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COMPLICATIONS DUE TO ACUTE RHINOSINUSITIS IN CHILDREN

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Karolinska Institutet

Stockholm 2021

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Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2021

ISBN 978-91-8016-199-2

Complications due to rhinosinusitis in children

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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The thesis will be defended in public at Rockefeller lecture hall, Karolinska Institutet Solna, Nobels väg 11, Friday May 7th 2021 at 9.00 am.

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To my mother Christina Hultman

POPULÄRVETENSKAPLIG SAMMANFATTNING

Akut rinosinuit är en inflammation i näs- och bihåleslemhinnan som ofta beror på en virusinfektion och är en del av en vanlig förkylning. Den akuta rinosinuiten orsakas ibland av bakterier, och i sällsynta fall kan den bakteriella infektionen i näsa och bihålor sprida sig till omkringliggande vävnader – komplikationer uppstår. Under barndomen tillväxer bihålorna gradvis. I takt med bihåleutvecklingen skiljer sig komplikationerna till akut rinosinuit åt i olika åldrar. Komplikationer kring och i ögat är vanligast hos mindre barn och komplikationer i hjärnan förekommer mest hos äldre barn. Akuta virusorsakade rinosinuiter eller förkylningar är mycket vanliga hos barn och vi vet att komplikationer är ovanliga. Siffror på förekomsten av komplikationer har dock saknats till stor del, och det finns få studier baserade på hela populationer. Diagnostiken av akut bakteriell rinosinuit samt komplikationer hos barn kan vara svårt och bygger till stor del på anamnes och klinisk undersökning. En av de vanligaste bakterierna vid infektioner i näsan hos barn är pneumokocker.

Flera faktorer som verkar ha en inverkan på akut bakteriell rinosinuit och relaterade komplikationer diskuteras i litteraturen. I denna avhandling analyserades pneumokock-vaccinets införande i Stockholm, förekomsten av olika typer av luftvägsvirus, allergi mot luftburna allergen och låga nivåer av immunglobuliner i blodet hos barn.

I alla delarbeten i denna avhandling undersöktes barn som var inlagda på sjukhus i Region Stockholm p.g.a. akut rinosinuit och relaterade komplikationer. Komplikationerna kategoriserades, och svåra komplikationer var de djupare ögon-komplikationerna, samt skallben- och hjärnkomplikationerna, som var bekräftade med skikt- eller magnetröntgen.

I det första delarbetet undersöktes barn upp till fem år, under de fyra sista åren innan införandet av pneumokock-vaccin (213 inläggningar, 203 individer). Vi fann att 44 barn per 100 000 barn i samma ålder i Stockholm, per år, behövde sjukhusvård. Av de barn som lades in hade över 80 % tecken till ögon-komplikation men endast 3 % hade en svår ögon-komplikation, de flesta förbättrades snabbt med behandling med antibiotika och endast ett barn behövde operation. Pneumokocker var den vanligaste bakterien vi fann i odlingar från näsa och blod.

I det andra delarbetet undersöktes barn upp till fem år, under åtta år efter införandet av pneumokock-vaccin (217 inläggningar, 215 individer). Vi fann att antalet barn i populationen som behövde inläggning minskade jämfört med första delarbetet, till 19 barn per 100 000 per år. Incidensen för svåra komplikationer och operationer ökade dock något. Vidare fann vi att pneumokocker inte längre dominerade i odlingar från näsa och blod, där bakterierna *Haemophilus influenzae* och *Streptococcus pyogenes* var mer framträdande. Inga pneumokocker fanns i operations-odlingarna.

I det tredje delarbetet undersöktes barn från fem upp till 18 år gamla under en 13-års period (310 inläggningar, 304 individer). Vi fann att: 8 barn per 100 000 per år behövde inläggning, 34 % hade en svår komplikation i öga, hjärna eller skallben, och att 17 % behövde opereras. Det fanns en trend relaterad till ålder, inläggning var vanligare i de yngre åldrarna, och en större andel av de äldre barnen hade en svår komplikation eller behövde operation. Resultaten från näs-odlingar liknade resultaten från delarbete II, och i operations-odlingarna var bakterien *Streptococcus milleri* helt dominerande. Pojkar lades in för sjukhusvård och hade svåra komplikationer i högre utsträckning jämfört med flickor i delarbete ett till tre.

I delarbete fyra samlade vi barn som lades in p.g.a. akut rinosinuit och komplikationer under en tre-års period i en s.k. prospektiv studie (55 individer). Odlingar och prover togs på dessa barn enligt ett förutbestämt protokoll. Vi fann skillnader mellan de två olika typer av näs-odlingar som togs. Odlingen som tas där bihålorna tömmer sig i näsan visade oftare växt av bakterier och en

annan sammansättning av bakterier, jämfört med odlingen tagen längre bak i näsan. När vi jämförde operations-odlingar tagna på de barn som opererades, såg vi att en relativt ny metod, bakterie DNA PCR, gav fler resultat jämfört med de traditionella odlingsmetoderna. I operations-odlingarna dominerade bakterien *Streptococcus milleri*, liksom i delarbete III. Vi fann att förekomst av vissa bakterier var associerat med kortare behandlingstid och lägre eller högre värden på laboratorie-prover.Vi fann att hälften av barnen hade förekomst av virus i näsan, att influensa virus var vanligast, och att virus i näsan var statistiskt associerat med mindre grad av komplikation. Vidare hade fler av barnen med bekräftat influensa virus en specifik bakterie i sina odlingar – *Streptococcus pyogenes* – jämfört med de övriga barnen. Vi fann att allergi testet som användes var positivt i 29 % av hela gruppen men i 50 % av de barn som behövde operation. Positivt allergitest var möjligen associerat med längre behandlings-tid med antibiotika. Vi fann inga avvikande immunglobulin resultat.

I alla delarbeten fann vi att antalet barn som var inlagda var som högst under vintermånaderna och som lägst under sommaren.

Sammanfattningsvis visar denna avhandling att komplikationer till akut rinosinuit är ovanligt hos barn. Barn upp till fem år får sällan svåra komplikationer men från fem år och uppåt löper barnen som läggs in en ansenlig risk att få svåra komplikationer. Vidare påvisar våra resultat de stora skillnader som finns v g komplikationer i olika åldrar, och att många olika aspekter måste vägas in i den kliniska bedömningen och behandlingen av dessa barn. Avhandlingen visar också på förändringar i samband med införandet av pneumokock-vaccin, och att luftburna virus och allergi kan ha en effekt på komplikationer till akut rinosinuit hos barn.

ABSTRACT

Background

There is a lack of population-based studies of complications to acute rhinosinusitis in children. Previous studies have demonstrated a possible effect of the conjugate pneumococcal vaccine (PCV) on hospital admissions and bacteriology in acute rhinosinusitis. There is a lack of prospective studies that investigate the possible association between complications to acute rhinosinusitis in children and: specific respiratory viruses, allergy sensitization and immunoglobulin levels in blood. The aim was to describe complications due to acute rhinosinusitis in children, 0-18 years old, in Stockholm, Sweden, including clinical presentation, incidence rates, results of bacterial cultures, and analyze factors that could have an effect on this rare disease – pneumococcal conjugate vaccine, concomitant virus infection, IgE-sensitization to airborne allergies and immunoglobulin levels.

Methods

All papers in this thesis included children hospitalized due to acute rhinosinusitis (ARS) and related complications in Stockholm Region. Paper I-III were population-based, observational cohort studies with retrospectively collected data. Paper I included children up to five years old, before the introduction of PCV, study period 2003-2007. Paper II included the same age but after PCV introduction, study period 2008-2016, and included a comparative data analysis with paper I. Paper III included children from five to 18 years old, study period 2003-2016. In paper I-III, hospital admissions of children with a discharge diagnosis of rhinosinusitis and related complications were included and reviewed.

Paper IV was a prospective cohort study of children up to 18 years old. The study period was april 2017 to april 2020. Inclusion criteria was acute bacterial rhinosinusitis and hospitalization. Data was gathered including: bacterial cultures from the nasopharynx, the nasal middle meatus, and bacterial cultures and broad-range 16s rDNA PCR from the surgical site; viral nasopharyngeal PCR; allergy sensitization IgE test; and immunoglobulins in blood.

In paper I-IV, the CT and MRI images were reviewed by a specialist in radiology. Incidence was expressed as cases per 100 000 children in studied age groups per year.

Results

There were 213 admissions (203 individuals) in paper I, 217 admissions (215 individuals) in paper II, 310 admissions (304 individuals) in paper III and 55 cases in paper IV. Comparing paper I and II, the incidence of hospital admission due to acute rhinosinusitis and related complications in children up to five years old decreased from 43.8 to 18.8 children per 100 000 per year, after the introduction of PCV. A CT/MRI verified postseptal orbital complication was found in 3.3% of admissions in paper I (mean incidence 1.51) and in 13.4% in paper II (mean incidence 2.54). Surgery increased from 0.5% of the admissions in paper I (mean incidence 0.22) to 4.1% in paper II (mean incidence 0.79). In paper III, the incidence of hospital admission due to ARS and related complications in children from five to 18 years old was 7.8 per 100 000 per year. A CT/MRI verified severe complication (postseptal orbital, intracranial or osseous) was found in 34%, representing an incidence of 2.6 per 100 000 per year. Surgery was performed in 17% of admissions (mean incidence 1.3). In paper I-III, between 80-96% of admissions had preseptal cellulitis. In paper I-II, males had a higher incidence of admission and postseptal complication compared to females. In paper III, males had a higher incidence of admission, all type of complications and surgery, compared to females. *S. pneumoniae* was the most common bacteria found in nasal and blood cultures in paper I, but was not dominant in the nasal or

blood cultures and absent in the surgical cultures in paper II. *H. influenzae* and *S. pyogenes* dominated in the nasal cultures in paper III, *S. milleri* was the dominating bacteria in surgical cultures (33%), and *S. pyogenes* dominated in blood cultures.

In paper IV, cultures from the middle meatus were positive for bacterial growth and displayed a wider range of bacteria compared to the nasopharyngeal cultures. There was a match of at least one type of bacteria in the MM and NPH culture in 36% of the cases. *M. catarrhalis* was possibly associated with a lower number of days with intravenous antibiotics (-1.3 p=0.055), *H. influenzae* and *S. pneumoniae* negatively associated with max CRP (-38.9 p=0.028 and -45.5 p = 0.023), and *S. pyogenes* positively associated with max CRP (57.5 p=0.007). *S. milleri* was found in the surgical culture in 58% of the cases that has surgery. The nasal cultures were negative in 58% of the cases that had surgery. In the surgical cultures, 16S rDNA PCR resulted in a higher number of positive results in comparison to the traditional swab and tissue cultures. The viral nasopharyngeal PCR was positive in 53% of the cohort in paper IV, and influenza A was most common. Positive viral PCR was associated with a lower grade of complication (-1.3, p=0.028) and CRP max (-36.2, p=0.05). Influenza virus was possibly associated with a lower grade of complication (-2.2, p=0.055). An association was found between *S. pyogenes* and influenza A/B positive cases (1.5 p=0.040). The cases with a positive viral PCR and total number of cases followed the same monthly distribution during the year. The allergy sensitization test was positive in 29% of the cohort and in 50% of the cases that had surgery, and possibly associated with a higher number of days with IV antibiotics (1.2, p=0.052). No cases had decreased immunoglobulins.

Conclusions

The incidence of complications to acute rhinosinusitis in children in the population is low. Children from five years and older that are hospitalized due to acute rhinosinusitis have a substantial risk of developing severe complications. There is a wide range of complications to rhinosinusitis, and many aspects needs to be considered in the caretaking of children with complications. The introduction of PCV, presence of certain airborne viruses and sensitization to airborne allergies seem to have an effect on acute rhinosinusitis and complications in children.

LIST OF SCIENTIFIC PAPERS

I. **Most preschool children hospitalised for acute rhinosinusitis had orbital complications, more common in the youngest and among boys**
Lina Schollin Ask*, Sofia Hultman Dennison*, Pär Stjärne, Anna Granath, Subhash Srivastava, Margareta Eriksson, Ann Lindstrand, Malin Ryd Rinder
*shared first authorship
Acta Paediatr. 2017 Feb;106(2):268-273.
doi: 10.1111/apa.13650. Epub 2016 Nov 24.

II. **Serious complications due to acute rhinosinusitis in children up to five years old in Stockholm, Sweden – Still a challenge in the pneumococcal conjugate vaccine era**
Sofia Hultman Dennison, Lina Schollin Ask, Margareta Eriksson, Anna Granath, Olof Hertting, Rutger Bennet, Ann Lindstrand, Patrick Masaba, Praxitelis Dimitriou, Pär Stjärne
Int J Pediatr Otorhinolaryngol. 2019 Jun;121:50-54.
doi: 10.1016/j.ijporl.2019.02.034. Epub 2019 Feb 25.

III. **A Swedish population-based study of complications due to acute rhinosinusitis in children 5 to 18 years old**
Sofia Hultman Dennison, Olof Hertting, Rutger Bennet, Margareta Eriksson, Mats Holmström, Lina Schollin Ask, Ann Lindstrand, Praxitelis Dimitriou, Pär Stjärne, Anna Granath
Submitted for publication

IV. **Complications to acute bacterial rhinosinusitis in children - a prospective study; bacterial cultures, virus detection, allergy sensitization and immunoglobulins**
Sofia Hultman Dennison, Anna Granath, Mats Holmström, Pär Stjärne, Olof Hertting
Manuscript

LIST OF ABBREVIATIONS

ABRS	Acute bacterial rhinosinusitis
ARS	Acute rhinosinusitis
CI	Confidence interval
CRP	C-reactive protein
CT	Computed tomography
ENT	Ear- nose- and throat
EPOS	European Position Paper on Rhinosinusitis and Nasal Polyps
ERS	Erythrocyte sedimentation rate
ICD-10	International Statistical Classification of Diseases and Related Health Problems - Tenth Revision
IRR	Incidence Rate Ratio
IV	Intravenous
MM	Middle meatus
MRI	Magnetic Resonance Imaging
MRSA	Methicillin-resistant Staphylococcus aureus
NPH	Nasopharyngeal
PCV	Pneumococcal conjugate vaccine
RS	Rhinosinusitis
WBC	White blood cell count

CONTENTS

1 INTRODUCTION

1.1 Rhinosinusitis

Rhinosinusitis (RS) is an inflammation of the mucosal membrane in the nasal cavity and the paranasal sinuses. RS is often viral and a part of an upper airway infection or common cold, but can develop into an acute bacterial rhinosinusitis (ABRS), and possibly to complications – a spread of the infection to the surrounding tissues. The term rhinosinusitis has largely replaced the term sinusitis since the morphology and the physiology of the mucosa of the nose and the sinuses are similar, and often engaged simultaneously, during an inflammation or infection.

The European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) defines pediatric acute rhinosinusitis as: an inflammation of the nose and the paranasal sinuses characterized by two or more symptoms one of which should be either nasal blockage/ obstruction/ congestion or nasal discharge (anterior/posterior nasal drip), and/or facial pain/pressure, and/or cough. Furthermore, when the diagnosis is given in clinical practice, there should be endoscopic signs of inflammation (nasal polyps, mucopurulent discharge primarily from the middle meatus, or edema/mucosal obstruction primarily in middle meatus) and/or computer tomography (CT) changes (mucosal changes within the ostiomeatal complex and/or sinuses) supporting the diagnosis. (1)

Acute rhinosinusitis (ARS) is defined as symptoms lasting less than 12 weeks. Viral rhinosinusitis often precedes acute bacterial rhinosinusitis (ABRS). ABRS is suggested when there is the presence of at least 3 symptoms/signs of: discolored discharge (with unilateral predominance), severe local pain, fever above 38°C, elevated erythrocyte sedimentation rate (ESR)/ C-reactive protein (CRP), and "double sickening" (i.e. a deterioration after an initial milder phase of illness). (1)

The incidence of acute, viral, and bacterial RS in the literature varies, depending on the definition criteria used, the setting (primary or tertiary care), and the study population. However, we know that upper airway infections are very common in children. There are studies estimating up to 14 episodes per year for preschool children (2) and 7-10 episodes per year for school children (3, 4) and studies estimate that between 4-10% of pediatric upper airway infections progress to ABRS (5-7). However, other studies report lower numbers, such as a Dutch study that presented an incidence of ABRS to 22-27 cases per 1000 children up to 17 years old (8).

1.2 Complications

A complication to acute bacterial rhinosinusitis occurs when the infection spreads to surrounding tissues and structures, such as skin, blood vessels, eye, bone or brain. The infection may spread through blood or directly through adjacent bone structures.
The paranasal sinuses develop gradually during childhood. The ethmoid and maxillary sinuses are partly developed at birth and reach full size around 15 years of age. The sphenoid and frontal sinuses start to develop later and are not fully developed until adulthood, see Table 1. There can be large individual variations in paranasal sinus development.

Table 1. Paranasal sinus development.

Sinus	Present at birth	First radiological evidence	Reaches adult size by
Maxillary sinus	Yes	0-5 months	15 years
Ethmoidal sinus	Yes	0-1 years	15-16 years
Sphenoidal sinus	No	4-5 years	After puberty
Frontal sinus	No	6-8 years	After puberty

The development of the paranasal sinuses throughout childhood has implications on the patterns of ABRS complications in different ages. Orbital complications from ABRS engaging the ethmoid and maxillary sinuses dominate in younger children (9). Intracranial and osseous complications with spread of infection from the sphenoid and frontal sinuses typically occur in older children and teenagers (10, 11).

Complications to ARS can be categorized as orbital, intracranial and osseous, examples are listed in Table 2. A grading system for orbital complications was developed by Chandler in the 1970s, see Table 3. (12). The classification according to Chandler is still widely used, although group 5 – cavernous sinus thrombosis – is now regarded primarily as an intracranial complication. Cavernous sinus thrombosis often originates from an infection in the sphenoid sinus rather than the ethmoid or frontal sinuses (13), and was categorized as an intracranial complication in this thesis.
The orbital complications can further be divided into preseptal (Chandler group 1) and postseptal complications (Chandler group 2-5), where the dividing anatomical structure is the orbital septum. The orbital septum is a membranous sheet that extends from the bone around the orbita into the eyelids, and creates a barrier protecting the orbital tissue. Postseptal complications are naturally considered to be more severe than the preseptal cellulitis.

Table 2. Complications of acute rhinosinusitis divided into orbital, intracranial and osseous complications.

Orbital	Preseptal cellulitis/abscess, orbital cellulitis, subperiosteal abscess, orbital abscess, myositis of extraocular muscles, optic neuritis
Intracranial	Meningitis, encephalitis, cerebritis, subdural or epidural empyema, epidural abscess, brain abscess, cavernous sinus thrombosis
Osseous	Osteomyelitis, subperiosteal abscess in the forehead (Potts puffy tumor), subgaleal abscess

Table 3. Chandler's classification of orbital complications (12)

1	Preseptal cellulitis	Inflammatory edema limited to eyelid
2	Orbital cellulitis	Inflammatory edema involving muscle and fat in the orbit
3	Subperiosteal abscess	Pus between bone and periosteum
4	Orbital abscess	Pus in orbital contents
5	Cavernous sinus thrombosis	Retrograde inflammation extending intracranially with bilateral symptoms, headache and cranial nerve palsy

Orbital complications are most common among the pediatric ARS complications, representing 60-75% in the literature, while intracranial complications account for 15-20% and osseous for 5-10% (1, 14).

There is no clear consensus of the incidence of complications to ARS in children. The results from studies vary, many are smaller, case or single institutional studies, and there is a lack of larger or population-based studies. Furthermore, studies differ in inclusion criteria, what type of complication is studied, age of subjects, and geographical setting. However, it is clear that only a small number of the cases with ARS progress and lead to a complication. Several of the population-based studies conducted internationally are based on register data. Two published population-based studies of orbital complications reported an incidence of 6.05 cases per 100 000 (15) children (USA) and 0.39-0.90 CT-verified or intraoperative cases per 1000 pediatric admissions (Canada) (16). Another publication, a register study with stratified sample data from four years (1997, 2000, 2003 and 2006), reported an

incidence of between 2.74 and 4.38 cases of sinusitis-caused intracranial abscesses in patients up to 17-20 years of age per million children per year (USA) (17). Yet another register study reported fewer ARS complications: only 25 children in a total pediatric population of 3.6 million (Netherlands) (9).

1.3 Diagnostics – bacterial cultures and radiology

The diagnosis of ARS and related complications in children is based on both medical history and clinical status, in order to verify the originating infection of ARS, evaluate the signs of ABRS, and to determine if a complication is present. Differential diagnoses to ARS that can cause preseptal cellulitis, such as skin infections, insect bites, eye infections, and dacrocystitis, need to be excluded. There are challenges in the diagnosis of ARS, especially in young children. There are difficulties related to clinical examination, such as the performance of endoscopy and the obtainment of representative bacterial cultures. Additionally, adequate radiology may include the need for anesthesia, which limits availability.

Radiology, either CT with contrast or Magnetic Resonance Imaging (MRI), is recommended if there are signs of a severe complication, insufficient clinical response to intravenous antibiotics, or to determine if surgery is needed (18). MRI is preferred when an intracranial complication is suspected, such as meningitis or intracranial abscess. CT is the most commonly used mode of radiology. CT without contrast or low-dose CT, as well as plain x-ray, do not provide sufficient information to determine the level of disease or complication. To determine if there is a cavernous sinus thrombosis, the CT with contrast has to be performed according to a certain protocol. Examples of symptoms that should make the clinician consider radiology are as follows:

- *Lack of improvement or deterioration of symptoms after 48 hours of treatment with intravenous antibiotics*
- *Decreased eye mobility, pain when test of eye mobility, ophtalmoplegia or ptosis*
- *Reduced visual acuity or reduced color vision*
- *Reduced vision or vision loss*
- *Reduced /affected afferent pupillary reflex (RAPD) or papillary edema*
- *Chemosis, exophtalmus*
- *Severe orbital pain, facial pain or headache*
- *Neurological deficits*
- *Convulsions, unconsciousness or somnolence*
- *Nausea, vomiting*
- *Stiff neck*
- *Vertigo*
- *Subcutaneous abscess formation in the forehead*
- *High fever*

To determine the predominant bacteria in ABRS, a culture specimen is preferably taken in the sinus that is affected. There are several difficulties associated with obtaining representative cultures from children. Sinus puncture (a needle puncture through the bony lower lateral wall of the nose) of the maxillary sinus is possible without anesthesia in adults, but rarely in children. The nasal mucosa is often swollen and an open drainage from the sinuses to the nasal cavity can be lacking. The rich nasopharyngeal flora in children leads to difficulties in interpreting the clinical representability of nasopharyngeal (NPH) cultures. Cultures taken during surgery due to complications of ABRS are often preceded by several doses of intravenous antibiotics and, thus, may be negative. Blood cultures are often negative.

However, there are studies in adults and one in children, that demonstrate a high similarity between cultures through sinus puncture and cultures of the nasal middle meatus, the area between the inferior and middle turbinate where the maxillary, ethmoid and frontal sinuses drain (19, 20).

Recently, new diagnostic methods have emerged to analyze presence of bacteria, including broad-range PCR and DNA sequencing. In this method, available bacterial DNA is first detected by PCR, and the bacterial identification is then obtained by DNA sequencing.

1.4 Treatment

Antibiotics that provide coverage of typical pathogens are recommended for a child that presents with ARS and any clinical signs of complication. Intravenous antibiotics are preferred if there is any risk of a complicated clinical course. Cefotaxime is currently the recommended choice of intravenous antibiotic at our institution. It provides coverage of *Staphylococcus aureus (S. aureus)*, *Streptococcus pyogenes (S. pyogenes)*, *Haemophilus influenzae (H. influenzae)*, *Streptococcus pneumoniae (S. pneumoniae)*, Gram-positive anaerobes and *Moraxella catarrhalis (M. catarrhalis)*, penetrates well to the CNS and has a bactericidal effect.

Surgery is recommended when there is abscess formation, in the orbita, intracranially or subcutaneously. However, some studies support that subperiosteal abscesses of smaller size can be treated successfully with intravenous antibiotics (21, 22). A worsening of symptoms or a lack of clinical improvement despite adequate treatment with intravenous antibiotics can also support the case for surgical intervention, with the aim of opening up the sinuses and reducing the infectious and inflammatory load. Based on current literature, EPOS has presented the following conditions for withholding surgical drainage: clear clinical improvement within 24-48 hours; no decrease in visual acuity; small (<0.5-1 ml in volume) medially located subperiosteal abscess; and no significant systemic involvement (23). Endoscopic surgery is considered to be a safe technique and is recommended first hand, with external approaches being used if necessary. Furthermore, one study found a reduced need for neurosurgical procedures with early endoscopic surgery (24).

1.5 Microbiology

The bacterial flora in the nasopharynx in children displays a wide variety of bacteria, and a viral upper respiratory infection alters the microbial profile and enables specific bacteria to flourish (25, 26). The composition of the nasal microbiome of children is still not fully understood, but several publications have emerged the last years in concordance with the new bacterial DNA/RNA sequencing techniques. Many of the studies include children under five years of age, and were designed to study nasal microbiota in relation to lower respiratory infections and asthma (27-31), based on the findings that the upper airway microbiota largely reflect the colonization of the lower airways (32, 33). The studies found that the nasopharyngeal microbiome in children consists of relatively few genus of bacteria, and can be categorized into profiles where one single bacterial genus dominates (27, 29, 31). The dominating genera varied to some degree between the studies but were mainly *M. catarrhalis*, *Haemophilus*, *Streptococcus, Staphylococcus*, *Corynebacterium*, and *Alloiococcus* (27, 29, 31). In the analysis of *Streptococcus* species in the nasal microbiome in one of the studies, *S. pyogenes* was only very sparsely found and *S. milleri* was not found at all (27). Changes in the nasal microbiome profile were found to occur both with age and during upper airway and lower airway infections. One study found the dominant bacteria during upper airway infections to be *Streptococcus* (predominantly Pneumococci), *Haemophilus* and *M. catarrhalis* (27).

The bacteria most commonly found in studies before the introduction of the pneumococcal conjugate vaccine (PCV), analyzing traditional bacterial culture swabs in children with acute bacterial rhinosinusitis, were *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. pyogenes* and anaerobes (34, 35).
Since the introduction of PCV, studies have reported a change in bacterial findings from airways and abscesses in children with ARS and related complications. While the presence of *S. pneumoniae* has decreased, the presence of *S. aureus*, methicillin-resistant *S. aureus (MRSA)*, *Streptococcus milleri (S. milleri)*, *S. pyogenes* and *H. influenzae* has increased (35-37). Additionally, a few studies, including one with a Stockholm cohort, found a nasopharyngeal pneumococcal serotype replacement, from more to less invasive serotypes, after the vaccine introduction (38, 39).

The antibiotic resistance patterns of bacteria in Sweden show that only 5-10% of the Pneumococci have a reduced susceptibility to penicillin. Around 30% of the *H. influenzae* and over 90% of *M. catarrhalis* are beta lactamase producing and resistant to penicillin. The level of multi-resistant *S. aureus* is low, 1-2%, and *S. pyogenes* is always sensitive to penicillin. (40, 41)

1.6 Pneumococcal conjugate vaccine

The 7-valent pneumococcal conjugate vaccine was introduced in Stockholm County/ Region on July 1st 2007, and replaced by the 13-valent vaccine in January 2010. The immunization series consists of three doses, given at three, five and 12 months of age. The coverage rate reached 96% within two years of introduction and 97% within three years (42).

It has been shown in international studies that PCV introduction has decreased the burden of invasive pneumococcal disease in children, most significantly septicemia and meningitis, but to a lesser extent also pneumonia and otitis media (43-46). Furthermore, after the introduction of the vaccine, a majority of the invasive pneumococcal diseases are due to serotypes not included in the vaccine (40).

Since PCV introduction, studies have found a decrease in hospital admissions for children with acute rhinosinusitis and related complications. Two were larger database studies from the USA (15, 47), and one was a cohort study from Sweden (48) that used part of the same cohort as in study I and II in this thesis. In spite of the decrease in hospital admissions, the same American studies reported a significant increase in the number of complications, with one showing an increase in surgeries (15, 47).

1.7 Virus

With the establishment of molecular viral detection methods, new and old respiratory viruses are more easily detected. In children, rhinovirus, coronaviruses, influenza virus, human metapneumovirus, parainfluenza virus, adenovirus and respiratory syncytial virus, are examples of common findings. Boca viruses and enteroviruses are also found. Virus detection in children does not automatically prove a causal relationship. Especially rhinoviruses are found also in asymptomatic children.

It is well understood that respiratory viruses are responsible for a majority of upper airway infections and common colds, and trigger mucosal responses that can lead to ABRS (1). When the nasal mucosa is invaded by a virus, an inflammatory cascade is started that is thought to form the basis of immunological defense, activating and attracting immune system cells (23). Several studies present interactions between upper respiratory viruses, immunological molecules and bacteria that could negatively affect the immune defense and increase risk of bacterial superinfection (49-54).

Examples are: an increase of bacterial adherence to human respiratory cells in the presence of rhinovirus (55), a decrease in the immune cells ability to take up and kill bacteria due to influenza virus, and an influenza-virus induced alteration of the gene expression of *S. pneumoniae* to enhance spread of the bacteria in the mucosa (56). The association between influenza virus and *S. pyogenes* infection has largely been studied with epidemiological data (52-54), and there are several reviews published (50, 51). Theories presented of the pathology have included increased fibronectin in the epithelial cells and delay of bacteria clearance (50).

Prospective studies of potential links between specific viruses and ABRS complications are lacking. One prospective study of ABRS found a correlation between the presence of rhinovirus and *M. catarrhalis* in children up to three years old (7).

1.8 Allergic rhinitis and sensitization

Allergic rhinitis is the most common form of non-infectious rhinitis and is an IgE-mediated immune response in the nasal mucosa. The IgE immune response is driven by allergens, most commonly airborne allergens, in the sensitized individual. Symptoms of allergic rhinitis include anterior or posterior rhinorrhea (non-purulent), nasal blockage, nasal itching and sneezing, and ocular symptoms are also common (itchy, red or watery eyes). (57)

Allergic rhinitis leads to mucosal responses and edema, and since the nasal mucosa is continuous with that of the paranasal sinuses and the changes due to allergic rhinitis also can involve the paranasal mucosa, allergic rhinitis can be considered to be an allergic rhinosinusitis (23). The pathogenesis of allergic rhinitis includes allergen-specific IgE that attach to the surface of mast cells and basophils in the nasal mucosa, which are activated and release mediators that attract inflammatory and immune cells. Thereafter, the response to subsequent allergen exposure becomes stronger and the mucosa can also become hyperresponsive to irritants. (58)

Allergic rhinitis can be persistent or intermittent, and mild, moderate or severe. The diagnosis is based on symptoms, a medical history of previous similar symptoms related to exposure, and can be confirmed with diagnostic tests. The kinds of diagnostic tests most widely used to verify IgE-mediated allergy are skin prick test or serum IgE tests (serum-specific IgE or total serum IgE). The Phadiatop test is a commercially available serum IgE test with a reported sensitivity between 70-80% (59), and specificity around 90% (60), in studies of adults.

In childhood, the prevalence of allergic rhinitis increases with age (61-63). Studies from an ongoing population-based prospective birth cohort of children in Stockholm, Sweden, have reported 16% sensitized to airborne allergens at four years of age, 26% at 8 years of age and 42% at 16 years of age. Thereafter, the sensitization rate was relatively stable up to 24 years of age. (64, 65)
However, asymptomatic sensitization is common and it seems that the sensitization occurs prior to the development of allergic rhinitis symptoms. In one of the Stockholm cohort studies mentioned, it was shown that only one third of the children that were sensitized at four years of age, had developed symptoms of allergic rhinitis. When the children in the cohort were eight years old, the percentage of sensitized cases with symptoms of allergic rhinitis had increased to approximately 50%. (64)

The dominating allergens differ in different parts of the world (66).The most common airborne allergens in children in Sweden are birch, timothy and cat, and mites are common in the southern parts of Sweden. (65)

The concept of united airway disease describes the relationship between the upper and lower airways as a unified morphological and functional unit (57, 67, 68). It is well documented that allergy in the upper airways is associated with asthma (57, 69) (70), and studies also associate non-allergic rhinitis and upper airway infections with the development of asthma (27, 70).

Allergic rhinitis has been discussed as a risk factor for ARS and ABRS, but the results are ambiguous (71-73), and only a few studies include children (74, 75). An increased risk of lower respiratory infection in children with allergy sensitization was found in one study (27). An association between allergy and chronic rhinosinusitis has been shown in adults (76), but the results from the few studies on children are inconclusive (77-80). To our knowledge, the relationship between ABRS complications and airborne allergy sensitization has not been studied.

1.9 Immunoglobulin levels

Immunoglobulins are important mediators for protection against microbes. In the general population, IgA deficiency is the most common immunoglobulin deficiency, and most patients are asymptomatic (81). Immunodeficiency has been studied as a risk factor for rhinosinusitis, implicating a correlation between immunodeficiency and chronic rhinosinusitis in adults (82, 83). A few studies of children with chronic or recurrent rhinosinusitis have demonstrated decreased levels of immunoglobulins and a poor response to vaccines (84, 85). However, less is known about immunoglobulin deficiency in ARS and ABRS.To our knowledge, there are no prospective studies of immunoglobulin levels in children with ABRS complications.

2 RESEARCH AIMS

The overall aim of the thesis was to increase the knowledge of complications due to acute rhinosinusitis in children, 0-18 years old, in Stockholm, Sweden. This included the clinical presentation of complications; the incidence of admission; the patterns of complications and outcome in different ages; what bacteria was found in bacterial cultures in these children; and an analysis of factors that could have an effect on this rare disease – pneumococcal conjugate vaccine, concomitant virus infection, IgE-sensitization to airborne allergies and immunoglobulin levels.

Specific aims:

Paper I: To determine the admission rate; incidence of different types of complications; results from bacterial cultures; and clinical presentation in children up to five years old, from 2003 to 2007, before the introduction of the pneumococcal conjugate vaccine.

Paper II: To determine the admission rate; incidence of different types of complications and surgery; results from bacterial cultures; and clinical presentation in children up to five years old, from 2008 to 2016, after the introduction of the pneumococcal conjugate vaccine. Furthermore, to compare the results to the results from paper I.

Paper III: To determine the admission rate, incidence of different types of complication and surgery; results from bacterial cultures; and clinical presentation in children from five to 18 years old, from 2003 to 2016.

Paper IV: To compare the bacterial findings in cultures from different sites (nasopharyngeal, middle meatus, and surgical) in children hospitalized due to acute rhinosinusitis from 2017 to 2020. Furthermore, to investigate the potential role that airborne allergy sensitization, concomitant viral infection and abnormal immunoglobulin levels could have on complications of acute rhinosinusitis.

3 MATERIALS AND METHODS

The setting for all papers in this thesis was Stockholm Region, Sweden (until 2019 named Stockholm County). Stockholm Region has the largest catchment area in Sweden, approximately 2.4 million inhabitants (2.2-2.4 during the study periods), and the hospitals from which data was collected are the only providers of emergency pediatric care, including all hospital admissions, in the region. Papers I-III were retrospective studies, with paper I and II including children 0-5 years old and paper III children 5-18 years old. Paper IV was a prospective study, see Figure 1.

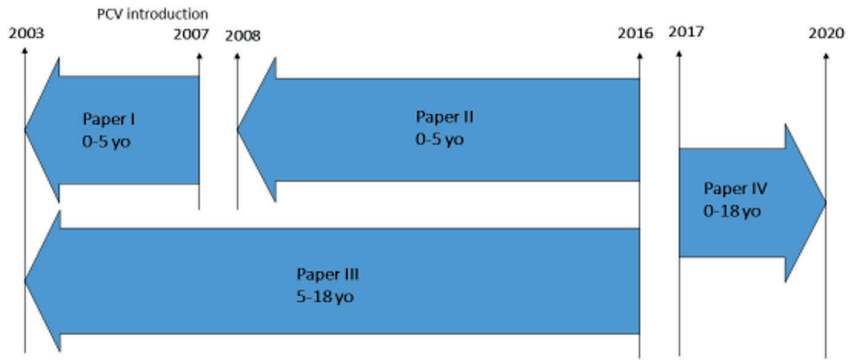

Figure 1. Paper I-IV, timeline, study ages, and arrows representing retrospective/prospective.

3.1 Paper I-III

Paper I-III were population-based, observational cohort studies with retrospectively collected data.

The study periods were:
Paper I: July 1st 2003 to July 1st 2007
Paper II: July 1st 2008 to July 1st 2016
Paper III: July 1st 2003 to July 1st 2016

In the analysis of the results, the study periods were divided into epidemic years with each study year starting July 1st and ending June 30th the following year.

The study period for paper II started one year after PCV was introduced in Stockholm. Furthermore, in paper II, an analysis was made to compare the results with the results from paper I. In order to match the four-year period in paper I, before the introduction of PCV (2003-2007), the results in paper II was divided into two four-year periods (2008-2012 and 2012-2016).

The data was collected from all pediatric and ear- nose- and throat (ENT) tertiary care units in Stockholm Region. The study population in paper I-III consisted of all admissions of children of study age with a main or secondary discharge diagnosis code of J01 (sinusitis) according to the International Statistical Classification of Diseases and Related Health Problems - Tenth Revision (ICD-10) from the regional hospital database, which covers 100% of hospital admissions in the area. Codes for related complications, orbital cellulitis/abscess (H050) and intracranial abscess (G060) were also identified and included to ensure inclusion of all cases of acute rhinosinusitis. The children that were included in the studies had been referred to, or directly sought care at, one of the hospitals' emergency departments and had been considered in need of inpatient tertiary care. Each admission was reviewed by an ENT surgeon and a pediatrician to verify the rhinosinusitis diagnosis. The criteria

for ARS listed according to the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) were used. Admissions that did not fulfill rhinosinusitis diagnosis, were incorrectly diagnosed, or residents of other regional areas in Sweden were excluded. All admissions were reviewed according to a schematic form that included demographics, medical history, symptoms, clinical status, laboratory results, culture results, performed radiology, surgery, treatment, length of treatment and hospital stay, etc.

The CT and MRI images were reviewed by a specialist in neuro- and head and neck- radiology. The orbital complications were classified according to Chandler to determine the degree of orbital complication (12). The Lund-Mackay score was used to determine the degree of paranasal sinus involvement in paper I (86). In the analysis of the results, the orbital complications were divided into preseptal (Chandler 1) or postseptal (Chandler 2-4). Each orbital complication was graded according to the most severe complication according to Chandler. In paper III, where the study population was older and the types of complications more diverse, the complications were categorized into orbital, intracranial and osseous complications. In the subanalysis of results, postseptal orbital, intracranial, and osseous complications were considered to be severe complications. Cavernous sinus thrombosis was categorized as an intracranial complication in this thesis.

To verify vaccine coverage in the study population in paper II, information regarding each child's pneumococcal vaccination status was retrieved from the Swedish National Vaccination Register from January 1st, 2013, when the register was introduced.

The population data that was used for incidence calculations in paper I-III was received from Statistics Sweden (SCB), with selected data from Stockholm Region, and then divided into age, gender, and year groups (87). Incidence rates were expressed as the number of cases per 100,000 children per year in the defined population.

The size of the source population increased during the study period in paper I-III and was as follows:

Paper I: from 112 717 children in 2003-2004 to 126 487 in 2006-2007 (female population from 54 964 to 61 783, male population from 57 753 to 64 704).
Paper II: from 134 003 children in 2008-2009 to 146 689 in 2015-2016 (female population range from 65 200 to 70 920, and male population range from 68 803 to 75 769).
Paper III: from 294 917 children in 2003-2004 to 338 998 in 2015-2016 (female population range from 143 697 to 164 706, and male population range from 151 220 to 174 292).

In paper I, confidence intervals (CI) were estimated with a 95% confidence level, p values of <0.05 were considered significant and significance tests were two sided. Fisher´s exact test was used to compare the incidence rates between age groups and boys versus girls, using conditional maximum likelihood estimates of the incidence rate ratio (IRR) (88, 89). To test the normal distribution, the Shapiro-Wilk test was used for the numeric variables: CRP; white blood cells count (WBC); the age expressed in months; number of days of intravenous antibiotics (IV); and days of total antibiotic treatment, and none were normally distributed ($p<0.001$). Statistical analyses were carried out in SPSS for Windows, version 23.0 (IBM Corp, New York, USA).

In paper II, Poisson regression analysis was used to compare the two PCV periods 2008-2012 and 2012-2016) with the pre-vaccine period (2003-2007), with the pre-vaccine period serving as a reference. To obtain adjusted incidence rate ratios, multivariable Poisson regression analysis (procedure GENMOD) was utilized. Confidence intervals (CI) were estimated with a 95% confidence level for incidence rates, p values of <0.05 were considered significant, and all significance tests were two sided. Statistical analyses were performed using SAS version 9.4 (SAS Campus Drive, Cary, NC, USA

27513). After review comments from the journal, the Poisson regression analysis was removed, and incidence rates were added to clarify the difference between the pre-PCV period and the two PCV study periods.

3.2 Paper IV

Paper IV was a prospective cohort study of children from birth to 18 years old, hospitalized due to suspected or confirmed acute bacterial rhinosinusitis, between April 1st 2017 to April 1st 2020. Patients were included by an ENT surgeon or a pediatrician, either at the emergency unit or when admitted to a ward. For inclusion, a written consent had to be signed by both caregivers, and participants older than 15 had to sign an additional informed written consent. The first case included was admitted September 6th 2017 and the last included case was admitted march 23rd 2020. The criteria for acute bacterial rhinosinusitis (ABRS) according to the EPOS were used to verify the rhinosinusitis diagnosis (18). The cases that had a different final diagnosis than rhinosinusitis or who were residents of region other than Stockholm were excluded.

Data was gathered according to a standardized study protocol including demographics, medical history, clinical status, laboratory test results, performed radiology, surgery, etc. Additional data gathered in paper IV was immunoglobulin levels (IgG, IgM and IgA), allergy screening test results (Phadiatop, fluoroenzymeimmunoassay FEIA, by Thermo Fisher Scientific), and multiplex viral nasopharyngeal PCR swab results. The bacterial cultures taken included a bacterial swab from the nasopharynx (NPH) and an aimed bacterial swab from the nasal middle meatus (MM) by an ENT surgeon. For the cases that had surgery, three different surgical cultures were obtained - a regular bacterial swab culture, a bacterial tissue culture, and samples for broad-range 16S rDNA PCR.

CT and MRI images were reviewed by a specialist in radiology, and the results categorized into orbital, intracranial or osseous complication. The orbital complications were classified according to Chandler (12), where each orbital complication was graded according to the most severe complication present.

In the statistical analysis of data in paper IV, the types of bacteria in the different cultures were described and categorized. The bacteria categorized as "others" were *Bacteroides fragilis* (MM culture), and five bacteria found in surgical cultures: *Campylobacter species*, Anaerobe mixed flora, *Fusobacterium nucleatum*, *Prevotella species* and *Klebsiella oxytoca*. Contaminated cultures were regarded as negative cultures in the statistical analysis. In the bacteria distribution analysis, each type of bacteria was compared to the cases without that bacteria, as a binary variable. Fisher's exact test was used to estimate whether the distribution of bacteria differed in the different cultures.

The exposures were: type of bacteria, viral swab positive for any virus, viral swab positive for influenza virus, and positive Phadiatop test. The outcomes were: grade of complication (ordinal variable), days of intravenous antibiotics (ordinal value) and maximum CRP value (continuous variable). Grade of complication was a scale 1-5, from least severe to most severe, as follows: 1 – preseptal cellulitis, 2- orbital cellulitis, 3- subperiosteal abscess, 4- orbital abscess, and 5- intracranial complication. The cases that did not have a CT or MRI performed and had clinical signs of preseptal cellulitis were considered as grade 1.

Ordered logistic regressions was used to estimate the associations between exposures and grade of complication and days of intravenous antibiotics. Linear regressions was used to estimate the associations between exposures and the maximum CRP value. The standard errors were obtained by robust estimator. P-values of <0.05 were considered significant. The analyses were conducted in Stata (MP 15.1, StataCorp LLC, College Station, TX).

3.3 Methodological considerations

The subjects in study I-III were extracted from the regional hospital database using the registered discharge diagnoses. It is theoretically possible that there were cases of ARS that were incorrectly registered according to ICD-10 in the discharge journal, did not receive the sinusitis diagnoses or related diagnoses, and therefore were not included in the cohort. Furthermore, the ICD-10 diagnoses for meningitis, G00 and G03, were not included in the database search. However, in the Swedish system, the discharge journal is written by the treating doctor, who summarizes the tertiary care stay in the hospital and determines the main and secondary discharge diagnoses according to ICD-10. Furthermore, the regional hospital database covers 100% of hospital admissions in the area and most diagnoses related to complications were included in the search. Therefore, the risk should be limited that rhinosinusitis cases were not included in the studies, especially the severe cases. There is a chance of selection bias, with a lean towards the more severe cases. We did not discover any other method that would have been superior.
The degree of complication, and especially the grading of orbital complications according to Chandler, can not be determined unless a CT with contrast or MRI is done. Plain x-ray does not enable degree of complication to be determined. CT with low-dose or without contrast provides insufficient imaging for adequate radiology analysis regarding complications. The calculated incidences of complications in the studies were based on the cases that had had the sufficient radiology performed. This was the chosen method in the studies, but included an inherent risk of undetected complications, due to a CT scan not being performed because of clinical status and/or quick improvement with antibiotic treatment. Another factor potentially affecting the occurrence of undetected complications was poor quality of radiology or omitted radiological evaluations. These issues were less likely to be present in the case of severe complications, which could entail a selection bias towards the more serious cases.

Paper I-III were retrospective studies with the limitations that this entails. There was missing data, and there is a risk of information bias if more data was collected from the admissions of children with more severe complications compared to the children with milder clinical status and symptoms. To reduce the risk of missclassification bias regarding the inclusion criteria, the same ENT surgeon and paediatrician reviewed all cases in each study. To reduce the risk of missclassification bias in the grading of complications, the same radiologist analyzed all the CT/MRI images in each study. Furthermore, the radiologist analyzed the images without looking at the assessment written when the CT/MRI was made, which should decrease the risk of bias. When the grading of the complication was uncertain, the lower grade of complication was chosen.

The analysis and presentation of the bacterial cultures from the nose differentiated between study I-IV. This was largely due to a change in thought regarding the clinical value of different types of cultures, and reflects the change that has taken place in the litterature parallell to the increased understanding of the microbiota of the nose. The results of the nasopharyngeal (NPH) cultures were not presented in paper I, only the bacterial samples obtained through sinus puncture. The reasoning behind this in the study design was that the NPH culture results would present the rich microbiome of small children instead of the disease-causing bacteria in the sinuses. In paper II, the NPH cultures and the aimed cultures taken in the nasal middle meatus were analyzed and presented together. In paper III the NPH, middle meatus and sinus tap cultures were analyzed and presented separately. In paper IV, the primary aim was to investigate the potential differences between the cultures. The difference between the papers in this regard makes a comparison more difficult.

In paper IV, the method used to evaluate IgE-sensitization in the subjects was a serum IgE test with a sensitivity between 70-80% (59) and a specificity around 90% (60). There is hereby a risk of false negative cases.

4. ETHICAL CONSIDERATIONS

Study I-III were approved by the Ethics Review Board in Stockholm, Sweden (application numbers 2011/44-31/1, 2011/1407-32/1, 2012/144-32/1, 2013/1429-32, 2015/1499-32 and 2016/1475-32).

In accordance with the approved ethics permissions, the study subjects and their guardians were not asked for permission for retrospective access to their personal medical records in study I-III, which was an invasion of privacy. Furthermore, in the journal review, medical data not related to the study could potentially be revealed to the researcher. However, the researchers that had access to the journals and the collected data were all ENT surgeons or pediatricians that are subject to the Secrecy Act, and the data was de-identified in data analysis and presentation of results. Therefore, the presented results were not derivable to the specific individuals included in the studies. No risk that the results would be used deleteriously in the future was anticipated. Overall, the scientific gains the studies could give were considered greater than the individual's potential harm.

Study IV was approved by the Ethics Review Board in Stockholm, Sweden (application number 2017/296-31).

Compared to the existing clinical guidelines of complications to rhinosinusitis in children, participation in study IV entailed more blood tests, and potentially more examinations by different specialists (ENT surgeon, pediatrician, ophtalmologist). To avoid any pain or discomfort du to the extra blood tests, they were taken at the same time as the standard laboratory tests included in the existing clinical guidelines. The methods used were standardized and did not imply any risks for the study participants.

The data gathered by the main researcher was de-coded and de-identified in the analysis and writing of results. Since severe complications to rhinosinusitis are rare on a population-level, it is possible that study objects and/or guardians could feel discomfort when the results are reported in a scientific paper, even if the results were presented anonymously and at group level. However, the study participants and their guardians were fully informed as to what participation in the study entailed, including publication of a scientific paper, and the participants over the age of 15 signed an additional informed consent. Participation was voluntary and could be canceled at any time.

Compared to the existing guidelines, the study participants were more thoroughly examined. There was no anticipated risk of the results being used in a negative way in the future. Overall, the gain for the individual study participant and for the patient-group as a whole was considered greater than the possible individual harm due to participation in the study.

5 RESULTS

5.1 Paper I

5.1.1 General data

A total of 213 admissions and 203 unique individuals up to five years old, hospitalized from July 1st 2003 to July 1st 2007, were included. Males represented 60.6% of admissions, median age was 1.5 years and chronic diseases were found in 10%.

Incidence of admission due to acute rhinosinusitis was 44 children per 100 000 per year, 53 per 100 000 per year for males and 36 for females. The IRR for admission for males compared to females was 1.5. Comparing the children below two years of age with children between two and five years of age, the IRR was 2.8. In the monthly distribution of admissions during the study period, the admission rate was the lowest in July and August.

The median value of maximum C-reactive protein (CRP) (mg/L) was 99 (interquartile range 42-150), and the median value of maximum WBC ($x10^9$/L) was 16 (interquartile range 12-22). Intravenous (IV) antibiotics were given in 97% of admissions and the median number of days with IV antibiotics was 2.0 (missing data in 22 admissions). Seven cases had received oral antibiotics before admission (3.3%). Within the first 48 hours of hospitalization, 96% improved clinically.

5.1.2 Radiology and complications

Preseptal cellulitis, redness and/or swelling around the eye, was found in 80.3% of admissions (n=171), representing an incidence of 36 per 100 000 per year (95% CI 26-49). A CT was performed in 9.9% (n=21) of admissions. A postseptal orbital complication (Chandler 2-4) was present in 3.3% of admissions (n=7), resulting in an incidence of 1.51 per 100 000 children per year. An orbital abscess was found in three cases. No intracranial or osseous complications were found and only one child required surgery.

5.1.3 Bacterial cultures

A nasopharyngeal culture was registered in 53 cases, and *M. catarrhalis* was the most common bacteria (n=21), followed by *H. influenzae* (n=18), *S. pneumoniae* (n=14), *S. pyogenes* (n=1), and negative for bacterial growth in four cases, see Table 4. Two cases had a middle meatus or sinus puncture registered, where one had growth of *S. pyogenes* and *S. pneumoniae* and the other one was negative. The most common bacteria in blood cultures was *S. pneumoniae*, found in 10/94 blood cultures. There were no cultures obtained from the child that had surgery.

Table 4. Number of admissions with growth of specified pathogens in cultures, children up to five years old, hospitalized due to acute rhinosinusitis in Stockholm Region 2003-2007. Data divided into type of culture: nasal, sinus puncture (Sinus), and blood, in the total cohort.

Number of admissions with type of culture positive for:	Nasal	Sinus	Blood
S. pneumoniae	14	1	10
M. catarrhalis	21	0	0
H. influenzae	18	0	0
S. pyogenes	1	1	0
S. aureus	0	0	1
Others #	0	0	3
Negative culture	4	1	80

Bacillus species, alpha-hemolytic Streptococci, coagulase-negative Staphylococci

5.2 Paper II

5.2.1 General data

The cohort consisted of 217 admissions and 215 unique individuals, children up to five years old, hospitalized between July 1st 2008 and July 1st 2016. The median age was 2.0 years, 62.7% were males (136/217) and 9% had chronic diseases. The immunization status could be controlled in 58 children in the National Vaccination Register and 97.2% had received at least one dose of PCV.

The mean incidence of admission during the whole study period was 18.8 per 100 000 children per year (14.6 for females and 22.8 for males), with the highest incidence of 27.6 for females and 42.1 for males the first study year, 2008-2009, see Figure 2.

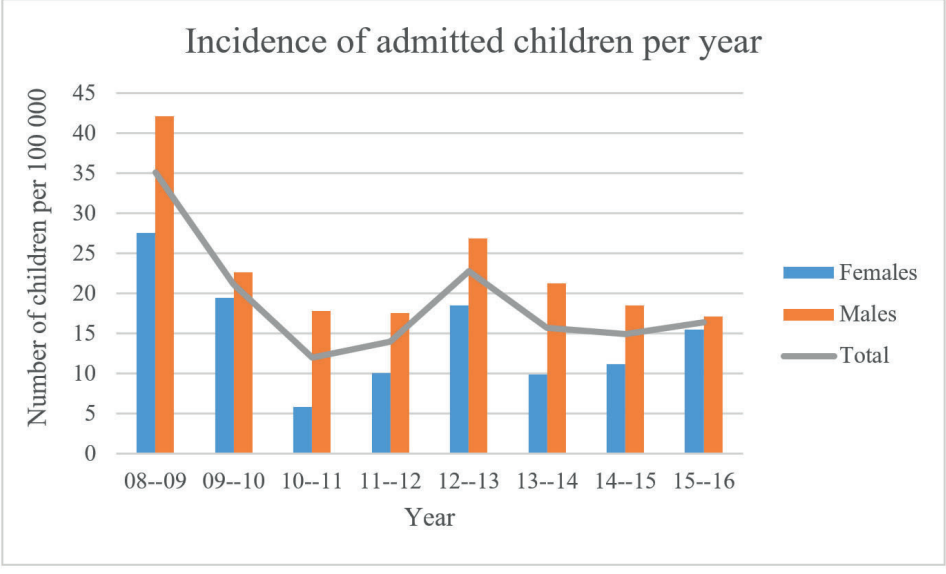

Figure 2. Incidence of hospital admission due to acute rhinosinusitis for females and males under five years old with acute rhinosinusitis per 100,000 children and study year in Stockholm county 2008-2016.

The mean maximum CRP was 76.1 mg/L and mean WBC was 16.3 x10^9/L. The mean number of days with IV antibiotics was 2.3. The child had received oral antibiotics before admission in 16 cases (4.6%), four of these had an orbital postseptal complication and one had surgery. The mean number of days with IV antibiotics in these 16 cases were 2.6, compared to 2.3 in the remaining cohort, in which there were 25 postseptal complications and eight cases of surgery.

5.2.2 Radiology and complications

Clinical signs of preseptal cellulitis, redness and/or swelling around the eye, was present in 96% of admissions, representing a mean incidence of 18.2 per 100 000 children per year. A CT was performed in 21.7% (n=47) of the admissions. A CT-verified postseptal orbital complication was found in 13.4% of admissions (n=29, all unique individuals), representing a mean incidence of 2.5 cases of postseptal complications per year (1.8 for females and 3.2 for males).

Nine children had surgery due to their orbital complications, which represented 4.2% of the total number of admitted children (n=215), and a mean incidence of 0.79 cases per 100 000 per year. The comparative data with paper I presented in paper II is displayed in Table 5.

Table 5. Data from the period before vaccine and the two periods with vaccine in Stockholm County from 2003 to 2016. The number of children that were admitted, had postseptal complication and surgery; the risk ratio for admission, postseptal complication and surgery; the rate ratio of admission, postseptal complication and surgery adjusted for time period and gender (95% CI).

	Pre-PCV 2003-07	**PCV 2008-12**	**PCV 2012-16**
Number of children			
Admitted	203*	113	102
Postseptal complication	7*	13	16
Surgery	1*	3	6
Comparison – incidence			
Admission (pseudo r2=0.71)	Ref	RR 0.47 (0,37-0,59) p=<0.001	RR 0.41 (0.32-0.51) p=<0.001
Postseptal complication (pseudo r2=0.38)	Ref	RR 3.32 (1.32-8.32) p=0.01	RR 4.54 (1.86-11.03) p=<0.001
Surgery (pseudo r2=0.40)	Ref	RR 5.45 (0.57-52.41) p=0.14	RR 12.01 (1.46-99.78) p=0.44
Male vs female			
Admission	Ref	RR 1.51 (1.24-1.84) p=<0.001	
Postseptal complication	Ref	RR 1.23 (0.61-2.45) p=0.55	
Surgery	Ref	RR 0.61(0.18-2.12) p=0.44	

*(90)

5.2.3 Bacterial cultures

The nasal culture was positive for bacterial growth in 41 admissions, and the blood culture positive for growth in eight admissions, see Table 6. The most common bacteria in the nasal cultures were *M. catarrhalis* (n=19), *H. influenzae* (n=13), *S. pneumoniae* (n=12), and *S. pyogenes* (n=11). In the sub-analysis of the children with CT-verified postseptal orbital complication, none of the cultures were positive for *S. pneumoniae*, but for *H. influenzae*, *S. aureus* and *S. pyogenes*. No surgical culture was taken in four cases that had surgery. Only two blood cultures had growth of *S. pneumoniae*.

Table 6. Growth of specified bacteria in number of admissions, in nasopharyngeal, surgical and blood cultures in the whole cohort of children up to five years old hospitalized due to ARS and related complications in Stockholm 2008-2016.

Bacteria	Nasopharyngeal	Surgical	Blood
S. pneumoniae	12		2
S. pyogenes	11	1	1
S. salivarius			1
H. influenzae	13	2	
M. catarrhalis	19		
S. aureus		1	
Bordetella pertussis	1		
High likelihood of contamination			
S. epidermidis			1
Undefined alpha-hemolytic streptococci and coagulase-negative staphylococci	1		
Corynebacterium species			2
Negative for bacterial growth	*15*	*1*	*118*

The serotypes of the *S. pneumoniae* positive blood cultures were 38 and 7F. Serotype 7F was found in a four-month-old boy that had received one dose of the 13-valent PCV, which includes serotype 7F.

5.3 Paper III

5.3.1 General data

A total of 310 admissions and 304 unique individuals, children from five to 18 years old, from July 1st 2003 to July 1st 2016, were included. The median age was 9.2 (SD 3.61), 61% of the cohort were males, and 17% had chronic diseases including allergies.

The mean incidence of admission was 7.8 children from five to 18 years old per 100 000 per year (9.2 for males and 6.2 for females). Mean CRP was 91.9 mg/L and mean WBC was 13.9 x10^9/L. The mean number of days with IV antibiotics was 4.1.

In 35 cases (11.3%), the child had received oral antibiotics before admission. The mean number of days with IV antibiotics (4.1), percentage of cases with CT/MRI-verified severe complication (10/35), or percentage of cases that needed surgery (6/35), was not higher in this subgroup compared to the rest of the cohort.

The incidences of admission, severe complication and surgery, per epidemic year and per age group, is shown in Figure 3 and Figure 4.

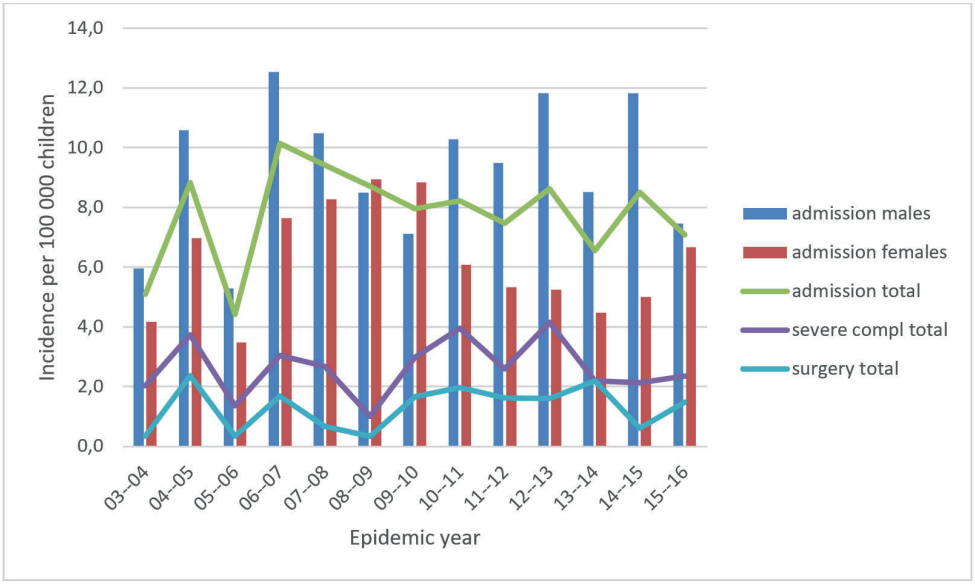

Figure 3. Incidence of hospital admissions, admissions with a severe complication, and admissions that had surgery, per 100 000 children, five to 18 years old, per epidemic year due to acute rhinosinusitis and related complications, in Stockholm Region, from 1st of July 2003 to June 30th 2016. Incidence of admission displayed in total and per gender.

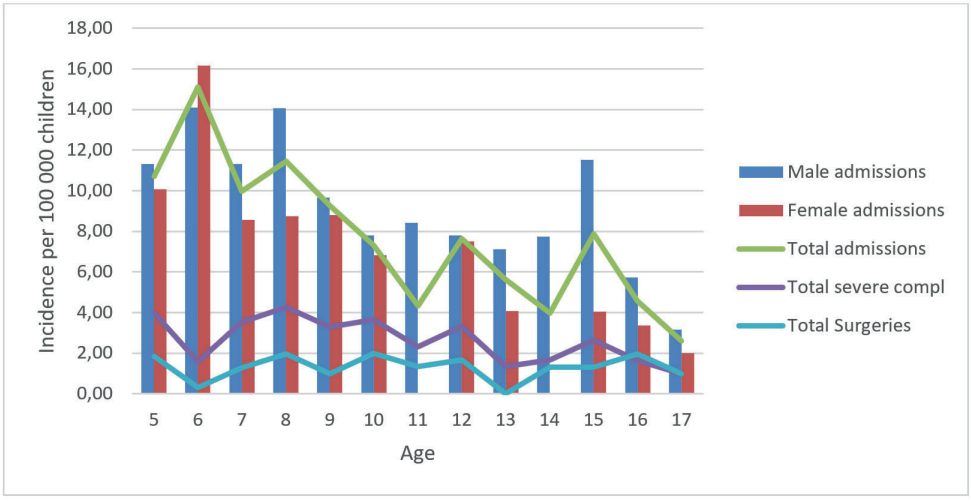

Figure 4. Incidence of hospital admissions and surgeries per 100 000 children, five to 18 years old, due to acute rhinosinusitis and related complications, in total and per gender, in Stockholm Region, from 1st of July 2003 to June 30th 2016, per age group.

5.3.2 Radiology and complications

Clinical signs of orbital preseptal cellulitis (redness and/or swelling around the eye) and/or CT/MRI-verified preseptal cellulitis were present in 87.7 % of the admissions, representing a mean incidence of 6.8 per 100 000 per year.

CT and/or MRI was performed in 174 admissions (56%), and 23 of them were low-dose CT and/or performed without contrast. Among children 5-7 years old, 40-60% had some kind of radiology performed (CT, MRI or plain x-ray), while the percentage in the age groups 8-17 was 65-85%.

A CT/MRI-verified postseptal orbital complication was found in 87 cases, representing a mean incidence of 2.2 per 100 000 per year, see Table 7. An intracranial complication was found in 19 cases and an osseous in 12. At least one severe complication – CT/MRI-verified postseptal orbital, intracranial and/ or osseous – was found in 105 admissions (33.9%), which represents an incidence of 2.6 severe complications per 100 000 children per year. The cases with CT/MRI-verified intracranial complication had the highest median age, mean CRP and WBC among the different types of complications. The incidence of admission and all types of complications were higher for males than for females, as was the median age. The admissions with a severe complication had 7.7 mean number of days with IV antibiotics (2.2 for admissions without severe complication).

The number of admissions that had surgery in each subgroup of complication is listed in Table 8. The incidence of admissions having surgery was relatively constant in the different age groups but due to the larger number of admissions in the younger age groups, a larger percentage of the admitted older children had surgery.

Table 7. Data collected regarding complications due to acute rhinosinusitis in children from five to 18 years of age in Stockholm Region 2003-2016. The severe, postseptal orbital, intracranial and osseous complications are CT/MRI verified.

Complication	Total cohort	Males	Females
Preseptal orbital cellulitis			
No of admissions with clinical signs and/or CT-verified Chandler 1 (% of total cohort/total males/ total females)	272 (87.7%)	167 (88.4%)	105 (86.8%)
Mean incidence per 100000 per year	6.8	8.2	5.4
Median age, years (SD)	8.9 (3.50)	9.9 (3.66)	8.1 (3.01)
Severe complication*			
No of admissions (% of total cohort/total males/ total females)	105 (33.9%)	73 (38.6%)	32 (26.5%)
Mean incidence per 100000 per year	2.6	3.6	1.6
Median age, years (SD)	10.1 (3.51)	10.7 (3.57)	9.0 (3.11)
Mean CRP, mg/L (mean WBC, x10^9/L)	115.5 (15.1)		
Mean no of days with intravenous antibiotics	7.7		
Postseptal orbital complication			
No of admissions (% of total cohort/total males/ total females)	87 (28.1%)	62 (32.8%)	25 (20.7%)
Mean incidence per 100000 per year	2.2	3.0	1.3
Median age, years (SD)	9.6 (3.63)	10.5 (3.66)	8.6 (3.21)
Mean CRP, mg/L (mean WBC, x10^9/L)	98.8 (14.9)		
No of admissions with:			
Chandler 2 orbital cellulitis	41	31	10
Chandler 3 subperiosteal abscess	44	30	14
Chandler 4 orbital abscess	2	1	1
Intracranial complication			
No of admissions (% of total cohort/total males/ total females)	19 (6.1%)	12 (6.4%)	7 (5.8%)
Mean incidence per 100000 per year	0.48	0.59	0.36
Median age, years (SD)	11.5 (3.19)	11.6 (3.28)	10.9 (2.59)
Mean CRP, mg/L (mean WBC, x10^9/L)	215.5 (16.1)		
Osseous complication			
No of admissions (% of total cohort/total males/ total females)	12 (3.9%)	7 (3.7%)	5 (4.1%)
Mean incidence per 100000 per year	0.30	0.34	0.26
Median age, years (SD)	10.9 (3.45)	11.5 (3.66)	9 (3.06)
Mean CRP, mg/L (mean WBC, x10^9/L)	165.9 (15.5)		

* CT/MRI verified postseptal orbital, intracranial and/ or osseous complication

There were 52 cases that had surgery (16.8 %), see Table 8. The percentage of males in the total cohort that had surgery was 19.0%, and 13.2% of the females. Total incidence of surgery was 1.3 per 100 000 per year for the total cohort (1.8 for males and 0.8 for females). One female had surgery three times, resulting in a mean incidence for surgery for unique individuals and females of 0.72 per 100 000 per year.

Table 8. Data collected regarding admissions that had surgery due to acute rhinosinusitis in children of five to 18 years of age in Stockholm Region 2003-2016.

	Total cohort	**Males**	**Females**
No of admissions that had surgery (% of total cohort/total males/ total females)	52 (16.8%)	36 (19.0%)	16 (13.2%)
Mean incidence per 100 000 per year	1.3	1.8	0.8
Median age, years (SD)	11.0 (3.85)	11.3 (4.0)	9.8 (3.39)
Mean CRP, mg/L	145.2	146.8	141.8
No of admissions with CT/MRI-verified complication that had surgery:			
Chandler 2 orbital cellulitis (%)	4 of 41 (9.8)		
Chandler 3 subperiosteal abscess (%)	29 of 44 (65.9)		
Chandler 4 orbital abscess (%)	2 of 2 (100)		
Intracranial complication (%)	16 of 19 (84.2)		
Osseous complication (%)	12 of 12 (100)		

In the sub-analysis of CT/MRI verified severe (postseptal orbital, intracranial and osseous) complications, the percentage of children having surgery increased in the older age groups compared to the younger, see Figure 5.

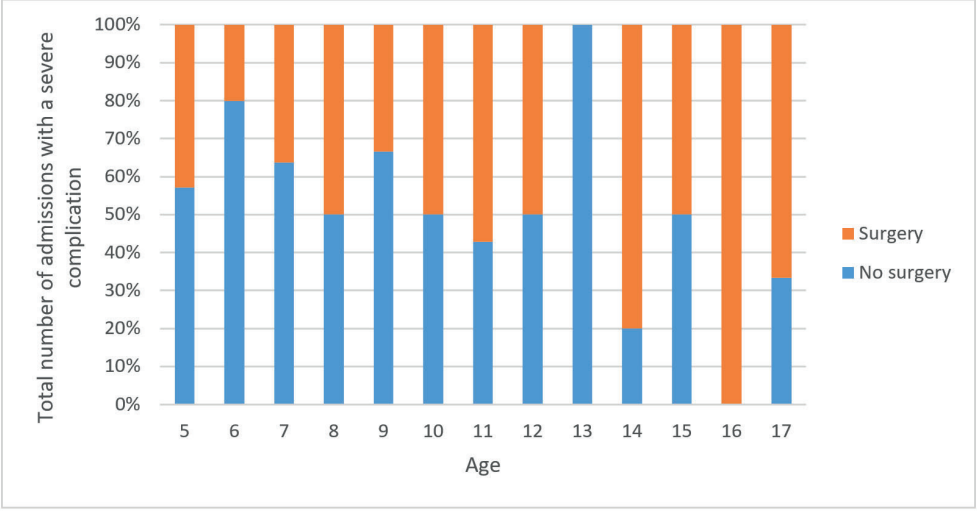

Figure 5. The total number of cases with a CT/MRI verified severe (postseptal orbital, intracranial and osseous) complication in each age group, divided into percentage that had surgery and not, hospitalized due to complications of acute rhinosinusitis in Stockholm Region 2003-2016.

5.3.3. Bacterial cultures

In 153 admissions there was at least one kind of culture taken from the nose, either nasopharyngeal, an aimed nasal culture taken by an ENT doctor or a culture from sinus puncture. No culture, either from the nose, blood, liquor or surgical site was recorded in 82 admissions. The number of cultures positive for bacterial growth were 20 for blood, 50 for nasal middle meatus, 21 for nasopharyngeal, and 37 for surgical. Results from cultures are displayed in Table 9.

There could be several cultures positive for bacterial growth for each admission, whereby the total number of positive cultures exceeded the number of culture-positive admissions.

Table 9. Number of admissions with growth of specified pathogens in cultures, children of five to 18 years of age, hospitalized due to acute rhinosinusitis in Stockholm Region 2003-2016. Expressed as number of admissions and divided into type of culture: nasopharyngeal (NPH), middle meatus (MM), sinus puncture (Sinus), all nasal (NPH, MM and sinus), surgical, and blood, in the total cohort. The last column displays the results from all different types of cultures in the total cohort.

Number of admissions with type of culture positive for:	NPH	MM	Sinus	All nasal	Surgical	Blood	All cultures
S. pyogenes	8	14	0	22	5	6	29
H. influenzae	6	18	1	25	0	2	27
S. milleri	0	2	2	4	19	5	23
S. pneumoniae	2	13	1	16	0	1	17
S. aureus (MRSA)	6(2)	3	0	9	8(1)	1(1)	15(2)
M. catarrhalis	1	9	0	9	0	0	9
Fusobacterium	0	0	0	0	2	2	3
Other Streptococci ¤	0	1	1	2	3	0	5
Others #	0	1	1	2	2	0	4
Contamination §	0	1	0		7	3	10
Negative culture	27	49	3		12	116	107

¤ alpha streptococci (two cultures), non-hemolytic streptococci, microaerophilic streptococci and *S. constellatus*
non-typeable anaerobic gramnegative rod, *Bacteroides species*, *Prevotella species*, *Bacillus species*
§ *Corynebacterium urealyticum*, *Corynebacterium pseudodiphteriticum*, *Corynebacterium Sp.*, *Micrococcus Sp.*, *Propionibacterium Sp.*, *Propionibacterium acnes*, *Staphylococcus epidermidis* (two cultures) and coagulase-negative staphylococcus (three cultures)

Cultures were positive for bacterial growth in 46% of the NPH cultures, in 56% of the aimed MM cultures and in 66% of the sinus punctures. *S. pyogenes* and *H. influenzae* were the most common bacteria found in the NPH and MM cultures, *S. pneumoniae* was more commonly found in the MM cultures than the NPH cultures and *S. aureus* was more common in the NPH cultures. *S. milleri* was found in two MM cultures and two sinus-puncture cultures, none in NPH cultures. *S. milleri* was the dominant bacteria in the surgical cultures, found in 33%. In the all the cultures (NPH, MM, sinus, surgical, and blood) that were positive for growth of *S. pyogenes*, it was the single growing bacteria. Both a NPH and a MM culture were recorded in four cases. In two cases, the MM culture was positive and the NPH culture negative, the reverse in one admission, and both were negative in one admission. In the admissions with a positive surgical site culture, 11 NPH and 11 MM cultures were taken. The pathogen matched in the surgical and NPH culture in four of the admissions and in the surgical and MM culture in one admission. *S. pyogenes*, *H. influenzae* and *S. milleri* were the three most common found pathogens in the whole cohort, followed by *S. pneumoniae* and *S. aureus*.

A subgroup analysis was made of the bacterial culture results, dividing the cohort into: admissions with CT/MRI verified severe complications but no surgery, non-severe complications, and admissions that had surgery. The results are displayed in Table 10.

Table 10. Number of admissions with growth of specified pathogens in cultures, children of five to 18 years of age, hospitalized due to acute rhinosinusitis in Stockholm Region 2003-2016. Expressed as number of admissions and divided into: total cohort; admissions without DT-verified severe complication (non-severe); admissions with a DT-verified severe complication without surgery performed (severe, surgery excluded); and in admissions in which surgery was performed (surgery). The percentage that each pathogen represents of the total number of cultures within each group is listed in parentheses.

No of admissions with culture positive for:	**Total cohort n (%)**	**Non-severe n (%)**	**Severe, surgery excluded n (%)**	**Surgery n (%)**
S. pyogenes	29 (11.6)	17 (12.0)	7 (16.3)	5 (7.8)
H. influenzae	27 (10.8)	19 (13.4)	6 (14.0)	2 (3.1)
S. milleri	23 (9.2)	2 (1.4)	2 (4.7)	19 (29.7)
S. pneumoniae	17 (6.8)	14 (9.9)	3 (7.0)	0
S. aureus	15 (6.0)	4 (2.8)	2* (4.7)	9* (14.1)
Fusobacterium	3 (1.2)	0	1 (2.3)	2 (3.1)
Other Streptococci*	5 (2.0)	0	1 (2.3)	4 (6.3)
M. catarrhalis	9 (3.6)	7 (4.9)	0	2 (3.1)
Others	4 (1.6)	1§ (0.7)	0	3# (4.7)
Contamination	10 (4.0)	2 (1.4)	1 (2.3)	7 (10.9)
Negative culture	107 (43.0)	76 (53.5)	20 (46.5)	11 (17.2)

* including 1 case of MRSA

§ non-typeable anaerobic gramnegative rod

one each of Bacteroides species, Prevotella species, Bacillus species

M. catarrhalis was found as part of mixed flora in the severe complication and surgery group, together with *S. milleri* and microaerophilic streptococci.

5.4 Paper IV

5.4.1 General data

The cohort consisted of 55 unique children under the age of 18, included between July 1st 2017 and July 1st 2020, and 67% were males. The median age was 7.6 years (7.5 years for males and 8.6 for females), and mean age was 7.8 years (SD 4.3). Chronic diseases were identified in 12 cases, of which five cases had asthma or wheezing. For descriptive data, see Table 11. For individual data of each case, see Table 12.

Table 11. Descriptive data of total cohort, prospective study of hospitalized children with acute bacterial rhinosinusitis in Stockholm 2017-2020, n= number of admissions.

Admissions, n	55
Gender, % males	67
Median age, years	7.6
Mean age, years (SD)	7.8 (4.3)
Mean CRP, mg/L, at admission	75.8
Mean CRP, mg/L, maximum	86.1
Mean WBC, x10⁹/L, maximum	13.8
Mean ESR, mm (missing, n)	55.5 (34)
CT/MRI, n	26
Surgery, n	12
Nasopharyngeal culture, n (missing)	50 (5)
Nasal middle meatus culture, n (missing)	52 (3)
Surgical site culture, 16s DNA test, n (missing)	12 (0)
Surgical site culture, tissue, n (missing)	11 (1)
Surgical site culture, swab, n (missing)	9 (3)
Blood culture, n (missing)	51 (4)
Viral nasopharyngeal PCR swab, n (missing)	53 (2)
Phadiatop test, n (missing)	48 (7)
Immunoglobulins, n (missing)	47 (8)

The mean number of days with common cold symptoms before admission was 6.8 days, the mean number of days with worsening symptoms before admission was 1.7, and mean number of days with IV antibiotics was 3.2.

Table 12. Individual data for whole cohort, prospective study of hospitalized children with acute bacterial rhinosinusitis in Stockholm 2017-2020, sex (M=male, F=female), days with intravenous antibiotics (IV AB), results from Phadiatop test and viral PCR, and results of cultures from nasopharynx (NPH), nasal middle meatus (MM), blood and surgical site (surgical).

Case no	Sex	IV AB (days)	Age (years)	Phadiatop	Viral PCR	NPH	MM	Blood	Surgical
1	F	7	12,0	neg	neg	neg	*S. milleri, Parvimonas micra* (cont)	neg	
2	M	2	10,7	pos	neg	neg	neg	neg	
3	M	2	2,4	neg	neg	*H. influenzae, S. pyogenes*	missing	neg	
4	M	2	6,9	neg	neg	missing	*M. catarrhalis, S. aureus*	neg	
5	M	5	13,5	missing	neg	missing	*S. pyogenes*	*S.salivarius*	
6	F	1	3,6	missing	missing	*M. catarrhalis*	*S. pyogenes, M. catarrhalis*	neg	
7*	M	4	2,8	neg	Influenza A	missing	Missing	neg	*S. pyogenes, M. catarrhalis, Corynebacterium species* (cont), *Dolosigranulum pigrum* (cont)
8*	M	1	1,6	neg	Boca	*S. pyogenes*	*S. pyogenes*	neg	
9*	M	2	8,8	neg	neg	neg	*S. pyogenes*	neg	
10	M	2	8,7	missing	Influenza B	*S. pyogenes*	*S. pyogenes, S. aureus*	neg	
11	M	1	8,6	missing	Adeno, RS	*H. influenzae*	neg	neg	
12	F	5	13,1	pos	neg	neg	neg	neg	*S. milleri*
13	M	2	12,6	pos	Influenza A	*S. pneumoniae*	*S. pneumoniae*	neg	
14	M	2	7,8	missing	neg	neg	*Staphylococcus epidermidis* (cont)	neg	
15	F	2	8,8	pos	Influenza A, Metapneumo	*S. pyogenes, S. aureus*	*S. pyogenes, S. aureus*	neg	
16	F	1	11,0	neg	Rhino	*M. catarrhalis*	*M. catarrhalis*	missing	
17	F	3	3,4	neg	Influenza A	*H. influenzae, S. pneumoniae*	*H. influenzae*	neg	
18	F	2	3,3	neg	Boca, Metapneumo, Rhino	neg	S. milleri, S. aureus	missing	
19	F	3	3,2	neg	Parainfluenza 1	neg	missing	neg	
20	F	3	10,8	pos	Influenza A	*H. influenzae*	*H. influenzae*	neg	
21	M	4	6,3	pos	neg	*S. pyogenes*	*S. pyogenes*	neg	
22	F	2	2,3	neg	Corona OC43 HKU1	*H. influenzae, S. pneumoniae*	*H. influenzae, S. pneumoniae*	neg	
23	M	4	7,3	pos	neg	neg	neg	neg	*S. milleri*
24	F	5	11,4	pos	missing	missing	neg	*S. milleri*	*S. milleri, Koagulas-negativ staphylococcus* (cont)

Case no	Sex	IV AB (days)	Age (years)	Phadia-top	Viral PCR	NPH	MM	Blood	Surgical
25	M	4	14,2	pos	neg	neg	Skinflora (cont)	neg	*S. milleri, Propionibacterium acnes* (cont)
26	M	3	5,3	neg	Parainfluenza 2	neg	*H. influenzae, S. pneumoniae*	neg	Neg
27*	F	4	6,0	neg	Adeno	neg	neg	neg	*S. milleri, Campylobacter species, Anaerobic mixed flora, Parvimonas micra* (cont)
28*	F	2	8,8	neg	neg	*M. catarrhalis*	*M. catarrhalis*	neg	
29	M	2	7,5	neg	Influenza A	*M. catarrhalis*	*M. catarrhalis*	neg	
30	M	3	3,3	neg	Influenza A	*S. pyogenes*	*S. pyogenes*	missing	
31*	M	3	16,0	pos	neg	neg	*Bacteroides fragilis*	neg	
32*	M	6	5,8	neg	RS	H. influenzae	H. influenzae	neg	*H. influenzae, M. catarrhalis, Corynebacterium fastidiosum* (cont)
33	M	2	1,5	neg	Boca	*M. catarrhalis*	*M. catarrhalis*	neg	
34	M	1	0,8	missing	Boca	*S. pneumoniae*	neg	neg	
35	M	3	16,4	neg	neg	neg	neg	neg	
36	F	2	5,7	neg	Influenza A	neg	*S. pyogenes*	neg	
37	M	2	13,7	neg	neg	neg	*S. milleri*	*S. milleri*	
38	M	2	2,7	neg	Rhino	*H. influenzae*	*H. influenzae, M. catarrhalis*	neg	
39	F	2	3,8	neg	neg	*S. pneumoniae*	*S. pneumoniae, M. catarrhalis*	neg	
40	M	2	1,7	neg	Rhino	*M. catarrhalis*	*M. catarrhalis*	missing	
41	M	3	5,7	neg	Rhino	neg	neg	neg	
42	F	9	16,9	neg	neg	neg	neg	*Micrococcus species* (cont)	
43	M	5	14,1	pos	Rhino	neg	neg	neg	*S. milleri, Prevotella species, Fusobacterium nucleatum, Staphylococcus epidermidis* (cont), *Staphylococcus lugdunensis* (cont)
44	M	6	12,7	pos	neg	*S. aureus*	neg	*S. milleri*	
45*	M	2	5,9	neg	neg	missing	*H. influenzae*	neg	*S. pyogenes, H. influenzae*
46	M	3	7,8	neg	neg	*S. pneumoniae*	*S. aureus*	neg	
47	M	14	9,6	pos	Rhino	neg	*S. pyogenes*	neg	
48	F	6	8,7	neg	Influenza A	neg	*S. pyogenes*	neg	

Case no	Sex	IV AB (days)	Age (years)	Phadia-top	Viral PCR	NPH	MM	Blood	Surgical
49	M	4	10,5	neg	neg	neg	neg	neg	
50	M	3	4,3	neg	Influenza A, Rhino, Entero	*H. influenzae*	neg	neg	
51	M	3	3,6	neg	neg	*S. pyogenes*	*S. pyogenes*	neg	
52*	M	2	13,6	pos	neg	neg	*Propionibacterium acnes* (cont)	neg	*S. pneumoniae, Klebsiella oxytoca*
53	F	3	8,5	neg	Rhino	*M. catarrhalis*	neg	neg	*S. milleri*
54	M	3	4,4	missing	neg	neg	neg	neg	
55*	M	2	7,6	neg	neg	*S. pyogenes*	*S. pyogenes*	neg	

*Cases that had received oral antibiotics before admission

Oral antibiotics was given before admission in ten cases (1-6 doses of Flucloxacillin, Phenoxymethylpenicillin or Amoxicillin/clavulanic acid). In this group, five had growth of *S. pyogenes* in at least one nasal or surgical culture; five had surgery; the average number of days with worsening symptoms before admission was 2.2 (1.6 days in the remaining cohort); four children tested positive for viral nasopharyngeal PCR and the average CPR at admission and maximum CRP during admission were 86 and 89 mg/L (74 and 86 mg/L in the remaining cohort).

5.4.2 Radiology and complications

At inclusion, all children but one had clinical signs of preseptal cellulitis, redness and/or swelling around the eye. A CT was performed in 26 cases (47.3%), and two cases also had an MRI. Five cases had orbital cellulitis (Chandler 2), 14 had a subperiosteal abscess (Chandler 3) and two had an orbital abscess (Chandler 4). Two children had an intracranial complication, one had a small intracranial abscess in addition to the orbital subperiosteal abscess, and another had meningitis and osteitis (osseous complication) in the skull base. Twelve children had surgery due to complications to ARS, and their median age was 7.9 years.

5.4.3 Bacterial cultures

At least one of the same type of bacteria was found in both the nasopharyngeal and middle meatus culture in 20 cases (36.4%). Both types of cultures were taken in 48 children, see Figure 6. The MM culture was positive for bacterial growth and NPH negative in nine individuals (16.4%), and NPH culture positive while MM culture negative in five individuals (9.1%). Different bacteria grew in the NPH and MM culture in one case and both were negative in 13 cultures (23.6%).

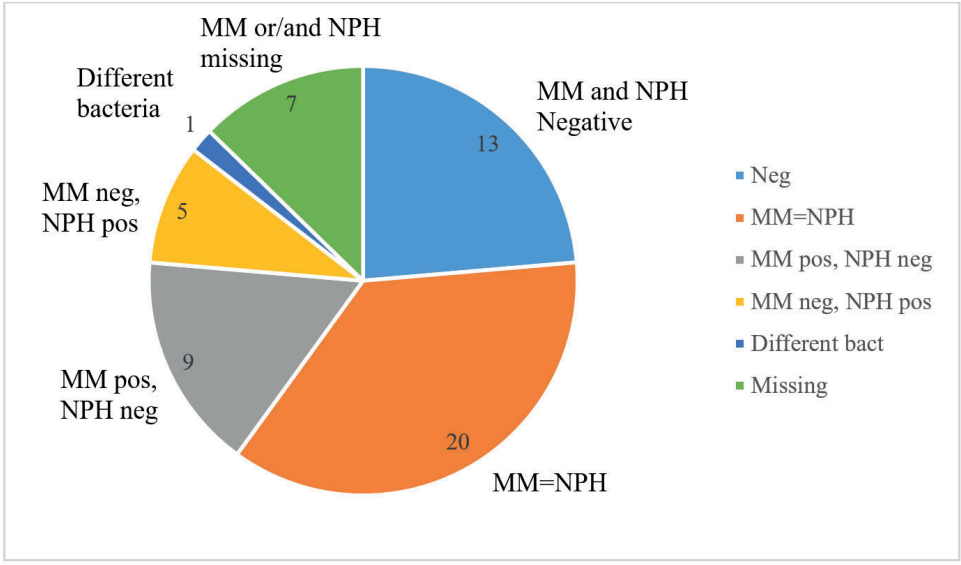

Figure 6. Coherence between results of bacterial cultures from nasopharyngeal (NPH) and middle meatus (MM) in each case, prospective study of hospitalized children with acute bacterial rhinosinusitis in Stockholm 2017-2020.

In the 20 children where the NPH and MM cultures had at least one bacteria in common, *S. pyogenes* and *M. catarrhalis* were the most common bacteria found (n=6 cases each), followed by *H. influenzae* (n=4), *S. pneumoniae* (n=2), *H. influenzae* and *S. pneumoniae* (n=1), and *S. pyogenes* and *S. aureus* (n=1). The most common bacteria in MM cultures were *S. pyogenes*, while the most common bacteria in surgical cultures was *S. milleri*, see Table 13. Among the NPH cultures there was a more even distribution of bacteria, primarily *S. pyogenes*, *H. influenzae*, *S. pneumoniae* and *M. catarrhalis*. In the 52 MM cultures obtained, 36 (69.2%) had growth of at least one type of bacteria. In the 50 NPH cultures obtained, 27 (54%) had growth of at least one type of bacteria.

Table 13. Number of admissions with culture positive for growth of specified bacteria (% of admissions with that specific type of culture), divided into nasopharyngeal culture (NPH), middle meatus culture (MM) and surgical culture, prospective study of hospitalized children with acute bacterial rhinosinusitis in Stockholm 2017-2020.

Number of admissions with culture positive for:	**NPH (%)**	**MM (%)**	**Surgical (%)**
S. pyogenes	8 (16.0)	13 (25.0)	2 (16.7)
H. influenzae	8 (16.0)	7 (13.0)	2 (16.7)
S. milleri	0	3 (5.8)	7 (58.3)
S. pneumoniae	6 (12.0)	4 (7.7)	1 (8.3)
S. aureus	2 (4.0)	5 (9.6)	0
M. catarrhalis	7 (14.0)	9 (17.3)	2 (16.7)
Other bacteria	0	1* (1.9)	5¤ (41.7)
Contamination#	0	4 (7.7)	6 (50.0)
Negative	23 (46.0)	16 (30.8)	1 (8.3)
Missing	5	3	0
Number of admissions with each type of culture obtained	50	52	12

* Bacteroides fragilis
¤ One each of Campylobacter species, Anaerob blandflora, Prevotella species, Fusobacterium nucleatum, Klebsiella oxytoca
Staphylococcus epidermidis (three cultures), Micrococcus Sp., Corynebacterium fastidiosum, Parvimonas micra (two cultures), Propionibacterium acnes (two cultures), Staphylococcus lugdunensis, Dolosigranulum pigrum, Corynebacterium pseudodiphtheriticum.

M. catarrhalis was possibly associated with a lower number of days with intravenous antibiotics (coefficient -1.3, p=0.055), H. influenzae and S. pneumoniae negatively associated with max CRP (coefficient -38.9, p=0.028 and coefficient -45.5, p=0.023),and *S. pyogenes* positively associated with max CRP (coefficient -57.5, p=0.007).

S. milleri was found in the MM culture in three children but none had surgery and the NPH cultures were negative. Including all types of cultures in the cohort, *S. pyogenes* was the bacteria found in the highest number of admissions (n=16), followed by *M. catarrhalis* (n=12), *S. milleri* (n=11), *H. influenzae* (n=10), *S. pneumoniae* (n=8), and *S. aureus* (n=6).

S. milleri was the most common pathogen found in the surgical cultures, see Table 13 and 14. In two cases, there was a match between the pathogens found in surgical cultures and nasal cultures, both showing *H. influenzae* in surgical and MM cultures. The nasal cultures were negative or regarded as contaminated in seven of the 12 children that had surgery, both nasal cultures missing in one case. The individual results of the different types of surgical cultures are displayed in Table 14. The broad-range 16S rDNA PCR was negative in one case, while there were more negative cultures and more data missing in the tissue and swab cultures.

Table 14. Individual results of surgical cultures, prospective study of hospitalized children with acute bacterial rhinosinusitis in Stockholm 2017-2020.

Case no	No days with IV Ab	Ab before admission	Bacterial DNA (16s)	Bacterial tissue culture	Bacterial swab culture
7	4	Phenoxymethyl-penicillin 1,5 days	*S. pyogenes, M. catarrhalis, Corynebacterium species (cont), Dolosigranulum pigrum (cont),*	*S. pyogenes*	*S. pyogenes, Corynebacterium pseudodiphtheriticum (cont)*
32	6	Phenoxymethyl-penicillin 0,5 days	*M. catarrhalis, Corynebacterium species (contt)*	neg	*H. influenzae*
45	2	Flucloxacillin 1 day	*H. influenzae, S. pyogenes*	*S. pyogenes*	*H. influenzae, S. pyogenes*
26	3		neg	neg	missing
52	2	Amoxicillin/clavulanic acid 2 days	*S. mitis*	Staphylococcus epidermidis (cont), Propionibacterium acnes (cont)	Klebsiella oxytoca, *S. pneumoniae,* Staphylococcus epidermidis (cont), Propionibacterium acnes (cont)
53	3		*S. milleri*	*S. milleri*	*S. milleri*
23	4		*S. milleri*	neg	missing
12	5		*S. milleri*	*S. milleri*	*S. milleri*
24	5		*S. milleri*	*S. milleri,* Koagulas-negative staphylococcuscont)	neg
27	4	Phenoxymethylpenicillin 1 day	*S. milleri, Campylobacter species*, Anaerobic mixed flora	*S. milleri*, Parvimonas micra (cont)	missing

Case no	No days with IV Ab	Ab before admission	Bacterial DNA (16s)	Bacterial tissue culture	Bacterial swab culture
43	5		*S. milleri, Prevotella species*, Fusobacterium nucleatum	*S. milleri, Staphylococcus epidermidis* (cont), *Staphylococcus lugdunensis* (cont)	*S. milleri*
25	4		*S. milleri*, Propionibacterium acnes (cont)	missing	*S. milleri*

Four children had positive blood cultures, three for *S. milleri* and one for *Streptococcus salivarius (S. salivarius)*. Of the cases with *S. milleri* in blood, one had the same pathogen found in the MM culture, another had growth of *S. aureus* in the NPH culture, and a third, undergoing surgery, had a negative MM culture. The child with *S. salivarius* in blood had growth of *S. pyogenes* in the MM culture.

5.4.4 Viral nasopharyngeal PCR

Viral nasopharyngeal PCR was obtained in 53 individuals and positive in 28. Influenza A was the most common virus found (n=10), followed by rhinovirus (n=9), bocavirus (n=4), metapneumovirus (n=2), adenovirus (n=2), parainfluenza (n=2), respiratory syncytial virus (n=2) and one each for coronavirus OC43 HKU1, enterovirus, and influenza B. More than one virus was identified in four cases. A positive viral PCR was associated with a lower grade of complication (coefficient -1.3, p=0.028) and CRP max (coefficient -36.2, p=0.05). Influenza virus was possibly associated with a lower grade of complication (coefficient -2.2, p=0.055).

In six of the 11 influenza A or B positive cases (54.5%), *S. pyogenes* was found in at least one culture (3 NPH, 5 MM, 1 surgical). In the total cohort, 16 cases had at least one culture positive for *S. pyogenes* (29.1%). Among these 16 cases, only two patients had a viral infection other than an influenza virus (boca- and rhinovirus), seven cases had a negative virus NPH swab, and a virus NPH swab was not obtained in one case. We found the association between *S. pyogenes* and influenza A/B positive cases to be statistically significant (coefficient 1.5, p=0.040). The cases with positive viral swabs correlated with the total number of cases in regard to month of the calendar year, see Figure 7.

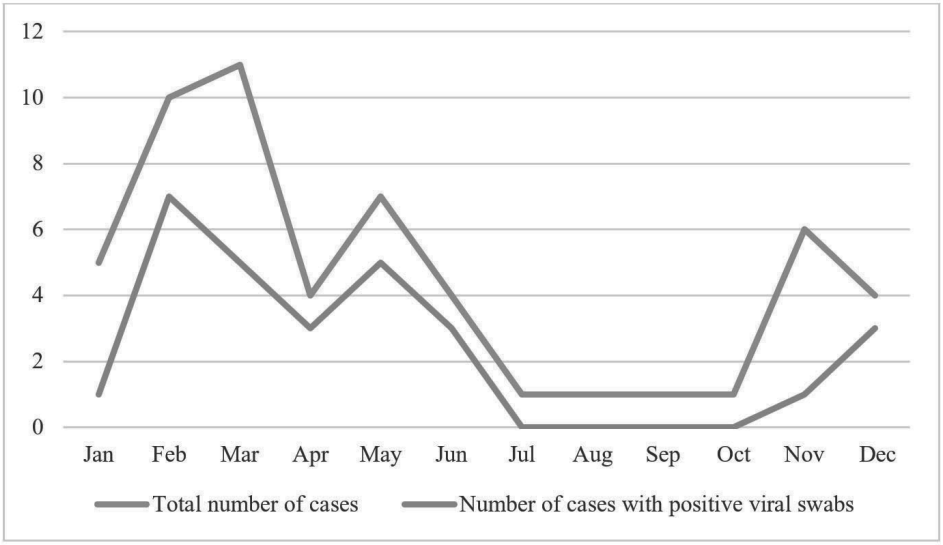

Figure 7. The total number of cases and number of cases with positive viral swab divided into month of admission, January through December, prospective study of hospitalized children with acute bacterial rhinosinusitis in Stockholm 2017-2020.

5.4.5 Immunoglobulins

Of the 47 cases where immunoglobulins (IgG, IgA and IgM) were obtained, only one case had a slightly low value of IgG (5.8 g/L, ref 6.1-14.5). Eight cases had elevated IgA levels and four cases had elevated IgM levels.

5.4.6 Allergy screening test

Of the 48 cases with obtained Phadiatop test, 14 were positive (29%). The mean age for the children with a positive test was 11.5 years, median 12.0 years. A positive allergy sensitization test was possibly associated with a higher number of days with IV antibiotics (coefficient 1.2, p=0.052).

Dividing the cohort into a surgery and non-surgery group, six of the 12 (50%) children in the surgery group, and 8 of the 36 children (22%) in the non-surgery group (7 missing) had a positive Phadiatop test. Median and mean age in the surgery group was 7.9 and 9.0, and 7.5 and 7.4 in the non-surgery group. In the group of children with a CT/MRI verified postseptal complication, 10 of 22 (45%) had a positive Phadiatop test (2 missing), the median age was 10.1 and mean age was 9.7. Among the children that had either only a preseptal cellulitis on CT/MRI or did not have CT/MRI performed, 4 of 26 (15%) had a positive Phadiatop test (5 missing). The median age was 5.7 and mean age was 6.3.

In five of the six children that had surgery and a positive Phadiatop test, *S. milleri* was found in surgery cultures. Out of the four children that had verified airborne allergy before admission, Phadiatop test was missing in two cases. Five of the cases with a positive Phadiatop test had a positive viral swab (36%).

6 DISCUSSION

Comparing paper I and II, we found that the incidence of hospital admission due to acute rhinosinusitis and related complications in children up to five years old decreased from 43.8 to 18.8 children per 100 000 per year, after the introduction of PCV. A CT/MRI verified postseptal orbital complication was found in 3.3% of admissions in paper I (incidence 1.51) and in 13.4% in paper II (incidence 2.54). Surgery increased from 0.5% of the admissions in paper I (incidence 0.22) to 4.1% in paper II (incidence 0.79). In paper III, the incidence of hospital admission due to ARS and related complications in children from five to 18 years old was 7.8 per 100 000 per year. A CT/MRI verified severe complication (postseptal orbital, intracranial or osseous) was found in 34%, representing an incidence of 2.6 per 100 000 per year. Surgery was performed in 17% of admissions (incidence 1.3). In paper I-III, between 80-96% of admissions had preseptal cellulitis. In paper I-II, males had a higher incidence of admission and postseptal complication compared to females. In paper III, males had a higher incidence of admission, all type of complications and surgery, compared to females. *S. pneumoniae* was the most common bacteria found in nasal and blood cultures in paper I, but was not dominant in the nasal or blood cultures and absent in the surgical cultures in paper II. *H. influenzae* and *S. pyogenes* dominated in the nasal cultures in paper III, *S. milleri* was the dominating bacteria in surgical cultures (33%), and *S. pyogenes* dominated in blood cultures.

In paper IV, cultures from the middle meatus were positive for bacterial growth and displayed a wider range of bacteria compared to the nasopharyngeal cultures. There was a match of at least one type of bacteria in the MM and NPH culture in 36% of the cases. *M. catarrhalis* was possibly associated with a lower number of days with intravenous antibiotics, *H. influenzae* and *S. pneumoniae* negatively associated with max CRP, and *S. pyogenes* positively associated with max CRP. *S milleri* was found in the surgical culture in 58% of the cases that had surgery. The nasal cultures were negative in 58% of the cases that had surgery. In the surgical cultures, 16S rDNA PCR resulted in a higher number of positive results in comparison to the traditional swab and tissue cultures. The viral nasopharyngeal PCR was positive in 53% of the cohort in paper IV, and influenza A was most common. A positive viral PCR was associated with a lower grade of complication and CRP max. Influenza virus was possibly associated with a lower grade of complication. *S. pyogenes* was found in at least one culture in 55% of the influenza A/B positive cases, and the association was significant. The cases with a positive viral PCR and total number of cases followed the same monthly distribution during the year. The allergy sensitization test was positive in 29% of the cohort and in 50% of the cases that had surgery, and possibly associated with a higher number of days with IV antibiotics.

The incidence of admission due to acute rhinosinusitis in children in Stockholm studied in paper I-III, showed that the youngest children were admitted the most and that admission decreases by age. Furthermore, study II showed that admission decreased compared to the pre-PCV study period in paper I. The unchanged admission rate in paper III after PCV is in line with international studies showing a vaccine effect primarily in young children and adults (45).

A higher percentage of the children in paper III had radiology performed compared to the cohort in paper I and II. This is in line with the increased number of severe complications among the older children in paper III. There was a temporal change in the mode of radiology in the studies. Plain x-rays were only performed during study I and in the first half of the study period in paper II and III, and was thereafter fully replaced by CT and MRI. There have been substantial advances in the technology of CT and MRI the last 10 years. CT has become more accessible, the radiation dose and examination time has decreased, the image quality has improved, and the possibility of creating 3D images has increased (91). Similar innovations have also been made regarding MRI, for example techniques that decrease motion artefacts.

The radiology analysis in all studies in this thesis highlight that if a CT is performed in a child with suspected complication to ARS, a full dose CT with contrast is required for an adequate assessment of the level of complication. Plain x-ray does not play a role in diagnosis of rhinosinusitis or complications. However, while CT/MRI is a crucial tool in the management of ARS complications, it is important to avoid overuse of radiology, and to perform the radiology at the right time in the disease process. Clinical signs of a developing subperiosteal abscess are ophtalmoplegia, exophtalmus and chemosis. Another important sign is patient deterioration and increased levels of CRP. An early CT scan may preclude the clinician from ordering a subsequent CT causing a delay in eventual surgical intervention. This is only one of the aspects that puts high demands on clinicians in the management of complications. The improved imaging creates new possibilities in diagnosing complications and may provide better circumstances for determining length of antibiotic treatment, follow-ups, and future research.

Based on the population-based results of paper I-III, we conclude that complications to ARS in children are rare. The results from all papers show that preseptal cellulitis is the most common complication, found in a great majority of the admitted children in all ages. In line with current literature, the orbital complications were the most common group of complications, followed by intracranial and osseous complications. Under the age of five, only orbital complications were found (paper I-II) and severe orbital complications (postseptal) were rare. The number of intracranial and osseous complications increased with age, most probably due to the development of the paranasal sinuses. In paper II, we found that the incidence of severe complications did not decrease in line with a decreased admission rate seen in the PCV cohort, but rather increased slightly. This trend is in accordance with two larger register studies from the USA (15, 47). However, there is a possibility that the increased number of severe complications found in paper II were due to an increase in number of CT/MRI performed. On the other hand, the clinical signs that indicate a subperiosteal abscess, and therefore requires imaging to evaluate if the child needs surgical drainage, has not changed significantly since 2003.

A comparison of the population-based incidence numbers of complications in paper I-III with international studies is difficult due to the differences in study design. Capra et al reported an incidence of hospital admission with an ICD-code of orbital cellulitis to 7.38 per 100 000 children, 18 years and younger, in the year 2000, and a decrease to 6.05 in 2009 (15). This is lower than the admission rate in paper I-III, but somewhat closer to our reported incidence of preseptal cellulitis. Capra et al also reported an increase in mean age between the two study years, which could be the same effect of PCV that we could see in the comparison between paper I and II. The incidence of intracranial complications in paper III is in line with the results from an American study by Piatt, who found an incidence of 0.43 per 100 000 children the year 2003 and 0.40 in 2006 (17).

The incidence of surgery was higher in study II compared to study I, and the percentage of children having surgery was higher among the older than the younger age groups in study III. There is a logical correlation between more severe complications and surgery in the older age groups. The increased incidence of surgery in study II could be associated with the effect of PCV, but the low number of cases precludes drawing strong conclusions. Furthermore, the increase could be due to changes in surgical techniques and trends. Capra et al reported an incidence of 0.8 per 100 000 children up to 18 years old due to orbital cellulitis in 2000 and 1.2 per 100 000 in 2009 (15). The results from 2009 are somewhat lower than our combined data from paper II and III. However, our data included all types of complications and was based on a hospital register covering all hospitals in the Stockholm area. In study III, we did not find a clear trend of increased number of severe complications or surgeries after the introduction of PCV. In line with no change in admission rate, this could be due to a vaccine effect primarily in young children.

The population based data in study I-III provides a contribution to the existing literature and should be representative of the Swedish pediatric population as a whole, and similar populations. Stockholm and the other regions of Sweden have an integrated pediatric care, with close cooperation between pediatric, ENT and ophthalmology departments. Furthermore, there is a long tradition of national registers and individual data gathered through the national social security number, based on date of birth.

S. pneumoniae was the dominant bacteria found in the nasopharyngeal and blood cultures in paper I. In paper II, *S. pneumoniae* was less common in the nasopharyngeal and blood cultures compared to in paper I. *S. pyogenes* was more commonly found in the nasal cultures in paper II compared to paper I. The reduction of S. pneumonia after PCV introduction in the younger children could theoretically have led to changes in the composition of nasal microbiota in favor of other bacteria. Theoretically, the PCV could also have led to changes in the complex interplay between viral, bacterial and immunological molecules in the nasal mucosa.

S. milleri was not found in any culture in paper II but the number of surgical cultures were few. The number of nasal cultures taken of the admitted children were low in paper I-II, and only somewhat higher in paper III. In all three papers, there were cases in which a surgical culture was not taken. This could be due to a lack of understanding of the importance of gathering bacterial cultures. In line with the questioned clinical value of nasopharyngeal cultures in younger children due to the rich nasal flora, the clinical value of obtaining a surgical culture is small if it often is negative after treatment of intravenous antibiotics, or if the result doesn't change the choice of treatment. However, the new available technologies of bacteria DNA create new possibilities to understand the role of nasal microbiome during infections, and henceforth give the bacterial analysis greater value.

In paper IV, when bacterial cultures were prospectively and systematically gathered, more results of bacterial cultures were obtained from traditional culture swabs. The comparison of different kinds of surgical cultures in paper IV showed that broad-range 16S rDNA PCR test resulted in a higher number of positive results in comparison to the traditional swab and tissue cultures, and should be recommended. The result of a higher percentage of positive cultures and a different display of bacteria in the MM compared to the NPH cultures in paper IV supports the theory that a MM culture may represent the bacteria in the sinuses, in line with the studies that have compared MM and sinus puncture cultures (19, 20). We could find several associations between bacteria and outcomes in the statistical analyses. *M. catarrhalis* was possibly associated with a lower number of days with IV antibiotics. *S. pneumoniae* was associated with a lower value of max CRP, as was *H. influenzae*. *S. pyogenes* was associated with a higher value of max CRP. These results implicates differences in the bacterial pathogenesis in ARS in children.

The dominant pathogen in the surgical cultures in paper III was *S. milleri*, and this result was confirmed in paper IV, which is in line with many other studies. *S. milleri* is most commonly found in the mouth and gastrointestinal tract and not in the nasal flora (27, 92), but was found in three MM cultures in paper IV. None of these cases had surgery. In paper IV, surprisingly, the nasal cultures of the children that had surgery were negative in 7/12 cases, and a match between nasal and surgical culture only found in two cases, both showing growth of *H. influenzae*. This could indicate an absence of drainage from the sinuses into the nose, and may partly explain how a severe complication develops. Furthermore, the absence of *S. milleri* in the nasal cultures in the surgery cases could be explained if *S. milleri* is established in the abscess after another pathogen initiated the infection.

In study IV, we found a positive viral nasopharyngeal PCR in 53% of the cases. There was a negative association between presence of virus and the outcomes: grade of complication and max CRP. Respiratory viruses play a substantial role in ARS, they are responsible for a majority of upper

airway infections and common colds, and have been shown to trigger mucosal changes involving complex immunological pathways and multiple bacteria. The virus-positive cases and total cases followed the same monthly distribution during the year, which indicates a close correlation between respiratory viruses and complications to ARS. In paper II, there was a peak of hospital admissions for the epidemic year of 2012-2013, and a possible explanation could be a high influenza activity in Stockholm recorded that season (93). We screened for multiple respiratory viruses in the study, and the shedding duration varies between them (94-97). A possible explanation to the negative association to grade of complication and why the number of positive viral PCR cases was not higher, could be that the virus had already disappeared from the nasopharynx. Another possible theory could be that there were changes in the nasal microbiota, and not a viral trigger, that induced the acute respiratory infection, as described in a pediatric nasal microbiome study (27).

There is a lack of studies of the impact of specific viruses on complications to ARS. One prospective study of ABRS found a correlation between the presence of rhinovirus and *M. catarrhalis* in children up to three years old (7). In our study, there was a possible negative association between influenza virus and grade of complication. We found a statistically significant association between *S. pyogenes* and influenza A/B. This supports the studies that suggest an association between influenza virus and secondary *S. pyogenes* infection (50-54). The results of paper IV can possibly shine some light on the intricate role of viruses in the development of ABRS. Further studies needs to be performed that investigate the role of specific viruses, such as influenza, and their impact on pathophysiological mechanisms of severe complications.

In paper IV, the number of children with verified sensitization for airborne allergens were congruent with pediatric population-based studies of sensitization rates in Stockholm (64, 65). Within the concept of united airways, associations have been found between allergy, airway infections and asthma (27, 57, 69-70). An association between allergic rhinitis and chronic rhinosinusitis have been found in adults (76), but the studies of children are few and inconclusive (77-80). Allergic rhinitis has been discussed as a risk factor for ARS and ABRS, but the results are ambiguous (71-73), and only a few studies regard children (74, 75). To our knowledge, the relationship between ABRS complications and airborne allergy sensitization has not been studied. In paper IV, we found a possible association between positive Phadiatop test and number of days with IV antibiotics, which could imply that sensitization is a risk factor for severe ARS. In our results, we cannot differentiate between the children that are only sensitized and the children that have a manifest allergic rhinitis. An interesting question is if the local inflammation caused by the IgE mediated allergic rhinitis is the pathological mechanism that predisposes for ARS, and not the allergic rhinitis.

We found no cases with decreased immunoglobulins in paper IV. The role of decreased levels of immunoglobulins in rhinosinusitis have primarily been studied in chronic rhinosinusitis in adults. However, it is intriguing to theorize around the potential relation between immunoglobulin deficiencies, a reduced vaccine-response, and nasal microbiome composition. It is likely that our sample size was too small to study a potential association between ARS and immunoglobulin deficiencies.

Our data shows that, from a population-perspective, complications are rare. However, the data also suggests that if admitted to hospital due to severe ARS or suspected complication, there is a substantial risk of developing a severe complication with possible serious consequences. This highlights the need in clinical practice to evaluate all the aspects of complications to ARS in children. This includes medical history and patient characteristics, symptoms, lab results, general/ local/ ENT/ neurological/ ophthalmological status, and to closely follow the clinical course. The medical evaluation and treatment of children with complications puts high demands on the cooperation between the specialties involved – pediatricians, ENT, ophthalmologists and neurosurgeons. The prospective study design in paper IV had a positive impact on this cooperation. The implementation of the study generated a

spread of information and increased awareness of complications to ARS in children, which alone can improve the clinical care of children with the disease. Due to the rarity and wide panorama of complications, the clinical data from studies such as paper I-IV in this thesis is of great value for clinicians who may not encounter many cases during their careers.

The strengths of paper I-III are that they are population-based, and therefore provide important data on the rare diagnoses of complications to ARS in children. However, the retrospective nature of the studies is a limitation, and valuable clinical information was missing in many cases, especially in regard to results of bacterial cultures. This was an important background in the planning of study IV, where the clinical data that we were missing in paper I-III was systematically and prospectively gathered. The strength of paper IV is the prospectively collected data from bacterial cultures from different sites, viral swabs, Phadiatop and immunoglobulin test. The prospective study design resulted in a lower number of cases, which is a limitation of paper IV. There was also some data missing. However, the prospective study design can hopefully be integrated into existing care programs and create better conditions for the gathering of clinical data and improved retrospective studies in the future.

7 CONCLUSIONS

Paper I: Complications due to ARS in children up to five years of age are rare. A majority of the admitted children are less than 2 years old and male. Clinical signs of preseptal cellulitis are present in most children but postseptal orbital complications are rare. Most children that are admitted due to complications to ARS are previously healthy and improve quickly with intravenous antibiotics. *S. pneumoniae* was the most common pathogen found in the bacterial cultures before the introduction of pneumococcal conjugate vaccine.

Paper II: Hospital admissions due to complications to ARS in children up to five years old have decreased after the introduction of pneumococcal conjugate vaccine, but the incidence of CT/MRI verified severe complications and surgeries seem to have increased slightly. Boys are admitted and have severe complications to a greater extent than girls. Clinical signs of preseptal cellulitis is found in most admitted children, but only few have a CT/MRI verified postseptal orbital complication and surgery is seldom needed. *S. pneumoniae* is not the most common bacteria found in nasal and blood cultures and is absent in surgical cultures. Regarding complications to ARS in children under the age of five, the pneumococcal conjugate vaccine has potentially had an effect on: admission rate, incidence of severe complications, and bacteriology.

Paper III: Complications due to ARS in children five to 18 years old are rare in the population, but among the hospitalized children, the risk of severe complication is high. The range of complications is wider among older children, who also have a higher percentage of severe complications and surgery, than in younger patients. Hospitalized males have a higher median age; and a higher incidence of admission, severe complication, and surgery, compared to females. The most common bacteria found in nasal cultures are *S. pyogenes* and *H. influenzae*, and *S. milleri* is the dominant bacteria in the surgical cultures. The findings in bacterial cultures from the surgical site and the nose seldom match. The correct mode of radiology is essential in the diagnosis of complications to ARS in children.

Paper IV: There are differences in results of middle meatus and nasopharyngeal cultures in children hospitalized due to ABRS. There seem to be associations between certain bacteria and length of treatment and CRP levels. Findings in cultures from the surgical site and the nose rarely match, and most nasal cultures from children that have surgery are negative for bacterial growth. Broad-range 16S rDNA PCR provide a higher number of positive results compared to traditional swab and tissue surgical cultures. Sensitization to airborne allergies and the presence of certain viruses seem to play a role in the development of complications to ABRS.

8 POINTS OF FUTURE PERSPECTIVES

During the work on this thesis, ideas and thoughts for future studies have emerged.

The datasets of paper I-III could be merged into one dataset. It would result in a larger cohort, a statistical analysis with more power, and results that cover all ages of childhood.

The research of this rare disease would benefit from larger study cohorts, national or international. A national Swedish study would be possible by adding register-based data from Sweden's remaining regions. Furthermore, like in Sweden, there is a long history of national registers in the other Nordic countries that could enable multi-national cohorts.

As described in this thesis, the clinical evaluation of a child with a possible complication to ARS is complex. It would be of interest to investigate the possibility of creating a risk assessment tool with a point system, to be used in clinical practice as guidance for risk of developing a severe complication to ARS.

The expanding field of nasal microbiome studies with new techniques of bacteria analysis is an area of great interest for the future. Further studies of changes in the nasal microbiome and microbiota that take place during viral and bacterial rhinosinusitis would increase the understanding of the development of complications, and the risk factors involved.

There is generally a need for more and larger prospective studies regarding complications to ARS.

The use of new broad-range 16S rDNA PCR techniques could be used to a greater extent in future studies. For example, it would be interesting to compare middle meatus cultures in children with ABRS with both traditional swab culture and rDNA PCR.

Although the number of studies that look at the immunological processes and cell changes that take place in the nasal mucosa in the presence of viruses are increasing, much knowledge is still to be gained in this area.

The advancements in radiology have been prominent the last few years, both regarding CT and MRI, with improved image quality and reduced radiation dosages, etc. This should enable a more detailed analysis of complications, and could possibly be used in future research to investigate potential radiological risk factors for complications to ARS.

9 ACKNOWLEDGEMENTS

I am most grateful to everyone, colleagues, friends, and family, that have contributed to this thesis. I would especially like to thank:

Main supervisor **Pär Stjärne**, for guiding me through this work. Thank you for making this project fun with your bursting enthusiasm, and for your unpretentious and inviting way of sharing your extensive knowledge in rhinology and research. Thank you for making our professional relationship work the last couple of years when you were my main supervisor and I was your manager. In the roller-coaster of writing a thesis, you have been a role model in leaving setbacks behind and getting on with it, a crucial quality in research.

Co-supervisors **Anna Granath**, **Olof Hertting** and **Mats Holmström**, for helping me through this thesis. You have all contributed immensely.
Anna, thank you for asking me to this join this project in the very beginning, for your efforts with data analysis and writing in all the studies, for being kind and caring, for sharing your knowledge within ENT and bacteriology, and for your wise feedback.
Olof, thank you for adding greatly to this thesis with your valuable expertise in pediatric infectious diseases, and for doing so in such a humble and pleasant way. Thank you for being thorough, for all your time and efforts in the extensive work with the prospective study, and for continuously checking in with me and asking what you can do to help.
Mats, thank you for believing in me as a resident, for your honest and timely feedback, and for sharing your vast experience in rhinology and research.

Margareta Eriksson and **Rutger Bennet**, for all the help with the collection of data and for cheering me on. The meetings we have had in your office have enlightened this work. Margareta, for your never-ending enthusiasm and unwavering "just do it" attitude, and Rutger, for introducing me to the wonders of Excel.

Lina Schollin Ask, friend and research buddy, for being such a great companion to write a scientific paper with, for your advice and kindness, and for caring about all components of my life.

Ann Lindstrand, for involving me in your research, introducing me to the topic of pneumococcal vaccine, and for being so very generous along the way. It was what laid the foundation to this project.

Malin Ryd Rinder, for your guidance, feedback and advice in the earlier phases of this project, when my beginner qualities needed it the most, and for your reliable support throughout.

Mentor **Catarina Almqvist Malmros**, thank you for all your support, encouragement, and practical tips. You are wise, calm and reassuring, and I can't imagine a better mentor. Our meetings have given me great joy, confidence and strength.

Professor **Lars-Olaf Cardell**, Head of Division of Ear, Nose and Throat Diseases at the Department of Clinical Science, Intervention and Technology, Karolinska Insitutet, for creating a scientific atmosphere and being a guardian of clinical research.

Division administrator **Agneta Wittlock**, for all the important help with forms, documents, deadlines and layouts.

Present and previous Heads of the Department of Otorhinolaryngology, Karolinska University Hospital - **Alexander Ahlberg**, **Sushma Nordemar**, **Bo Tideholm**, and **Mats Holmström**, for making it possible to do this research. A special thank you to **Alexander Ahlberg** for being generous and encouraging towards my research in my role as a manager.

All ENT-colleagues at Karolinska University Hospital, thank you for making work fun and interesting. An extra, very grateful, thank you for including patients to paper IV in spite of the busy reality of clinical work. This thesis would not have been possible without your help.

My colleagues at the rhinology department, **Babak Alinasab**, **Ola Fridman Bengtsson**, **Jon Flodström**, **Laila Hellkvist**, **Åsa Kågedal**, **Pär Stjärne** and **Karin Toll**, for being "my family" at work, for sharing your knowledge and skills and making me a better clinician.
Rusana Bark, **Henrik Smeds**, **Magnus Starkhammar** and **Malin Wendt**, for sharing thoughts and giving advice of how to fit research into one's work life, against all odds.
Laila Hellkvist, **Lina Nygren**, and **Karin Åberg**, for the support and sense of community since we started at the ENT-department in 2009, thank you for giving a couple of extra minutes here and there to check how things are with work, research and life in general.

Marit Westman, for staying in touch, and giving me your expert help in the tricky field of allergology.

Praxitelis Dimitriou, **Babak Flahat**, **Patrick Masaba**, **Alice Odenrick** and **Subhash Srivastava**, for all the discussions and invaluable help with radiology analysis.

Helena Thulin, for adding friendship and humor to research school, and for the good lunches with entertaining reflections of our place in research.

Jenny Alkén, **Helene Bodegård**, and **Maria Schultz**, my dear friends, thank you for all the laughs and adventures, and for always being there for me, during the ups and downs in life.

My childhood friends **Anna**, **Annicka**, **Linda F**, **Linda W**, **Lisa, Sara** and **Ulrika**, for contributing to a happy childhood, laying the foundation for academic thinking, and for still being in my life.

My mother and father, **Christina** and **Jan Hultman**, for your unlimited love and care, and for being my sounding board for thoughts and ideas about research, work and life. Thank you pappa for inspiring me to be a doctor. Thank you mamma for showing me that everything becomes easier and more fun with ingenuity. Thank you both for teaching me to never give up.

My brothers **Johan** and **Gustav**. Thank you for being great supporters and listeners. Johan, for being so present even though you're far away. Gustav, for being reliable and for helping me in your expert areas, where my knowledge is lacking.

My lovely family, **Tyler**, **Iris** and **Louise**. Tyler, thank you for being my life companion and greatest supporter, and for lifting me up when things are hard. And thank you for the hours of proof-reading. Iris and Louise, our wonderful daughters, your sweet and smart personalities make me happy and proud every day.

This work was financially supported by grants from: The Samariten foundation for paediatric research, The Acta Oto-laryngologica foundation, Swedish Association for Otorhinolaryngology Head and Neck Surgery (SFOHH), the regional agreement on clinical research (ALF) between Stockholm County Council, Karolinska Institutet and Karolinska University Hospital.

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