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PERIOPERATIVE MANAGEMENT AND MOLECULAR PATTERNS IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA

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Cover illustration: Recording of a home sleep apnea testing showing obstructive apneas with subsequent desaturation and maintained respiratory movement of the thorax and abdomen. Illustration received from Carin Sahlin.

PERIOPERATIVE MANAGEMENT AND MOLECULAR PATTERNS IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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To my family

POPULAR SCIENCE SUMMARY OF THE THESIS

Obstructive sleep apnea (OSA) is commonly occurring, but most are undiagnosed. Patients with OSA have an increased risk of complications during anesthesia and surgery. Many of the complications are linked to breathing and the upper airway, but there is also an increased need for intensive care and longer hospital care. In patients with OSA, the upper airway repeatedly collapses during sleep, which in turn leads to declining oxygen saturation in the blood, short awakenings and activation of the sympathetic nervous system. The most common symptoms are excessive daytime tiredness, snoring and apneas. Moderate-to-severe OSA is usually treated with continuous positive airway pressure (CPAP). Air with a constant pressure is delivered to the airways through a face or nose mask and in this way the airways are kept open and the apneas disappear. Patients with OSA also often have other comorbidities, such as obesity, high blood pressure and diabetes.

In situations of acute shortage of oxygen, ie hypoxia, the body's immediate response is to increase ventilation – the so-called hypoxic ventilatory response (HVR). It is known that patients with OSA have an elevated HVR compared to healthy individuals. In the first study of this thesis, we examined how the HVR is affected by residual effects of a neuromuscular blocking drug, rocuronium, in patients with untreated sleep apnea. We found that residual effects of rocuronium cause a reduction of the HVR by a third compared to a situation without the drug. Notably, the ventilatory response to increased levels of carbon dioxide was maintained, i.e. there seems to be a disturbance of the hypoxic control of ventilation. The same response is found in healthy volunteers when given a neuromuscular blocking drug in the same dose. This means that sleep apnea patients are just as vulnerable to hypoxia as healthy people when affected by a muscle relaxant drug and that they are not protected by the fact that they otherwise have an elevated HVR.

In the second study, we examined how well the STOP-Bang questionnaire identifies OSA in patients referred to a sleep clinic. The questionnaire consists of eight yes/no questions. In the surgical population, it has previously been shown that a score of three or more yes answers indicate a high risk of OSA. We found that six or more yes answers gave a sensitivity of 91% of having at least moderate OSA and that less than two yes answers can rule out at least moderate OSA with a 95% probability in sleep clinic patients.

Sleep apnea is considered a low-grade chronic inflammatory disease caused by repeated apneas and micro-awakening during sleep. We therefore investigated whether the genetic expression, measured as mRNA in whole blood, as well as inflammatory biomarkers in the blood, were affected in patients with untreated OSA and if any changes occurred after three and/or twelve months of CPAP treatment. In the third study, we were able to show that untreated patients with OSA have a down-regulated immune-related gene expression compared to matched controls. After three months of CPAP treatment, the gene expression was similar to what matched controls show. Surprisingly, gene expression returned to the untreated state after twelve months of CPAP treatment.

In the fourth study, we found that six inflammatory biomarkers changed after three and/or twelve months of CPAP treatment. The inflammatory biomarkers caspase 8 and glia cell-line derived neurotrophic factor were downregulated, while monocyte chemoattractant protein 1, fibroblast growth factor 21, neutrophils and the neutrophil-to-lymphocyte ratio were upregulated. These changes occurred mostly after twelve months of CPAP treatment. No inflammatory biomarkers were altered in the untreated sleep apnea patients compared to matched controls.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Obstruktiv sömnapné (OSA) är vanligt förekommande, men de allra flesta är odiagnostiserade. Patienter med OSA har en ökad risk för komplikationer under anestesi och kirurgi. Många av komplikationerna är kopplade till andningen och övre luftvägen, men även ett ökat intensivvårdsbehov och en förlängd sjukhusvård förekommer. Vid OSA faller de övre andningsvägarna samman upprepat under sömn, vilket i sin tur medför sjunkande syrgasmättnad i blodet, korta uppvaknanden och en aktivering av det sympatiska nervsystemet. De vanligaste symptomen är uttalad dagtrötthet, snarkning och andningsuppehåll. De flesta får en så kallad continuous positive airway pressure (CPAP)-behandling. Luft med ett övertryck leverans via en ansikts- eller näsmask och på detta sätt hålls luftvägarna öppna och andningsuppehållen försvinner. Personer med OSA har ofta även andra sjukdomar, som tex övervikt/fetma, högt blodtryck och sockersjuka.

Vid akut syrebrist är kroppens naturliga försvarsreaktion att omedelbart öka andningen, vilket kallas för den hypoxiska ventilatoriska reaktionen (HVR). Man vet sedan tidigare att patienter med OSA har en förhöjd HVR jämfört med friska kontrollpersoner. I avhandlingens första studie undersöktes hur HVR påverkas av resteffekter av ett neuromuskulärt blockerande läkemedel, rocuronium, hos personer med obehandlad sömnapné. Vi fann att resteffekter av rocuronium orsakar en minskning av HVR med en tredjedel jämfört med när man inte har läkemedlet i kroppen. Noteras bör att andningssvaret på en ökad mängd inandad koldioxid var oförändrat vilket innebär att muskelkraften att andas inte var påverkad men sannolikt andningsregleringen vid syrebrist. Friska försökspersoner har ett likartat svar vid motsvarande försök. Detta betyder att sömnapnépatienter är lika sårbara vid en eventuell syrebrist som friska personer vid samtidig påverkan av neuromuskulärt blockerande läkemedel och att de därmed inte är skyddade av att de annars har ett förhöjt HVR svar.

I den andra studien studerades hur väl STOP-Bang frågeformuläret identifierar OSA hos patienter remitterade till en sömnklinik. Frågeformuläret består av åtta ja/nej frågor och hos kirurgiska patienter har man tidigare funnit att vid tre eller fler ja-svar, har man en ökad risk för sömnapné. Vi fann hos patienter på en sömnklinik att sex eller flera ja-svar har en känslighet på 91% för minst måttligt svår OSA samt att färre än två ja-svar medför att en minst måttlig OSA kan uteslutas med 95% sannolikhet.

Sömnapné betraktas som en kroniskt låggradig inflammatorisk sjukdom orsakad av upprepade andningsuppehållen samt mikrouppvaknande under sömn. Vi undersökte därför huruvida det genetiska uttrycket, mät som mRNA i helblod, samt inflammatoriska biomarkörer i blod var påverkade hos patienter med obehandlad OSA samt om detta påverkades av tre och/eller tolv månaders CPAP-behandling. I den tredje studien kunde vi påvisa att obehandlade patienter med OSA har ett nedreglerat immunologiskt genuttryck jämfört med matchade kontroller. Efter tre månaders CPAP-behandling påminner genuttrycket om det som matchade kontroller uppvisar. Något förvånande återgick genuttrycket till det obehandlade tillståndet efter tolv månaders CPAP-behandling.

I den fjärde studien fann vi att sex inflammatoriska biomarkörer förändrades efter tre och/eller tolv månaders CPAP-behandling. De inflammatoriska biomarkörerna caspase 8 och glia cell-line derived neurotrophic factor var nedreglerade, medan monocyte chemoattractant protein 1, fibroblast growth factor 21, neutrofiler och kvoten neutrofiler/lymfocyter var uppreglerade. Dessa förändringar skedde framför allt efter tolv månaders CPAP-behandling. Inga inflammatoriska biomarkörer var förändrade hos de obehandlade sömnapné patienterna jämfört med matchade kontroller.

ABSTRACT

Obstructive sleep apnea (OSA) is a common disorder, both in the general and surgical population. While there is a steadily increased awareness of the disorder both in the society as a whole and within health care, unfortunately, most individuals with OSA still go undiagnosed. The repeated upper airway obstructions causing hypoxias, microarousals and increased sympathetic activation do not only contribute to the classical symptoms of excessive daytime tiredness and nightly snoring but also to increased cardiovascular and metabolic comorbidity. Patients with OSA are found to have an increased risk for perioperative pulmonary and cardiovascular complications, but also increased risk for intensive unit care and prolonged hospital stay after surgery.

The aim of this thesis was to investigate the effect of partial neuromuscular blockade on the hypoxic ventilatory regulation in patients with OSA, to evaluate the STOP-Bang questionnaire in a sleep clinic population and to explore whole blood transcriptome and circulating inflammatory biomarkers in patients with OSA compared to matched controls and after three and twelve months of continuous positive airway pressure (CPAP) treatment.

It has previously been shown that the hypoxic ventilatory response (HVR) is reduced by a third during partial neuromuscular blockade in healthy volunteers and that sleep apnea patients have an increased HVR compared to healthy controls. We found that the HVR is reduced by 36% in untreated sleep apnea patients at a train-of-four ratio of 0.7, whilst the hypercapnic ventilatory response was unaffected.

The STOP-Bang questionnaire is designed as a simple screening tool to identify OSA in the surgical population. It consists of eight dichotomous (yes/no) questions, each yes giving one score. In the sleep clinic population, we found that the optimal cut-off for identifying OSA is a score of 5 and to identify at least moderate OSA is a score of 6. In addition, we also showed that a score of ≥6 has a sensitivity of 91% to detect moderate-to-severe OSA and that a score <2 can exclude moderate-to-severe OSA by 95%. There was a good correlation between the apnea-hypopnea index and the oxygen desaturation index.

Obstructive sleep apnea is considered a chronic low-grade inflammatory disease and together with increased sympathetic activation and oxidative stress may cause many of the associated comorbidities. To better understand the pathophysiology of the disease, there has been an intense search for biomarkers. We showed that untreated patients with OSA have a downregulation of immune-related genes, including light and heavy chain immunoglobulins and interferon-inducible genes compared to matched controls. However, after three months of CPAP treatment, the gene expression resembled that of the matched controls and finally, after twelve months of treatment, the gene expression returned to the initial untreated state. When exploring circulating inflammatory biomarkers we found that capase 8 and glia cell-line derived neurotrophic factor were downregulated and that monocyte chemoattractant protein 1, fibroblast growth factor 21, neutrophils and neutrophil-to-lymphocyte ratio was upregulated by 3 and/or 12 months CPAP treatment. No inflammatory biomarker was changed in untreated patients with OSA compared to matched controls. However, interleukin 1 alpha, c-reactive protein and erythrocyte sedimentation rate were increased in untreated sleep apnea patients compared to normal body mass index controls.

In conclusion, untreated patients with OSA are as vulnerable to acute hypoxia during partial neuromuscular block as healthy volunteers with a reduced HVR by one-third. They are not

protected by their typically increased HVR. The STOP-Bang questionnaire can be an effective screening tool in the sleep population, where nearly all patients with a score of ≥6 have at least moderate OSA and a score <2 almost excludes at least moderate OSA. For intermediate scoring (2-5) nightly pulse oximetry can add extra information. There is a difference in the genetic and protein molecular pattern in patients with OSA before and after CPAP treatment in the sense that changes in the transcriptome were found in the untreated state compared to matched controls but not in circulating inflammatory biomarkers. Howevere there was a normalisation of the genetic expression after three months of treatment and a return to the untreated state after twelve months whereas the changes of inflammatory biomarkers mainly appeared after 12 months of CPAP treatment.

LIST OF SCIENTIFIC PAPERS

I. **Hypoxic ventilatory response after rocuronium-induced partial neuromuscular blockade in men with obstructive sleep apnoea**
Eva Christensson, Anette Ebberyd, Anna Hårdemark Cedborg, Åse Lodenius, Åsa Österlund Modalen. Karl A Franklin, Lars I Eriksson, Malin Jonsson Fagerlund
Anaesthesia, 2020;75(3):338-347

II. **Can STOP-Bang and pulse oximetry dectect and exclude obstructive sleep apnea?**
Eva Christensson, Karl A Franklin, Carin Sahlin, Andreas Palm, Jan Ulfberg, Lars I Eriksson, Eva Lindberg, Eva Hagel, Malin Jonsson Fagerlund
Anestesia and Analgesia, 2018,127(3):736-743

III. **Whole blood gene expression signature in patients with obstructive sleep apnea and effect of continuous positive airway pressure treatment**
Eva Christensson*, Souren Mkrtchian*, Anette Ebberyd, Åsa Österlund Modalen, Karl A Franklin, Lars I Eriksson, Malin Jonsson Fagerlund
Respiratory Physiology and Neurobiology, 2021;294:103746

IV. **Effect of CPAP treatment on inflammatory biomarkers in patients with obstructive sleep apnea**
Eva Christensson, Souren Mkrtchian, Anette Ebberyd, Karl A Franklin, Lars I Eriksson, Malin Jonsson Fagerlund
Manuscript

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LIST OF ABBREVIATIONS

AHI	Apnea Hypopnea Index
ARNT	Artemin
ASA	American Society of Anesthesiologist
BMI	Body Mass Index
CASP-8	Caspase 8
CO_2	Carbon dioxide
CPAP	Continuous positive airway pressure
CRP	C-reactive protein
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
$ETCO_2$	End-tidal pressure of carbon dioxide
ETO_2	End-tidal pressure of oxygen
FGF21	Fibroblast growth factor 21
$FiCO_2$	Inspired fraction of carbon dioxide
FiO_2	Inspired fraction of oxygen
GABA	Gamma-aminobutyric acid
GDNF	Glia cell-line derived neurotrophic factor
HCVR	Hypercapnic ventilatory response
HVR	Hypoxic ventilatory response
IL	Interleukin
MAC	Minimal alveolar concentration
MCP-1	Monocyte chemoattractant protein 1
mRNA	Messenger ribonucleic acid
NPX	Normalized Protein Expression
ODI	Oxygen desaturation Index
OSA	Obstructive sleep apnea
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
SpO_2	Peripheral oxygen saturation
TOF	Train-of-Four
TNF-α	Tumor necrosis factor alpha

1 INTRODUCTION

Life is pretty easy. Breathe in and breathe out, then repeat. – Author Unknown

Breathing is vital to mankind. It is essential to have stable and regular respiration and it is one of the very first questions asked in an emergency situation – is he or she breathing? This is also one of the first things we focus on at the very beginning and end of life, that is, at birth and death. Breathing is essential to provide adequate and continuous gas exchange and hereby the prerequisite for the supply of oxygen and removal of carbon dioxide from all body organs. Breathing is delicately governed and orchestrated by central mechanisms in order to adjust to various physiological states and metabolic needs, to ultimately maintain the homeostasis of oxygen and carbon dioxide.

Sleep provides the body with rest and is critical to many body functions. During tranquil sleep the metabolic rate is reduced, and energy is being restored. Lack of or disturbed sleep has a high impact on the brain and cognitive functions resulting in for example difficulties in learning and concentration. Sleep deprivation also has negative consequences on other vital organ functions, like the cardiovascular system, renal system and immune system. It may also cause microsleep, which is a brief moment of sleep that suddenly occurs during periods of wakefulness and that cannot be controlled.

Obstructive sleep apnea (OSA) a disease that involves both disturbed sleep and intermittent hypoxias and has during the last decade been identified as a major co-morbidity in the perioperative period causing an increased risk for postoperative pulmonary and cardiovascular complications, intensive care unit admittance and increased length of hospital stay [1-7].

This thesis is based on four studies that explore different aspects of OSA using a wide range of methods and techniques. The main goal has been to increase the pathophysiological knowledge of the disease with the ultimate goal to improve the perioperative outcome in these patients.

2 LITERATURE REVIEW

2.1 OBSTRUCTIVE SLEEP APNEA

Symptoms and consequences

Obstructive sleep apnea is a condition when the upper airway repeatedly, either completely or partially, obstructs during sleep with maintained respiratory movements. Even though there is the triad of classical symptoms; excessive daytime tiredness, loud snoring and observed apneas during sleep, symptoms are not always present, and the condition is unfortunately not well recognised by either the general population or by medical staff [8,9]. The severity of the disease can range from five to more than one hundred apneas or hypopneas per hour of sleep. These apneas and hypopneas cause hypoxia, arousals and an increased sympathetic activation which depending on the severity and length of untreated disease give rise to a variety of comorbidities.

Obstructive sleep apnea is an increasingly growing health problem with a reported increase in prevalence during the last decades. The prevalence is dependent on the population studied, the equipment used and the scoring model of hypopneas. Studies published before the year 2000 reported a lower prevalence of moderate-to-severe OSA of 3-17%, whereas modern studies have found a much higher prevalence of 25-50% [10-13]. The prevalence is dependent on both unmodifiable and modifiable risk factors. Unmodifiable risk factors are for example, being male even though menopausal women are close to the same prevalence as men, age (becoming older), race (black or Hispanic compared to white), genetics (approximately 40% of the variance in apnea-hypopnea index (AHI) may be explained by inherited factors) and craniofacial anatomical differences [14,15]. Obesity, smoking and medication decreasing musculature tone, for example sedatives and alcohol belong to the modifiable risk factors [16] and it has been shown that each percent change in weight may result in a three percent change of AHI [17]. On the basis of these observations and with a larger proportion of elderly and obesity within the general population, it is most likely that the prevalence of OSA will continue to increase. While there is a steadily increased awareness of the disorder both in the society as a whole and within health care, unfortunately, > 80% of individuals with OSA still go undiagnosed [8]. Patients with cardiovascular diseases, for example hypertension, atrial flutter, congestive heart failure coronary artery disease and stroke, have been shown to have an increased prevalence of OSA, 20-83% depending on which cardiovascular disease and AHI >5 or AHI >15 [15].

The repeated nocturnal chronic intermittent hypoxias and arousals do not “only” cause excess daytime tiredness, patients with OSA have been shown to have an increased risk of developing additional disease(s), in particular cardiovascular disease and metabolic disorders, but also cognitive decline, cancer, motor vehicle accidents and an increased mortality [18-22]. As many as 59-67% of patients with OSA, depending on the severity, also suffer from hypertension [23].

Diagnostics

For the diagnosis of OSA close patient monitoring of breathing and oxygenation during sleep is essential. Overnight polysomnography is at present the gold standard for sleep apnea investigations, and it is usually performed at a staffed laboratory of a sleep clinic. It

includes continuous recordings of electroencephalography, electrooculography, electromyography, body position, electrocardiography, pulse oximetry, airflow and thoracic and abdominal respiratory movements [24]. A full overnight polysomnography investigation is both rare and costly.

As sleep apnea is a common disorder, most investigations are therefore done with more simplified recordings at home, so-called home sleep apnea testing (Figure 1). These are mainly done without electroencephalography, electrooculography and electromyography for objective sleep scoring. Sleep time is instead estimated from respiratory recordings or from subjective measures of sleep. Sleep studies are often divided into four different types, where type I is a full overnight polysomnography, type II provides the same recordings as a full overnight polysomnography but is performed at home and is therefore not supervised by staff. Type III is the most common at-home recording with two respiratory variables, oxygen saturation and one cardiac variable and type IV is also an at-home device that measures only one or two variables, most commonly oxygen saturation and heart rate. The sensitivity and specificity for simplified sleep scoring including airflow, respiratory monitoring, pulse oximetry and body position is high [24,25].

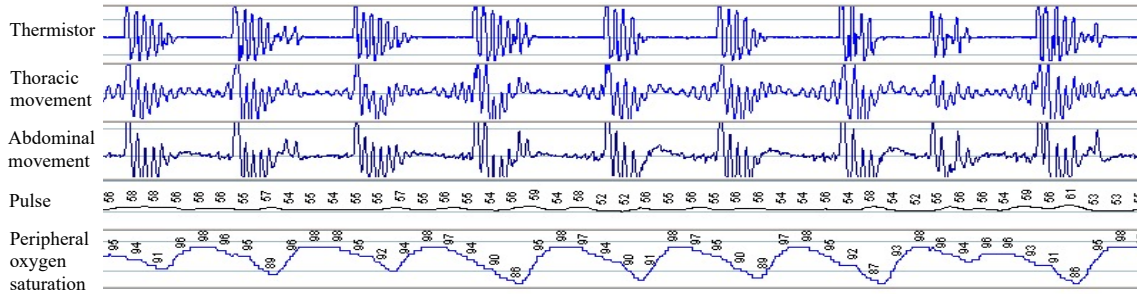

Figure 1: *Readings of a home sleep apnea test showing repeated apneas and desaturations with maintained respiratory efforts. The different channels have recorded airflow, thoracic and abdominal movement, pulse and peripheral oxygen saturation. The time course of the picture is 10 minutes.*

The apnea-hypopnea index is the average number of apneas or hypopneas per hour for one night's sleep (Table 1). An apnea is defined as a ≥90% ceased airflow from baseline lasting for ≥10 sec and a hypopnea is defined as ≥30 % reduction of airflow compared with baseline lasting ≥ 10 sec and with ≥3 % desaturation or an arousal according to the American Academy of Sleep Medicine [26]. However, according to an earlier definition of a hypopnea a ≥4% reduction in saturation was needed (in addition to ≥ 30% reduced airflow for at least 10 sec) [27]. The oxygen desaturation index (ODI) is defined as the average number of oxygen desaturations of ≥3% per hour compared to baseline [26]. The OSA syndrome (OSAS) is classified as an AHI >5 with associated symptoms such as daytime sleepiness, fatigue or impaired cognition or an AHI >15 regardless of associated symptoms [28].

Table 1: *Classification of OSA according to measured AHI value.*

	No OSA	**Mild OSA**	**Moderate OSA**	**Severe OSA**
AHI	0-4.9	5.0-14.9	15.0-30.0	>30

OSA=obstructive sleep apnea, AHI=apnea-hypopnea index

Treatment

Treatment of OSA can be divided into either lifestyle changes or interventions by means of medical devices or by surgery. Lifestyle and behavioural changes include weight reduction, physical exercise, reduced (or no) alcohol intake and sleeping in the lateral position [29]. All of these changes have the potential to reduce but rarely normalise the AHI. Continuous positive airway pressure (CPAP) devices are the first in line treatment option for patients with at least moderate OSA. It works by providing a positive pressure to the air being inspired and exhaled which prevents the airway to collapse during sleep and has been shown to normalise the AHI in >90% of the patients [30]. This minimizes the intermittent hypoxia and hypercapnia and gives the patients a better quality of sleep since microarousals in order to restore breathing diminishes. Oral appliance is more common in patients with mild-to-moderate OSA but can also be an alternative device if CPAP treatment fails. It works by the advancement of the mandible in relation to the maxilla and thereby prevents airway collapse. Surgical procedures, mainly uvulo-palatopharyngoplasty, are sometimes offered to patients with CPAP failure and have been shown to reduce, but not normalize AHI [31]. A relatively new surgical procedure is electrical therapy such as hypoglossal nerve stimulation, which increases pharyngeal dilator muscle activity during sleep and thereby keeps the upper airway open [32]. To date, there are no medical drugs for OSA registered.

Treatment of OSA, in particular with CPAP, has been shown to increase daytime wakefulness and quality of life, but also reduce both systolic and diastolic blood pressure [30]. A recent meta-analysis of randomized trials has not shown a reduction of either cardiovascular events or mortality in patients receiving CPAP treatment [30].

2.2 OBSTRUCTIVE SLEEP APNEA AND THE PERIOPERATIVE PERIOD

It is estimated that approximately 310 million people annually undergo surgery worldwide [33] and OSA is at least as common in the surgical population as it is in the general population. Approximately 22% of adults undergoing general non-upper airway surgery have been found to have OSA, of which >70% go undetected at the preoperative assessment [34] and as many as 70% of the morbidly obese patients undergoing bariatric surgery have OSA [35]. Needless to say, the clinical diagnosis of OSA is alarmingly often missed by physicians. Singh and colleagues found that in patients without a previous OSA diagnosis but with preoperative polysomnography showing a moderate-to-severe OSA, that the condition was missed 60% of the time by anaesthetists and 92% of the time by surgeons [9]. The same study also reported that in patients with pre-existing OSA diagnoses it was missed 15% of the time by anaesthetists and 58% of the time by surgeons [9].

Several studies have shown that OSA patients undergoing non-upper airway surgery have an increased risk for difficult intubation and postoperative complications, such as respiratory and cardiovascular complications, increased intensive care unit admissions and hospital length of stay [2,6,36-39]. The increasing numbers of patients with OSA have warranted international guidelines for safe perioperative management of this group of patients, however, there is still a lack of randomised control trials and therefore not all recommendations are evidence-based and some are based on expert opinion or retrospective studies [40-44].

Surgical patients with undiagnosed OSA may run an increased risk for serious postoperative complications, primarily related to the respiratory and circulatory systems. There is a current controversy as to whether or not patients with OSA will do better postoperatively if they have good compliance with their prescribed CPAP treatment and

whether or not postoperative CPAP treatment can improve outcomes. Guidelines by the Society of Anesthesia and Sleep Medicine do not recommend postponing elective surgery to initiate further evaluation or testing in patients with a high suspension of OSA unless there is also evidence of other uncontrolled comorbidities. They also recommend that CPAP results and settings should be obtained beforehand, and that CPAP therapy should be considered perioperatively in patients with diagnosed OSA and prior treatment [40]. Recent meta-analysis shows that although postoperative CPAP treatment in patients with OSA significantly reduces AHI, there was no significant decrease in postoperative complications in patients receiving CPAP compared to the control group. There was however, a trend towards a shorter length of hospital stay in the CPAP group [45,46].

2.3 PERIOPERATIVE SCREENING FOR OBSTRUCTIVE SLEEP APNEA

The most widely used screening tools in the perioperative setting are the Berlin questionnaire, the American Society of Anesthesiologists (ASA) checklist and the STOP-Bang questionnaire. The Berlin questionnaire was created in 1996 in Berlin, Germany for primary care [47]. The ASA checklist was published in 2006 and the American Society of Anesthesiologists recommends the routine screening of surgical populations with this checklist [48]. Both have later been validated in the surgical population [49]. However, both the Berlin questionnaire and the ASA checklist consist of many questions, three sections, of which two sections must turn out positive in order for the patient to be classified as having a high risk of OSA. These screening tests are therefore quite cumbersome to use. Recently, several new screening tools have been developed with the goal to simplify the scoring, for example the B-APNEIC score, the BOAH scale, the NoSAS score, the No-Apnea score and the GOAL questionnaire [50-54]. These different scoring systems are validated in sleep clinic populations and seem to have similar sensitivity and sensibility to at least moderate OSA or severe OSA as the STOP-Bang questionnaire.

2.4 STOP-BANG QUESTIONNAIRE

The STOP-Bang questionnaire is a screening tool that was originally designed to recognize patients scheduled for surgery with preoperatively undiagnosed OSA [55]. The tool is based on eight yes/no answer questions (**S**noring loudly, daytime **T**iredness, **O**bserved stop breathing, high blood **P**ressure, **B**ody mass index >35, **A**ge >50 years, **N**eck circumference >40 cm and male **G**ender). Each “yes” generates one score (Figure 2).

STOP-Bang questionnaire

Snoring

Do you snore loudly (louder than talking or loud enough to be heard through closed doors)?

Yes/No

Tired

Do you often feel tired or sleepy during daytime?

Yes/No

Observed

Has anyone observed you stop breathing during your sleep?

Yes/No

Blood Pressure

Do you have or are you being treated for high blood pressure?

Yes/No

BMI

BMI more than 35 kg/m^2

Yes/No

Age

Age over 50 years old?

Yes/No

***Neck* Circumference**

Neck Circumference greater than 40 cm

Yes/No

Gender

Male gender?

Yes/No

Figure 2: *The eight questions that constitute the STOP-Bang questionnaire*

A STOP-Bang score of ≥3 gives a high sensitivity, but rather low specificity for moderate (93% and 47% respectively) and severe (100% and 37% respectively) OSA [55]. This results in a fairly high false-positive rate. However, a score <3 helps to rule out patients having moderate-to-severe OSA. The STOP-Bang questionnaire is considered the most accurate and user-friendly screening tool available.

The above-mentioned study by Singh and co-workers demonstrate that in the patients with no prior diagnosis of OSA, but where polysomnography identified moderate-to-severe OSA, the STOP-Bang questionnaire would have classified the patient as high risk of OSA in 93% of the times when neither the anesthesiologist nor the surgeon clinically could identify the disease [9].

Recent meta-analyses have demonstrated that surgical patients with a high risk of OSA according to STOP-Bang have an increased risk for pulmonary and cardiac complications and prolonged hospital stay after surgery [56,57].

Because the STOP-Bang questionnaire is easy to use it has also found its way into the sleep clinic population. A recent meta-analysis found that 47 studies now have been performed in four different continents [58]. The overall (all four continents) sensitivity of a score ≥3 for OSA was 91% and the false-negative rate was 8%. As the score of STOP-Bang increased, so did the specificity. This can help sleep clinicians with their limited resources and long waiting lists to prioritize patients. Patients with a STOP-Bang <3 have a very little risk of suffering from moderate-to-severe OSA, whereas patients with STOP-Bang ≥5 have a high probability of severe OSA. In summary, STOP-Bang has a high sensitivity but a moderate specificity and therefore an improved screening questionnaire would be of value.

2.5 REGULATION OF BREATHING

Adequate breathing is essential for oxygen delivery and removal of carbon dioxide and is therefore strictly regulated. Breathing is regulated through a complex integration of central and peripheral neuronal information converging into respiratory neuronal circuits located in the brain stem [59]. In brief, each breath and the integration of voluntary and autonomic requirements of breathing is regulated by the central respiratory pattern generator, located in the medulla in the brain stem. The central pattern generator coordinates input from higher levels of the cerebrum (pons, hypothalamus and cortex), the chemoreceptors (central and peripheral), pulmonary stretch receptors, pharyngeal and laryngeal mechanoreceptors, vagal and other afferents. Breathing is also affected by wakefulness, emotions and temperature. Three different groups of motor neurons originating from the central pattern generator allow for the different requirements during breathing a) autonomic rhythmic inspiratory and expiratory output b) autonomic non-rhythmic control of breathing – like hiccups and sneezing, c) voluntary control of breathing – like speech and sniffing. Even though ventilation is mainly governed by the autonomic nervous system, it can (for some time) voluntarily be overridden by direct actions on the respiratory muscles.

Ventilation is modulated by the partial pressure of carbon dioxide in the blood and during hypoxia by the partial pressure of oxygen in the blood. The central chemoreceptors in the ventrolateral medulla rapidly respond to changes in cerebrospinal fluid hydrogen ion concentration (CSF-pH), causing immediate augmented ventilation. Carbon dioxide can diffuse across the blood-brain-barrier (unlike hydrogen ions) and is subsequently hydrolysed and ionized causing a decrease of the pH of the blood and cerebrospinal fluid.

The exact mechanism by which the central chemoreceptors sense a change of pH is not known.

The peripheral chemoreceptors are situated outside the blood-brain-barrier in the carotid bifurcation, the carotid body. The carotid body is the major oxygen sensor of the body. When exposed to hypoxia the oxygen sensing peripheral chemoreceptors, the carotid bodies immediately, within seconds, respond with increased minute ventilation. This is called the hypoxic ventilatory response (HVR). The carotid bodies contain oxygen-sensitive cells that increase their afferent input via the carotid sinus nerve and the glossopharyngeal nerve to the medulla to modulate ventilation during hypoxia [60]. The carotid bodies also have the ability to respond to an increase of carbon dioxide or a fall in their perfusion.

2.6 THE HYPOXIC AND HYPERCAPNIC VENTILATORY RESPONSE

The HVR is typically divided into three phases (Figure 3). The first phase being the acute hypoxic response, which is elicited within seconds and reaches a steady state in 5-10 minutes. The minute ventilation, both respiratory rate and tidal volume are rapidly increased, approximately two times the basic ventilation, when performed under isocapnia (maintaining expired carbon dioxide constant). There is a large inter-individual response to acute hypoxia and some people do not exhibit the acute HVR [61]. The acute HVR can also vary due to the circadian rhythm, gender, hormones (menstrual cycle) [62] and between days in the same individual [63]. The first acute part is followed by a second phase of decline in ventilation reaching a plateau after 20-30 minutes. Even though there is a decline in ventilation, it still is above the resting ventilation. This second phase is called the hypoxic ventilatory decline and is thought to be mediated by central gamma-aminobutyric acid (GABA) inhibition [64]. If hypoxia is still maintained during isocapnia, ventilation begins to increase even further during a third HVR phase that may last for several hours.

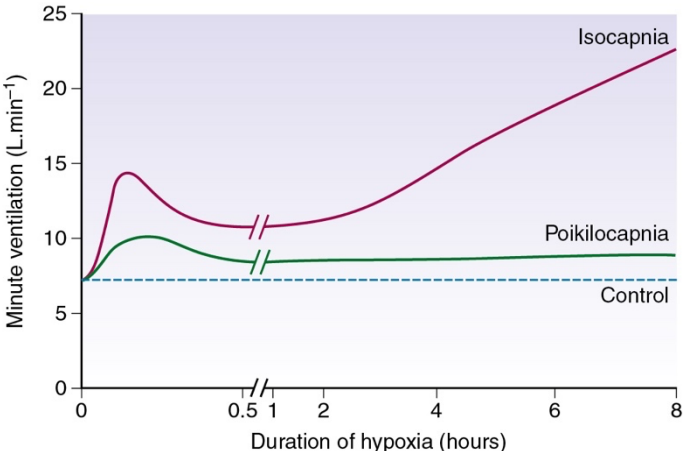

Figure 3: *The hypoxic ventilatory response during isocapnia and poikilocapnia. From Nunn`s Applied Respiratory Physiology, 7:th Edition, 2012, Copyright Elsevier [65]. Illustration used with permission from Elsevier.*

The hypercapnic ventilatory response (HCVR) causes an immediate increase of both tidal volume and respiratory rate when an individual is exposed to carbon dioxide due to stimulation of the central chemoreceptors in the medulla and a subsequently increased discharge in the phrenic nerve. Steady state is achieved after a few minutes, at this time point 75% of the maximum increase is reached. If the hypercarbia is still maintained ventilation is further increased for the next hour [66]. As with the HVR the HCVR is

influenced by circadian rhythm, hormones and within individuals. The slope of the HCVR curve steepens as the fraction of inspired oxygen decreases.

2.7 THE HYPOXIC AND HYPERCAPNIC VENTILATORY RESPONSE AND DRUGS USED IN ANESTHESIA

Many of the drugs used in anesthesia or sedation cause a respiratory depression, either by direct action on the respiratory centre or by interfering with the peripheral chemoreceptors.

It is shown that different classes of non-depolarising neuromuscular blocking agents reduce both the isocapnic and poikilocapnic HVR in healthy volunteers by approximately 30% at an adductor pollicis train- of-four (TOF) ratio of 0.70 [67-69]. A recent publication has shown that the HVR was still reduced by 18% after recovery to TOF ratio 1.0 and this was regardless of the partial neuromuscular blockade was reversed with a reversal drug or reversed spontaneously [70]. The reduction of HVR has been suggested to originate from inhibition of nicotinergic chemoreceptor neurotransmission within the carotid bodies and may be recovered by anticholinesterase or sugammadex reversal [71-75]. The HCVR has not been shown to be affected by partial neuromuscular blockade at TOF ratio 0.70 [67-69,76].

Propofol is a widely used drug in general anesthesia or for sedation that has its effects by binding to the $GABA_A$ receptors in the brain and potentiating the GABA effect. Propofol has also been shown to reduce the HVR in humans [77-80]. The mechanism seems to be through inhibition of neuronal nicotinic receptor transmission within the carotid bodies [81]. Propofol has also been shown to reduce the HCVR in humans and is believed to be mediated through central chemoreceptors [80,82].

A meta-analysis of the HVR and HCVR on different volatile anesthetics, with recordings taken from the carotid sinus nerve in cats or rabbits concluded that volatile anesthetics cause a moderate reduction of HVR, by 24%, at 0.75 minimal alveolar concentration (MAC), but that similar doses of volatiles, 0.81 MAC, did not cause any reduction of the HCVR [83]. Another review, of humans showed that subanesthetic doses of volatiles (<0.2 MAC) reduced the HVR by 44%. Halothane had the greatest reduction of HVR, followed by enflurane, isoflurane and finally sevoflurane [84].

Dexmedetomidine is a specific α2 receptor agonist mainly used for sedation in the intensive care setting. Dexmedetomidine has until recently been thought of as a drug not affecting breathing. Data from our group show that dexmedetomidine decreases the acute HVR by approximately 40% and to the same extent as propofol in healthy volunteers [80]. In addition, both propofol and dexmedetomidine reduce the HCVRin volunteers [77-80].

In summary, many of the drugs used in anesthesia impair ventilatory responses to hypoxia and hypercapnia in healthy volunteers.

2.8 THE HYPOXIC VENTILATORY RESPONSE AND OBSTRUCTIVE SLEEP APNEA

Most reports, from animal studies to experimental studies on healthy volunteers and patients with OSA indicate that the HVR is increased in patients with OSA [15,53,85-89]. Fung et al suggested that OSA and its chronic intermittent hypoxia directly, or indirectly via the induction of oxidative stress pathways, give rise to carotid body inflammatory response affecting the cytokine signalling pathways and subsequently increases the carotid chemoreceptor activity [90].

An increase of the HVR has also been demonstrated in patients with OSA [86,89]. Even though there are conflicting data in humans reporting both increase and decrease in HVR [85,86,89,91], the most stringent studies report an increase. Also, studies on healthy volunteers that were exposed to chronic intermittent hypoxia for four days had an increase in the acute HVR with a corresponding increase in reactive oxidative species [92]. Furthermore, treatment of OSA patients with one month of CPAP reduces (normalises) the response to the acute HVR [93].

Chronic intermittent hypoxia in animals has repeatedly demonstrated an increase in carotid body sensitivity to acute hypoxia [87,88]. Experiments in rats exposed to intermittent hypoxia have demonstrated an increase of long-term facilitation in the phrenic nerve, which was prevented by superoxide dismutase (an antioxidant). Also, an increase of the hypoxic but not the hypercapnic sensitivity of both the in vivo and in vitro carotid body was found, which was suppressed by antioxidant defense mechanism. However, this was reversed by normoxia. Even though there were functional changes of the carotid bodies there were no macroscopic morphological changes [61].

2.9 UNDERLYING MECHANISMS OF OBSTRUCTIVE SLEEP APNEA

During the last two decades, there has been a search for biomarkers for OSA. The purpose of finding a biomarker of OSA is screening, diagnostic, for evaluation of treatment and risk scoring in patients with OSA. The ideal biomarker should have a high sensitivity and specificity for the disease, have a clear cut-off between normal values and disease, correlate to the severity of the disease, reverse to treatment, be detectable before symptoms and foresee co-morbidities. In addition, it should also be inexpensive, easy to collect and painless.

There are several biological areas of interest regarding biomarkers in patients with OSA, in particular inflammation, oxidative stress and metabolism. This can give us an understanding of the underlying mechanisms of the disease, but also an understanding of subsequent complications, for example cardiovascular, atherosclerotic and metabolic events. However, one difficulty is that many of these biomarkers are also altered by obesity in itself, a comorbidity that many patients with OSA suffer from. A reduction of sleep time in healthy volunteers can also affect biomarkers that are of interest in patients with OSA [94,95]. In earlier studies of biomarkers for OSA this has not always been accounted for and thus the results might be influenced by both obesity and sleep deprivation.

In a meta-analysis, by De Luca Canto and co-workers, they found 141 published studies on biomarkers for well-defined OSA in children and adults up until March 2014. Notably, only nine studies of which five were in adults reported the specificity and sensitivity of the investigated biomarker(s) and were included in the meta-analysis. Biomarkers were mainly investigated in blood, but also in exhaled breath condensate and urine. The conclusion was that plasma interleukin (IL)-6 and IL-10 are promising candidates for becoming a good biomarker for OSA in adults [96].

There is a growing body of evidence that the repeated episodes of hypoxia and reoxygenation in OSA result in an increased level of oxidative stress in patients with OSA [97]. Oxidant factors or a reduction of antioxidant capacity can damage biomolecules, alter signalling pathways, activate inflammatory responses and cause cellular dysfunction or death. Many studies report an increase in different oxidative stress markers in untreated OSA patients and a reduction after CPAP treatment. Yet other studies have not been able to

demonstrate any differences after treatment. Several studies are relatively small and not all have matched controls. A biomarker that seems to present reproducible results is malondialdehyde, a product of lipid peroxidation. Several studies have demonstrated increased malondialdehyde in untreated OSA patients compared to matched healthy controls and it was reduced after CPAP treatment [98-101].

Obstructive sleep apnea-induced oxidative stress may give rise to inflammation. Many of the inflammatory biomarkers that have been investigated have in most studies been found to be affected in patients with OSA and normalisation has occurred after CPAP treatment. However, some studies have not observed any difference between OSA patients and matched (age, sex and body mass index (BMI)) healthy controls. A meta-analysis by Nadeem and co-workers have reported that the following inflammatory biomarkers: C-reactive protein (CRP), tumor necrosis factor-α (TNF- α), IL-6, IL-8, intercellular adhesion molecule, vascular cell adhesion molecule and selectins were significantly increased in OSA patients compared to controls and that they correlate to an increase in AHI. However, there was a modest but significant effect of age and BMI on all above-mentioned inflammatory biomarkers [102].

A summary of studies investigating potential biomarkers in patients with confirmed OSA by polysomnography or in-home monitoring has been made by De Luca Canto and co-workers [103]. Eighty-two studies were performed in adults, of which 58 found biomarkers considered as potential diagnostic biomarker(s). However, not all studies had a control group. The most commonly found potential diagnostic biomarkers were; IL-6, TNF- α, CRP, 8-isoprostane, high-sensitive CRP and intercellular adhesion molecule-1.

In summary, so far, no specific biomarker for OSA has been found since those described above are general markers of inflammation.

2.10 DIFFERENTIALLY EXPRESSED GENES IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA

In order to better understand the molecular pattern of OSA and maybe find an alternative way of diagnosing OSA there has been an interest in genetic signatures of patients with OSA the last decade. A recent systematic review of epigenetics in patients with OSA found a total of 65 publications, including both human and animal studies, on RNA expression/transcriptome in relation to OSA (of which 12 had published epigenetic data) [104]. An array of different methodologies has been used in these studies, for example PCR, qPCR, microarray and RNA-sequencing making it difficult to compare results. Also different selections of patients with OSA or healthy volunteers exposed to intermittent hypoxia have been used. In some studies, the sleep apnea patients are their own control, ie genetic studies before and after one night sleep, in other studies there is a comparison to healthy controls and occasionally to matched controls and in some studies the OSA patients are allowed to have other diseases than OSA. A few studies have analyzed the effect of CPAP treatment on the transcriptome in sleep apnea patients. Yet another difference is the tissue being analyzed. In human studies the most common is blood, but it can be subdivided between whole blood or specific blood cells. However, both adipose tissue, saliva and upper airway tissue have been used. A variety of different tissues have been used in animal studies.
Previous RNA sequencing studies in patients with OSA or healthy volunteers exposed to intermittent hypoxia have found genetic expression to be changed in many different biological pathways, like systemic and vascular inflammation, induction of apoptosis, neoplastic processes and cell communication and adhesion.

Altogether, despite numerous studies on gene expression in relation to OSA, the data is scattered and there is to date no clear picture of the changes taking place.

2.11 INFLAMMATORY BIOMARKERS IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA

There has been an intense search for biomarkers in the field of OSA for the last two decades. Searching the terms “biomarkers obstructive sleep apnea” in PubMed yields 1204 publications, of which 956 have been published in the last decade. In the field of OSA there has been a special focus on inflammatory, oxidative stress and metabolic biomarkers. But there has also been a search of biomarkers for specific co-morbidities in patients with OSA for example arteriosclerosis, cardiac or depression. Various fluids have been explored in the search for a specific OSA biomarker however, blood seems to have been the most dominant.

In the field of circulating inflammatory biomarkers CRP, IL-6 and TNF-α have been extensively studied however with conflicting results. Recently meta-analyses that are exclusively dedicated to one of the aforementioned biomarkers have been published.

IL-6 is generally considered as the prototypical proinflammatory cytokine with an array of biological functions, primarily related to the immune system, such as the induction of acute phase hepatic proteins, triggering of blood-borne B and T cells and inducing the production of immunoglobulins by B cells [105]. A recent meta-analysis by Imani et al including 37 studies shows that adults with OSA have an increased plasma and serum IL-6 compared to healthy controls [106]. They could also show that IL-6 increased with age [106]. However, no effect was seen after CPAP treatment in a meta-analysis of six randomised control trials [107].

CRP is an acute phase protein and is synthesised in the liver in response to IL-6. It is considered to be a more stable biomarker in the same individual under 24 hours compared to other cytokines [102]. Meta-analyses have shown that both CRP and high sensitive CRP are elevated in sleep apnea patients [108,109] and that both surgical treatment, in particular when managing to reduce AHI >20 events/hour and CPAP treatment reduces CRP levels [107,110,111]. Interestingly, in a meta-analysis by Imani et al including 96 studies of adults with OSA showed that the levels of plasma and serum high sensitive CRP and serum CRP were higher in comparison to healthy controls, but not in plasma CRP [112].

Tumor necrosis factor-α is a rapidly responding (within minutes) proinflammatory cytokine secreted by macrophages. It is also involved in physiological sleep regulation, in particular non-rapid eye movement sleep [113]. A meta-analysis of 50 publications found that TNF-α is increased in patients with OSA compared to healthy controls and that there is a correlation between TNF-α levels and severity of OSA [114]. CPAP therapy has not been shown to decrease TNF-α levels in a meta-analysis containing three randomised controlled trials[107]. However, meta-analysis by Xie et al including 12 studies found that CPAP therapy did reduce TNF-α [115].

3 RESEARCH AIMS

The overall aim of this thesis was to gain further knowledge about the regulation of breathing, the usability of the STOP-Bang questionnaire and molecular patterns in patients with OSA.

The specific aims were:

- To investigate the effect of a partial neuromuscular block on hypoxic and hypercapnic ventilatory responses in patients with untreated OSA.

- To investigate if the STOP-Bang questionnaire and/or oxygen desaturation index can be a useful screening tool in the sleep clinic population and to access the independent contribution of variables included in STOP-Bang.

- To investigate the gene expression signature in whole blood of untreated patients with moderate-to-severe OSA and after three and twelve months of CPAP treatment.

- To identify the temporal effect of CPAP treatment on inflammatory plasma biomarkers in patients with moderate-to-severe OSA, and the untreated condition compared to matched controls.

4 MATERIALS AND METHODS

4.1 ETHICAL CONSIDERATIONS

All studies were approved by the Regional Ethics Committee on Human Research at the Karolinska Institutet, Stockholm, Sweden and were conducted according to the standard of the Declaration of Helsinki and Good Clinical Practice. All study subjects were given both oral and written information regarding the study, thereafter all participants gave both oral and written consent.

The studies raised several ethical considerations. In Study I, a main concern and focus was to avoid overdosing of the muscle relaxant drug. Therefore, rocuronium was diluted to a low concentration of 0.5 mg/ml and continuous monitoring of the TOF was performed every 12 seconds. As even low doses of rocuronium can reduce the pharyngeal muscle tone the breathing tests were performed with a CPAP of 5 cmH_20 to assure an open airway throughout the experiment. If there were to be any signs of an obstructive airway the infusion of rocuronium was to be stopped and the experiment terminated. As with any drug administration there is always a potential risk of an allergenic reaction. For safety, the antidote sugammadex and anti-allergenic drugs were always available during the study along with a minimum of two medical doctors specialized in anesthesia and intensive care together with equipment needed for assisting ventilation or intubation.
During this study, the participants were alternately breathing a hypoxic or hypercarbic gas mixture for three minutes after the breathing pattern reached a steady state. As the study subjects were healthy apart from their OSA, these hypoxic or hypercarbic periods were not considered harmful to the study subjects. In addition, the study subjects had been desaturating to these levels every night because of their OSA, in most cases for many years. During the experiment, it was always possible to administer pure oxygen immediately, if necessary.

In Study II, the participants received the regular sleep apnea investigation with the addition of the STOP-Bang questionnaire. The participants were not considered to either benefit nor caused any harm by taking part of the study.

The same study subjects with OSA contributed to Study III and IV. Home sleep apnea testing was performed in each participant to either confirm OSA diagnosis or the absence of OSA. Seven matched or normal BMI controls who perceived that they were fully healthy received a sleep study showing that they had an AHI >5 and thus OSA. These individuals were referred to a medical doctor specialized in sleep medicine for further evaluation.

All patient data from the four studies were pseudonyminized according to ethical regulations in Sweden and are kept in a locked storage at the Clinical Research Unit at the department of Perioperative Medicine and Intensiv Care, Karolinska University Hospital, Stockholm, Sweden.

4.2 STUDY DESIGN AND OUTCOMES

Table 2: *Study design and outcomes for the four studies.*

	Study I	**Study II**	**Study III**	**Study IV**
Design	Prospective, interventional study	Prospective observational multicentred study	Prospective longitudinal observational study	Prospective longitudinal observational study
Study period	October 2012 - May 2014	November 2014 - January 2016	October 2013 - April 2017	October 2013 - April 2017
Total number of subjects included	10 patients with OSA	460 patients referred for OSA investigation	30 patients with OSA, 20 matched controls and 15 normal BMI controls	
Total number of subjects with complete results	8 patients at 0 months 4 patients at 3 months	449 patients	10 patients with OSA 19 matched controls 11 normal BMI controls	17 patients with OSA at 0, 3 and 12 months. 19 patients with OSA, 19 matched controls
Interventions	HVR-test HCVR-test Neuromuscular monitoring Home sleep apnea testing	STOP-Bang questionnaire Home sleep apnea testing	Blood samples for RNA-sequencing Home sleep apnea testing	Blood samples for circulatory biomarkers Home sleep apnea testing
Outcome	Effect of partial neuromuscular block on HVR and HCVR on patients with OSA before and after three months of CPAP treatment	Correlation between AHI vs STOP-Bang, AHI vs ODI and ODI vs STOP-Bang Optimal STOP-Bang cut-off scores	Peripheral whole blood gene expression in patients with OSA before and after 3 and 12 months of CPAP treatment	Change in inflammatory biomarkers in patients with OSA before and after 3 and 12 months of CPAP treatment
Analyses	Wilcoxon signed rank test	Spearman correlation coefficient Receiver operating characteristic (ROC) curves correlation Sensitivity Specificity Positive and negative predictive value	The fold change threshold was ≥±1.5, false discovery rate, ≤0.05. Heatmaps visualizing hierarchical cluster analyses of gene expression and principal component analyses plots	Repeated measure ANOVA and post hoc analyses using Bonferroni corrections Friedman´s test and post hoc analysis with Wilcoxon signed-rank test Independent sample t-test

OSA=Obstructive sleep apnea, BMI=Body mass index, HVR=Hypoxic ventilatory response, HCVR=Hypercapnic ventilatory response, RNA=Ribonucleotide acid, CPAP=Continuous positive airway pressure, AHI=Apnea-hypopnea index, ODI=Oxygen desaturation index.

4.3 PARTICIPANTS

The study subjects with OSA in Study I, III and IV were all recruited from Sweden's at the time, largest outpatient sleep clinic, Aleris FysiologLab in Stockholm. At this clinic approximately 100 first-time appointments were scheduled with the question at issue being obstructive sleep apnea. Despite the large number of new patients, it was difficult to find study subjects with no other medical condition apart from OSA and well-treated hypertension with no change in medication in the last three months. Five volunteers with OSA participated in Study I, III and IV.

The matched controls and the normal BMI controls to Study III and IV came from the Stockholm population.

In Study II the volunteers were consecutively included at their first visit at four different sleep clinics (Gävle, Umeå, Uppsala and Örebro) in Sweden.

4.4 HOME SLEEP APNEA TESTING

All home sleep apnea testing in patients with OSA was performed and scored manually by trained sleep physicians at the five different sleep clinics that took part in the different studies. Home sleep apnea testing of the matched and normal BMI controls were performed by Karolinska Institutet, and manually scored by trained staff. All home sleep apnea testing (Embletta®, Embla, Reykjavik, Iceland or NOX T3™, Nox Medical, Reykjavik, Iceland) provided a continuous recording of thoracic and abdominal movements, nasal airflow through a nasal cannula connected to a pressure transducer, peripheral saturation, pulse and body position through a built-in sensor and were performed during at least one night (Figure 4). An AHI and ODI were calculated from estimated total sleep time and these indexes were scored according to the current guidelines by the American Academy of Sleep Medicine [26,27]. In this thesis, Study I used the scoring rules of 2007 [27], whereas Study II, III and IV used those of 2012 [26].

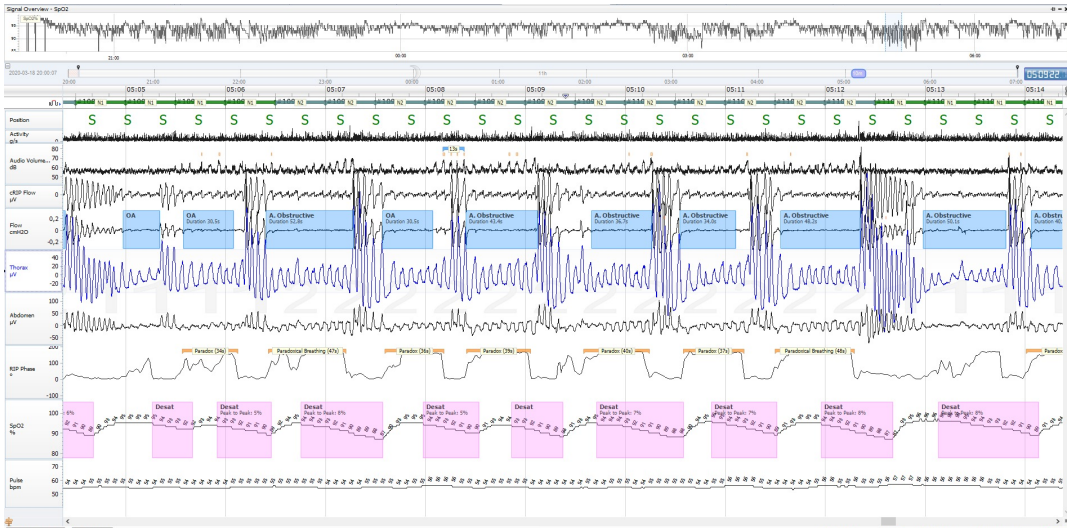

Figure 4: *Data obtained from a home sleep apnea test showing several obstructive apneas with subsequent desaturations and maintained breathing efforts, ie chest and abdominal movements. The most upper curve shows the peripheral oxygen saturation throughout the night. For the remaining picture, the time frame is 10 min and the obstructive sleep apneas are 20-52 sec long. The channel readings are from top to bottom: Position, Activity, Audio volume (snoring), Respiratory Inductance plethysmography (RIP) flow, Airflow, Thoracic and Abdominal movements, RIP phase, Peripheral oxygen saturation and Pulse.*

4.5 CONTINUOUS POSITIVE AIRWAY PRESSURE

All patients with OSA in Study I, III and IV received CPAP treatment. No sham CPAP was used in any of the studies. In Study I the experiment was repeated after three months of CPAP treatment, and in Study III and IV after three and twelve months of CPAP usage. Every participant with OSA received an auto-titrating home CPAP device (S9 AutoSet™, ResMed, Sydney, Australia) through the outpatient clinic and regular follow-up by the clinic was made to assure effective and comfortable treatment. Data of usage (hours per night and number of nights in use since the last reading) was collected from the CPAP software at regular intervals (ResScan™, ResMed, Sydney, Australia).

4.6 SPIROMETRY

A spirometer (D-lite™, Datex-Ohmeda AS/3TM GE Medical Systems, Madison, W1, USA) was used to measure inspiratory and expiratory tidal volumes just distal to the Y-piece in Study I. Using the principle of the Pitot tube, the total pressure and the static pressure over a known resistance with laminar flow was measured by the spirometer, giving a pressure difference. Inspiratory and expiratory airflow was determined every 40 milliseconds from the pressure difference and gas concentration information using the built-in computer calculations. Using the manufacture´s software (Datex-Ohmeda S/5™ Collect) sampling of airflow measurements was performed at 100Hz. Later, flow curves for each breath during the predefined three minutes interval of either HVR or HCVR were visually inspected, and inspiratory and expiratory tidal volumes were calculated through integration. Calibration of the breathing system was done before each experiment with a 500- and 1000-ml high precision calibration syringe and the accuracy of inspiratory volumes were found to be 96-99% of calibration volume and precision.

4.7 RESPIRATORY MOVEMENTS

Respiratory movements were assessed by monitoring thoracic and abdominal respiratory inductance plethysmography/impedance (Bio-Radio™, Great Lakes Neuro Technologies, Valley View, OH, USA) in Study I. The technology is based on magnetic fields and electricity generation. Soft belts with sinusoid wire coils are placed around the thorax and abdomen and an alternating current is passed through the wires creating a magnetic field. With inspiration and expiration, the belts expand or contract causing a fluctuation of the magnetic field, which creates an electric current opposite of the applied current and the change can be calculated.

4.8 THE HYPOXIC VENTILATORY RESPONSE TEST

In Study I, the hypoxic ventilatory response tests were performed at four occasions and always with its own control breathing test (Figure 5).

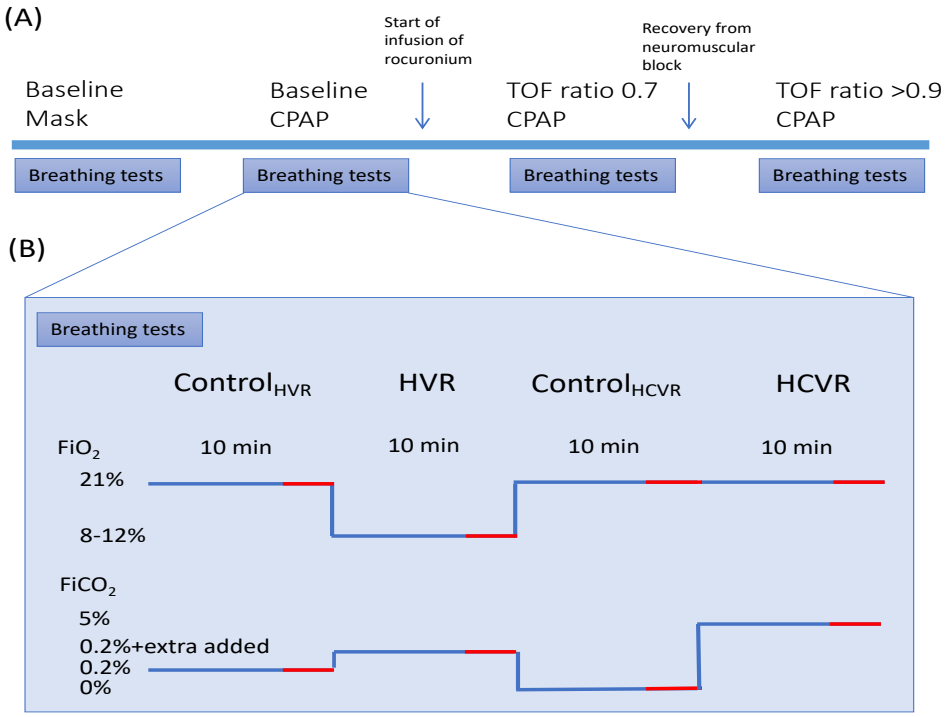

Figure 5: *Schematic overview of the test protocol. The uppermost timeline shows the different four occasions. The first was a baseline test with mask breathing, followed by three tests with CPAP for the remainder of the study. Within each of these four occasions was a set of breathing tests, First, there was resting ventilation at room air with the addition of 0.2% carbon dioxide, followed by isocapnic hypoxia (carbon dioxide was added to the inspirate to maintain the EtCO2), next air breathing and finally normoxic hypercapnia. Each breathing test was 10 min and recordings were made in the last 3 min during steady state (marked in red). FiO_2=Inspired fraction of oxygen, ETO_2=End-tidal pressure of oxygen, $FiCO_2$=Inspired fraction of carbon dioxide, $ETCO_2$=End-tidal pressure of carbon dioxide, SpO_2=Peripheral oxygen saturation, CO_2=Carbon dioxide, HVR=Hypoxic ventilatory response, HCVR=Hypercapnic ventilatory response, CPAP=Continuous positive airway pressure*

The first occasion was a baseline HVR recording with a normal facemask and the second HVR occasion was a baseline HVR recording with a CPAP of 5 cm H_2O. The CPAP was thereafter maintained at 5 cm of H_2O throughout the test period to ensure an open airway during the partial neuromuscular blockade. The third HVR test was performed at a partial neuromuscular blockade of TOF 0.7 and the fourth HVR test was performed after recovery at a TOF ≥0.9.

During resting control breathing test the volunteers were breathing room air with an inspired fraction of oxygen of 21%. The end tidal level of CO_2 was increased by 0.2% in order to stimulate background central drive. During the HVR tests the inspired fraction of oxygen was reduced to 8-12% by a step introduction of nitrogen to achieve a peripheral saturation of 80%. Additional CO_2 was manually titrated into the circuit by a microflow meter to maintain the same end tidal CO_2 level as during the control situation, ie isocapnia. After reaching a steady state increase in minute ventilation caused by the hypoxia, the hypoxic period was continued for three further minutes for data sampling. An average of the minute ventilation and peripheral saturation during the three minutes were calculated.

The HVR was defined as:

$$\text{HVR}\ (l \cdot \text{min}^{-1} \cdot \%^{-1}) = \frac{\text{VE hypoxia} - \text{VE control}}{\text{SpO}_2\ \text{control} - \text{SpO}_2\ \text{hypoxia}} = \frac{\Delta \text{VE}}{\Delta \text{SpO}_2}$$

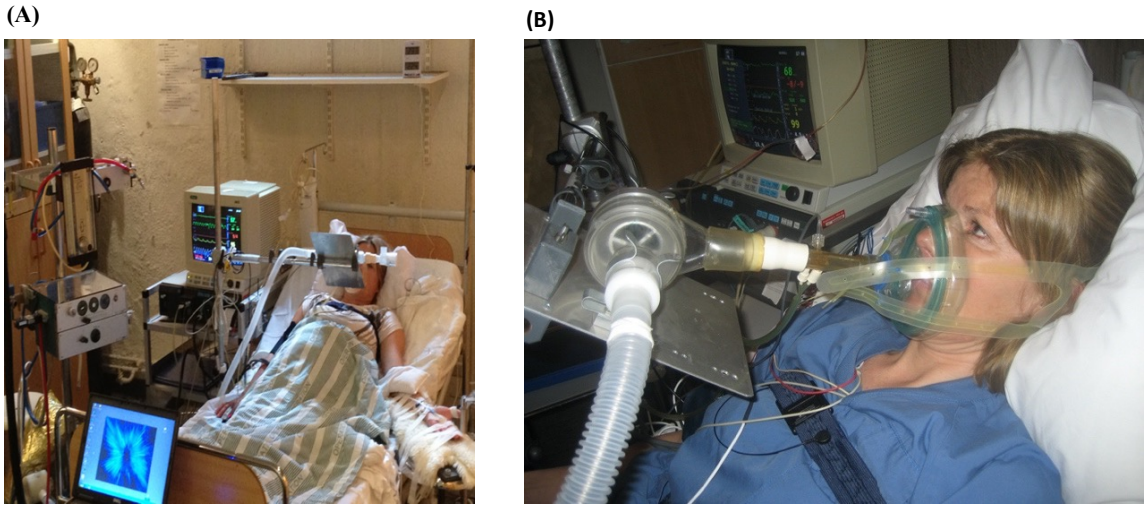

Figure 6: *The laboratory set-up for Study I (measurements of HVR and HCVR). (A) Datex-Ohmeda monitor with continuous perioperative monitoring (left corner). Engström open-breathing circuit (left). The thumb, hand and arm in place for neuromuscular monitoring (right). Collect software on the computer (at the end of the bed). (B)Volunteer breathing into a tightly fitted facemask.*

4.9 THE HYPERCAPNIC VENTILATORY RESPONSE TEST

After each HVR test the volunteers had a resting period of >10 min before starting a normoxic HCVR test. Each test started with a control breathing test where the volunteers were breathing room air with an inspired fraction of oxygen of 21%. During the normoxic HCVR test CO_2 was manually titrated into the breathing circuit through a microflow meter in order to achieve an inspired fraction of CO_2 of 5% in air. After reaching a steady state in the breathing pattern, recordings were made for three minutes. An average of the minute ventilation and end tidal carbon dioxide during the three minutes were calculated.

The HCVR was defined as:

$$\text{HCVR}\ (l \cdot \text{min}^{-1} \cdot \text{kPa}^{-1}) = \frac{\text{VE hypercapnia} - \text{VE control}}{\text{PETCO}_2\ \text{hypercapnia} - \text{PETCO}_2\ \text{control}} = \frac{\Delta \text{VE}}{\Delta \text{PETCO}_2}$$

4.10 MECHANOMYOGRAPHY RECORDINGS

Mechanomyography Myograph 2000® (Biometer, Odense, Denmark) was used to monitor adductor pollicis neuromuscular function by means of TOF response according to international guidelines [116]. Surface electrodes were placed after careful skin cleaning above the ulnar nerve distal to the wrist and connected to a Myotest® nerve stimulator (Biometer, Odense, Denmark). The forearm and hand, except the thumb, were immobilised with thick tape to a specially designed table with a metal bar on which the crotch of the thumb and forefinger rested. The thumb was placed in a ring with an adjustable preload set

at 0.25-0.3 kg. A temperature probe was placed in the palm of the hand for continuous monitoring and a heated pad was placed on the forearm in order to securely maintain a skin temperature above 32 °C. The Myotest® was set at 1 Hz, single-twitch stimulation and the current was slowly increased to 15-20% above maximal response in order to achieve supramaximal ulnar nerve stimulation. The stimulation was then changed to a TOF stimulation with 0.3 millisecond impulses at 2 Hz for 2 sec every 12 sec with maintained preload. Calibration of the settings were performed after 20 minutes of warmup. TOF stimulus pattern was maintained during the entire experiment.

4.11 STOP-BANG QUESTIONNAIRE

An evaluation of the STOP-Bang questionnaire in patients referred to sleep clinics for suspected OSA was performed in Study II. The questionnaire is based on eight acronyms (yes/no answer questions) - **S**noring loudly, daytime **T**iredness, **O**bserved stop breathing, high blood **P**ressure, **B**ody mass index >35, **A**ge >50 years, **N**eck circumference >40 cm and male **G**ender. Each "yes" generates one score. The patients answered the STOP questions whereas the staff filled in the Bang questions.

4.12 RNA SEQUENCING

Messenger RNA sequencing of whole blood was performed in Study III. Blood samples were collected into the specialised test tubes, PAXgene^{TM} blood RNA tubes (Qiagen, Hombrechtikon, Switzerland). Total RNA was extracted according to manufactures instructions. In order to maximise RNA purity, the samples were treated with DNase and thereafter RNA clean-up according to the manufactures protocol. RNA qualitative and quantitative control were performed by NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA) and Bioanalyzer (Bioanalyzer, Agilent). The RNA Integrity Number as measured by Bioanalyzer was 6.2 – 7.5. The RNA library and RNA sequencing were performed by the Science for Life Laboratory, Stockholm, Sweden. For the RNA library, ribosomal RNA and globin mRNA were removed using Illumina TruSeq® Stranded Total RNA Library Prep Globin (Illumina Inc., San Diego, California, USA). RNA sequencing, with a read length of 2x50 base pairs and > 40 million reads per sample, was performed by the NovaSeq6000 system (Illumina Inc., San Diego, California, USA). Quality check of the raw RNA sequencing data was done using MultiQC v.1.6 and normalisation and differential gene expression analysis were carried out with Bioconductor R software package edgeR [117] using Degust (http://victorian-bioinformatics-consortium.github.io/degust/). The cut-off margin for low expression genes was set at 0.5 counts per million.

4.13 QUANTITATIVE POLYMERASE CHAIN REACTION

In order to validate the results of the RNA sequencing five different genes and glyceraldehyde 3-phosphate dehydrogenase as the house-keeping reference gene were chosen for quantitative polymerase chain reaction (qPCR) analysis. Complementary DNA was synthesized by the SuperScript First-Strand Synthesis System for Real-Time PCR (Invitrogen, Eugene, OR, USA) and oligo (dT) primers. Amplification of the complementary DNA was done by Applied Biosystems 7500 Real-Time PCR System using the following TaqMan probes: interleukin 3 receptor, RNA component of mitochondrial RNA processing endoribonuclease, interferon alpha-inducible protein 27, interferon alpha-inducible protein 6, interferon-induced protein with tetratricopeptide repeats 1 and glyceraldehyde 3-phosphate dehydrogenase (Fisher Scientific, Gothenburg, Sweden). The relative abundance of each transcript was estimated according to the $2^{-\Delta\Delta Ct}$ method.

4.14 ROUTINE BIOCHEMISTRY RESULTS

All blood samples in Study IV were taken with the participants fasted during the morning at 7-9 AM. Test tubes for routine biochemistry analysis were immediately sent off for analysis to Karolinska University Laboratory, Karolinska University Hospital, Stockholm, Sweden for automatic biochemical analysis according to standard and accredited procedures at the hospital in a consecutive order. Twenty-seven different analyses were performed.

4.15 PROXIMITY EXTENSION ASSAY

Fasting blood samples were taken at 7-9 AM in lithium-heparin test tubes. The test tubes were immediately stored in slushed ice and within 30-60 min centrifugated at 1000xG at 4°C for ten minutes. Isolated plasma samples were stored at - 80°C for later analysis. Plasma samples were analyzed simultaneously on two plates using the proximity extension assay technology by Olink Proteomics AB (Uppsala, Sweden). The inflammation panel consisting of 92 different inflammatory proteins was chosen. The proximity extension assay technology uses 96 oligonucleotide antibody pairs containing unique DNA sequencing for each protein, which only allows hybridization with each other. This gives a double-stranded DNA molecule for each protein. These unique DNA reporter sequences are then amplified by real-time quantitative PCR. Any antibody cross-reactivity will not be detected as only matched DNA reporter sequences can be amplified. Data are given as normalized protein expression, NPX, which is an arbitrary unit in log 2 scale.

4.16 STATISTICS

For all studies
Continuous data were presented as mean and standard deviation or as median and interquartile range as appropriate. Ordinal data were presented as median and interquartile range. Nominal data were presented as frequencies and percentages. Statistical significance was set at 0.05. Power analyses were performed for all studies.

Study I
For the primary and secondary aims baseline HVR and HCVR with CPAP were compared with HVR and HCVR at TOF ratio 0.7 using Wilcoxon matched-pairs signed rank test. The same test, Wilcoxon matched-pairs signed rank test, was also used to compare baseline HVR and HCVR with ordinary facemask compared to baseline HVR and HCVR with CPAP. Data required to calculate HVR and HCVR, ie respiratory data, minute ventilation, peripheral saturation and expired fraction of CO_2 were averaged during three minutes of steady state ventilation in each of the four parts of the protocol. Statistical analysis and graphs were made using Prism 6.0 (GraphPad, Software Inc., La Jolla, CA, USA).

Study II
Spearman rank correlation coefficient was used to determine the monotonic correlation between AHI and STOP-Bang, ODI and STOP-Bang and AHI and ODI. Spearman rank correlation coefficient is a non-parametric test and was used as both AHI and ODI are skewed variables and STOP-Bang is an ordinal variable. Multiple logistic regression models were used to assess the individual contributions of each STOP-Bang variable for AHI ≥15 and AHI ≥5. Receiver operating curves were made and together with Youden´s index identified the optimal STOP-Bang score for AHI ≥15 and AHI ≥5.
Sensitivity, a test´s ability to correctly identify patients who do have the disease, and specificity, a test´s ability to correctly identify patients who are without the disease, was calculated for each STOP-Bang score. As sensitivity and specificity are not affected by the

prevalence of the disease, the positive predicted value and negative predicted values were also calculated for each STOP-Bang score. Graphs were made using SPSS software (version 23, IBM, Armonk, NY) and statistics were calculated using SPSS or R (Vienna, Austria).

Study III

Bioconductor R software package edgeR [117] using Degust (http://victorian-bioinformatics-consortium.github.io/degust/) was used for data normalization and differential gene expression analysis. For filtering out the low-expression genes the value of 0.5 counts per million was chosen as a cut-off margin. Fold change threshold was set at ≥±1.5 and false discovery rate, ≤0.05 for all the comparison groups. Two on-line tool - Enrichr (https://amp.pharm.mssm.edu/Enrichr/) [118] and David (david.ncifcrf.gov), v. 6.8 [119] were used for the gene ontology and pathway analysis. Qlucore Omics Explorer software 3.5 (Qlucore, Lund, Sweden) were used for heat map visualization of differentially expressed genes and principal component analysis plots. One-way ANOVA was used for qPCR data. Statistical analysis of qPCR data and graphs were made using GraphPad Prism (version 8.0, San Diego, CA, USA).

Study IV

For comparison of the three different timepoints of CPAP treatment repeated measure ANOVA with a post hoc Bonferroni correction was used for normally distributed data and Friedmans test with a post hoc Wilcoxon signed-rank test with a Bonferroni correction was used for skewed data. For comparison between two groups independent sample t-test or Mann-Whitney U was used for continuous data as appropriate. Statistics were calculated using SPSS version 24 (IBM, Armonk, USA).

5 RESULTS

5.1 STUDY I

In Study I we investigated the HVR and HCVR in untreated adult patients with OSA during partial neuromuscular blockade induced by rocuronium. Ten male patients with untreated OSA were recruited to the study with a mean±SD age of 52.2±15.0 years, BMI 29.9±2.8, AHI 30.0±13.6 and ODI 25.5±11.4. We demonstrated that the HVR was reduced by 32.2%±37.5% at a TOF ratio of 0.75, whilst the HCVR was unchanged at a TOF ratio of 0.70 (Figure 7). The HVR and HCVR after recovery (TOF ratio >0.90) were equipotent to baseline HVR and HCVR with CPAP. No difference was found in either baseline HVR or HCVR with a facemask compared to baseline measurements with CPAP.

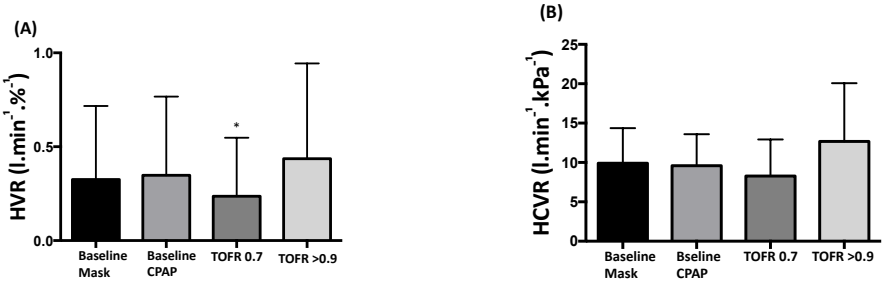

Figure 7: *Hypoxic and hypercapnic ventilatory responses during two baseline conditions, TOF ratio 0.7 and after recovery TOF ratio >0.9 in untreated patients with obstructive sleep apnea. (A) The hypoxic ventilatory response was reduced at TOF ratio 0.7, with n=8 for control and TOF ratio 0.75, and n=6 for TOF ratio >0.90. *= p=0.016 between baseline CPAP and TOF ratio 0.75. (B) The hypercapnic response was maintained, with n=7 for baseline and TOF ratio 0.70 and n=3 for TOF ratio >0.90. Data are analysed with Wilcoxon matched-pairs signed rank test and presented as mean±SD. HVR=Hypoxic ventilatory response, HCVR=Hypercapnic ventilatory response, CPAP=Continuous positive airway pressure, TOFR=Train-of-four ratio*

5.2 STUDY II

Study II was a prospective multicentre study where we evaluated the performance of the STOP-Bang questionnaire in the sleep clinic population and the impact of each item of the questionnaire. A total of 460 adult patients with suspected OSA at their first visit to a sleep clinic were enrolled in the study, of which 449 had complete STOP-Bang and AHI results. Their mean±SD age was 53.5±13.8 years, BMI 30.3±5.7 kg/m^2 and 61% were males. The median (interquartile range) AHI was 9.6 (3.3-23.9) and 39% had at least moderate OSA. We found that a STOP-Bang score of 6 or 5 for AHI ≥15 or AHI ≥5 respectively, maximized the trade-off between specificity and sensitivity according to receiver operating curves for STOP-Bang scores (Figure 8).

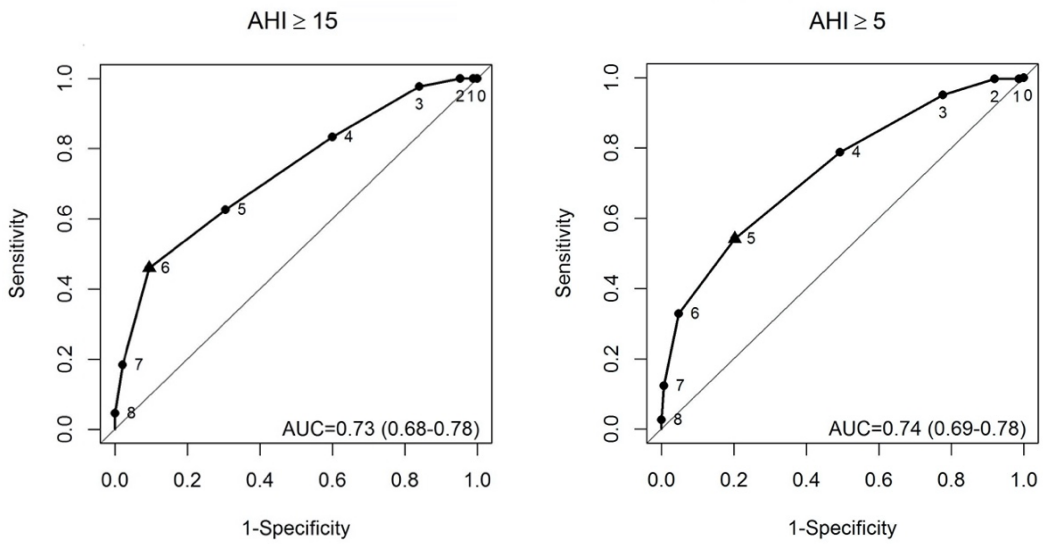

Figure 8: *Receiver operating curves for STOP-Bang scores and AHI ≥15 or AHI ≥5. Area under the curve was 0.73 for AHI ≥15 and 0.74 for AHI ≥5. AHI=Apnea-hypopnea index.*

In addition, we found a moderate positive correlation between AHI and STOP-Bang scores and a high positive correlation between AHI and ODI (Figure 9).

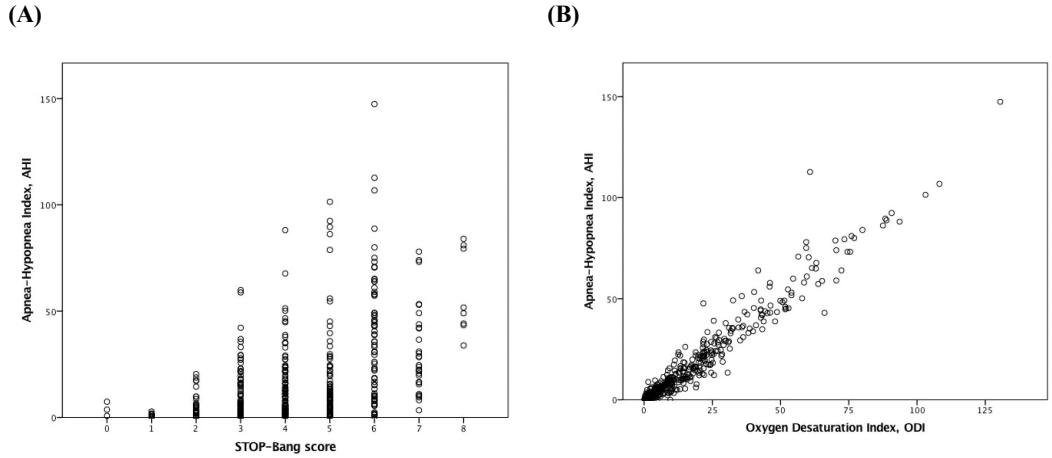

Figure 9: *(A) Correlations between AHI and STOP-Bang, Spearman rho of 0.50 (95% CI 0.43-0.58) and (B) Correlations between AHI and ODI, Spearman rho of 0.96 (95% CI 0.94-0.97). Each data point represents an individual patient. AHI=Apnea-hypopnea index, ODI=Oxygen desaturation Index*

An in-depth analysis revealed that a STOP-Bang score of 6-8 had a specificity of 91% (95% CI 0.87-0.94) for AHI ≥15 and a STOP-Bang score 0-1 had a probability of excluding an AHI ≥15 by 95% (95% CI 0.92-0.98).

Multiple logistic regression treating all STOP-Bang items as binary explanatory variables found that P (high blood pressure), B (BMI), A (age) and N (neck circumference) significantly contributed to an increased odds ratio both for an AHI ≥15 and AHI ≥5 (Table 3). O (observed apneas) also contributed significantly to an increased odds ratio for AHI ≥5.

Table 3: *Multiple logistic regression of the different items in the STOP-Bang questionnaire and their respective odds ratio and p-values for AHI ≥15 and AHI ≥5.*

STOP-Bang item	AHI ≥15 n=174 (39%)		AHI ≥5 n=301 (67%)	
	OR (95% CI)	P-value	OR (95% CI)	P-value
S - Snoring	1.32 (0.83-2.12)	0.24	1.41 (0.88-2.25)	0.16
T - Tiredness	1.52 (0.89-2.63)	0.13	1.12 (0.63-1.95)	0.70
O – Observed Apnea	1.43 (0.91-2.28)	0.13	1.75 (1.09-2.81)	0.02
P – Pressure (high BP)	2.95 (1.89-4.63)	<0.0001	2.45 (1.50-4.07)	<0.0001
B – BMI >35 kg/m^2	2.49 (1.43-4.36)	0.001	3.85 (1.90-8.44)	<0.0001
A – Age >50 years	3.12 (1.92-5.17)	<0.0001	3.28 (2.04-5.36)	<0.0001
N – Neck circumference >40 cm	2.06 (1.25-3.41)	0.005	2.29 (1.35-3.91)	0.002
G – Gender (male)	1.57 (0.92-2.69)	0.10	1.33 (0.78-2.28)	0.29

AHI= Apnea-hypopnea index, OR=Odds ratio, CI=Confidence interval, BP=Blood pressure, BMI=Body mass index

5.3 STUDY III

In Study III we investigated differential gene expression in whole blood and their associations with biological pathways in untreated adult patients with moderate-to-severe OSA and after three and twelve months of CPAP treatment compared to an individually BMI, age and sex matched control. A total of 30 patients with untreated OSA, 20 matched non-OSA controls and 15 non-OSA controls with a normal BMI were enrolled. The final analysis included 10 patients with OSA having RNA sequencing at the three time-points and good CPAP compliance throughout the year and 10 matched non-OSA controls. The median (interquartile range) AHI in patients with OSA at 0, 3 and 12 months was 22.5 (23.0), 2.1 (4.2) and 2.0 (3.1) respectively and in the matched controls 3.4 (2.3). There was no significant difference in neither age nor BMI at 0 months for the OSA patients and matched controls.

The main finding in this study was that the gene expression in untreated patients with OSA was changed compared that of matched controls but resembled that of the matched controls after three months of CPAP treatment, followed by a return of the gene expression to the untreated state after twelve months of CPAP treatment. This was shown both by principal component analysis and hierarchical clustering where two main clusters could be found – one containing matched controls and patients with OSA after three months of CPAP treatment and the other containing untreated patients with OSA and after twelve months of treatment (Figure 10). There was a down-regulation of immune related genes, light and heavy chain immunoglobulins and interferon-induced genes, in the untreated state.

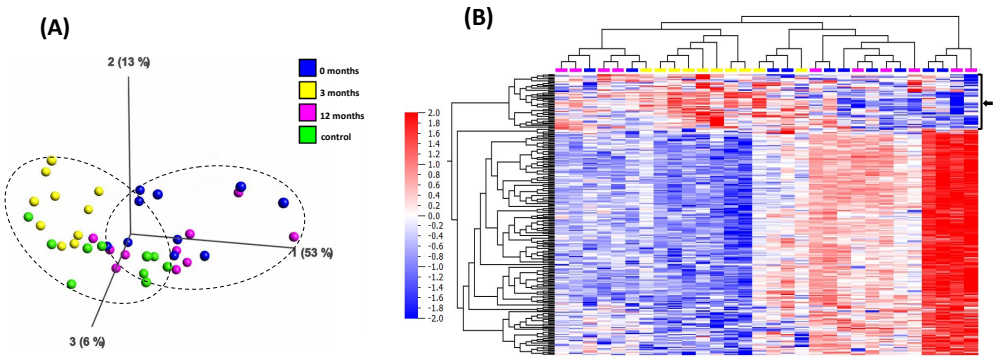

Figure 10: *(A) Principal component analysis of differentially expressed genes (gene counts per million) in 10 patients with OSA at 0, 3 and 12 months of CPAP treatment and 10 matched controls. Despite some heterogenicity, two distinct clusters are found. one containing matched controls and OSA patients after 3 months treatment and the other containing untreated patients and patients after 12 of treatment. (B) Heat maps of differentially expressed genes (based on fold change ≥±1.5 and false discovery rate ≤0.05) showing hierarchical clustering in 10 patients with OSA. Colour codes indicate red for up-regulated genes and blue for down-regulated genes. The arrow indicates a group of immunoglobulins and interferon-inducible genes.*

We also showed that the differential gene expression between BMI, age and sex matched controls and normal BMI controls are almost completely separated.

5.4 STUDY IV

The same subjects who participated in Study III (30 untreated patients with moderate-to-severe OSA receiving CPAP treatment, 20 BMI, age and sex matched controls and 15 normal BMI controls), also participated in Study IV. 17 patients with OSA had blood samples at all three time-points (0, 3 and 12 months) and a good reduction of AHI with CPAP treatment. There was a good CPAP usage throughout the year with a mean±SD 6.2±1.6 hours/night and 90.6±10.5 % of possible nights. We found that the circulatory inflammatory biomarkers caspase 8 (CASP-8) and glia cell-line derived neurotrophic factor (GDNF) were downregulated and monocyte chemoattractant protein 1 (MCP-1), fibroblastic growth factor 21 (FGF21), neutrophils and neutrophil-to-lymphocyte ratio were upregulated in patients with OSA after 3 and/or 12 months of CPAP treatment (Figure 11). However, most changes occurred after 12 months.

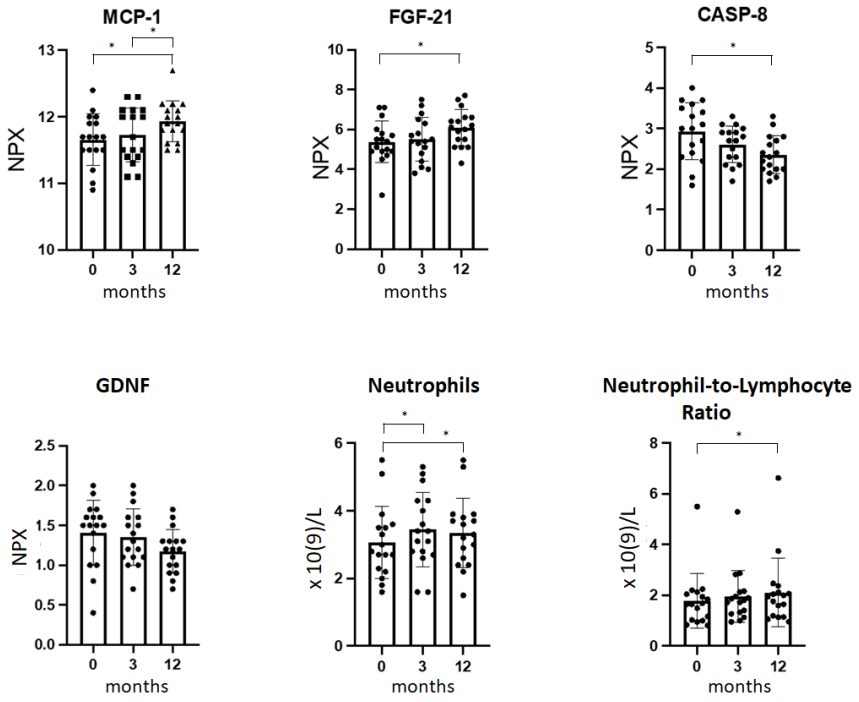

Figure 11: *Change in circulatory inflammatory biomarkers in 17 patients with obstructive sleep apnea after 0, 3 and 12 months of CPAP treatment. Data analysed with repeated measures ANOVA with post hoc Bonferroni corrections. * = p<0.05. MCP-1=Monocyte chemoattractant protein 1, FGF-21=Fibroblastic growth factor 21, CASP-8=Caspase 8, GDNF=Glia cell-line derived neurotrophic factor, NPX=Normalised protein expression, an arbitrary unit which is in log2 scale.*

There was no change in any of the 101 inflammatory biomarkers tested for when comparing 19 untreated patients with OSA and 19 matched controls, but there was a trend for an upregulation of artemin (ARTN) (p=0.05) (Figure 12). However, interleukin 1 alpha, c-reactive protein and erythrocyte sedimentation rate were increased in 19 untreated patients with OSA compared to 15 normal BMI controls (Figure 12).

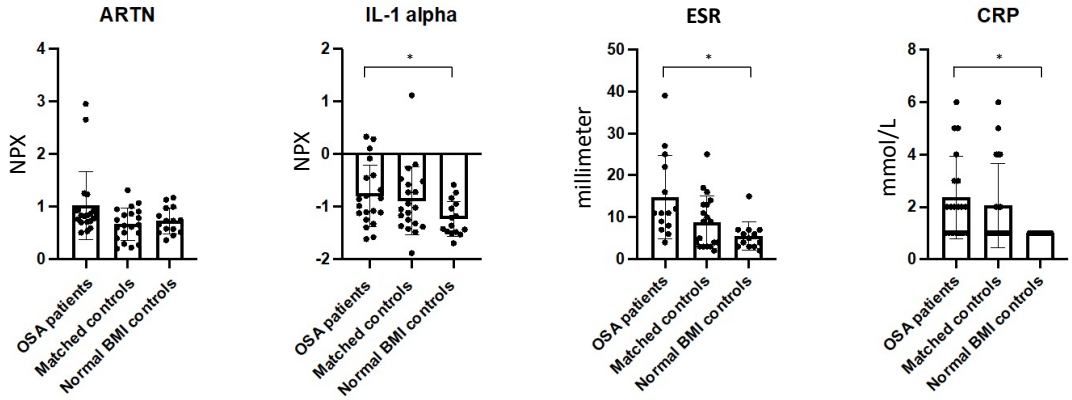

Figure 12: *Changes in circulating inflammatory biomarkers in 19 untreated patients with OSA, 19 BMI, age and sex matched controls and 15 normal BMI controls. Data analysed with Independent sample t-test. * = p<0.05. ARTN=Artemin, IL-1alpha=Interleukin 1 alpha, ESR=Erythrocyte sedimentation rate, CRP=C-reactive protein. NPX=Normalised protein expression, an arbitrary unit which is in log2 scale*

6 DISCUSSION

In this thesis it has been shown that the hypoxic ventilatory response is affected to the same extent by a partial neuromuscular blockade in untreated patients with OSA as in healthy individuals. The STOP-Bang questionnaire is a useful tool, not only in the surgical population but also in the sleep clinic population where a score of ≥6 has a sensitivity of 91% of detecting at least moderate OSA and a score <2 has a probability of excluding at least moderate OSA by 95%. We also show that there is a high correlation between AHI and ODI and therefore it could add valuable information in patients with a score of 3-5. Moreover, novel observations were made when examining the molecular pattern in OSA patients after 3 and 12 months of nocturnal CPAP treatment compared to BMI, age and sex matched controls. The whole blood transcriptome in untreated OSA patients revealed a down-regulation of immune-specific genes, light and heavy chain immunoglobulins compared to matched controls. This change decreased after 3 months of CPAP treatment and instead, the gene expression was reminiscent of that of the matched controls. Surprisingly, the gene expression returned to the untreated state after 12 months of continued treatment with good compliance. Six inflammatory biomarkers were found to be changed in patients with OSA after 3 and/or 12 months of CPAP treatment. MCP-1, FGF 21, neutrophils and neutrophil-to-lymphocyte ratio were upregulated, whereas CASP-8 and GDNF were downregulated. No change in inflammatory biomarkers was found when comparing untreated patients with OSA and matched controls. However, IL-1α, CRP and erythrocyte sedimentation rate were increased in untreated OSA patients compared to normal BMI controls.

As stated previously, patients with OSA seem to be at increased risk for postoperative pulmonary complications, in particular desaturation but also aspiration, pneumonia, ARDS and intubation/mechanical ventilation [2-5,120]. Even though OSA is a risk factor for postoperative pulmonary complications there were no studies on how drugs in anesthesia affect vital respiratory functions in these patients. In line with OSA patients, patients exposed to residual neuromuscular block in the post-anesthetic care unit are at increased risk for postoperative pulmonary complications [121]. Studies on patients with OSA have shown an increased HVR compared to healthy controls, although there are some studies showing conflicting results [85,86,89,91]. This enhancement of the HVR is also supported by studies where animals and humans have been exposed to intermittent hypoxia [87,88,92]. Interestingly, the HVR has been shown to decrease during sleep in both men and premenopausal women, in particular during REM sleep. However, the reduction of the HVR was more pronounced in men and in all stages of sleep, whereas in women the reduction was only found in REM sleep [122,123].

With this knowledge, it may be argued that patients with sleep apnea are protected or have a less pronounced decrease of the HVR during partial neuromuscular blockade since they have an increased HVR compared with healthy controls. On the other hand, it may also be argued that OSA patients are at increased risk of a further decrease in HVR (compared to healthy volunteers) during partial neuromuscular blockage due to their disturbed respiratory regulation. In Study I we showed for the first time that the HVR during partial neuromuscular blockade was reduced in untreated patients with OSA and that this reduction was very similar to that of healthy volunteers. Additionally, a recent systematic review found that patients with OSA are at higher risk of postoperative pulmonary complications when given neuromuscular blocking agents during surgery compared to non-OSA patients [124]. This means that at least untreated sleep apnea patients require vigilant observations of both the neuromuscular recovery and their respiratory function postoperatively when they

have received a neuromuscular blocking agent. We originally aimed to re-assess the HVR and HCVR during neuromuscular blockade in the same patients after three months of nightly CPAP treatment. As previous study by Spicuzza et al showed that HVR in patients with OSA decreased after one month of CPAP treatment [93], the question raised was whether this could lead to a further reduction in HVR during partial neuromuscular blockade in treated sleep apnea patients compared to the untreated state.
However, only four study subjects participated after three months CPAP treatment. Reasons given for dropping out were discontinuation of treatment or personal reasons. No statistical analysis was performed of the results obtained after three months CPAP treatment, but the HVR seemed to be reduced at the same magnitude as in the untreated state. Given the results by Spicuzza and the results that we received from the participants who returned after three months of CPAP treatment, I would recommend attentive postoperative management for all sleep apnea patients, regardless of whether they are on treatment or not.

Importantly, the HCVR in Study I was not affected by partial neuromuscular block and thus confirms previous observations in healthy individuals [69] which means that the decrease in HVR was not caused by a reduction in respiratory muscle function per se. The attenuated HVR is rather a direct effect of the acetylcholine-dependent nicotinic chemotransmission in the carotid bodies [71,72,74,75].

The respiratory tests during partial neuromuscular blockade were made with a CPAP of 5 cm H_2O to ensure an open airway throughout the experiment, as patients with OSA have been shown to have an instability of the respiratory control system [59]. In addition, these patients have a profound decrease of the pharyngeal dilator muscles activity during sleep (however, a compensatory increase during wakefulness) [125] and elevated P_{CRIT}, the pressure at which the airway collapse [126], which increases the likelihood of a further narrowed airway during partial neuromuscular blockade.

As we are aware of the increased perioperative risks in patients with OSA it is of most importance to identify these individuals before surgery in order to give optimal care with the goal to reduce complications. However, two major obstacles are encountered, the first one being the low awareness of OSA and the other is, in case of clinical suspicion of OSA, the long waiting lists and limited resources of most sleep clinics. To overcome this, different screening tools for predicting patients with OSA have been created. The screening tools have the advantage of being user-friendly, easily available and cheap. Ideally, the screening tool has a high sensitivity to rule out OSA and a sufficient specificity and is used for every patient at the pre-evaluation/anesthetic clinic scheduled for, at least, major surgery.

The STOP-Bang questionnaire was developed in 2008 by the anesthetist Frances Chung to identify OSA preoperatively in surgical patients [55]. It consists of eight yes/no questions, each yes creating one score. In Study II we evaluated the STOP-Bang score in the sleep clinic population. When the study was conducted, eleven studies had been performed on sleep clinic patients and the STOP-Bang questionnaire [127-136]. However, ten of these studies were performed on a small number of patients, the larger being retrospective and only two of the studies were performed in Europe. We therefore identified a need for a larger prospective European study of the STOP-Bang questionnaire in this population. Since then, there has been an intense progress in this field and a recent meta-analysis included 47 studies, including >26 000 participants from most continents [58].

There was a high prevalence of OSA in Study II, 67% for AHI ≥5 and 39% for AHI ≥15 in comparison to the prevalence of the general population [11]. However, the prevalence for any

severity of OSA was lower in our study, compared to what was found by Pivetta et al where both the general (worldwide) and European prevalence for AHI ≥5 was 80% [58] (Table 4). Although, the prevalence for at least moderate OSA was the same in both studies.

A STOP-Bang score of ≥3 generated nearly the same sensitivity, specificity, positive and negative predictive values for both any severity of OSA or moderate-to-severe OSA in the general (worldwide) and the European population, with a high sensitivity and moderately low specificity [58]. The sensitivity and specificity found in our study very much resemble the European results. Our positive and negative predicted value is respectively lower and higher compared to both the general and European values which reflects the lower prevalence in our cohort (Table 4).

Table 4: *Summary of sensitivity, specificity, PPN and NPV from our Swedish study and from a meta-analysis of 9 (AHI ≥5) or 10 (AHI ≥15) European studies and 45 (AHI ≥5) or 38 (AHI ≥15) studies performed in four continents.*

	Can STOP-Bang and pulse oximetry detect and exclude obstructive sleep apnea? Christensson et al, 2018 [137]		**Use and Performance of the STOP-Bang Questionnaire for Obstructive Sleep Apnea Screening Across Geographic Regions: A Systematic Review and Meta-Analysis Pivetta et al, 2021 [58]**			
	Sweden		Europe		General/world-wide	
	AHI ≥5	AHI ≥15	AHI ≥5	AHI ≥15	AHI ≥5	AHI ≥15
Sensitivity	95 (92-97)	98 (94-99)	95 (90-97)	97 (93-99)	92 (89-94)	95 (93-96)
Specificity	22 (16-30)	16 (12-21)	24 (13-39)	25 (14-40)	33 (26-41)	28 (22-34)
PPV	71 (67-76)	42 (38-47)	81 (80-82)	67 (66-69)	86 (85-86)	66 (65-66)
NPV	69 (54-81)	72 (80-98)	41 (36-46)	78 (75-81)	47 (45-49)	77 (75-78)

Data are presented as percentages and 95% confidence intervals. AHI=apnea-hypopnea index, PPV=positive predictive value and NPV=negative predictive value.

Our results regarding the increased probability of at least moderate OSA, but also decrease of sensitivity and increased specificity as the STOP-Bang score increases from 3 to 8 were also confirmed by the large meta-analyses [58]. This knowledge may help sleep clinicians prioritize patients.

In the original STOP-Bang study, a cut-off score of ≥3 was established for patients having a high risk for OSA [55], but with the trade-off of including many false positives. In our study we found that the optimal cut-off STOP-Bang score for detecting AHI ≥5 was 5 and for AHI ≥15 it was 6. This is in line with other studies. In a later and more in-depth analysis of the STOP-Bang score in a sleep patient population Chung et al conclude that a score of 0-2 can be regarded as a low risk and a score of 5-8 as a high risk of at least moderate OSA and the intermediate scores 3-4 further criteria should be assessed [138]. A Brazilian study found that the optimal STOP-Bang score was 3, 5 and 6 to identify mild, moderate and severe OSA respectively, and a Turkish study demonstrated that the optimal cut-off was 4 to identify any severity of OSA [139,140].

Recently, several new screening tools have been developed with the goal to either simplify the scoring or to increase the specificity, for example the B-APNEIC score, the BOAH scale, the NoSAS score, the No-Apnea score and the GOAL questionnaire [50-54]. These new

scoring systems are validated in sleep clinic populations and seem to have similar sensitivity and sensibility for at least moderate OSA or severe OSA as the STOP-Bang questionnaire. All have reduced the number of questions compared to the STOP-Bang questionnaire and the number of questions varies from two to five. In contrast to the STOP-Bang questionnaire where each yes generates one point, some of the questions in the new screening tools (for example BMI, age and neck circumference) are weighted and thus give different points depending on the answer. One item that is included in many of the different screening tools which is not measured "by default" during a medical visit is neck circumference. Neck circumference has been shown to have a large contribution in many tests, but thus requires extra work effort. The importance might not be which screening tool is used, as long as it has been validated, has a high sensitivity and a fair (or high as possible) specificity and the clinic is familiar with the tool and uses it regularly.

We could also demonstrate that a score ≥6 include nearly all patients with at least moderate OSA (sensitivity of 91% (95% CI 0.87.0.94)) and a score <2 almost excluded at least moderate OSA (probability of 95% (95% CI 0.92-0.98)) and a high correlation (Spearman rho of 0.96 (95% CI 0.94-0.97)) between AHI and ODI (see figure 9 in Results). With such a high correlation between AHI and ODI, a peripheral pulse oximetry could add additional information to patients scoring 2-5 whether they are at risk of having OSA. One might argue that since the peripheral oxygen saturation probe is much smaller compared to the tools needed for a home sleep apnea testing or a complete polysomnography the quality of sleep during such a registration is better and therefore has the potential to give a more correct value. The drawback of only monitoring the ODI is of course the lack of both respiratory movements/efforts and the confirmation of sleep and any arousals during sleep. Recent studies in both the surgical population and in patients with long-term opioid treatment for chronic pain show a high correlation between AHI and ODI [141,142].

The pathophysiology and the underlying molecular mechanisms of OSA is still unclear. Clinically OSA consists of several components and not all patients present with all of them, ie patients can present with different phenotypes. These components are for example repeated hypoxias which vary in both length and severity, sleep fragmentation, daytime tiredness, changes in intrathoracic pressures and increased sympathetic activation. There are also various underlying factor, endotypes, for OSA such as craniofacial and airway anatomy, pharyngeal dilator muscle activity, ventilatory control and arousal threshold [143]. In other words, there are several subgroups or factors to take into account when in search of, for example a biomarker. In addition, OSA has been shown to be closely linked to obesity and with it comes the challenge of distinguishing the different molecular patterns of these two conditions. [144,145]. It has been shown that approximately 70% of patients with OSA are obese [146]. Both OSA and obesity are known to cause inflammation [23,144]. Therefore, efforts should be made to make comparisons between sleep apnea patients and well-matched controls but also between obese patients with OSA and matched non-obese patients with OSA.

In Study III we showed that the gene expression of untreated patients with OSA differed to that of matched non-OSA controls with 105 genes being upregulated and 32 genes downregulated. The most striking pattern was found amongst the downregulated genes which contained several immune-related genes, mostly immunoglobulins – both light and heavy chains, but also interferon-inducing genes. The pattern of up and downregulated genes changed with CPAP treatment, showing a decrease of upregulated genes and an increase of downregulated genes at 3 months and then again, the reverse pattern at 12 months treatment. Both principal component analysis and hierarchical clustering with heatmaps revealed the same pattern with two different clusters – one containing sleep apnea

patients receiving 3 months of treatment and matched controls and the other cluster containing untreated patients and patients after 12 months of treatment.

There are an increasing number of publications on the genetic profile of patients with OSA. The field is continuously developing with many different methodologies which make comparisons between studies challenging. Many different tissues have been analysed, in humans for example blood (whole blood or peripheral blood mononuclear cells), adipose tissue (subcutaneous and visceral), saliva and upper airway tissue and in animals many different organ-specific tissues, for example brain and heart. Some have investigated specific genes of interest, whilst others have used microarray or complete sequencing. The assessment of microRNA and epigenetics have just recently begun to be explored [145,147].

Despite throughout search, no other publication has been found showing a differential expression of immune-related genes like immunoglobulins and interferon-induced genes on sleep apnea patients. An attempt to validate our mRNA results using the hospital's laboratory was performed with a small sample of untreated sleep apnea patients and matched controls to measure the levels of different subclasses of immunoglobulins however the results were inconclusive and the analysis available on different subclasses were not found to be sufficiently specific.

Inflammatory differential gene expression and pathways have been shown in whole blood leukocytes in patients with OSA. A recent publication by Turnbull et al found an upregulation of several NFkB signalling genes and inflammatory pathways after withdrawal of CPAP treatment onto sham (supplementary air) [148]. Interestingly in this study there was no change in gene expression after withdrawal of CPAP treatment onto supplementary oxygen (ODI was unaffected with supplementary oxygen compared with CPAP treatment) [148].

After 3 months of CPAP treatment the gene expression was similar to that of matched controls in our study. Only a few other studies have investigated changes in RNA sequencing after CPAP treatment. Perry et al, showed that 32 genes were differentially expressed in patients with severe OSA after 6 months of CPAP treatment compared to the untreated state and network analysis showed two functional networks - the first network included genes associated with cell death, cell cycle and cellular growth and the second included genes associated with embryonic and organ development [149]. A study by Gharib et al did not find any changes in gene expression in circulating leukocytes in patients with severe OSA after two weeks of CPAP treatment. However, gene set enrichment analysis and network analysis found downregulated expression patterns in cancer-related pathways [150]. In neither of these two studies were any comparisons made between CPAP treated sleep apnea patients and controls.

In our study, the transcriptome surprisingly returned to the untreated state after a year of good CPAP treatment. The CPAP compliance was not inferior at 12 months compared to 3 months. One possible mechanism to this could be an annual cycle of gene expression. This has previously been shown in whole blood, peripheral blood mononuclear cells and adipose tissue [151-153]. However, we do not believe that this is the reason. Half of the patients were recruited in the autumn and the other half was evenly distributed among the remaining nine months of the year. We have evaluated other explanations but so far none can explain the reversal after 12 months.

Historically many studies have used controls that differ in for example BMI and age, compared to the OSA individuals being studied. The matched and normal BMI controls in

our study had different BMI, but there was no difference in age or sex. Our data demonstrate the importance of choosing BMI-matched controls and not normal BMI controls as principal component analysis and hierarchical clustering of the differentially expressed genes showed an almost perfect separation between the two groups.

In Study IV we could not find any difference in circulatory inflammatory biomarkers in patients with untreated OSA compared to matched controls, but there was a tendency of an increase of ARNT in the untreated OSA patients. ARNT is a neurotrophic factor belonging to the GDNF family ligands and is involved with the development of both the central and peripheral nervous system and its differentiation [154]. In a recent thesis it was shown that ARNT was increased in untreated patients with OSA compared to controls and this increase was persisted after multivariate adjustment [155] which is in line with our results.

C-reactive protein, erythrocyte sedimentation rate and IL-1α were increased in the untreated OSA patients compared to normal BMI controls. These findings may reflect the importance of choosing matched controls and not only healthy controls. Obesity as well as OSA is found to be a disease with low chronic inflammation and has been shown to have elevated CRP compared to controls [156-158]. But it can also reflect the importance of choosing which medium (serum or plasma) the biomarker should be analysed in. A meta-analysis by Imani et al showed that both serum and plasma high sensitive CRP and serum CRP was elevated in untreated patients with OSA compared to healthy controls, but not plasma CRP [112]. In our study we analysed plasma CRP.

Six different circulatory inflammatory biomarkers were found to be affected by nightly CPAP treatment. Four biomarkers, MCP-1, FGF 21, neutrophils and neutrophil-to-lymphocyte ratio were upregulated, whereas CASP-8 and GDNF were downregulated. Most changes occurred after 12 months of CPAP treatment, only for neutrophils did a change occur after 3 months.

The upregulated inflammatory biomarkers (MCP-1, FGF 21, neutrophils and neutrophil-to-lymphocyte ratio) have previously been found to be changed in patients with OSA compared to healthy controls or being affected by CPAP treatment, but not the downregulated biomarkers (CASP-8 and GDNF).

MCP-1 is a chemokine (also known as CCL2) mainly derived from monocytes and macrophages, but is expressed by a wide range of cell types. Through chemotaxis MCP-1 directs the migration and infiltration of monocytes, microglia, natural killer cells and memory T lymphocytes towards injury and inflammation. Several studies have shown that increased levels of MCP-1 is associated with oxidative stress but also with arteriosclerotic disease [159,160]. It has been shown that MCP-1is increased after one night's sleep in patients with OSA compared to non-OSA, but also that the evening MCP-1 was increased in severe OSA compared to both patients with mild-moderate OSA and non-OSA [161]. It has also been demonstrated that serum MCP-1 is reduced in patients with OSA after 3 months of CPAP treatment but remained unaltered in a group of patients with OSA that used subtherapeutic CPAP treatment [162]. This contrasts with our findings, where MCP-1 increased with CPAP treatment.

FGF21 is a hormone that regulates metabolism by reducing blood sugar, lipids and improving insulin resistance, but also affects the heart, bones and brain. Normally the level of FGF21 is low in humans, but it is found to be increased in obese subjects, patients with atherosclerotic disease or metabolic syndrome. Lately FGF21 has also been shown to inhibit inflammation, reduce oxidative stress and apoptosis of cardiomyocytes and

endothelial cells [163]. In our study, FGF21 increased after CPAP treatment which is in line with a previous study that showed that patients with OSA and type II diabetes mellitus had a lower serum FGF21 than non-OSA patients with type II diabetes mellitus and that it was negatively correlated to AHI and ODI [164].

Neutrophils are a part of the innate immune response and are rapidly mobilised to sites of inflammation. Patients with at least moderate OSA have been shown to have reduced apoptosis of neutrophils and increased expression of adhesion molecules and this was reversed by CPAP [165]. Two large studies have found that neutrophils are increased in patients with OSA compared to controls and that it is correlated with AHI [166,167]. The results of these two large studies are not in agreement with our study. However, in the study by Giovanini [167] severe OSA patients were compared with non-to-moderate OSA patients and the severe OSA patients had significantly more diabetes mellitus and in the study by Fan [166] only males were included and there was a significant difference of BMI between OSA patients and controls.

Data on neutrophil-to-lymphocyte ratio and patients with OSA have been inconsistent, however a recent metanalysis on neutrophil-to-lymphocyte ratio found a significant difference between patients with OSA and controls and the difference in neutrophil-to-lymphocyte ratio increased gradually with OSA severity [168]. In addition, that metanalysis also concluded that there was no significant difference in neutrophil-to-lymphocyte ratio before and after CPAP treatment [168] which contrasts with our findings.

Caspase 8 is the principle initiator of the extrinsic receptor-mediated apoptotic pathway, but is also found to mediate in the intrinsic pathway. Moreover, CASP-8 has an important function in inhibiting necroptosis and thereby protects against inflammation [169]. Although no prior reports of CASP-8 and patients with OSA has been found, previous studies have shown that exposure to intermittent hypoxia causes an increase of CASP-8 in rat hippocampus and heart cells [170,171] which is in agreement with our findings.

Glia cell-line derived neurotrophic factor is synthesised and secreted by many cell types including astrocytes, oligodendrocytes, Schwann cells, motor cells and skeletal muscle cells. It mainly affects dopamine and sympathetic neurons, but also has an important effect upon the peripheral nervous system [172]. Neither have there been any reports on the GDNF protein in patients with OSA. A study based on participants from the Cleveland Family Study [173] showed that single nucleotide polymorphism of GNDF was associated with OSA in European Americans and it persisted after adjustment of BMI. This finding could not be confirmed in a large Icelandic OSA cohort [174]. A recent study found that the GDNF gene was downregulated in patients with OSA compared to controls in peripheral blood mononuclear cells and that one of the key hub genes in adipose tissue was GDNF [175]. According to Larkins et al variations of GDNF could be associated with OSA via the influence of abnormal ventilatory control, impaired arousal response or contribute an imbalanced activation of airway muscle tone relative to that of the chest wall. GDNF plays a major role in the development of neural pathways vital for normal respiration, the development of noradrenergic neurons which play a vital role in normal respiration, and it might influence the hypoxic ventilatory response as it seems to have a trophic role for sensory afferent neurons in the carotid body. Additionally knockout of GDNF gene results in abnormal central breathing and several mutations in GDNF are associated with congenital hypoventilation syndrome. [173]

In summary, we found that six inflammatory biomarkers were affected by CPAP treatment and this effect mainly came after 12 months of treatment. It was a surprise that there was no

differential expression of the inflammatory biomarkers in sleep apnea patients compared to matched controls.

6.1 METHODOLOGICAL CONSIDERATIONS

There have been several methodological considerations during the work with the four studies included in the theses. Some of which will be discussed in more detail below.

In all four studies home sleep apnea testing has been performed in both of the untreated patients with sleep apnea at the different sleep clinics and of matched or normal BMI controls in order to objectively confirm or exclude OSA diagnosis. Every home sleep apnea testing have been manually scored by trained staff and assessed in accordance to international guidelines [26]. While polysomnography is still considered gold standard when diagnosing OSA, home sleep apnea testing have been shown to have a good correlation to laboratory polysomnography [176,177] and in Europe the majority (67%) of diagnostic testing are performed with cardiorespiratory polygraphy [178].

As partial neuromuscular blockade has been shown to decrease inspiratory upper airway dimensions and function in healthy volunteers without causing respiratory symptoms [179] and patients with OSA have an inherent tendency to obstruct the airway we decided to perform the ventilatory tests in Study I with the addition of a CPAP of 5 cmH_2O as an extra measure of safety. In addition, we also had continuous measures of both airflow, thoracic and abdominal movements for close monitoring, if any obstructions were to happen. We did observe some obstructions during the experiment with untreated OSA patients before the start of the neuromuscular blocking agent (rocuronuim), however no recording was made during these obstructions. All obstructions ceased spontaneously or upon verbal stimulation within a short period of time (seconds). An interesting observation is that these spontaneous obstructions were no longer present after three months of CPAP treatment. We originally planned to study the HVR in patients with OSA during dexmedetomidine-induced sedation. However, as the majority of healthy, normal BMI volunteers showed obstructions during sedation with the drug this planned study was postponed [80].

The peripheral chemosensitivity was measured by the HVR test. This test can be performed in different ways. Currently two different techniques are used, either the progressive (ramp) or step technique. The main difference is how long it takes to reach the desired inspired fraction of oxygen, but also the depth and duration of hypoxia and how isocapnia is maintained. During the ramp technique rebreathing from a circuit/bag cause a gradual decrease of the inspired fraction of oxygen over a period of 5-15 minutes controlling the carbon dioxide with an absorber. Whilst during the step technique the inspiratory fraction of oxygen is momentarily changed either with computer-controlled dynamic end-tidal forcing or manually. Isocapnia is maintained by adding carbon dioxide into the inspired gases. In Study I we used the step technique and manually added nitrogen or carbon dioxide to the desired concentrations.

There was a slight difference in the achieved TOF ratio between the HVR and HCVR tests. TOF ratio during HVR was 0.75 and during HCVR 0.70. This difference is explained by the time differences between the tests and small adjustments of the rocuronium infusion that had to be done during the experiment even when a steady state was perceived. We do not believe that this difference has a major impact on the results obtained. If any changes in HVR were to occur between TOF ratio 0.75 and 0.70 our belief is that there would be an even more pronounced decrease compared to the baseline.

The second study was a multicentre study involving four different sleep clinics in Sweden evaluating the STOP-Bang questionnaire. In this study, the patients answered the STOP questions and the staff filled in the Bang questions. However, it is possible for the patient may answer more of the questionnaire, ie A (age) and G (gender). Help from staff is likely to be needed for answering B (BMI) and N (neck circumference), but most likely even for P (blood pressure). Not all patients are aware of that they have an elevated blood pressure, but it should be a compulsory part of the medical examination and thereafter answered in the questionnaire.

It took a year and a half to complete data collection for Study I and three and a half years to complete Study III and IV. It was more difficult than anticipated to find patients with OSA and no other medical condition apart from well-treated hypertension, despite the fact the sleep clinic received more than one hundred new patients per week. However, in Study III and IV it was most difficult to find BMI, age and sex matched controls. Even though hundreds of notifications arrived from people who volunteered to participate as controls it was not possible to find a matched control for every patient with OSA. It was notoriously difficult to find over-weight/obese middle-aged or older men as matched controls. There were no obstacles in recruiting healthy normal BMI controls.

In Study III and IV data was collected at three time points (0, 3 and 12 months) from patients with OSA and twice (0 and 12 months) from the matched and normal BMI controls. The data from the matched and normal BMI controls were primarily used to confirm that there were no differences in the data over time. If data from the matched and normal BMI controls also had been collected after three months, we would have been able to make comparisons between the sleep apnea patients and the controls at all three time points. As we did not find any differences between the data collected at 0 and 12 months for the matched controls, only data from 0 months were used for comparison with the three different time points for the OSA patients.

In Study III and IV seven patients with OSA discontinued their CPAP treatment. No further data or samples were taken after this occured. In retrospect, it would of course have been interesting to have these samples and have them analysed. What changes of the differential gene expression and circulatory inflammatory biomarkers would appear as intermittent hypoxia returned?

6.2 CLINICAL IMPLICATIONS

Obstructive sleep apnea is a common disease however, the majority of patients are undiagnosed [8,11]. In addition, many clinicians fail to identify the condition during a medical visit [9]. Therefore reliable screening tools, like the STOP-Bang questionnaire, are needed to identify patients at increased risk of OSA. In the Swedish sleep clinic population, we found that a STOP-Bang score of 6-8 had a specificity of 91% for detecting at least moderate OSA and a score of 0-1 could exclude at least moderate OSA by 95%. With limited resources sleep clinics could make it mandatory for the referral to include STOP-Bang data. The STOP-Bang score could then be of valuable information helping to priorities among the referred patients. For patients with a STOP-Bang score of 3-5 a nocturnal peripheral saturation measurement resulting in an ODI would add extra information as AHI and ODI correlates well.

In the perioperative setting, a patient with a STOP-Bang score ≥6 can be assumed to have at least moderate OSA and precaution and special preparations should be taken. For example during induction of anesthesia and an awareness of the possibility of difficulties with both

mask ventilation and intubation, but also postoperatively with cardiorespiratory complications. It is important to remember that sleep apnea patients do not have any protection of their normally increased HVR during partial neuromuscular blockade. In contrast, respiratory control during hypoxia show the same sensitivity to partial neuromuscular blockade as in healthy individuals (which is a reduction of the HVR by one-third at a TOF ratio of 0.7). This reduction in HVR during partial neuromuscular blockade remain despite 3 months of CPAP treatment. When administering a neuromuscular blocking drug it is very important to routinely use an objective quantitative intraoperative neuromuscular monitoring of the depth of the blockade [180]. If partial neuromuscular blockade persists at the end of surgery either a reversal agent should be administered, or additional time is needed for spontaneous recovery. However, regardless of the option chosen the neuromuscular status must be monitored until acceptable levels are achieved before anesthesia is ended and extubation can be performed. What is considered an acceptable level has been changing with time and there are relatively new data supporting that extubation is preferred at a TOF ratio > 0.95 instead of the current policy of 0.9 [121]. In clinical practice, a neuromuscular blocking agent is very rarely given on its own, but rather in conjunction with other anesthetic drugs which might have an additional effect on the HVR.

Obstructive sleep apnea is characterized by repeated nocturnal hypoxias and microarousals. This causes an activation of the sympathetic system, oxidative stress, endothelial dysfunction and chronic inflammation which in turn is believed to be the foundations of associated comorbidities. However, the molecular mechanisms of OSA are still not clear. In this thesis, we found that the transcriptome of untreated patients with OSA differs from that of BMI, age and sex matched controls with a significant downregulation of immune-related genes, and that this was recovered after 3 months of CPAP treatment but returned to the untreated state after 12 months of CPAP treatment. However, it cannot be concluded that any changes found at the mRNA level will generate the corresponding changes at the protein level. We also demonstrated that inflammatory biomarkers MCP-1, FGF 21, neutrophils and neutrophil-to-lymphocyte ratio were upregulated and that CASP-8 and GDNF were downregulated in CPAP treated OSA patients compared to the untreated state and that the majority of changes came first after 12 months of treatment. Inflammatory biomarkers previously shown to be increased in untreated OSA patients and to be reduced after CPAP treatment such as CRP, IL-6 and TNF-α were not affected in our study population compared to matched controls However, CRP was elevated in patients with OSA compared to normal BMI controls. Further research is needed in both genetics and potential biomarkers of sleep apnea patients in comparison with well-matched controls before it can have clinical use, such as facilitating diagnostics or evaluating initiated therapy.

7 CONCLUSIONS

The following conclusions were found in this thesis:

- The hypoxic ventilatory response in untreated patients with obstructive sleep apnea is reduced by approximately a third during peripheral neuromuscular blockade (train-of-four ratio 0.7) which is equivalent to that seen in healthy volunteers, whilst the hypercarbic ventilatory response is unaffected.

- The STOP-Bang questionnaire and pulse oximetry are useful tools in the sleep patient population. The optimal cut-off for identifying patients with an AHI ≥15 was found to be a score of 6 and for patients with an AHI ≥5 a score of 5. A score ≥ 6 has a sensitivity of at least moderate OSA of 91% and a score <2 has the probability to exclude at least moderate OSA by 95%.

- Whole blood gene expression in patients with untreated obstructive sleep apnea shows a down-regulation of immune-related genes compared to body mass index, age and sex matched controls. After three months of nocturnal continuous positive airway pressure treatment the gene expression resembled that of the matched controls. However, after twelve months of continuous positive airway pressure treatment the gene expression returned to the untreated state.

- The inflammatory proteins: monocyte chemoattractant protein 1, fibroblast growth factor 21, neutrophils and neutrophil-to-lymphocyte ration are upregulated whereas caspase 8 and glia cell-line derived neurotrophic factor are downregulated in patients with obstructive sleep apnea after three and/or twelve months of continuous positive airway pressure treatment compared to the untreated state. Most changes came after 12 months of treatment. There is no differential expression in inflammatory biomarkers in untreated patients with obstructive sleep apnea compared with body mass index, age and sex matched controls.

8 POINTS OF PERSPECTIVE

In this thesis, we have shown that the HVR during partial neuromuscular blockade is decreased by approximately one-third and that it seems like this reduction is unaffected by three months of CPAP treatment. During surgical procedures other anesthetic drugs are usually administered, either to achieve sedation or a general anesthetic. In some instances, usually at out-patient clinics, it is the surgeon who is responsible for both the sedation, monitoring of the patient and the surgery. It would therefore be important to continue to investigate how the HVR is affected by other sedative drugs, for example propofol, benzodiazepines, volatiles, dexmedetomidine and nitrous oxide in patients with OSA. It is of course most important to consider the depth of sedation (clinically relevant for surgical procedures or as estimated postoperative concentrations) and possible airway obstructions in this vulnerable population.

The STOP-Bang questionnaire is a well-known screening tool for identifying OSA and has been validated in numerous populations (ie surgical, sleep, general population and commercial drivers). Other, more recently invented, screening tools claiming to be more user-friendly as they consist of fewer questions should also be validated in the surgical population. So far, the B-APNEIC score, the BOAH scale, the NoSAS score, the No-Apnea score and the GOAL questionnaire have only been validated in the sleep population.

It is important to identify patients with OSA preoperatively in order to minimize any adverse events in the perioperative period. The question of what the most appropriate measures are still remains. For example, should patients with different phenotypes of OSA be treated and/or monitored in the same way? Since ODI and AHI correlate well, it would be interesting to evaluate whether preoperative ODI measurement (in the form of nocturnal peripheral oxygen saturation) could provide additional valuable information about surgical patients having a STOP-Bang score ≥3 together with predefined interventions and if this could reduce perioperative complications. Another question is whether patients with OSA will do better if compliant with treatment and if that were the case, what is the cut-off for treatment needed before surgery to have an optimal effect?

The genetic expression returned to the untreated state after 12 months of good compliance to CPAP treatment. This finding was very surprising and deserves further exploration. A first step would be to validate/confirm this finding. Even though we had difficulties finding matched controls and the final analysis included ten patients with OSA and their corresponding matched control it would be of great value to increase the number of participants in order to get a more robust result. In our study, the gene expression in whole blood was analysed which has its benefits, but also some drawbacks. The majority of other mRNA sequencing studies in patients with OSA have analysed peripheral blood mononuclear cells. In addition, it would be interesting to increase the sampling rate during the treatment year, for example at 0, 3, 6, 9 and 12 months. This would allow a more detailed investigation in changes of gene expression during CPAP treatment, ie when does the gene expression return to the untreated condition and, of course, any other changes in gene expression during the year. It would be of great value if patients deciding to terminate their CPAP treatment could continue with the study protocol and do home sleep apnea tests and have blood samples collected at the predefined intervals in order to evaluate how the gene expression changes as the apneas/hypopneas return.

Even though there is an increasing interest in OSA, both in general and in the perioperative setting, this area needs and deserves more research and spotlight in order to reach as far as

other areas in medicine. The aims of physicians are to cure, when this is not possible to relieve and always, at the very least, to comfort our patients. However, prevention is always preferred before cure. We must therefore continue in our thrive to identify patients with OSA and keep our minds and eyes open for how we can provide a safer perioperative environment for these patients by always evaluating and investigating the current practice in the continuous search for novel and even better preventive and therapeutic strategies.

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