
8. Nos últimos 7 dias, durante quanto tempo sentiu **mais dificuldade em se concentrar** *por causa dos seus sintomas nasais*?					
9. Nos últimos 7 dias, durante quanto tempo sentiu **mais dificuldade em fazer as coisas que normalmente faz** *por causa dos seus sintomas nasais*?					
10. Nos últimos 7 dias, durante quanto tempo se sentiu **embaraçado(a)** *por causa dos seus sintomas nasais*?					
11. Nos últimos 7 dias, durante quanto tempo se sentiu **frustrado(a)** *por causa dos seus sintomas nasais*?					
12. Nos últimos 7 dias, durante quanto tempo se sentiu **irritável** *por causa dos seus sintomas nasais*?					
13. Nos últimos 7 dias, durante quanto tempo se sentiu **triste ou deprimido(a)** *por causa dos seus sintomas nasais*?					
14. Nos últimos 7 dias, durante quanto tempo **pensou acerca** dos seus sintomas nasais?					

In Cerejeira R, Veloso-Teles R, Lousan N, Moura CP. The Portuguese version of the RhinoQOL Questionnaire: validation and clinical application. *Braz J Otorhinolaryngol*. 2015;81(6):630-635.

APPENDIX 10

O seu nome:_____

Data de hoje:_____

Como está a sua DPOC (Doença Pulmonar Obstrutiva Crónica)? Faça o Teste de Avaliação da DPOC (COPD Assessment Test™– CAT)

Este questionário irá ajudá-lo a si e ao seu profissional de saúde a medir o impacto que a DPOC (Doença Pulmonar Obstrutiva Crónica) está a ter no seu bem estar e no seu quotidiano. As suas respostas e a pontuação do teste podem ser utilizadas por si e pelo seu profissional de saúde para ajudar a melhorar a gestão da sua DPOC e a obter o máximo benefício do tratamento.

Para cada um dos itens a seguir, assinale com um (X) o quadrado que melhor o descreve presentemente. Certifique-se que selecciona apenas uma resposta para cada pergunta.

Por exemplo: Estou muito feliz [0] [X] [2] [3] [4] [5] Estou muito triste

			PONTUAÇÃO
Nunca tenho tosse	0 1 2 3 4 5	Estou sempre a tossir	☐
Não tenho nenhuma expectoração (catarro) no peito	0 1 2 3 4 5	O meu peito está cheio de expectoração (catarro)	☐
Não sinto nenhum aperto no peito	0 1 2 3 4 5	Sinto um grande aperto no peito	☐
Não sinto falta de ar ao subir uma ladeira ou um lance de escadas	0 1 2 3 4 5	Quando subo uma ladeira ou um lance de escadas sinto bastante falta de ar	☐
Não sinto nenhuma limitação nas minhas actividades em casa	0 1 2 3 4 5	Sinto-me muito limitado nas minhas actividades em casa	☐
Sinto-me confiante para sair de casa, apesar da minha doença pulmonar	0 1 2 3 4 5	Não me sinto nada confiante para sair de casa, por causa da minha doença pulmonar	☐
Durmo bem/profundamente	0 1 2 3 4 5	Não durmo bem/ profundamente devido à minha doença pulmonar	☐
Tenho muita energia	0 1 2 3 4 5	Não tenho nenhuma energia	☐

PONTUAÇÃO TOTAL ☐☐

Available online: https://www.catestonline.org/patient-site-portuguese_portugal.html
In CAT Development Steering Group. Healthcare Professional User Guide - CAT 2012:5-12.

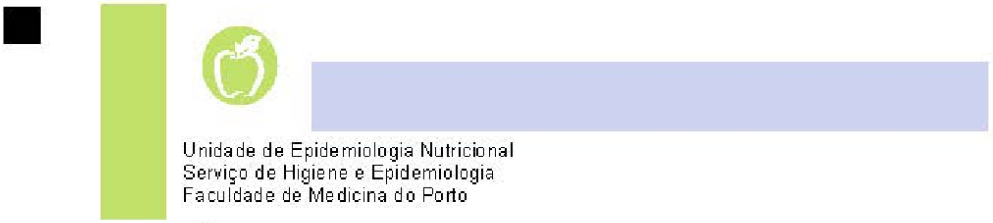
APPENDIX 11

QFA- Questionário de Frequência Alimentar

Available in: http://higiene.med.up.pt/freq.php

Lopes C, Oliveira A, Santos AC, Ramos E, Gaio AR, Severo M, Barros H. Consumo alimentar no Porto. Faculdade de Medicina da Universidade do Porto; 2006. Disponível em: www.consumoalimentarporto.med.up.pt

Lopes C. Reprodutibilidade e validação do questionário semi-quantitativo de frequência alimentar. In: Alimentação e enfarte agudo do miocárdio: um estudo caso-controlo de base comunitária. [PhD]. Porto: Faculdade de Medicina da Universidade do Porto; 2000.

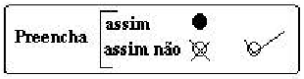


Unidade de Epidemiologia Nutricional
Serviço de Higiene e Epidemiologia
Faculdade de Medicina do Porto

INSTRUÇÕES (PARA ENTREVISTADOR)

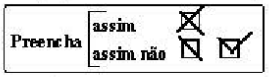
- As questões devem ser "neutras", isto é, não devem influenciar de qualquer forma o tipo de respostas

- O questionário pretende identificar o consumo de alimentos do ano anterior. Assim para cada alimento, deve assinalar, preenchendo o respectivo círculo, quantas vezes, em média, por dia, semana ou mês o inquirido consumiu cada um dos alimentos referidos nesta lista, **ao longo do último ano**. Não se esqueça de assinalar no círculo respectivo os alimentos que o inquirido nunca come, ou come menos de 1 vez por mês.



- Na coluna correspondente à quantidade assinale se a porção que habitualmente o inquirido come é igual, maior ou menor do que a referida como porção média.

- Para os alimentos que só são consumidos, em determinadas épocas do ano (por ex: cerejas, diospíros, etc.), assinale as vezes em que o inquirido consumiu o alimento nessa época, e coloque uma cruz (x) na última coluna (Sazonal).



- Não se esqueça de ter em conta as vezes que o alimento é consumido sozinho e aquelas em que é adicionado a outros alimentos ou pratos (ex: café com leite, os ovos das omeletas, etc).

- No grupo III - **Óleos e Gorduras** - pergunte apenas os que são **adicionados** em saladas, no prato, no pão, etc, e **não** aos utilizados para cozinhar

- No grupo VI - **Hortaliças e Legumes** - pergunte pensando nos que são consumidos no prato (cozidos ou em saladas) e **não** nos que entram na confecção da sopa.

- No item nº 86, anote a frequência com que o inquirido come sopa de legumes. No caso da sopa consumida ser caldo verde, canja ou sopa instantânea, com uma frequência de **pelo menos 1 vez por semana**, deve assinalar este consumo separadamente no quadro existente para outros alimentos, tendo o cuidado em o subtrair à frequência que foi referida anteriormente para a sopa de legumes.

- Se houver algum alimento não mencionado na lista de alimentos e que consuma pelo menos 1 vez por semana, assinale, no quadro que existe para **outros alimentos**, a respectiva frequência e indique ainda a porção média de consumo. ***Por ex: frutos tropicais, sumos de fruta natural, bebidas espirituosas, café de mistura, alheiras, farinheiras, frutos secos (figo, ameixa, damasco), produtos dietéticos, rebuçados, etc.***

ID

Por favor, **antes de iniciar o questionário leia as instruções da página anterior.**
Pense durante o último ano quantas vezes por dia, semana ou mês, em média, consumiu cada um dos alimentos referidos. Na coluna referente à quantidade deverá assinalar se sua porção é igual, menor ou maior do que a referida como porção média. Para os alimentos consumidos só em determinadas épocas do ano, anote a frequência com que o alimento é consumido nessa época e assinale com uma cruz **(x)** na última coluna (Sazonal).

I. P. LÁCTEOS	Frequência alimentar									Quantidade				Sazonal
	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia	Porção Média	A sua porção é: Menor	Igual	Maior	
1. Leite gordo	O	O	O	O	O	O	O	O	O	1 chávena = 250 ml	O	O	O	☐
2. Leite meio-gordo	O	O	O	O	O	O	O	O	O	1 chávena = 250 ml	O	O	O	☐
3. Leite magro	O	O	O	O	O	O	O	O	O	1 chávena = 250 ml	O	O	O	☐
4. Iogurte	O	O	O	O	O	O	O	O	O	Um =125g	O	O	O	☐
5.Queijo (de qualquer tipo incluindo queijo fresco e requeijão)	O	O	O	O	O	O	O	O	O	1 fatia = 30g	O	O	O	☐
6. Sobremesas lácteas: pudim, aletria e leite creme , etc	O	O	O	O	O	O	O	O	O	Um ou 1 prato sobremesa	O	O	O	☐
7. Gelados	O	O	O	O	O	O	O	O	O	Um ou 2 bolas	O	O	O	☐

II. OVOS, CARNES E PEIXES	Frequência alimentar									Quantidade				Sazonal
	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia	Porção Média	A sua porção é: Menor	Igual	Maior	
8.Ovos	O	O	O	O	O	O	O	O	O	Um	O	O	O	☐
9.Frango	O	O	O	O	O	O	O	O	O	1 porção ou 2 peças=150g	O	O	O	☐
10.Peru, coelho	O	O	O	O	O	O	O	O	O	1 porção ou 2 peças=150g	O	O	O	☐
11.Carne vaca, porco,cabrito	O	O	O	O	O	O	O	O	O	1 porção =120g	O	O	O	☐
12. Fígado de vaca, porco, frango	O	O	O	O	O	O	O	O	O	1 porção = 120g	O	O	O	☐
13 Língua, mão de vaca, tripas, chispe, coração, rim	O	O	O	O	O	O	O	O	O	1 porção =100g	O	O	O	☐
14.Fiambre, chouriço, salpicão, presunto, etc	O	O	O	O	O	O	O	O	O	2 fatias ou 3 rodelas =20g	O	O	O	☐
15. Salsichas	O	O	O	O	O	O	O	O	O	3 médias	O	O	O	☐
16. Toucinho, bacon	O	O	O	O	O	O	O	O	O	2 fatias=50g	O	O	O	☐
17. Peixe gordo: sardinha, cavala, carapau, salmão,	O	O	O	O	O	O	O	O	O	1 porção =125g	O	O	O	☐
18. Peixe magro: pescada, faneca, dourada, etc	O	O	O	O	O	O	O	O	O	1 porção =125g	O	O	O	☐
19.Bacalhau	O	O	O	O	O	O	O	O	O	1 porção =125g	O	O	O	☐
20.Peixe conserva: atum, sardinhas,etc	O	O	O	O	O	O	O	O	O	1 lata	O	O	O	☐
21.Lulas, polvo	O	O	O	O	O	O	O	O	O	1 porção =100g	O	O	O	☐
22.Camarão, amêijoas, mexilhão, etc	O	O	O	O	O	O	O	O	O	1 prato sobremesa =100g	O	O	O	☐

III. Óleos e Gorduras	Frequência alimentar									Quantidade				Sazonal
	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia	Porção Média	A sua porção é: Menor	Igual	Maior	
23. Azeite	O	O	O	O	O	O	O	O	O	1 colher sopa	O	O	O	☐
24. Óleos: girassol, milho, soja	O	O	O	O	O	O	O	O	O	1 colher sopa	O	O	O	☐
25. Margarina	O	O	O	O	O	O	O	O	O	1 colher chá	O	O	O	☐
26. Manteiga	O	O	O	O	O	O	O	O	O	1 colher chá	O	O	O	☐

Unidade de Epidemiologia Nutricional
Serviço de Higiene e Epidemiologia - FMUP

30295

ID

IV.PÃO, CEREAIS E SIMILARES	Frequência alimentar									Quantidade				Sazonal
	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia	Porção Média	A sua porção é: Menor	Igual	Maior	
27. Pão branco ou tostas	O	O	O	O	O	O	O	O	O	Um ou 2 tostas = 40g	O	O	O	□
28. Pão (ou tostas), integral,centeio, mistura	O	O	O	O	O	O	O	O	O	Um ou 2 tostas= 50g	O	O	O	□
29. Broa, broa de avintes	O	O	O	O	O	O	O	O	O	1 fatia = 80g	O	O	O	□
30. Flocos cereais (muesli, corn-flakes, chocapic,etc.)	O	O	O	O	O	O	O	O	O	1 chávena =40g	O	O	O	□
31. Arroz	O	O	O	O	O	O	O	O	O	½ prato = 100g	O	O	O	□
32. Massas: esparguete, macarrão, etc.	O	O	O	O	O	O	O	O	O	½ prato = 100g	O	O	O	□
33. Batatas fritas caseiras	O	O	O	O	O	O	O	O	O	½ prato = 100g	O	O	O	□
34. Batatas fritas de pacote	O	O	O	O	O	O	O	O	O	1 pacote pequeno =30g	O	O	O	□
35. Batatas cozidas, assadas, estufadas e puré	O	O	O	O	O	O	O	O	O	2 batatas médias =160 g	O	O	O	□

V.DOCES E PASTÉIS	Frequência alimentar									Quantidade				Sazonal
	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia	Porção Média	A sua porção é: Menor	Igual	Maior	
36. Bolachas tipo maria, água e sal ou integrais	O	O	O	O	O	O	O	O	O	3 bolachas	O	O	O	□
37. Outras bolachas ou biscoitos	O	O	O	O	O	O	O	O	O	3 bolachas	O	O	O	□
38. Croissant, pasteis, bolicao, doughnut ou bolos	O	O	O	O	O	O	O	O	O	Um; 1 fatia = 80g	O	O	O	□
39.Chocolate (tablete ou em pó)	O	O	O	O	O	O	O	O	O	3 quadrados; 1 colher sopa	O	O	O	□
40. Snacks de chocolate (Mars, Twix, Kit Kat, etc)	O	O	O	O	O	O	O	O	O	Um	O	O	O	□
41. Marmelada, compota, geleia, mel	O	O	O	O	O	O	O	O	O	1 colher sobremesa	O	O	O	□
42. Açúcar	O	O	O	O	O	O	O	O	O	1 colher sobremesa; 1 pacote	O	O	O	□

VI. HORTALIÇAS E LEGUMES	Frequência alimentar									Quantidade				Sazonal
	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia	Porção Média	A sua porção é: Menor	Igual	Maior	
43. Couve branca, couve lombarda	O	O	O	O	O	O	O	O	O	½ chávena =75g	O	O	O	□
44. Penca, Tronchuda	O	O	O	O	O	O	O	O	O	½ chávena = 65g	O	O	O	□
45. Couve galega	O	O	O	O	O	O	O	O	O	½ chávena =65g	O	O	O	□
46. Brócolos	O	O	O	O	O	O	O	O	O	½ chávena =85g	O	O	O	□
47. Couve-flor, Couve-bruxelas	O	O	O	O	O	O	O	O	O	½ chávena =65g	O	O	O	□
48. Grelos, Nabiças, Espinafres	O	O	O	O	O	O	O	O	O	½ chávena =72g	O	O	O	□
49. Feijão verde	O	O	O	O	O	O	O	O	O	½ chávena =65g	O	O	O	□
50. Alface, Agrião	O	O	O	O	O	O	O	O	O	½ chávena =15g	O	O	O	□
51. Cebola	O	O	O	O	O	O	O	O	O	½ média=40g	O	O	O	□
52. Cenoura	O	O	O	O	O	O	O	O	O	1 média=80g	O	O	O	□
53. Nabo	O	O	O	O	O	O	O	O	O	1 médio=78g	O	O	O	□
54. Tomate fresco	O	O	O	O	O	O	O	O	O	½ médio=63g	O	O	O	□
55. Pimento	O	O	O	O	O	O	O	O	O	½ médio=68g	O	O	O	□
56. Pepino	O	O	O	O	O	O	O	O	O	¼ médio=50g	O	O	O	□
57. Leguminosas: feijão, grão de bico	O	O	O	O	O	O	O	O	O	1 chávena	O	O	O	□
58. Ervilha grão, Fava	O	O	O	O	O	O	O	O	O	½ chávena	O	O	O	□

Unidade de Epidemiologia Nutricional
Serviço de Higiene e Epidemiologia - FMUP

30295

ID

VII. FRUTOS	Frequência alimentar									Quantidade				
	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia	Porção Média	A sua porção é: Menor	Igual	Maior	Sazonal
59. Maça, pêra	O	O	O	O	O	O	O	O	O	uma média	O	O	O	□
60. Laranja, Tangerinas	O	O	O	O	O	O	O	O	O	1 média; 2 médias	O	O	O	□
61. Banana	O	O	O	O	O	O	O	O	O	uma média	O	O	O	□
62. Kiwi	O	O	O	O	O	O	O	O	O	um médio	O	O	O	□
63. Morangos	O	O	O	O	O	O	O	O	O	1 chávena	O	O	O	□
64. Cerejas	O	O	O	O	O	O	O	O	O	1 chávena	O	O	O	□
65. Pêssego, Ameixa	O	O	O	O	O	O	O	O	O	1 médio; 3 médios	O	O	O	□
66. Melão, Melancia	O	O	O	O	O	O	O	O	O	1 fatia média = 150g	O	O	O	□
67. Diospiro	O	O	O	O	O	O	O	O	O	1 médio	O	O	O	□
68. Figo fresco, Nêsperas, Damascos	O	O	O	O	O	O	O	O	O	3 médios	O	O	O	□
69. Uvas frescas	O	O	O	O	O	O	O	O	O	1 cacho médio	O	O	O	□
70. Frutos conserva pêssego, ananás	O	O	O	O	O	O	O	O	O	2 metades ou rodelas	O	O	O	□
71. Amêndoas, avelãs, nozes, amendoins, pistachio, etc.	O	O	O	O	O	O	O	O	O	½ chávena (descascado)	O	O	O	□
72. Azeitonas	O	O	O	O	O	O	O	O	O	6 unidades	O	O	O	□

VIII. BEBIDAS E MISCELANEAS	Frequência alimentar									Quantidade				
	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia	Porção Média	A sua porção é: Menor	Igual	Maior	Sazonal
73. Vinho	O	O	O	O	O	O	O	O	O	1 copo=125ml	O	O	O	□
74. Cerveja	O	O	O	O	O	O	O	O	O	1 garrafa ou 1 lata=330 ml	O	O	O	□
75. Bebidas brancas: whisky, aguardente, brandy, etc	O	O	O	O	O	O	O	O	O	1 cálice = 40 ml	O	O	O	□
76. Coca-cola, pepsi-cola ou outras colas	O	O	O	O	O	O	O	O	O	1 garrafa ou 1 lata=330 ml	O	O	O	□
77. Ice-tea	O	O	O	O	O	O	O	O	O	1 garrafa ou 1 lata=330 ml	O	O	O	□
78.Outros refrigerantes, sumos de fruta ou néctares embalados	O	O	O	O	O	O	O	O	O	1 garrafa ou 1 copo = 250 ml	O	O	O	□
79.Café (incluindo pingo, meia de leite e outras bebidas com café)	O	O	O	O	O	O	O	O	O	1 chávena café	O	O	O	□
80. Chá preto e verde	O	O	O	O	O	O	O	O	O	1 chávena	O	O	O	□
81. Croquetes, rissóis, bolinhos de bacalhau, etc.	O	O	O	O	O	O	O	O	O	3 unidades	O	O	O	□
82. Maionese	O	O	O	O	O	O	O	O	O	1 colher sobremesa	O	O	O	□
83. Molho de tomate, ketchup	O	O	O	O	O	O	O	O	O	1 colher sopa	O	O	O	□
84. Pizza	O	O	O	O	O	O	O	O	O	Meia pizza-normal	O	O	O	□
85. Hambúrguer	O	O	O	O	O	O	O	O	O	Um médio	O	O	O	□
86. Sopa de legumes	O	O	O	O	O	O	O	O	O	1 prato	O	O	O	□

Existe algum alimento ou bebida que eu não tenha mencionado e que tenha consumido pelo menos 1 vez por semana mesmo em pequenas quantidades, ou numa época em particular. Por ex: **frutos tropicais, sumos de fruta natural, bebidas espirituosas, café de mistura, alheiras, farinheiras, frutos secos (figo, ameixa, damasco), produtos dietéticos, rebuçados, etc.**

Outros Alimentos	Frequência alimentar									Quantidade	
	Nunca ou <1 mês	1-3 por mês	1 por sem	2-4 por sem	5-6 por sem	1 por dia	2-3 por dia	4-5 por dia	6 + por dia	Porção Média	Sazonal
	O	O	O	O	O	O	O	O	O		□
	O	O	O	O	O	O	O	O	O		□
	O	O	O	O	O	O	O	O	O		□

Unidade de Epidemiologia Nutricional
Serviço de Higiene e Epidemiologia - FMUP

30295

APPENDIX 12

Centro Hospitalar Cova da Beira, E.P.E.

Deliberação n.º: 82/2015	**Data:** 18/12/2015	**Órgão:** Reunião plenária
Título: "Etiopatogenia da Rinossinusite Crónica com Pólipos Nasais: foco na interação ambiente-hospedeiro"		**Nº. EudraCT:** ----------
Versão da documentação aprovada: 16/12/2015	**Promotor:** ----------	**Investigadores principais:** Rafaela da Cruz Vieira Veloso Teles

Membros da CES do CHCB:

Prof. Doutor Manuel Passos Morgado (Presidente, Farmacêutico)

Dr.ª Ana Paula Torgal Carreira (Vice-Presidente, Assistente Social)

Enf. João José Carvalhão Ramalhinho (Enfermeiro)

Dr.ª Maria Fátima Jesus Simões (Psicóloga)

Dr. Luís Manuel Almeida Alves Ribeiro (Médico)

Dr. Luis Manuel Carreira Fiadeiro (Jurista)

Dr. António Luciano (Teólogo)

A Comissão de Ética para a Saúde do Centro Hospitalar Cova da Beira, em reunião realizada na data 18/12/2015, apreciou a fundamentação do relator sobre o pedido de parecer para a realização do estudo acima referenciado.

Ouvido o relator, o processo foi votado pelos membros da CES do CHCB presentes:

Presidente: Prof. Doutor Manuel Passos Morgado
Vice-Presidente: Dr.ª Ana Paula Torgal Carreira
Dr. Luís Manuel Almeida Alves Ribeiro
Enf. João José Carvalhão Ramalhinho
Dr. Luís Manuel Carreira Fiadeiro

Resultado da votação:
Apreciado o projecto, concluiu esta Comissão de Ética nada ter a opor à realização do mesmo.

Data: 28/12/2015

O Presidente da CES do CHCB:

(Prof. Doutor Manuel Morgado)

Capital Social: 24 000 000 € - Nº Contribuinte 506 843 679 - E. E. C. Covilhã (RP)

Sede: Quinta do Alvito 6200 – 251 Covilhã ☎ 275 330 000 Fax: 275 330 001
Av. Adolfo Portela 6230 – 288 Fundão ☎ 275 330 000 Fax: 275 751 057

APPENDIX 13

Centro
Hospitalar
Cova da Beira, E.P.E.

Exmo(a) Sr(a). Dr.(a)
Rafaela da Cruz Vieira
Veloso Teles

Data: 28/12/2015

Assunto: Parecer da CES relativo ao estudo "Etiopatogenia da Rinossinusite Crónica com Pólipos Nasais: foco na interação ambiente-hospedeiro".

Exmos(as). Senhores(as),

Em resposta ao V. pedido de parecer para realização do estudo supramencionado, a Comissão de Ética para a Saúde do Centro Hospitalar Cova da Beira, ao abrigo do disposto na Lei nº 21/2014, de 16 de Abril, e em sessão plenária no dia 18/12/2015 deliberou emitir parecer, conforme documento em anexo.

A CES do CHCB opera dentro do exigido pelas boas práticas clínicas.

Na execução deste estudo qualquer informação/comunicação relevante para a segurança dos participantes tem de ser imediatamente comunicada à CES do CHCB.

Com os melhores cumprimentos,

Pela Comissão de Ética para a Saúde
do Centro Hospitalar Cova da Beira

(Prof. Doutor Manuel Morgado – Presidente da CES)

Sede: Quinta do Alvito 6200 – 251 Covilhã ☎ 275 330 000 Fax: 275 330 001
Av. Adolfo Portela 6230 – 288 Fundão ☎ 275 330 000 Fax: 275 751 057

APPENDIX 14

FACULDADE CIÊNCIAS DA SAÚDE

PARECER

Processo: CE-FCS-2016-020

Tema Projecto/Proponente: "Exposição ocupacional e prevalência de rinossinusite crónica com pólipos nasais" – Exma. Senhora Dra. Rafaela Veloso-Teles

Exmo. Sr. Presidente da Faculdade de Ciências da Saúde

Apreciado o pedido referente ao processo acima mencionado esta Comissão não detectou matéria que ofenda os princípios éticos.

Covilhã, 4 de Julho de 2016

O Presidente da Comissão de Ética
Prof. Doutor José Martínez de Oliveira

Universidade da Beira Interior | Faculdade de Ciências da Saúde
Avenida Infante D. Henrique
6200-506 Covilhã | PORTUGAL
Tel.: +351 275 319 700 | E-mail: pcabral@ubi.pt
www.ubi.pt