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Pediatric Obstructive Sleep Apnea

Evaluation of surgical treatments and immunological aspects

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Abstract

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Pediatric obstructive sleep apnea (OSA) is a sleep-related breathing disorder often caused by enlargement of the adenoid and the tonsils. The peak prevalence is among children who are two to six years of age, when the lymphatic tissue is most active. Clinical diagnosis can be challenging, since the symptoms are often unspecific. Polysomnography, the diagnostic gold standard is a complex procedure with limited availability.

The standard surgical treatment for OSA is removal of the tonsils (tonsillectomy) together with removal of the adenoid (adenoidectomy). Partial removal of the tonsils together with adenoidectomy, known as adenotonsillotomy, is a treatment accompanied with less postoperative morbidity but a higher risk of reoperation. The aims of this thesis are to evaluate different treatment methods for treating pediatric OSA and to investigate immunological aspects of tonsil hypertrophy.

In Paper I, tonsil tissue from children with large tonsils and moderate to severe OSA was compared with tonsil tissue from children with small tonsils and milder OSA. The tissue was analyzed with flow-cytometry using markers for T cells, B cells and ILCs. Patients with enlarged tonsils and more severe OSA had a significant increase of naïve B cells without a difference in the Ki67 proliferation marker. This indicates an impaired differentiation and/or migration of B cells in the larger tonsils.

In Paper II, behavior and mental health after adenotonsillectomy or adenotonsillotomy were compared. Together with polysomnography and the questionnaire OSA-18, a strength and difficulties questionnaire (SDQ) was filled out both before and one year after treatment. No significant differences between the treatment groups were seen after one year, in terms of mental health and behavior.

Paper III is a long-term follow-up study of a previously published randomized controlled trial of adenotonsillectomy and adenotonsillotomy. Five years after surgery, a new polysomnography was performed on the patients. The results showed a high dropout rate, and six children were excluded because of re-operation with adenotonsillectomy. No difference in the obstructive apnea hypopnea index was found between the groups five years after surgical intervention.

Paper IV is a prospective cohort study of children treated for OSA. The correlations between subjective data from OSA-18, patient-reported outcome measures, and objective data from polysomnography were compared. Significant correlations were shown between changes in objective polysomnography data and changes in OSA-18, with the strongest correlation in the sleep disturbance subscale. The measure of patient-reported outcomes showed a significant but weak correlation.

Keywords: Obstructive sleep apnea, pediatric sleep disordered breathing, tonsillar surgery, Polysomnography, adenotonsillotomy, adenotonsillectomy, Strength and Difficulties Questionnaire, OSA-18

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For every child, every right

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Carrasco A., Sjölander I., Van Acker A., Dernstedt A., Fehrm J., Forsell M., Friberg D., Mjösberg J., Rao A. (2021) The Tonsil Lymphocyte Landscape in Pediatric Tonsil Hyperplasia and Obstructive Sleep Apnea. *Frontiers in Immunology*, Oct 22;12:674080.

II. Sjölander, I., Borgström, A., Larsson, J., Smedje, H., Friberg, D. (2020) Randomised trial showed no difference in behavioral symptoms between surgical methods treating paediatric obstructive sleep apnoea. *Acta Paediatrica*, 109(10):2099-2104

III. Sjölander, I., Borgström, A., Nerfeldt, P., Friberg, D. (2022) Adenotonsillotomy versus adenotonsillectomy in pediatric obstructive sleep apnea: A 5-year RCT. *Sleep Medicine:X,* 4:100055

IV. Sjölander, I., Borgström, A., Nerfeldt, P., Fehrm, J., Friberg, D. (2022) Correlations between subjective and objective outcomes after adenotonsillar surgery in children with OSA. Submitted.

Contents

Abbreviations

AASM	American Academy of Sleep Medicine
ADHD	Attention-deficit/hyperactive disorder
AHI	Apnea-hypopnea index
APP	Adenopharyngoplasty
ATE	Adenotonsillectomy
ATT	Adenotonsillotomy
BMI	Body mass index
CD	Cluster of differentiation
CHAT	Childhood adenotonsillectomy trial
CI	Confidence interval
CPAP	Continuous positive airway pressure
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electrooculogram
HRQoL	Health-related quality of life
ITT	Intention to treat
MHC	Major histocompatibility complex
OAHI	Obstructive apnea-hypopnea index
OSA	Obstructive sleep apnea
OSDB	Obstructive sleep-disordered breathing
PATS	Pediatric Adenotonsillectomy Trial for Snoring
PG	Polygraphy
POSTA	Pre-school OSA Tonsillectomy Adenoidectomy study
PROM	Patient-reported outcome measures
PSG	Polysomnography
PSQ	Pediatric Sleep Questionnaire
RCT	Randomised controlled trial
RDI	Respiratory disturbance index
RERA	Respiratory-effort related arousal
SD	Standard deviation
SDS	Sleep disturbance subscale
SDQ	Strengths and Difficulties Questionnaire
TE	Tonsillectomy
TSS	Total symptom score
TT	Tonsillotomy

Introduction

Sleep disordered breathing (SDB) is an umbrella term for several forms of abnormal respiratory patterns during sleep. The term encompasses central breathing disorders, but is most commonly used for obstructive sleep disordered breathing (OSDB), which is the focus of this thesis. OSDB varies from habitual snoring to the most serious form, known as obstructive sleep apnea (OSA).

OSA is characterized by repeated events of upper airway obstruction during sleep, causing decreased airflow and hypoxemia despite continuous respiratory effort (1). Hyperplasia of the adenoid and/or tonsils is the most common cause of OSA, which affects about one to five percent of all children from the ages of two to eight years (2). This is the period of development when immunological activity in this tissue is also at its peak (3).

The surgical treatment for OSDB is one of the most common procedures in Sweden, with about 5,000 performed yearly (4). It is also among the first surgical procedures you are taught to perform independently, as an ear, nose and throat doctor. Nevertheless, many unanswered questions remain, such as when to do surgery, what kind of surgical procedure to choose, and how to evaluate the treatment given.

When I met my supervisor Danielle and her team in 2017, I was unaware of what a great research field sleep apnea constitutes. Despite it being a common disease, the level of knowledge of OSDB among physicians is somewhat limited.

Pediatric OSDB is prevalent worldwide, with different diagnostic tools, treatment alternatives, and follow-up guidelines available according to the different countries' health care systems, cultural factors and economic opportunities. The more I have learned about the condition, the more complex, interesting and unclear the science behind it has become. This is not to mention the fascinating world of immunology, which is not only connected to many such health problems but also treatments of them, as became evident during the SARS-CoV-2 pandemic.

In brief, the present thesis focuses on evaluating the long-term effects of surgical techniques for treating pediatric sleep apnea. It also examines the correlation between objective and subjective outcomes. Moreover, it includes an immunological assessment of the complex mystery of why some children develop difficulties in breathing because of enlarged lymphatic tissue.

Normal sleep and sleep stages in childhood

Sleep-wake regulation and sleep stages evolve rapidly during childhood, especially during the first year of life. Newborns do not have an established circadian rhythm, as this starts to appear around 10 to 12 weeks of age.

Sleep is divided into two stages: rapid eye movement (REM) and non-rapid eye movement (NREM). During the night at the age of five years, sleep oscillates between REM and NREM throughout the night with an average period of 90-120 minutes cycles, of which REM accounts for about 20-25 percent (5, 6). NREM is further divided into four stages:1, 2, 3 and 4, where stage 3 and 4 are similar and have lately been merged into one stage known as deep sleep or slow-wave sleep. The characteristics of different sleep stages are shown in Table 1.

Table 1. Characteristics of different sleep stages

Sleep stage	Characteristics
NREM	
Stage 1	During this stage, slow-rolling eyes can be seen and theta brain waves (>13 Hz) are dominant in the electroencephalogram (EEG).
Stage 2	This stage is characterized by spindles (10-13 Hz) and K-complex.
Stage 3-4	Defined by delta waves (high amplitude). This is the stage where deep sleep is present. It is often difficult to wake a person up and children can experience enuresis, night terrors or sleep walking during this stage.
REM	Rapid eye movement (REM) stage resembles an awake EEG-pattern, with low voltage and fast frequency. In contrast to the awake state, the electromyogram (EMG) shows inactivity since all the muscles are inactive except the ocular muscles and the diaphragm.

Normal sleep architecture continues to change significantly over the first two decades of life (7). The most significant shifts are the distribution of REM-sleep from about 50 percent from birth to 25 to 30 percent in adulthood. Furthermore, the amount of slow-wave sleep peaks in early childhood, which is clinically significant, with higher prevalence of partial arousal parasomnias such as sleepwalking during preschool and early school aged children. The normal sleep architecture, i.e., the distribution of sleep across different sleep stages, is visualized for different ages as an hypnogram in Figure 1.

An arousal is an abrupt shift in EEG frequency of at least three seconds in duration, following at least 10 seconds of sleep. It may be a sign of disturbed sleep caused, for example, by breathing problems, but may also be a normal consequence of the ultradian rhythm of the sleep cycle.

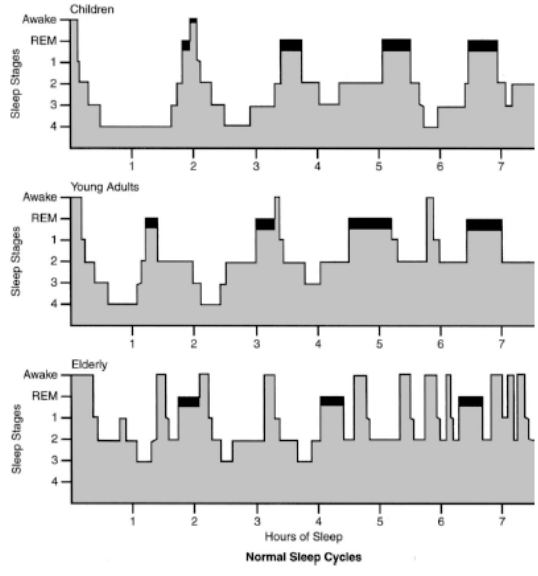


Figure 1. Hypnogram of normal sleep cycles of children compared with young adults and the elderly. Published with permission from Wolters Kluwer.

The sleep-wake pattern is driven by a complex interplay between biological, environmental, behavioral and social factors (8). However, a good night's sleep is essential for children's well-being and health. Problems with sleep duration, timing and quality as well as the variability of sleep patterns have shown an association with a range of health problems (9). One of these, is obstructive sleep disordered breathing and obstructive sleep apnea in particular.

Obstructive sleep apnea

Etiology

The underlying causes of pediatric OSA are complex. Habitual snoring is a prominent symptom defined as loud snoring ≥3 nights per week. The cause of this noise is mainly vibrating tissues or the walls of the upper airway collapsing during inspiration when asleep (10). A total collapse of the airways results in apneas.

The most common cause of this collapse among children is lymphoid hypertrophy of the palatine tonsils and the nasopharyngeal adenoid. But the etiology of the obstruction may be defined by other anatomical factors, such as neuromuscular disorders making an adequate muscle tension around the airway difficult to maintain.

These repetitive obstructive episodes result in oxygen desaturation, disturbed sleep, and the risk of many other significant health consequences if left untreated. The importance of early diagnosis and treatment is seen both for the children's health and from a health economic point of view (11).

Epidemiology

The prevalence of OSDB is estimated to vary widely from four to 15 percent in the pediatric population, depending on the criteria used (12, 13). Within the same age range, OSA prevalence ranges between 1 to 5 percent (14, 15). The peak is at preschool age, when the enlargement the of tonsils and adenoid are at their highest ratio in terms of size versus airway.

Comorbidities and risk factors

Other factors associated with an increased risk of OSDB and OSA are obesity (12), asthma (16), and allergic rhinitis (17), especially poorly controlled (18), as well as preterm birth (19), craniofacial anomalies (20), and neuromuscular disorders. African American ethnicity and familiar clustering have also been mentioned, but the cause of these connections is not clear (15, 21). Even social and economic disparities have been shown to affect the prevalence of OSDB (22).

Obesity is a major risk factor for OSDB. Habitual snoring has been shown to occur in up to 40 percent among obese children and adolescents (23) while the prevalence of OSA in the same group is 19–61 percent (24). It is well known that weight issues and obesity are a growing problem among adults, adolescents and children in Sweden as well as in other countries around the world (25, 26). The mechanisms are multiple: obese children have a narrower oropharynx because of fat infiltration, and they have a more pronounced adenoid and tonsils as well as retropharyngeal lymph nodes (27). The infiltration of fat not only narrows the airway but also reduces the ventilating capacity by increased visceral fat in the thorax and abdomen. In addition, a connection between disturbed metabolic biomarkers in children with concurrent obesity and OSAS has been shown, shining a light on the importance of treating the OSA that also might have an effect on the obesity (28).

Other diagnoses with a high risk of OSA include children with neuromuscular diseases (29) (e.g., cerebral palsy (30), congenital muscular dystrophies), craniofacial abnormalities (31) (e.g., Craniosynostoses, Pierre Robin sequence, cleft palate), Downs syndrome (32), Prader Willi syndrome (33), mucopolysaccharidoses (34), and sickle cell anemia (35) among others.

Consequences and sequelae

OSA is associated with significant morbidity for children and their families, especially if left untreated. The most prominent negative consequences are neurobehavioral dysfunction (36), growth and developmental delay as well as cardiovascular complications (37).

Neurocognitive and behavioral problems related to OSA were first described extensively in the early 1980's, when studies showed that children with attention deficit hyperactivity disorder who were treated for their sleep-disordered breathing, also experienced fewer behavioral symptoms (38). Newer studies support these findings (39, 40), and even suggest that not only OSA but even habitual snoring can have a large impact on children's behavior (41, 42). The reasons for these effects are not clear but one hypothesis is that the increased work of breathing and sympathetic activation can cause neurobehavioral deficits. Magnetic resonance (MR) studies of the brain of children with OSA suggest that it may affect the cortical thickness and grey matter in the brain (43-45). These findings account not only for children with OSA but also for habitual snoring children (46, 47).

Children with OSA and habitual snoring are at higher risk for cardiovascular sequelae such as autonomic dysfunction, endothelial dysfunction and hypertension both in children and adults (37, 48).

Last but not least, having a child with persistent OSDB has a negative impact on the whole family (49) with more frequent hospital visits, higher consumption of drugs (11), and, in the worst cases, increased mortality rates in children with comorbidities (50).

Symptoms

Pediatric OSA was first described in 1976. Five years later, doctor Guilleminault from Stanford University, demonstrated that the clinical presentation among children differed substantially from adults. Children show more disturbed nocturnal sleep and daytime symptoms such as hyperactivity and behavioral problems compared with daytime excessive sleepiness, which is more common among adolescents and adults with OSA (51).

The manifestations of OSDB and OSA differ greatly depending on the age of the child when the disease is most present. Nightly symptoms described by parents include: frequent snoring, apneas, disturbed sleep, abnormal sleep positions, and nocturnal enuresis. Daytime symptoms include hyperactivity, fatigue, difficulties with eating, and mouth-breathing. Untreated, OSDB is associated with serious complications, such as failure to thrive, hyperactivity, cognitive disturbances, lower quality of life and cardiovascular disorders (37, 50, 52, 53).

A bidirectional relationship between OSA and asthma has been shown, and the treatment of OSA appears to improve asthma symptoms (54).

Neurocognitive dysfunction is a commonly reported consequence of OSA and can include hyperactivity, aggressive behavior, and behavioral disturbances. These symptoms are similar to neuropsychiatric disorders such as attention-deficit/hyperactive disorder (ADHD) and children with this condition should therefore be asked about their sleep and potential snoring habits.

Diagnosis of OSA

History and clinical examination

Early recognition and treatment of OSA is important in moderate to severe cases. The guidelines from the American Academy of Pediatrics from 2012, recommend screening for snoring among all children and adolescents (14). If snoring is present, a more extensive evaluation of OSDB symptoms should be initiated, with a patient's history and a physical examination as the first steps.

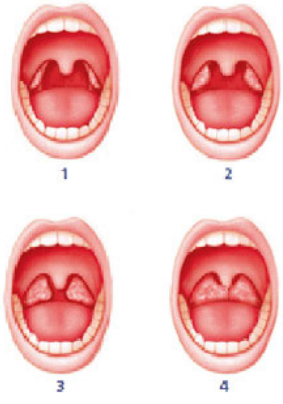
The history is most often given by the parents or caregiver and is of high importance. Important conditions from past medical and family history include prematurity and parents or siblings having a history of OSA or adenotonsillar hypertrophy (55).

Tonsil size is most often assessed using a headlamp and a tongue depressor and a quick look into the child's throat. To be able to make an inter-examiner comparison a systematic assessment such as that established by Brodsky can be used (Figure 2) (56). However, clinical assessment of tonsillar size is a weak predictor of the presence or severity of OSDB (55). The adenoid is best evaluated with flexible fiberoptic endoscopy through the nose, an examination not always easily performed on children.

To better evaluate a child's breathing problems during sleep, the parents can record the child during sleep, on their smartphone, when they hear or see what they are concerned about and show the video to the clinician. An adequately recorded video makes it easier to understand the circumstances for the child's problems and the parents' concern. A standardized scoring system can even be used to predict the severity of OSDB (57).

History and clinical evaluation have shown a poor positive predictive value for OSA compared with polysomnography (PSG), which is the gold standard method (58).

European guidelines state that all children with symptoms of OSDB and the presence of obesity, complex abnormalities such as Downs syndrome, or neuromuscular disorders should be referred for further investigation, which includes a full in-laboratory PSG, an ambulatory PSG or a respiratory polygraphy (55). This should also apply when the treatment is unclear (e.g., no visual adenotonsillar hypertrophy).



Degree of Brodsky	Ratio of tonsils in the oropharynx
1	Tonsils occupy less than 25%
2	Tonsils occupy from 25 to 50%
3	Tonsils occupy from 50 to 75%
4	Tonsils occupy more than 75%

Figure 2. Brodsky tonsil size.

Polysomnography

As mentioned, the gold standard for an OSA diagnosis is polysomnography (PSG), according to the American Academy of Sleep medicine (AASM). PSG measures sleep quality together with the respiration. The child spends a whole night in a sleep laboratory (level 1) where he or she is connected to six EEG-electrodes to monitor brain activity, an EMG to register muscle activity in the limbs and chin, an EOG to register eye movements, a pulse oximeter to register the child's pulse and oxygen saturation, a nasal cannula to register the air flow, and piezoelectric belts to register the thoracic and abdominal movements and body position. The night is also video and sound recorded. An acceptable night (at least four hours of sleep) is scored in accordance with the AASM manual (59). An example of a short section of a PSG-curve from a patient with OSA is seen in Figure 3.

A PSG can also be conducted at home, when it is referred to as an "unattended" PSG (level 2). This is mostly recommended for adults and teenagers (60); the nasal cannula is often irritating for small children and lost when unattended, which causes a high risk of underscoring the severity of OSA.

One of the most important parameters is the obstructive apnea/hypopnea index (OAHI), which is the mean number of apneas or hypopneas caused by obstruction, per hour. To identify the apnea or hypopnea as an obstructive, the cessation of airflow must be accompanied by continued respiratory effort or increased work of breathing seen by chest and abdominal movement. The OAHI is obtained from calculating the number of apneas and hypopneas

during the whole night, and divide it by the hours of sleep. The criteria for scoring respiratory events are defined in Table 2.

Table 2. Criteria for scoring respiratory events as obstructive apneas and hypopneas according to AASM

Apnea (have to meet all criteria)
• The event lasts for at least two missed breaths (or the duration of two breaths as determined by baseline breathing pattern). • The event is associated with a ≥90% drop in amplitude of airflow • The event is associated with the presence of respiratory effort throughout the entire period of absent airflow.
Hypopnea (have to meet all criteria)
• The event is associated with a ≥30% drop in amplitude of airflow • The duration of the event lasts for at least two missed breaths • There is a >3% desaturation or the event is associated with an arousal

Other important information obtained from a PSG is the apnea hypopnea index (AHI), which also includes the central apneas caused by the central nervous system and may be normal among some children. Central apneas are, contrary to obstructive apneas, seen when the child simply stops breathing and does not even try to take a breath.

The oxygen saturation index (ODI) tells us how many desaturation episodes are present on average per sleep hour.

The respiratory disturbance index (RDI) is an index of all the respiratory events per hour (central and obstructive) but also adds respiratory-effort related arousals (RERA), which is when a child's sleep is disturbed by a respiratory event but the event does not technically meet the criteria of an apnea or hypopnea.

In this thesis, both OAHI and AHI are used. To repeat, the OAHI measures only obstructive events, while AHI also includes any central apneas. Both OAHI and AHI are used in the literature to determine the severity of OSA. The following cut-offs in children are the most widely used (55, 61):

Table 2. Cut-offs for OSA-severity among children <16 years of age (62)

Degree of OSA	**OAHI**
Mild	≥ 1 to < 5
Moderate	≥ 5 to < 10
Severe	≥ 10

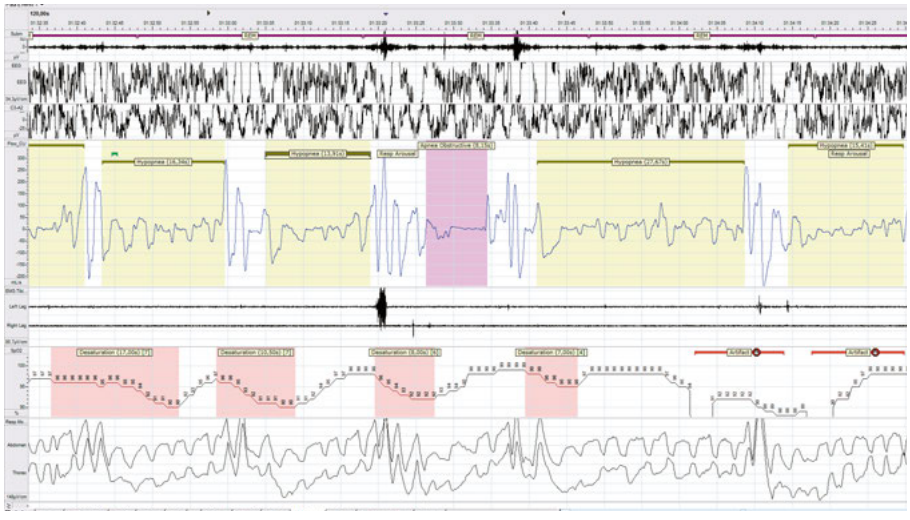


Figure 3. A typical polysomnographic curve from a patient with OSA, showing the nasal airflow with a high number of hypopneas (in yellow) and an obstructive apnea (in lilac) followed by desaturations (in pink) during REM sleep. From P. Murphy.

Respiratory polygraphy

Another type of objective tool used in the diagnostic work-up of OSA is a respiratory polygraphy test (RP or simply polygraphy) during sleep at night. Polygraphy only measures respiration and not sleep stages, the sleep time is estimated by the observers (parents or staff), and arousals are not registered. Polygraphy can be performed either at a hospital or at home. Respiratory polygraphy is not recommended as a reliable tool by AASM, but if PSG is not available, for example due to a lack of resources, it is considered as an alternative by the European Respiratory Society (55, 63). Recently, a guideline for diagnosing and monitoring pediatric sleep disordered breathing have been available from the British thoracic society (62). In that guideline, Polygraphy is considered as level 2 if undertaken attended with video recording and staff in hospital, and level 3 if undertaken at home unattended.

Polygraphy is cheaper and is widely used among adults. Even among adults, however, polygraphy has been shown to underestimate the numbers of AHI by up to 30 percent compared with PSG (64). Respiratory polygraphy is even more difficult to interpret among children since they have different sleeping patterns from adults. If the investigation is conducted at home, unattended and without video-recording the child, any result may be misleading. The scorer needs to be experienced and the score should be analyzed with caution (65). Therefore, a level 1 PSG is recommended in cases with low quality of respiratory polygraphy.

Questionnaires

OSA-18

There are several questionnaires available that score the symptoms connected with OSA. OSA-18 is a disease-specific tool that was developed in 2000 by Franco et al (66) and was translated and validated to Swedish in 2009 (67). OSA-18 consists of 18 questions regarding symptoms of OSDB during the past four weeks. The questions are divided into five subdomains that concern sleep disturbance, physical suffering, emotional distress, daytime problems and caregiver concerns (Figure 4). Each question is scored on a seven-point Likert scale ranging from 1= “none of the time” to 7 = “all of the time”.

A total symptom score (TSS) is calculated (score 18-126) where a score less than 60 suggests to be normal with a small impact on quality of life, while scores between 60 and 80 indicate abnormal and a moderate impact, and scores above 80 suggests a large impact (66).

Compared with PSG-outcomes, this questionnaire has not shown a good diagnostic value (68, 69), though instead it can be used to assess quality of life or a change of symptoms after treatment. The Swedish version (Figure 5) includes a general health-related quality of life (HRQoL) scale, where the caregiver is asked to “estimate the child’s quality of life given the troubles of OSDB-symptoms” giving a score from 0 to 10, where 0 = worst thinkable and 10 = best thinkable.

OSA-18 Quality of Life Survey

Evaluation of Sleep-Disordered Breathing

Instructions. For each question below, please circle the number that best describes how often each symptom or problem has occurred during the past *4 weeks* (or since the last survey if sooner). Thank you.

	None of the time	Hardly any of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time
SLEEP DISTURBANCE							
During the past *4 weeks*, how often has your child had…							
…loud snoring?	1	2	3	4	5	6	7
…breath holding spells or pauses in breathing at night?	1	2	3	4	5	6	7
…choking or gasping sounds while asleep?	1	2	3	4	5	6	7
…restless sleep or frequent awakenings from sleep?	1	2	3	4	5	6	7
PHYSICAL SUFFERING							
During the past *4 weeks*, how often has your child had…							
…mouth breathing because of nasal obstruction?	1	2	3	4	5	6	7
…frequent colds or upper respiratory infections?	1	2	3	4	5	6	7
…nasal discharge or runny nose?	1	2	3	4	5	6	7
…difficulty in swallowing foods?	1	2	3	4	5	6	7
EMOTIONAL DISTRESS							
During the past *4 weeks*, how often has your child had…							
…mood swings or temper tantrums?	1	2	3	4	5	6	7
…aggressive or hyperactive behavior?	1	2	3	4	5	6	7
…discipline problems?	1	2	3	4	5	6	7
DAYTIME PROBLEMS							
During the past *4 weeks*, how often has your child had…							
…excessive daytime drowsiness or sleepiness?	1	2	3	4	5	6	7
…poor attention span or concentration?	1	2	3	4	5	6	7
…difficulty getting out of bed in the morning?	1	2	3	4	5	6	7
CAREGIVER CONCERNS							
During the past *4 weeks*, how often have the above problems…							
…caused you to worry about your child's general health?	1	2	3	4	5	6	7
…created concern that your child is not getting enough air?	1	2	3	4	5	6	7
…interfered with your ability to perform daily activities?	1	2	3	4	5	6	7
…made you frustrated?	1	2	3	4	5	6	7

Figure 4. OSA-18 Questionnaire in English

OSA-18 Livskvalitetsinstrument

Utvärdering av Sömnrelaterade andningsstörningar

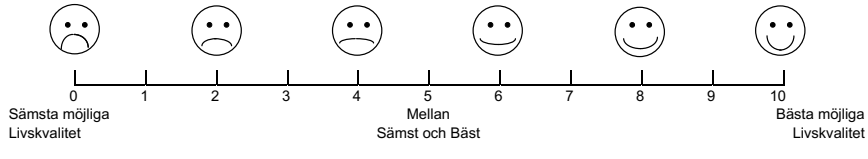
För varje fråga nedan rita en ring kring siffran som bäst beskriver hur ofta varje symptom eller problem har inträffat någon gång under den senaste 4 veckorna. Var snäll och ringa in endast en siffra per fråga. Tack!

	Aldrig	Nästan aldrig	Lite grand	Ibland	Ganska ofta	Ofta	Alltid
SÖMNSTÖRNING							
Under de senaste *4 veckorna*, hur ofta har ditt barn haft …							
…ljudlig snarkning?	1	2	3	4	5	6	7
…perioder med andningsuppehåll?	1	2	3	4	5	6	7
…stryp- eller kvävnings-ljud under sömn?	1	2	3	4	5	6	7
…orolig sömn eller täta uppvaknanden?	1	2	3	4	5	6	7
FYSISKA SYMTOM							
Under de senaste *4 veckorna*, hur ofta har ditt barn haft …							
…munandning p.g.a. nästäppa?	1	2	3	4	5	6	7
…förkylning eller annan infektion i övre luftvägarna?	1	2	3	4	5	6	7
…rinnande näsa?	1	2	3	4	5	6	7
…svårigheter att svälja mat?	1	2	3	4	5	6	7
EMOTIONELLA SYMTOM							
Under de senaste *4 veckorna*, hur ofta har ditt barn haft …							
…humörsvängningar eller vredesutbrott?	1	2	3	4	5	6	7
…aggressivt eller hyperaktivt beteende?	1	2	3	4	5	6	7
…disciplinproblem?	1	2	3	4	5	6	7
DAGTIDSFUNKTION							
Under de senaste *4 veckorna*, hur ofta har ditt barn haft …							
…ovanligt mycket dagsömnighet?	1	2	3	4	5	6	7
…uppmärksamhets- eller koncentrationsproblem?	1	2	3	4	5	6	7
…svårt att gå upp på morgonen?	1	2	3	4	5	6	7
OMHÄNDERTAGARENS BEKYMMER							
Under de senaste *4 veckorna*, hur ofta har ditt barn …							
…orsakat dig oro angående barnets allmänna hälsotillstånd?	1	2	3	4	5	6	7
…orsakat oro för att ditt barn inte får tillräckligt med luft?	1	2	3	4	5	6	7
…stört dina möjligheter att utföra dagliga aktiviteter?	1	2	3	4	5	6	7
…gjort dig frustrerad?	1	2	3	4	5	6	7

Total poäng =

HUR VILL DU TOTALT SKATTA DITT BARNS LIVSKVALITET I RELATION TILL OVANSTÅENDE PROBLEM?

(sätt en cirkel runt ett nummer)



Översatt av Elisabeth Ericsson m.fl. Avd. för Oto-rhino-laryngologi, Hälsouniversitetet, Linköping och Hälsohögskolan, Jönköping, Sverige, 2009

Kontakt: Elisabeth.Ericsson@hhj.hj.se

Figure 5. The OSA-18 Questionnaire in Swedish

SDQ – Strengths and Difficulties Questionnaire

The Strength and Difficulties Questionnaire (SDQ) is a well-validated, brief questionnaire with a focus on children's mental health. The SDQ is not connected to sleep apnea, but is used as a general tool to assess both young children's and adolescents' behavioral issues as well as their resources and life quality. It was first published by the British child psychiatrist Robert Goodman in 1997, described as "a brief behavioural screening questionnaire that provides balanced coverage of children and young people's behaviours, emotions and relationships." (70)

The original intended application of the SDQ was for children aged four to 16 years old; this range has lately been broadened with slightly different versions available for different age groups. The information is derived from parents and teachers/pedagogues who know the child, as well as from the children themselves from 11 years of age onward. The questionnaire includes 25 statements about the child's behavior during the past six months and the informant is asked to assess whether it is "not true" "somewhat true" or "certainly true" (Figure 6). "Somewhat true" gives a score of 1 and the other two statements are rated as 0 or 2 respectively, depending on the statement. The questions are divided according to five subscales (emotional, conduct, hyperactivity/inattention, peer-relationships and prosocial) with an extra range of questions used to assess the impact of the child's behavior. Four of these subscales are calculated to provide a total score using a scoring syntax available for free on the SDQ homepage (71).

The SDQ was translated into Swedish and the parental version validated for older children (six to 10 years) in the 1990's by Hans Smedje in the county of Gävleborg (72). In 2016, the teacher version for preschool children (one to five years) was validated (73). However, the normative data for SDQ differ according to age, countries, and the subscales. Cut-off scores indicating that children are in need of psychiatric support often concur with the 90th percentile in the general population sample. In the Swedish validation study of children from six to 10 years, a cut-off of the total scale was put at 14. In the Swedish validation of younger children it was concluded that a preschool teacher SDQ can be used as a valid instrument for identifying early signs of distress/behavioral problems. However, the subscales of emotional and peer problems were difficult to use for preschool teachers. In a Danish study the normative scores for children aged two to six years show a high score (above the 90th percentile) if the total difficulties score was between 16–17 for boys, 15–16 for girls and 15–17 for both gender (74). But the scores are more often used as an evaluation of deviation from the mean than as a cut-off for establishing disease or not.

Previous studies have shown significant improvement in behavioral problems among children with OSA treated with tonsil surgery, when assessed with other scores and questionnaires other than SDQ (75-77).

Strengths and Difficulties Questionnaire P 4-17

For each item, please mark the box for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain or the item seems daft! Please give your answers on the basis of the child's behaviour over the last six months.

Child's Name ..... Male/Female

Date of Birth.....

	Not True	Somewhat True	Certainly True
Considerate of other people's feelings	☐	☐	☐
Restless, overactive, cannot stay still for long	☐	☐	☐
Often complains of headaches, stomach-aches or sickness	☐	☐	☐
Shares readily with other children (treats, toys, pencils etc.)	☐	☐	☐
Often has temper tantrums or hot tempers	☐	☐	☐
Rather solitary, tends to play alone	☐	☐	☐
Generally obedient, usually does what adults request	☐	☐	☐
Many worries, often seems worried	☐	☐	☐
Helpful if someone is hurt, upset or feeling ill	☐	☐	☐
Constantly fidgeting or squirming	☐	☐	☐
Has at least one good friend	☐	☐	☐
Often fights with other children or bullies them	☐	☐	☐
Often unhappy, down-hearted or tearful	☐	☐	☐
Generally liked by other children	☐	☐	☐
Easily distracted, concentration wanders	☐	☐	☐
Nervous or clingy in new situations, easily loses confidence	☐	☐	☐
Kind to younger children	☐	☐	☐
Often lies or cheats	☐	☐	☐
Picked on or bullied by other children	☐	☐	☐
Often volunteers to help others (parents, teachers, other children)	☐	☐	☐
Thinks things out before acting	☐	☐	☐
Steals from home, school or elsewhere	☐	☐	☐
Gets on better with adults than with other children	☐	☐	☐
Many fears, easily scared	☐	☐	☐
Sees tasks through to the end, good attention span	☐	☐	☐

Do you have any other comments or concerns?

Please turn over - there are a few more questions on the other side

Overall, do you think that your child has difficulties in one or more of the following areas: emotions, concentration, behaviour or being able to get on with other people?

No	Yes- minor difficulties	Yes- definite difficulties	Yes- severe difficulties
☐	☐	☐	☐

If you have answered "Yes", please answer the following questions about these difficulties:

• How long have these difficulties been present?

Less than a month	1-5 months	6-12 months	Over a year
☐	☐	☐	☐

• Do the difficulties upset or distress your child?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

• Do the difficulties interfere with your child's everyday life in the following areas?

	Not at all	Only a little	Quite a lot	A great deal
HOME LIFE	☐	☐	☐	☐
FRIENDSHIPS	☐	☐	☐	☐
CLASSROOM LEARNING	☐	☐	☐	☐
LEISURE ACTIVITIES	☐	☐	☐	☐

• Do the difficulties put a burden on you or the family as a whole?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

Signature ..... Date .....

Mother/Father/Other (please specify:)

Thank you very much for your help

Figure 6. Strengths and difficulties questionnaire (SDQ) used in Paper II. ©Robert Goodman 2005 from sdqinfo.org

Treatment of pediatric OSA

Tonsil surgery

> "Perhaps there is no operation in surgery of the same magnitude, that has been attended with greater difficulties, than the removal of enlarged tonsils. The situations of the tonsils, their connexion with large vessels of the neck, and the spasmodic contraction of the surrounding muscles, have at all times rendered their excision a difficult, and sometimes a fatal operation."
>
> BY DAVID L. Rogers, M.D. OF NEW-YORK.
> From the New-York Medical Journal May, 1831 (78).

The first mentioned tonsil surgery (tonsillotomy) is found in Hindu medicine around 1000 BC. About a millennium later, around 30 AD, Celsus, a roman aristocrat gave the first description of a complete removal of tonsils done by finger and (if necessary) a scalpel (79). A snare or guillotine was developed for the procedure, but because of the serious risk of hemorrhage, and later the idea that tonsils were an important absorber of secretions from the nose, the tonsillotomy was the dominant technique until 1897 (80). In 1909. George Waugh described in the Lancet his technique using fine dissection forceps and curved scissors. With the arrival of new anesthesiologic techniques, such as intubation, introduced by Magill after the First World War, it became possible to conduct the operation much as it is done today, with the patient lying on their back and mouth open with a gag (80, 81).

During 2019, 11,237 tonsil surgical interventions were registered in the National Tonsil Surgery Register in Sweden (NTSRS). Out of these, 7,098 were primarily done for the indication of obstructive sleep apnea/snoring (82).

Today, tonsillectomy together with adenoidectomy, called adenotonsillectomy (ATE), is considered the first-line treatment for pediatric OSA worldwide. It is also one of the most common surgical procedures among children (83). The procedure of tonsillectomy (TE, Figure 7) involves dissecting out the whole tonsils with the capsule, ideally leaving no parts behind. Tonsillectomy can be performed with cold steel dissection and different hot techniques such as electrocautery, ultracission or controlled ablation. The cold steel technique is recommended due to the lower risks of postoperative hemorrhage (84).

An adenoidectomy involves removal of the adenoid tissue and is further described below.

Tonsillectomy and ATE are surgical interventions with risks such as hemorrhage, infections and postoperative pain. Lethal post-operative bleeding has occurred even though this is uncommon (85, 86). The level of OSA is important to take into consideration when treating a child with surgical

procedures. It is recommended that children with severe OSA or of <3 years of age with comorbidities should be observed for 23 hours after surgery (37). Even though ATE is a common procedure, a careful selection of surgical candidates has to be made, where the pros and cons of the intervention are considered.

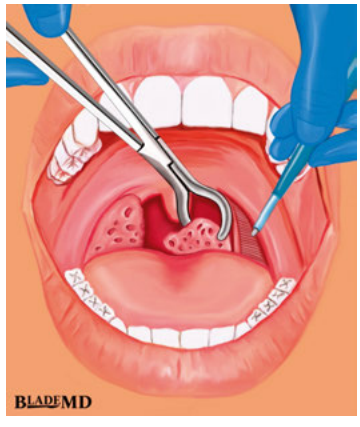


Figure 7. Tonsillectomy, extracapsular total removal of tonsils. Published with permission from Blade MD.

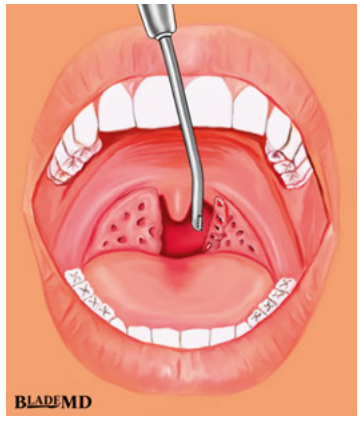


Figure 8. Tonsillotomy, intracapsular partial removal of the tonsil tissue. Published with permission from Blade MD.

Tonsillotomy (TT) or, adenotonsillotomy (ATT) when combined with removal of the adenoid, is a more conservative procedure with only a reduction of the tonsil tissue. This procedure preserves the lateral tonsillar capsule, which offers protection of the underlying pharyngeal muscles, vessels and nerves (see Figure 8). Tonsillotomy can be performed with various surgical devices, such as microdebrider, controlled ablation (coblation), and radiofrequency.

Adenotonsillotomy is accompanied with less bleeding and postoperative pain, as well as decreased costs for society (87, 88). This method has not been shown to be inferior to ATE when assessed with PSG parameters and quality of life (89). But since you're leaving the capsule and some tonsil tissue are left, there is a risk of regrowth and future infectious problems. ATT is indicated when the surgery is conducted because of tonsillar hyperplasia and obstruction.

Adenopharyngoplasty (APP) is another method where the tonsils are removed through a tonsillectomy. In addition, two sutures are placed on each side, closing the tonsillar pillars to enlarge the air-flowing passage.

Adenoid surgery

Surgical removal of the adenoid (adenoidectomy) is often accompanied with tonsil surgery when treating pediatric OSA. The American Academy of Pediatrics recommends that both the adenoid and tonsils should be removed since residual lymphoid tissue may contribute to persistent obstruction (90). However, the adenoid surgery alone is accompanied with less morbidity and could be recommended for non-obese children under 7 years old with moderate OSA and small tonsils (defined as Brodsky <3) (91).

The adenoidectomy is performed under general anesthesia, using either a cold technique e.g., cold curettage, or an electrosurgical method such as electrocautery, microdebrider and coblator (91, 92). The most common surgical technique for adenoidectomy in Sweden is using a curettage (ring knife) and cold hemostasis with pressure.

Non-surgical treatment

The alternatives to tonsil surgery for pediatric OSA will only briefly be described here since this is not the immediate subject matter of this thesis.

Medical treatments have been studied to find other options for surgery. Anti-inflammatory drugs, such as corticosteroid nasal spray, can decrease adenoid size, improve AHI and reduce symptoms. A six-week treatment period with intranasal corticosteroid has shown an effect on OSA-symptoms at least eight weeks after the end of the treatment. It is therefore recommended while waiting for surgery and for mild cases of OSA (93, 94). Montelukast, a leukotriene-receptor antagonist is another anti-inflammatory medicine that has shown a short-time effect by reducing the number of apneas among otherwise healthy children with mild OSA (95).

CPAP (continuous positive airway pressure) is a treatment method using air pressure through a mask to keep the airway open during the breathing cycle. It is an effective alternative when surgery is not suitable or not effective e.g., in cases of obesity or craniofacial disorders. CPAP is a common treatment for adult OSA but is not common in Sweden for children with OSA. Adherence to the mask is a barrier and CPAP is not recommended as a first-line therapy when tonsillar surgery is an option (58).

The immune system

The immune system is a remarkable organization of cells, tissues, and molecules in the body that mediate reactions to pathogens. The most important function of this system is to prevent and eradicate infections. In addition, the system can prevent growth of some tumors but may also react to harmless molecules, causing inflammatory diseases.

The body is exposed to microorganisms in the environment. Despite this continual exposure, we rarely become ill. Our immunity is grouped into *innate* immunity which provides immediate protection through physical and chemical barriers as well as cellular defenses, and *adaptive* immunity, which develops more slowly and provide more specialized defense.

The first protection against microorganisms is the local barrier of epithelial tissue such as the skin, the intestine, the urogenital tract, and the airways. These closely connected cells play an important protective role with their mechanical, dynamic and chemical functions, for example through secretion. Leukocytes (white blood cells), on the other hand constitute a mobilized system of cells specialized to protect against infections in the whole body. The leukocytes originate from the bone marrow hematopoietic stem cell and evolve in general along two different cell lines: the myeloid progenitor, and the lymphoid progenitor (Figure 9).

The myeloid lineage comprises most of the cells of the so-called innate system, e.g., macrophages, granulocytes, mast cells, and dendritic cells (96). The innate system reacts quickly but is unspecific due to the fact that the cells do not react to a specific antigen, although some of the cells can form a memory. The adaptive system, on the other hand, is highly specific and can evolve a memory lasting for years. It is composed of certain lymphocytes known as B cells and T cells, both of which develop from a common lymphoid precursor. In addition, lymphocytes known as natural killer cells (NK-cells) and innate lymphoid cells (ILCs) evolve from a lymphoid precursor, but since they lack re-arranged antigen-specific receptors they do not belong to the adaptive immune system (97). Lymphocytes constitute about 20–50 percent of the leukocytes in the blood of which T cells make up around 70–85 percent, B cells 10–15 percent and ILC/NK-cells 5–15 percent (97).

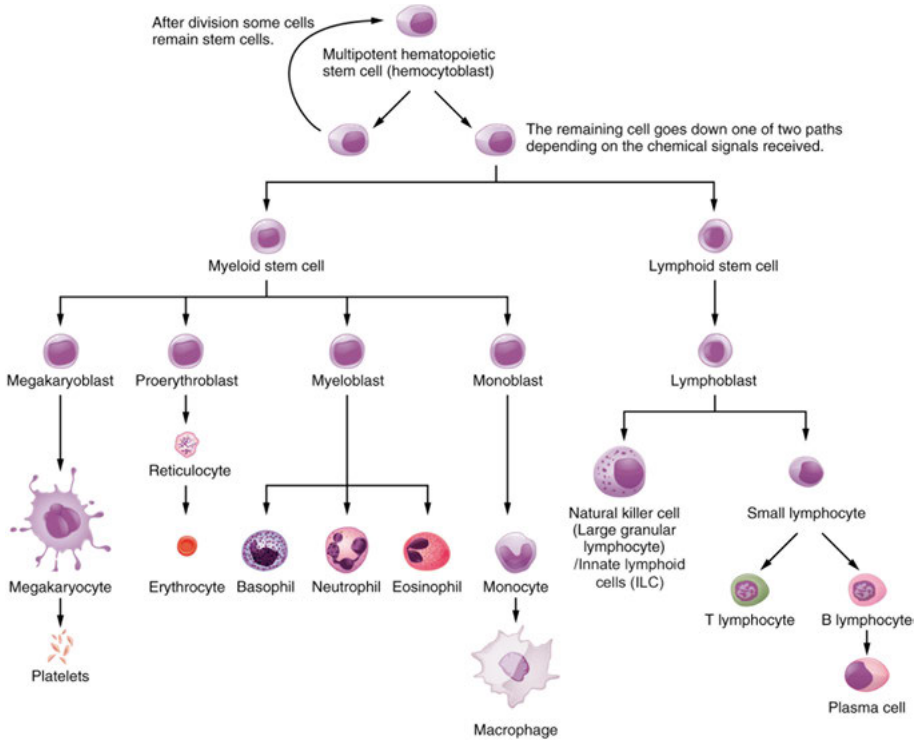


Figure 9. Hematopoiesis, the development of the different leukocytes involved in the immune system. Image from Openstax.

T cells

T cells leave the bone marrow in an immature state since they are dependent on the thymus to develop. This has given them their name, with the T standing for "Thymus dependent" (97). The precursor T lymphocytes migrate to the thymus and differentiate in several important steps to become mature naïve T cells, i.e., T cells that have not met an antigen yet. There are different subtypes of T cells such as the cytotoxic T cells (Tc-cells or CD8+ T cells) which can identify infected cells by their receptor and eliminate them. Another type are known as "helper" T cells (Th-cells or CD4+ T cells) because they help to activate and recruit other cells in the immune system by producing cytokines and binding molecules to other cells (e.g., B cells, Tc cells and macrophages).

To activate the naïve T cells, the cells have to meet the specific ligand that matches the cell's specific receptor, the so-called T cell receptor (TCR). The specific receptors are coded by highly recombined TCR genes in the development of each T cell, creating a vast diversity of T cell antigen-specificities. For T cells the specific antigen must be presented by an antigen presenting cell (APC) such as a B cell, a dendritic cell or a macrophage that encounter antigens in tissue-draining lymph nodes. The APC presents the

antigen through its major histocompatibility complex (MHC), a membrane protein that displays the peptide antigen for recognition by T lymphocytes. There are two main classes of MHC, class I and class II. The TCR on a cytotoxic CD8+ T helper cell binds to the peptide presented on an MHC class I and the TCR on a CD4+ T helper cell binds to an MHC class II. When the T cell is activated by an antigen they grow in size and amount by cloning themselves to strengthen the body's defense against that antigen of interest.

Another type of T cells, which can suppress activation and proliferation of many types of immune cells are called regulatory T cells (T reg) (98). Some of these become regulatory T cells directly after the maturation in the thymus while others seem to be generated in peripheral sites from CD4+ T helper cells during an immunological response (97, 99).

B cells

In contrast to T cells, B cells in human beings mature completely in the bone marrow. However, since B cells were first discovered in birds when it was demonstrated that they are dependent on a specialist organ known as the bursa of Fabricius, they were given the name B cells.

B cells, like T cells have a specific receptor, composed of immunoglobulins for a specific antigen, but, unlike T cells, the B cell does not demand an antigen-presenting cell but can bind to both antigen on other cells and soluble antigen in body fluids. In addition, the B cell after activation can produce soluble immunoglobulins that can bind to infected cells and disarm them directly or mark them for other immune cells to give action. This is why activity in this part of the immune system is referred to as humoral immunity (from the Latin term *humor* for fluid). When the B cells are mature and ready to meet antigens, they go as mature naïve B-lymphocytes to secondary lymphoid organs in the body such as the spleen, lymph nodes, and mucosa-associated lymphoid tissues (MALT) (100).

Within these peripheral lymphoid organs, the B lymphocytes are segregated into different anatomical compartments called follicles. If the B cells receive signals from the correct T helper cell they will become activated to proliferate and migrate to form a germinal center. This is named so because it was once falsely believed to be the site where new lymphocytes were generated, or germinated (98). A germinal center reaction is when the B cell undergoes mutation and isotype switching. The B cells with highest affinity to the antigen are selected to survive and leave the germinal center as plasma cells or memory cells (Figure 10). Plasma cells secrete antibodies even after the antigen is eliminated and tend to survive for years in the bone marrow or mucosal tissues. The circulating antibodies thus reflect each human body's history of antigen exposure.

The activated and differentiated plasma cells can be further divided into subgroups: follicular B cells, marginal zone B cells and B-1-cells. Follicular B cells demand help from T helper cells to become activated and bind primarily to protein antigens. They are located in follicles in secondary lymphoid organs. The marginal zone B cells and B-1-cells bind primarily to carbohydrate-based antigens and can activate without Th cells. Marginal zone B cells are located in the spleen and the B-1 cells are numerous in mucosal tissue and the peritoneal cavity (97, 98, 101).

A proportion of the activated B cells become memory cells, which are found in mucosal tissue and circulate in the blood to rapidly respond if the specific antigen reenters the body years later (98). Lately, a new subpopulation of B cells called atypical memory B cells have been described. This is a heterogenic population of B cells harboring memory potential that have been described as a phenomenon common in several chronic diseases such as malaria, HIV and tuberculosis (102).

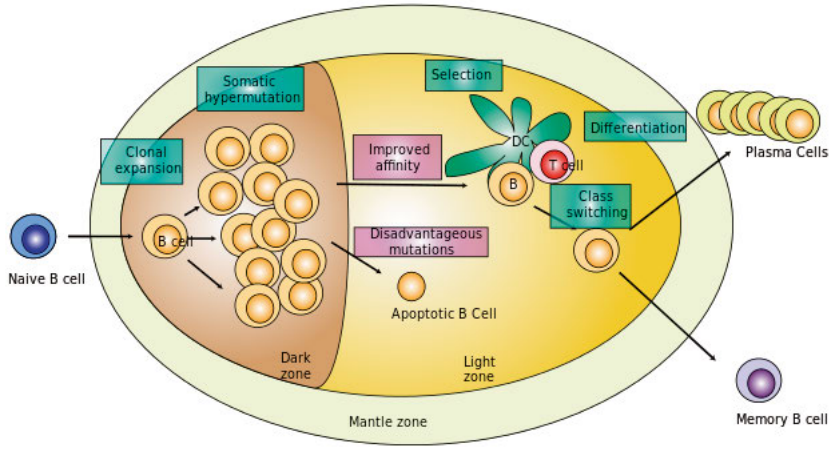


Figure 10. Schematic picture of Germinal center where B cell differentiation occur after B cell activation by T helper cells. Picture from Wikimedia Commons.

Innate lymphoid cells

Innate lymphoid cells (ILCs) are lymphocytes that stem from the common lymphoid progenitor (Figure 9) but lack antigen-specific receptors. The ILC family encompasses the cytotoxic “natural killer” cells (NK cells), which were discovered in 1975. They are involved in protection against viruses and

tumors, where several subpopulations of ILCs have been found based on their phenotype and function: ILC1, ILC2, ILC3 and lymphoid-tissue inducer (LTi) cells (103).

The similarities between T-helper cells and ILCs are numerous and some studies even suggest that ILCs could be redundant in the presence of T cells (104). ILC1 and NK cells are involved in cytotoxic functions and react to intracellular pathogens such as viruses as well as to tumors. ILC2 cells are involved in the defense to large extracellular parasites and when dysregulated diseases such as asthma and chronic rhinosinusitis. ILC3 cells are involved in the innate immune response to extracellular bacteria and are abundant at mucosal sites. LTis are involved in the formation of the secondary lymphoid structures (103). Human tonsils contain all populations of ILCs described so far (105, 106).

Table 3. Selected cell-surface molecules (98, 103, 105).

Cell-surface molecules	**Main cellular expression**	**Known or proposed function**
CD3	Mature T cells	Cell surface expression of T cell antigen receptor
CD4	Class II MHC-restricted T cells	Coreceptor in class II MHC-restricted antigen -induced T-cell activation
CD8	Class I MHC-restricted T cells	Coreceptor in class I MHC-receptor antigen-induced T cell activation
CD19	Most B cells	B-cell activation
CD21	Mature B cells	
CD27	Activated B cells	
CD45	Hematopoietic cells	Tyrosine phosphate that regulates T and B cell activation
CD45RA	Naïve B cells or naïve T cells	
CD127	Majority of ILCs	
Ki67	Expressed during the S-phase of cell division	Marker for proliferation
NRP1	ILC3	

Cell-surface molecules

All lymphocytes are morphologically similar so their heterogeneity in lineage, function and activity are distinguished by the expression of cell-surface proteins. Using panels of monoclonal antibodies, the different surface molecules can be identified and cluster of differentiation (CD) numbers are

used as nomenclature for some of these. With this CD-numeric designation, different cell types and stages can be identified. A list of CD numbers and other cell-surface molecules that are important for this thesis, are outlined in Table 3. A complete and up-to-date list is found at www.hcdm.org

Lymphoid organs and tissue

The organs and tissues involved in specialized functions within the immune system are divided into primary lymphoid tissue, which include the thymus and the bone marrow, and the secondary lymphoid organs and tissue which includes the spleen, lymph nodes and mucosa-associated lymphoid tissues (MALT).

In the primary lymphoid organs, lymphocytes mature and develop their specific receptors. The secondary lymphoid organs, on the other hand, are the platforms for communication between lymphocytes and antigens, as well as between different leukocytes. Barriers of mucosa play an important part of the innate immune system wherein many immunological cells wait for an infectious antigen to react to. Every breath we take contains antigenic material. Our first site for immunological reactions for these pathogens is represented by MALT in the Waldeyer's ring, which includes the palatine tonsils, the adenoid, and the lingual tonsil in the pharynx (Figure 11) (107).

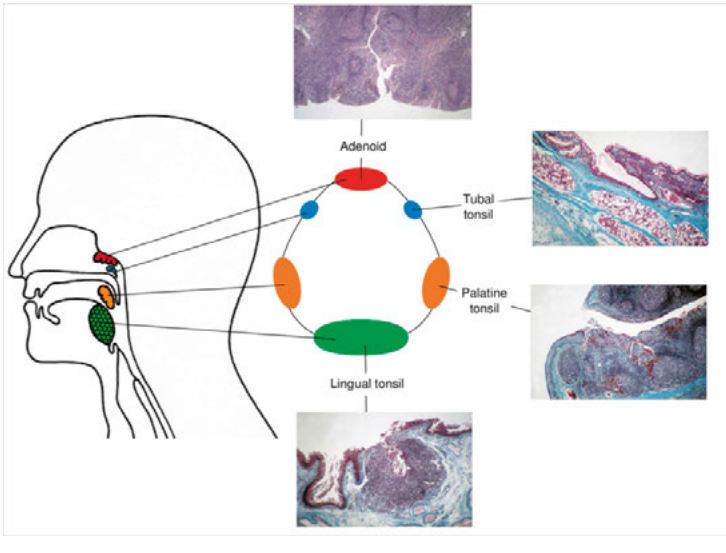


Figure 11. Waldeyer's ring, lymphoid tissue in the pharynx which includes the palatine tonsils and the adenoid. Reproduced with permission from Elsevier (3)

Immunology of tonsils and adenoid

The tonsils and the adenoid are, as part of MALT and secondary lymphoid tissue, a strategic first line of defense against microorganisms in the upper respiratory tract. The tonsils are also an easily accessible lymphoid organ and are therefore often taken as samples for lymphoid organs (108). The lymphoid compartments of the tonsils consist of subepithelial lymphoid follicles where mature naïve B cells are predominant. Extrafollicular regions surrounding these follicles are predominantly populated by T cells, mostly as follicular CD4+T helper cells (T_{FH}). The presence of helper ILCs have been described more recently in human tonsillar tissue (105, 109, 110).

The tonsillar surface area is considerably increased by macroscopically visible narrow epithelial channels, called crypts (107). The crypts are designed to entrap foreign material and it is also here that the immune response is initiated (107). Within the tonsillar epithelium, there is a rich network of blood vessels to provide the area with its metabolic needs and increase the interaction between antigens and leukocytes (3). When an antigen has entered the throat, it is taken up by specialized membranous (M)-cells in the epithelium of tonsils and brought to the extrafollicular region. An antigen presenting cell (APC), such as dendritic cells or macrophages, co-stimulate the T_{FH}. The T_{FH} then stimulate and activate the specific follicular B cells to start to proliferate and migrate from the germinal center dark zone to the light zone, differentiating to become a B memory cell and an antibody-producing plasma cell (108).

Tonsil hyperplasia has for many hundreds of years been best treated with surgical methods. Still, we do not have the answer as to why this hyperplasia is so large in some individuals, making it difficult to breathe normally when asleep. The greatest immunological activity and growth of tonsil tissue is between three and 10 years of age (3, 108). Studies have been made to find an immunopathological explanation, suggesting increased T cell proliferation and elevated levels of proinflammatory cytokines (111), Substance P and upregulation neurokinin 1 receptors (112), as well as cysteinyl leukotrienes receptors (113), as contributing mechanisms of the hyperplasia of the tonsillar tissue. A hypothesis has also been raised that viral infections are a possible contributor to hyperplasia (114). The immunopathology of tonsil hyperplasia in children with OSA is not clear and therefore needs to be further understood.

Flow cytometry

The multicolor flow cytometry is a widespread and powerful tool used to enumerate cells, biomarker detection and cell sorting(115). The early prototype of a flow cytometer was described by Andrew Moldavan in 1934. Since then the cytometer has developed in various aspects, and in the late sixties it became similar to the ones we use today (116). In brief, the technique

is based on a procedure where cells are illuminated by laser and analyzed one by one in front of a beam light on a photodetector to identify signals from the cells (Figure 12). A flow cytometer that can separate the identified cells is called a fluorescence-activated cell sorter, often abbreviated to FACS.

To be able to detect cells from the tissue that you are interested in, a single-cell suspension must be prepared. Thereafter, the cells are incubated with monoclonal antibodies specific for your cells of interest. The antibodies are labeled with a fluorescent dye that emit light of different colors when excited by the appropriate wavelengths.

A flow cytometer is similar to a microscope but instead of producing an image of the cell it quantifies specific optimal parameters on cell basis. Two important parameters are the volume of the cell measured by forward scatter light and the granularity of the cells measured by side scatter light. Modern flow cytometers are able to analyze many thousands particles per second.

When the cells are analyzed in the flow cytometer, the data need to be interpreted in a method of sequential identification and refinement called "gating". This is where the cells of interest are being selected and the others will be "gated out" (Figure 13).

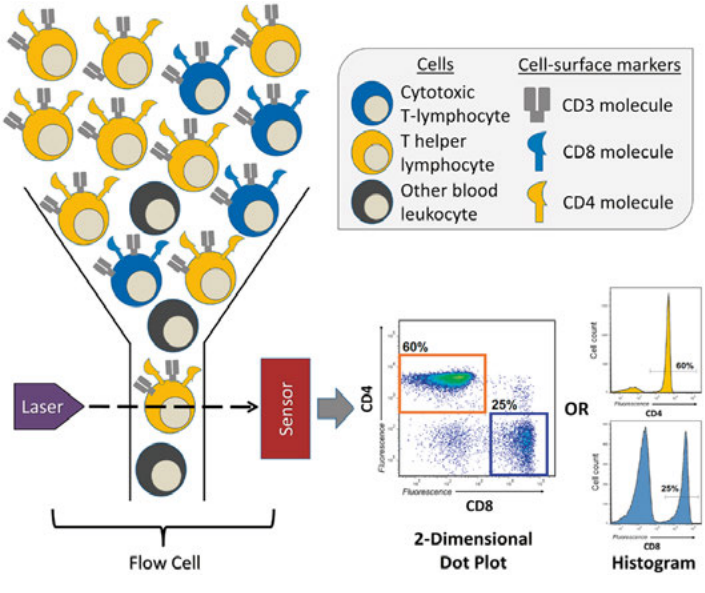


Figure 12. Overview of flow cytometry identifying proportions of CD4+ T helper and CD8+ Cytotoxic T lymphocytes. Adapted with permission from authors (117)

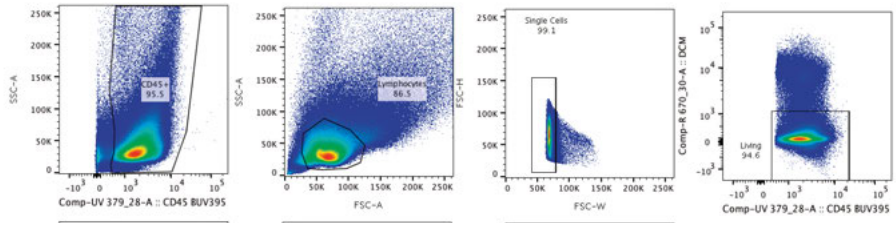


Figure 13. Example of gating where CD45+ living cells are identified

Aims

This thesis focuses on immunological aspects and treatment methods in children with tonsil hypertrophy and obstructive sleep apnea, or OSA. The aims of the thesis are to evaluate:

I. Whether children with large tonsils/very severe OSA and children with smaller tonsils/milder OSA differ in the composition of the innate and adaptive cells in their tonsils, analyzed using flow cytometry (Paper I).

II. Whether children with obstructive sleep apnea treated with adenotonsillotomy versus adenotonsillectomy show any differences in mental health and behavioral difficulties, using a validated Strengths and Difficulties Questionnaire (Paper II).

III. Whether adenotonsillotomy is as effective as adenotonsillectomy as a treatment method for children with obstructive sleep apnea in a five-year perspective (Paper III).

IV. Whether there is a correlation between subjective data (OSA-18 questionnaire and patient-reported outcome question) and objective data from polysomnography, in patients treated surgically for obstructive sleep apnea (Paper IV).

Summary of papers

Significance

The overall aim of this thesis is to increase our understanding of pediatric OSA from a broad perspective. By focusing on immunological aspects, surgical treatment options and quality of life, hopefully the results of these studies can contribute to the research field of the common and potentially harmful disease of pediatric OSA. Since the etiology of tonsil hyperplasia, the most common cause of OSA, is still unknown, the immunological study may shed some light on the possible causes. It is important to evaluate one of the most common surgical treatment for OSA in Sweden today, adenotonsillotomy, to compare its long-term effects with standard adenotonsillectomy. Furthermore, investigating the validity of a patient-reported outcome measure (PROM) question, can help us to make a better follow-up of all these patients operated on every year for OSDB and OSA.

For a detailed description of patient material and methods, please see the separate article/manuscript (I-IV). A summary is presented below.

Materials and Methods

Paper I

Paper I is an immunological mapping of tonsils from children who were operated on with a tonsillectomy between 2014 and 2017 because of OSA, at the otorhinolaryngological department of Karolinska University Hospital in Huddinge, Sweden. They had previously been included in other clinical studies. The aim of this paper was to investigate the presence of immunological cells among children with large tonsils and severe OSA compared with small tonsils from children with mild to moderate OSA.

The exclusion criteria were the presence of craniofacial abnormality, neuromuscular disease, chromosomal abnormality, previous adenotonsillar surgery, bleeding disorders and cardiopulmonary disease. All remaining 19 children had before surgery undergone polysomnography (PSG) overnight in a sleep laboratory at Karolinska, to diagnose their degree of OSA.

Tonsil hyperplasia was scored according to the Brodsky scale (56). To be able to compare two tonsil types, patients were selected and divided into two

groups according to tonsil size and severity of OSA. The first group included patients with small tonsils (Brodsky size 2, except one with size 2.5) and moderate OSA (all patients had OAHI ≤11) (n = 6), the other group included patients with large tonsils (Brodsky size 4) and very severe OSA (OAHI ≥33) (n=13) (Table 4).

Table 4. Baseline characteristics of the patient cohort in Paper I

Parameter	**Small (n=6)**	**Large (n=13)**	**p**
Age at surgery, mean (SD), months	38 (8)	34 (8)	0,4
Sex, n (%)			
Men	2 (33)	8 (38)	
Women	4 (67)	5 (62)	
Height, mean (SD), cm	94 (7) [a]	92 (5)	0,9
Weight, mean (SD), kg	14 (2) [a]	14 (3)	0,9
BMI z-score, mean (SD)	-0,7 (2,0)	-0,4 (1,8)	0,6
Tonsil size[b], median (IQR)	2 (2-2,5)	4 (4-4)	<0,001
OAHI, median (IQR)	9,5 (7-11)	35 (33-36)	<0,001

[a]One missing value in group Small (n=5). [b]Tonsil size scored according to Brodsky. P-value calculated with Mann-Whitney U test

Adenotonsillectomy was conducted with cold steel technique. The tonsils were cut into small pieces, ground through a cell strainer and washed with phosphate buffered saline (PBS). The mononuclear cell suspension was prepared to be frozen in freezing medium and stored in gas phase of liquid nitrogen. When it was time to analyze, the mononuclear cells were thawed and washed. For flow cytometry analysis, the cells were stained with dye and markers (antibodies) targeting surface molecules at room temperature for 30 minutes.

Multi-color flow cytometry was performed to analyze the composition of different T cells, B cells and, ILCs including NK cells, as well as their phenotype, to investigate their differentiation. A description of the principles of flow cytometry is found in the introduction section of this thesis. FlowJo v.10.6.1 (BD Biosciences) was used to analyze the data. Cell numbers were reduced and samples from the same patient group were concatenated. The cell numbers were adjusted to be equal between the groups.

Algorithms from the FlowJo plugins Uniform manifold approximation and projection (UMAP v. 2.1) (118) and Phenograph (v. 1.8) (119) were applied for further separate cell populations based on their maturation state and lineage and to identify distinct lymphocyte clusters as well as visualizing data (Figure 14 and Figure 15).

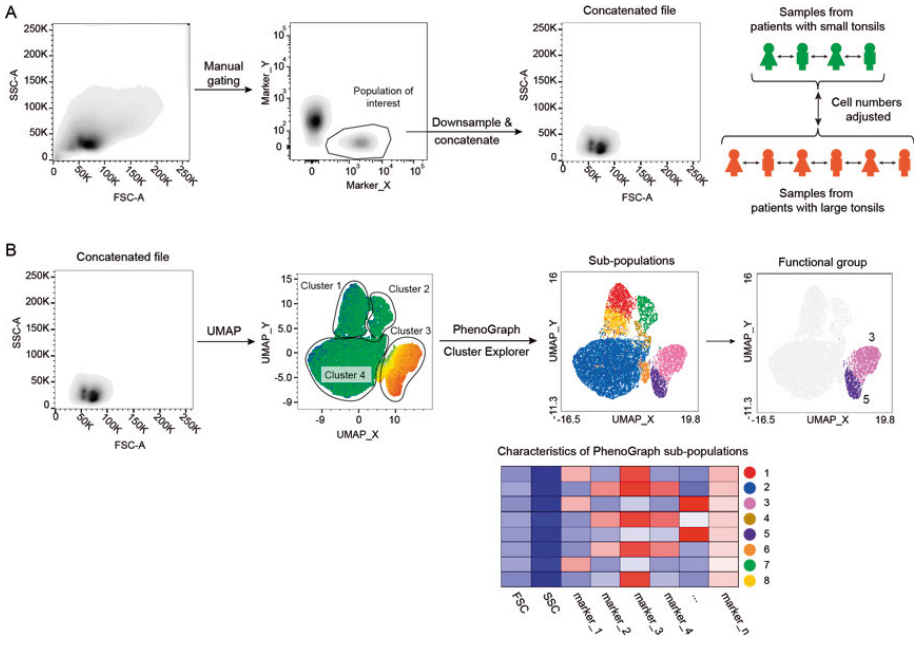


Figure 14. Flow cytometric data analysis workflow. (A) Schematic of concatenation of cell populations derived from the same patient group. (B) UMAP and Phenograph analysis workflow of concatenated samples.

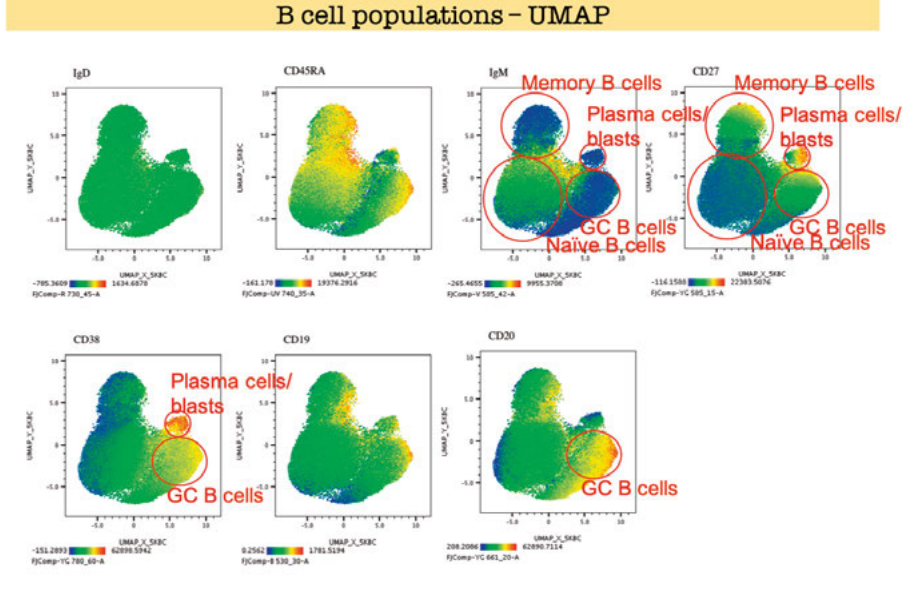


Figure 15. Example of UMAP gating of B cell populations.

Paper II and Paper III

Papers II and III are based on the same cohort participating in a randomized controlled trial (RCT) (89). Children with OSA between two to five years of age were randomly assigned to two surgical study arms (ATE or ATT) at the Otorhinolaryngological department Karolinska University Hospital Huddinge from 2011 to 2015. Inclusion criteria were age two to six years, symptoms of OSA, apnea hypopnea index (AHI) of ≥5 and ≤30 and tonsil hypertrophy 3 or 4 according to Brodsky (56). Exclusion criteria were craniofacial abnormality, obesity (BMI z score >1.67), previous adenotonsil surgery, bleeding disorder, cardiopulmonary diseases, history of recurrent tonsillitis or parents with insufficient knowledge of Swedish (89).

Forty children were assigned to ATE, and 39 to ATT. The surgical procedure was randomized using envelopes in the operating room, with only the surgeon and the staff knowing which surgical method was performed. All children had undergone PSG before and one year after surgery. All patients and caregivers were blinded to method, as was the technologist interpreting the PSGs. In conjunction with the sleep studies, one parent per child filled out the Strengths and Difficulties Questionnaire (SDQ) to assess the child's psychiatric health and evaluate any behavioral differences. In addition, the OSA-18 questionnaire was filled out at the same time. The SDQ score was calculated according to the algorithm presented by SDQ developers (71).

The aim of Paper II was to investigate whether there were any differences in the child's psychiatric health depending on the surgical method, one year after surgery. For this study, one inclusion criterium was added; children with a complete first page of SDQ before and/or after surgery, were included. The total score and subscales of SDQ, the AHI and OSA-18 total symptom score, were compared between the two groups. Baseline characteristics of Paper II is seen in Table 5 and a Flowchart of patients in Figure 16.

Table 5. Baseline characteristics Paper II

	ATE (n = 37)	**ATT (n = 32)**	**p**
Age, months (range)	44 (29–74)	42 (25–80)	0.38
Boy/Girl (%)	26/11 (70/30)	21/11 (66/34)	0.30
Length, cm	98 (14)	98 (10)	0.99
Weight, kg	15.6 (3.0)	15.2 (3.4)	0.38
Tonsil size	3.3 (0.6)	3.5 (0.6)	0.13
Adenoid size	2.7 (0.8)	3.0 (0.7)	0.06
SDQ Total	11 (4.8)	10.2 (5.3)	0.52
AHI	14.5 (7.3)	15.2 (7.3)	0.65
OSA-18	60.9 (18)	65.0 (19)	0.30

Values are mean (SD) unless otherwise noted. p calculated with Mann-Whitney U test.

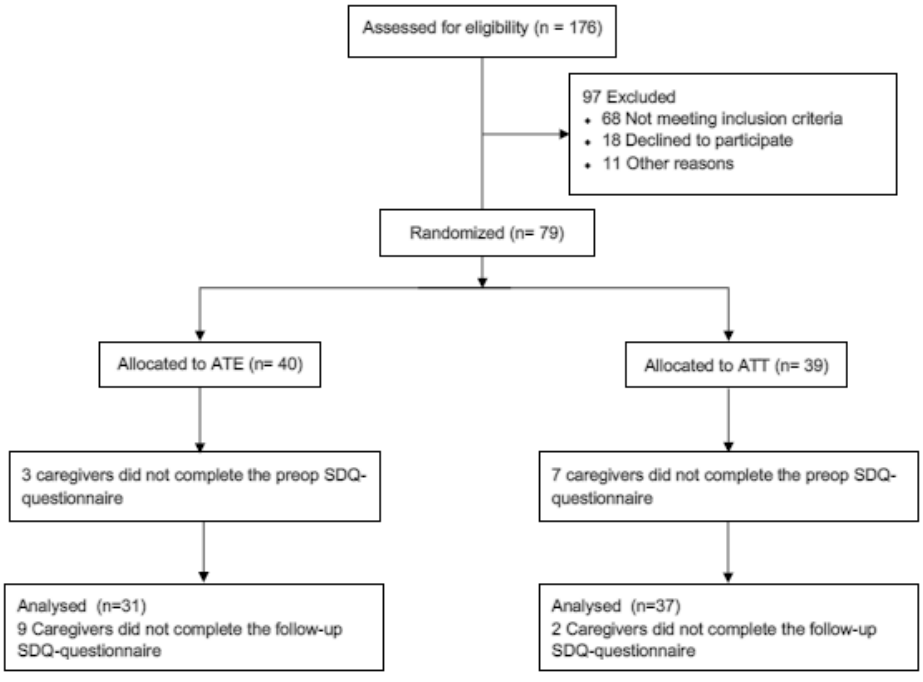


Figure 16. Flowchart of patients included in Paper II

In Paper III, the RCT was followed up with a new sleep study and OSA-18 questionnaire five years after surgery. This long-term follow-up focused on differences in PSG-parameters and OSA-18 results between these two treatment arms. The aim was also to analyze the risk of reoperation, with focus on the group operated with ATT. No routinely visits were planned during this follow-period but the caregivers were urged to contact clinic if the child had signs of recurrence of OSA-symptoms.

Primary outcome was difference in the postoperative values of OAHI between the groups. The previous follow-up, after one year, compared the apnea hypopnea index (AHI) but in this study focus was exchanged to the obstructive AHI (OAHI).

Secondary outcomes were other PSG variables and OSA-18 score, compared between groups, to baseline and to the previous one year follow-up. The reoperation rate was also evaluated. Children who had undergone ATT was recommended reoperation with ATE if they showed signs of tonsil regrowth and symptoms of OSA, AHI≥5 or recurrent tonsillitis. Patients reoperated with ATE within the follow-up period were excluded from the per-protocol analysis but included in the sensitivity analysis.

Baseline characteristics of the children included in Paper III are shown in Table 6. A flow-chart of patients included in Paper III is shown in Figure 17.

Table 6. Baseline characteristics Paper III

Parameter	ATE (n=40)	ATT (n=39)
Age, months	47 (15)	45 (15)
Sex n (%)		
Men	29 (72.5)	24 (62)
Women	11 (27.5)	15 (38)
Length, cm	98 (13)	99 (10)
Weight, kg	15.7 (3.1)	15.3 (3.3)
Tonsil size, 1–4, median (iqr)[a]	3 (3-4)	4 (3-4)
Adenoid size, median (iqr)	3 (2-3)	3 (3-4)
AHI	14.5 (7.3)	15.4 (7.3)
OAHI	12.5 (7.8)	13.4 (7.3)

Values are mean (SD) unless specified otherwise. [a]Tonsil size according to Brodsky, occlusion of the oropharynx (%) 1: 0–25%; 2: 26–50%; 3: 51–75%; 4:76–100%. AHI = apnea hypopnea index. OAHI = obstructive apnea hypopnea index.

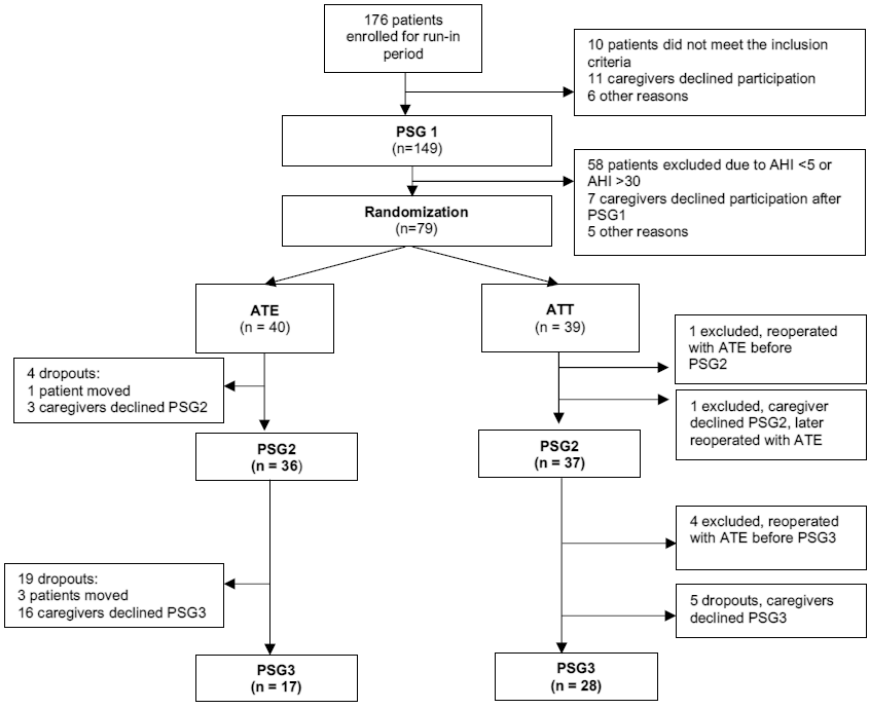


Figure 17. Flowchart of patients included in Paper III.

Paper IV

In this prospective cohort study, an existing database containing information on children who had undergone tonsil surgery for OSA at the Karolinska Institute's Department of Otorhinolaryngology between 2011–2017 was used. The children were aged two to six years at the time of surgery and had been operated on with either ATT, ATE, or adenopharyngoplasty. A registration with PSG was done before and six to 12 months after surgery; on both occasions, the OSA-18 questionnaire was filled out.

During the latter PSG, the parent/caregiver was asked to answer a patient-related outcome measure question (PROM): "How have your child's symptoms changed after surgery?" They were asked to answer with one of four alternatives that best describes their child's situation:

(1) The symptoms are gone.
(2) My symptoms are almost gone.
(3) My symptoms remain.
(4) My symptoms have worsened.

The PROM question was asked verbally at a clinical visit, or over the telephone, or through a questionnaire. We then compared PSG data from this database with OSA-18 and PROM answers six to 12 months after surgery. Baseline characteristics of the patients in Paper IV are seen in Table 7.

Table 7. Baseline characteristics

Variable	**Value (n = 201)**
Age, mean (SD) years	3.2 (1.0)
Sex, No (%)	
Women	80 (40)
Men	121 (60)
BMI z-score mean (SD)	-0.2 (1.4)
Surgical procedure, No (%)	
Adenotonsillectomy	126 (63)
Adenotonsillotomy	39 (19)
Adenopharyngoplasty	36 (18)
Tonsil size[a], 1–4 median (range)	3 (1–4)
OAHI, mean (SD)	15.9 (11.3)
OSA-18 TSS[b], median (range)	64.5 (25–108)
OSA-18 SDS[b], median (range)	18 (6–28)
HRQoL[c], median (range)	7 (2–10)

[a]Tonsil size according to Brodsky(56) [b]n = 194, [c]n = 186

Statistical analysis

Statistical analyses in Paper I were performed using Prism software v. 6 (GraphPad) where the statistical difference between data sets was assessed using the Mann-Whitney U test. In Paper II–IV Stata/SE 15.1 (StataCorp, College Station, Texas, USA) was used, while in Paper III-IV R Studio Version 1.1.463 was also used for statistical analyses.

In Paper I, boxplot bars and error bars indicate mean ±Standard error of the mean. In papers II – IV, boxplots are illustrated with the median (line in box) and quartile values; whiskers are within the 1.5 interquartile range; and dots represent the outliers. Two-tailed *P*-values < 0.05 were considered significant.

In Paper II, the non-parametric Mann-Whitney U test was used to compare ordinal data between groups and Wilcoxon sign ranks were used to compare within groups (before and after treatment). Intention-to-treat analyses were performed using "baseline carried forward." A spearman correlation test was performed to correlate AHI with total SDQ score as well as between OSA-18 total score and the SDQ at follow-up. A multivariable regression model was used to evaluate the impact of gender.

In Paper III, the primary analysis was per protocol. A sensitivity analysis of intention-to-treat was also performed regarding the primary outcome: OAHI at follow-up, including dropouts from both groups and the six reoperated-on children from the ATT-group, using their last OAHI-value before reoperation. Missing values were imputed using last observation carried forward (LOCF). In addition, a simulative negative scenario was made for the ATT-group with a replacement of LOCF OAHI <5 with OAHI = 5. A dropout analysis was conducted comparing baseline characteristics between patients included and patients excluded or dropouts.

The PSG variables are numerical data and results presented as mean (standard deviation). A parametrical two-sample t-test was used to compare the difference between the two groups. The OSA-18 score is categorical data and presented as a median with interquartile range (IQR). These results were compared between the two groups using a non-parametric Mann-Whitney U test.

In Paper IV, per protocol analysis was performed primarily. In addition, intention-to-treat analyses were performed by imputing missing values using last observation carried forward and backward. Quantitative data were presented as mean (standard deviation) and ordinal data as median (range) or (interquartile range). Changes between preoperative and postoperative ordinal data are sometimes presented as median (SD). The Kruskal-Wallis test was used to test differences between >2 groups. A spearman rank correlation coefficient was used for correlations between objective PSG-parameters and subjective OSA-18 parameters as well as PROM. The correlation was interpreted as $0.1 < r < 0.39$ being weak, $0.4 \leq r < 0.69$ being moderate, and $r \geq 0.7$ being strong (120).

To evaluate the PROM question for cured OSA, we used Chi-square with the dichotomized variable of postoperative OAHI <2 as cured and OAHI ≥2 as not cured, with the PROM answer "the symptoms are gone" considered as cured and all the other answers as not cured. Sensitivity, specificity as well as positive predictive value and negative predictive value were calculated.

Ethical approvals and considerations

Written informed consent was obtained from the parents/guardians of the patients in Paper I. Sample collection was approved by the regional ethical board in Stockholm (Dnr 2014/1000-31/1 NCT02315911, registered at www.clinicaltrials.gov) and (Dnr 2011/925-32 with complementary number of patients Dnr 2013/2274-32).

Paper II and III were approved by regional ethical board in Stockholm (Dnr 2011/925-32 with complementary number of patients Dnr 2013/2274-32). Both were based on a randomized clinical trial, which was registered at www.clinicaltrials.gov (Trial Registration Number: NCT01676181). All caregivers of the children included in the trial gave their written informed consent to participate in the study.

All patient's caregivers in Paper IV gave their consent to be included in each study and ethical approval was given by Swedish Regional Ethics Board, Stockholm (ref 2014/1000-31/1 and 2011/333-31/4).

Results

Paper I

The final cell numbers for ILCs were 7,200 and 7,205 in the small and large tonsil group respectively. The cell numbers for T cells and B cells were 18,000 and 18,005 in the small and large tonsil group, respectively.

Similar frequencies of ILC2 and ILC1 were detected in both groups of those small and large tonsils. A tendency toward a lower percentage of ILC3 among large tonsils was seen (Figure 18).

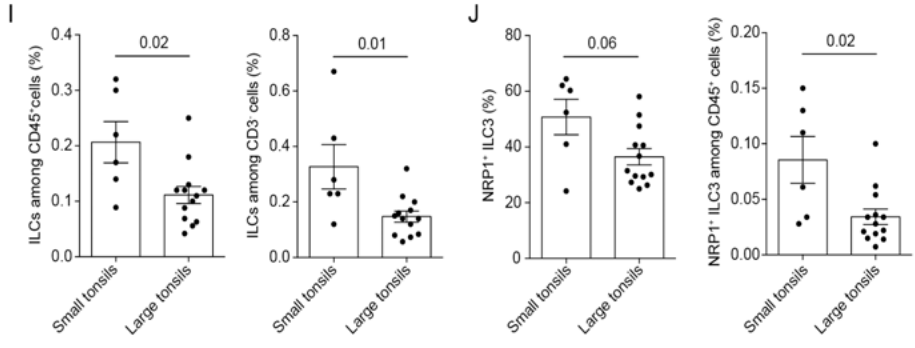


Figure 18. Frequency of ILCs (I) among total living CD45+ cells (I), Frequency of (J) NRP1+ ILC3 subset of all ILC.

The two groups showed a similar composition of T_{FH} cells. Three non- T_{FH} memory helper T cell subpopulations were identified in the small-tonsils group, and five subpopulations in the large-tonsils group (Figure 19).

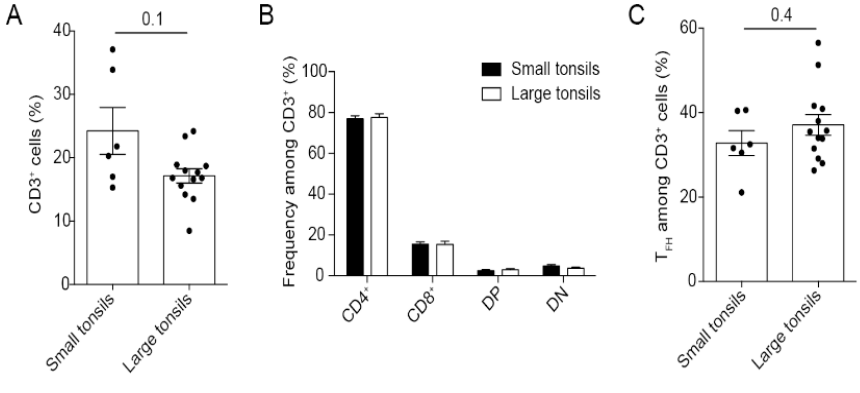


Figure 19. Frequency of CD3+ T cell clusters in the two groups (A) among total living CD45+, (B) frequency of CD4+, CD8+, double positive (DP) and double negative (DN) among total CD3+, and (C) frequency of T_{FH} cells among total CD3+ cells.

The large tonsil-group showed a tendency for a higher frequency of total B cells (Figure 20 – 22). A greater frequency of CD27+ B cells was found in the group with small tonsils whereas a higher frequency of CD27- B cells was identified in the group of large tonsils and very severe OSA. A strong enrichment of $CD27^{-}$ $CD21^{hi}$ naïve B cells was seen in patients with large tonsils, at the same time the expression of Ki67 was not increased in the same group.

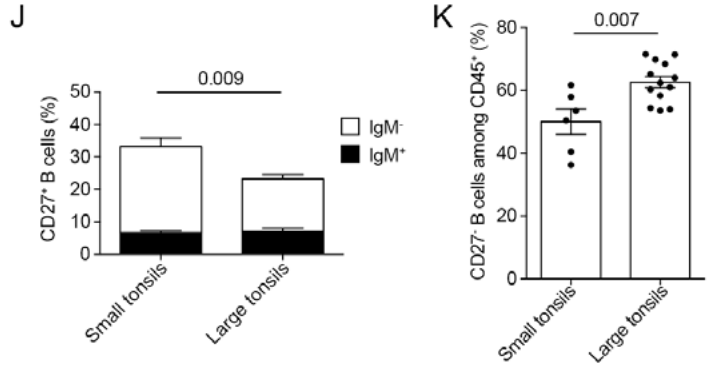


Figure 20. Difference of B cells between the groups, CD27+ memory B cells (J) Black bars indicate IgM+ and white bars indicate IgM- subsets. Difference of CD27- (naïve) B cells among all living cells CD45+(K)

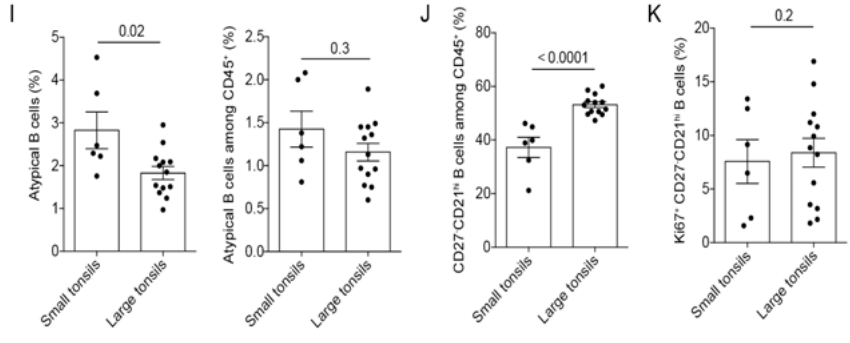


Figure 21. Differences of percentage Atypical B cells (I), naïve B cells (J) and Ki67 (K) between the groups.

In summary, the most interesting findings in this study are the smaller frequency of ILC3 and higher naïve B cell frequency in patients with large tonsils compared with those with small ones. The enrichment of naïve B cells could not be explained by cellular proliferation since the $Ki67^{+}$ expression was not in proportion. The causative mechanism of these findings may be a defect differentiation of naïve B cells and/or migration into the germinal center.

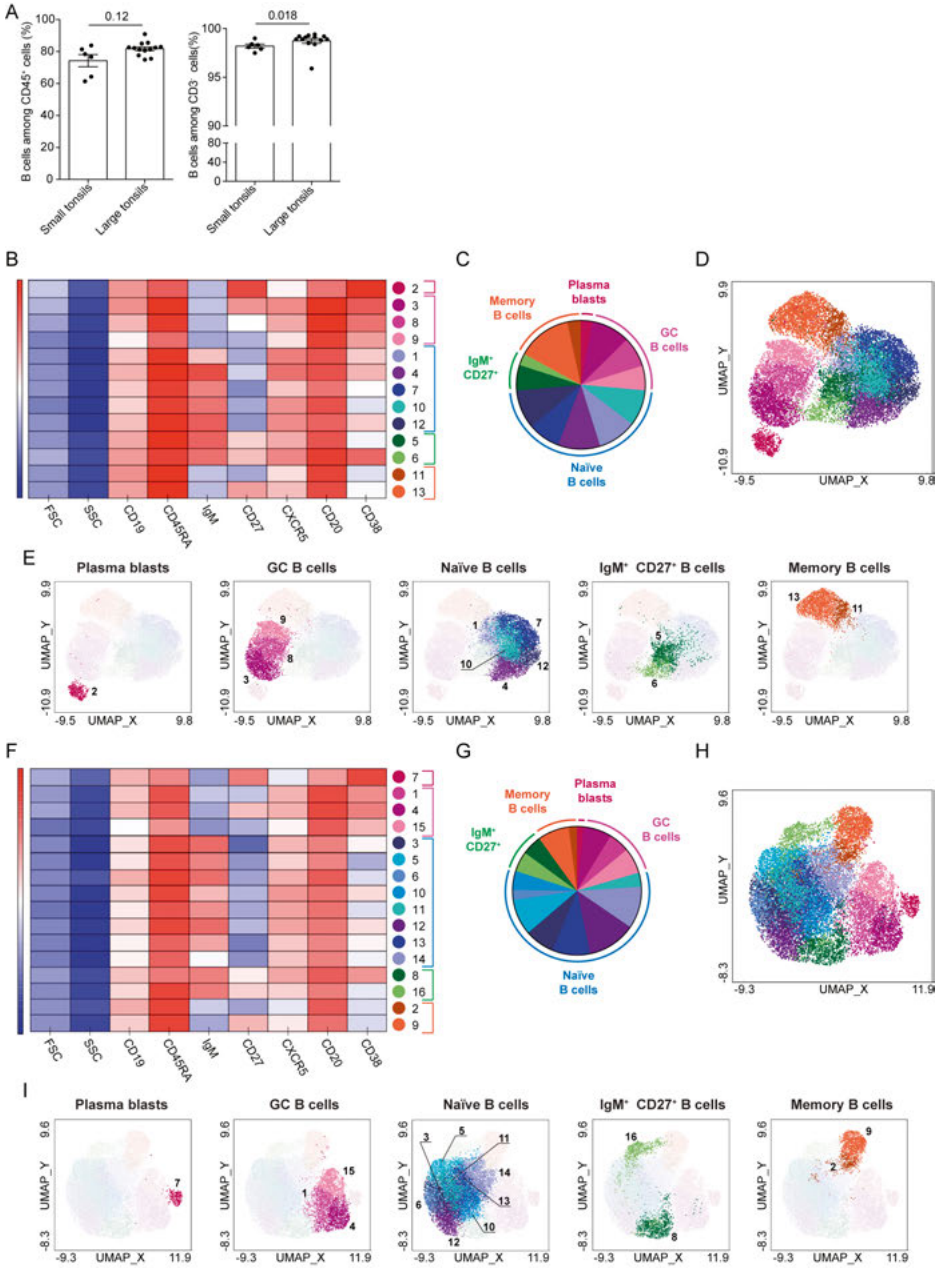


Figure 22. Heatmap of B cells (A) frequency of B cells among total living CD45^{+} and CD3^{-} cells. B cell clusters in small (B – E) and large tonsils (F – I). Heatmap of marker expression for each marker (B, F) Diagram showing proportions of PhenoGraph clusters and combined functional groups (C, G). Phenograph clusters on the corresponding UMAP (D, H) and separated by functional groups (E, I).

Paper II

A total of 79 patients were included in the original RCT. The mean age was 3.6 years. At baseline, 69 patients (87 percent) had filled out the SDQ. Of these, 32 children belonged to the ATE group and 37 children to the ATT group (Table 5). At follow-up after one year, the SDQ was filled out by 68 patients (86 percent), 31 treated with ATE and 37 operated on with ATT . Characteristics at follow-up is seen in Table 8. Mean time from baseline to follow-up was 15.1 months (a range of 9–19 months) in the ATE-group and 14.3 months (a range of 9–21 months) in the ATT-group. The median total SDQ-score before surgery was 11.0 (range of 4–22) in the ATE group and 9.5 (1–22) in the ATT group ($p = 0.52$). The median total SDQ-score at follow-up was 9 (2–29) in the ATE-group and 7 (0–35) in the ATT-group ($p = 0.09$) shown in Figure 23.

Subscales of SDQ were compared between the groups and there was no significant difference at baseline or at follow-up. There was no significant correlation between AHI and total SDQ score after surgery, with $r = -15$ and $p = 0.22$. A significant correlation was seen between OSA-18 and total SDQ score after surgery, $r = 0.55$, $p = 0.00$. When comparing preoperative and postoperative data in the whole population, the preoperative median total SDQ was 10 (range 1–22) and the postoperative figure was 8 (0–35). Among girls there was a modest decrease in total SDQ-score (mean 9.9–9.6, $p = 0.26$) but among boys there was a significant difference (mean 11.0–8.4, $p = 0.0001$). No difference was seen between girls and boys after surgery ($p = 0.17$)

Table 8. Characteristics at follow-up

	ATE (n = 31)	**ATT (n = 37)**	**p**
Age, months (range)	59 (39–90)	57 (39–97)	
Boy/Girl (%)	22/9 (71/29)	23/14 (62/38)	
Length, cm	110 (11)	111 (8)	
Weight, kg	19.4 (3.6)	19.6 (4.1)	
SDQ Total	9.6 (5.1)	8.2 (6.7)	0.09
AHI	2.5 (2.0)	4.5 (6.3)	0.11
OSA-18	31.8 (11.7)	36.5 (11.6)	0.11

Data are mean (SD) unless otherwise noted. p calculated with Mann-Whitney U test between groups.

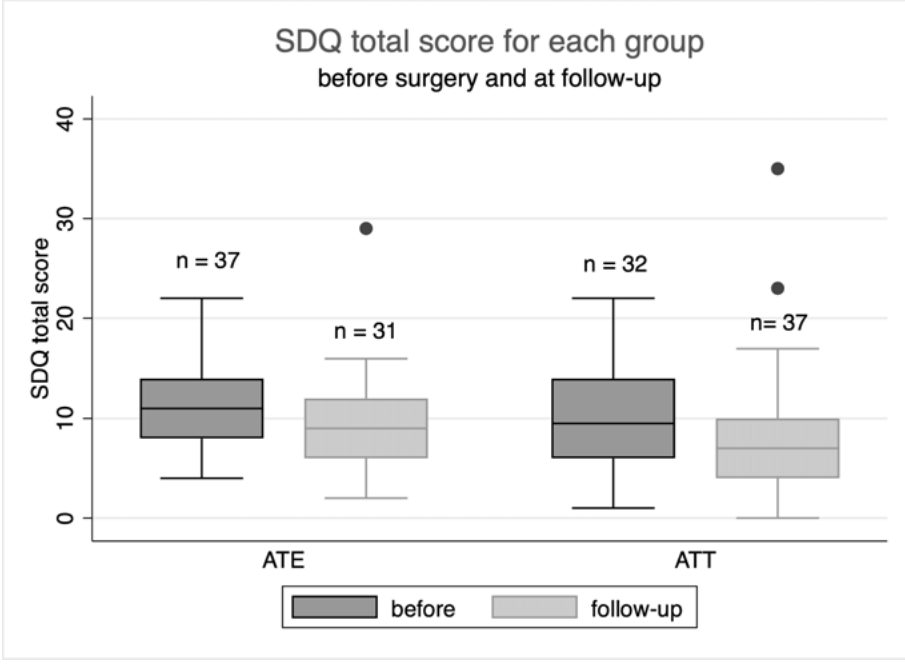


Figure 23. SDQ Total score before and after surgery for each group.

Paper III

Out of the 79 randomized patients, the follow-up PSG five years after surgical treatment was undergone by 51 children (65 percent). The dropout rate was 35 percent (28 children) and six children from the ATT-group were excluded due to reoperation with ATE. In total, 45 children (57 percent) were included in the per-protocol analysis, 28 from the group treated with ATT and 17 treated with ATE. Mean OAHI was 0.56 for all children at follow-up. The median age at follow-up PSG was nine years and six months (114 months, interquartile range 107–120).

In the ATT-group, 28 of 39 were included (72 percent); the mean OAHI decreased from 12.6 (SD 7.4) to 0.5 (0.6), a 96 percent mean reduction. In the ATE-group, 17 of 40 (43 percent) were included; the mean OAHI decreased from 12.3 (8.0) to 0.6 (0.7), a 95 percent mean reduction. Five children in each group had mild OSA (defined as ≥1 OAHI <5), while the rest had OAHI<1. The difference between the groups' mean OAHI five years after surgical treatment was 0.1 (95 percent CI -0.3–0.5) (Table 9). A boxplot illustrating mean OAHI for all patients included in the five-year follow up, at baseline, after one year, and at five years follow-up for each group is seen in Figure 24.

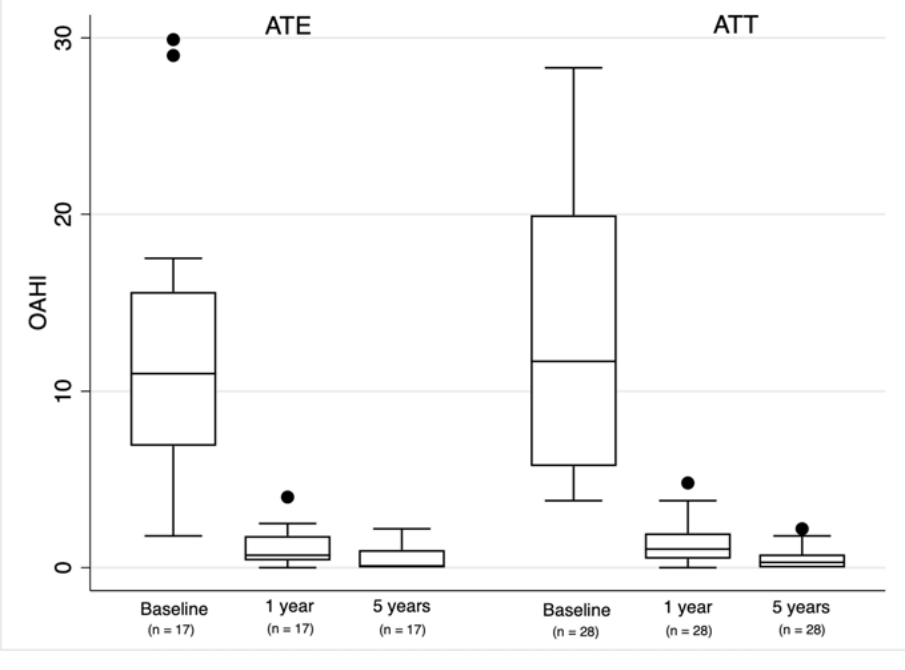


Figure 24. Boxplots illustrating obstructive apnea-hypopnea index (OAHI) at baseline, 1-year, and 5-years follow-up for each group.

Table 9. Results from PSG and OSA-18 variables – baseline (PSG1), one year (PSG2), and five years (PSG3).

	ATE PSG1 n = 17	ATE PSG2 n = 17	ATE PSG3 n = 17	ATT PSG1 n = 28	ATT PSG2 n = 28	ATT PSG3 n = 28	Group diff PSG3 Mean Difference (95% CI)
AHI	13.6 (7.3)	2.4 (1.8)	1.1 (1.4)	14.4 (7.7)	3.3 (2.3)	0.8 (1.2)	0.3 (-0.5 – 1.1)
OAHI	12.3 (8.0)	1.2 (1.0)	0.6 (0.7)	12.6 (7.4)	1.3 (1.2)	0.5 (0.6)	0.1 (-0.3 – 0.5)
ODI	3.7 (4.0)	1.3 (1.1)	0.7 (0.5)	3.9 (3.9)	1.7 (1.7)	0.9 (1.1)	-0.2 (-0.8 – 0.4)
RDI	16.3 (7.2)	2.5 (2.0)	0.6 (0.7)	16.2 (7.8)	2.7 (2.3)	0.5 (0.6)	0.04 (-0.4 – 0.4)
Mean SaO2	96.5 (1.4)	97.0 (0.5)	96.7 (0.7)	96.7 (0.7)	97.1 (0.8)	96.6 (0.8)	0.06 (-0.4 – 0.5)
Nadir O2	87 (7.4)[a]	90.9 (3.5)[a]	91.1 (4.3)	86.4 (8.0)[a]	89.6 (5.0)	91.3 (4.3)	-0.1 (-2.8 – 2.6)
TSS	57(47–79)	31 (29–34)	27 (22–36)	67 (53 – 79)	31 (26–47)[a]	32 (25–44)	6.9 (-0.8 – 14.7)
SDS	18(16–22)	6 (4 – 8)	6 (4–8)	16 (12 – 21)	6 (5–8)[a]	6.5 (5–9)	0.9 (-0.8 – 2.6)
HRQoL	7 (6–9)	10 (9–10)	9 (8–10)	7 (5 – 8)[a]	9 (8–10)	8 (7 – 10)	-0.6 (-1.5 – 0.2)

Data are mean (SD) except OSA-18 median (iqr). Group diff PSG3 = Difference between the groups in postoperative mean values at PSG3; ODI = oxygen desaturation index; RDI = respiratory disturbance index; SaO2 = oxygen saturation; TSS = OSA-18 total symptom score; SDS = OSA-18 sleep disturbance score; HRQoL= OSA-18 general health related quality of life; [a] = missing data for less than 5 of the group participants.

The intention-to-treat using LOCF resulted in a mean OAHI 1.9 (SD 5.1) in the ATT-group (n = 39) and OAHI 2.4 (SD 4.9) in the ATE-group (n = 40). The difference between the groups' mean OAHI using ITT was -2.8 (95 percent CI -10.4 – 4.8). When using a simulative negative scenario for the ATT-group, the difference of the mean OAHI was -0.2 (CI -2.5 – 2-0).

Results from the OSA-18 questionnaire showed for the ATT-group a total score that decreased from 67 (iqr 53–79) to 32 (iqr 25–44) and in the ATE-group from median 57 (iqr 47–79) to 27 (iqr 22–36). Compared with a Mann-Whitney U assessment, no differences were seen between the groups for a total score of OSA-18 (p = 0.10), sleep disturbance subscale (p = 0.18), or health related quality of life (p = 0.17).

Five of the patients who underwent tonsillar surgery a second time (ATE) were operated on because of persistent OSA, one within six months and the other four within 22 months. The sixth patient was operated on with ATE because of recurrent tonsillitis 36 months after ATT. The median age at first surgery was 34 months (iqr 30–37) for the six patients reoperated on, compared with 43 months (iqr 35–54) for the other children.

The dropout analysis revealed a significant difference in the distribution of patients belonging to each surgical technique, otherwise the baseline characteristics were similar between included and dropouts/excluded (Table 10).

Table 10. Comparing parameters between patients included and dropout/excluded.

Parameter	Included	Dropout/Excluded	P value
N (%)	45 (57)	28/6 (35/8)	
Age at intervention, months	42 (15)	45 (14)	0.3[a]
Surgical treatment ATE/ATT	17/28	23/11	0.009[b]
Sex boy/girl (%)	28/17 (62/38)	25/9 (74/26)	0.3[b]
OAHI			
Baseline	12.5 (7.5)	13.1 (7.9)	0.7[a]
1 year	1.3 (1.1)	2.5 (5.2)	0.15[a]
OSA-18, median (range)			
Baseline	65 (50–79)	63 (55–74)	0.8[c]
1 year	31 (26–39.5)	30 (23–43)	0.6[c]

Values are mean (SD) if not specified otherwise. Probability calculated with [a]t test, [b]chi-square test or [c]Mann Whitney U.

A total of 201 patients were included. The mean OAHI at baseline was 15.9 (SD 11.3). Pre- and postoperative data from PSG and OSA-18 were obtained from 173 children (86 percent). The mean age at follow-up was 4.2 years with a significant difference between the treatment arms. The group operated with APP was slightly younger with a mean age of 3.6 (SD 0.7) years compared to 4.7 (1.2) years in the ATT-group and 4.2 (1.1) in the ATE group. The mean OAHI at follow-up was 2.0 (3.2), no differences between the groups. The mean change in the OAHI was -13.8 (SD 11.5) with a higher reduction in the group operated with APP. The median change in OSA-18 TSS was -27.5 (iqr -42 – -15). All characteristics at follow-up are seen in Table 12.

The HRQoL was answered by 165 parents (94 percent) and the PROM question by 136 (78 percent). The median of the PROM question at follow-up was 1 (range 1–3). No parent had answered that the symptoms were worse, while four patients answered that the symptoms were the same, 31 patients were better, and 102 had no symptoms at follow-up (Table 12).

The changes in the OAHI as well as changes in the OSA-18 TSS and SDS were divided into groups according to the answer to the PROM question. There were significant group differences for changes in the OAHI and OSA-18 SDS as illustrated in the boxplots in Figures 25 to 27.

A chi-square table of “cured patients” using the cut-off value of postoperative OAHI <2 and the PROM answer “the symptoms are gone” resulted in a sensitivity of 38 percent, a specificity of 82 percent, a positive predictive value of 53 percent, and a negative predictive value of 70 percent (Table 11).

Table 11. Chi-square of Postop OAHI and PROM question p = 0.018

Postop OAHI	**PROM 2-3**	**PROM 1**	
<2	16	71	87
≥2	18	31	48
	34	102	136

The correlation between changes in the OAHI and OSA-18 total score was significant but weak: r = 0.29, p <0.001, illustrated with a scatterplot in Figure 28. The correlation between changes in the OAHI and the OSA-18 sleep disturbance scale (SDS) was moderate: r = 0.53, p < 0.001, illustrated with a scatterplot in Figure 29. The correlation between changes in the OAHI and HRQoL was also significant but weak: r = -0.16, p = 0.045. Intention-to-treat analysis did not change the results (Table 13).

The correlation between the PROM answers and changes in the OAHI was r = 0.36, p < 0.001. The correlation between the PROM answers and changes in OSA-18 TSS was r = 0.24, p = 0.0006 and changes in the OSA-18 SDS were also weak r = 0.34, p < 0.001. For all correlations, see Table 13.

Table 12. Characteristics at evaluation divided into each surgical treatment group

Variable	treatment	n	Value	P
Age, years mean (SD)		182	4.2 (1.0)	0.0004
OAHI, mean (SD)		183	2.0 (3.2)	0.4
	ATE	114	2.0 (3.0	
	ATT	39	2.2 (4.5)	
	APP	30	2.1 (1.7)	
OSA-18 TSS, median (range)		178	31 (18 –82)	0.7
	ATE	111	31 (18–78)	
	ATT	38	31.5 (20–59)	
	APP	29	30 (18–82)	
OSA-18 SDS, median (range)		178	6 (4–24)	0.2
	ATE	111	6 (4–24)	
	ATT	38	6.5 (4–24)	
	APP	29	6 (4–20)	
HRQoL, median (range)		176	9 (4–10)	1.0
	ATE	111	9 (4–10)	
	ATT	37	9 (5–10)	
	APP	28	9 (5–10)	
ΔOAHI, mean (SD)		183	-13.8 (11.5)	0.0001
	ATE	114	-12.6 (11.2)	
	ATT	39	-11.2 (9.1)	
	APP	30	-21.7 (12-2)	
ΔOSA-18, TSS, median (SD)		174	-27.5 (18.5)	0.4
	ATE	107	-28 (18.7)	
	ATT	38	-26.5 (17.7)	
	APP	29	-31 (18.7)	
ΔOSA-18, SDS, median (SD)		174	-11 (6.1)	0.3
	ATE	107	-11 (6.2)	
	ATT	38	-9.5 (6.2)	
	APP	29	-11 (5.4)	
ΔHRQoL, median (SD)		167	2 (2.1)	0.5
	ATE	106	2 (2.3)	
	ATT	34	2 (1.9)	
	APP	27	2 (2.3)	
PROM question		136	100%	
The symptoms are gone (1)		102	75%	
My symptoms are almost gone (2)		30	22%	
My symptoms remain (3)		4	3%	
My symptoms have worsened (4)		0	0%	

Δ = changes between preoperative and postoperative values. P-value calculated with Kruskal Wallis-test for differences between surgical treatment groups.

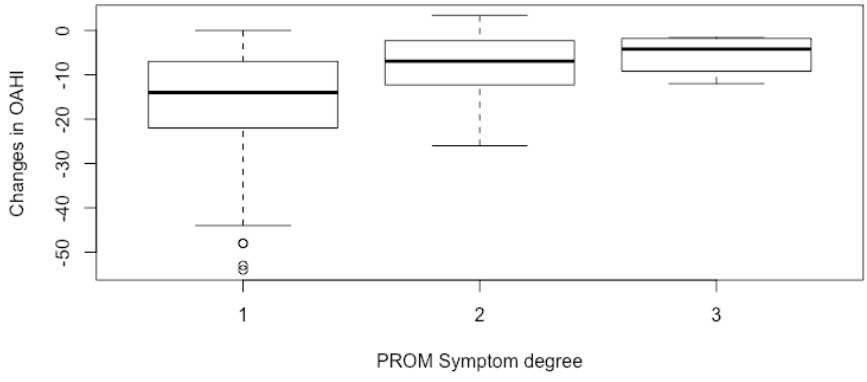


Figure 25. Boxplot of changes in OAHI divided into groups according to answer to PROM.

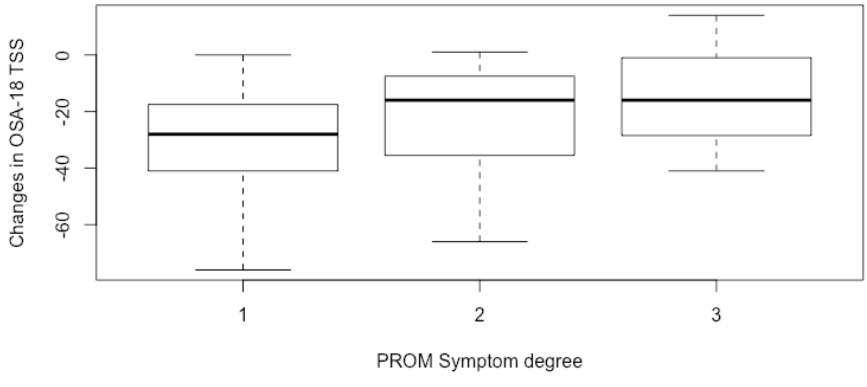


Figure 26. Boxplot of changes in OSA-18 total symptom score (TSS) divided into groups according to answer to PROM.

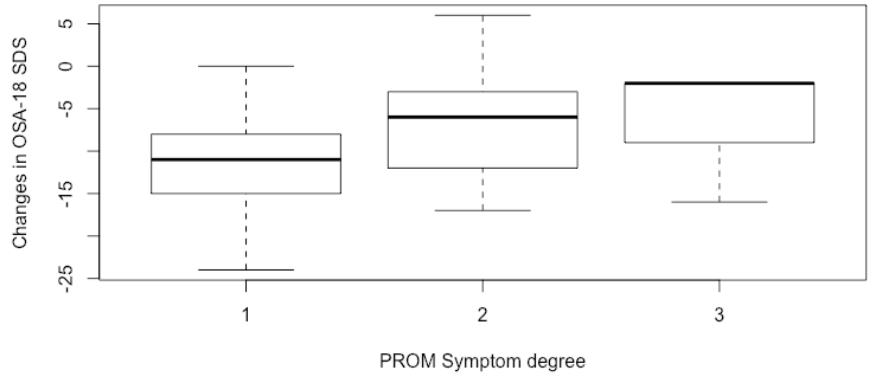


Figure 27. Boxplot of changes in OSA-18 sleep disturbance subscale (SDS) divided into groups according to answer to PROM.

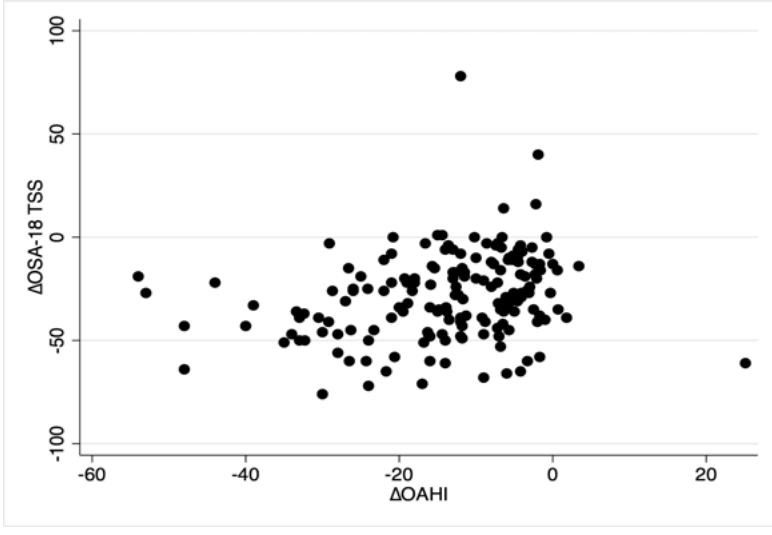


Figure 28. Scatterplot of differences between the pre- and postoperative values of OAHI and the OSA-18 total symptom score (TSS). Correlation r = 0.29, p <0.001

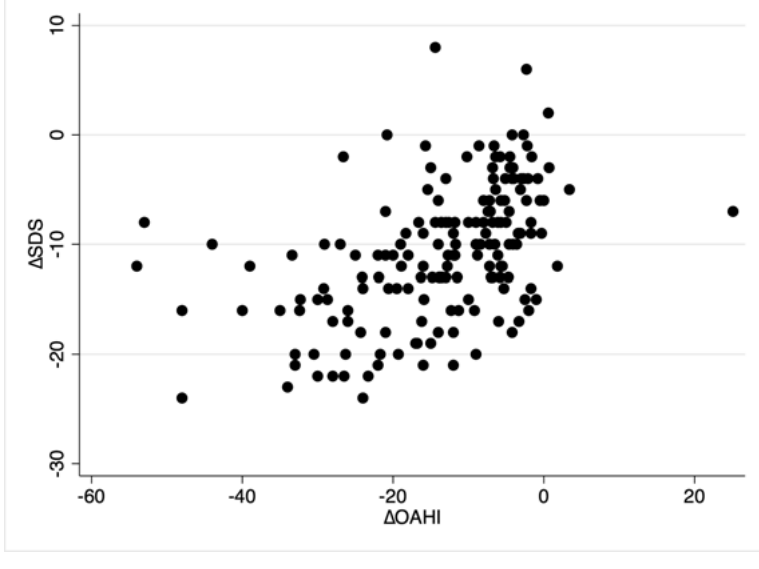


Figure 29. Scatterplot of differences between the pre- and postoperative values of OAHI and the OSA-18 sleep disturbance subscale (SDS). Correlation r = 0.53, p <0.001.

Table 13. Correlations between objective and subjective data from polysomnography and questionnaires.

Correlations	**Per protocol**			**Intention to treat**		
Preoperative	N	R	P	n	R	P
OAHI ~ OSA-18 TSS	194	0.23	0.002	198	0.35	<0.001
OAHI ~ OSA-18 SDS	194	**0.4**	**<0.001**	198	**0.51**	**<0.001**
OAHI ~ HRQoL	186	-0.07	0.33			
ODI ~ OSA-18 TSS	194	0.17	0.02			
ODI ~ OSA-18 SDS	194	0.35	<0.001			
RDI ~ OSA-18 TSS	193	0.23	0.001			
RDI ~ OSA-18 SDS	193	0.37	<0.001			
Postoperative						
OAHI ~ OSA-18 TSS	177	0.04	0.6	198	0.17	0.01
OAHI ~ OSA-18 SDS	177	0.17	0.02	197	0.28	0.001
OAHI ~ PROM	136	0.19	0.03	136	0.19	0.03
OAHI ~ HRQoL	175	-0.08	0.28			
ODI ~ OSA-18 TSS	174	0.03	0.7			
ODI ~ OSA-18 SDS	174	0.06	0.5			
ODI ~ PROM	133	0.22	0.01			
RDI ~ OSA-18 TSS	171	0.004	0.96			
RDI ~ OSA-18 SDS	171	0.21	0.007			
RDI ~ PROM	132	0.36	0.003			
Changes pre- and postoperatively						
ΔOAHI ~ ΔOSA-18 TSS	175	0.29	<0.001	198	0.36	<0.001
ΔOAHI ~ ΔOSA-18 SDS	175	**0.53**	**<0.001**	198	**0.51**	**<0.001**
ΔOAHI ~ ΔHRQoL	165	-0.16	0.045			
ΔOAHI ~ PROM	136	0.36	<0.001	136	0.36	<0.001
ΔODI ~ ΔOSA-18 TSS	173	0.20	0.01			
ΔODI ~ ΔOSA-18 SDS	**174**	**0.42**	**<0.001**			
ΔODI ~ ΔHRQoL	166	-0.03	0.7			
ΔODI ~ PROM	136	0.24	0.004			
ΔRDI ~ ΔOSA-18 TSS	174	0.25	<0.001			
ΔRDI ~ ΔOSA-18 SDS	**175**	**0.46**	**<0.001**			
ΔRDI ~ ΔHRQoL	167	-0.1	0.2			
ΔRDI ~ PROM	136	0.34	<0.001			
PROM ~ ΔHRQoL	133	-0.34	<0.001			
PROM ~ ΔOSA-18 TSS	131	0.24	0.006	136	0.24	0.004
PROM ~ ΔOSA-18 SDS	131	0.34	<0.001	136	0.37	<0.001

Correlations calculated with the Spearman's rank correlation. Moderate correlations (r-value ≥ 0.4) and significant p-values are marked in bold.

Discussion

In the everyday clinical life, children with sleep-related breathing disorders are common. On the contrary, investigations using polysomnography or polygraphy are uncommon due to a lack of resources, time or competence in interpreting the results. A shift from the classical OSA-disease, with its close connections to the objective PSG-findings, to a snoring symptom-based diagnosis, has emerged during the work on this thesis. Changes in behavior and brain imaging as well as cardiovascular risk factors have been highlighted among children with mild OSA or habitual snoring (≥3 nights per week) (42, 47, 48). But even if the consequences are evident, the children are snoring, the parents are worried and the surgeons are ready, there are still many questions to answer.

Why do the tonsils cause obstruction for some children?

In Paper I, our aim was to investigate if any immunological differences could answer this question by mapping the tonsil's immunological cells and compare between one group of patients with severe OSA and large tonsils and another with milder OSA and small tonsils. The study sample was small, and there were no significant differences in ILCs or T cells between the groups but there were some interesting differences in B cells in the tonsil tissue. Naïve $CD27^{-}CD21^{hi}$ B cells showed a significant enrichment in the tonsil tissue from patients with larger tonsils and severe OSA. This enrichment was not accompanied with increased proliferation since Ki67 was not different between the groups. Ki67 is a protein expressed during the S-phase of cell division and is a marker for proliferation, which is an important finding, in this case looking at naïve B cells that could have been recruited days before the surgery was done. The cause of this infiltration may be a consequence of an impaired differentiation of B cells. The reduction of ILC3 with LTi phenotype could have this effect since ILC3 has been shown to support B cell differentiation in the spleen (121) but the role of ILC3 and LTi in human B cell differentiation in tonsils is not well known and need to be further explored.

Other studies looking at tonsils from patients with OSA often use them as controls to compare with tonsils extracted from patients with recurrent tonsillitis (RT). One limitation in our study is of course that our "controls" are patients with mild OSA and some tonsil hyperplasia instead of normal tonsil tissue from patients without OSA, which would have been ideal. But a strength, on the other hand, is that the tonsils are not surgically removed because of recurrent tonsillitis, which would have given us a skewed immunological picture in this comparison.

It has been demonstrated in adults, that tonsils from patients with OSA have larger germinal center areas with increased frequency of B cells compared with tonsils from patients with recurrent tonsillitis (122). The same study also

showed a functional impairment concerning T_{FH} that were higher in number but inferior in providing help to B cells. A study of children with OSA compared with children with group-A streptococcus RT showed that the latter had smaller germinal centers and a lower frequency of T_{FH} cells but higher frequencies of naïve B cells (123).

Why hypertrophic tonsils cause obstructive sleep apnea for some children is still not clear but the impaired function of the naïve B cells could indicate this is a part of the immunological explanation.

Mental health among children with OSDB

The prevalence of sleep disturbances are higher among children with neurodevelopmental disorders than in the general population (124). In addition, studies have shown a connection between OSA and neurobehavioral sequelae as well as improvement after surgical treatment (125, 126). Some published results seem to indicate that the connection between OSA and attention-deficit/hyperactive disorder (ADHD) are reciprocal, one exacerbating symptoms of the other (36). Nevertheless, the relationship between behavioral consequences and disease severity of OSA is unclear.

An ongoing study called pediatric adenotonsillectomy trial for snoring (PATS) compared children with mild OSA (OAHI<3) with children from a previous large study called childhood adenotonsillectomy trial (CHAT) with moderate OSA (OAHI 2–30), and found an increased frequency of abnormal executive function among the group with milder disease (41). However, some studies show no difference in these symptoms when comparing surgical treatment and watchful waiting. For example, the large CHAT study from 2013 evaluated children from five to nine years of age randomized to either ATE or watchful waiting. A total of 464 children were included but no differences were found when assessing the children's attention and executive function seven months after treatment using a Developmental Neuropsychological Assessment (NEPSY). On the other hand, differences were found using Conner's rating scale which also is used to assess ADHD symptoms (127).

Since young children from two to six years old are among those most affected by OSA, any behavioral consequences may be somewhat difficult to evaluate. Maturation differs greatly between individuals in this age range, while at the same time different neurodevelopmental disorders could overlap and require assessment from a range of specialists (128).

A preschool obstructive sleep apnea tonsillectomy and adenoidectomy (POSTA) study from 2020 examined 190 children between three and five years old with mild to moderate OSA (OAHI<10). The primary endpoint was to evaluate global IQ using the Woodcock Johnson III method, comparing early ATE with watchful waiting. Furthermore, sleep-related endpoints from polysomnography and questionnaires of behavior and executive functions

were also assessed after 12 months (129). The conclusion after the first year was that no differences could be seen in global IQ between the groups, but differences in the apnea index and day sleeps were found. Interestingly, after 24 months, when both groups had been operated on with ATE, improvements were seen in sleep quality, apneas and behavioral measurement, as reported by parents. The conclusion was drawn that children with mild to moderate OSA may benefit from surgical treatment with ATE regardless of the timing of surgery (40).

A further analysis from the POSTA study looked at a cohort of 91 children (52 with mild to moderate OSA vs. 39 with primary snoring) and compared their neurocognition and behavior using the Woodcock Johnson III method and the Brief Intellectual Ability (BIA) approach for neurocognition, the Behavior Assessment System for Children, second edition (BASC-2) and the Behavior Rating Inventory of Executive Function–Preschool (BRIEF-P) for behavior (42). No differences were found between the two groups and the authors conclude that PSG parameters alone are not sufficient for treatment decisions for pediatric OSA.

In the PATS, children from three to 12 years of age with habitual snoring (OAHI<3 and obstructive apnea index<1) were included. Neurobehavioral outcomes were assessed using BRIEF, the child behavior checklist (CBCL) and Conners 3rd edition. The aim is described as to evaluate the effect of adenotonsillectomy versus watchful waiting for treating mild OSA. The results from this study will be of high interest and are expected within the next year (130).

The Strengths and Difficulties Questionnaire used in this thesis is also a widely used tool in clinical settings. For example in a study exploring the associations between disturbed sleep and behavioral difficulties among 635 children, six to eight years (131), as well as to assess the risk of functional impairment because of ADHD (132). But this questionnaire has not previously been used in a larger scale to assess the mental health among patients with OSA. In Paper II, the children's total difficulties score was in the normal range both before (median 11 vs. 9.5) and after surgery (median 9 vs. 7).

What questionnaire to use differs between countries and cultures but the Swedish Agency for Health Technology Assessment and Assessment of Social Services (SBU) presented a report in 2022 on standardized methods for the evaluation of children and youths in social services. In the SBU-report, SDQ together with the lengthier child behavior checklist (CBCL) were the two recommended for assessing psychiatric disorders. SDQ was described as a short questionnaire to screen for behavior with good reliability and validity but somehow deficient concordance between different informants. One large Swedish study (n = 17.752) had found a good validity for a preschool (age 3 to 5) population (133). One important difference between the SDQ and the instruments mentioned from other studies, is that SDQ is free for anyone to download and use.

The aspects of behavior and mental health among children with OSA and OSDB are important to address and SDQ seems to be a reliable and brief tool to do so.

ATE or ATT, that is the question

The most important finding in Paper II is that there was no difference between the groups treated with either ATE or ATT when comparing SDQ total score one year after surgery. In addition, the most important findings in Paper III are that there were no differences seen when comparing PSG-parameters and OSA-18 scores five years after surgery in Paper III.

To be able to decide which surgical method to choose for whom, when, and why, a randomized controlled trial (RCT) is the best way to evaluate scientifically the outcomes over the short and long term. By randomizing patients to one of the treatment arms, the patients' different characteristics are distributed evenly and therefore do not affect the outcome in a skewed manner. The patients or caregivers should be blinded to method but the surgeon cannot be. This type of study is time and resource-consuming and often difficult to perform when comparing surgical treatment methods.

Paper II and III are based on an RCT of 79 children operated on with either ATE or ATT. This is a unique study and the long-term effects of this kind have been requested (88). Even so, the results showing a high dropout rate and high rate of reoperations could, and should be discussed. A large study from the national tonsil surgery register in Sweden showed a risk of reoperation that was seven times higher after tonsillotomy compared with tonsillectomy among 27,535 children aged one to 12 years (134). The same author concluded in another study of 35,060 children in the same age interval, that tonsillotomy entails less risk of postoperative hemorrhage and postoperative complications resulting in any type of contact with health services compared with tonsillectomy (135).

The evidence behind the assertion that tonsillotomy is accompanied with less postoperative complications such as pain, bleeding and faster return to normal activity has been shown in many other studies but these are often small (77, 88). What is well known, however, is that in the operation room, a tonsillotomy is preferable in many ways: the procedure is more controlled with less bleeding and a better view, and is more quickly performed. At the same time, when performing a tonsillectomy in a child who has not experienced many tonsillar infections, the tonsils can be very easy to peel out and the bleeding is much less than if the tonsils have been infected and inflamed over the course of several years. Additionally, the risk of readmission due to postoperative hemorrhage increases with age, especially after ATE (136), which could be a reason not to wait for reoperation as an indication for ATE.

In Paper III, the reoperation rate was 15 percent since six out of 39 patients treated with ATT were operated on with ATE during the follow-up period, one because of recurrent infections and the others due to regrowth of tonsils and relapse of OSA symptoms. No patient in the group treated with ATE needed second surgery, but one had postoperative bleeding, which needed surgical treatment immediately (89). These results show that the risk of reoperation is higher after ATT and, as shown in the population-based study, is highest among the youngest children (134), most likely because they have more time for regrowth and more active lymphatic tissue.

Limitations of Paper III include the high dropout rate of 35 percent from a quite small sample of patients. The largest dropout was in the ATE group where only 17 children of the 40 originally included (42.5 percent) underwent the third PSG. This could possibly be explained by pleased caregivers of children without problems not taking part or by tired caregivers whose children still had problems of some kind but for whom the procedure of undertaking another in-laboratory PSG proved too demanding. Furthermore, the homogenous population without obese patients and other comorbidities limits the generalizability of any findings.

So, which is the best choice of ATE or ATT, for pediatric OSA? For otherwise healthy children, one could argue for both. But to start with tonsillotomy, if the child is three years or older this option seems reasonable and evidence-based since the risk of postoperative hemorrhage and other morbidities are lower. If a reoperation is needed, ATE is recommended.

Do objective and subjective parameters correlate?

The main findings of Paper IV are the significant moderate correlation between the changes in PSG-parameters OAHI, ODI and RDI with the changes in subscale of sleep disturbance (SDS) from OSA-18 as well as preoperative OAHI and SDS. The sleep disturbance scale (Figure 4) includes questions about snoring, frequent awakenings, and choking or gasping sounds while asleep, as well as breath-holding pauses at night. These are all frequently seen symptoms among children with OSA and the improvement in these symptoms are somewhat expected with an improved PSG. At the same time, the correlation was only moderate (not strong).

The other questions in the OSA-18 questionnaire are focused on physical suffering, daytime symptoms and behavior. These symptoms are sometimes seen but not as frequently in all ages as the sleep-related breathing symptoms. The correlation between changes in the total score of OSA-18 and PSG data was significant but weak. Mitchell et al. found a poor correlation ($r = 0.09$) between changes in AHI and OSA-18 (137). A Norwegian study from 2021 including 56 children found no significant correlations ($r = 0.26$, $p = 0.05$) between changes in OAHI and the OSA-18 total score (138). Kang et al. presented a study from 2014 involving 119 children which showed a

significant but weak correlation between changes in the OSA-18 total score and AHI ($r = 0.26$, $p = 0.004$) (139).

Significant but weak correlations were found between the OSA-18 total symptom score and preoperative OAHI but not postoperatively. However, postoperative correlation between OAHI and SDS, respectively was significant but weak. Previous studies have focused on OSA-18 as a screening tool more than for follow-up and investigated the correlations preoperatively, finding significant correlations between OAHI and SDS but weaker or even no significant correlation between OAHI and TSS (140-142).

It is important to note the limitation of this study that the selected cohort have a moderate to severe OSA with median OAHI of 16 at baseline. This is not typical for the OSA patient in this relatively young age group and together with the small number of obese patients that makes the generalizability and external validity somewhat limited.

At the same time, obstructive sleep-disordered breathing is more complex than only looking at PSG and OAHI values. As previously mentioned, habitual snoring has been shown to have an impact on behavior and brain structure without an OAHI level of OSA. These results suggest that PSG parameters and parameters evaluating snoring and breathing during sleep are connected. On the other hand, symptoms such as behavior, cognition and mental health issues are expressing something else also important among children with OSDB and OSA. These symptoms might have to be evaluated separately from PSG findings among certain children.

Furthermore, a high specificity of 82 percent of the PROM question was found when using an OAHI of <2 and the answer “the symptoms are gone” was taken as cured. A high specificity is desirable if we want to accurately identify people who do not have the condition. So the PROM question is somewhat better for that but not as good as identifying children having persistent OSA with OAHI>2. That said, another important limitation in this study is the small variation of PROM answers with only 22 percent answering “symptoms almost gone” and 3 percent answering “my symptoms remain”. In addition, the PROM question was not posed in the same way for all caregivers, some answered in a questionnaire, some answered verbally at the clinic, while other answered over the phone, which could have affected the answers.

Conclusions

I. The heterogeneity of lymphocyte populations in the tonsils of pediatric patients with mild to moderate obstructive sleep apnea and small tonsils, compared with severe obstructive sleep apnea and large tonsils, revealed a difference in lymphocyte composition regarding ILC and naïve B cells.

II. No differences were found between patients treated with either adenotonsillotomy or adenotonsillectomy regarding children's mental health and behavior using a Strengths and Difficulties Questionnaire (SDQ).

III. Adenotonsillotomy is a non-inferior treatment method for pediatric obstructive sleep apnea compared with adenotonsillectomy regarding polysomnographic parameters such as obstructive apnea-hypopnea index and quality of life after five years. But the risk of reoperation after adenotonsillotomy is considerable and warrants follow-up.

IV. The objectively measured obstructive apnea-hypopnea index, oxygen desaturation index and respiratory disturbance index showed a significant moderate correlation to subjectively measured changes in the OSA-18 among children treated for obstructive sleep apnea with tonsillar surgery. The strongest correlation was seen in the subscale of sleep disturbance symptoms.

Future perspectives

An overarching aim, in medicine, regardless of discipline, is to improve our ability to correctly diagnose and treat our patients. As physicians, we also aspire to be empathetic to our patients concerns and problems. Within the field of pediatric OSA, practitioners also need to play a close attention to the concerns and worries of the parent. That said, communication with the parent and the child does not always provide the clearest picture of what is happening. Therefore, a recorded video can be of great value when assessing a child's breathing pattern during sleep. What is it that the parent is hearing, seeing, and worrying about? As previously mentioned, studies have started to investigate video recording as a reliable method, and evidence suggests that it may, in the era of smartphones offer a valuable source of information, and can serve as a diagnostic tool in the future.

Personally, I feel strongly for children's rights, and I fear that these rights are sometimes compromised since they cannot make their voices heard. In November 1989, a historic commitment to the world's children was made by the adoption of the United Nations Convention on the Rights of the Child (143). This is the most widely ratified human rights treaty that also became part of Swedish law in January 2020 (144). The 54 articles describe how every child is an individual with their own rights, and not objects that belong to their parents. The convention says that childhood lasts until 18 years of age and that children must be allowed to grow, learn, play, develop, and flourish with dignity. Article 24 recognizes the right to the highest attainable standard of health and health care services. This commitment to the most honest and vulnerable people cannot only be words but must be something we all carry with us and demand from world leaders and our surroundings, to ensure that every child, has every right.

In addition to working on this thesis, I have had the privilege to be part of a national working group, consisting of various researchers who work on devising a national care process for children with OSDB in Sweden. One of the recommendations from these guidelines is to encourage nurses and physicians in primary health care to ask caregivers if their child regularly snores three nights per week or more. Moreover, in the course of this work, we have analyzed another available questionnaire often used to diagnose OSA called the Pediatric Sleep Questionnaire (PSQ), and translated it into Swedish. The PSQ was introduced in 2000 by Chervin et al. and consists of 22 questions

(145). The answers are given as yes (Y=1), no (N=0) or don't know (DK=missing). A ratio of total score divided into all answers is measured and a ratio above 0.33 (at least 8 positive answers if all none are missing) is considered a high risk of OSA. The PSQ has been validated in Norway and Spain, where it seems to have a higher sensitivity than OSA-18 (146, 147). Some recommend it as an acceptable screening tool and suggest it should be used together with nocturnal pulse oximetry, measuring the child's oxygen saturation during the night (148). The Swedish version of PSQ needs to be validated and this work could hopefully be carried out in the near future.

The research of pediatric OSA and OSDB will continue to assess a child's symptoms. In the future it may also be compulsory to briefly assess neurocognitive functions and mental health, in addition to the gold standard polysomnography. Although, polysomnography offers an objective recording of the sleep, it is not a feasible procedure for all children. Moreover, it seems like polysomnography does not fully capture the whole truth of the disease of pediatric OSA.

A validated questionnaire regularly used, in combination with a structured way of recording a film, together with a higher level of knowledge in the population might help the children and the clinician in the right direction in the future.

Populärvetenskaplig sammanfattning

Bakgrund

Obstruktiv sömnrelaterad andningsstörning, på engelska betecknat som obstructive sleep disordered breathing (OSDB) är en övergripande term innefattande barn med obstruktiva andningsbesvär nattetid. Man uppskattar att mellan fyra till elva procent av barn i åldrarna två till sex år har OSDB i olika grad. Prevalenssiffrorna varierar mycket beroende på definitioner och metoder. Den allvarligaste formen av OSDB heter obstruktiv sömnapné (OSA) och drabbar cirka en till fem procent av barn i samma ålder. Förutom störd sömn och oroliga nätter kan besvären leda till dålig tillväxt, förändringar i hjärnan, beteendestörningar som koncentrationssvårigheter och i värsta fall hjärt- & kärlsjukdom.

Den vanligaste orsaken till OSA är förstorad adenoid (körteln bakom näsan) och/eller förstorade tonsiller (halsmandlar). Adenoiden och halsmandlarna är lymfoid vävnad som tillväxer kraftigt under förskoleåldern, och då barn har en liten näsa och ett litet svalg kan det leda till andningssvårigheter som behöver åtgärdas. Anledningen till att det påverkar barnet mest nattetid eller vid sömn är att då slappnar muskulaturen i svalget av och luftströmmen blir på så vis tilltäppt. Orsaken till varför denna lymfoida vävnad tillväxer så pass mycket att det för vissa barn blir ett fysiologiskt problem är idag okänd. Man vet att barnets immunförsvar mognar under förskoleåldern, och i den lymfoida vävnaden finner man B celler, T celler och medfödda lymfoida celler (ILC). Svalget och näsan är även en bra ingång för nya patogener (virus och bakterier), och när immunförsvaret möter dessa kan nya försvarsceller mogna och förbereda sig för framtida infektioner.

För att diagnosticera barn med OSDB krävs att man har upprepade snarkningar minst tre nätter i veckan den senaste månaden, utan att man är förkyld eller har någon annan förklaring till snarkningarna. För att kunna ställa diagnosen OSA behöver man göra en analys av andningen under sömn och detta görs med en nattlig andningsregistrering (NAR) eller polysomnografi (PSG), där PSG även mäter sömnstadier och exakt sömntid vilket leder till ett säkrare resultat avseende gradering av besvär än med NAR. PSG är i dagsläget gold standard för utredning av OSA, men är både en resurskrävande och obekväm undersökning att göra för barn. Tillgängligheten på PSG och NAR för barn är dessutom dålig i Sverige varför PSG endast används för utredning av OSDB i vissa fall och i forskningssammanhang.

Behandlingen av OSDB består i första hand av kortisonnäspray för de mildare fallen, där man endast behöver minska på adenoiden, men i många fall behöver man även kirurgiskt ta bort adenoiden och/eller tonsillerna. Denna behandling som görs när barnet är sövt, har förfinats över åren. Det finns två metoder: tonsillotomi och tonsillektomi, vilka kan kombineras med skrapning av adenoiden (adenoidektomi) om det behövs. Tonsillotomi är den äldsta metoden där man tar bort den vävnaden som syns utanför gombågarna för att skapa bättre plats i svalget medan tonsillektomi innebär att man tar bort hela halsmandeln med dess kapsel för att få bort så stor del som möjligt för att förhindra återväxt, infektion och immunologisk aktivitet.

Delarbete I – Lymfoida celler i förstorade halsmandlar

Delarbete I är ett deskriptivt arbete där vi analyserat tonsillvävnad från barn med förstorade halsmandlar och grav sömnapné och jämfört dessa med barn med små halsmandlar och mild till måttlig OSA. Syftet var att studera om det finns någon skillnad på halsmandlarnas uppbyggnad av olika immunologiska celler. För att kvantifiera de olika celltyperna i vävnaden användes flödescytometri. Vi fann då en tydlig skillnad vad gäller omogna (naiva) B-celler som verkar anhopa sig i de stora tonsillerna. Det är dock inte klart vad som orsakar denna skillnad vad gäller B-celler och studiematerialet var litet (11 barn med stora tonsiller och 6 barn med små). Vi fann ingen tydlig skillnad mellan grupperna avseende ILC eller T-celler.

Delarbete II – Strengths and Difficulties Questionnaire

Delarbete II är en uppföljningsstudie av en tidigare utförd randomiserad kontrollerad studie, som utvärderar mental hälsa och beteendestörningar hos 79 barn mellan 2 till 6 år behandlade för sin OSA. I studien använde vi oss av en validerad enkät för beteende som heter Strengths and Difficulties Questionnaire (SDQ) för att utvärdera barnens uppskattade beteende före och ett år efter operation med antingen adenotonsillektomi eller adenotonsillotomi. Resultaten visade inga tydliga skillnader mellan grupperna ett år efter operation vilket talar för att dessa behandlingsmetoder är likvärdiga i detta avseende. Båda grupperna blev klart förbättrade efter operation vilket också är förväntat som del i den normala utvecklingen för barn.

Delarbete III – ATT eller ATE, uppföljning efter fem år

Detta delarbete är också en randomiserad kontrollerad studie med samma population som studerades i delarbete II. Här utvärderas effekterna fem år efter operation med antingen adenotonsillotomi (ATT) eller adenotonsillektomi (ATE). Fokus i delarbete III var att studera om det blir någon skillnad avseende kvarvarande OSA eller symptom efter fem år och om

ny kirurgisk behandling behövdes. Vi utförde polysomnografi på barnen fem år efter att de opererats och utförde uppföljning med ett validerat frågeformulär (OSA-18) för att se om det var någon skillnad på objektiva eller subjektiva symptom mellan grupperna. Det var inte alla barn som inkluderades i studien från början som var med efter fem år, men av de vi undersökte (57 procent) fanns det inte någon skillnad mellan grupperna i de analyser som utfördes. Det var dock sex patienter som tidigare hade gjort adenotonsillotomi som fick reopereras med adenotonsillektomi, fem av dem på grund av återväxt av halsmandlarna och en på grund av återkommande halsinfektioner.

Delarbete IV – Korrelation mellan subjektiva och objektiva data

Det finns idag ingen strukturerad uppföljning efter halsmandelsoperation förutom ett nationellt tonsillregister som skickar ut en icke validerad fråga till patienter/vårdnadshavare sex månader efter operation. Det betyder att mycket få barn följs upp inom sjukvården avseende kvarvarande symptom eller ytterligare behov av behandling av sin OSA. I delarbete IV studeras korrelationen mellan förändring i objektiva parametrar mätt med polysomnografi och subjektiva symptom mätt med tidigare nämnt frågeformulär, OSA-18, samt en fråga angående kvarstående symptom sex till tolv månader efter operation. Vi fann en signifikant korrelation mellan de objektiva sömndata och subjektiva parametrar, vilken var starkast i en specifik del av OSA-18 enkäten, som just innehåller frågor rörande snarkning och andningsproblem nattetid. Vi såg även att frågan angående kvarstående symptom hade en högre specificitet än sensitivitet vilket gör den bättre på att se vilka som blivit bra i sina symptom. Begränsningarna i detta material innefattar just att det var så många av barnen som blev bra och därför svårt att generalisera resultaten.

Samtidigt som det är viktigt att se att en förbättring av symptom stämmer överens med förbättring i objektiva mått, har senare forskning visat att det är av särskild betydelse att fokusera på just barnens symptom och inte objektiva data. Det är också oftast en sådan subjektiv bild vi bygger vår bedömning på i kliniken och dessa specifika sömnrelaterade frågor kan därför ligga till grund för en strukturerad uppföljning efter tonsilloperation.

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