Rôle des exotoxines de Staphylococcus aureus dans la rhinosinusite chronique

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Rôle des exotoxines de *Staphylococcus aureus* dans la rhinosinusite chronique

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Résumé

Les exotoxines du staphylocoque doré (SD) pourraient être un facteur aggravant dans l'inflammation des muqueuses rhino-sinusiennes observées dans la rhino-sinusite chronique (RSC) avec polypose ethmoïdale.
Nous avons effectué une étude prospective incluant 236 patients provenant de 5 grandes villes européennes. Les patients ont été divisés en 3 groupes : RSC sans polypose ethmoïdale, RSC avec polypose ethmoïdale et un groupe contrôle. Des prélèvements bactériologiques ont été effectués sous contrôle endoscopique au niveau du méat moyen et 93 porteurs de SD ont été identifiés. Nous n'avons trouvé aucune corrélation entre la présence ou non des 22 gènes codants pour les exotoxines de SD, la gravité de la rhino-sinusite chronique ou les patients contrôles. Ces observations semblent indiquer que la sévérité de la RSC n'est pas associée avec la présence ou non d'exotoxines de SD.

INDEX

ABBREVIATIONS

agr: accessory gene regulator

CRS: chronic rhinosinusitis

CRSsNP: chronic rhinosinusitis without nasal polyposis

CRSwNP: chronic rhinosinusitis with nasal polyposis

egc: enterotoxin gene cluster *(minuscule, italic: international gene nomenclature)*

MLVA: multilocus variable number of tandem repeats assay

NP: nasal polyposis

NP1 / 2 / 3: nasal polyposis stage 1 / 2 / 3 (Mackay and Nacleiro classification)

NSAID: non-steroid anti-inflammatory drugs

PCR: polymerase chain reaction

PFGE: Pulse Field Gel Electrophoresis

PVL: Panton-Valentine leukocidin

QB: quantity of bacteria

SA: *Staphylococcus aureus*

SE: *Staphylococcus aureus* exotoxins

se: *Staphylococcus aureus* exotoxin gene(s), including enterotoxin gene(s) subgroup

(minuscule, italic: international gene nomenclature)

Th2 cell: type 2 helper T lymphocyte

TSST-1: Toxic Shock Staphylococcal Toxin-1

VNTR: Variable Number of Tandem Repeats

SUMMARY

Chronic rhinosinusitis (CRS) is a common chronic condition and its pathogenesis remains poorly understood. Recent research has provided evidence that *Staphylococcus aureus* exotoxins (SE) worsen inflammation in chronic rhinosinusitis with nasal polyposis (CRSwNP). To investigate the clinical relevance of this finding, a prospective study of 236 subjects was performed at several European centres. Subjects were divided into 3 groups: CRSwNP, chronic rhinosinusitis without nasal polyposis (CRSsNP) and controls. Middle meatal swabs were collected under endoscopic guidance from the subjects and 93 *Staphylococcus aureus* (SA) carriers were identified. A broad variety of SA strains were identified. The 22 currently-known SE genes (*se*) were identified in strains from all subjects. CRSwNP and CRSsNP did not correlate with the presence of *se*, the overall numbers of *se* or any individual *se*, with the exception of *sep* and *seq*, which were present in CRSwNP in significantly greater amounts when compared to the other study groups. The severity of CRSwNP did not correlate with the presence of *se*, the overall numbers of *se* or any individual *se*. While other studies have shown that SE contribute to inflammation in mucosa from CRSwNP in a minority of patients, the findings from the present study may cause questions about the clinical relevance of SE to the majority of CRS patients.

1. INTRODUCTION

1.1. Definitions

1.1.1. Chronic rhinosinusitis

Chronic rhinosinusitis is defined as inflammation of the nose and paranasal sinuses for 12 weeks or more. This is symptomatically characterised by nasal blockage or nasal discharge, plus one other symptom, including facial pressure or pain or loss of smell. In addition, endoscopic nasal examination should reveal mucopurulent discharge or mucosal oedema of the middle meatus or polyps and CT scanning should reveal mucosal changes in the middle meatus or sinuses [1].

Chronic rhinosinusitis without nasal polyposis (CRSsNP) is defined as above, with endoscopic examination revealing no middle meatal polyps or, in patients with prior nasal surgery, no bilateral pedunculated lesions on the lateral nasal wall [1].

1.1.2. Chronic rhinosinusitis with nasal polyposis

Chronic rhinosinusitis with nasal polyposis (CRSwNP) is defined as per 1.1.1., with endoscopic examination revealing middle meatal polyps, in patients with no prior history of nasal surgery. Six or more months after surgery has been performed, polyps are defined as bilateral pedunculated lesions on the lateral nasal wall [1].

1.2. Epidemiology of CRS

It is difficult to estimate the true prevalence of CRS, partly due to a lack of standardised definitions in the literature. The EPOS 2007 paper attempted to address this issue by stating clear definitions of CRS and CRSwNP, as determined by a panel of European experts [1]. These definitions have been used in this thesis (see 1.1.).

Having acknowledged these problems, CRS has a reported prevalence of 15.5% in USA and is the second commonest chronic medical condition [2]. It is more common in females (with a female:male ratio of 6:4) [2]. Its highest prevalence is in young and middle-aged adults [2, 3].

CRSwNP has a reported prevalence of 2.7% when diagnosed by nasal endoscopy. It is more common in males, (with a male:female ratio of 2.2:1,), patients over 60 years of age and asthmatics [4].

1.3. Pathophysiology of CRS

CRS is thought to represent a heterogeneous group of conditions, in which different pathogenic mechanisms ultimately lead to the common result of inflammation of the nose and paranasal sinuses [1]. The American Rhinosinusitis Task Force report described the involvement of systemic host factors, local host factors and environmental factors in the pathogenesis of CRS. Systemic factors included allergy, immunodeficiency, systemic mucociliary dysfunction, genetic, neurological and endocrine disturbances. Local host factors included acquired mucociliary dysfunction, dental disease and anatomical anomalies. Environmental factors included micro-organisms (viral, bacterial, fungal), inhaled irritants, trauma or iatrogenic factors (medication or surgery) [5].

EPOS 2007 distinguished between CRSwNP and CRSsNP because of evidence of major differences in their pathophysiologies [1].

1.3.1. Pathophysiology of CRSsNP

Sinonasal secretions in CRSsNP show a predominance of neutrophils [6]. Mucosal histopathology exhibits goblet cell hyperplasia, thickening of the basement membrane, subepithelial oedema and a mononuclear cell infiltrate [7]. CD4+ helper T cells, macrophages, neutrophils, mast cells and eosinophils are present in greater numbers than in controls [1].

Biofilms have become a topic of much interest in the study of the pathogenesis of CRS, as well as tonsillitis, cholesteatoma and otitis media [8]. Biofilms are specialised communities of micro-organisms which exist within a three-dimensional structure of extracellular polymeric substance. They adhere to biological surfaces [9]. Biofilm-specific genes are responsible for resistance to both antimicrobial agents and host immune defences [10]. Several studies have found biofilms to be present in the mucosa of CRS, but their role in its pathogenesis is yet to be defined [11].

1.3.2. Pathophysiology of CRSwNP

Mucosal histopathology in CRSwNP exhibits damaged epithelium with a thickened basement membrane and oedematous, sometimes fibrotic stroma characterised by an inflammatory infiltrate around empty areas of pseudocysts [12]. The infiltrate shows a predominance of eosinophils in 80% of Caucasian (but not Chinese) polyps [13]. Neutrophils, T cells and plasma cells are also present [14]. See Figure 1, below. A likely pathogenic mechanism for polyp formation is that the eosinophilic stromal infiltrate regulates a deposition of plasma proteins into the pseudocystic areas, leading to greater stromal oedema and enlargement into polyps [1]. What causes the eosinophilic infiltrate, however, is unclear. IL-5 appears to play a key role in the activation of eosinophils in CRSwNP, along with RANTES and eotaxin [1].

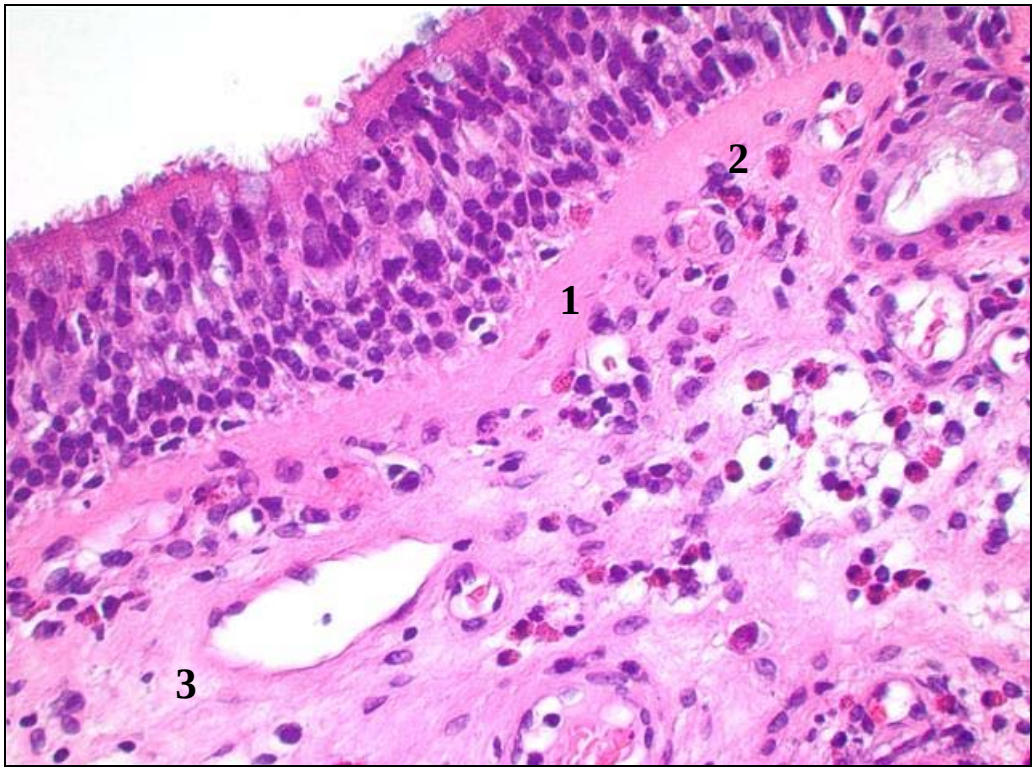

Figure 1: Histopathology of Nasal Mucosa in Chronic Rhinosinusitis with Nasal Polyposis.
1: basement membrane thickening; 2 : eosinophilic infiltration; 3: stromal oedema.

1.4. *Staphylococcus aureus* exotoxins

Staphylococcus aureus (SA) is a Gram positive bacteria commonly found on human skin, including the anterior nares. It is capable of producing various toxins, which are pathogenic in humans: enterotoxins cause food poisoning, exfoliatin causes generalised exfoliative syndrome and bullous impetigo, Panton-Valentine Leukocidin (PVL) causes furunculosis and necrotising pneumonia, Toxic Shock Staphylococcal Toxin-1 (TSST-1) causes the potentially lethal toxic shock syndrome [15]. Because these proteins are secreted from the SA bacteria, they are called “exotoxins” (SE).

There are currently 22 known SE. Some *Staphylococcus aureus* exotoxin genes (*se*) may co-transfer between bacteria, so that certain genes are usually present in a cluster. For example, the SE genes *seg, sei, sem, sen, seo seu* form a cluster named *egc* (enterotoxin gene cluster). *sed* and *sej* form another cluster.

Generally, SE are pathogenic because they are pore-forming molecules which insert into a human cell and lead to its lysis. However, SE are also able to act as superantigens: they bind to the V beta-region of the human T cell receptor, which is distinct from the classical antigen binding site [16]. This leads to an intense polyclonal activation and proliferation of T cells and an immune response, independent of classical antigen-receptor interaction. There are about 50 human V beta-domains and certain SE bind to certain of these domains.

1.5. Exotoxins in the pathophysiology of CRS

Numerous studies have implicated SEA, SEB, SEC and TSST-1 in the pathogenesis of CRSwNP [17-22]. Interest in SE was particularly stimulated by the finding that IgE antibodies to SE were present in nasal-associated lymphoid tissue (NALT) localised in the sinonasal mucosa of subjects with CRSwNP. This was interpreted to mean that a local immune response could be stimulated by the presence of SE. In one study, IgE antibodies to SE were found in 25% of CRSwNP subjects and were not found in any of the controls [20]. Another study found rates of 28% in CRSwNP subjects and 6% in CRSsNP subjects; in patients with Widal syndrome, the prevalence increased to 80% [21]. The relevance of these findings to the pathogenesis of CRSwNP is still uncertain. It is apparent however, that only a minority of CRSwNP patients exhibit IgE antibodies to SE and no large, controlled studies have yet been performed.

Interactions between SE and T cells have also been established in 35% of CRSwNP patients [23]. In another study, SEB was found to polarise inflammation to a Th2 pattern and suppress induced T-regulatory lymphocytes, thus potentially prolonging the inflammation [18].

There is evidence that SE may affect the sinonasal mucosa directly, as well as via their immunomodulatory effects. For example, in an *in vitro* study on rabbit sinus mucosa, low dose SEA produced histological changes of CRS and high dose SEA lowered respiratory epithelial cells' ciliary beat frequency, a feature of CRS [22].

Previous epidemiological studies have investigated a correlation between SE and CRSwNP. One study found SE were present in half of CRSwNP patients and no SE was found in any of the control patients [24]. Another study found SEA, SEB or TSST-1 in 60% of CRSwNP patients, but there was no control group with which to draw comparisons [19]. These data were considered to be epidemiological evidence of a role for SE in the pathogenesis of CRSwNP.

2. AIM

The main aim of this thesis was to investigate whether there was a correlation between the presence or overall numbers of *Staphylococcus aureus* exotoxin genes (*se*) and CRSwNP or CRSsNP. A secondary aim was to examine whether there was a correlation between the presence or overall numbers of *se* and the severity of CRSwNP. The research was conducted in the setting of a multi-centred, prospective trial containing the largest patient numbers of any published data on this subject.

3. METHODS

3.1. Study Design

The study was a blinded, prospective, multi-centred European trial. Patients were enrolled into one of three groups: CRSwNP, CRSsNP or controls. Inclusion criteria for patients in the CRSwNP and CRSsNP groups were: disease diagnosis by the EPOS definition, failure of maximal medical treatment (oral antibiotics and oral and topical steroids) and need for endoscopic sinus surgery as further treatment, and consent to participate in the study. Inclusion criteria for control patients were: need for nasal surgery unrelated to CRS and consent to participate in the study. Exclusion criteria for all groups were: history of nasal allergy, current upper respiratory tract infection and systemic corticosteroid or antibiotic therapy within the 6 months prior to specimen collection.

3.2. Data and specimen collection

A total of 236 subjects were enrolled in the study. Demographic and clinical data were collected prospectively and informed consent was obtained. A history of asthma was noted and categorised into mild, moderate or severe. The presence of Widal syndrome (also known as Samter's triad of CRSwNP, asthma and aspirin intolerance) was noted. All patients were examined endoscopically, as per EPOS. CRSwNP severity was categorised using the Mackay and Nacleiro classification, reported by Malm: stage 1 (NP1) = polyps present in the middle meatus; stage 2 (NP2) = polyps extending below the middle turbinate; stage 3 (NP3) = polyps reaching the floor of the nasal cavity [25].

Each patient had an endoscopically-guided microbiological swab taken from their middle meatus at the time of their surgery. All specimens were then promptly sent to

the same microbiology laboratory in Geneva, where the staff was blinded as to the patient group of each specimen.

3.3. Specimen processing

3.3.1. Quantity of bacteria

A Gram stain was performed on each specimen. The quantity of SA bacteria (QB) was categorised into 3 groups: “low” was defined by the presence of colonies only in the first streaking quadrant on a Petri dish, “moderate” by their presence in the second streaking quadrant and “abundant” by their presence in the third streaking quadrant.

3.3.2. Identification of SA and their genotypes

Identification of SA was performed according to CLSI recommendations [26]. Each sample was tested for the presence of the *femA* gene, a SA-specific gene, in order to confirm species identification and to assess the quality of further polymerase chain reaction (PCR) amplifications. Using this method, 93 of the 236 enrolled subjects were positive for intranasal SA. Their ages ranged between 16 and 82 years. There were 39 females and 54 males. The locations of the subjects were as follows: 49 from Geneva (Switzerland), 27 from Paris (France), 9 from Bruges (Belgium), 6 from Zagreb (Croatia) and 2 from London (UK). In the trial groups, CRSwNP contained 55 subjects, CRSsNP contained 16 subjects and there were 22 controls. See Table 1 below, for the characteristics of the subjects overall and by group.

Patient Group	Overall Number	Males	Females	Median Age (Range) in years	Widal Syndrome	Asthma
Control	22	13	9	45 (16-63)	0	0
CRSsNP	16	11	5	53 (21-70)	0	4
CRSwNP	55	30	25	56 (21-82)	13	39
NP1	*25*	*14*	*11*	*56 (21-72)*	*4*	*19*
NP2	*24*	*13*	*11*	*56 (40-82)*	*6*	*16*
NP3	*6*	*3*	*3*	*52 (46-62)*	*3*	*4*
Total	**93**	**54**	**39**	**56 (16-82)**	**13**	**43**

Table 1: Characteristics of Study Subjects. CRSwNP: chronic rhinosinusitis with nasal polyposis; CRSsNP: chronic rhinosinusitis without nasal polyposis. NP1 – 3: nasal polyp severity, stages 1 – 3 of Mackay and Nacleiro classification.

3.3.3. Identification of *se*

The 93 specimens that were positive for SA were subsequently tested, using PCR, for the presence of all of the 22 currently-known *se* and 2 pseudo-genes: this includes genes for 18 enterotoxins, PVL, TSST-1 and exfoliatins A and B. See Table 2, below, for details of primer names and sequences used in PCR.

Primer Name		**Sequence *(5'-->3')***
seh	Forward	TTCACATCATATGCGAAAGC
	Reverse	TTTTCTTTAATGAATGGGTGA
yent1	Forward	TTTATCCGAAAGGCTTTTATT
	Reverse	GAACAATTGAATAAAGCGAGTG
seq	Forward	ATACCTATTAATCTCTGGGTCAATG
	Reverse	AATGGAAAGTAATTTTTCCTTTGT
seg	Forward	TGAGGTTAAAACTGAATTAGAAAA
	Reverse	AGAATCAACWACTTTATTATCTCCGT
sec	Forward	TTTTTGGCACATGATTTAATTT
	Reverse	CAACCGTTTTATTGTCGTTG

sek	Forward	TGGACATAACGGCACTAAAA
	Reverse	TTGGTARCCCATCATCTCCT
yent2	Forward	TTTACCAGATTCAGGCATCA
	Reverse	AAGAAAAACATAACAGCACAAG
seo	Forward	AGTTTGTGTAAGAAGTCAAGTGTAGA
	Reverse	ATCTTTAAATTCAGCAGATATTCCATCTAAC
sem	Forward	TTTAGTATCAATTTCTTGAGCTGTT
	Reverse	AAAATCATATCGCAACCGC
sen	Forward	ATGAGATTGTTCTACATAGCTGCAAT
	Reverse	AACTCTGCTCCCACTGAAC
sep	Forward	CTGAATTGCAGGGAACTGCT
	Reverse	ATTGGCGGTGTCTTTTGAAC
seb	Forward	TTCGGGTATTTGAAGATGGT
	Reverse	AGGCATCATGTCATACCAAA
sel	Forward	AGACAAAAATTCACCAGAATCA
	Reverse	TTGACATCTATTTCTTGTGCG
sea	Forward	TTATGGTTATCAATGTGCGG
	Reverse	TACTGTCCTTGAGCACCAAA
sei	Forward	ACMGGTAYCAATGATTTGAT
	Reverse	CTTACAGGCASWCCATSTCC
seu	Forward	ATGGAGTTGTTGGAATGAAGT
	Reverse	TTTTTGGTTAAATGAACTTCTACA
see	Forward	GGAGGCACACCAAATAAAAC
	Reverse	GGACCCTTCAGAAGAATGAA
ser	Forward	GTGCTAAACCAGATCCAAGG
	Reverse	AAGGGAACCAAATCCTTTTTA
sed	Forward	CGTTAAAGCCAATGAAAACA
	Reverse	TGAAGGTGCTCTGTGGATAA
sej	Forward	CTGATTTTCTCCCTGACGTT
	Reverse	TCGATATGCATGTTTTCAGA

pvl	Forward	AAAATGCCAGTGTTATCCAGAGGTA
	Reverse	TTTGCAGCGTTTTGTTTTCG
exfoA (eta)	Forward	CCATATGGAGAGTATGAAGTCAAAGAAA
	Reverse	TGAAACACCGTTTTGATCTGGTT
exfoB (etb)	Forward	GAGGTAGGAAACTCTGGATCAGGTAT
	Reverse	TCTATTGAAAAACACTCCTATTGGAAGA
TSST-1	Forward	TCATCAGCTAACTCAAATACATGGATTA
	Reverse	TGTGGATCCGTCATTCATTGTT

Table 2: Names and Sequences of Primers used in Polymerase Chain Reaction for Detection of Exotoxin Genes. *sea* – *seu*: Stapylococcal enterotoxin genes a – u; *pvl*: Panton-Valentine leukocidin gene; *exfoA* – *B*: exfoliatin toxin A – B genes; *TSST-1*: toxic shock syndrome toxin-1 gene. Sequence is written in five prime to three prime direction. A: adenine; C: cytosine; G: guanine; T: thymine.

The 2 non-functional pseudogenes, *yent1* and *yent2*, can rearrange together to yield the functional enterotoxin gene *seu* [27]. Their presence was not considered in the final analysis – instead the presence of the functional gene, *seu*, was considered.

3.3.4. Laboratory method

Genomic DNA was extracted using the DNeasy kit (QIAGEN, Hilden, Germany) from isolated SA colonies freshly grown on Mueller Hinton agar plates (Difco). Each strain was suspended in 500 µl Tris-EDTA buffer (TE, 10mM Tris and 1 mM EDTA), then washed twice by centrifugation for 10 minutes at 4000 g. The pellet was suspended in 200 µl TE supplemented by 100 µg of lysostaphin (Ambicin, Applied Microbiology, Tarrytown, NY), and incubated for 10 minutes at 37°C. DNeasy spin columns were then used following the manufacturer's recommendations. DNA concentration and purity were assessed by using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Rockland, DE). For clinical isolates tests, fast DNA extraction was performed using glass beads (in Tris-EDTA buffer) and vortex agitation [27]. 5 µl of crude lysate were used to perform PCR amplifications. Control experiments showed that rapid extraction yielded similar results to purified DNA samples [27].

SA genotyping was performed according to accessory gene regulator (*agr*) groups, using a previously published real-time multiplex PCR assay [28]. This consists of a quadruplex PCR amplification allowing discrimination of the 4 different alleles which define the *agr* groups of SA (*agr* I – IV) [29]. Rapid genotyping was performed using a recently published multilocus variable number of tandem repeats assay (MLVA) using 10 primer pairs [30, 31] and a microcapillary electrophoresis system for the rapid evaluation of the MLVA profile. MLVA genotyping is a rapid methodology

allowing the identification of the bacterial genetic background and the relatedness between isolates with a discrimination power at least comparable to that of Pulse Field Gel Electrophoresis (PFGE) [30].

Enterotoxin genes were identified by using 4 PCR multiplex amplifications [32]. 1µl of each PCR mixture was loaded in a BioAnalyzer 2100 device using DNA 1000 chip, yielding to an outstanding resolution from 25-1000 bp. Results were analyzed using specific software previously described [30]. Briefly, BioAnalyzer output files containing raw fluorescence data were exported and processed to discriminate peaks from background signals. The exfoliatin A and B genes (*eta* and *etb*), TSST-1 gene (*tsst-1*) and PVL gene (*pvl*) were identified as formerly described [28].

3.4. Statistical analysis of data

The "Fisher Exact Test" was used to calculate p-values to evaluate any statistically significant difference between groups containing categorical data. The "Mann Whitney U Test" was used for the same purpose with quantitative data.

4. RESULTS

4.1. Identification of SA and their genotypes

SA was demonstrated in 39.4 % of all specimens (93/236), by detection of *femA*. Whilst the most abundant *agr*-type identified overall was *agr* I, there was a similar distribution of all 4 alleles across all study groups. Likewise, there was no correlation between geographical origin and SA strain genotype. Hence, the study population of SA consisted of a large diversity of unrelated isolates, derived from various genetic backgrounds.

4.2. Identification of *se*

4.2.1. Identification of *se* in SA carriers overall

Overall, at least one *se* was detected in 79 of the 93 subjects (84.9%) in whom SA were detected. Hence, there were 14 subjects carrying SA with no *se* detected - all of these SA were in the *agr* I and II groups (7 in each group). There was no geographical predilection for carrying any particular *se*. The different types of *se* were equally distributed between women and men. Refer to Figure 2, below.

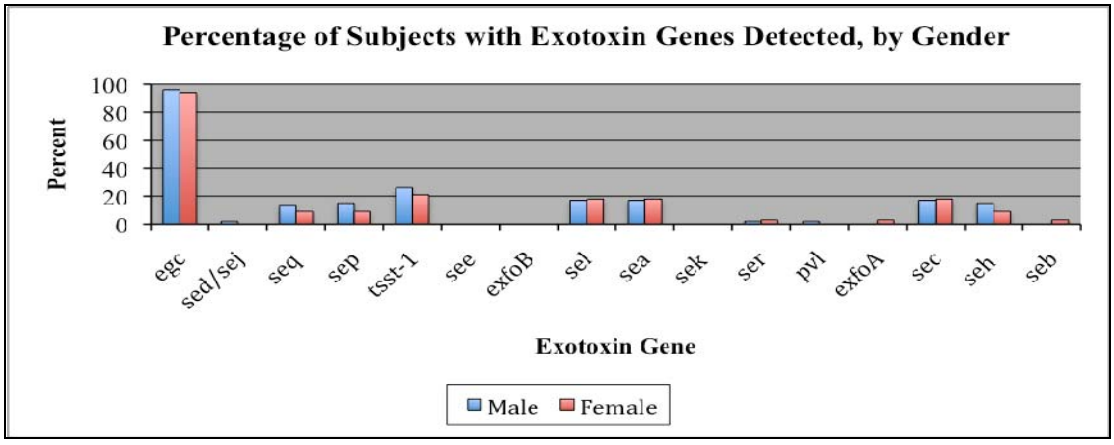

Figure 2: Percentage of Subjects with Exotoxin Genes Detected, by Gender. *egc*: enterotoxin gene cluster of *seg, sem, sen, sei, seo, seu*; *sea – r*: Staphylococcal enterotoxin genes a – r; *pvl*: Panton-Valentine leukocidin gene; *exfoA – B*: exfoliatin toxin A – B genes; *TSST-1*: toxic shock syndrome toxin-1 gene.

No single *se* was present in all of the 79 subjects in which an *se* was detected. The most frequently detected *se* were those of the *egc* (enterotoxin gene cluster: *seg, sem, sen, sei, seo, seu*): present in 75/79 (94.9%). Indeed, all subjects in the CRSsNP and control groups who had an *se* detected demonstrated the *egc*, while the only four patients without genes of this cluster (but with other *se* present) were in the CRSwNP group. Other commonly detected *se* were *tsst-1* (19/79; 24%), *sea, sec, sel* (14/79; 17.7%), *sep, seh* (10/79; 12.7%) and *seq* (9/79; 11.4%). In contrast, *see*, *sek* and *etb* were not detected in any subject. All other exotoxins were detected in only 1 or 2 subjects.

4.2.2. Comparison of *se* between study groups

Within the study groups, at least one *se* was detected in 46/55 subjects with CRSwNP (83.6%), 15/16 with CRSsNP (93.7%) and 18/22 controls (81.2%). Using the Fisher exact test, there were no statistically significant differences between these rates: comparing CRSwNP and controls (p= 0.253), CRSwNP and CRSsNP (p= 0.22), or CRSsNP and controls (p= 0.233).

The rates of each individual *se* detected in the study groups were compared by the Fisher exact test. There were significantly more *sep* (p= 0.039) and *seq* (p= 0.033) detected in the CRSwNP group than in the controls: indeed, these genes were present in 8 and 9 CRSwNP subjects (7 had both), respectively, but not found in any control. In contrast, there was no difference in the detection rates of any other *se* between any of the study groups, including those most commonly studied in CRSwNP in the past: *sea*, *seb*, *sec* and *tsst-1*. Refer to Figure 3, below.

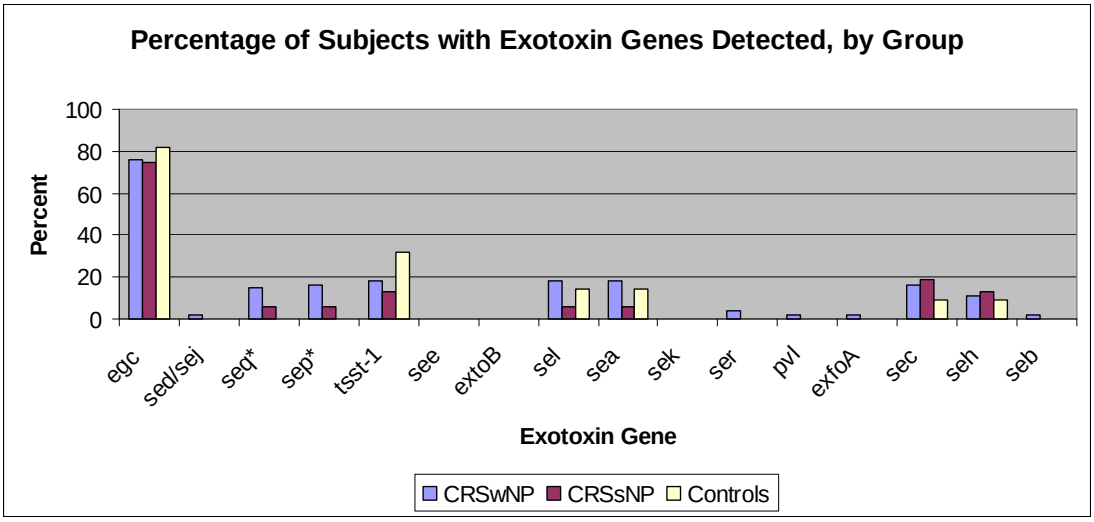

Figure 3: Percentage of Subjects with Exotoxin Genes Detected, by Study Group. CRSwNP: chronic rhinosinusitis with nasal polyposis; CRSsNP: chronic rhinosinusitis without nasal polyposis; *egc*: enterotoxin gene cluster of *seg, sem, sen, sei, seo, seu*; *sea – r*: Staphylococcal enterotoxin genes a – r; *pvl*: Panton-Valentine leukocidin gene; *exfoA – B*: exfoliatin toxin A – B genes; *TSST-1*: toxic shock syndrome toxin-1 gene.

Overall, there were 318 *se* detected amongst the 55 subjects with CRSwNP, 85 detected amongst the 16 subjects with CRSsNP and 125 detected amongst the 22 controls. Using the Mann Whitney U Test, there was no statistically significant difference between the overall numbers of *se* detected in the CRSwNP and control groups (p= 0.532), the CRSwNP and CRSsNP groups (p= 0.259) and the CRSsNP and control groups (p= 0.492). The median number of *se* per subject was 7 in the CRSwNP group (range 0 – 11), 6 in the CRS group (range 0 – 8) and 6 in the controls (range 0 – 8) [see Figure 4, below]. Hence, none of the SA strains detected carried all of the 22 currently known *se*.

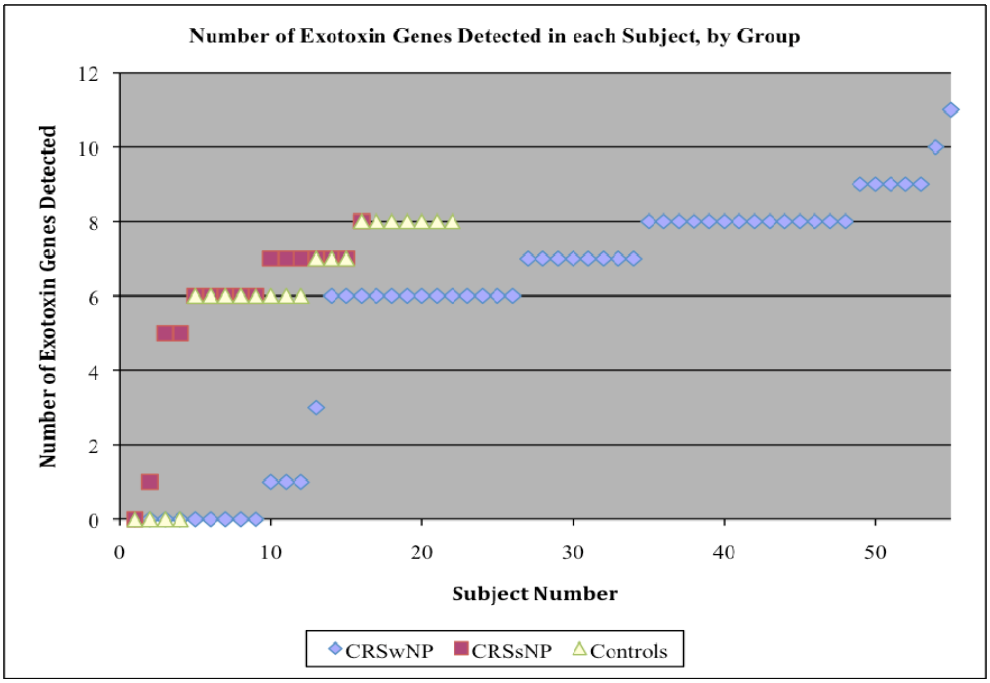

Figure 4: Number of Exotoxin Genes Detected in each Subject, by Study Group. CRSwNP: chronic rhinosinusitis with nasal polyposis; CRSsNP: chronic rhinosinusitis without nasal polyposis. Median values of exotoxin genes detected per subject were: 7 for CRSwNP, 6 for CRSsNP , 6 for Controls.

4.2.3. Comparison of *se* between CRSwNP subgroups

Within the CRSwNP group, at least one *se* was detected in 24/25 subjects with NP1 (96%), 20/24 with NP2 (83%) and 2/6 with NP3 (33%). There was no statistically significant difference between the rates in the NP1 and NP2 groups (p= 0.15), but there were significantly more NP2 subjects with at least one *se* detected, compared to NP3 subjects (p= 0.029). Likewise, there were significantly more NP1 subjects with at least one *se* detected, compared to NP3 subjects (p= 0.002). In other words, there were relatively more NP3 subjects without any *se* detected, when compared to NP1 or NP2 subjects.

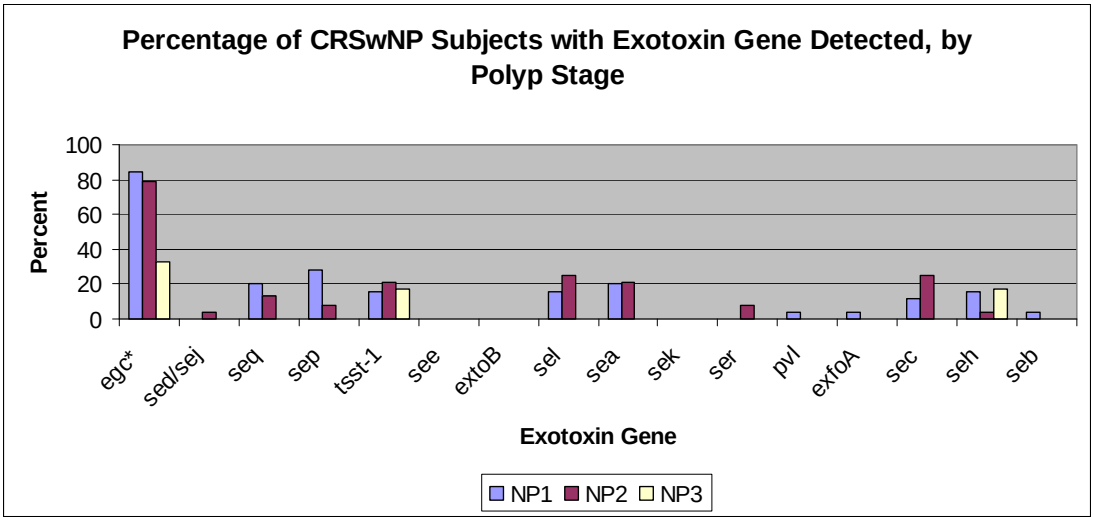

Figure 5: Percentage of Subjects with Chronic Rhinosinusitis with Nasal Polyposis who had Exotoxin Genes Detected, grouped by stage of polyp disease. *egc*: enterotoxin gene cluster of *seg, sem, sen, sei, seo, seu*; *sea – r*: Staphylococcal enterotoxin genes a – r; *pvl*: Panton-Valentine leukocidin gene; *exfoA – B*: exfoliatin toxin A – B genes; *TSST-1*: toxic shock syndrome toxin-1 gene. NP1 – 3: nasal polyp severity, stages 1 – 3 of Mackay and Nacleiro classification.

The rates of each individual *se* detected in the CRSwNP subgroups were compared by the Fisher exact test. There was no difference in the detection rates of any individual *se* between the NP1 and NP2 groups. However, NP3 subjects had a significantly lower rate of detection of the *egc* genes, compared to NP1 (p= 0.026) and NP2 (p= 0.049) subjects. All other *se* showed similar rates in all CRSwNP subgroups. Refer to Figure 5, above.

When the CRSwNP subgroups were analysed for overall numbers of *se* detected, there were 161 *se* detected in the 25 NP1 subjects (median = 7 *se* per subject), 144 in the 24 NP2 subjects (median = 7 *se* per subject) and 13 in the 6 NP3 subjects (median = 0 *se* per subject). Using the Mann Whitney U test, there was no significant difference between the overall numbers of *se* detected in the NP1 and NP2 (p= 0.275). However, there were significantly fewer *se* detected in the NP3 group, when compared to both the NP1 and NP2 groups (p= 0.009 and p= 0.036 respectively). As stated previously, only 2 of the 6 NP3 subjects had at least one *se* detected.

When analysing the CRSwNP subjects for Widal syndrome, there was a greater proportion in the NP3 group (3/6: 50%), compared to the NP1 (4/25: 16%) and NP2 groups (6/24: 24%), but these differences were not statistically significant. Of the 3 NP3 subjects with Widal syndrome, 2 had no *se* detected.

4.3. Quantification of bacteria

Of the CRSwNP subjects, 6/55 (11%) had low, 11/55 (20%) had moderate and 38/55 (69%) had abundant bacteria. Of the CRSsNP subjects, 5/16 (31%) had low, 5/16 (31%) had moderate and 6/16 (38%) had abundant bacteria. Of the control subjects, 14/22 (64%) had low, 6/22 (27%) had moderate and 2/22 (9%) had abundant bacteria. When comparing study groups, CRSwNP subjects had significantly more abundant bacteria compared to both controls ($p < 0.0001$) and CRSsNP ($p = 0.016$) subjects. Also, CRSsNP subjects had significantly more abundant bacteria than controls ($p = 0.013$). Refer to Figure 6, below.

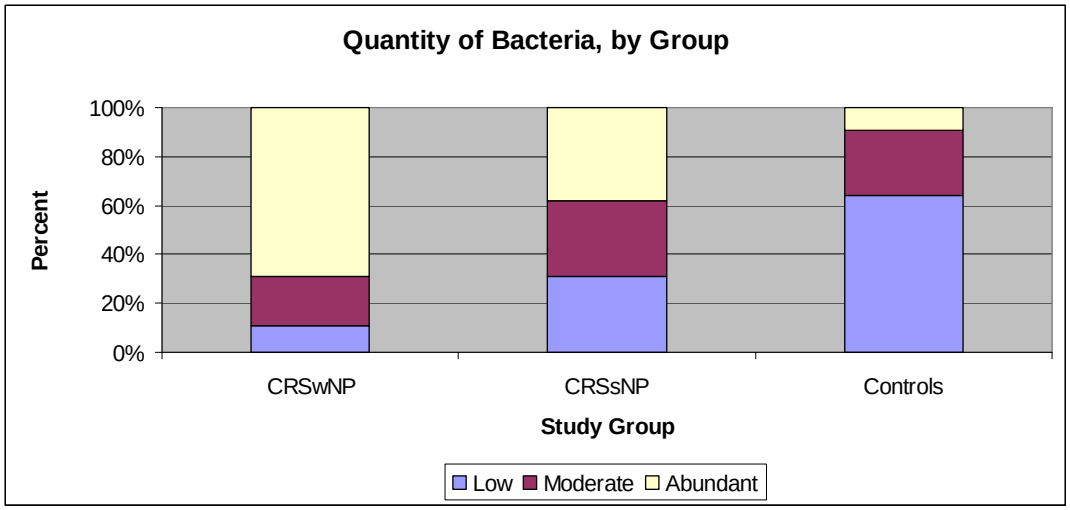

Figure 6: Quantity of Bacteria detected, by Study Group. CRSwNP: chronic rhinosinusitis with nasal polyposis; CRSsNP: chronic rhinosinusitis without nasal polyposis.

The CRSwNP subgroups were analysed with regards to quantity of bacteria. Of the NP1 subjects, 1/25 (4%) had low, 5/25 (20%) had moderate and 19/25 (76%) had abundant bacteria. Of the NP2 subjects, 2/24 (8%) had low, 6/24 (25%) had moderate and 16/24 (67%) had abundant bacteria. Of the NP3 subjects, 3/6 (50%) had low, 0/6 had moderate and 3/6 (50%) had abundant bacteria. There was no statistically significant difference in the quantity of bacteria in the NP1 and NP2 groups (p= 0.258), but there were significantly fewer bacteria in the NP3 group than in the NP1 (p= 0.0008) and NP2 (p= 0.005) groups. See Figure 7, below.

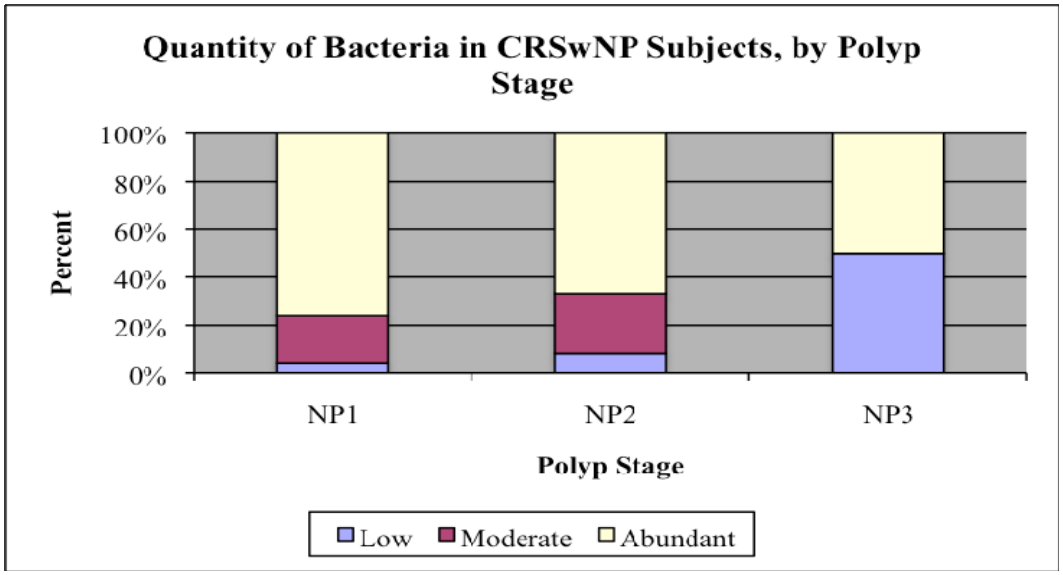

Figure 7: Quantity of Bacteria detected in Chronic Rhinosinusitis with Nasal Polyposis Subjects, by Study Group. CRSwNP: chronic rhinosinusitis with nasal polyposis; CRSsNP: chronic rhinosinusitis without nasal polyposis. NP1 – 3: nasal polyp severity, stages 1 – 3 of Mackay and Nacleiro classification.

5. DISCUSSION

The role of SE in the pathogenesis of CRSwNP has received much attention in recent years [17-19, 23, 24, 33]. Unfortunately, despite being a common chronic disease, the causes of CRS remain poorly understood. If treatments are to be improved, a better knowledge of its pathogenesis is crucial.

5.1. Self-criticism of this study

To date, this present study contains the largest patient numbers of any published work on *Staphylococcus aureus* exotoxins and CRS. The inclusion and exclusion criteria were designed to limit the effects of other pathologies or treatments upon the study findings. The laboratory staff was blinded as to which study group each specimen belonged, in attempt to reduce bias. The multi-centred enrolment of subjects was aimed at minimising geographical bias – collection at a single centre may have led to analysis of only limited SA strains. Instead, the current results showed that a variety of SA genotypes were collected and analysed. MLVA analysis suggested that neither the geographical origin, nor the genetic background of isolates, were associated with a specific population.

It is imperative to note that this present study identified the genes which encode exotoxins, rather than the exotoxins themselves. This was essential because, currently, no accurate and reliable test is available to detect and quantify all known SA exotoxins, once they have been synthesised or excreted. By identifying exotoxin genes, the aim was to explore which individual exotoxins might potentially be present in CRSwNP, CRSsNP and controls and compare the results. In effect, by identifying the gene, rather than the exotoxin, the maximum potential of the exotoxin being present is measured. The actual amounts of exotoxin present in nasal mucosa will be

influenced by the presence of the exotoxin gene, its expression and subsequent exotoxin production and secretion from the bacteria, and the quantity of bacteria present. In this study, only the presence of the gene and the quantity of bacteria have been measured, due to the aforementioned difficulties of measuring the exotoxins itself.

5.2. Pathogenesis of CRS and SE

A major finding of the present study was that there was no correlation between the presence of at least one *se* and the presence of CRSwNP or CRSsNP: results for these groups were similar to the control group. Furthermore, there was no correlation between the overall numbers of *se* detected in each swab and the presence of CRSwNP or CRSsNP. Indeed, 9/55 subjects with CRSwNP had no *se* detected at all, suggesting that it is not essential in the pathogenesis of the disease. This contrasts with findings of some previous studies which found SE to be have high prevalence in CRSwNP [19, 24].

The prevalence of the 22 currently-known SE was individually compared between the study groups. There was no correlation between the presence of any individual *se* and the presence of CRSwNP or CRSsNP, with 2 exceptions: significantly higher numbers of *sep* and *seq* were detected in CRSwNP. In fact, these two *se* were not found in any control subjects. However, they were only detected in a small minority of CRSwNP subjects (16% and 14.5% respectively), so that this finding is difficult to interpret with current data. Interestingly, 7/55 CRSwNP subjects (12.7%) had SA carrying *sep* and *seq* simultaneously, reflecting two distinct genetic transfers in a single bacteria – they are not known to form a cluster. While recent studies suggest a role for SEP [34] and SEQ [35] in food poisoning, there is no publication yet on their

potential role in CRS. Hence, further research into the effect of SEP and SEQ on sinonasal mucosa appears justified.

SEB has been reported as potentially important in the pathogenesis of CRSwNP because it polarised inflammation to a Th2 pattern and suppressed induced T-regulatory lymphocytes, thus potentially prolonging the inflammation [18]. The relevance of this finding to the majority of CRSwNP patients must now be questioned, given that, in the present study, only 1/55 subjects with CRSwNP had the gene for SEB. Likewise, other SE which have been proposed as important to worsening inflammation in CRSwNP, were only found in a small minority of such subjects: SEA and TSST-1 were each present in only 10/55 subjects with CRSwNP, a similar rate to that of controls.

One recent study had a similar method to this present study [36]. The major differences were that the other study had fewer patients with SA strains detected (26 CRSwNP, 14 controls) and fewer *se* were examined for (11 instead of our 22: *sep* and *seq* were not tested). In that study, 75% of the SA strains contained at least one *se*, in contrast to 85% in this present study. That may be because this present study tested for a greater number of *se*. The similar results in both studies were that genes of the *egc* were the most frequently identified and that there was no difference in prevalence of *se* in controls and CRSwNP subjects. Essentially the studies show similar results, but the present study is more comprehensive in its review of all known *se* and contains larger subject numbers. Also, both studies produced results which refute earlier claims that SA, if present in the nose, always produces SE [19].

5.3. Severity of CRSwNP and SE

Some studies have found that exotoxins changed the balance of inflammatory mediators to worsen inflammation [18, 19, 37]. While this may be true *in vitro*, the data from this present study suggested that the degree of these inflammatory changes is not clinically relevant: there was no significant difference in the quantity of *se* detected between NP1 and NP2 subjects groups. Indeed, there were significantly fewer *se* detected in NP3 subjects, when compared to both NP1 ($p= 0.009$) and NP2 ($p= 0.036$). Interpretation of this result remains limited by the small number of subjects in NP3 group. However, it should be noted that of the 9 CRSwNP subjects with no *se* detected, a greater proportion were in the more severely-diseased subgroups: 1 in NP1, 4 in NP2 and 4 in NP3. A possible explanation for this lies in the fact that 50% of the NP3 group had Widal syndrome and the pathogenesis of CRSwNP in Widal syndrome is distinct from non-Widal CRSwNP: it is related to anomalies in the biosynthesis of eicosanoid mediators and eicosanoid receptor expression [48]. Hence, the contribution of SE to the severity of the mucosal inflammation of CRSwNP in Widal syndrome may be less relevant than in non-Widal disease.

There was no difference in the prevalence of individual *se* in the CRSwNP subgroups, except for *egc*, which was significantly less prevalent in NP3 compared to both NP1 and NP2. As *egc* genes were the commonest group, this most likely reflects the general result for *se* overall.

5.4. CRSwNP and bacterial quantity

SA colonisation has been previously reported as 60% in CRSwNP, 27% in CRSsNP and 33% in controls [17]. In the present study, all analysed subjects had SA colonisation and there was a correlation between the severity of the inflammatory

changes and the abundance of the bacteria present: CRSwNP subjects had significantly more abundant bacteria compared to both controls ($p< 0.0001$) and CRSsNP ($p= 0.016$) subjects, while CRSsNP subjects had significantly more abundant bacteria than controls ($p= 0.013$). In other words, not only do a larger proportion of CRSwNP patients have SA colonisation, but once colonised, they have more abundant bacteria than controls or CRSsNP.

However, the same correlation did not exist within the CRSwNP subgroups: there was no statistical difference in the quantity of bacteria present in NP1 and NP2 subjects, while there were actually significantly fewer bacteria in the NP3 group than in the NP1 ($p= 0.0008$) and NP2 ($p= 0.005$) groups. This means that in CRSwNP, disease severity did not correlate with more abundant bacteria. It is important to remember that 50% of the NP3 group had Widal syndrome, which most likely has a pathogenesis based on metabolic anomalies, distinct from non-Widal CRSwNP. While there were no statistically significant differences in the presence of Widal syndrome between CRSwNP subgroups, the NP3 group had a much smaller number of subjects than the other 2 groups and this may have affected the results.

It is unclear whether the higher rate of SA colonisation and the greater numbers of bacteria found in CRSwNP lead to worsening inflammation or, instead, the impaired mucociliary clearance due to pre-existing inflammation from some other cause, allows greater numbers of bacteria to accumulate in a larger proportion of patients. A third possibility is that both situations exist simultaneously. *In vitro* work has shown that SA-derived protein A, lipoteichoic acid and SEB all worsen inflammation in CRSwNP [18]. High dose SEA acted to lower ciliary beat frequency in rabbit sinuses [22], suggesting that it may contribute to the reduced mucociliary clearance found in CRS. However, ciliary beat frequency and its role in mucociliary clearance in CRSwNP is a

complex issue and likely to be influenced by multiple factors, rather than a single agent [38].

5.5. Future directions in study of pathogenesis of CRS

More research is needed into the systemic host factors thought to be important in the pathogenesis of CRS. Recent findings suggested that the genetic background of the patient is relevant [39]. Also, an abnormal immune response to bacterial components present in the nasal mucosa may play a role – hence both bacteria and abnormal host immune factors may be essential for disease development [36]. Moreover, data on other chronic inflammatory diseases has led to the concept of a "neuroimmune" system, to acknowledge that the neurological and neuroendocrine systems work simultaneously with the immune system to restore homeostasis in the setting of an inflammatory challenge [40, 41]. Future research on the genetics of CRS patients and abnormalities in their neuroimmune system may prove fruitful.

Research is also needed into the environmental factors thought to be important in the pathogenesis of CRS. Biofilms are an area of particular interest. One study found that 44% of CRS patients had biofilms, whereas no controls exhibited biofilms [42]. Another study found biofilms in 14/18 CRS patients and 2/5 controls [43]. Post-operatively, 50% of CRS patients had biofilms in their sinus mucosa and these patients tended to have worse outcomes than post-operative CRS patients without biofilms [44]. Also, there is evidence that current culture techniques only reliably detect planktonic (free-floating) bacteria. This means that non-planktonic bacteria in biofilms may be present in CRS but are not detected by swab and culture [42, 43]. Instead, confocal scanning microscopy and fluorescent in situ hybridisation should be used to identify microbial biofilms in CRS mucosa [10].

In addition to its ability to produce exotoxins, there is evidence that SA can survive intracellularly for various periods of time, within epithelial and endothelial cells, fibroblasts, osteoblasts, keratinocytes and even macrophages and neutrophils [45]. It has been suggested that this intracellular reservoir may be responsible for chronic or relapsing infection. In human nasal mucosa, SA has been detected within epithelial, glandular and myofibroblastic cells [46]. In a study of CRS patients followed over a 12 month period, the presence of intracellular SA was found to be a risk factor for recurrent episodes of CRS, refractory to medical and surgical therapy [47]. Certain varieties of SA, called small-colony variants, can arise during antimicrobial treatment and can cause subacute, antibiotic-resistant infections [45]. These troublesome variants may actually be an integral part of the life-cycle of SA. Further research into the host and bacterial mechanisms allowing SA intracellular persistence may provide insights into the pathogenesis of CRS.

6. CONCLUSION

This blinded, prospective, multi-centred trial of 93 *Staphylococcus aureus* nasal carriers showed no correlation between the presence or overall numbers of *Staphylococcus aureus* exotoxin genes (*se*) and CRSwNP or CRSsNP. Moreover, there was no correlation between the presence or overall numbers of *se* and the severity of CRSwNP. This study does not support the assertion that *Staphylococcus aureus* exotoxins worsen inflammation in CRS.

7. REFERENCES

1. Fokkens W, Lund V, Mullol J. European position paper on rhinosinusitis and nasal polyps 2007. *Rhinol Suppl.* 2007(20):1-136.

2. Collins JG. Prevalence of selected chronic conditions: United States, 1990-1992. *Vital Health Stat 10.* Jan 1997(194):1-89.

3. Chen Y, Dales R, Lin M. The epidemiology of chronic rhinosinusitis in Canadians. *Laryngoscope.* Jul 2003;113(7):1199-1205.

4. 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.* Jul 2003;112(7):625-629.

5. Benninger MS, Ferguson BJ, Hadley JA, et al. Adult chronic rhinosinusitis: definitions, diagnosis, epidemiology, and pathophysiology. *Otolaryngol Head Neck Surg.* Sep 2003;129(3 Suppl):S1-32.

6. Georgitis JW, Matthews BL, Stone B. Chronic sinusitis: characterization of cellular influx and inflammatory mediators in sinus lavage fluid. *Int Arch Allergy Immunol.* Apr 1995;106(4):416-421.

7. Stierna P, Carlsoo B. Histopathological observations in chronic maxillary sinusitis. *Acta Otolaryngol.* Nov-Dec 1990;110(5-6):450-458.

8. Post JC, Hiller NL, Nistico L, Stoodley P, Ehrlich GD. The role of biofilms in otolaryngologic infections: update 2007. *Curr Opin Otolaryngol Head Neck Surg.* Oct 2007;15(5):347-351.

9. Hall-Stoodley L, Stoodley P. Biofilm formation and dispersal and the transmission of human pathogens. *Trends Microbiol.* Jan 2005;13(1):7-10.

10. Hunsaker DH, Leid JG. The relationship of biofilms to chronic rhinosinusitis. *Curr Opin Otolaryngol Head Neck Surg.* Jun 2008;16(3):237-241.

11. Harvey RJ, Lund VJ. Biofilms and chronic rhinosinusitis: systematic review of evidence, current concepts and directions for research. *Rhinology.* Mar 2007;45(1):3-13.

12. Kakoi H, Hiraide F. A histological study of formation and growth of nasal polyps. *Acta Otolaryngol.* Jan-Feb 1987;103(1-2):137-144.

13. Stoop AE, van der Heijden HA, Biewenga J, van der Baan S. Eosinophils in nasal polyps and nasal mucosa: an immunohistochemical study. *J Allergy Clin Immunol.* Feb 1993;91(2):616-622.

14. Van Zele T, Claeys S, Gevaert P, et al. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. *Allergy.* Nov 2006;61(11):1280-1289.

15. Floret D. [Clinical aspects of streptococcal and staphylococcal toxinic diseases]. *Arch Pediatr.* Sep 2001;8 Suppl 4:762s-768s.

16. Alouf JE, Muller-Alouf H. Staphylococcal and streptococcal superantigens: molecular, biological and clinical aspects. *Int J Med Microbiol.* Feb 2003;292(7-8):429-440.

17. Bachert C, Zhang N, Patou J, van Zele T, Gevaert P. Role of staphylococcal superantigens in upper airway disease. *Curr Opin Allergy Clin Immunol.* Feb 2008;8(1):34-38.

18. Patou J, Gevaert P, Van Zele T, Holtappels G, van Cauwenberge P, Bachert C. Staphylococcus aureus enterotoxin B, protein A, and lipoteichoic acid stimulations in nasal polyps. *J Allergy Clin Immunol.* Jan 2008;121(1):110-115.

19. Bernstein JM, Kansal R. Superantigen hypothesis for the early development of chronic hyperplastic sinusitis with massive nasal polyposis. *Curr Opin Otolaryngol Head Neck Surg.* Feb 2005;13(1):39-44.
20. Gevaert P, Holtappels G, Johansson SG, Cuvelier C, Cauwenberge P, Bachert C. Organization of secondary lymphoid tissue and local IgE formation to Staphylococcus aureus enterotoxins in nasal polyp tissue. *Allergy.* Jan 2005;60(1):71-79.
21. Van Zele T, Gevaert P, Watelet JB, et al. Staphylococcus aureus colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. *J Allergy Clin Immunol.* Oct 2004;114(4):981-983.
22. Min YG, Oh SJ, Won TB, et al. Effects of staphylococcal enterotoxin on ciliary activity and histology of the sinus mucosa. *Acta Otolaryngol.* Sep 2006;126(9):941-947.
23. Conley DB, Tripathi A, Seiberling KA, et al. Superantigens and chronic rhinosinusitis: skewing of T-cell receptor V beta-distributions in polyp-derived CD4+ and CD8+ T cells. *Am J Rhinol.* Sep-Oct 2006;20(5):534-539.
24. Seiberling KA, Conley DB, Tripathi A, et al. Superantigens and chronic rhinosinusitis: detection of staphylococcal exotoxins in nasal polyps. *Laryngoscope.* Sep 2005;115(9):1580-1585.
25. Malm L. Assessment and staging of nasal polyposis. *Acta Otolaryngol.* Jul 1997;117(4):465-467.
26. Institute. CaLS. *Performance standards for antimicrobial susceptibility testing: 15th informational supplement.* Wayne. PA: Clinical and Laboratory Standards Institute; 2005.
27. Letertre C, Perelle S, Dilasser F, Fach P. Identification of a new putative enterotoxin SEU encoded by the egc cluster of Staphylococcus aureus. *J Appl Microbiol.* 2003;95(1):38-43.
28. Francois P, Harbarth S, Huyghe A, et al. Methicillin-resistant Staphylococcus aureus, Geneva, Switzerland, 1993-2005. *Emerg Infect Dis.* Feb 2008;14(2):304-307.
29. Francois P, Koessler T, Huyghe A, et al. Rapid Staphylococcus aureus agr type determination by a novel multiplex real-time quantitative PCR assay. *J Clin Microbiol.* May 2006;44(5):1892-1895.
30. Francois P, Huyghe A, Charbonnier Y, et al. Use of an automated multiple-locus, variable-number tandem repeat-based method for rapid and high-throughput genotyping of Staphylococcus aureus isolates. *J Clin Microbiol.* Jul 2005;43(7):3346-3355.
31. Koessler T, Francois P, Charbonnier Y, et al. Use of oligoarrays for characterization of community-onset methicillin-resistant Staphylococcus aureus. *J Clin Microbiol.* Mar 2006;44(3):1040-1048.
32. Fischer A FP, Holtfreter S, Broker BM, Schrenzel J. Development and evaluation of a rapid strategy to determine enterotoxin gene content in *Staphylococcus aureus*. *J Microbiol Methods.* 2009;in press.
33. Conley DB, Tripathi A, Seiberling KA, et al. Superantigens and chronic rhinosinusitis II: analysis of T-cell receptor V beta domains in nasal polyps. *Am J Rhinol.* Jul-Aug 2006;20(4):451-455.
34. Bania J, Dabrowska A, Bystron J, Korzekwa K, Chrzanowska J, Molenda J. Distribution of newly described enterotoxin-like genes in Staphylococcus aureus from food. *Int J Food Microbiol.* Apr 15 2006;108(1):36-41.

35. Chiang YC, Liao WW, Fan CM, Pai WY, Chiou CS, Tsen HY. PCR detection of Staphylococcal enterotoxins (SEs) N, O, P, Q, R, U, and survey of SE types in Staphylococcus aureus isolates from food-poisoning cases in Taiwan. *Int J Food Microbiol.* Jan 15 2008;121(1):66-73.
36. Van Zele T, Vaneechoutte M, Holtappels G, Gevaert P, van Cauwenberge P, Bachert C. Detection of enterotoxin DNA in Staphylococcus aureus strains obtained from the middle meatus in controls and nasal polyp patients. *Am J Rhinol.* May-Jun 2008;22(3):223-227.
37. Dohin B, Gillet Y, Kohler R, et al. Pediatric bone and joint infections caused by Panton-Valentine leukocidin-positive Staphylococcus aureus. *Pediatr Infect Dis J.* Nov 2007;26(11):1042-1048.
38. Chen B, Antunes MB, Claire SE, et al. Reversal of chronic rhinosinusitis-associated sinonasal ciliary dysfunction. *Am J Rhinol.* May-Jun 2007;21(3):346-353.
39. Pinto JM, Hayes MG, Schneider D, Naclerio RM, Ober C. A genomewide screen for chronic rhinosinusitis genes identifies a locus on chromosome 7q. *Laryngoscope.* Nov 2008;118(11):2067-2072.
40. Straub RH, Besedovsky HO. Integrated evolutionary, immunological, and neuroendocrine framework for the pathogenesis of chronic disabling inflammatory diseases. *Faseb J.* Dec 2003;17(15):2176-2183.
41. Steinman L. Elaborate interactions between the immune and nervous systems. *Nat Immunol.* Jun 2004;5(6):575-581.
42. Psaltis AJ, Ha KR, Beule AG, Tan LW, Wormald PJ. Confocal scanning laser microscopy evidence of biofilms in patients with chronic rhinosinusitis. *Laryngoscope.* Jul 2007;117(7):1302-1306.
43. Sanderson AR, Leid JG, Hunsaker D. Bacterial biofilms on the sinus mucosa of human subjects with chronic rhinosinusitis. *Laryngoscope.* Jul 2006;116(7):1121-1126.
44. Psaltis AJ, Weitzel EK, Ha KR, Wormald PJ. The effect of bacterial biofilms on post-sinus surgical outcomes. *Am J Rhinol.* Jan-Feb 2008;22(1):1-6.
45. Garzoni C, Kelley WL. Staphylococcus aureus: new evidence for intracellular persistence. *Trends Microbiol.* Feb 2009;17(2):59-65.
46. Clement S, Vaudaux P, Francois P, et al. Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent Staphylococcus aureus rhinosinusitis. *J Infect Dis.* Sep 15 2005;192(6):1023-1028.
47. Plouin-Gaudon I, Clement S, Huggler E, et al. Intracellular residency is frequently associated with recurrent Staphylococcus aureus rhinosinusitis. *Rhinology.* Dec 2006;44(4):249-254.
48. Palikhe NS, Kim JH, Park HS. Update on Recent Advances in the Management of Aspirin Exacerbated Respiratory Disease. *Yonsei Medical J.* Dec 2009; 50(6): 744-750.

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