NON-INVASIVE RESPIRATORY SUPPORT IS A PRO-INFLAMMATORY STIMULUS TO THE UPPER AND LOWER AIRWAYS

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DECLARATION

"I, Mohammed Dhafer AlAhmari, confirm that the work presented in this thesis is my own except for the help listed in the acknowledgements. Where information has been derived from other sources, I confirm that this has been indicated in the thesis"

Signuture: ……

Date: ……

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ABSTRACT

Non-invasive respiratory support (NIV) is associated with a high prevalence of local side-effects which may be associated with induction of upper airway inflammation. This thesis examines the effect of NIV on the upper and lower airway by examining bronchial epithelial cell cultures, healthy subjects, obstructive sleep apnoea (OSA), and chronic obstructive pulmonary disease (COPD) patients. The rationale for this thesis is based on reports suggesting that continuous positive airway pressure (CPAP) may induce early upper airway inflammation, and discusses the relationship between upper and lower airways in COPD.

To examine the addressed rationale we used both an *in vitro* and *in vivo* approach. *In vitro,* we examined the release of the inflammatory cytokines from a human bronchial epithelial cell line over time-intervals using CPAP therapy. *In vivo* studies investigated whether induction of nasal inflammation was associated with the development of systemic inflammation, nasal symptoms, and changes in nasal mucociliary clearance after a short period of CPAP therapy. Results were investigated further in OSA by investigating whether induction of nasal inflammation was associated with the development of systemic inflammation, nasal symptoms, airway obstruction, and impaired adherence to CPAP therapy and quality of sleepiness over a six months follow-up period. Additional pilot data obtained involved a comparison of local and systemic inflammatory indices in COPD patients using and not using NIV.

The key finding was that CPAP is pro-inflammatory. The *in vitro* data showed that CPAP resulted in the release of inflammatory mediators from cultured human bronchial epithelial cells, in a time-and-pressure-dependent manner. Meanwhile, *in vivo* data from healthy control subjects showed CPAP was associated with dose (pressure) response changes in nasal and systemic inflammatory markers, reduced nasal function, and the development of nasal symptoms. The development of nasal symptoms was associated with the degree of functional impairment and nasal inflammatory response. These *in vitro* and *in vivo* results were novel in reporting the effects of CPAP in this way, providing new data on the mechanisms of CPAP intolerance in the crucial, early phase of therapy. Furthermore, the long-term CPAP study with OSA resulted in nasal inflammation, reduction in nasal

mucociliary function, and significant other adverse effects. However, sleep quality and the perceived benefits of therapy improved over time, despite the presence of side-effects.

These results have important implications for clinical practice, since it demonstrates a relationship between nasal symptoms, mucociliary clearance, inflammation and compliance in patients with OSA initiating and continuing CPAP therapy. Further investigation of strategies to combat the initial side-effects and nasal inflammation associated with this treatment modality might target the epithelial lining of the nose in an attempt to address the origin of the inflammatory response. In addition, educational and support strategies to improve patients' tolerance of side effects may further increase compliance with nasal CPAP treatment for OSA patients.

ABBREVIATIONS

°C	Degree Celsius
AASM	American Academy of Sleep Medicine
AAT	$Alpha_1$-antitrypsin
AECOPD	Acute exacerbation of chronic obstructive pulmonary disease
AHI	Apnoea-Hypopnoea Index
AHR	Airway hyperresponsiveness
ALI	Acute lung injury
ANOVA	Analysis of Variance
AR	Acoustic rhinometry
ARDS	Acute Respiratory Distress Syndrome
BEAS-2B	Bronchial epithelial cell line
BMI	Body-mass index
BODE	Body mass index, obstruction, dyspnea, exercise capacity index
cm	Centimeter
CO	Carbon monoxide
CO_2	Carbon dioxide
COPD	Chronic Obstructive Pulmonary Disease
CPAP	Continuous positive airway pressure
CRP	C-reactive protein
CSA	Cross sectional area
CT	Computed Tomography
CV	Coefficient of variation
CXCR	Chemokine receptor
D-MCA	Distance of cross-sectional area from nasal orifice
EBC	Exhaled breath condensate
ECM	Extracellular matrix
ECP	Eosinophil cationic protein

EDS	Excessive daytime sleepiness
EEG	Electroencephalography
ELISA	Enzyme-linked immunosorbent assay
EPAP	Expiratory positive airway pressure
EPO	Erythropoietin
ESS	Epworth Sleepiness Scale
ETDA	Ethylenediaminetetraacetic acid
FeNO	Fractional exhaled nitric oxide
FEV_1	Forced expiratory volume in 1 second
FRC	Functional residual capacity
FVC	Forced vital capacity
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GPRD	General Practice Research Database
GSH	Glutathione
H_2O	Water (hydrogen dioxide)
ICAM-1	Inter-Cellular Adhesion Molecule-1
ICC	Intraclass correlation coefficients
IF	Inflammatory factors
IFN-γ	Interferon-gamma
IL	Interleukin
IPAP	Inspiratory positive airway pressure
IPPV	Intermittent positive pressure ventilation
Kg	Kilogram
L	Liter
MCA1	First minimum cross-sectional area
MCA2	Second minimum cross-sectional area
MMP	Matrix metalloproteinases
MPO	Myeloperoxidase
MRI	Magnetic Resonance Imaging
MSLT	The multiple sleep latency test

N	Number
NaCl	Sodium chloride
nCPAP	Nasal continuous positive airway pressure
NHS	National Health Service
NIV	Non-invasive ventilation
MCC	mucociliary clearance
NO	Nitric oxide
O_2	Oxygen
OSA	Obstructive sleep apnoea
P	Pressure
$PaCO_2$	Partial arterial pressure of carbon dioxide
P_{aw}	Airway pressure
PBS	Phosphate buffered saline
PEEP	Positive end-expiratory pressure
PEFR	Peak expiratory flow rate
PND	Post-nasal drip
R	Pearson correlation co-efficient
RDI	Respiratory disturbance index
Rho	Spearman rank correlation co-efficient
RMM	Rhinomanometry
rpm	revolutions per minute
S	Second
SaO_2	Blood oxygen saturation
SGRQ	St. George's Respiratory Questionnaire
STT	Saccharin transit time
SD	Standard deviation
STT	Saccharin transit time
T	Temperature
TIMP	Tissue inhibitors of matrix metalloproteinases
TCC	Total cell count

TGF-β	Transforming growth factor-beta
TNF-α	Tumour necrosis factor-alpha
UARS	Upper airway resistance syndrome
UK	United Kingdom
μm	Micrometer
USA	United States of America
V	Volume
v/v	volume for volume
V_{2-5}	Volume of nasal cavity between the 2^{nd} and 5^{th} cm from nasal orifice
VEGF	Vascular endothelial growth factor
VIP	Vasoactive intestinal peptide
VT	Tidal volume
w/v	Weight/volume
WHO	World Health Organization
WOB	Work of breathing
VEGF	Vascular endothelial growth factor

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1

INTRODUCTION

This thesis concerns the pro-inflammatory effects of non-invasive respiratory support (NIV) *in vivo* in healthy subjects, obstructive sleep apnoea (OSA) and chronic obstructive pulmonary disease (COPD), and *in vitro* of human bronchial epithelial cell-culture. The aim of this introductory chapter is to explain the rationale behind the research topic, by reviewing and discussing the existing literature.

1.1 OVERVIEW OF THE RESPIRATORY SYSTEM

The human respiratory tract is the major gas exchange system of the body. The system consists of complex organs and tissues that mainly include the nose, pharynx, trachea, and lungs. These organs help to capture oxygen from the environment and transport it into the lungs [1]. The primary function of the respiratory system is to supply air into the lungs, thereby facilitating the diffusion of oxygen into the blood stream so that the oxygen can be further distributed to all body parts. It also receives the waste CO_2 from the blood and exhales it. This is achieved through breathing, during the inhalation of oxygen and exhalation of CO_2 [2]. This gaseous exchange is called respiration.

The breathing mechanisms are facilitated by the respiratory system. The term respiration consists of two processes: internal and external respiration. External respiration (breathing) is the process of inhaling oxygen from the air and exhaling carbon dioxide into the air. This process occurs in the respiratory tract, which starts at the nose and mouth. Oxygen enters through the nose and the mouth to the respiratory system, passing through the larynx and the trachea, which become narrower, shorter and more numerously branching airways as they penetrate deeper into the lung. The trachea divides into right and left main bronchi, which divide into lobar, then segmental bronchi that continuous down to the terminal bronchioles.

The terminal bronchioles divide into respiratory bronchioles that lead to tiny sacs called alveoli (**Figure 1**) [3]. The inhaled oxygen passes into the alveoli and then diffuses into the arterial blood through the capillaries. The CO_2 follows the same route out of the lungs during exhalation. During breathing, the diaphragm contracts and passively relaxes, thereby drawing air into the lungs and exhaling the CO_2 out of the lungs.

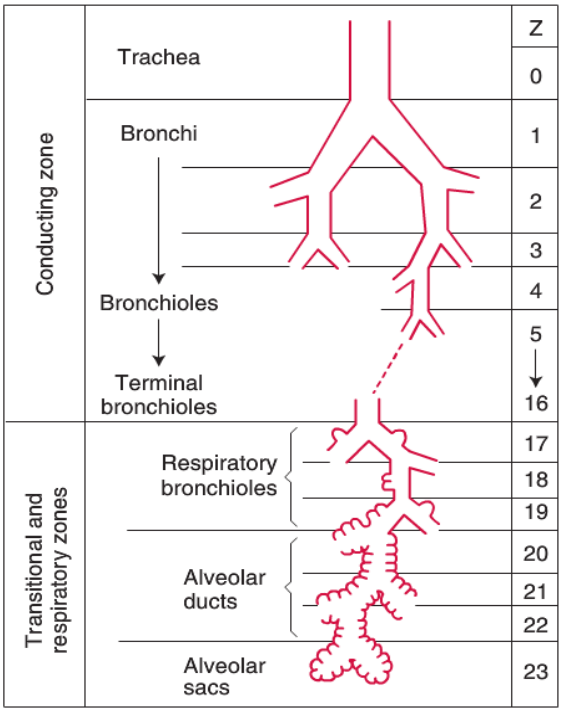


Figure 1: The first 16 generations (Z) make up the conducting airways, and the last 7, the respiratory zone (or the transitional and respiratory zones).

1.1.1 Structure of Upper Airway

The upper airway is an essential and vital part of the respiratory tract, as it helps in conducting air into the lungs [4]. Its anatomical structure and functional features influence the properties of the inhaled air. Additionally, the upper airways also play an important role in the protection of the lower airways [5]. The upper respiratory tract mainly consists of three valve-like structures: the nasal cavity, pharynx, and larynx (**Figure 2**). These particular regions regulate the airflow by a phenomenon known as airway collapse. It has also been described as a complex organ concerned with digestive, defensive, and vocalisation processes [6].

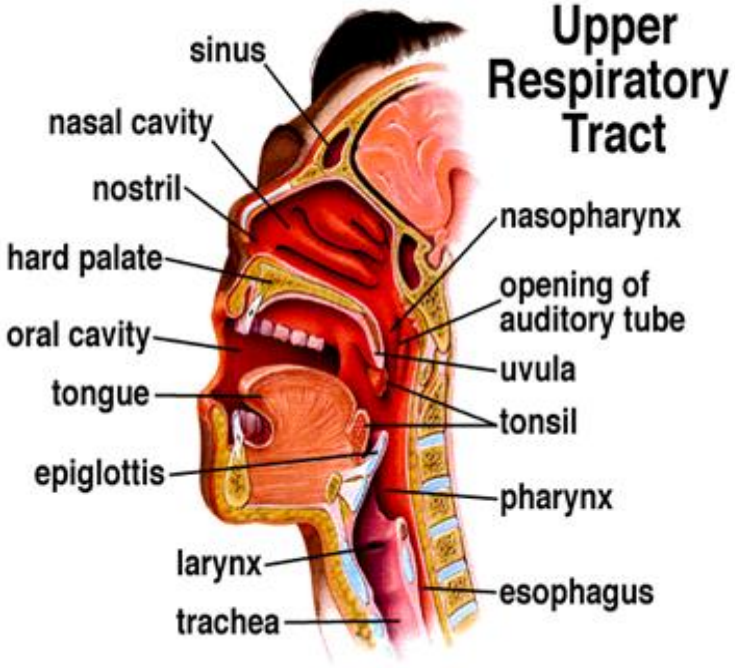


Figure 2: Basic Structure of the Upper Respiratory Tract.

1.1.1.1 Parts of the Upper Respiratory Tract:

The upper respiratory airways extend from the mouth and the nose to the larynx. The upper respiratory system is responsible for filtering airborne particles, humidify and warm the

inspired gasses inhaled. It is also used for olfaction. The following are brief descriptions for the important parts of the upper airway:

• **Nasal cavity:** The nasal cavity comprises of the nose, nasal fossa, and paranasal sinuses. The nose is the primary structure of entry for inhaled air entering the respiratory tract, therefore, the mucosal epithelium lining (pseudostratified ciliated columnar epithelium with goblet cells) the nasopharyngeal airways are exposed to high concentrations of inhaled allergens and other particulate matters. The normal respiratory epithelium is coated with mucus, which lubricates and humidifies the epithelium and acts as a protective system by entrapping bacteria and other particulates for removal by mucociliary clearance [7].

• **Pharynx:** This is a fibromuscular tube connecting the nasal cavity and the mouth with the lower respiratory tract and the esophagus. The pharynx interior is often separated into three sections: the nasopharynx, oropharynx, and laryngopharynx. Here, the throat divides into the trachea and oesophagus and a small flap of cartilage, called the epiglottis, separates both, preventing food from entering into the trachea [8].

• **Larynx:** This tube connects the pharynx and the trachea, and is known as the voice box, as it consists of vocal cords that are responsible for speech. It protects the trachea using the glottis, which closes during swallowing [8].

1.1.2 Structure of Lower Airway

The lower respiratory airways extend from the trachea to the alveoli. The entire lower tract is protected by the ribcage, vertebral column, and sternum bone. Lower respiratory airway structures include the trachea, right and left mainstem bronchi, segmental bronchi, and terminal bronchioles through the many branches of the respiratory tree to the level of alveolar system, where the gas exchange occurs. The gas exchange process takes place in the alveoli, small air sacs at the end of the respiratory bronchioles.

1.1.2.1 Parts of the Lower Respiratory Tract:

The lower respiratory airways consist of a series of branching tubes, which become narrower and shorter as they penetrate deeper into the lung (**Figure 1**). The following are brief descriptions for the important parts of the lower airway:

• **Trachea:** A flexible tube that has an inner diameter of about 2 cm and that is approximately 12 cm long. It extends from the edge of the larynx, conducting air from the larynx into the lungs where it divides into two main bronchi [9]. Its inner membrane is covered with tiny hair-like structures called cilia that help to eliminate dust particles out of the respiratory tract through coughing before it enters the lungs [10]. The trachea is a cartilaginous and membranous tube surrounded by 15-20 C-shaped semi-circular cartilage rings at the front and side that protects the trachea and regulates its opening to allow the expansion of the oesophagus during the swallowing process.

• **Bronchi:** The trachea divides into two main tubes called bronchi, one entering the left and one entering the right lung. Each main bronchus will further divide into lobar, then segmental bronchi. This process will continue down to the end, to the terminal bronchioles, which are the smallest conducting airways without alveoli. The main function of the conducting airways is conducting inhaled air to the gas exchange regions of the lung [8].

• **Bronchioles:** Terminal bronchioles divide into respiratory bronchioles and alveolar ducts. Respiratory bronchioles sometimes have alveoli resulting from its walls and alveolar ducts are completely contains alveoli, as illustrated in **Figure 2**. Bronchioles are smaller airways that carry the inspired air on to the inside walls of the lungs, where the alveoli allow the oxygen to be absorbed by the blood cells, and oxygenate the blood for transfer throughout the body [11-12].

• **Alveoli:** These are individual hollow cavities contained within the alveolar sacs (or ducts). Alveoli have very thin walls that allow the gaseous exchange of oxygen and CO_2 for the body's metabolisms. They are surrounded by a network of capillaries, into which the inspired gasses pass. The number of alveoli is a key structural determinant of the lung

architecture. Human lungs have a mean number of 480 million alveoli with a mean size of $4.2 \times 106\ \mu m^3$ (roughly a diameter of 200 μm) [13].

• **Lungs:** The lungs are the vital organs for the respiration process. They are located in the thoracic cavity and are covered with pleural membranes. The right lung has three lobes, while the left lung has only two lobes [14]. The lungs include the bronchi, the alveoli, connective tissues, blood vessels, lymph vessels and nerves [15]. Lungs are responsible for respiration, since the exchange of gasses takes place in them. The lungs supply the heart with oxygenated blood through the pulmonary veins and take off the deoxygenated blood through the pulmonary arteries. The elasticity of the lungs facilitates the inhalation and exhalation of air [10].

• **Diaphragm:** The diaphragm is a dome-shaped, broad band of internal skeletal muscle, which lies underneath the lungs, attached to the lower ribs, sternum and lumbar spine, forming the base of the thoracic cavity [16]. The diaphragm separates the thoracic cavity (heart, lungs and ribs) from the abdominal cavity, and performs an important function in respiration. The diaphragm functions in breathing by contracting and relaxing itself during inhalation and exhalation, respectively [17].

• **Accessory muscles of respiration:**

The accessory muscles are responsible for assisting the diaphragm during breathing in patients suffering from breathing difficulties (e.g COPD), or in individuals who have undertaken vigorous exercise. The major accessory muscles of inspiration include the scalenes and the sternocleidomastoid muscles in the neck, the serratus anterior, and the pectoral muscles, the upper trapezius and latissimus dorsi muscle, and the erector spinae muscles. However, during expiration, the accessory muscles assist in exhalation when airway resistance becomes elevated. These muscles include rectus abdominis, external and internal abdominis obliquus muscles, transverses abdominis, and internal intercostals muscles [8].

1.2 CONTINUOUS POSITIVE AIRWAY PRESSURE (CPAP)

1.2.1 Definition and History

CPAP is a supportive, positive pressure throughout the entire respiratory cycle (inspiration and expiration), when breathing spontaneously [18]. The CPAP system is delivered using a tight-fitting face or nasal mask and a valve, usually at a pressure of 5 - 10 cm H_2O, against which the patient exhales [19-21]. The CPAP valve should be a low resistance type [22].

CPAP has been demonstrated as an effective treatment for patients with OSA [23]. Other treatment options are generally less effective, including oral-appliance devices [24] and uvulopalatopharyngoplasty [25]. OSA is a prevalent condition closely associated with the global obesity epidemic, and is characterised by repetitive, partial or complete collapse of the upper airway during sleep, causing impaired gaseous exchange and sleep disturbance [26].

CPAP is a treatment that uses air pressure to keep the airways patent (**Figure 3**) [32]. It was introduced by Sullivan and his colleagues in 1981, and has become the initial choice for the management of OSA, and can completely abolish apnoeas when effective pressure levels are used during sleep [27]. It has now become the standard treatment for OSA [28]. It prevents intermittent pharyngeal obstruction (a major pathogenetic mechanism in OSA) by acting as a “pneumatic splint”, thus maintaining a patent airway throughout the respiratory cycle in order to maintain an open airway during sleep, which obviates the effects of airway collapsibility [29-31].

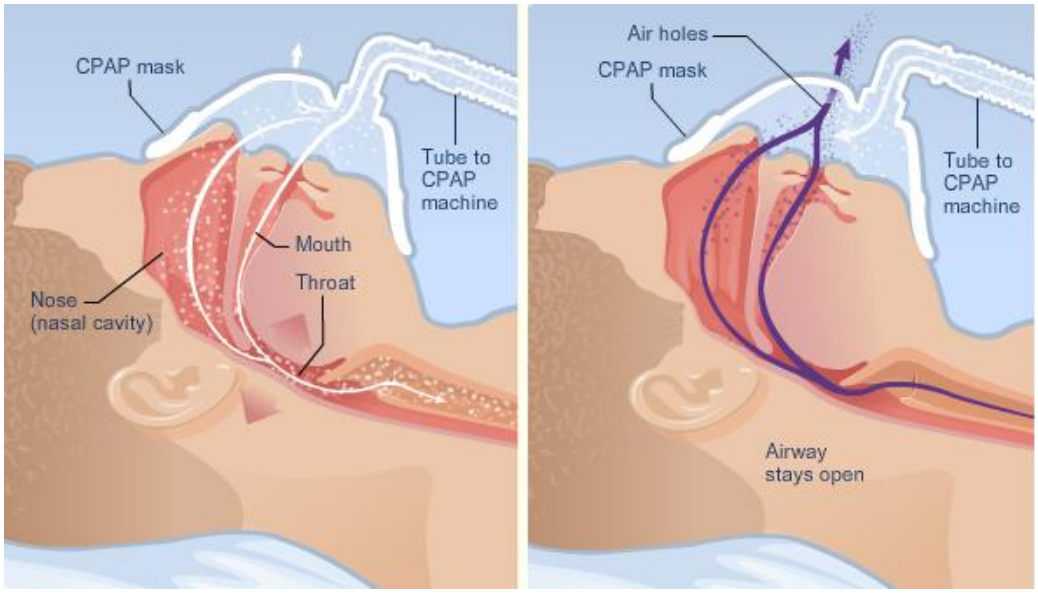


Figure 3: CPAP Device: the principle of CPAP operation in resolving the airway obstruction during sleep.

Before the 1960s, nearly all ventilation techniques for mechanical ventilation were non-invasive. In 1980, clinical evidence began to show that the benefits of intermittent positive pressure ventilation (IPPV) were often exaggerated and could be replaced with other simpler and more cost-effective therapies [33]. As IPPV lost favour in clinical practice, nasal mask CPAP started to emerge as a highly effective therapy for the treatment of OSA [34].

CPAP comes in two forms. Each form has different indications and applications in the clinical setting. The first form is a CPAP generated by a flow generator in the acute setting, which is used as a form of respiratory support in the treatment of type I respiratory failure. The second form is generated by a small, portable electrically powered compressor, and used in the treatment of obstructive sleep apnoea. The focus of this thesis is on the second form of CPAP with OSA.

1.2.2 Indications and Contraindications of CPAP Therapy

1.2.2.1 Indications of CPAP Therapy

It is essential that CPAP is applied in an appropriate form of therapy in patients whom clinically beneficial to them. CPAP is not suitable for all patients with respiratory failure and any inappropriate use of this therapy may lead to suboptimal treatment. The following are indications for CPAP:

- Treatment of choice for patients with OSA [30].
- Effective therapy in patients with cardiogenic pulmonary oedema [35-37].

1.2.2.2 Contraindications of CPAP Therapy

Although CPAP is a safe and effective form of therapy, it requires patient to be alert and breathing. If the patient is unable or fails to cooperate with fitting or wearing mask, then CPAP therapy is not appropriate. Most of the contraindications to CPAP are listed below [35, 38]:

- Cardiac or respiratory arrest.
- Severe gastrointestinal obstruction and/or bleeding.
- Severe haemodynamic instability with or without unstable cardiac angina.
- Facial surgery or trauma.
- Fixed upper airway obstruction.
- Inability to protect the airway: risk of aspiration, viscous or copious secretions.
- Untreated pneumothorax.

1.2.2.3 CPAP-Related Side Effects

Many side effects can occur during the first few weeks of CPAP use and that leads to poor adherence and discontinuation of treatment [39-40]. The most reasons for poor compliance in adults include inconvenience, side effects, discomfort, claustrophobia, and expense, **Table 1** [41].

Table 1: Possible CPAP-related side effects.

CPAP-RELATED SIDE EFFECTS	POSSIBLE CAUSE
Interface related	Mask leak, skin abrasion/ulceration, mask allergy, conjunctivitis/sore eyes, facial irritation, claustrophobia.
Pressure-Related (Airway)	Rhinitis, rhinorrhoea, sneezing, sinusitis, headache, epistaxis, ear pain, air swallowing/aspiration.
Pressure-Related	Mouth leak (dry mouth) or mask leak, pressure intolerance, sense of suffocation or difficulty in exhaling, tinnitus, aerophobia, pneumoencephalus.
Equipment-Related	Noise, smell, tubing condensation, cumbersome equipment, spousal intolerance/less intimacy, ramp abuse, equipment maintenance and cleaning.
Equipment Failure	Machine lifespan, tubing and mask, OSA recurrence.
General	Periodic limb movements, anxiety, insomnia, headache, fatigue/feeling tired, chest discomfort.

1.3 BILEVEL POSITIVE PRESSURE AIRWAY THERAPY

1.3.1 Definition and History

Bilevel positive pressure airway therapy is a form of NIV, which is defined as "the application of positive pressure without airway intubation for the purpose of augmenting alveolar ventilation" [42]. This technique is distinguished from invasive ventilatory support, which bypasses the upper airway with a tracheal tube, laryngeal mask, or tracheostomy (**Figure 4**) [35, 43]. It has increasingly been used to avoid intubation [44], and eliminates the need for intubation or tracheostomy, preventing problems such as injury to the vocal cords or trachea, and lower respiratory tract infections [45]. Evidence supports the use of NIV with exacerbated COPD, acute cardiogenic pulmonary oedema, and may reduce mortality [38, 46].

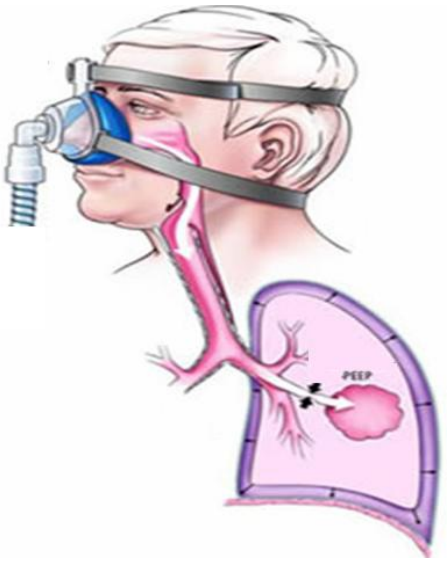


Figure 4: NIV as an effective therapy in acute exacerbations of COPD [43].

The concept of mechanical ventilation first evolved as a negative-pressure ventilation when the first workable 'iron lung' was developed in 1876 [47], and was used extensively during the polio epidemics of the 1930s and 1960s (**Plate 1**). The iron lung works by augmenting the tidal volume by applying negative extrathoracic pressure. These earliest forms of NIV

fell out of favour as the use of invasive positive pressure ventilation increased during the 1960s. In the early 1980s, proliferations of NIV began with the introduction of the nasal CPAP mask for the treatment of OSA [48]. Moreover, the past decade has seen continuity in the use of NIV, largely because of the development of nasal ventilation, which has the potential for providing ventilatory assistance with greater convenience, more comfort, safety, and less cost than invasive positive ventilation [49]. NIV currently has a definite and important role in the management of acute and chronic respiratory failure of many aetiologies [43].

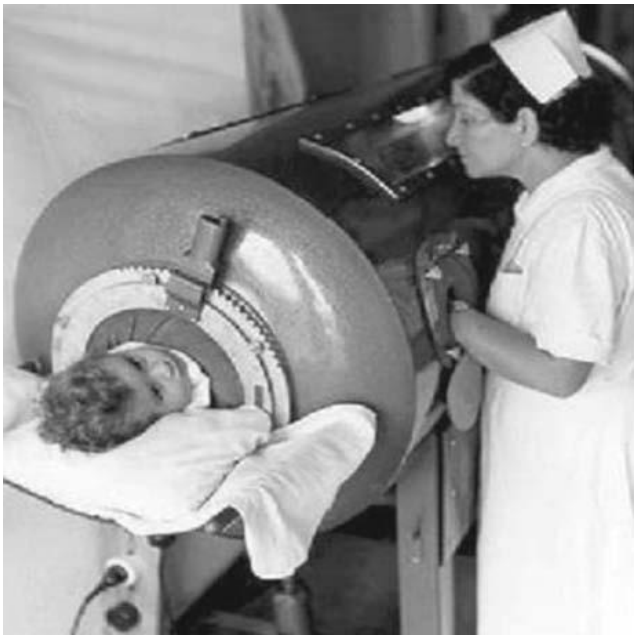


Plate 1: An iron lung: Typical equipment available for respiratory failure in 1952: (Image of child in iron lung reproduced courtesy of the WHO Global Polio Eradication Initiative) [50].

1.3.2 Physiological Effects of NIV

Of different aetiologies, the pathophysiology of acute respiratory failure include an imbalance between an increased respiratory mechanical load and a decreased capacity of the respiratory muscles, decrease in lung gas exchange due to either ventilation-perfusion mismatch or intrapulmonary shunt, and deteriorations in cardiovascular function [51]. NIV is an effective supportive therapy in unloading the respiratory muscles, decreasing the work of breathing and improve lung mechanics in numerous diseases. **Table 2** summarises goals and indications of NIV in acute and chronic settings [43].

Table 2: Goals and indications of NIV in acute and chronic settings.

GOALS OF NIV	
ACUTE CARE	**CHRONIC CARE**
Relieve symptoms	Relieve & improve symptoms
Decrease work of breathing	Improve quality of life
Improve gas exchange	Enhance functional status
Improve patient comfort	Avoid hospitalisation
Decrease length of hospitalisation	Increase survival
Avoid intubation	Improve mobility
NIV ACUTE INDICATIONS	**NIV CHRONIC INDICATIONS**
COPD exacerbations	Restrictive chest diseases:
Acute cardiogenic pulmonary oedema	Neuromuscular diseases
Respiratory infection in immunocompromised state	Chest wall deformities
Hypoxemic respiratory failure	Kyphoscoliosis
Postoperative respiratory failure	COPD
Difficulty weaning	Nocturnal hypoventilation

1.4 PRINCIPLE OF OPERATION FOR NON-INVASIVE VENTILATION

Noninvasive positive ventilation works by delivering a pressurised gas via external interfaces. There are two forms of noninvasive positive ventilation, which is CPAP and bilevel positive pressure airway therapy, as mentioned earlier. These devices are relatively portable, quiet, and often come with a variety of features designed to enhance patient comfort. In thesis, both forms of noninvasive positive ventilation have been used with healthy subjects, using CPAP and OSA, and bilevel positive pressure airway therapy, with COPD patients.

CPAP

A basic CPAP device provides the patient with fixed pressure therapy throughout both inspiration and expiration. Many of these CPAP devices are equipped with a ramp feature that allows a gradual increase in the length of time of the pressure from a low level until reaching the final prescribed pressure.

In obstructive sleep apnoea, application of CPAP acts as a pneumatic "splint" which prevents upper airway collapse during sleep. During CPAP therapy, the patient breathes from a pressurised circuit against a threshold resistor (water-column, weighted, or spring loaded) that maintains consistent pre-set airway pressures from 5 to 20 cm H_2O during both inspiration and expiration. The expiratory pressures are set above atmospheric pressure and therefore both inspiratory and expiratory pressures are increased (**Figure 5**). This form of CPAP is generated by a small and portable electric compressor [52].

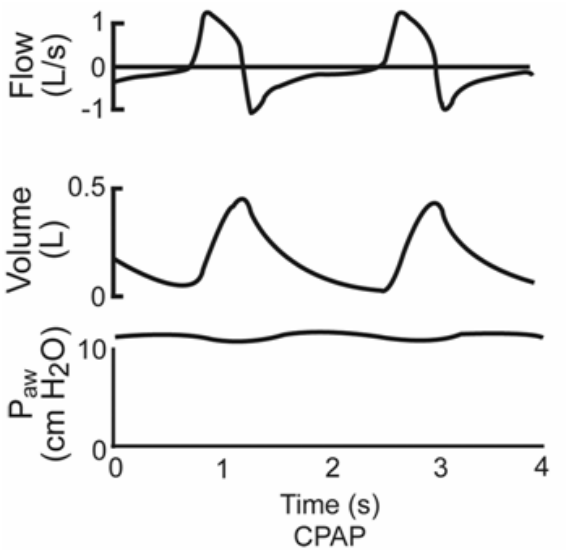


Figure 5: A representative tracings of flow, tidal volume, and airway pressure (P_{aw}) during administration of CPAP.

BILEVEL POSITIVE PRESSURE AIRWAY THERAPY

A bilevel positive pressure airway device delivers a separately adjustable higher pressure level on inspiration, (IPAP) and cycles to a lower pressure on expiration (EPAP) [52]. This therapy may be appropriate therapy for the patient who requires a high level of CPAP to resolve all respiratory events and for some patients with difficulty exhaling [52-55]. The level of pressure difference between the IPAP and EPAP settings provides a pressure-supported augmentation of tidal volume and unloading of the respiratory muscles that can benefit patients who have underlying hypoxemia or hypoventilation, or in patients with neuromuscular disorders or COPD [56].

Such a variable pressure setting between the inspiratory and expiratory cycles would help in decreasing the amount of pressure against which the patient exhales, thereby decreasing abdominal muscle recruitment and consequent respiratory discomfort during the expiratory cycle [57]. However, the greater level of pressure during the inspiratory cycle helps in combating the inspiratory flow limitation suffered by the upper airway (**Figure 6**) [58].

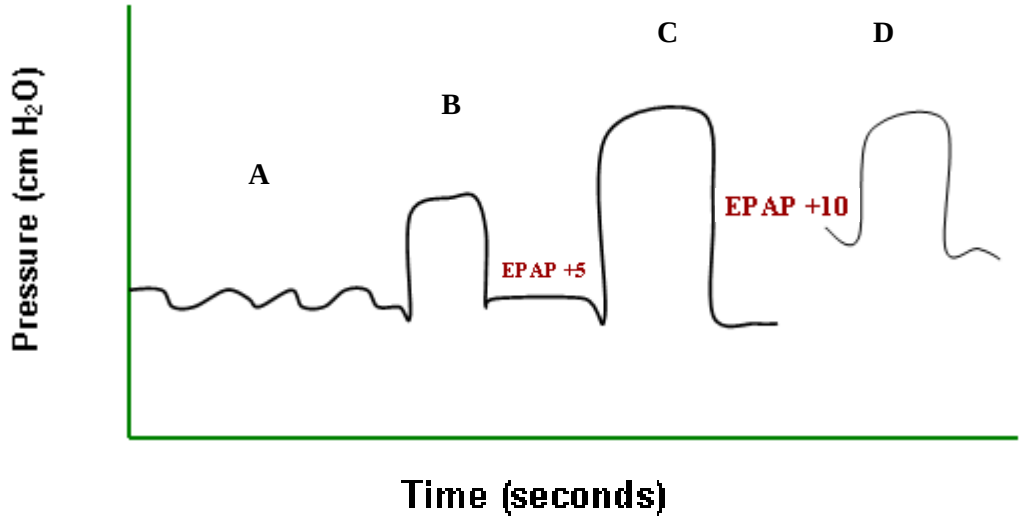


Figure 6: A. continuous positive airway pressure of 5 cm H_2O with a patient breathing spontaneously. **B.** patient-triggered breath during NIV with an IPAP of 10 cm H_2O and EPAP of 5 cm H_2O. **C.** inspiratory positive airway pressure has been increased to 1 cm H_2O. D. inspiratory positive airway pressure remains 15 cm H_2O, but EPAP has been increased to 10 cm H_2O. [Modified from reference 58].

Figure 7 below explains and illustrates how obstructive sleep apnoea occurs and the principle of CPAP operation in resolving the airway obstruction during sleep [32].

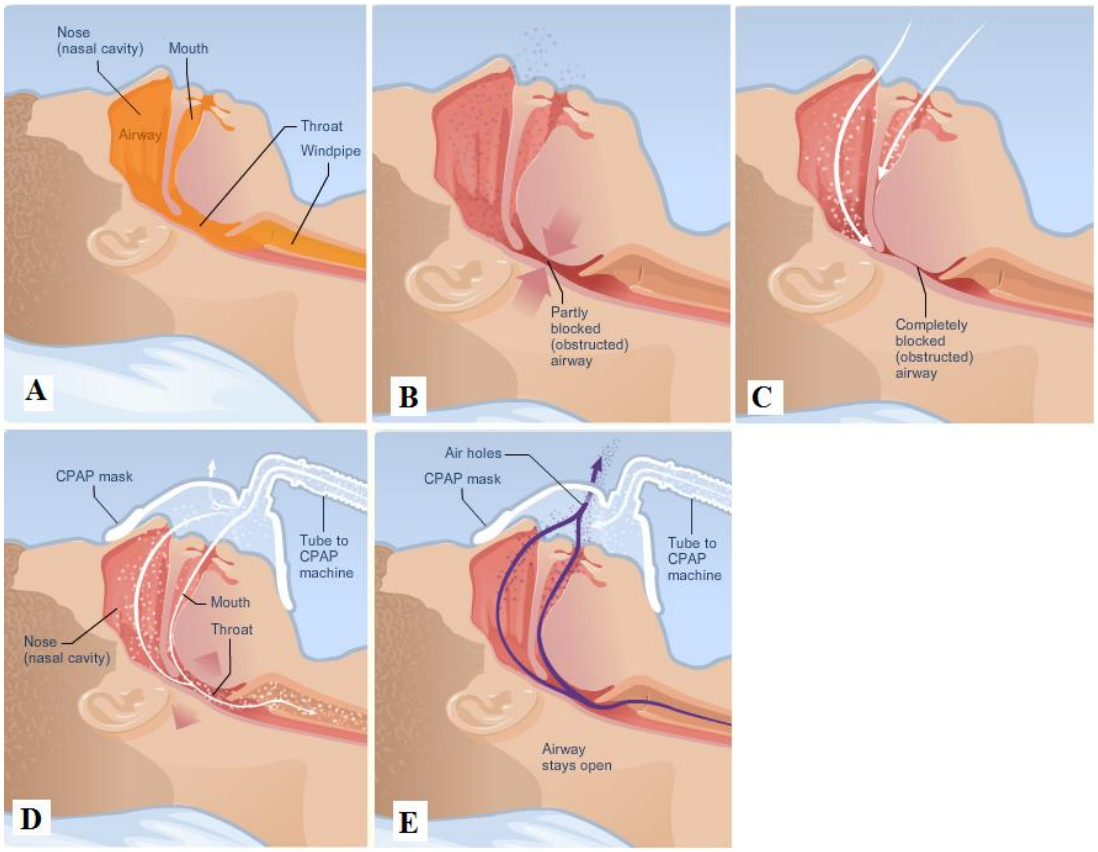


Figure 7: The principle of CPAP operation in resolving the airway obstruction with OSA patient.

A. Normal airways without obstructive sleep apnoea. Patent airways allow the flow of air and oxygen into the lungs during inhalation and the flow of carbon dioxide out of the lungs during exhalation.

B. OSA may be mild to severe. When the airway is partially blocked, it becomes difficult for the air to pass though the upper airways to the lung, which results in snoring.

C. At times when the OSA becomes more severe, the airway may become completely blocked, and this results in no airflow. This is called apnoea.

D. CPAP is the most common treatment for moderate to severe OSA. Here, a CPAP mask fits over the nose and mouth. During inhalation, the CPAP machine works by delivering air through the tube and mask into the nose, mouth, and throat. The pressurised air presses on the walls of the upper airway, keeping it open. This prevents airways from collapsing.

E. During exhalation, the CPAP machine continues to gently deliver air to keep the airway open. The continuous airflow also washes out the exhaled air and carbon dioxide through the mask holes. This process continuous throughout the night with each breath, allowing the patient to breathe uninterrupted during sleep.

1.5 Patient Interfaces for NIV

Patient interfaces have a major impact on patient comfort, clinical effectiveness, and compliance to NIV therapy. Several factors need to be considered when offering interface options to the patient to ensure the interface will feel comfortable and fit properly. **Table 3** summarises the characteristics of an ideal NIV interface [59].

Table 3: Characteristics of an ideal non-invasive ventilation interface and securing system.

Ideal interface
Leak-free
Good stability
Nontraumatic
Light-weight
Long-lasting
Nondeformable
Nonallergenic material
Low resistance to airflow
Minimal dead space
Low cost
Easy to manufacture (for the moulded interfaces)
Available in various sizes
Ideal securing system
Stable (to avoid interface movements or dislocation)
Easy to put on or remove
Nontraumatic
Light and soft
Breathable material
Available in various sizes
Works with various interfaces
Washable, for home care
Disposable, for hospital use

Facial anatomy differs dramatically from patient to patient, therefore, proper selection of the interface size is mandatory to achieve the best clinical results. A variety of NIV interfaces are available, including [59]:

- Mouthpiece: placed between the patients lips and held in place by lip-seal

- Nasal mask: covers the nose but not the mouth
- Nasal pillows: plugs inserted into the nostrils
- Oronasal: covers the nose and mouth
- Full-face: covers the mouth, nose, and eyes [49].
- Helmet: covers the whole head and all or part of the neck; no contact with the face or head [60].

- **Nasal masks:**

The nasal mask is widely used for the administration of CPAP or bilevel positive pressure airway therapy, machines. The standard nasal mask is a triangular or cone-shaped clear plastic device that fits over the nose. This mask utilises a soft cuff to form an air seal over the skin (**Plate 2**).

An alternative type of nasal interface is nasal "pillows" or "seals," which consist of soft rubber or silicone pledgets that are inserted directly into the nostrils (**Plate 3**). Because they exert no pressure over the bridge of the nose, nasal pillows are useful in patients who develop redness or ulceration on the nasal bridge while using standard nasal masks [43].

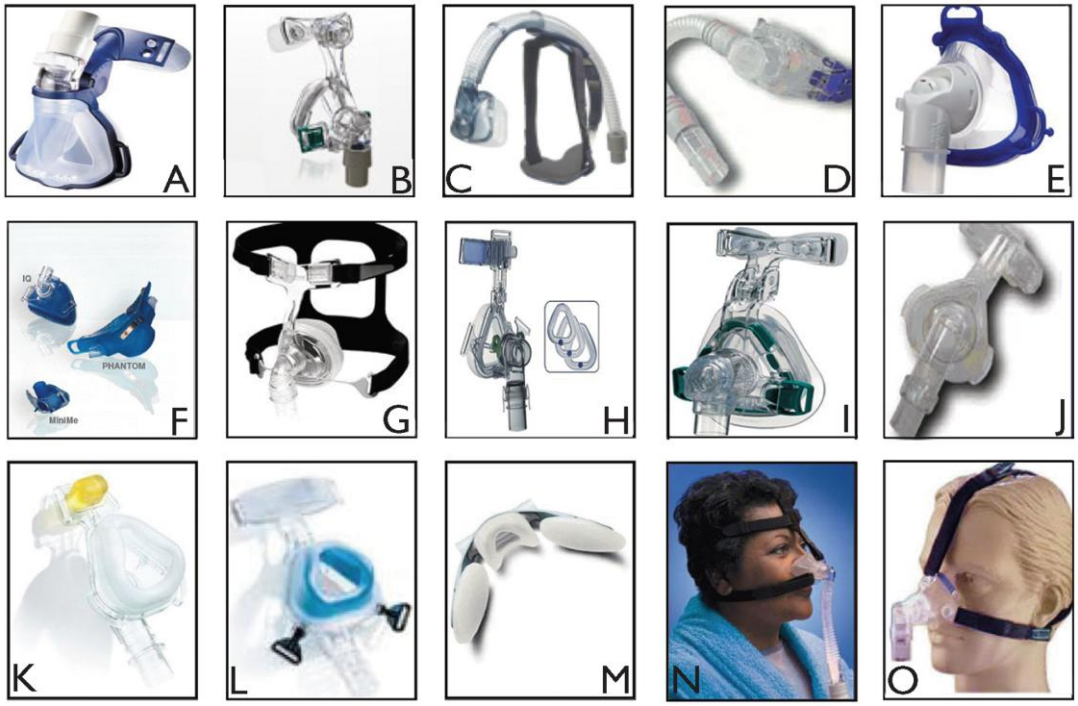


Plate 2: Different types of nasal masks [59].

Plate 3: Different types of Nasal pillows [59].

- **Oronasal and full-face masks:**

Oronasal or full-face masks cover both the nose and the mouth (**Plate 4**). They have been used mainly on patients with acute respiratory failure, as these patients generally breathe through the mouth to overcome or bypass nasal resistance [61].

A full-face mask (**Plate 5**) is made of a soft cuff that seals around the perimeter of the face; therefore there is no pressure on areas that an oronasal mask touches. The frame of the full-face mask includes an anti-asphyxia valve that opens to room air in case of malfunction of the machine when airway pressure falls below 3 cm H_2O [59].

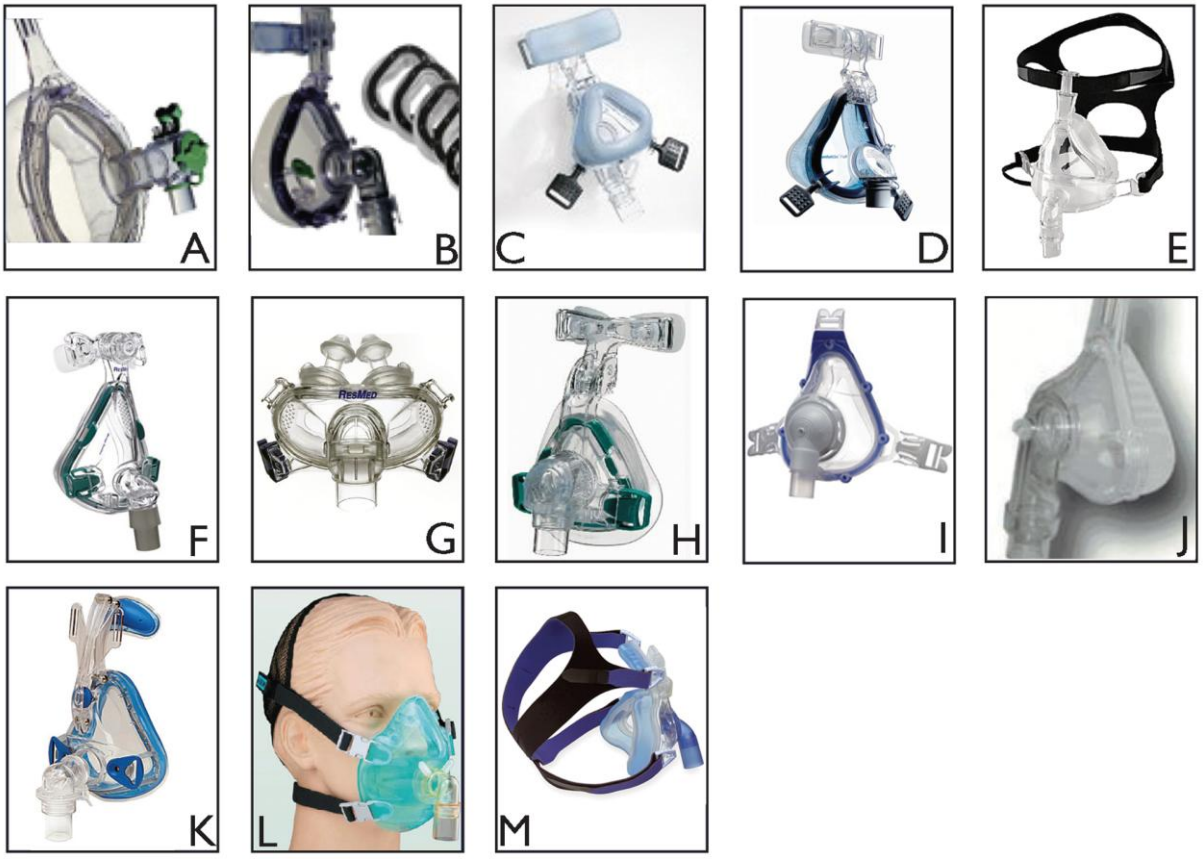


Plate 4: Different types of Oronasal masks [59].

Plate 5: Different types of Full-face masks [59].

- **Mouthpieces:**

Mouthpieces held in place by lipseals have been used since the 1960s to provide NIV therapy with chronic respiratory failure (**Plate 6**) [43]. Mouthpieces are simple and inexpensive. The drawback of this interface is that nasal air leaking affects the efficacy of NIV. Mouthpieces have been successfully used in patients with neuromuscular disease and those with negligible vital capacity, and have enabled tetraplegic patients shift from tracheostomies to NIV [62-63].

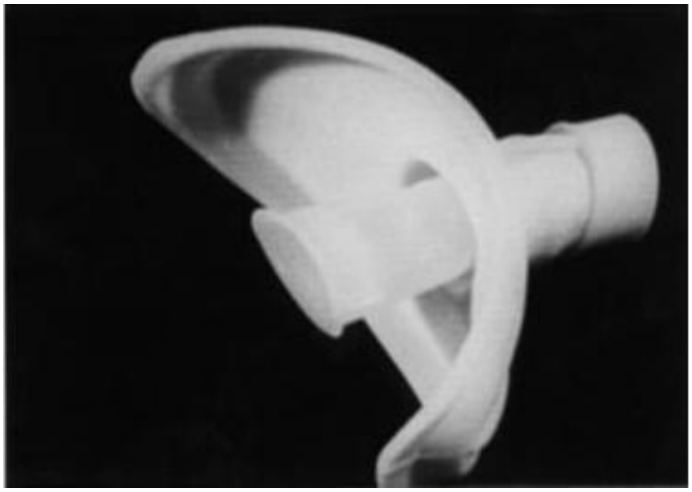


Plate 6: Mouthpiece with lipseal (Mallinkrodt).

Complications Related to the Mask

Studies have been conducted to compare the efficacy of various patient interfaces. Navalesi et al. [64] found that while nasal masks were better tolerated than nasal pillows or oronasal masks, they were less effective at reducing $PaCO_2$. This was attributed to greater air leakage. However, McCormick et al. [65] demonstrated that use of nasal and oronasal masks resulted in comparable decrease in Pa_{CO2} and respiratory rate.

Among all these interfaces, nasal masks are the most widely used for both NIV and CPAP. Nasal masks are preferable for long term ventilation, but have also been used for acute hypercapnic [66] and hypoxemic respiratory failure [67].

Table 4 lists NIV complications related to masks [68]. The incidence of patient complaint due to masks may be as high as 50%, but in most cases, this can be treated with mask adjustment or change of mask [68].

Table 4: Problems related to non-invasive ventilation interfaces.

Problem	Incidence	Solutions
Nasal Mask		
Mask discomfort	30–50%	Decrease strap tension Re-seat mask Try different mask size or type
Skin rash	10–20%	Topical steroids or clindamycin Dermatologic consultation
Nasal-bridge sores	5–10%	Minimize strap tension Use forehead spacer Artificial skin Switch to different mask type
Nasal obstruction	Occasional	Topical decongestant Try oronasal mask
Oronasal Mask		
Mask discomfort	30–50%	Minimize strap tension Try different mask size or type
Claustrophobia	10–20%	Reassure patient Try different mask type
Skin rash, nasal bridge sores	10–20%	Same as for nasal mask sores
More dead space	Depends on mask	Insert foam rubber to reduce dead space

Aspiration of vomit	Rare	Anti-asphyxia valve Quick-release straps
Mouthpiece		
Discomfort	Common	Reassure patient Diminishes with adaptation
Hypersalivation, salivary retention	Common	Reassure patient Diminishes with adaptation
Aerophagia	Common	Reassure patient Simethicone
Pressure sores on lips, gums	Infrequent	Decrease strap tension Consider custom fitting
Orthodontic problems	After prolonged use	Remodel mouthpiece Consult orthodontist
Head Straps		
Discomfort	10–30%	Try different strap system
Unstable mask	Common with 2-strap system	Try different mask or strap system

For this thesis, the Respironics REMstar CPAP device was used for *in vivo* and *in vitro* studies (**Plate 7**). All CPAP was delivered at a fixed pressure of 7.5 or 12.5 cmH_2O for *in vivo* and a fixed pressure of 4 or 7 cmH_2O for *in vitro* studies. This machine is designed for the treatment of adult Obstructive Sleep Apnoea only.

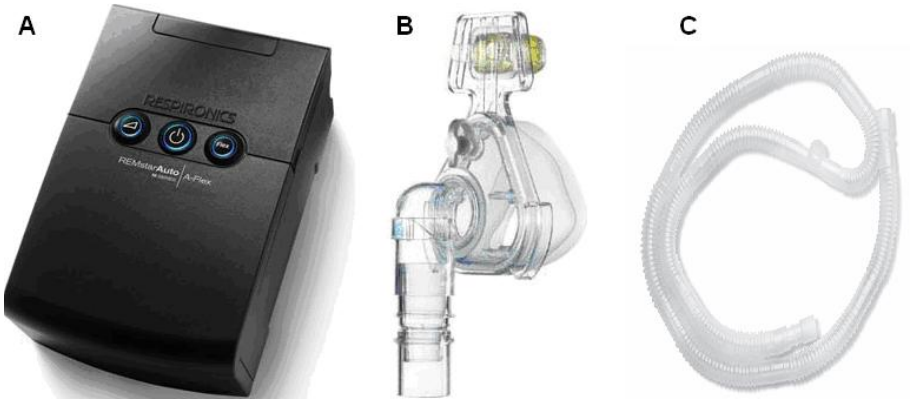


Plate 7: Non-invasive respiratory support modalities used for this thesis. (**A**) Respironics REMstar CPAP. (**B)** Respironics Comfort Classic Nasal mask. (**C)** Disposable air-flow circuit.

1.6 Obstructive Sleep Apnoea (OSA)

1.6.1 Definition

Obstructive sleep apnoea (OSA) is a form of the common disease of sleep apnoea. It has been found to affect 2 to 4% of adult men and about 1 to 2% of adult women [69]. It is characterised by repetitive episodes of upper airway partial or complete pharyngeal obstruction during sleep [70]. **Figure 8** shows the pharyngeal obstruction during sleep and a schematic trace for a polysomnography test of OSA [71-72].

The repetitive closure and opening of airways with this disease causes impaired gaseous exchange [28], recurrent arousals, intermittent hypoxaemia, sleep fragmentation, poor sleep quality, daytime sleepiness, an increase in cardiovascular risk [73-75] and an increased risk of injuries from motor vehicle crashes and industrial injuries [76-77].

The American Academy of Sleep Medicine (AASM) defines obstructive sleep apnoea as 'cessation of airflow during sleep for more than 10 seconds, five or more apnoea episodes per hour of sleep (apnoea–index), and oxygen desaturation of at least 4%' [78].

Severity is assessed by the apnoea-hypopnea index (AHI). OSA can be classified based on the number of partial (hypopnoea) or complete (apnoea) airway obstructions per hour, and AHI is categorised by the AASM as [78]:

- Mild (5–14 events per hour)
- Moderate (15–30 per hour)
- Severe (>30 per hour)

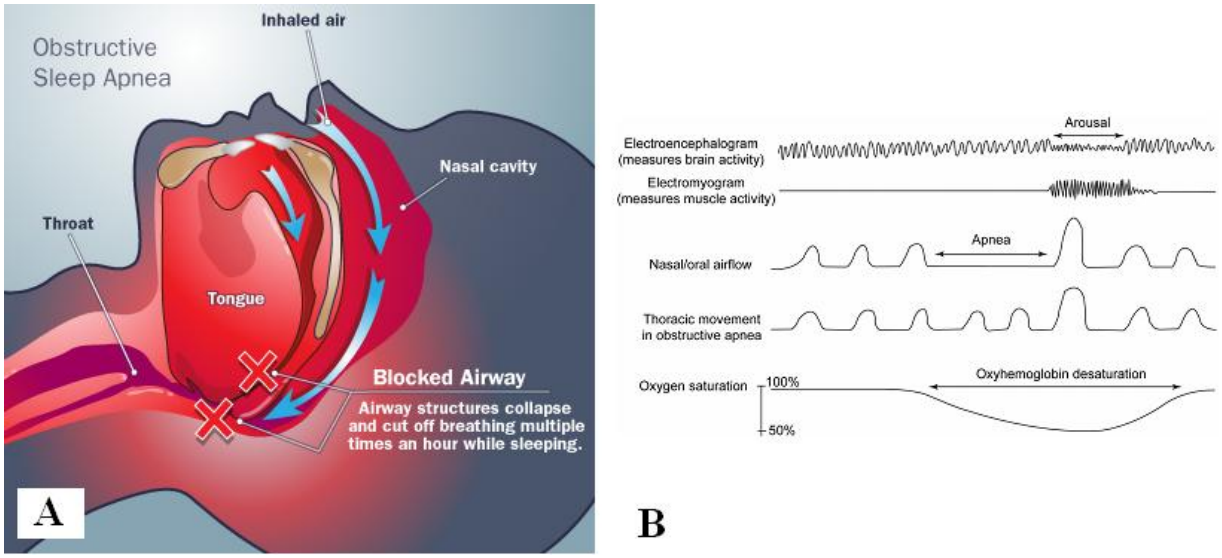


Figure 8: Obstructive Sleep Apnoea. (**A)** upper airway pharyngeal obstruction during sleep [71]. (**B)** Polysomnography tracing illustrating obstructive sleep apnoea events [72].

Mild OSA appears not to be associated with increased risk of mortality, whereas some, but not all, data indicate than moderate OSA is associated with an increased mortality risk. Severe OSA, however, has consistently been associated with a two- to six-fold increase in all-cause mortality [79-80].

OSA has a broad spectrum of signs and symptoms, summarized in **Table 5**. Snoring and excessive daytime sleepiness appear to be the most prominent symptoms.

Table 5: Broad spectrum of signs and symptoms of OSA.

Snoring
Excessive daytime sleepiness
Apnoeas during sleep
Excessive day-time sleepiness
Choking episodes during sleep
Arousals
Irritability and personality change
Headaches
Loss of concentration and impaired memory

1.6.2 Incidence

OSA is a common problem, but an underdiagnosed condition [81]. The magnitude of this problem is underestimated by many health care providers in the medical field. However, the OSA incidence rate is increasing due to its association with obesity, and increased public awareness, resulting in more patients and family members bringing symptoms to the attention of health care providers [82].

OSA is now recognized as a major health issue. There are considerable amounts of prevalence data on OSA from Western countries, but little is known about the incidence (i.e. the occurrence of new cases over a given time interval) or progression (i.e. worsening over time) [83]. The incidence of OSA has never been formally investigated, but it was recently shown that it is the most common diagnosis among patients referred to diagnostic sleep laboratories in the United States, and that it might be especially prevalent among the aged [84-86]. More recently, it was shown that about 1% of an unselected inpatient population admitted to a general hospital in Italy during a 1-year period had OSA [87].

There is considerable variation in the estimates of OSA population due to population heterogeneity, differences in methodology in measuring sleep, and variation in thresholds that differentiate abnormal from normal subjects [88]. The prevalence rates of OSA have been estimated within the range of 2 to 10 percent worldwide [26]. Young estimated that the prevalence of OSA in a group of 602 middle-aged males was 4 per cent, and 2 per cent for middle-aged females, and this is reported to increase with age [83]. Those patients were at the minimal diagnostic criteria for sleep apnoea, that is, an AHI > 5 and 3 symptoms of daytime hypersomnolence. Excessive daytime sleepiness, not feeling refreshed after awakening, and uncontrollable daytime sleepiness, were the 3 symptoms of daytime hypersomnolence used in this study. The prevalence of OSA with daytime symptoms is in the range of 5% [84]. A large epidemiological survey of people aged between 30 to 60 years in the United States showed the prevalence of sleep-related breathing disorder. The disorder, defined by an AHI of 5 or more, was 24% for males and 9% for females [83]. However, the prevalence rates are higher among elderly people, with up to 60% showing disturbances

during sleep [89]. Several studies have shown a prevalence of 60 percent among patients with stroke [90-93], as compared with 4 per cent in the middle-aged adult population [83].

Any attempt to determine the prevalence of OSA in the general population is complicated by the fact that there is no definition of the syndrome upon which there is general agreement. Although it has been suggested that OSA be defined by the occurrence of at least 30 apnoeas, with each apnoea of at least 10s duration in 7h of sleep, or by the occurrence of at least 5 apnoeas in each hour of sleep [94], there are data suggesting that these criteria are too permissive, since multiple sleep apnoeas can occur in normal individuals who are completely asymptomatic [95]. Much more information about the incidence of sleep apnoea among normal individuals is needed before an adequate definition of OSA can be provided [74].

The health system in Saudi Arabia relies on the referral system, where the patient is usually first examined by the primary health care physician, who assesses and decides the patient's plan of management. Therefore, early detection and management of patients with sleep disorders depends on the significant knowledge and awareness of physicians.

In Saudi Arabia, sleep medicine as a specialty is relatively new, hence the prevalence of sleep disorders among the Saudi population is not well investigated and studies that address the size of this problem are limited. The available data indicate that sleep disorders are prevalent among Saudis [96]: 3 out of 10 Saudi men and 4 out of 10 Saudi women are at high risk of OSA [97]. Other reported sleep disorders are snoring in 17.9% of children, narcolepsy in 40/100,000 Saudis, and 5.2% for restless legs syndrome [97].

Previous studies have reported high incidences of side effects during long-term therapy, which approached 97% in a large series [98].

1.6.3 Risk Factors for OSA

There are several risk factors that could predispose an individual to OSA. There is increasing evidence that OSA is being considered as an independent risk factor for hypertension, glucose intolerance/diabetes mellitus, cardiovascular diseases and stroke, leading to increased cardiometabolic morbidity and mortality. The risk factors for OSA include advanced age, male sex, obesity, family history, craniofacial abnormalities, smoking, alcohol consumption [26], and depression [99]. Moreover, there is increasing evidence that OSA is an independent risk factor for an adverse cardiometabolic profile [100]. It has been associated with vascular risk factors [87] and increased cardiovascular and cerebrovascular morbidity and mortality, although much of the causal role and mechanisms are still poorly understood [101].

A summary of the different risk factors for OSA is given below:

- **Snoring:** It is a risk factor that has been examined in association with OSA. A study by Woodhead showed that 35 patients with a history of loud snoring were diagnosed with OSA after being examined by a sleep study. It was found that 16 (46%) of the 35 participants demonstrated OSA [102].

- **Obesity and body-mass index (BMI) greater than 30 kg/m^2**: It is a primary risk factor for OSA. Several studies have reported that there were association between obesity and OSA. According to the Wisconsin Cohort Sleep study, a 10% increase in weight is associated with a 6-fold risk of developing OSA [103]. About two-thirds of patients with OSA are more than 20% above their ideal body weight.

- **Male gender:** There is a two- to three-fold increased risk for OSA in men, related to anatomical and sex hormone differences [104].

- **Familial association/genetics**: Mendelian and familial inheritance patterns of upper airway craniofacial structures predisposing to OSA have been reported [105].

- **Alcohol consumption**: Acute consumption may induce apnoeic events in normal subjects and worsen the severity of OSA in established patients [104].

- **Cranial facial structure/craniofacial abnormalities**: High and narrow hard palate, elongated soft palate, small chin, and abnormal overjet [106]. Imaging techniques have described the craniofacial features of OSA patients. These include a reduction in the length of the mandible, an inferiorly positioned hyoid bone, a retroposition of the maxilla, retrognathia, nasal septal deviation, and narrowing of the hard palate [107].

- **Upper airway soft tissue abnormalities**: Macroglossia, lateral peritonsillar narrowing, soft palate enlargement, and tonsillar hypertrophy alter the configuration of the luminal airway, increasing the risk of OSA [108].

- **Age:** Older subjects (i.e. > 45 years of age) have more than three times the increased risk of having OSA. The relationship between age and OSA has been studied by various researchers, yielding disparate results with a suggestion of a rise in the prevalence of OSA with age [109]. Some researchers [83,110-111] have concluded that this effect occurs only up to middle age and that age ceases to be an independent risk factor for OSA beyond middle age. However, population studies indicate a higher prevalence of OSA in older subjects (aged 60 years and above) compared with middle-aged groups [104].

    - **Endocrine system:** OSA is more common in hypothyroid (especially myxedema) and acromegaly patients [112]. In cases of hypothyroidism and acromegaly, OSA is mainly related to UA narrowing due to reversible thickening of the pharyngeal walls [113].

    - **Race:** OSA is more common among African Americans, Mexican Americans, Pacific Islanders, and East Asians [114-115]. Moreover, when compared with white men, Far East Asian men were less obese but had greater severity of OSA [116].

According to the AASM, the following risk factors that increase the likelihood of having obstructive sleep apnoea are [82]:

- Obesity
- Congestive heart failure
- Atrial fibrillation
- Treatment refractory hypertension
- Type 2 diabetes
- Nocturnal dysrhythmias
- Stroke
- Pulmonary hypertension
- High-risk driving populations
- Preoperative for bariatric surgery
- Patients undergoing upper airway surgery for snoring

1.6.4 Geography

Geographically, OSA prevalence has been established in few populations other than the Western nations. Therefore, the worldwide importance of OSA and its importance racially or ethnically prevalence patterns of OSA, is poorly understood. Epidemiologists have investigated geographical distributions of disease occurrence to find aetiologic answers, but it is always hard to disentangle environmental risk factors, including cultural differences in diet and lifestyle, from genetic factors. At current, avaliable data from studies of groups other than white subjects are too sparse even to determine with confidence if prevalence differs worldwide [84].

However, in spite of the limitations, different studies have concluded that OSA is prevalent in the United Kingdom [117], Northern Europe [118-120], Australia [110], and the United States [83, 121]. The estimated prevalence of symptomatic OSA in the United States in the early 1990s by Young et al. [83] was 4% among adult men and 2% among adult women. Since then, the adult prevalence rates of sleep-disordered breathing have become available in many different countries after large-scale epidemiological studies (**Table 6**) [83, 99, 109-

110, 122-128]. Interestingly, the prevalence of OSA in developing countries such as India and China is in the same order of magnitude as that in the developed countries, despite less obesity [129]. Moreover, the prevalence of OSA is similar in both Caucasians and Asians; this indicates that OSA is not only common in developed countries, but also in developing countries. Inter-ethnic studies suggest that African-American ethnicity may also be a significant risk factor for OSA [26]. The increased prevalence of OSA among American Indians and Hispanic adults, and increased severity among Pacific Islanders and Maoris, have mainly been explained by the increased obesity indices [130].

Table 6: Recent studies on the prevalence of OSA in different ethnic groups.

Reference	Study population	Age, yr	Prevalence (%)
Young et al. [83]	American men and Women	30-60	Men: 4*-25[#] Women: 2*-19[#]
Bixler et al. [131]	American men	20-100	17[#]
Bixler et al. [123]	American men and women	20-100	Men: 3.9* Women: 1.2*
Duran et al. [124]	Spanish men and women	30-70	Men: 14*-26[#] Women: 7*-28[#]
Ip et al. [132]	Chinese men	30-60	4.1*-8.8[#]
Ip et al. [125]	Chinese women	30-60	2.1*-3.7[#]
Kim et al. [126]	Korean men and women	40-69	Men: 4.5*-27[#] Women: 3.2*-16[#]
Udwadia et al. [127]	Indian men	25-65	7.5*-19.5[#]
Sharma et al. [109]	Indian men and women	30-60	Men: 4.9*-19.7[#] Women: 2.1*-7.4[#]

*OSA syndrome is defined as apnoea-hypopnoea index ≥ 5 with excessive daytime sleepiness; [#] OSA is defined as apnoea-hypopnoea index ≥ 5. All these prevalence studies were assessed with standard polysomnography.

However, in Saudi Arabia sleep medicine specialty is relatively new; hence, the prevalence of sleep disorders among the Saudi population is not well investigated and studies that address the size of this problem are limited.

Table 7 shows the status of sleep medicine activity in Saudi Arabia compared to other countries [97]. Four sleep facilities have the complete setup and staffing to perform the sleep studies. One of those facilities had certified technicians, but the other facilities had different backgrounds (respiratory therapists, nurses, and electroencephalography (EEG) technicians).

Table 7: Sleep Medicine in Saudi Arabia compared to other countries.

Country	Population	No. of sleep facilities	No. of sleep beds	No. of beds/100,000	No. of studies/ year	No. if studies/year/ 100,000
Saudi Arabia	21,500,000	9	14	0.06	1,536	7.1
United States	280,000,000	1,292	-	-	1,170,000	427.0
Canada	31,400,000	100	440	1.4	116,000	370.4
Australia	18,970,000	65	244	1.3	53,500	282.0
Belgium	10,000,000	50	150	1.5	17,716	177.2
Spain	40,341,462	63	-	0.29	17,270	45.6
United Kingdom	58,800,000	84	170	0.3	25,000	42.5
Japan	126,686,000	146	-	-	23,184	18.3

Bahammam et al. [96] demonstrated that the lack of trained sleep technicians able to perform polysomnography tests was the most important obstacle to the provision of sleep facilities for 80% of the surveyed hospitals. Other important reasons are shown in **Figure 9** from the same study, where the data were collected from 53 major governmental and private hospitals and medical centres in Saudi Arabia. This study also showed that only 19 qualified sleep medicine physicians were located in a few hospitals in three major cities in Saudi Arabia. This number is considered extremely low in a large country geographically considered as the world's 13th largest state, with an estimated population of 27 million.

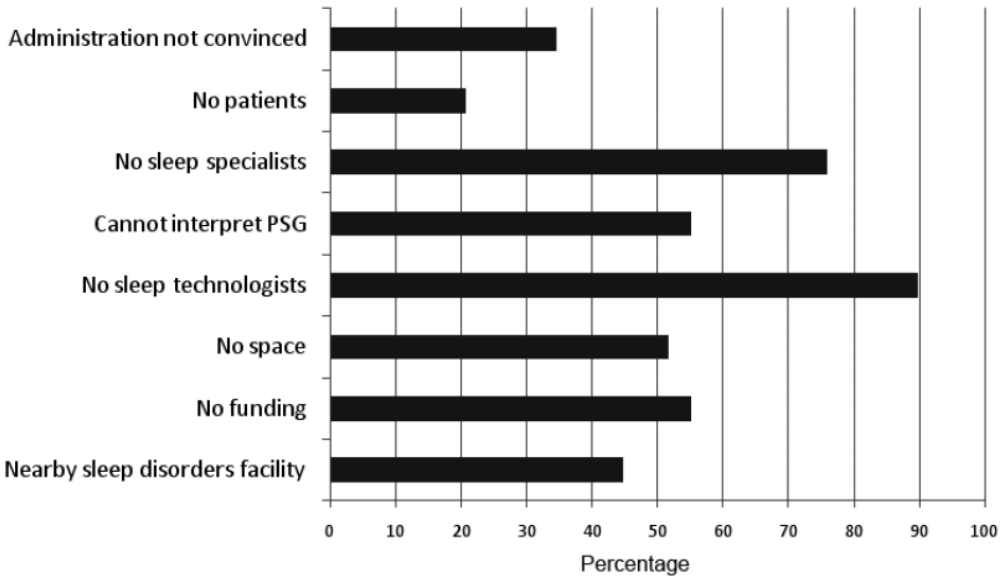


Figure 9: Important factors contributing to lack of sleep medicine practice in Saudi Arabia [96].

Sleep medicine specialty is still underdeveloped in Saudi Arabia in comparison with other countries. More serious effort needs to be made at all levels in order to overcome the challenging obstacles (addressed in the current studies) to the progress of sleep medicine in Saudi Arabia.

1.6.5 Aetiology

The aetiology of OSA is somewhat complex and misunderstood, but is thought to arise from a combination of anatomical and pathophysiological factors, which predispose to narrowing of the upper airway [133].

OSA results from a physical obstruction occurring in the upper airway (during which there are still attempts to breathe during sleep), as a result of anatomical and physiological abnormalities in pharyngeal structures [99]. The spectrum of obstructive sleep-disordered breathing has historically been described as ranging from simple snoring, through upper airway resistance syndrome (UARS), to OSA. This concept also implies that, over time, an

individual progresses from being normal to developing simple snoring, then UARS and then finally OSA. The repeated occurrence of obstructive apnoeas overnight leads to a predictable pattern of events: a reduction of inspiratory airflow leading to episodic hypoxemia and hypercarbia, an increase in respiratory effort, arousals in order to restore normal respiration, fragmented sleep, and in most cases excessive daytime sleepiness [134]. The recurrent episodes also increase the output of the sympathetic nervous system activities [135], the effect of which is to restore pharyngeal muscle tone and reopen the airway. These events, which are mediated by a variety of chemoreceptors in the carotid body and brainstem, trigger pathophysiological changes that occur not only in the obstructive sleep apnoeas, but also extend into wakeful states during the daytime. For example, during daytime wakefulness, an individual with OSA has higher sympathetic activity [136] and heightened chemoreflex sensitivity, which generates an increased ventilatory response [137]. However, the full pathophysiology of OSA remains somewhat unclear, although the relationships between OSA and a range of other long-term health effects are being pieced together [99].

1.6.6 Pathogenesis

It has been known for some time that sleep apnoea is characterised by recurrent collapse of the upper airway during sleep [138]. Most evidence would suggest that a variety of features can lead to OSA, and that the combination of these features leading to this disorder in any particular patient may vary considerably (**Table 8**) [139].

Table 8: Phenotypic traits contributing to sleep apnoea pathogenesis.

Anatomic/Physiologic Trait	Apnoea Predisposition
Upper airway anatomy	Small pharyngeal airway
Upper airway motor control	Poor muscle responsiveness during sleep
Ventilatory control stability	High loop gain
Lung volume	Low sleeping lung volume
Arousal threshold	Low respiratory arousal threshold

Patients with OSA have smaller and more collapsible airways than normal. In the awake state, the airway is patent because of pharyngeal dilator muscle activation. However, in the sleep state, the activity of those muscles is markedly diminished [140] and lung volume decreases in the supine position, causing substantial restriction in airflow and resulting in hypopnea or apnoea [141]. This mechanical load and CO_2 retention activate the pharyngeal muscles in order to restore upper airway patency [142-143]. Recruitment of pharyngeal dilator muscles can be achieved and upper airway patency is restored without a cortical arousal in some individuals, whereas, in others, the arousal is required for effective muscle recruitment; this phenomenon has been described as compensatory effectiveness [144]. Indeed, after each apnoea, patients with OSA hyperventilate, CO_2 drops to the apnoeic threshold, expiratory time becomes prolonged, and the upper airway may collapse again. Moreover, several reports have described decreased responsiveness of the pharyngeal muscles to negative upper airway pressure, due to damage to the sensory nerves in the upper airway or to the muscle itself. This damage may be due to inflammation caused by vibration, snoring, or trauma [145-148]. Data also support the notion of ventilatory variability at sleep-wake transitions as a predictor of OSA severity [149]. In addition, humoral and inflammatory mediators released from the visceral adipose tissue may play a role in ventilatory regulation. The most studied is leptin, which binds to its receptors in the hypothalamus, causing reduced satiety and increased ventilation [150]. Leptin stimulates respiratory drive, as shown in leptin-deficient or resistant mice that exhibited features of central hypoventilation and obesity [151]. Overall, there are a variety of ventilatory control mechanisms that might predispose to an unstable upper airway, including alterations in loop gain, impairments in upper airway motor and neural control, and responsiveness, as well as humoral mechanisms.

Hypercapnia at night is an important matter in OSA, especially if chronic obstructive pulmonary disease **(**COPD**)** is present. This is an overlap syndrome, and there is increasing evidence that both diseases cause inflammation and create oxidative stress. McNicholas et al. [152] showed how both diseases cause systemic inflammation consequences resulting in cardiovascular disease (**Figure 10**).

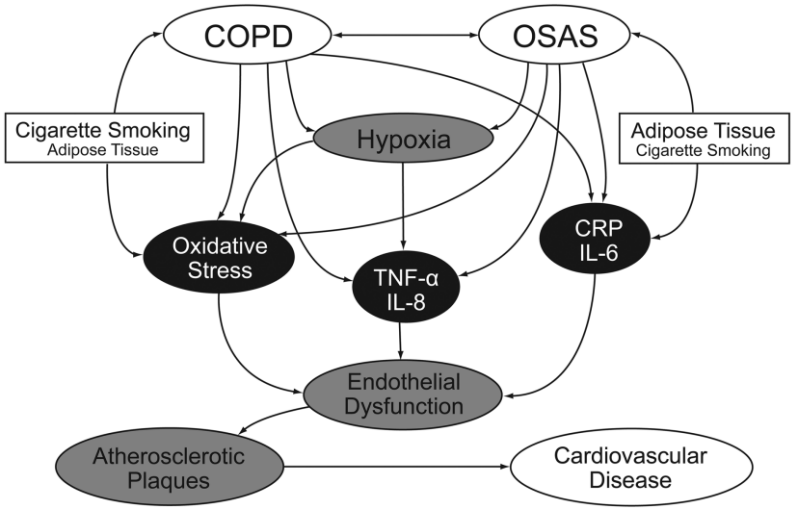


Figure 10: Both COPD and OSA are systemic inflammatory disorders that cause cardiovascular disease via various common pathways.

1.6.7 Pathology

The primary pathophysiologic event that occurs in OSA is complete obstruction or partial obstruction of the pharynx during sleep. When an individual is awake, pharyngeal muscles are sufficiently activated to maintain patency of the upper airway, while, during sleep, there is decreased activation of this musculature [153]. During sleep, the uvula and soft palate collapse on to the back wall of the upper airway, then the tongue falls backward causing a collapse on the back wall of the upper airway. The uvula and soft palate cause a tight blockage and prevent inspired air from entering the lungs. The effort of the diaphragm, chest, and abdomen cause the blockage to seal tightly. To breathe, the individual must arouse or awaken, causing tension in the tongue, which results in opening of the airway and allowing air to pass to the lungs [154].

Moreover, in OSA patients, during normal breathing, the narrowing of the upper airway progresses to a complete collapse during inspiration, or at end-expiration. When patients exert increased breathing effort to inspire against the obstructed airway, it makes the situation worse by creating more negative airway pressure. Airway obstruction continues until arousal occurs and the result is increased tone of the pharyngeal muscles, which then reopen the airway. These 'micro-arousals' may occur at rates of more than 90 per hour, whenever an apnoeic or hypopnoeic event disrupts sleep, with patients usually unaware of these events. When these apnoeic events occur, they can result in significant oxygen desaturation and decrease the amount of sleep (necessary for physical and psychological restoration) [153].

Sleep apnoea causes a drop in the blood oxygen saturation (SaO_2) and an increase in the blood's CO_2. The drop in SaO_2 causes the heart rate to decrease. If the SaO_2 continues to drop, the heart rate will slow until hypoxia is corrected. However, as the CO_2 increases, the brain will try to increase the drive to breathe. **Figure 11** illustrates the sequence of typical pathophysiological events occurring with OSA [155].

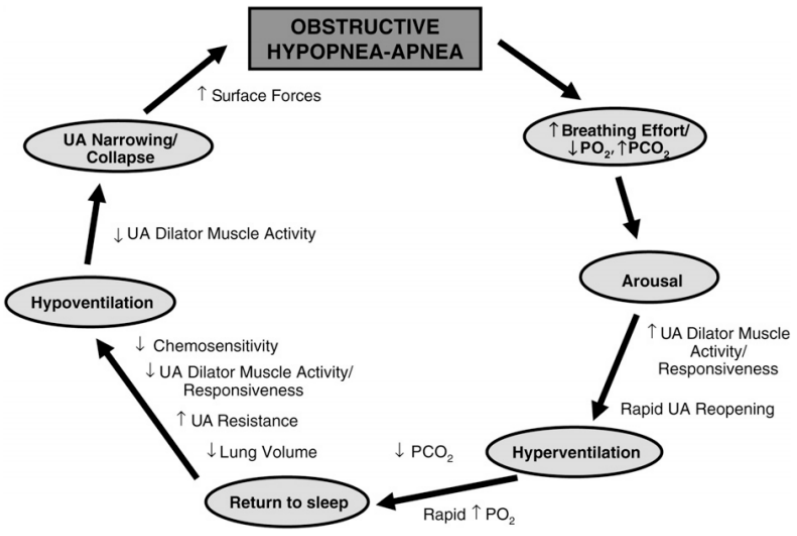


Figure 11: Schematic representation of the typical pathophysiological sequence that occurs in OSA (shown in grey) and the associated physiological processes.

1.6.8 Prognosis

The prognosis of treated OSA is unclear [156]. Studies on the short-term prognosis for OSA have revealed better results in daytime sleepiness and snoring in regular users of continuous positive airway pressure (CPAP). These studies showed improvement in cognitive function and general health status after four to eight weeks of CPAP treatment [157-159]. However, the long-term prognosis of untreated severe OSA is increased mortality, poor quality of life, an increase in the likelihood of motor vehicle accidents, hypertension, and cardiovascular consequences [160].

Limitations of the evidence on OSA prognosis include bias in the selection of participants, short follow-up duration, and variation in the measurement of smoking history, alcohol consumption and other cardiovascular risk factors.

Observational studies support a causal association between the increased severity of OSA and systemic hypertension [160]. OSA patients with developed cardiovascular disease had a significantly higher respiratory disturbance index (RDI) than patients with normal RDI [161-163]. Campos-Rodriguez et al. [164] showed that severe OSA is associated with cardiovascular death in women, and adequate CPAP treatment might help to reduce the cardiovascular consequences. The increased risk of motor vehicle accidents has also been associated with OSA as three- to seven-fold, and OSA has been associated with increased risk of premature mortality and cardiovascular diseases [160].

Campos-Rodriguez et al. [165] showed that for patients who used CPAP for more than 6 hours per night there was an increased survival rate at 5 years (96.4%), compared with those who used CPAP for 1 to 6 hours per night (91.3%) and 85.5% for those patients who used CPAP for less than 1 hour per night. OSA patients with a history of ischemic stroke who regularly used CPAP had decreased mortality, compared with those who did not use their CPAP treatment [166]. Baguet et al. [167] reported that left ventricular diastolic dysfunction is common in these patients and is related to the severity of oxygen desaturation.

OSA could trigger an inflammatory metabolic syndrome (increased IL-6, CRP, tumour necrosis factor, adhesion molecules, leptin, insulin, and nitrotyrosine) independently of

obesity or age [168-171]. Some data showed that these endocrine and cytokine alterations are improved with CPAP treatment [172]. However, we showed in our short-term use of CPAP with normal subjects that three hours is enough to induce airway and systemic inflammation [173].

With all the evidence suggesting that OSA is an independent risk factor for the development of cardiovascular disease and mortality, no definitive randomized studies have investigated the effect of CPAP in preventing the potential cardiovascular risks and consequences.

1.7 CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

1.7.1 Definition

COPD is a heterogeneous disease that is characterised by poorly reversible expiratory airflow limitation. The expiratory airflow limitation tends to be progressive and is associated with abnormal inflammatory consequences due to exposure to noxious particles or gases [174].

COPD has been recently defined by the Global Initiative of Obstructive Lung Disease (GOLD) [174] as:

> 'a common preventable and treatable disease […] Characterised by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients.'

COPD is a leading cause of morbidity and mortality in the world. According to the WHO's Global Burden of Disease and Risk Factors project in 2001 [175], COPD was the fifth leading cause of death in high income countries, accounting for 3.8% of deaths, and the sixth in low and middle income countries, accounting for nearly 5% of total deaths. In the United Kingdom, COPD is the fifth most common cause of death [176] and a major cause of morbidity due to exacerbations of the disease [177].

COPD is most commonly caused from tobacco smoking, which triggers an abnormal inflammatory response in the lung [178]. It is usually observed in patients who are heavy cigarette smokers. COPD is more common in men than in women, probably because there are greater number of men smokers than women smokers. COPD has a lifetime risk and its comorbidities include ischemic heart disease, coronary artery disease, hypertension, anaemia, chest infection, diabetes, congestive heart failure, active pulmonary tuberculosis, and lung cancer [179-180]. Patients with COPD normally present with daily symptoms of cough and sputum production, and/or breathlessness.

Assessment of COPD severity is based on the acuteness of its symptoms, spirometric abnormalities, and the presence of complications. GOLD classify the severity of COPD according to the spirometric results (**Table 9 A-B**) [174].

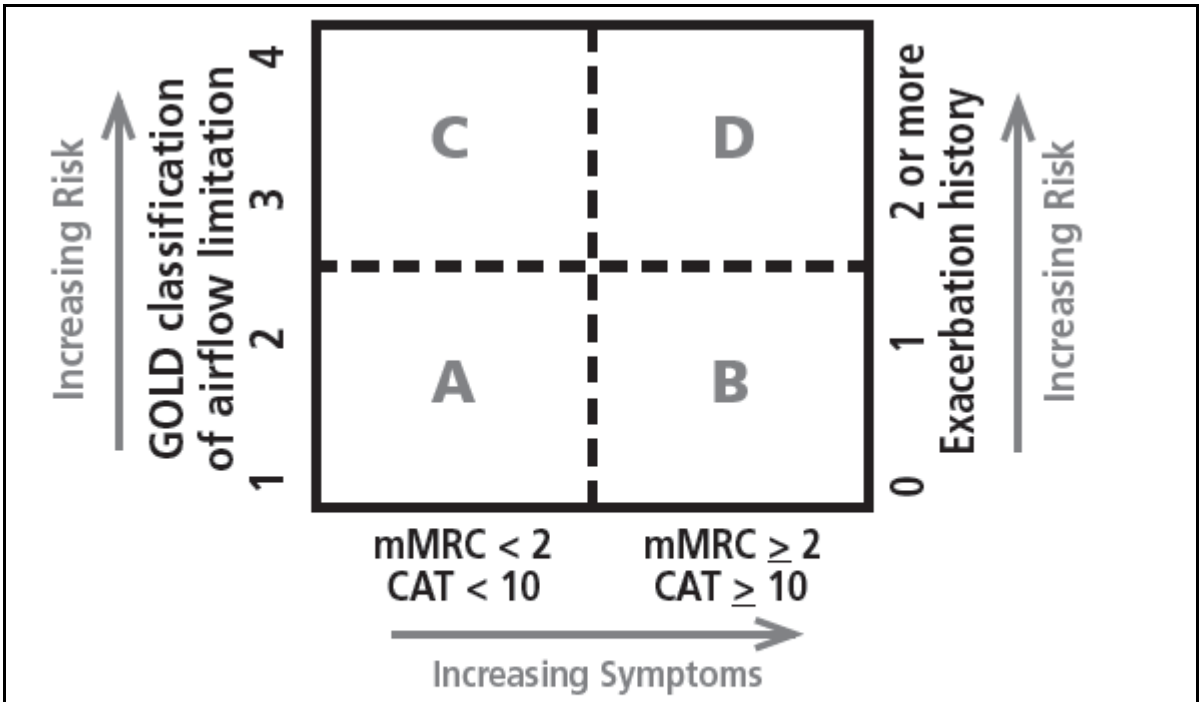
Table 9-A: GOLD classification of COPD Severity.

STAGE	CRITERIA
GOLD 1: Mild	FEV_1[#]/FVC* <70%, FEV_1 ≥80% predicted
GOLD 2: Moderate	FEV_1/FVC <70%, 50% ≤ FEV_1 <80% predicted
GOLD 3: Severe	FEV_1/FVC <70%, 30% ≤ FEV_1 <50% predicted
GOLD 4: Very Severe	FEV_1 < 30% or FEV^1 < 50% and the presence of chronic respiratory failure

[#] FEV_1 = Forced Expiratory Volume in One Second; *FVC= Forced Vital Capacity

However, GOLD has provided an update to the COPD strategy by introducing a more complex multi-dimensional classification. It is not only a spirometric classification. The new model of symptom/risk evaluation of COPD adds two additional variables for assessments: exacerbation history and a modified Medical Research Council dyspnoea score. Based on these factors and the GOLD severity grade, patients fall into one of four quadrants (A, B, C, or D) on a 3-axis grid (**Table 9-B**) [174].

Table 9-B: A new model of the GOLD classification of COPD.



Patient Category	Characteristics	Spirometric Classification	Exacerbations per year	mMRC	CAT
A	Low Risk, Less Symptoms	GOLD 1-2	≤ 1	0-1	<10
B	Low Risk, Less Symptoms	GOLD 1-2	≤ 1	≥ 2	≥10
C	High Risk, Less Symptoms	GOLD 3-4	≥ 2	0-1	<10
D	High Risk, Less Symptoms	GOLD 3-4	≥ 2	≥ 2	≥10

COPD is associated with increased upper airway inflammation. Both upper and lower airway inflammation has shown a proportional relationship in those patients. This relationship has provided further evidence of pan-airway involvement in COPD [181]. Bilevel positive pressure airway therapy has been shown to be an effective modality and a recognised intervention in the treatment of acute hypercapnic respiratory failure due to exacerbations of COPD, in addition to some stable conditions [57, 62, 182]. However, the effect of this modality with stable COPD patients was not investigated. This thesis will investigate and discuss the effect of bilevel positive pressure airway therapy with stable COPD in Chapter Seven.

1.7.2 Incidence

COPD is a leading cause of chronic morbidity and mortality worldwide. The available data for the incidence rates of COPD vary [183-184]. This could be due to differing disease criteria [185], and to population differences and environmental factors. The incidence and prevalence rates of COPD show alarming statistics [186-188].

Few studies have shown the estimated incidence numbers for COPD. In the United Kingdom (UK), a study [189] that examined newly diagnosed COPD patients aged between 60–85 years, using the UK General Practice Research Database (GPRD), found the overall annual incidence of COPD to be 7.2 per 1000 persons in the general population. Another recent study [190] established the overall incidence of COPD among patients aged between 40-89 years to be 2.6 per 1000 persons per year. This study showed that the risk of COPD diagnosis is higher for current smokers and those with low BMI, and is reduced by 40% for former smokers compared with current smokers.

In the United States, recent data estimate that 16 million Americans are affected by COPD: 14 million have chronic bronchitis, and 2 million have emphysema [174, 186]. It is estimated that there may be in addition an equal number of American citizens with undiagnosed COPD.

The incidence of COPD varies greatly between countries as estimates are reported in different units and over different lengths of time. Most studies reported that the incidence of COPD was greater in men than in women [191-194] and the incidence rate was also greater in older people, particularly in those aged 75 years and older [190, 195].

In Saudi Arabia, unfortunately the COPD incidence rate is not known because of the lack of population-based studies. AlGhobain et al. [196] reported that 14.2% of the population of smokers in a study had COPD (71 out of 501); this incidence rate is similar to other reported data in other countries. Smoking is the main risk factor in Saudi Arabia and the rate of smoking is steadily increasing among Saudis. Data are lacking not only for the incidence rates of COPD in Saudi Arabia, but also for the severity of the disease.

1.7.3 Risk Factors for COPD

The risk factor for the development of COPD is related to an interaction between environmental exposures and genetic factors. The two most common risk factors for COPD are cigarette smoking, which accounts for 80% to 90% of all COPD related mortality, and $alpha_1$-antitrypsin (AAT) deficiency [197].

The COPD risk factors are of two types: exposure to noxious stimuli (external environmental factors) and host factors (inherent in the patient) (**Table 10**) [174]. Cigarette smoking is the most well-known important risk factor identified for COPD; however, it is not the only factor, as there is suggestive evidence from epidemiologic studies that non-smokers may also develop this disease [198-199]. Data from the Lung Health Study highlighted the accelerated decline in FEV_1 in smokers, compared with former smokers who achieved 'sustained quitting' [200-201].

The second well-studied (genetic) risk factor causing COPD is AAT deficiency. AAT deficiency plays a major role as a circulatory serine protease inhibitor whose deficiency or imbalance with respect to serine proteases in human body causes COPD [197]. Other host factors that contribute to the increased risk of COPD include asthma, airway hyperresponsiveness, respiratory illness during early childhood, and reduced lung growth [202].

Although host factors cannot be modified, exposure to noxious stimuli can be prevented and controlled. This includes cigarette smoking, occupational and chemical irritants, indoor and outdoor pollution, infections, and socioeconomic status [203-205]. Poor socioeconomic conditions play an important role in causing a substantial health risk for COPD, as they reduce patients' access to healthcare, reduce treatment availability, offer fewer opportunities for high levels of education, and reduce the standards of housing and related utilities [206-207].

Table 10: Risk factors for COPD [174].

Exposure Factors	Host Factors
Tobacco smoke, especially cigarette smoking	Genetic factors such as *alpha$_1$-antitrypsin deficiency*
Occupational and chemical irritants	Airway hyperresponsiveness such as immunoglobulin E and asthma
Indoor and outdoor air pollution	Reduced lung growth
Infections	
Socioeconomic status	

1.7.4 Geography

Globally, COPD is a major cause of morbidity and mortality [208]. Geography plays an important role in determining the risk factors of COPD, and provides an understanding of a population's general health, and the environment's effect on health and disease. In the general population, the incidence of COPD is estimated to be approximately 1% across all ages, rising from 8 to 10% or higher in ≥40 years of age [209]. However, many others remain large regions in the world with no actual data contributing to these estimates [210].

Geography can also be a factor in accessibility to health care [211]. However, not all diseases are geographically specific. Diseases such as COPD are not restricted by particular geographies, as they occur as a result of common factors such as smoking, air pollution, diet and oxidative stress [174]. Potential smokers and polluting agents are found in almost all parts of the world, and thus the incidence of COPD worldwide is of no surprise. In the UK, geographic and temporal variations in smoking do not entitely account for variations in COPD mortality over the last century [212-214]. In the United States, geographic data showed that there is discrepancy between population smoking prevalence and COPD hospitalization rates [215]. This discrepancy suggests that environmental risk factors could have geographically differential relationships with severe COPD hospitalization risk. In

western states of the US (e.g. North Dakota, Montana and Wyoming), the prevalence of smoking was found to be high, but hospitalization rates for COPD were low [216]. However, Brown et al. [217] have described significant state-level geographic variations in COPD mortality across the United States, which could be consequences of different regional environmental exposures.

In Saudi Arabia, there are limited data on the prevalence of COPD across the country. Despite the high rate of smoking in Saudi Arabia, no study has shown the magnitude of COPD prevalence among the Saudi population. The only available data are small and hospital-based studies, which are not geographically extensive enough to conclude significant results on the extent of the problem [218-219].

1.7.5 Aetiology

COPD as a disease is heterogeneous, as are the exacerbations. Despite the wide scientific interest in these episodes, there is still debate regarding how exacerbations should be defined, their aetiology, and their prognostic significance [220]. Tobacco smoking is the main risk factor for COPD. It is responsible for 90% of COPD cases and its effects lead to an inflammatory response, cilia dysfunction, and oxidative injury.

Occupational exposure, air pollution and genetic predisposition are other common aetiologies for COPD [174]. Many studies have shown indirect evidence of a viral aetiology [221]. These data suggest that 50–70% of exacerbations are due to respiratory infections (including bacteria, atypical organisms and respiratory viruses), 10% are due to environmental pollution (depending on season and geographical placement), and approximately 30% are of unknown aetiology [221-222].

Despite the importance of infections, other factors may induce exacerbations in COPD. In a study of 1,016 patients with severe COPD, infection was shown as the cause in 51% of exacerbation cases. Heart failure was thought to account for 26% of episodes of increased symptoms, but about 30% of exacerbations had no identified aetiology [223].

Fletcher and Peto et al. [202] showed that there is a close relationship between the amount of tobacco smoked and the decline rate in forced expiratory flow in one second (FEV_1), although COPD patients vary in susceptibility [224].

COPD has a variable natural history, which is characterised by progressive deterioration in symptoms, with increased episodes due to acute exacerbations. Jones et al. [225] showed in a large study of 4,951 COPD patients from 28 countries that health-related quality of life, measured by the St. George's Respiratory Questionnaire (SGRQ), deteriorated faster in patients with more severe disease.

1.7.6 Pathogenesis

COPD is mainly caused by cigarette smoking and affects up to 25% of smokers [226], although other factors such as air pollution and occupational exposure to dust and fumes may contribute to its development. This causes an inflammatory response in the lungs and leads to the pathological characteristic of COPD. This inflammation to the COPD lung is often caused initially by cigarette smoke and the increased infiltration of immune cells into the lung, but it is not clear why the inflammation persists even after cessation of smoking, while other pathologies partly reverse [227]. This inflammation appears to be an amplification of the normal inflammatory response of the respiratory tract to chronic irritants such as cigarette smoke. Some patients develop COPD even without cigarette smoking. The nature of the inflammatory response in these patients is still unclear. Lung inflammation is further induced by oxidative stress and imbalance of proteinases in the lung [228]. All these mechanisms together lead to the characteristic pathological changes in COPD such as mucous hypersecretion, ciliary dysfunction, airflow limitation, gas exchange abnormalities, and systemic effects [229-230].

A wide variety of inflammatory cells and mediators such as cytokines have been shown to increase in COPD patients [231-232]. These inflammatory cells, in association with infiltration of macrophages, neutrophils and T-cells, release pro-inflammatory mediators in the lung, which induce structural changes in the airways and correlate with the degree of progression of COPD (**Figure 12**) [233-234].

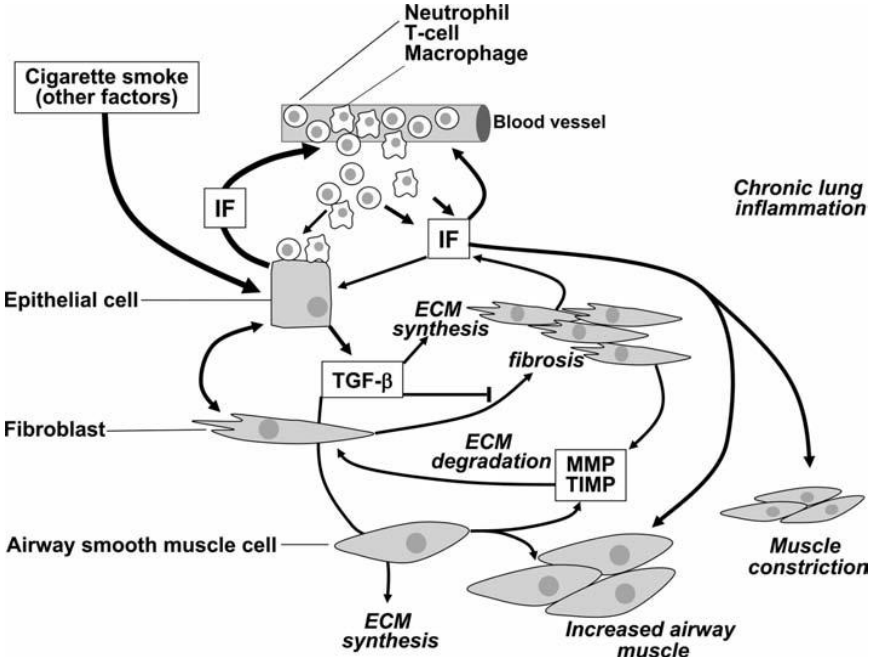


Figure 12: Effect of cigarette smoke and other triggers on the lung inflammatory response. IF: inflammatory factors such as IL-6, IL-8, tumour growth factor beta (TGF-β). ECM: extracellular matrix; MMP: matrix metalloproteinases; TIMP: tissue inhibitors of MMP [227].

1.7.7 Pathology

The pathological hallmarks of COPD are chronic airway inflammation and airways obstruction. This inflammatory response and the morphological changes of COPD affect the central airways in the proximal and peripheral airways, lung parenchyma and pulmonary vasculature [235].

These pathological changes lead to increased numbers of specific inflammatory cells in different parts of the lung, and structural changes resulting from repeated injury and repair [236]. Narrowing of the airways causes airflow limitation to and from the alveoli, and hypersecretion of the viscous mucus, mucosal and submucosal oedema; hypertrophy and airway remodelling, and spasm of the bronchial smooth muscles also occur [237-239].

More studies have confirmed that a chronic inflammation is an important component of the pathology in both small airway obstruction and emphysematous destruction in COPD [237, 240]. In a case of exacerbation of COPD, these inflammatory responses are more affected

and marked with increased neutrophils and eosinophils, which in turn accelerate the progression of lung function deterioration [241].

1.7.8 Prognosis

COPD is a heterogeneous disease with comorbidities that could have a great impact on prognosis [174, 242-244]. Because of the heterogeneousness of the disease, there is no single measure that can give an adequate assessment of the severity of the disease in COPD patients. Prognosis depends on a number of factors, including environmental exposures genetic predisposition, acute exacerbations, and comorbidities [174]. Although short-term survival for COPD patients depends on the overall severity of acute illness, long-term survival is primarily influenced by the severity of COPD and the presence of comorbidities. Commonly, prognosis of COPD depends on the rate of decline in airflow as examined by the FEV_1. An FEV_1 of less than 30% of predicted value means very severe disease; certain studies have estimated that more than half of those patients with very severe disease may not be expected to survive for four years [245]. In addition to the FEV_1, BMI predicts prognosis (very low weight is a negative prognostic factor), distance walked in six minutes, and degree of shortness of breath with activities. These factors, known as the BODE index (body mass index, obstruction, dyspnoea, exercise capacity) can be used to provide data on prognosis for 1-year, 2-year, and 4-year survival [246]. Frequent COPD exacerbations and the need for multiple intubation and mechanical ventilation for acute respiratory failure in COPD patients are other critical markers of poor prognosis [247].

Recurrent episodes of acute deterioration caused by exacerbations have been shown to significantly contribute to impaired health status in COPD patients [248-249]. Moreover, several studies have suggested that the occurrence of exacerbations may influence lung function, quality of life, and survival in the long-term [250-253].

1.8 Inflammation in COPD

COPD is a heterogeneous disease [254], and the most recent definition emphasizes that it is a systemic disease [244].

Because cigarette smoking is the major risk factor, studies have associated the level of cigarette smoking with the increase of pro-inflammatory cytokines (IL-6 and IL-8) [255-256]. Keating et al. [257] have demonstrated that cigarette smoking induces the recruitment of neutrophils to the lung. Airway neutrophils have been shown to increase in chronic bronchitis [258] and neutrophil recruitment has been related to airflow reduction [259]. However, not all smokers develop COPD and the observed inflammation in COPD is still present after patients stop smoking cigarettes [260].

In mild COPD, airway inflammation is characterised by T-lymphocytes and macrophages, but with increasing severity of the disease, lymphocytes decline and the macrophages are accompanied by neutrophils [261]. Systemically, Hurst et al. [262] have reported increased inflammation with COPD as the blood CRP, IL-6, IL-8, and sICAM-1 increased. There was a significant relationship between neutrophilic lower airway inflammation and the systemic inflammatory response at exacerbation [262], even in mild/moderate COPD patients [263].

The effect of NIV on the inflammatory markers in COPD is poorly studied. The available data have shown inconsistency. A recent study, which examined the impact of bilevel positive pressure airway therapy on systemic inflammatory response and lung structure in patients undergoing subarachnoid block for small or medium surgical procedures [264], found no difference between the inflammatory response of patients receiving a single lung hyperinflation manoeuvre and a control group. This suggests that lung hyperinflation did not further amplify the systemic inflammatory response triggered by the surgical procedure. Similarly, Puls and colleagues et al. [265] were unable to detect changes in the systemic levels of either inflammatory or anti-inflammatory cytokines in patients with atelectasis following lung hyperinflation. In contrast, a recent study in patients with ARDS found an association between the use of mechanical ventilation and an increase in cytokine levels, both locally in the lung and systemically [266].

To date, clinical studies have focused on the inflammatory response to NIV in healthy subjects without evidence of inflammatory activity. It is not currently known whether the use of bilevel positive pressure airway therapy in COPD, a disease that is associated with local airway and systemic inflammation, causes further up-regulation of the inflammatory response. For this reason, this thesis will further introduce a pilot study on the effect of NIV on inflammatory markers.

1.9 Inflammation in OSA

Systemic and airway inflammation associated with OSA may be the cause of cardiovascular complications, neurocognitive and metabolic syndrome [267-268].

Upper airway inflammation can arise as a result of environmental and physiological factors [269]. Nevertheless, OSA inflammation is characteristically distinctive and vital to the progression of this condition. Airway inflammation is present in 90% of OSA patients at larynx level, and the level of inflammation positively correlates with AHI [270]. Furthermore, the intermittent hypoxia that is a hallmark of OSA has been shown to stimulate cytokine production [271], which can itself act as a stimulus to systemic inflammation [272]. These inflammatory cytokines can lead to compromised endothelial function systemically [273] and can induce cardiovascular morbidities [274-275].

Studies have demonstrated the association of OSA with nasal inflammation [276-278]. Rubinstein et al. [276] have demonstrated a local mucosal increase in polymorphonuclear leukocytes and the concentrations of bradykinin and vasoactive intestinal peptide (VIP), thereby indicating the presence of nasal inflammation in patients with OSA. It was also suggested that nasal inflammation exacerbated upper airway obstruction in OSA. Studies have demonstrated increased thickness of uvula mucosa, interstitial oedema and increased numbers of leukocytes in the lamina propria of the soft palate of OSA patients, and have suggested that inflammation of upper airway soft tissues is significant in the pathogenesis of upper airway narrowing during sleep in patients with OSA [277, 279].

The potential cause of inflammation in the airways of OSA patients is unclear. However, it has been suggested that the combination of mechanical trauma due to snoring, airway vibration and forced suction collapse during apnoea is responsible for a local inflammatory response [280-281].

CPAP has been associated with substantial improvement in disease-specific quality of life [282], eliminates upper airway collapse during sleep, and improves sleep fragmentation and daytime symptoms [283]. Nevertheless, undesirable side effects are frequent during the

application of non-invasive ventilation and make CPAP compliance a major clinical challenge.

More than 50% of the patients complain of nasal symptoms, such as nasal congestion, dry nose, mouth or throat, and discomfort associated with cold air [284-287]. Such symptoms may appear within a few hours of CPAP application, causing the therapy to be discontinued prematurely [285, 288].

The non-invasive continuous application of the positive pressure to the nasal wall would cause mechanical tension to the nasal anatomy. This mechanical tension may aggravate the biological reaction in a variety of cells and tissues [289-290], and in the airways [291]. Such mechanical tension will eventually lead to an inflamed airway.

An early upper airway inflammation response has been reported with OSA patients. Negative mechanical pressure events were applied to the intraluminal of the upper airways in a rat model, which revealed recurrent upper airway collapse, and reopening induces an early upper airways pro-inflammatory cascade [292]. Another report stated that mechanical stimuli could induce an early upper airway inflammation by the application of a vibration akin to snoring in human airway epithelial cells [293], and in the rate soft palate [294].

Mechanical stress, however, has been also examined during breathing inspirations in the lower airways of the lung. Recurrent inspirations with an increased upper airway resistance lead to exerting more forceful negative pressure in order to overcome the high resistance in the upper airways. Bronchial inflammation has been observed with those patients, and CPAP has demonstrated an increase in the airway hyperresponsiveness (AHR) in bronchial mucosa in obstructive sleep apnoea syndrome (OSA) [295].

Another important example of mechanical stress is the overdistension that tends to occur during invasive mechanical ventilation in adult respiratory distress syndrome (ARDS) [296]. With regard to the non-invasive ventilation (NIV) pressure levels, it is interesting to find from literature that there is no correlation between the side effects and level of pressure used during nasal CPAP with OSA [98, 288]. The mechanical stress to the upper airway

induces inflammatory mediators that result in structural and functional consequences, thickening of the pharyngeal wall, local myopathy or neuropathy [293, 297].

Unfortunately, CPAP itself induces an early nasal, neutrophil-rich, inflammatory response in rats (due to the mechanical pressure exerted by the face mask), but this is localised to the nasal wall [27]. In a clinical study, it was reported that nasal CPAP resulted in an increased total inflammatory cell count, thereby indicating local nasal inflammation in patients with obstructive sleep apnoea [298]. These findings illustrate the development of local hyperacute inflammation due to CPAP treatment at the onset of therapy that is independent of the inflammatory process of the disease. The systemic inflammatory effects of CPAP therapy are dependent on the duration of therapy. Malbouisson et al. [264] established that the lung hyperinflation in healthy individuals, with up to 20 cm H_2O CPAP administered by a face mask for a short time, resulted in increased cytokine levels, including tumour necrosis factor alpha (TNF-α) and interleukins (IL-1β, IL-6, IL-8, IL-10, and IL-12), which returned to normal after 12 hours. Steriopolos et al. [299] reported a marked improvement after 6 months in inflammatory markers, including lymphocyte count, $CD4^+$ cells, TNF-α levels and uric acid levels, in patients with good compliance to CPAP therapy; this effect was not observed in patients with poor compliance to the treatment. However, Kohler et al. [300] did not report a difference in systemic inflammatory markers, high sensitive C-reactive protein (CRP), plasma IL-6, interferon gamma (IFN-γ) or adiponectin levels between patients receiving therapeutic and sub-therapeutic CPAP treatment for four weeks. Significantly lower levels of CRP and IL-6 levels in newly diagnosed OSA patients on CPAP treatment for a month (compared to obese control patients) established that nasal CPAP decreased inflammatory markers [301].

Almendros et al. [294] examined the effects of vibratory stimuli on the upper airway in a live-rat model. They subjected the upper airway to vibratory trauma. They then assessed the level of expression of the genes coding for tumour necrosis factor-alpha and macrophage inflammatory protein 2 in the soft palate. The conclusion was that vibratory stimuli induce at least some form of early inflammation in mucosal tissue.

Another important example of mechanical stress is the overdistension that tends to occur during invasive mechanical ventilation in ARDS [296]. There is evidence that the mechanical factor during invasive positive ventilation shows up-regulation of inflammatory responses. **Figure 13** describes the potential injurious effects and shear force on the lung epithelial and endothelial cells [302].

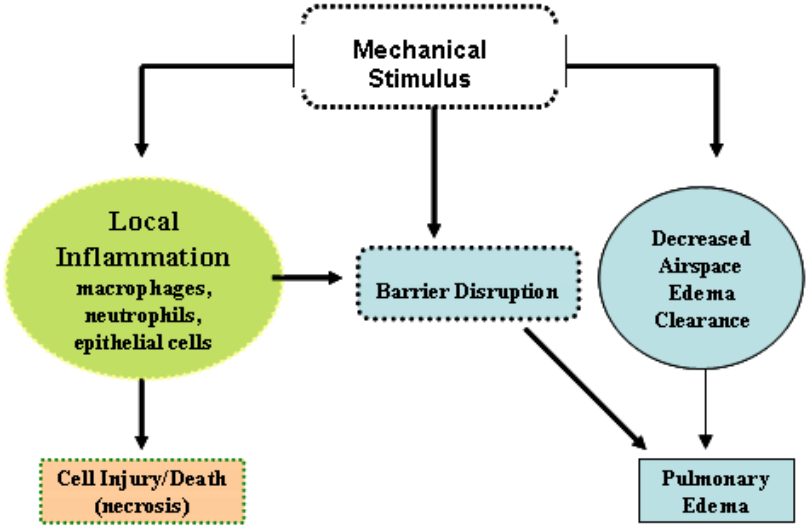


Figure 13: Potential mechanisms of ventilator-induced lung injury. Mechanical ventilation induces tensile strain and shear forces in the lung. These forces result in increased permeability and disruption of the alveolar–capillary barrier. Mechanical forces also induce an increase in the concentrations of pro-inflammatory mediators (including IL-1β, tumor necrosis factor alpha, IL-8 and IL-6) in the distal airspaces of the lung [Modified from reference 302].

Systemic inflammation in OSA is characterised by increased plasma levels of inflammatory markers such as TNF-α, IL-6, CRP, IL-1b, and adhesion molecules (**Table 11**) [303-304]. The systemic inflammation seen in OSA patients may be independent of obesity [301, 303, 305].

Local airway inflammation has been reported using inflammatory markers in exhaled breath and sputum samples [306-307].

Table 11: Identified inflammatory markers in obstructive sleep apnoea.

Systemic inflammation	Local inflammation
↑ C-reactive protein	↑ IL-6
↑ IL-6	↑ 8-isopentane
↑ TNF-α	↑ neutrophils
↑ VEGF	↑ CD4+ T cells
↑ EPO	
↑↓ Adiponectin	
↑ Reactive oxygen species	
↓ Nitric oxide	

VEGF, Vascular endothelial growth factor; EPO, erythropoietin; ↓, decrease in; ↑, increase in.

There are very limited data on the effects of nasal CPAP in inducing local inflammation on the upper airway (**Table 12**).

Table 12: Summary for the papers reporting changes in inflammatory indices after application of CPAP therapy.

Paper	Ref	Increased after CPAP	Decreased after CPAP	Unchanged after CPAP	Design	N=	Comments
Almendros et al. Sleep, 2008	[294]	Neutrophils in nucleated cells (%); mRNA of MIP-2	NA	Gene expressions of TNF-alpha, nerve growth factor, tachykinin -1 receptor	Prospective controlled animal study	32	Male Sprague-Dawley rats
Skoczynski et al. Rhinology, 2008	[298]	Total inflammatory cell count		Nasal patency	Prospective	42	Clinical study
Steiropoulos et al. Sleep, 2009	[299]	Good compliance group: Compliance Improved	Total Lymphocytes, CD4+ cells, TNF-alpha levels, uric acid levels	Good Compliance	Prospective	52	Hospitalised patients. Samples obtained at baseline and at 6-month follow up.
		Poor compliance group: Not improved	NA	Poor compliance – Total lymphocytes, CD4+ cells, TNF-alpha levels, uric acid levels.			

Kohler et al. Thorax, 2009	[300]	NA	NA	Highly sensitive CRP, IL6, interferon gamma, adinopectin	Randomised	100	Subjects were randomised to therapeutic and sub-therapeutic CPAP treatment for 4 weeks
Malbouisson et al. Braz J Med Biol Res, 2010	[264]	TNF-alpha, IL-1beta, IL-6, Il-8, Il-10, Il-12	NA	NA	Randomised	10	Healthy subjects were studied
Yokoe et al. Circulation, 2003	[301]	NA	CRP, IL-6, spontaneous production of IL-6 by monocytes	NA	Randomised	30	

1.10 UPPER AND LOWER AIRWAY INVESTIGATION

1.10.1 Background

The aim of the current section in this thesis is to introduce the upper and lower airways investigations, highlight their importance, and to discuss the selective methods that are important for assessing inflammation in the upper and lower airways in healthy subjects, OSA, and COPD patients using NIV.

The upper and lower airways behave similarly in many ways, including common triggers, pathogenic mechanisms, many of the inflammatory cells and mediators, and the treatment modalities [308-309]. COPD patients have shown increased nasal symptoms, suggesting a link between the upper and lower airways beyond asthma and allergy-associated inflammation [310]. In nonasthmatic allergic rhinitis patients, studies have demonstrated a significant relationship between the upper and lower airways, evidenced by impaired FEV_1 and increased bronchial responsiveness [311-313]. Moreover, a nasal allergen challenge in allergic rhinitis patients experiencing nasal symptoms alone can result in bronchial inflammation [314], and segmental bronchial provocation has been shown also to induce nasal inflammation [315]. This inflammatory association between upper and lower airway is reflected in a continuous nasobronchial airway inflammation process from healthy to diseased airways. This process was supported by the work of Chawes et al. [316] who showed that there were independent associations between blood eosinophil count, nasal eosinophilia, and upper airway patency. In OSA adult patients, evidence of interactions between upper and lower airways is a poorly researched field. Data available suggest that both local airway and systemic inflammation are implicated in the pathophysiology of this seemingly all-mechanical condition [317].

Loss of the protective functions of the nose may be responsible for nasobronchial inflammation, in which the absorption of inflammatory mediators from inflammatory sites into the systemic circulation has been shown to induce the release of eosinophils and thereby possible systemic propagation of inflammation from nose to lung, and vice versa

[318]. All these clinical findings suggest that nasal inflammation is not a local, but that the entire respiratory tract is involved.

Therefore, the phenomenon of inflammatory “cross-talk” between the nose and lung [319], and the concept of a “united airways disease” in which rhinitis and asthma are the upper and lower airway manifestations of the same disease process were clinically stated [320]. In contrast, this phenomenon was not clinically studied with COPD patient until recently. Hurst et al. [321] suggested that a correlation between the degree of upper and lower airway inflammation in COPD patients exist. His results suggested that in COPD patients there is also a pan-airway inflammatory response, reflecting the pan-airway exposure to cigarette smoke.

The rationale of this thesis is to examine the pro-inflammatory effect of NIV to the airways, and further examine the interactions between the upper and lower airway in these conditions. The pros and cons of a few of the techniques used in this thesis have been discussed in detail in the subsequent sections.

1.10.2 Methods of Assessing Upper Airway

1.10.2.1 Nasal Lavage vs. Other Techniques

The nasal cavity is affected by many conditions including inflammatory diseases. Since it is the most accessible part of the respiratory system, the cellular and biochemical composition of nasal specimens can be readily evaluated. Obtaining surgical biopsy specimens is an option that gives excellent information on cellular events. However, it is invasive and associated with a risk of bleeding, and artefacts may occur from the infiltration of local anaesthetic. In addition, repeated procedures are problematic, limiting the biopsy's use for follow-up purposes. Alternatively, the composition of nasal secretions may also aid in the diagnosis and follow-up of conditions affecting the upper or lower airways, in particular inflammatory and allergic disorders [322-324].

A variety of methods for obtaining nasal secretion samples have been reported which fall into three main categories [325]. The first is the collection of spontaneous secretions, with or without stimulation, which can be accomplished with the aid of nose blowing, suction or through the collection of dripping secretions. The second category includes dilution techniques in which the nasal cavity is washed or sprayed with a fluid then a lavage is recovered for examination. In the third category are techniques in which an absorbent material such as cotton wool, filter paper in the form of strip or disc, polyurethane foam, or cellulose sponge is placed into the nasal cavity.

Nasal washing (nasal lavage) is a commonly used technique. However, unpredictable amounts of fluid losses through swallowing, absorption by nasal mucosa and leakage from the nasal orifice all contribute to a variable degree of dilution, resulting in concerns related to the repeatability of the technique. Concentrations of interleukin (IL)-6 and IL-8 in nasal secretions accurately demonstrate the degree of inflammation since both take part in the inflammatory process. Their concentrations in respiratory secretions increase in disease states characterised by airway inflammation [322, 326]. High sputum concentrations of these two cytokines, for example, have been associated with increased frequency of COPD exacerbations [326]. In addition, COPD patients have been found to have an increased IL-8 concentration in nasal secretions compared to controls [322].

In light of the disadvantages discussed here, when using conventional nasal lavage techniques, we developed in our Department a novel and practical method for the collection of nasal secretion. The method utilises a paediatric tracheostomy tube with a good seal at the nasal orifice. It is less time consuming can be reprocessed for usage, and provides comfortable test conditions [327]. This nasal lavage technique is the standardised technique in this thesis for upper airways nasal lavage sampling and is described in detail in chapter four.

1.10.2.2.1 Acoustic Rhinometry vs. Rhinomanometry vs. Medical Imaging

Acoustic Rhinometry (AR)

The clinical use of acoustic rhinometry was first described by Hilberg in 1989 as an objective method for examining the patency of the nasal cavity [328]. This technique is non-invasive, reliable, convenient, easy and quick to perform, requires little patient cooperation, and can aid in the assessment of nasal obstruction. AR works by estimating the intranasal volume between predefined segments, and cross-sectional areas, through the identification of local changes in acoustic impedance using a sound pulse propagating in the nasal cavity (**Figure 14**). Since nasal obstruction is common in a wide range of sinonasal disorders, the technique may play an important role in the diagnosis and follow-up of conditions altering intranasal dimensions, either permanently or transiently. Nasal mucosal inflammation is the most common pathologic cause and, besides viral upper respiratory tract infections, allergic rhinitis is the most frequent cause of nasal obstruction [329]. Since nasal obstruction is common in a wide range of sinonasal disorders, AR technique was used in this thesis on all studied groups to assess the impact of NIV in causing altering intranasal dimensions.

Since its introduction, AR has been investigated and used in many conditions in adults and children including allergic rhinitis, vasomotor rhinitis, nasal, pharyngeal and maxillofacial procedures and conditions, and sleep disorders [330]. AR is not only able to detect irreversible structural changes but also reversible dynamic changes, providing information

on both the location and reversibility of the pathological condition. Correlation and/or agreement of the technique with other diagnostic methods including rhinomanometry, computed tomography, optical rhinometry, magnetic resonance imaging, direct X-ray examination, fiberoptic nasal endoscopy, and clinical findings, has been well-established in both clinical and experimental settings [331-339], although AR seems to have some limitations in the posterior part of the nose [249, 337, 340] and interchangeable use of different methods is not appropriate [341].

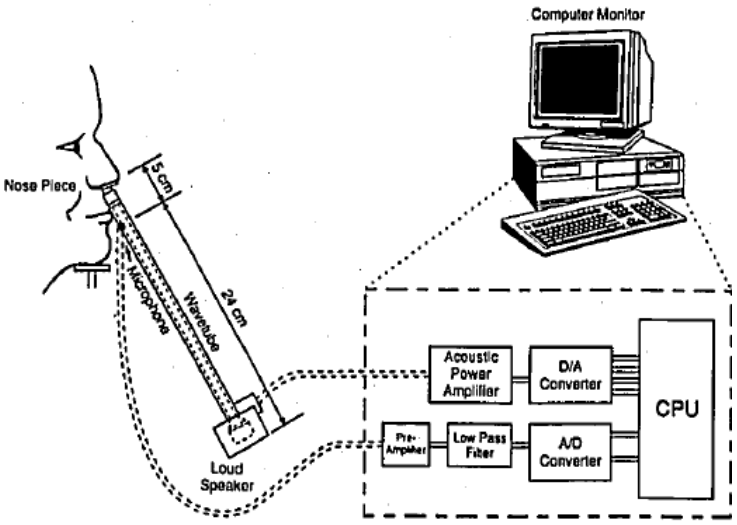


Figure 14: Diagram of the Acoustic Rhinometry System [342].

Common clinical and practical uses of AR for the rhinologic surgeon include assessment of "mixed" nasal blockage, documentation of nasal alar collapse, and preoperative planning for reduction rhinoplasty [342]. It can also be used to examine the positive effect of surgery on nasal airway obstruction [329]. As an accurate method for measuring the nasal cavity geometry, AR is easy to perform and is potentially useful for the investigation of physiological and pathological changes in the nose. In many cases the technique is even used for clinical assessments before and after interventions (cg turbinectomy, septoplasty, and rhinoplasty) [343-345]. The volume of mucovascular swelling can be quantified

expeditiously in a manner that is particularly useful for measuring rapidly changing airway dimensions as with nasal challenge tests. Precise measurements pre- and post-intervention of nasal valve dimensions can be obtained and recorded. The area-distance graph obtained allows visualization of the site of obstruction [331]. The technique offers particular advantages in terms of accurate dimensional evaluation of the nasal valve region and the structural and mucovascular components of nasal obstruction [342].

A study by Morris et al. [346] showed that a small, constricted, minimal and cross-sectional area 1 and 2 was predictive of non-adherence to CPAP therapy in OSA patients. He also suggested using acoustic rhinometry for evaluating nasal obstruction in OSA [347].

AR has been used as an aid for the diagnosis and treatment of OSA. Houser described the use of AR findings in mild sleep apnoea [348]. In 2003, Virkula et al. [349] also studied the body positions on snorers in sleep apnoea and sleep-disordered breathing using AR.

Since the repeatability of AR remains unclear, holding important clinical implications, particularly for when the method is to be used for the purpose of follow-up, this technique has been validated in our Department before using it with all other studied subjects in this thesis [350]. The validation is outlined in detail in chapter four.

Rhinomanometry (RMM)

Rhinomanometry (RMM) is a test of nasal function that measures air pressure and the flow rate in the nasal airway during the breathing cycle [351], which is considered dynamic. RMM uses an intranasal closed loop system to measure nasal airway resistance. The measured values are then used to calculate the resistance of the nasal airway. It quantifies nasal breathing parameters and indicates how hard breathing is from the nose. Many factors affect nasal airflow. These factors include nose length, the cross-sectional area, transnasal pressure, and flow type, whether this be turbulent or laminar [352].The method enables the mucovascular and structural components of nasal obstruction to be differentiated and quantified and the anterior, posterior or adenoid site and severity of the obstruction to be determined [331]. A series of measurements with RMM can determine the response to a

topical decongestant of the mucovascular contribution at the time of examination and in the decongested nose, which is the degree of severity of structural nasal obstruction [353]. RMM is not 'medically necessary' when assessing clinical cases of nasal obstructive symptoms, but, in many clinical situations, it can provide valuable objective information in compliance with the requirements of evidence-based medicine [353].

A rhinomanometer is a device that can be used to measure nasal inspiratory flow and pressure (**Figure 15**). The device has been designed for the purpose of ascertaining whether slight degrees of nasal obstruction are present. Thus, the device will indicate both inspiratory and expiratory obstruction. The device works by measuring the distance through which a column of water can be moved in a definite time period. During the test, the mouth is kept wide open. The device is a glass tube with a wide nose-piece, and a resistance at the distal end [354]. It enables accurate measurement of nasal airflow resistance.

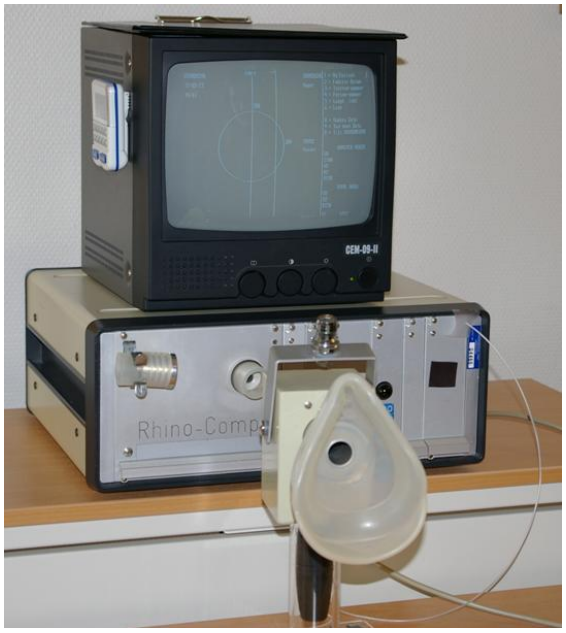


Figure 15: Rhinomanometer device (Rhino Comp®, Sweden).

It is important to state that there is inadequate evidence of the clinical utility of RMM and AR. These tests have not been shown and demonstrated to be superior to physical examination, nasal endoscopy or CT imaging in selecting patients who would benefit from

surgical management due to nasal obstruction, and do not correlate with a patient's subjective perception of nasal obstruction [323, 355]. Published clinical studies suggest that both RMM and AR are used in research studies in which objective measurements of nasal obstruction may be important to determine treatment effects. However, no data provided a detailed analysis of how these two diagnostic studies would be used in the clinical management of the patient.

Computed Tomography (CT) Scan and Magnetic Resonance Imaging (MRI)

CT is an imaging technique based on x-rays. It produces an enormous of data that can be manipulated, through a process known as "windowing", in order to demonstrate various bodily structures based on their ability to block the X-ray beam [356].

Clinically, CT and Magnetic resonance imaging (MRI) usually reserved for recurrent or complicated diseases and the benefit that the disease severity may be quantified. MRI is a method that can be used to illustrate the anatomy of the nasal cavities with no known adverse effects on patients. In clinical rhinotology, the use of MRI remains limited due to the inability of the technique to directly visualize bone. MRI, however, is useful in mucosal structures imaging that are important factors in nasal patency and volume. CT scans have been used earlier as a reference in the evaluation of AR accuracy [357]. CT and MRI can be used to assess cross-sectional areas and volumes of the nasal airways: however, it is an expensive method for evaluating nasal disease in routine use, and patients will be exposed to unnecessary radiation. Although the correlation between imaging scores and symptoms is not significant [358], Lund and Mackay et al. [359] made the most frequent use of quantitative assessment of chronic rhinosinusitis imaging, using CT scanning which scores each of the five sinuses to give bilaterally a total score between zero and 24. Lund reported that CT scan data provided more supportive evidence of symptom scores in chronic sinusitis patients [360], while MRI technique has the ability to reflect the nasal mucosa status and bony anatomic abnormalities within the nose with certain limitations. MRI is more better to CT in evaluating mucosal appearance and has been used successfully to demonstrate the nasal cycle [361].

Hilberg et al. [362] was the first to use CT and MRI to assess the accuracy of the CSA and volume measurements of AR in a single cadaver head. His results have shown a significant correlation between CT and AR findings when the imaging was obtained perpendicular to the acoustic wave direction. Due to the limitations of CT in comparison with MRI, particularly in detecting airway fat because of its poor resolution, both techniques are not frequently used for upper airway evaluation of OSA patients.

Available clinical studies showed that AR measurements have shown reliability readings, and demonstrated a good correlation between them and both CT and MRI findings, but poorer results further posterior [335, 340, 357, 363-364]. However this data cannot reflect the dynamic aspects of anatomical structure of the nasal cavity.

1.10.2.3 Mucociliary Clearance: Saccharin Transit Time

Mucociliary clearance (MCC) is an important host defence mechanism, from the nose and upper airways to the lower respiratory tract. It protects the respiratory mucosa against inhaled particles and microorganisms by enacting a clearance mechanism and producing unidirectional mucus flow toward the oropharynx [365]. The nose has an epithelium similar to the lower respiratory airway, and the nose is an important site to assess inflammatory events and other pathophysiologic mechanisms in the lower respiratory tract [366-367]. This importance has been reported as the MCC in the nose and bronchi were correlated [368]. Many factors have been shown to interfere with mucociliary clearance such as cigarette smoking and acute infection [369-372].

Mechanical ventilation as mechanical stimulus may influences mucociliary clearance work. It results in disrupting the equilibrium of the mucous membrane and consequently leads to inflammation of the nasal mucosa [373]. Konrad showed that mucociliary clearance is impaired in patients receiving mechanical ventilation at the level of the main bronchus and trachea [374]. However, the observed affect may be secondary to the tracheal intubation, mechanical ventilation, and method of artificial airway humidification [375]. Therefore, it is

necessary to examine the influence of mechanical therapy such CPAP with OSA, since the underlying mechanism impairing MCC may differ from one illness to another.

A number of ways to measure mucociliary clearance have been shown to be effective. Although the gold standard for mucociliary clearance measurement is the radiolabeled method first introduced by Proctor [376], a simpler and efficacious alternate method is the saccharin test [377]: Saccharin Transit Time (STT) has been used to assess mucociliary clearance in normal subjects as well as in those with various sinonasal disorders, such as allergic rhinitis and chronic rhinosinusitis [378]. As mentioned above, numerous studies have reported a good correlation between the STT in the nose and various tests of MCC in the tracheobronchial tree. Verra et al. [368] have reported on ciliary dyskinesia with situs inversus, bronchiectasis, chronic sinusitis and sterility. His results revealed that abnormal nasal cilia samples correlated with the bronchial biopsies. Thus, by using the easily performed STT to measure MCC in the nose, we have an indicator for the lower airways of the lung and can treat accordingly [368, 379].

Nasal mucociliary clearance was measured using *in vivo* STT. The modified technique described by Rutland and Cole was used [380]. STT is a simple and reliable method to determine the mucociliary clearance [366, 381]. The use of nasal mucociliary clearance avoids the need for more invasive procedures such as bronchoscopy and is feasible in stable, conscious, and cooperative patients. Saccharin was applied on the inferior turbinate of the nasal cavity under direct visualization and the time at which the subject reported a sweet taste was recorded in seconds using a stopwatch. During the procedure, the patient was asked to sit quietly with the head in a neutral position, and avoiding sniffing, sneezing, coughing, and drinking.

Oliviera et al. [382] has demonstrated that in the short-term, STTs decreased significantly after 20 minutes of nasal CPAP in healthy individuals. This may be attributed to differences in the duration of nasal CPAP treatment, and suggests that nasal CPAP may provide an initial improvement in nasal clearance that is followed by impairment due to inflammation. Inflammatory and functional changes may contribute to the high incidence of symptoms and adverse effects associated with nasal CPAP treatment. Previous studies have reported high

incidences of side effects during long-term therapy, with as much as 97% occurring in a large series [98].

In this thesis, an overall measurement of mucociliary clearance was assessed using STT before and nasal CPAP therapy with healthy and OSA subjects.

1.10.3 Methods of Assessing Lower Airway

1.10.3.1 Spirometry

Spirometry is the most objective test for measuring the airflow limitation in pulmonary diseases [174]. The measurements of spirometry are based on age, height, sex and race in comparison with reference value [383]. The GOLD report [245] states: "COPD is a disease state characterised by airflow limitation that is not fully reversible … Airflow limitation is measured by spirometry, as this is the most widely available, reproducible test of lung function." Spirometry test is recommended in the diagnosis and evaluation of COPD [174, 384-385]. The test is performed by asking the patient to inspire before fully exhaling as fast as possible for at least six seconds. The test is repeated at least three times, and the FEV_1 or FVC values should vary by no more than 5% or 150 ml [386].

The importance of spirometry is recognized in early detection of COPD, the prevalence and consequent burden of which are rapidly increasing globally [387]. However, population-based studies relating to the relationships between lung function and OSA have rarely been performed, and findings are sparse [388]. In OSA patients, the abnormalities of the flow-volume loop consistent with inspiratory flow limitation and upper airway instability during wakefulness are common, but their role is controversial in predicting OSA [389-391].

Several studies have examined pulmonary function in OSA patients [392-393]. Interestingly, OSA has been found to closely correlate with lower airway obstruction, although it is defined as an upper airway disease [394]. Chaouat et al. [395] have shown in their large series of unselected OSA patients that the presence of an associated chronic

obstructive pulmonary disease (COPD), defined by an FEV_1/VC ratio <60%, occurred in 11% of cases.

CPAP has been widely established in the treatment of OSA [27]. However, few studies have evaluated the effects of this treatment on lung function [396-398]. In patients with COPD, CPAP has been shown to reduce the work of breathing [399], while in asthmatic patients; CPAP tends to reduce inspiratory muscle work [400]. But in OSA, Bonay et al. [401] has shown that lung function may be impaired by long-term, nasal CPAP treatment.

Young et al. [397] has observed a reduction of total lung capacity, functional residual capacity in a group of OSA after 1 year of CPAP treatment. On the other hand, in patients with overlap syndrome, Vázquez et al. [402] have reported that CPAP treatment caused an improvement in FVC and maximal inspiratory pressure. These findings did not show significant variation in the degree of bronchial obstruction, and for this reason, these findings may be due to an improvement in the function of respiratory muscles.

1.10.3.2 Sputum Sampling

The purpose of sputum sampling is to obtain an adequate amount of secretions from the lower airways and to use this sample for assessing airway inflammation in respiratory disorders [403]. For many years, inhalation of hypertonic saline has been one of the most widely used methods for assessing a number of airway inflammatory conditions in sputum induction [404]. It has been advocated as the only direct, relatively non-invasive, and safe method for assessing airway inflammation indices [404].

The study of cells and inflammatory mediators in airway secretions from patients with lung diseases has been performed in samples of spontaneously expectorated sputum obtained by bronchoscopy and lavage [405]. These sampling methods are limited in their applicability. Bronchoscopy can only be used on patients who tolerate the procedure and cannot easily be performed repeatedly to examine inflammatory changes in airway secretions over short periods of time [406]. The invasiveness of the procedure, associated costs, and restriction of

its use to exacerbated patients make bronchoscopy unsuitable for monitoring airway inflammation in clinical practice [407-408]. Furthermore, changes in induced sputum such as increased neutrophils and IL-6 concentration has been shown to be similar to those occurring in nasal lavage [409]. Sputum sampling, either spontaneous or induced, was obtained and processed according to published guidelines [410-412], has been used as an alternative assessment for lower airway inflammation used in this study.

The importance of measuring airway inflammation in COPD has been emphasised [413]. The pathophysiology of COPD is an inflammatory disorder which is characterised by neutrophilic inflammation with the presence of macrophages and lymphocytes on airway tissue [405]. Few studies reported on the presence of bronchial inflammation in OSA patients. Olopade et al. [414] was the first to report on the increase of pentane and nitric oxide in the exhaled air of OSA patients after sleep. Interleukin-6 in exhaled breath condensate (EBC) has been also shown to increase in OSA as a characteristic of airway inflammation [415]. However, Salerno has demonstrated the presence of lower neutrophilic airway inflammation using the analysis of induced sputum (**Table 13**) [306, 416].

Table 13: Summary for the papers reporting the effect of CPAP on inflammatory markers in OSA.

Inflammatory marker	Main study	Ref	Data available in OSA	Effects on CPAP	Comments
Induced sputum					
Neutrophils	Rubinstein, 1995 Salerno et al. 2004	[276] [306]	High percentage in IS	No change	Data about baseline are reliable. The role of obesity is unclear, poor data on effects of CPAP
Lymphocytes	Boyd et al. 2004	[148]	Increase in IS	No data	Poor evidence, few studies
Eosinophils	Kalpaklioglu et al. 2009	[417]	Poorly investigated	No data	Few data
Exhaled gases					
FENO	Olopade et al. 1997 Depalo et al. 2008	[414] [307]	High concentration	Unclear	Studies use often different methods
Exhaled pentane	Agusti et al. 1999	[418]	High concentration	No data	Few studies, small sample
Exhaled CO	Petrosyan et al. 2008	[419]	High concentration	Decrease of exhaled CO level	Few studies, small sample
Exhaled breath condensate					
pH	Petrosyan et al. 2008 Antonopoulou et al. 2008	[419] [420]	Lower in EBC	Increase of pH	Few studies
LT B4	Petrosyan et al. 2008 Goldbart et al. 2006	[419] [421]	High concentration	Decreases of LT B4 concentration	Few studies
Nitrite/nitrate	Petrosyan et al. 2008	[419]	High concentration	Decrease of nitrite/nitrate concentration	Few studies
8-Isoprostane	Carpagnano et al. 2002	[415]	High concentration	Data unclear	Few data about effects of CPAP

CO, carbon monoxide; CPAP, continuous positive airway pressure; EBC, exhaled breath condensate; FENO, fractional exhaled nitric oxide; LT, leukotriene; OSAS, obstructive sleep apnoea syndrome.

With all these methods, sputum sampling is the most validated technique and thoroughly discussed in the literature, in terms of appropriate methodologies discussed and published guidelines [420-422]. Published work in this Department has previously shown that induced and spontaneous samples contain similar total cell counts and IL-8 concentration [423], and therefore samples were treated identically.

1.10.4 Epworth Sleepiness Scale

The common complaint symptom with OSA patients is Daytime sleepiness [424-425]. Approximately 78% of OSA patients have been reported to have excessive daytime sleepiness (EDS) and fatigue as major complaints [426]. The cause of excessive daytime sleepiness results from sleep fragmentation due to repeated central nervous system arousals, in response to disordered breathing events. OSA may be significantly under-recognised as causes of excessive daytime sleepiness. One study estimated that 93 per cent of women and 82 per cent of men with moderate to severe OSA are undiagnosed [84].

To evaluate daytime sleepiness, all OSA patients were measured according to the Epworth Sleepiness Scale (ESS). ESS is a simple, convenient and self-administered questionnaire in which patients rate their propensity to fall asleep in eight different situations [427]. The subject is required to score for the likelihood of falling asleep in eight different situations on a scale of 0–3 (0 being little or no chance of dozing and 3 representing a high probability of dozing). A score of ≥ 10 indicates abnormality and a score of > 14 suggests a significant degree of EDS [428-429]. Numerous studies using the ESS have demonstrated the high validity and reliability of the test [430-431]. Moreover, it is an inexpensive evaluation and has been translated into more than five different languages, and numerous studies have vouched for its validity and reliability in different languages [432-436].

Although the ESS is brief and simple to administer, the score is subjective and may indicate symptoms of fatigue rather than EDS exclusively. Due to its subjective nature, ESS alone cannot be used as a diagnostic tool for OSA. However, the ESS attempts to avoid biases that may occur during the test by asking respondents to gauge their responses based on propensity to sleep over the past few weeks rather than just at the time of testing [437].

The multiple sleep latency test (MSLT) is regarded as the gold standard for objective determination of daytime sleepiness [438], but is not used routinely because it is expensive and requires the patient to remain in the sleep study all day.

To measure the improvement of quality of sleepiness with OSA in this thesis, we have used ESS before CPAP therapy and as a follow-up measure over a six-month period (Appendix Seven).

1.11 Cytokines and Mediators Relevant to This Thesis (IL-6, IL-8 & MPO)

One of the factors involved in sleep regulation is the immune system, consisting of humoral and cellular components. Molecules that transport the information are, beside others, the cytokines. Cytokines are low molecular weight protein mediators which control cell growth, inflammation and repair. Cytokines can be defined as the following:

"…regulatory proteins secreted by white blood cells and a variety of other cells in the body; the pleiotrophic actions of cytokines include numerous effects on cells of the immune system and modulation of inflammatory responses". [439]

In the lung, the dynamic balance is maintained by pro-inflammatory and anti-inflammatory cytokines. For example, COPD and Asthma are examples of conditions where the balance of cytokines in the lung is directed to a pro-inflammatory phenotype [440-441]. However, in OSA the levels of inflammation of pro-inflammatory cytokines are commonly elevated in OSA. There is increased inflammatory cell infiltration within the tissue of upper airway in OSA [88, 281]. Such cytokines could alter tissue structure and function, and impair muscle contractility [442-443]. Therefore, evaluation of inflammatory mediators with OSA patients may provide insights into the mechanisms underlying inflammation, some of such cytokines and mediators that have active participation in the inflammatory process that has been selected in this thesis are discussed below:

Interleukin-6

Interleukin IL-6 is a pro-inflammatory cytokine. It is secreted by macrophages, monocytes, T cells, B cells, fibroblasts, bone marrow stromal cells, keratinocytes, and endothelial cells (**Figure 16**) [444]. Primary basic protein secreted from eosinophils can interact with IL-1 or TGF to increase IL-6 release from fibroblasts [445].

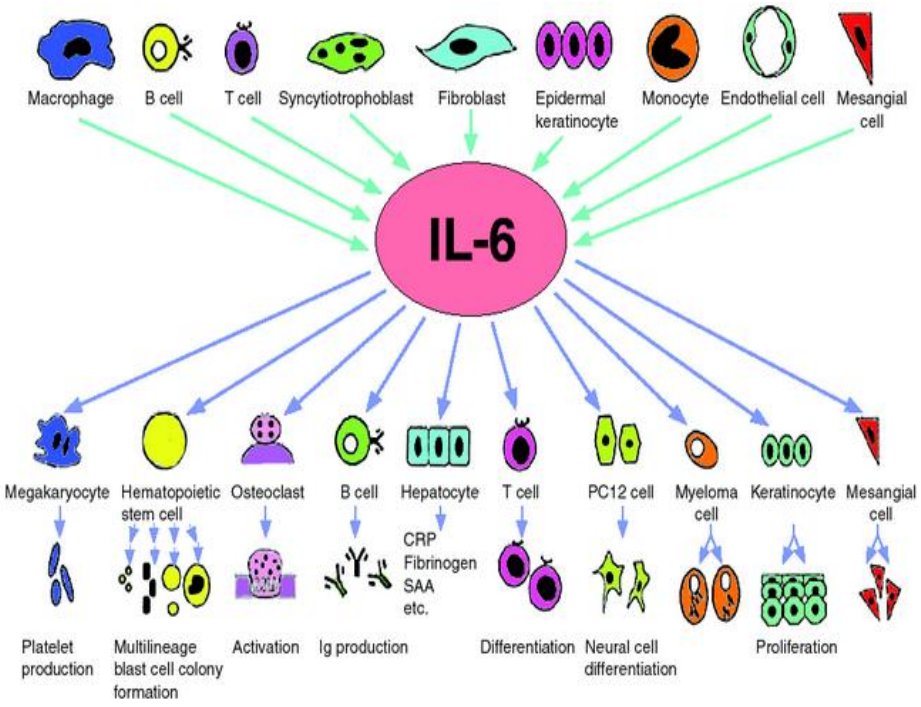


Figure 16: Cells producing interleukin-6 (IL-6) and the actions of IL-6 in the body.

IL-6 is important in the induction of the acute phase and augmentation of antibody production and is released in early inflammatory response [446]. IL-6 is a key-cytokine of the pro-inflammatory immuneresponse, is involved in different functions and diseases, and plays a role in sleep apnoea and responds to sleep deprivation [447]. In COPD, serum IL-6 levels were elevated compared to the baseline of exacerbated COPD patients [448].

Interleukin-8

IL-8 is a chemokine and also known as CXCL8 [449]. IL8 is produced by stimulated monocytes, fibroblasts, macrophages, endothelial cells. In humans, the interleukin-8 protein is encoded by the IL8 gene [450]. The main function of IL-8 is the induction of chemotaxis in its target cells and activator of neutrophils. It binds to CXCR1 and CXCR2 receptors, resulting in an increased intracellular calcium concentration, exocytosis of neutrophil granules and a respiratory burst [451]. CXCR2 appears to mediate the chemotactic response. The primary IL-8's function is to recruit neutrophils to phagocytose the antigen, which triggers the antigen pattern toll-like receptors (**Figure 17**) [452].

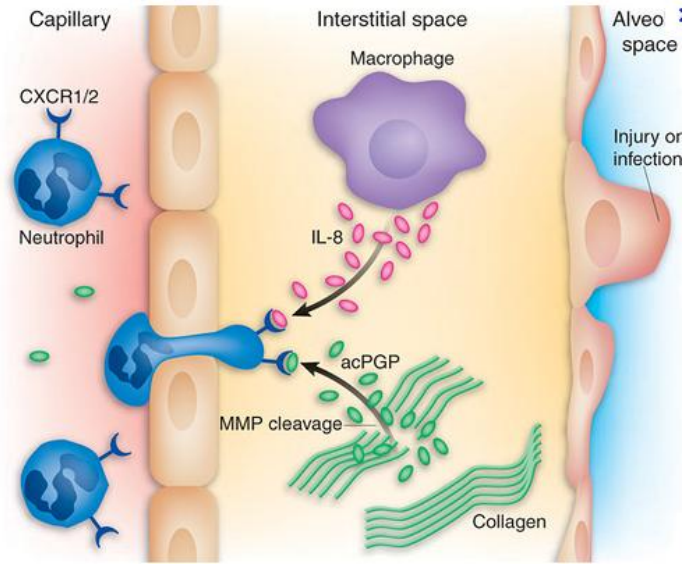


Figure 17: Functioning of IL- 8 in the body.

IL-8 is a chemokine which serves as a chemical signal that attracts neutrophils at the site of inflammation, and therefore is also known as neutrophil chemotactic factor. IL-8 differs from all other cytokines in its ability to specifically activate neutrophil granulocytes. High concentrations of IL-8 levels have been reported in the airways of COPD patients [453-454], which is significantly higher when compared to asthmatic patients [455]. IL-8 has been found to increase in adult OSA patients [301 ,456-457], and a cause of elevated cardiovascular risk [458].

Myeloperoxidase (MPO)

Myeloperoxidase is most expressed in neutrophil granulocytes. It is a lysosomal protein stored in azurophilic granules of the neutrophil, which causes green color in secretions rich in neutrophils, such as pus and some forms of mucus [459]. This enzyme is predominantly found in neutrophils, monocytes and some of the subtypes of macrophages. It represents more than 5% of total protein content of the cell in neutrophils [460]. MPO retain potent pro-inflammatory properties and may contribute directly to tissue injury. In the airway, MPO is a reflection of neutrophil activation. Increased level of serum MPO was link between with cardiovascular disease [461]. It was demonstrated that an increased MPO level in patient's blood serves as a risk marker for atherosclerosis [462] and coronary artery disease [463]. In OSA, increased salivary MPO levels were reported as may be useful as oropharyngeal local inflammatory marker in OSA patients [464].

2

AIMS AND HYPOTHESIS

HYPOTHESIS

This thesis tests the hypothesis that non-invasive respiratory support is a pro-inflammatory stimulus to the upper and lower airways. This hypothesis is tested in healthy subjects, patients with OSA and COPD, and on a cultured human bronchial epithelial cell line (BEAS-2B).

Aims

(1). At the outset of the investigation, we aimed to validate two relevant methodological techniques used in this thesis:

1. To examine the validity of intersession repeatability of acoustic rhinometry measurements of unilateral and combined nasal parameters in a group of healthy subjects.

2. To establish the repeatability and acceptability of a novel upper airway sampling technique we have developed, employing a paediatric tracheostomy tube (TT).

All details and results of this aim are introduced and discussed in **Chapter 3**.

(2). Is CPAP a pro-inflammatory stimulus in healthy subjects?

- To investigate the effect of 3 hours of 7.5 cmH_2O and 12.5 cmH_2O, non-humidified nasal CPAP on airway and systemic inflammation, and the effect of physiological function and thus upper airway symptoms in healthy subjects.
- To determine any relationships between the subsequent effects on the upper airway and lower airways, and whether there are differences between the two groups due to the dose-response effect.
- To investigate whether nasal CPAP escalates the nasal symptoms, and airway and systemic inflammation when effect-dose of nasal CPAP is increased from 7.5 cm H_2O to 12.5 cmH_2O in healthy subjects groups, and whether there are differences between the two groups at baseline and at 3 hrs of continuous CPAP use.

All details and results of this aim are introduced and discussed in **Chapter 4**.

(3). Is CPAP a pro-inflammatory stimulus in OSA patients?

- To investigate the effect of non-humidified nasal CPAP on the airway and systemic inflammation, and the effect of physiological function in OSA at the following intervals:
    - Baseline (no nasal CPAP)
    - One month (with nasal CPAP)
    - Three months (with nasal CPAP)
    - Six months (with nasal CPAP)

- To investigate the impact of nasal CPAP therapy on the Epworth sleep score, compliance & periodicity questionnaire, and six-point score.
- To investigate if symptomatic OSA patients are more likely to have airway and/ or systemic inflammation, and thus show less compliance to the nasal CPAP therapy.

All details and results of this aim are introduced and discussed in **Chapter 5**.

(4). *In vitro* study: Is CPAP a pro-inflammatory stimulus to human bronchial epithelial cell-lines in a culture model?

We used an *in vitro* cell-culture model to fulfil the following objective:

To investigate the *in vitro* effects of 4 and 7 cmH_2O CPAP exposure on interleukin (IL)-6 and (IL)-8 production by a human bronchial epithelial cell line (BEAS-2B) over 4 time intervals.

All details and results of this aim are introduced and discussed in **Chapter 6**.

(5). Is NIV pro-inflammatory stimulus in a matched group of COPD patients?

In the final part of this thesis, we conduct a pilot study with stable COPD patients to achieve the following specific aim:

To investigate the effect of using bilevel positive pressure airway therapy on inflammatory indices with stable COPD, comparing this to a COPD group, and by not using the bilevel positive pressure airway therapy.

All details and results of this aim are introduced and discussed in **Chapter 7**.

3

VALIDATION OF THE NASAL ASSESSMENTS

This chapter describes the validation process for methodologies used for the nasal assessments adopted in this thesis. To reiterate the content of the previous chapter, this is a study of nasal obstruction using acoustic rhinometry, and upper airway sampling using a dilutional wash via a paediatric tracheostomy in healthy subjects and OSA. The data have been presented at meetings of the American Thoracic Society 2010 and European Respiratory Society 2010. The validation of both methodological studies has been accepted for publication in the Translational Research, and Clinical and Experimental Otorhinolaryngology [327, 350], attached as Appendix Two.

PART A

VALIDATION OF ACOUSTIC RHINOMETRY

3A.1 INTRODUCTION

The nose is the natural and preferred respiratory passageway. Nasal obstruction is common symptom and the etiology of nasal obstruction may be anatomical, physiological or pathological. Nasal mucosal inflammation is the most common pathologic cause and besides viral upper respiratory tract infections, allergic rhinitis is the most frequent cause of nasal obstruction [465]. Since nasal obstruction is common in a wide range of sinonasal disorders, a technique to assess this work have an important role in the diagnosis and follow-up of conditions altering intranasal dimensions, either permanently or transiently. Acoustic rhinometry (AR) objectively defines nasal cavity dimensions by acoustic reflections as described in the introduction. AR is a static test and independent of airflow [466]. It estimates intranasal volume between predefined segments, and cross-sectional areas through identification of local changes in acoustic impedance using a sound pulse propagating in the nasal cavity.

AR is able to detect irreversible structural changes and reversible dynamic changes, which has been used in many conditions in adults and children including sleep disorders [467].

The automated nature of the technique and dependency of measurements on position necessitates the confirmation of its repeatability in serial measurements over consecutive sessions only by understanding repeatability can we assess change with disease and or intervention. The intersession repeatability of AR remained to be clarified and this has important clinical implications, particularly when the method is to be used for the purpose of follow-up. Importantly, no previous study had examined the intersession repeatability of

AR using the intraclass correlation coefficient, comparing combined (mean) and individual nares, in healthy subjects [468-473]. The present study was therefore designed to assess the inter-session repeatability using the statistical approach of intra-class correlation co-efficient on averaged combined and separate nostril minimum cross-sectional areas and nasal volumes in healthy subjects.

This study aimed:

1. to define the intersession repeatability of acoustic rhinometry measurements of unilateral and combined nasal parameters in a group of healthy subjects.

And to test the following hypothesis:

2. that combined nasal parameters (according for changes with nasal cycle) are more reproducible estimates of nasal geometry over time than assessing each nostril separately.

3A.2 METHODs

3A.2.1 Study Subjects

Twenty healthy subjects were enrolled into the study from the London Respiratory Medicine Research clinic at Royal Free London NHS Foundation Trust. Inclusion criteria to the healthy group comprised no active respiratory or nasal diagnosis, and no nasal medications.

3A.2.2 Measurement Protocol

AR measurements were performed in accordance with a previously published protocol [362]. The AR device was A1 Acoustic equipment with an updated software version 0.5 (GM Instruments, Kilwinning, UK). Measurements were obtained when the subject was in a sitting position and under close supervision of the operator. Each subject was instructed to open the mouth and stop breathing during the measurements. The sound tube was handheld by the subject to obtain a sealed nostril. **Plate 8** shows the handling and positioning of the wave tube by the subject. All measurements were obtained by the same operator in the same air-conditioned room to provide similar conditions with regard to temperature, humidity and ambient noise level. Multiple recordings were taken for each subject at each visit and curves with obvious artefact were discarded. For each subject, measurements were repeated on five consecutive days.

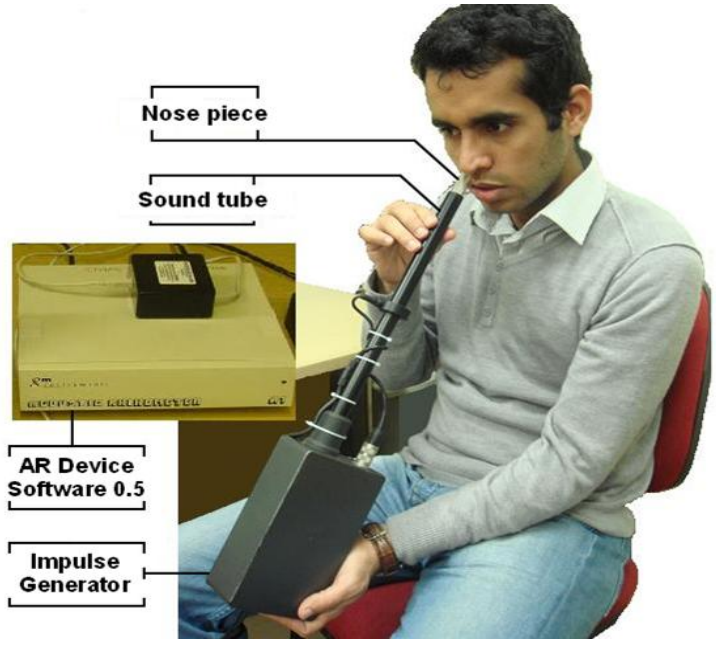
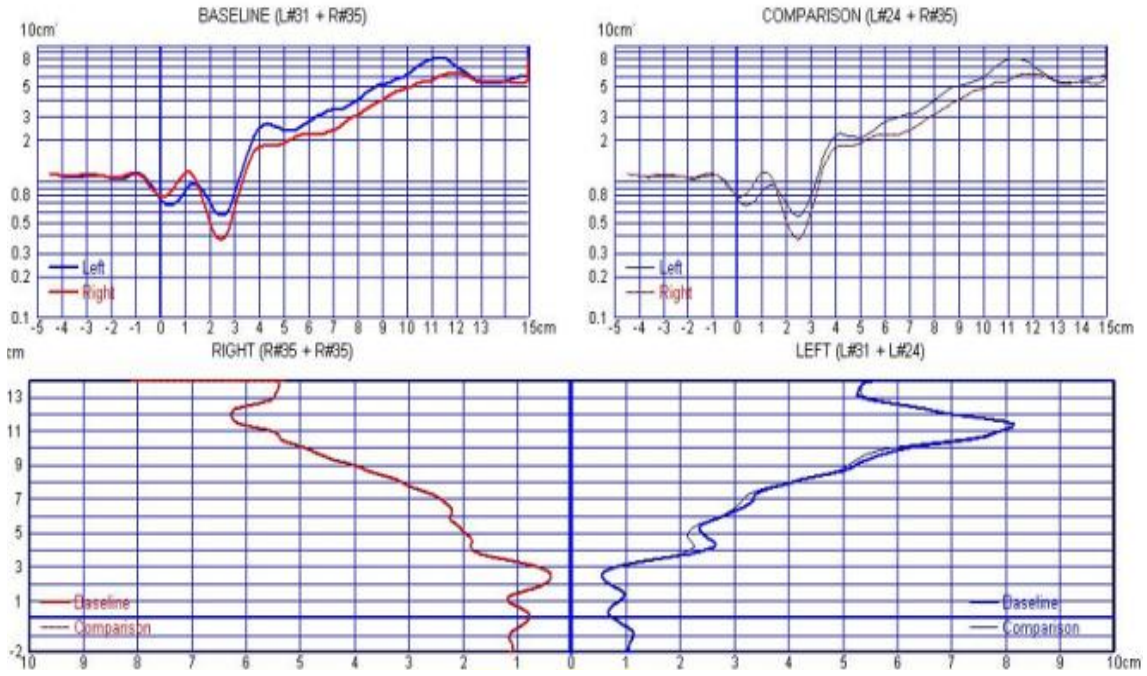


Plate 8: Handling and positioning of the device by the subject (Reproduced with permission of the volunteer).

3A.2.3 Variables

Five important AR variables were assessed and examined separately to test the intersession repeatability of the technique: outermost minimum cross-sectional area (MCA1, cm^2), the distance of MCA1 from the nasal orifice (D-MCA1, cm), innermost minimum cross-sectional area (MCA2, cm^2), the distance of MCA2 from the nasal orifice (D-MCA2, cm), and the volume of the nasal segment between the 2^{nd} and 5^{th} cm from the nasal orifice (V_{2-5}, cm^3). MCA1 corresponds to the level of nasal valve, whereas MCA2 corresponds to the anterior half of the inferior turbinate. In each subject on each day, areas, distances and volume values for each separate nostril were averaged (mean), and combined values were obtained by calculating the mean of the mean results from the right and left nostrils to correct for the variation between nostrils during the nasal cycle. **Plate 9** illustrates a typical rhinometry trace from one of the subjects.



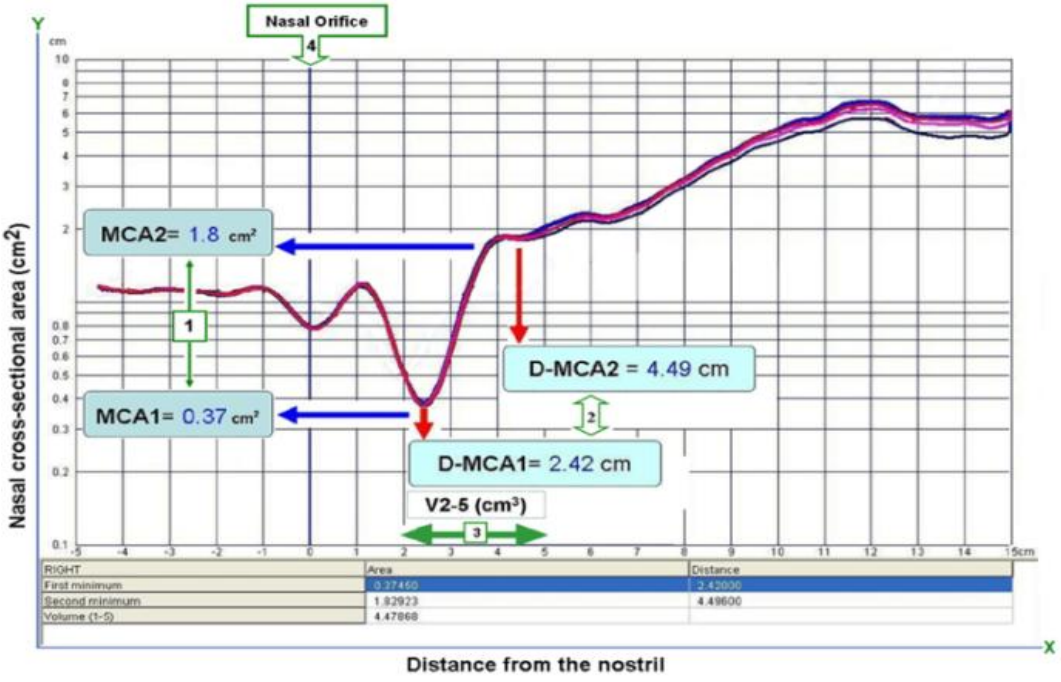


Plate 9: An example of an acoustic rhimonetry trace. The x-axis represents distance from the nostril (at 0 cm), and the y-axis represents the nasal cross-sectional area (cm^2). MCA1 and MCA2 (arrow 1) correspond to the nasal valve and anterior half of the inferior turbinate, respectively. D-MCA1 and D-MCA2 (arrow 2) correspond to the distance of MCA1 and MCA2 from the nasal orifice (x=0cm), respectively. The green arrow (arrow 3) indicates the segment used for the calculation of $V_{2\text{-}5}$, and arrow 4 the nasal orifice.

3A.2.4 Statistical Analysis

SPSS version 18.0 was used for the analysis of data. Intraclass correlation coefficients (ICC) were used to examine intersession repeatability of each parameter measured over five separate occasions for unilateral and total (combined right and left) values. ICC values were interpreted as follows: 0.41-0.60, moderate agreement; 0.61-0.80, substantial agreement; > 0.80, "almost perfect agreement" [474]. In addition, the mean coefficient of variance and inter-item correlations were calculated for each parameter. A p value <0.05 was considered indicative of statistical significance.

3A.3 RESULTS

Twenty healthy subjects (13 males, 7 females) without a history of nasal symptoms or disease were included in this study. The mean (SD) age of the subjects was 38 ± 7.1 years.

Table 14 reports the repeatability estimates of combined (mean of left and right) values for the five different acoustic rhinometry measurements. Intraclass correlation coefficients and inter-item correlation coefficients indicated excellent agreement for all parameters across five separate sessions. All intraclass correlations were ≥0.80 confirming almost perfect agreement. All intraclass correlations and inter-item correlations were associated with p values < 0.001. The mean coefficient of variation was low (<10%) for all but MCA1 measurements (14.5%). The coefficient of variation was particularly low for D-MCA1 (7.4.2%), D-MCA2 (2.8%), and nasal volume (5.4%).

Unilateral data from the right and left nostrils are also presented in **Table 14**. Agreement was less good (lower intraclass correlation) than when using data from both nostrils combined.

Table 14: Repeatability for the combined (mean of left and right) and separate nostril values for each acoustic rhinometry variable over five days.

	Variable* Combined values	Item mean±SD	Mean CV (%)	Inter-item correlation coefficient	Intraclass correlation coefficient
COMBINED	MCA1 (cm^2)	0.54±0.07	14.5	0.87	0.89
	D-MCA1 (cm)	2.17±0.15	7.4	0.86	0.88
	MCA2 (cm^2)	1.61±0.16	9.1	0.86	0.86
	D-MCA2 (cm)	4.28±0.12	2.8	0.87	0.88
	V_{2-5} (cm^3)	4.63±0.24	5.4	0.94	0.93
RIGHT NOSTRIL	MCA1 (cm^2)	0.53±0.07	13	0.88	0.84
	D-MCA1 (cm)	2.07±0.06	9.2	0.82	0.83
	MCA2 (cm^2)	1.59±0.18	11.1	0.83	0.84
	D-MCA2 (cm)	4.20±0.18	4.8	0.79	0.80
	V_{2-5} (cm^3)	4.60±0.25	6.5	0.92	0.91
LEFT NOSTRIL	MCA1 (cm^2)	0.52±0.08	14.2	0.88	0.84
	D-MCA1 (cm)	2.12±0.13	6.4	0.83	0.81
	MCA2 (cm^2)	1.64±0.24	15.2	0.76	0.75
	D-MCA2 (cm)	4.37±0.16	3.8	0.82	0.81
	V_{2-5} (cm^3)	4.57±0.33	7.2	0.81	0.78

*Combined variables were calculated as the average (mean) values for the right and left nostrils. CV, coefficient of variation; SD, standard deviation; MCA, minimum cross sectional area; D-MCA, distance of MCA from nasal orifice; V_{2-5}, volume of nasal cavity between the 2^{nd} and 5^{th} cm from nasal orifice.

3A.4 DISCUSSION

This is the first study to examine the intersession repeatability of acoustic rhinometry using ICC on averaged combined and separate nostril minimum cross-sectional areas and nasal volumes in healthy subjects. We showed that acoustic rhinometry provides excellent reproducible results, best over different sessions when combined nasal parameters values are used than right or left nostrils separately. This degree of repeatability has clinical implications with regard to the utilisation of this rapid and easy-to-use technique in the follow-up of conditions associated with impaired nasal patency. We reassured to the technique would be valid in this thesis.

The measurements obtained in this study included a wide range of clinically relevant parameters: minimum cross sectional area at two distinct points and their corresponding distances from the nostrils, and the volume of the nasal cavity between the 2^{nd} and 5^{th} centimetres. The outermost minimum cross sectional area (MCA1) corresponds to the nasal valve and is a good indicator of structural obstruction, whereas the innermost minimum cross sectional area (MCA2) corresponds to the anterior half of the inferior turbinate, which contains erectile tissue and therefore is a reflection of functional obstruction. Similarly, the region of the nasal cavity between the 2^{nd} and 5^{th} centimetres is the site of mucosal changes; thus this volume is also considered a reflection of functional obstruction.

Several previous studies had examined the intersession repeatability of acoustic rhinometry measurements in both healthy individuals and in patients with nasal obstruction, but each had limitations, which we have attempted to address in the present study design. Similar to the current study, except not using combined values for nostrils, Silkoff et al. [471] examined the repeatability of acoustic rhinometry measurements performed on five separate occasions and found relatively low variation in minimal cross sectional area and nasal volume (0-5 cm). Measurements of minimal cross sectional area showed 8.1% and 9.7% variation for the right and left sides, whereas variations were even lower for nasal volumes (4.8% and 5.5%, respectively). Intraclass coefficients for right and left MCA were 0.91 and 0.87, whereas corresponding figures for nasal volume were 0.86 and 0.69, respectively. This study, unlike ours, did not combine values from both nostrils and therefore does not account

for variation with nasal cycle. We hypothesised, and showed that combined results were more repeatable than those from individual nostrils. In a separate study, Castano et al. [468] examined the repeatability of acoustic rhinometry measurements in patients with occupational rhinitis and found low variation in both minimal cross sectional area and nasal volume measurements. Intersession intraclass correlation coefficients ranged from 0.80 to 0.88 and from 0.83 to 0.94 for nasal volume and MCA, respectively. These figures are in agreement with the findings of this study on healthy individuals.

Harar et al. [469] investigated the repeatability of acoustic rhinometry measurements on day-to-day basis using the minimal cross sectional area at the nasal valve level (MCA1). They made measurements on just two separate days and found a relatively high mean intersession difference (12.9%) when only one measurement was performed at each visit and this mean intersession difference could only be reduced to 10.85% when ten recordings were done for each subject at each visit. We report a slightly higher variation in minimal cross sectional area measurements at the nasal valve; however, intraclass correlations calculated using measurements on five consecutive days showed better agreement. The higher variation in MCA1 measurements (14.5%) may be attributed to the malpositioning of the device by the patient. However, intraclass correlation takes into account correlations between every pair of observation and thus may be regarded as a more accurate means for examining the repeatability of the measurements over time. Similarly, Ognibene et al. [470] found lower agreement between measurements obtained on two different sessions one week apart, with intraclass coefficient of 0.67. The high intraclass correlation coefficients obtained in the present study may be attributed to the relatively short duration between measurements in contrast to the Ognibene study, which may cause bias due to carry-over effect.

Most previous studies have assessed parameters for the individual nasal cavity (either right or left) separately; however, in our study we used combined (mean) parameters from the two nostrils to correct for the variations between nostrils during the nasal cycle. This may also account for the strong agreement found in this study. Similarly, Roithman et al. [472]

found less intersession coefficient variation for combined parameters when compared to unilateral parameters, but this data does not employ the assessment of intraclass correlation.

Acoustic rhinometry is a rapid, convenient and reliable technique to examine nasal patency. Although there are several techniques that can be utilised for this purpose, acoustic rhinometry has several advantages. The benefits of rhinomanometry have been demonstrated in many clinical settings; however, it does not give an idea on the localization of the obstruction. Nasal endoscopy on the other hand is not able to produce quantitative results, although it provides excellent visual topography. Similarly, nasal examination provides only subjective information on the degree of obstruction. Acoustic rhinometry has the ability to localize and quantify both reversible and irreversible obstruction of the nasal cavity. However, since it is a position dependent procedure, concerns related to the repeatability of measurements have arisen. Current evidence suggests that repeated measurements should be made in each session to obtain a reasonable level of reproducibility [474]. In addition, Harar et al. [469] reported better intersession repeatability when multiple measurements were done in each session. Intersession repeatability itself is important particularly when the technique is to be used for follow-up purposes. Therefore, multiple recordings were made in each session of this study to avoid the effects of intra-session variability.

The accuracy of acoustic rhinometry has shown to be higher for the anterior part of the nose when compared to posterior part [337, 340, 475]. Tarhan et al. [475] reported that acoustic rhinometry overestimates cross-sectional area beyond the paranasal sinus ostia. In this study, both minimal cross sectional areas measured at two different levels showed similar repeatability, suggesting that acoustic rhinometry provides reproducible results at as far as the level of anterior half of the inferior turbinate.

3A.5 CONCLUSION

Using intraclass correlations in healthy subjects, combined acoustic rhinometry measurements (mean of left and right nostrils) provide excellent and more reproducible estimates of nasal geometry over time than assessing each nostril separately, in terms of both localization and reversibility of pathological obstructions. The support of its use for the follow-up of conditions associated with structural or functional obstruction, in any to be described in the remainder of the thesis.

PART B

VALIDATION OF NASAL LAVAGE SAMPLING TECHNIQUE

3B.1 INTRODUCTION

This chapter describes repeatability, importance in understanding changes with time in dilutions. The nose is the natural and preferred respiratory passageway. The distinction between the upper and lower airway is artificial and many lung diseases are associated with upper airway manifestations. There is therefore the need for a simple, repeatable, acceptable method for sampling the upper airway.

As discussed earlier in section “methods of assessing upper airway” of this thesis, the nasal cavity is affected by many conditions including inflammatory diseases such as OSA and COPD. Since it is the most accessible part of the respiratory system, the cellular and biochemical composition of nasal specimens can be readily evaluated. To avoid obtaining surgical biopsy specimens that gives excellent information on cellular events, this technique is invasive and associated with bleeding risk, and artifacts may occur from the infiltration of local anesthetic, therefore, nasal secretions was considered in this thesis which may also aid in the diagnosis and follow-up of conditions affecting upper or lower airways, in particular inflammatory and allergic disorders [322-324].

Nasal washing (nasal lavage) is a commonly used technique. However, unpredictable amounts of fluid losses through swallowing, absorption by nasal mucosa and leakage from the nasal orifice all contribute to a variable degree of dilution resulting in concerns related to the repeatability of the technique.

Concentrations of interleukin (IL)-6 and IL-8 in nasal secretions are good markers for the degree of inflammation since both take part in the inflammatory process. Cell counts provides information on the inflammatory status and are a good indicator of the effectiveness of nasal sample collection technique, which has been used in previous studies examining the repeatability and reliability of sample collection methods [476-477]. Finally, recovery volume and its variability is the main determinant for the degree of dilution.

Considering these disadvantages of conventional nasal lavage techniques, we developed a novel and practical method for the collection of nasal secretion. The method utilises a paediatric tracheostomy tube with a good seal at the nasal orifice, is less time consuming, can be reprocessed for usage and provides comfortable test conditions.

This study aimed:

1. To introduce a novel concept of utilising a paediatric tracheostomy tube with a good seal, less time consuming, sterilised and can be reprocessed for usage and provides comfortable test conditions.

2. To investigate the repeatability of our novel method with regards to several clinically and technically relevant parameters, in healthy subjects across sessions performed on five consecutive days.

3B.2 METHODS

3B.2.1 Study Subjects

Fourteen healthy subjects were enrolled in this study. Subjects were clearly instructed before and during the entire experimental period to follow their normal activities. Inclusion criteria to the healthy group comprised no active respiratory or nasal diagnosis, and no nasal medications.

3B.2.2 Materials for Nasal Lavage Technique

The nasal lavage was performed by using a Bivona Fome-Cuf Pediatric Silicone Tracheostomy Tubes, size I.D 2.5 mm (UK). The arrangement is illustrated in **Plate 10 and 11.**

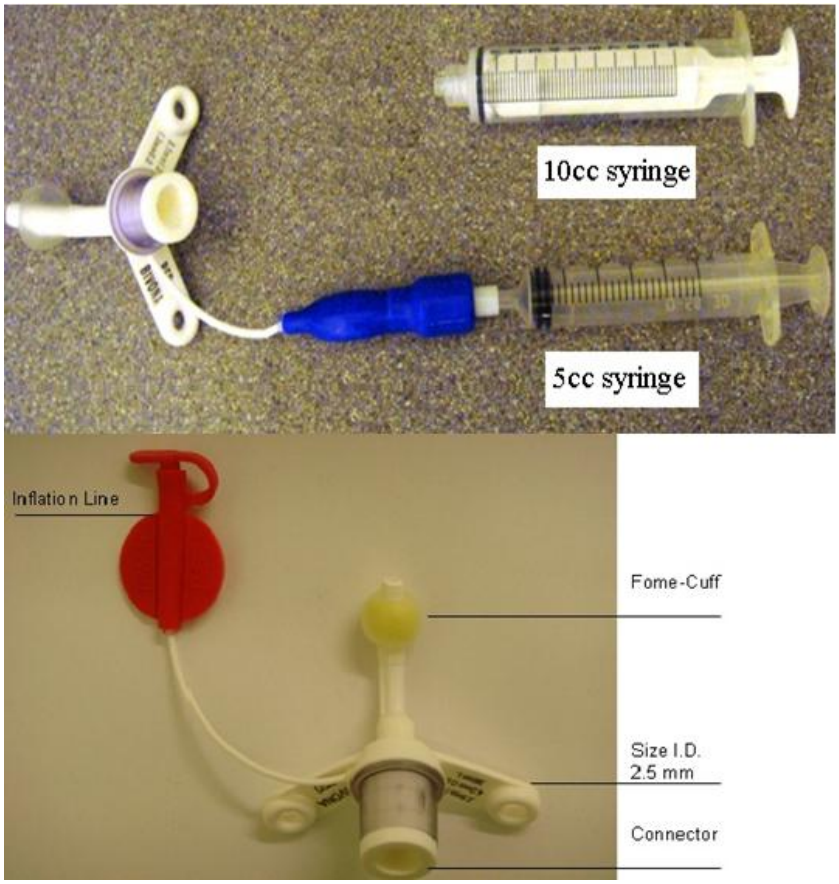


Plate 10: Shows the components of tracheostomy tube (Bivona Fome-Cuff Tracheostomy Tube, Smiths Medical, UK).

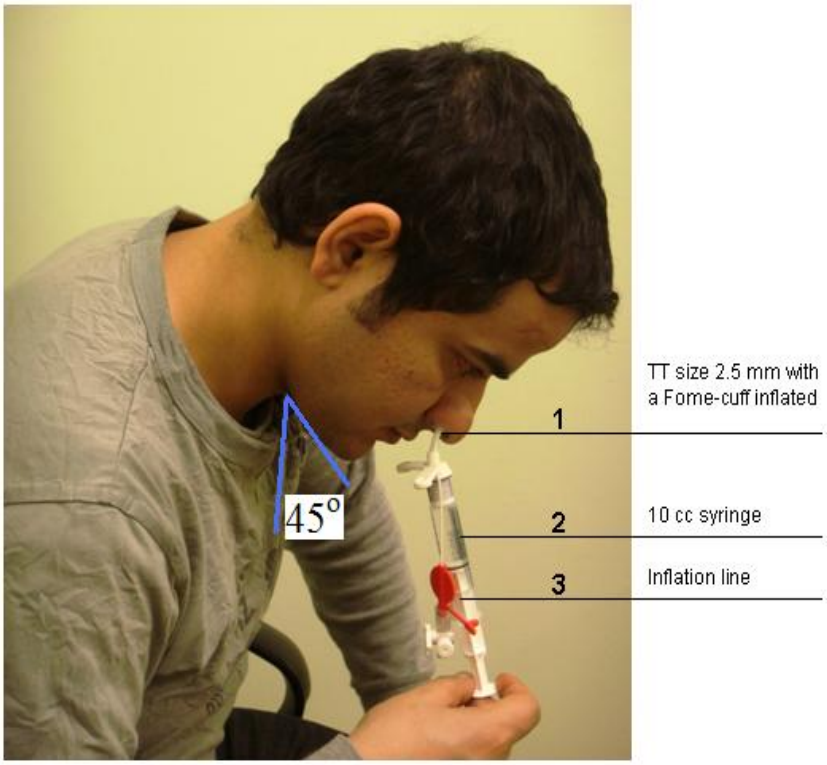


Plate 11: The tracheostomy tube is inserted into the nasal cavity and the Fome-cuff is inflated (arrow 1). Arrow 2 indicates the 10 ml syringe that contains 7 ml of 0.9% saline and arrow 3 shows the inflation line for the cuff. Written consent was obtained from the subject for the photograph.

Processing of the TT describing the reprocessing protocol as recommend from the Smiths Medical Company (Appendix Three).

3B.2.3 Collection of Nasal Lavage Samples

After explaining the procedure, the patient was asked to bend forward, seating with approximately 45 degrees of angle between the chin and the chest. The paediatric tracheostomy tube was inserted into one of the nasal orifices and the Fome-cuff was inflated to achieve comfortable sealing. Then 7 ml of saline solution (0.9% NaCl) at room temperature was slowly instilled through the tracheostomy tube using a syringe, and then the solution was drawn back (**Plate 11**). This process was repeated once more; thus, the

nasal cavity was washed in and out twice. A clean funnel tube was used to collect any leaked fluids during the removal of the tube from the nose and this fluid was also added to the sample. The technique was repeated for the other nasal cavity using another syringe and the two recovered solutions were pooled for analysis. For each subject, measurements were repeated on five consecutive days. To ensure standard conditions, all sampling procedures were performed by the same person. Nasal wash recovery volume, cell count and cytokine concentrations (IL-6 & IL-8) were used to assess the intersession repeatability of the technique.

3B.2.4 Measurements

The nasal wash from both left and right nostrils was measured and recorded as the total pooled recovery volume (maximum 14 ml). The pooled sample was then centrifuged for 10 minutes at 1000rpm at 4°C to yield a cell pellet for a total percentage of cell types found within the collected sample. Aliquots of supernatant were collected and stored at – 80°C until required for the analysis of inflammatory cytokines. The cell pellet was re-suspended in 400 to 1200 µl with phosphate-buffered saline (PBS) solution and gently mixed. A 50 µl aliquot was removed and stained with Trypan blue stain and counted using a Neubauer haemocytometer. Measurement of the supernatant inflammatory cytokines (IL-6 and IL-8) was carried out by a standard ELISA technique. The ELISA kits used was obtained commercially from R&D Systems Europe. The limits of detection were 0.70 pg/ml (IL-6), and 3.5 pg/ml (IL-8).

3B.2.4 Statistical Analysis

SPSS version 18.0 (SPSS Inc, Chicago, Ill) was used for the analyses of data and data are expressed as mean (SD). Kolmogorov-Smirnov test was used to test normality of distribution. Intraclass correlation coefficients (ICCs) were used to examine intersession repeatability of recovery volume, cell count, and IL-6 and IL-8 levels over 5 separate occasions. Logarithmic transformation was performed for cytokine concentrations and cell count. ICC values were interpreted as follows: 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; and .0.80, "almost perfect agreement" [474]. In addition, the mean coefficient of variance values and interitem correlations were calculated for each parameter. A P value <0.05 was considered indicative of statistical significance.

3B.3 RESULTS

Fourteen healthy subjects (9 males, 5 females) without a history of nasal symptoms or disease were included in this study. The mean ± SD age of the subjects was 35 ± 7.6 years. **Table 15** shows the repeatability estimates for cell count, cytokine concentrations and recovery volume over a 5 day period. Intraclass correlation coefficients and inter-item correlation coefficients indicated strong agreement for cell count and IL-8 concentrations. However, recovery volume and IL-8 levels were more variable. Nevertheless, all intraclass correlations and inter-item correlations were associated with $p < 0.001$. The mean coefficient of variation was very low for cell count (2%), IL-8 concentration (3%), and recovery volume (3%), whereas it was quite higher for IL-6 (48%). The mean percentage of recovery was high (87%).

Table 15: Repeatability of nasal wash parameters over five consecutive days in 14 subjects.

Variable*	mean±SD	Inter-item correlation coefficient	Intraclass correlation coefficient
Cell count (log_{10} cells/ml)	4.15±0.26	0.88	0.87
IL-6 concentration (log_{10} pg/ml)	0.44±0.34	0.79	0.78
IL-8 concentration (log_{10} pg/ml)	1.71±0.37	0.98	0.98
Recovery volume (ml)	12.19±0.54	0.63	0.60

Abbreviation: CV, coefficient of variation. *Data are expressed as mean 6 SD.

3B.4 DISCUSSION

This study examined the intersession repeatability of a newly developed nasal lavage technique utilizing a paediatric tracheostomy tube. Highly repeatable results for IL-8 and cell count data were achieved across five separate sessions. To our knowledge only few studies have examined the repeatability of different nasal lavage techniques for clinically relevant parameters and varying results have been obtained. Our data support the use of this technique in subsequent chapters.

Nikasinovic-Fournier et al. [324] examined a method utilizing a transparent paediatric Foley catheter and tested the repeatability of the method across three different measurements for a number of parameters including the ones used in this study. Although ICC for recovery volume was quite high (0.81), the mean percentage of recovery volume was somewhat less than that observed in our study (75% versus 87%). Repeatability of their method with regard to total cell count (ICC, 0.73) indicate a substantial agreement but repeatability of IL-6 and IL-8 levels were quite low making the method unsuitable for the detection and follow-up of these two parameters in inflammatory conditions. Better sealing of the cuff using our paediatric tracheostomy tube may provide more comfortable conditions for the patient and prevent fluid loss, both from the nasal opening and through nasopharynx. Thus, less variation in interleukin levels, particularly in IL-8 concentrations, observed in this study may be attributed to more effective sampling with this novel technique.

Several studies have compared nasal lavage with other techniques for the collection of nasal samples [325, 476, 478-479]; however, none of them reported ICCs to evaluate repeatability. In addition, none of these studies has used a specific instrument for nasal lavage to aid in sample collection such as the paediatric tracheostomy tube used in this study. Riechelman et al. [325] compared four different sampling techniques: nasal lavage, nasal spray blow technique, filter paper method, and polyurethane foam sampler technique. Intra-individual variations of total protein concentration over three measurements were high for all four methods: 43%, 48%, 34%, and 39%, respectively. Melillo et al. [476] examined nasal lavage and a technique utilizing ultrasonic nebulisation of hypertonic solution and

tested the repeatability of ultrasonic nebulisation technique, but not the repeatability of nasal lavage, using the measurements obtained on two occasions in each individual. They reported significant and high correlations for total cell count (TCC) (r=0.81), differential cell count (r=0.72-0.90), and eosinophil cationic protein (ECP) (r=0.85) between two measurements from each individual. However, TCC and ECP results obtained by the two methods did not correlate and absolute TCC and ECP values were lower with nasal lavage. Walsh and Falsey et al. [479] compared nasal wash and nasal swab techniques in the detection of several virus specific IgAs and found that both techniques were reproducible with test-retest correlation coefficients ranging between 0.71-0.75 for nasal wash and 0.85-0.86 for nasal swab technique. Ours is the first study using ICC, repeated on five consecutive days, reporting both cell counts and inflammatory markers.

A concern of nasal lavage methodology is the unpredictable degree of dilution since unknown fractions of the instilled fluid may be swallowed, lost from the nasal opening or absorbed through nasal mucosa. This uncertainty may interfere with the reproducibility of the method and preclude its use for follow-up purposes. Accordingly, analyte concentrations were generally lower with nasal wash technique when compared to other techniques in the previous studies employing a classical nasal wash technique without the aid of a catheter and seal [325, 476]. The fine catheter used in this study provides comfortable conditions for the patients, may alleviate swallowing reflex, and through prevention of fluid loss allows the recovery of a relatively standard amount of lavage fluid as shown with moderate agreement for recovery volume and the high mean percentage of recovery. We believe that the high repeatability of IL-8 concentrations in our work reflects the high, and reliably reproducible volumes of wash fluid returned. In addition, whilst assessing dilution may be desirable, there is no accepted way of achieving this and methods assessing total protein may indeed prevent the detection of differences between health and disease and inflammatory nasal conditions are also associated with protein leak into nasal lavage fluid as previously reported from this laboratory [321].

Nasal secretions reflect the inflammatory status of the nasal mucosa and changes have been shown to parallel the course of inflammatory conditions [321, 326, 480]. In addition, the

nasal cavity is the first part of the respiratory system to come in contact with allergens and airbone pollutants. Thus, examination of nasal secretions is particularly valuable in conditions associated with inflammation. One method for sampling nasal secretions is the collection of spontaneous secretions. It is practicable in patients with hypersecretion as a result of nasal disease. However, the amount of fluid is often insufficient for analysis, particularly in healthy individuals [481-483]. Absorption techniques involve the placement of a sampler with absorptive properties into the nasal cavity and they provide sufficient amounts of undiluted nasal secretions for analyses. Although several studies showed that these techniques are sensitive, reliable and reproducible in several settings [325, 484-485], placement of a foreign substance for a period of time may traumatise the mucosa and alter the concentrations of analytes under investigation. Nasal lavage is a useful technique for biochemical and cytological exploration of nasal secretions since it is a non-invasive, cheap and easy-to-perform method not only suitable for use in clinical trials but also in large-scale epidemiological studies. In addition, nasal lavage is suitable for the examination of nasal cytology owing to its good inter-observer variability [486]. However, the unpredictable degree of dilution represents a major obstacle resulting in inconsistent results across repetitive measurements. In addition, the degree of dilution may be problematic when measuring analytes in low concentrations, which often will be well below the detection limit of the assay [325].

Biopsy sampling of nasal mucosa may represent another method suitable for the evaluation of cellular and biochemical events in inflammatory conditions [487-488]. Dilution is not an issue and cellular composition may be better evaluated. However, it is invasive, associated with bleeding risk, and artefacts may occur due to infiltration of local anaesthetic. In addition, repeated procedures are problematic, limiting its use for follow-up purposes.

One important finding of this study is that we confirmed the repeatability of measurements obtained on five separate occasions in each individual, which is higher than the number of sessions used in previous studies evaluating the repeatability of nasal sampling techniques. Several attempts have been made to correct for the degree of dilution including the use of inulin (an inert polysaccharide), lithium, urea or total protein; and the latter has been

reported as having the least inter-subject variation repeatability [481, 489-491]. However, due to protein leakage through the inflamed mucosa in the inflammatory conditions of the respiratory system, correcting for total protein concentration while estimating cytokine levels may not be reliable [321, 492]. In addition, several other variables including the position of the subject and the duration of contact between the fluid and the nasal mucosa may affect the concentrations of analytes in nasal secretions. The new technique presented in this study attempted to standardize these conditions to improve repeatability. Very high intersession repeatability was found for consecutive IL-8 measurements and cell counts. IL-6 measurements were also repeatable but the coefficient of variation was higher, and this is discussed below. However, there was moderate agreement between recovery volumes, which may be due to different amount of absorption and amount of secretion already present in the nasal cavity. Nevertheless, this variation does not seem to interfere with the repeatability of cytokine concentrations and cell counts substantially, although it may partly account for high variation in IL-6 measurements. In different disease states there may be different recovery and this will need studying. Nasal inflammatory conditions may reduce recovery.

The greater variability of IL-6 compared to IL-8 and cellular measurements are interesting, and important for future studies. Dilution is unlikely to be a factor as this would have affected all the parameters, and our recovery volumes were reproducible. Nikasinovic-Fournier et al. [324] also found lower intraclass correlation coefficients for IL-6 than IL-8 (0.33 versus 0.44), suggesting greater variability for IL-6 levels in nasal secretions. To date, no other study has directly compared the variability of IL-6 and IL-8 concentrations in nasal wash samples. Thus, this high variation may be due to higher intra-individual variations of IL-6 in nasal secretions in normal subjects between days, possibly due to environmental factors, which may be a subject for further studies. This also implies that in future nasal studies, researchers may wish to assess IL-8 responses which appear to be inherently more stable.

3B.5 CONCLUSION

The main findings of this study suggest that the newly developed nasal lavage technique utilizing a paediatric tracheostomy tube provides highly repeatable cytokine and cell count measurements over successive sessions. This may be attributed to better sealing of the nasal opening to avoid fluid loss, to comfortable conditions alleviating swallowing reflex and fluid loss through the nasopharynx, and to accurate and precise installation of a standard amount of fluid. Nasal IL-8 concentration appears inherently more stable than IL-6. Our method may prove valuable in the diagnosis and follow-up of inflammatory conditions involving the upper (and lower) respiratory tract and support as of the technique in the studies of this thesis.

4

DOSE-RESPONSE EFFECT OF NASAL CPAP ON SYMPTOMS, PHYSIOLOGY, AND INFLAMMATION IN HEALTHY SUBJECTS

This chapter presents an investigation and analysis of short-term, dose-response effects of nasal CPAP on airway and systemic inflammatory indices, nasal symptoms and airway obstruction in healthy subjects. The results of this work have been published in the European Respiratory Journal in 2012 [173], as attached in Appendices One and Two. The data were previously presented in the 2010 and 2012 meetings of the American Thoracic Society.

4.1 INTRODUCTION

As described in the introduction, nasal CPAP has become the gold-standard management of clinically significant OSA [27]. Despite its beneficial effects on airway patency, CPAP treatment is associated with a high prevalence of side effects [98, 285]. Some patients adapt to the treatment within a few weeks, others struggle for longer periods, and some discontinue treatment entirely with consequent detriment to their health. Although the long-term compliance rate is generally good, 8- 15% of OSA patients refuse treatment after a single night of use in the laboratory setting [98, 493-495]. There are reports of many adverse symptoms occurring with CPAP use, including nasal congestion, sneezing, anosmia, itchy nose, dry nose, mouth, throat and eyes, blocked ears, and dizziness [98]. Thus, the initial experiences of the patient with nasal CPAP may be of great importance in long-term treatment compliance.

The development of nasal symptoms with CPAP treatment may be related to the induction of nasal inflammation. Several clinical and experimental studies have reported on local and systemic inflammatory outcomes with ventilator support [298, 264, 496-500], but little is known about the early induction of nasal inflammation with CPAP and how this relates to changes in nasal physiology, symptoms and therefore compliance. In addition, reported symptoms and inflammatory changes may be influenced by pre-existing conditions, such as OSA. The investigations reported in our previous *in vitro* study demonstrated that continuous pressure applied to the airway epithelial triggers early inflammatory reaction originated from bronchial cells as evidenced by the increased secretion of inflammatory markers IL-6 and IL-8 by epithelial cells.

We hypothesised that examining early symptoms and inflammatory changes after a short period of CPAP in nCPAP-naïve healthy individuals *in vivo* would provide complementary insights into the mechanisms associated with the development of adverse symptoms and the inflammatory response. This hypothesis forms the basis of the following two chapters. This chapter comprises a dose-response analysis under two different nasal CPAP pressures.

This study primarily aimed:

- to investigate *in vivo* the short-term effects of 3 hours of 7.5 cm H_2O of non-humidified nasal CPAP on nasal mucociliary clearance time and upper airways symptoms in healthy individuals.
- to investigate the short-term effects of 3 hours of 7.5 cm H_2O of nasal CPAP on airway inflammatory indices in nasal CPAP-naïve, healthy individuals.
- to investigate the short-term effects of 3 hours of 7.5 cm H_2O of nasal CPAP on upper and lower airway obstruction.

Secondly, after the first nasal CPAP therapy at 7.5 cm H_2O pressure was administered, we aimed to investigate further the effects of nasal CPAP on the same clinical parameters but this time with nasal CPAP of 12.5 cm H_2O that aimed to confirm the results and demonstrate a dose-response.

The second study aimed

- to examine the same aims of the first study but at 12.5 cm H_2O.
- to investigate further whether the increase of nasal CPAP pressure from 7.5 to 12.5 cm H_2O would result in increased inflammatory effect or none-effect.

4.2 METHODS

4.2.1 Control Subjects and Protocol

Thirty-one healthy non-smokers (21 male and 10 female) with no prior history of nasal symptoms or disease were recruited for the study.

The subjects were divided into two groups for the study. One higher and one lower nasal CPAP fixd pressure (within the range of clinical use) were selected for the *in vivo* component of the study: 7.5 cm H_2O and 12.5 cm H_2O. The first group (n=22) was subjected to three hours of standard nasal CPAP (Respironics REMstar®) at 7.5 cm H_2O pressure, without humidification, and through a nasal mask. The second group (n=31, 11 of whom had received the 7.5 cm H_2O protocol six months previously) was subjected to three hours of nasal CPAP treatment at 12.5 cm H_2O, also without humidification. A six-month gap was specifically chosen between the two studies to ensure that the effect of 7.5 cm H_2O CPAP did not persist during the 12.5 cm H_2O CPAP treatment.

None of the subjects had clinical findings suggestive of upper and lower respiratory problems. Subjects with respiratory problems were excluded from the study. On being chosen to participate in the study a full medical history was ascertained and an examination was performed. Subjects then attended research clinics before the three hours of nasal CPAP therapy to have the following measurements taken before and after intervention: interleukin IL-(6), IL-8 and myeloperoxidase (MPO) levels in serum and nasal wash samples, nasal wash leukocyte count, lung function test, acoustic rhinometry measurements and nasal mucociliary clearance test using saccharin. In addition, six nasal score and other symptoms were questioned before and after the intervention.

4.2.2 Ethics and Consent

The protocol was approved by the Research Ethics Committee at Royal Free Hampstead NHS Trust (study reference 09/H0720/24). Informed written consent was obtained from all subjects prior to their inclusion in the study.

4.2.3 Study Device and Patient Interface

The non-invasive respiratory support device used for the (*in vivo* and *in vitro*) studies in this thesis was a standard non-humidified nasal CPAP (Respironics REMstar®), and the interface to deliver the supportive ventilation for *in vivo* studies was nasal mask (**Plate 7**). The CPAP device was designed for the treatment of adult obstructive sleep apnoea (OSA) only.

However, the mask that was intended to provide a subject interface for the application of non-invasive ventilation was nasal mask. The nasal mask is called Contour Deluxe^{TM,} which is designed to provide subjects or OSA patients with an enhanced comfort and leak control. The mask comes assembled with a unique one-size-fits-most head strap to enable quicker and easier set-up on a patient.

4.2.4 Measurements

4.2.4.1 Nasal and Systemic Sampling and Processing

The aim of this section is to introduce the measurements and the processing techniques used to assess nasal and systemic inflammation in all study groups in this thesis. These measurements were of the amounts of nasal wash and serum samples used.

4.2.4.2 Nasal Wash Sampling

Nasal wash samples were obtained using a dilution method [327] modified from a technique described by Hilding [501] as discussed and validated for repeatability in chapter three. In brief, a paediatric tracheostomy tube (Bivona Fome-Cuf, size I.D 2.5 mm; Smiths Medical, Kent, UK) was used to collect the nasal lavage. The patient was asked to bend forward, sitting with an approximately 45^{o} angle between the chin and the chest. The paediatric tracheostomy tube was inserted into one of the nasal orifices, and the cuff was inflated to achieve a comfortable seal. Then, 7 ml 0.9% room-temperature saline solution was instilled slowly through the tracheostomy tube using a syringe and drawn back. This process was repeated once more; thus, the nasal cavity was washed in and out twice. A clean funnel tube was used to collect any leaked fluids during the removal of the tube from the nose, and this fluid was added to the sample. The technique was repeated for the other nasal cavity using another syringe, and the two recovered solutions were pooled for analysis. To ensure standard conditions, all sampling procedures were performed by the same investigator.

4.2.4.3 Nasal Wash Sampling Process for Analysis of Inflammatory Mediators

The total portion of the pooled nasal wash fluid from both nostrils was collected in a 15 ml centrifuge tube and processed for the analysis of inflammatory mediators and leukocyte count as below. The total pooled nasal wash sample was mixed for 15 seconds on the vortex mixer and then centrifuged for 10 minutes at 2000 rpm at 4°C for a supernatant analysis of inflammatory cytokines, and the remainder to yield a cell-pellet for leukocyte count. Aliquots of supernatant were collected and stored at -80°C for interleukins IL-(6), IL-8 and MPO assay. All samples in this thesis were processed within a maximum of two hours from collection.

4.2.4.4 Nasal Wash Sampling Process for Leukocyte Count

The cell pellet was re-suspended in 1 ml of phosphate-buffered saline (PBS) solution and gently mixed. A 50 µl aliquot was taken out into a 1.5 ml Eppendorf tube and 50 µl of trypan blue stain was added and carefully mixed. This suspension was then used for the leukocyte count using a Neubauer haemocytometer, a specimen slide which is used to determine the concentration of cells in a liquid sample. It is basically designed for the counting of blood cells but now also used to count other types of cells. A grid of perpendicular lines is etched into the glass of the hemocytometer chamber. This grid, an arrangement of squares of different sizes, allows for an easy counting of cells. This way it is possible to determine the number of cells in a specified volume, and thereby calculate the concentration of cells in the fluid overall.

4.2.4.5 Serum Sampling and Processing

Peripheral venous blood samples were obtained for serum measurements. A 5 milliliters of sample of venous blood was collected in to a sterile vacutainer, centrifuged at 2000 rpm for 10 minutes at 4°C, and the supernatant was stored at -80°C for later analysis of inflammatory mediators.

4.2.4.6 Samples Analysing and Reporting

Measurements of the inflammatory cytokines (IL-6, IL-8 and MPO) in nasal wash supernatants and serum were performed by a standard ELISA technique. The ELISA kits were obtained from R&D Systems, Abingdon, UK.

The limits of detection of the kit were 0.70 pg/ml for (IL-6), 3.5 pg/ml for (IL-8), and 1.5 ng/ml for (MPO).

4.2.5 Physiological assessments

The aim of this section is to introduce the measurements used in this thesis to assess the upper and lower airways. For nasal airway obstruction, acoustic rhinometry was used and for pulmonary airway obstruction, spirometry was used. For nasal mucociliary clearance, saccharin transit time (STT) was used to assess the function of nasal clearance. All measurements were conducted according to the same standardised protocols used in throughout this thesis, (Appendix Four).

4.2.5.1 Acoustic Rhinometry

Acoustic rhinometry measurements were performed in accordance with a previously published protocol [362], as discussed and validated in chapter four. Briefly, it allows one to measurement the relationship between a cross-sectional area and the distance of the nasal cavity by using a sound-pulse propagating in the nasal cavity, which reflects local changes in acoustic impedance: this method calculates a nasal cross-sectional area. All measurements were performed by the same operator in the same air-conditioned room to provide similar conditions with regard to temperature, humidity and ambient noise levels. The following five important acoustic rhinometry variables were assessed and examined separately: (i) outermost, minimum cross-sectional area (MCA1), (ii) D-MCA1, (iii) innermost, minimum cross-sectional area (MCA2), (iv) D-MCA2, and (v) V2-5. Area and volume values from the right and left nostrils were added together to obtain a combined value to account for variations in the nasal cycle.

4.2.5.2 Spirometry

Spirometry was measured and recorded using a Vitalograph 2160 (Maids Moreton, Buckingham, UK). A bronchodilator was not administered. The best of three attempts at

spirometry was measured and recorded. We recorded forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio and peak expiratory flow rate (PEFR).

4.2.5.3 Mucociliary Clearance Time: *in vivo*

Several factors affect the mucociliary function such as smoking, respiratory disorders and ambient pollutant gases [502-504]. Konrad et al. [374] showed that bronchial mucociliary clearance is impaired in patients receiving mechanical ventilation. Nasal CPAP as a noninvasive therapy might modify nasal mucociliary clearance and can be a risk factor for the defence of the respiratory airways, especially in OSA patients [285, 505-507]. The intolerance of CPAP would presumably occur because this therapy would lead to epithelium alterations and changes in the nasal mucociliary clearance.

As discussed in the upper airway assessment in the introduction, although the gold standard for measuring mucociliary clearance is the radiolabeled method [376] saccharin transit time is an inexpensive, safe, simple technique, and results are similar to those obtained when using radioactively labelled particles [377]. STT has been documented in the literature to assess mucociliary clearance with various sinonasal disorders, such as allergic rhinitis and chronic rhinosinusitis, as well as in normal subjects [378, 508-509].

In this thesis, nasal mucociliary clearance was measured in healthy subjects and OSA using *in vivo* saccharin transit time as described by Rutland and Cole [380] which is a modification of Andersen's original description of the test.

Therefore, we aimed to investigate *in vivo* the short- and long-term effect of nasal CPAP on the mucociliary clearance time in healthy subjects and OSA patients, and to correlate the adverse effects of this therapy with the pressure level used in this study.

Procedure Protocol

During the procedure, subjects were asked to sit quietly in an upright position with the head in a neutral position. Subjects were asked to refrain from sniffing, sneezing, coughing, and drinking during the procedure. A saccharin particle, 0.5 mm diameter under direct visualization, was gently placed on the medial surface of the inferior turbinate of one nasal cavity at least 7 mm behind the turbinate's anterior end to avoid the area of mucosa where cilia beat in an anterior direction.

The saccharin clearance time was recorded at the first sensation of sweet taste. The time from particle placement until the patient reports the first sensation of a sweet taste was measured with a stop-watch (**Figure 18**). If no taste sensation occurred within 60 minutes, then the ability to taste saccharin was confirmed by placing saccharin directly on the tongue. The saccharin transit time protocol is detailed in Appendix Four.

There is no standardization in the mucociliary clearance time. Therefore, for the purpose of analysis, mucociliary clearance time was classified as follows: normal (up to 20 minutes); prolonged (21 to 30 minutes); severely prolonged (31 to 60 minutes); and failure to clear (over 60 minutes). These standardized times will be utilised in this thesis for other study groups.

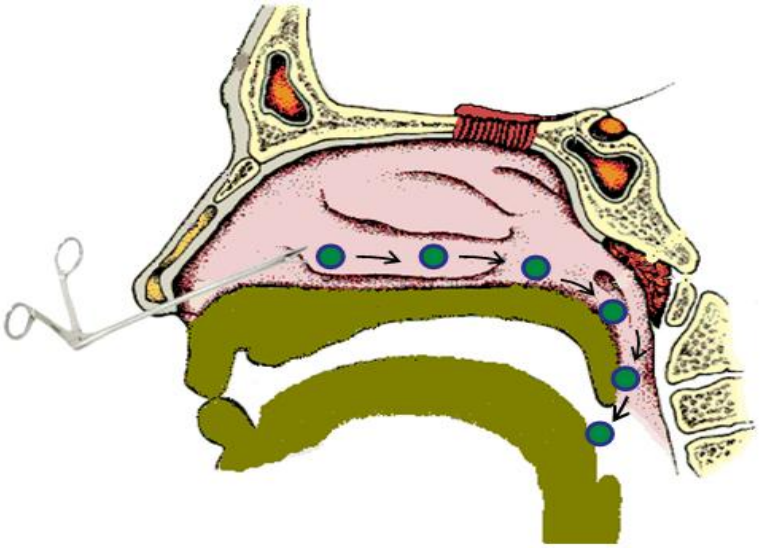


Figure 18: Illustration of the saccharin particle placement and motion in the inferior nasal turbinate of a nostril.

4.2.6 Questionnaire

4.2.6.1 Assessment of Nasal Symptoms

Subjects were asked to report any respiratory symptoms experienced on a regular basis during their visits to the research clinic before any interventions.

A six-point nasal score, as used in previous work in the Department [321], was used to assess the presence or absence of nasal symptoms. The nasal score consists of the principal nasal symptoms of rhinorrhoea, which are postnasal drip, nasal congestion, sneezing, reduced smell and itchy nose. Other symptoms were assessed in order to determine the presence of nasal symptoms before and after the intervention of nasal CPAP. Results were binary coded as 1 or 0, respectively, and the scores were summed to yield a total score between 0 and 6. An example of a six-point nasal score is given in Appendix Five.

4.2.7 Statistical Analysis

Data were analysed using GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA) and SPSS version 18.0 (SPSS Inc, Chicago, Ill). The Kolmogorov–Smirnov test of normality was applied. Paired t-tests were used to examine differences between baseline and post-CPAP therapy measurements. A one-way ANOVA was run to examine dose-response differences between treatments, followed by post-hoc Tukey's multiple comparison tests. For the subgroup analysis, one-way repeated measures ANOVA for parametric data and Friedman test for nonparametric data were run to examine dose-response differences between treatments as appropriate, followed by post-hoc Tukey's multiple comparison tests. Pearson (r) and Spearman (rho) correlations were conducted as appropriate to examine relationships between variables. A Chi-squared ($\mathbf{x}^2$) test was used to compare nasopharyngeal symptoms at baseline and after CPAP therapy. A P value < 0.05 was considered statistically significant.

4.3 RESULTS

The baseline characteristics of the subjects enrolled in the study are reported in **Table 16**. Both groups were healthy non-smokers, with a mean age between 33 and 34 years.

Table 16: Baseline information of subjects enrolled in the study. Eleven patients took part in both protocols.

Details of subjects studied	**7.5 cm H_2O (n=22)**		**12.5 cm H_2O (n=31)**	
	Mean	**SD**	**Mean**	**SD**
Age, years	33.8	5.8	33.4	5.4
Weight, kg	70.1	6.6	71.5	7.5
Height, cm	168.8	7.1	169.7	7.1
BMI, kg/m^2	25.12	1.84	24.82	2.01
Smoking history	Never-smoking		Never-smoking	
Nasal symptoms	None		None	
Medications	None		None	

BMI, body mass index; SD, standard deviation

4.3.1 Changes in Nasal and Systemic Inflammation with CPAP

The changes in serum and nasal wash inflammatory markers in response to three hours of nasal CPAP are reported in **Table 17**. Both CPAP pressures resulted in significant increases in nasal inflammation as assessed by nasal wash leukocyte and MPO measurements. The increase in nasal IL-6 and IL-8 concentrations following CPAP was only statistically significant at the higher pressure. Both pressures also resulted in changes in systemic

inflammatory markers, with significant increases in serum IL-6 concentrations and decreases in serum IL-8 concentrations following CPAP. There was no change in serum MPO concentration. The ANOVA test highlights the observed dose (pressure) responses for changes in inflammation with CPAP, illustrated in **Figure 19**. When one-way repeated measures ANOVA was conducted for the subset of subjects (n=11) that underwent both experiments (7.5 and 12.5 cm H_2O), the changes in nasal wash IL-6 (p=0.038) and nasal wash MPO (p=0.027) remained statistically significantly.

Table 17: Changes in inflammatory markers in serum and nasal wash fluid from baseline to post-three hours of CPAP treatment.

Marker	**7.5 cm H_2O (n = 22)**			**12.5 cm H_2O (n = 31)**			
	Baseline	**After CPAP**	**P value***	**Baseline**	**After CPAP**	**P value***	**ANOVA****
Inflammatory markers in serum							
IL-6, pg/ml	4.6 (3.5)	6.3 (3.5)	0.010	8.7 (4.2)	11.3 (5.8)	0.037	<0.001
IL-8, pg/ml	20.9 (7.8)	16.5 (7.3)	<0.001	14.9 (5.9)	13.0 (5.6)	0.002	0.022
MPO, ng/ml	10.3 (8.4)	9.3 (9.4)	0.805	11.5 (8.5)	9.1 (7.2)	0.138	0.858
Inflammatory markers in nasal wash							
Leukocyte count, 1000 cells/ml	18.8 (4.1)	20.6 (6.4)	0.024	18.2(4.7)	21.5 (6.2)	<0.001	0.039
IL-6, pg/ml	2.4 (2.8)	2.4 (3.1)	0.670	3.7 (1.5)	4.9 (1.8)	0.001	0.012
IL-8, pg/ml	86 (114)	81 (98)	0.689	164 (90)	206 (164)	0.281	0.006
MPO, ng/ml	1.9 (5.2)	3.4 (6.6)	0.006	2.0 (4.3)	3.8 (5.5)	0.002	0.006

Data are expressed as the geometric mean (SD). *versus baseline, paired samples *t* -test.
**ANOVA: compares the baseline (mean of the two baselines in the 11 subjects who had both pressures) with the 22 results at 7.5 cm H_2O and the 31 subjects at 12.5cmH_2O nCPAP. IL, interleukin; MPO, myeloperoxidase; CPAP, continuous positive airway pressure.

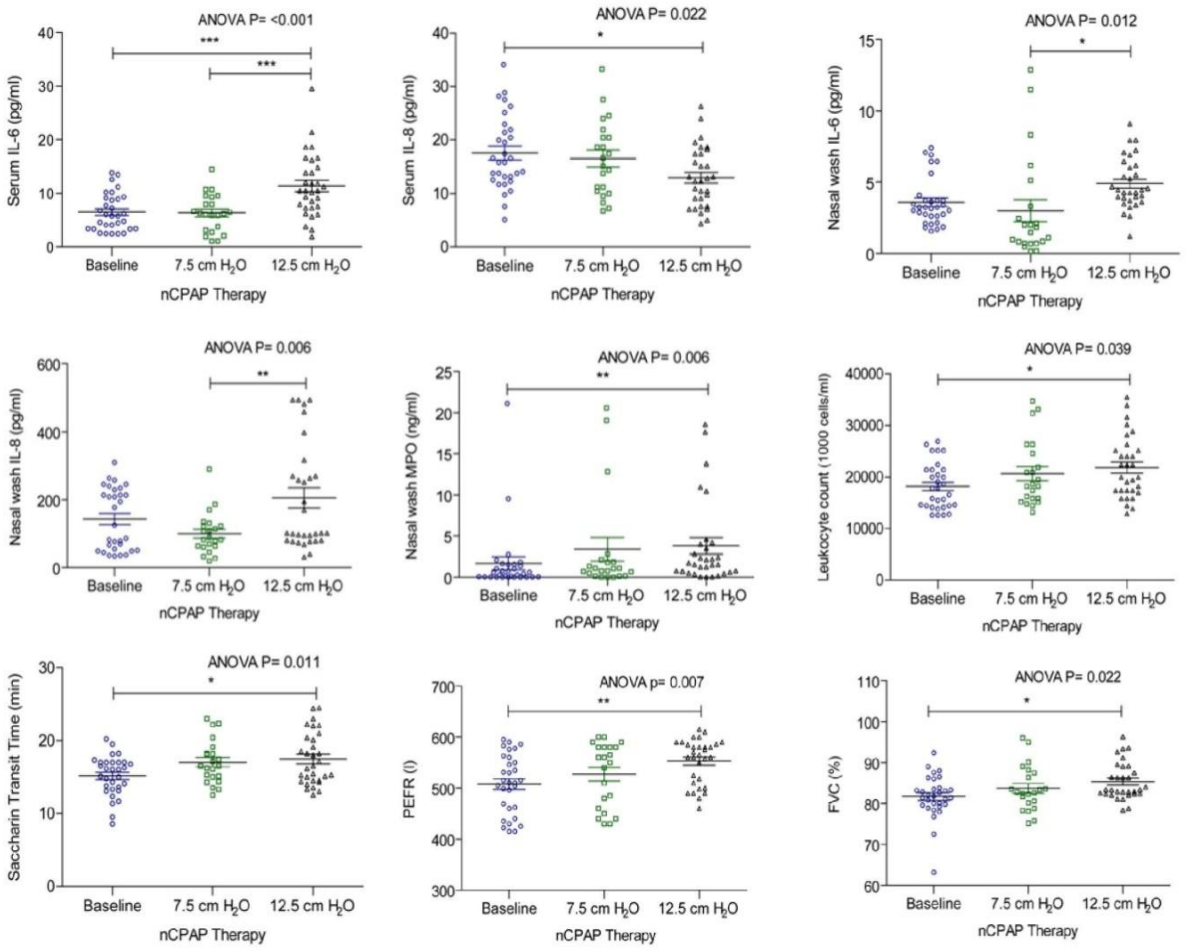


Figure 19: CPAP is associated with a pressure-dependent alteration in nasal and systemic inflammatory markers, and nasal mucociliary clearance. Lines represent mean and standard error. Significant differences illustrated using ANOVA with post-hoc analysis.

4.3.2 Changes in Physiology with CPAP

The changes in spirometry, rhinometry and nasal mucociliary clearance are reported in **Table 18**. At both pressures, three hours of nasal CPAP treatment resulted in a significant slowing of nasal clearance (i.e., increased saccharin transit time) without significant changes in rhinometry variables. At both pressures, CPAP was associated with small but significant changes in FVC and PEFR (but not FEV_1).

Table 18: Changes in lung function, rhinometry findings and nasal mucociliary clearance from baseline to after three hours of nCPAP treatment.

Parameter	7.5 cm H_2O (n = 22)			12.5 cm H_2O (n = 31)			
	Baseline	**After CPAP**	**P value***	**Baseline**	**After CPAP**	**P value***	**ANOVA****
Lung function test							
FEV	3.03 (0.52)	3.05 (0.50)	0.093	3.07 (0.50)	3.09 (0.51)	0.596	0.751
FEV1 %	86.31 (5.94)	86.22 (5.75)	0.506	85.74 (5.58)	86.90 (4.62)	0.091	0.370
FVC	3.54 (0.63)	3.58 (0.63)	0.030	3.59 (0.63)	3.64 (0.61)	0.026	0.647
FVC %	83.06 (5.62)	83.71 (5.53)	0.052	83.01 (6.18)	85.36 (4.54)	0.071	0.022
FEV1/FVC ratio	85.99 (6.00)	85.76 (6.10)	0.615	85.90 (6.49)	86.35 (6.79)	0.354	0.625
PEFR (l)	519.00 (71.53)	527.27 (62.79)	0.024	523.81 (64.44)	553.06 (43.71)	0.002	0.007
Acoustic rhinometry							
MCA1	0.57 (0.31)	0.60 (0.38)	0.527	0.61 (0.31)	0.59 (0.31)	0.175	0.894
DMCA1	2.15 (0.38)	2.15 (0.34)	0.947	2.06 (0.43)	2.04 (40)	0.529	0.501
MCA2	1.62 (0.54)	1.58 (0.45)	0.488	1.55 (0.53)	1.53 (0.50)	0.531	0.938
DMCA2	4.23 (0.39)	4.24 (0.32)	0.884	4.22 (0.40)	4.23 (0.36)	0.568	0.823
V2-5	4.43 (1.05)	4.51 (1.08)	0.482	4.73 (0.93)	4.82 (0.89)	0.113	0.362
Nasal mucociliary clearance							
STT, minutes	16.31 (2.71)	17.41 (3.31)	0.035	15.30 (3.56)	16.35 (3.34)	0.045	0.011

Data are expressed as the means (SD). *versus baseline, paired samples *t* -test. **ANOVA: compares the baseline (mean of the two baselines in the 11 subjects who had both pressures) with the 22 results at 7.5 cm H_2O and the 31 subjects at 12.5cmH_2O CPAP. FEV, forced expiratory volume; FVC, forced vital capacity; PEFR, peak expiratory flow rate; MCA1, outermost minimum cross-sectional area; DMCA1, the distance of the MCA1 from the nasal orifice; MCA2, innermost minimum cross-sectional area; DMCA2, the distance of the MCA2 from the nasal orifice; V2-5, the volume of the nasal segment between the 2nd and 5th cm from the nasal orifice; STT, saccharin transit time.

4.3.3 Changes in Nasopharyngeal Symptoms with CPAP

Nasopharyngeal symptoms before and after nasal CPAP treatment are presented in **Table 19**. None of the subjects had any upper airway symptoms before CPAP. The median number of nasopharyngeal symptoms increased significantly from 0 at baseline to 1 (0-3) after 7.5 cm H_2O CPAP ($P = 0.002$) and to 2 (1-3) after 12.5 cm H_2O CPAP ($P < 0.001$). After CPAP at 7.5 cm H_2O, 12/22 subjects (55%) experienced at least one nasal symptom; after CPAP at 12.5 cm H_2O, 21/31 subjects (68%) experienced at least one nasal symptom. There was an overall increase in the frequency of all of the symptoms during nasal CPAP treatment; the most common nasal symptom at both pressures was itchy nose. The higher the pressure, the more symptoms were recorded: x^2 ($P = 0.041$).

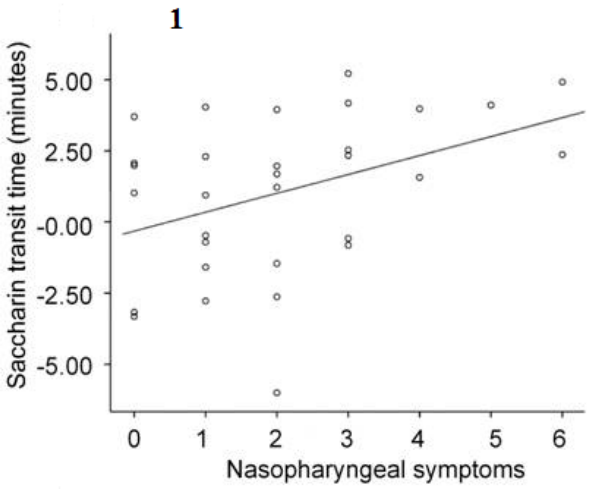
Table 19: Changes in nasopharyngeal symptoms following CPAP application to healthy subjects *in vivo*.

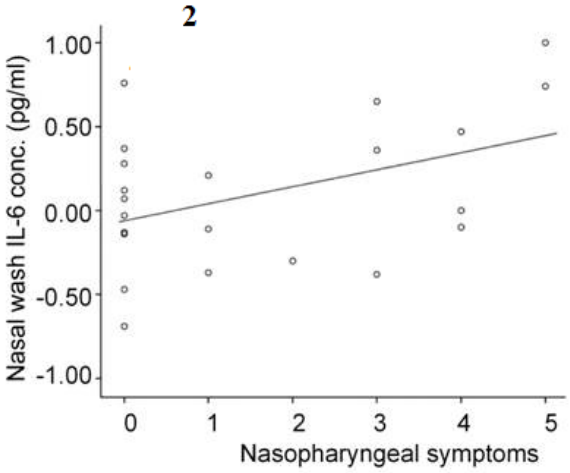
CPAP pressure	Nasopharyngeal symptoms								
	Rhino-rrhoea	PND	Sneezing	Congestion	Anosmia	Itchy nose	Nose Dryness	Dry mouth/ throat	Blocked Ears
Baseline (Pre-CPAP)	0	0	0	0	0	0	0	0	0
7.5 cm H_2O (n = 22)	2 (9%)	0 (0%)	5 (23%)	3 (14%)	0 (0%)	12 (54%)	7 (32%)	4 (18%)	1 (4%)
12.5 cm H_2O (n = 31)	5 (16%)	2 (6%)	5 (16%)	7 (23%)	0 (0%)	17 (55%)	15 (48%)	11 (35%)	2 (6%)

* Data are expressed as numbers (%) of subjects.

4.3.4 Relationships between Symptoms and Changes in Nasal Physiology and Inflammation

The data demonstrate relationships between the development of nasal symptoms, nasal inflammation and impaired nasal function *in vivo*. The greater the nasal symptoms with CPAP, the slower the nasal mucociliary clearance (r=0.40 p=0.025) and the greater the nasal inflammation as assessed by nasal wash IL-6 (r= 0.43 p=0.045), **Figures 20.1** and **20.2** respectively. The significant slowing in nasal clearance was also associated with the degree of nasal inflammation, as assessed by nasal MPO concentrations (r = 0.42, P = 0.049), illustrated in **Figure 20.3**.





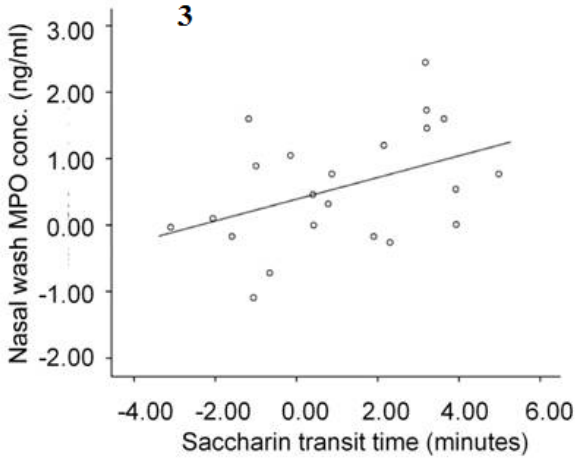


Figure 20: (1) Relationship between nasal mucociliary clearance (saccharin transit time) and nasopharyngeal symptoms from 31 healthy subjects after 3 hours of CPAP treatment at 12.5 cm H_2O ($r = 0.40$; $P = 0.025$). **(2)** Relationship between nasopharyngeal symptoms and nasal wash IL-6 concentration in 22 healthy subjects after 3 hours of CPAP treatment at 7.5 cm H_2O CPAP treatment ($r = 0.43$; $P = 0.045$). **(3)** Relationship between nasal mucociliary clearance (saccharin transit time) and nasal wash MPO concentration in 22 healthy subjects after three hours 7.5 cm H_2O CPAP application ($r = 0.42$; $P = 0.049$).

4.4 DISCUSSION

We report that CPAP was associated with dose (pressure)-response changes in nasal and systemic inflammatory markers, reduced nasal function and the development of nasal symptoms. The development of nasal symptoms related to the degree of functional impairment and nasal inflammatory response. To the best of our knowledge, this is the first report to examine the *in vitro* and *in vivo* effects of CPAP in this way, providing new data on the mechanisms of CPAP intolerance in the crucial early phase of therapy.

IL-6 is an important pro-inflammatory cytokine. IL-8 is a chemokine that attracts neutrophils to the site of inflammation. Our *in vivo* findings showed a neutrophilic nature of inflammation. Neutrophil chemotaxis following epithelial IL-8 release is the likely explanation of increased leukocyte count and elevated myeloperoxidase (MPO) activity in nasal wash fluid samples since MPO is an enzyme abundantly present in neutrophils. The neutrophilic nature of CPAP-induced local inflammation has also been shown in a rat model in which early nasal inflammation was mediated by macrophage induced inflammatory protein-2 and manifested as neutrophil extravasation following five hours of 10 cm H_2O CPAP [500]. Paradoxically, in nasal wash fluid samples, IL-8 levels remained unchanged in this study at both pressures and an increase in IL-6 levels was only evident in response to the higher pressure. This may arise from rapid consumption and binding of interleukins before they cross the nasal epithelium. Our study therefore suggests that CPAP itself may be pro-inflammatory and that this effect occurs early after initiation of therapy.

In this study, even a brief period of CPAP application resulted in increased IL-6 levels in serum suggesting a systemic inflammatory response. However, we did not observe an increased systemic IL-8 concentration or MPO activity. The decrease in serum IL-8 levels may be due to local recruitment of leukocytes and increased consumption. Unaltered systemic MPO activity is plausible since MPO may predominately increase at the site of inflammation. Studies in OSA are even more complex as the condition itself is associated with up-regulated upper airway inflammation [510-511] and in such circumstances CPAP

may not up-regulate this further [512]. The work complements previous findings in patients with OSA, where CPAP is known to increase nasal inflammation [298].

This *in vivo* nasal inflammatory response was associated with clinical and functional consequences in that we also demonstrated CPAP reduced nasal clearance and was associated with a high prevalence of new nasal symptoms. It has been suggested that the presence of nasal inflammation predicts patients at greater risk of discontinuing CPAP therapy [513] and IL-8, a potent neutrophil chemoattractant that we have shown is up-regulated by CPAP *in vitro,* causes rhinorrhoea when directly instilled to the nose [514]. Three hours of CPAP decreased mucociliary clearance at both 7.5 and 12.5 cm H_2O in healthy individuals. Our findings are contrary to those reported by de Oliviera et al. [382], who found significantly decreased STT after 20 minutes of CPAP in healthy individuals. This may be attributed to differences in the duration of CPAP treatment and suggests that CPAP may provide an initial improvement in nasal clearance that is followed by impairment due to inflammation. These inflammatory and functional changes may contribute to the high incidence of symptoms and adverse effects associated with CPAP treatment. In this study, more than half of the subjects experienced at least one nasal symptom after a single session. Previous studies have reported high incidences of side effects during long-term therapy, which approached 97% in a large series [98]. To assess the duration of symptom changes, in a subsequent pilot experiment we assessed nasal symptoms prior to and after 3 hours of CPAP at 12cm H_2O, then again at three, six, nine and 24 hours later. None of the subjects had nasal symptoms prior to CPAP and all had one or more symptom after. A three, six, nine and 24 hours post CPAP the numbers remaining symptomatic were 4/5, 1/5, 1/5 and 0/5 respectively suggesting that acute nasal symptoms typically last for between three and six hours following initiation of CPAP.

In this study, CPAP treatment did not result in any change in acoustic rhinometry parameters in healthy individuals; thus, it did not alter nasal patency. This was unexpected given the apparent nasal inflammatory response. Nasal geometry has been reported to affect CPAP tolerability [346]. Although the effect of short- or long-term CPAP on acoustic rhinometry parameters has not been investigated previously, several studies have reported

unaltered rhinomanometry results after long-term CPAP therapy [346, 515]. One study reported a reduction in airway resistance after an acute exposure to nasal CPAP for 6 hours in healthy CPAP-naïve individuals [282]. In this study, a small but statistically significant improvement was identified in lung function parameters (i.e., FVC and PEFR). This suggests that the CPAP applied in our subjects had a demonstrable biological effect.

OSA is associated with increased cardiovascular risk [100, 516], as is increased systemic inflammation [517]. Whether CPAP reduces cardiovascular risk remains controversial [300-301, 518-520], but the finding that CPAP itself, at least in the acute setting, is pro-inflammatory is potentially important regarding the timing of the initiation of therapy. In the long-term, Steiropoulos et al. [299] reported significant improvements in systemic inflammatory markers, including total lymphocyte counts, $CD4^+$ cells, TNF-α levels and uric acid levels after six months in patients with good compliance to CPAP therapy; however, no such improvements were identified in patients with poor compliance. In contrast, Kohler et al. [300] did not find any differences in systemic inflammatory markers, high-sensitive C-reactive protein (CRP), plasma IL-6, IFN-γ, and adiponectin levels between patients receiving therapeutic and sub-therapeutic CPAP treatments for four weeks. Thus, the relationships between CPAP, systemic inflammation, and the duration of therapy are complex. We report small but statistically significant elevation in systemic IL-6 with CPAP and it is known that even small changes in long-term IL-6 concentration can be associated with excess cardiovascular risk [521]. The effects of acute changes are less well studied.

The mechanisms by which CPAP may be pro-inflammatory include airway drying (i.e., not using humidification) or direct distension. The possible benefits of humidification have been controversial; an experimental study in rats failed to demonstrate any beneficial effects of heated humidification on nasal inflammation [522], whereas clinical and experimental studies have reported conflicting results on the benefits of humidification [523-524]. Most previous *in vitro* work has used direct distension [525-527], and a mouse model of airway stretch for ventilator-associated lung injury was associated with increased expression of the murine equivalent of IL-8 [497]. Stretch may affect inflammation via oxidative stress, as

stretch-induced IL-6 and IL-8 production can be reduced by the use of anti-oxidants to increase intra-cellular glutathione; production can be increased with glutathione depletion [528]. Our data add to the literature by reporting a direct effect of pressure rather than stretch. It is unlikely that the nasal epithelium is able to accommodate stretch given the confines of the nose within the bony structures of the skull.

The strengths of our study include the careful and comprehensive assessment of symptoms, upper and lower airway function, and nasal and systemic inflammation, which demonstrated a dose response in healthy subjects. Our results have important implications for clinical practice. In particular, by demonstrating a relationship between nasal symptoms, mucociliary clearance and inflammation, it should be possible to investigate strategies to reduce the nasal inflammation associated with CPAP treatment, which may reduce symptoms and, therefore, aid compliance. Approaches include anti-inflammatory agents or humidification (discussed above), and we have provided further rationale for the development of strategies to mitigate nasal inflammation during CPAP therapy. This is particularly important as existing nasal corticosteroids appear clinically ineffective [529]. An alternative strategy might involve dose titration at the beginning of the therapy (i.e., a gradual increase of the pressure until attaining optimal clinical benefits with minimal side effects), as we have provided evidence that suggests that the inflammatory effects are dose (pressure)-dependent.

This study also has several limitations that need to be considered. Ours was a relatively small sample. Associations observed in the *in vivo* study do not provide direct evidence for a causal relationship between CPAP and airway inflammation. The design of the study may have been more robust with the inclusion of a sham CPAP arm but we were concerned that even sham CPAP might affect nasal inflammation. We cannot comment on the timing of resolution of inflammation associated with CPAP as we were interested primarily in induction of this response. Whilst we elected to measure IL-6 and IL-8 to provide consistency across the *in vivo* work, there are alternative markers including CRP and TNF-alpha that may have provided additional insight into cardiovascular risks associated with

CPAP. Finally, as many of our analyses are hypothesis-generating we have not attempted to correct analyses for multiplicity and the results should be interpreted in the light of this.

4.5 CONCLUSION

In conclusion, we report a high prevalence of nasal symptoms following CPAP therapy in healthy subjects associated with changes in nasal function and an inflammatory response in the nasal and systemic compartments. This study also suggests that CPAP triggers an early pressure-dependent inflammatory reaction, as evidenced by the increased secretion of inflammatory markers by cultured bronchial epithelial cells. These findings have implications for the adherence of patients to CPAP therapy, especially during the important initiation phase. Strategies to combat the initial side effects of this treatment modality and to improve compliance and retention might target the epithelial lining of the respiratory system in an attempt to address the origin of the inflammatory response.

5

LONG-TERM EFFECTS OF NASAL CPAP ON SYMPTOMS, PHYSIOLOGY, INFLAMMATION, AND HEALTH-STATUS IN PATIENTS WITH OSA

This chapter comprises an analysis of the effects of nasal CPAP on airway and systemic inflammatory indices, airway obstruction and nasal symptoms in OSA patients. The chapter also assesses the impact of nasal symptoms, machine side effects and inflammatory effect on daytime sleepiness and compliance of CPAP therapy. The results of this work were previously presented at the 2010 and 2012 meetings of the American Thoracic Society (Appendix One), and a manuscript is currently under review by a peer-reviewed journal.

5.1 INTRODUCTION

Nasal CPAP is the established treatment for clinically significant OSA [27]. It is a distending mechanical split pressure applied at a continuous level throughout the respiratory cycle to maintain an open airway, preventing airway collapse during sleep [31, 35]. However, CPAP treatment is associated with a high prevalence of side effects [98, 530]. Adaptation period is variable ranging from weeks to longer periods and some patients discontinue treatment due to side effects. Although the long-term compliance rate is generally good, 8- 15% of OSA patients refuse treatment after a single night of use in the laboratory setting [493-495, 530]. Nasal congestion, sneezing, anosmia, itchy nose, dry nose, mouth, throat and eyes, blocked ears, and dizziness are among the adverse symptoms occurring with CPAP use [98].

High frequency of nasal symptoms suggests that CPAP treatment may be related to the induction of nasal inflammation. Several clinical and experimental studies have reported on local and systemic inflammatory outcomes of nasal CPAP treatment [298, 300-301, 373, 500, 512, 524]. However, a comprehensive study investigating both nasal and systemic inflammatory parameters, clinical symptoms together with structural and functional outcomes is lacking. Our previous data in previous chapter in this thesis in *in vivo* studies demonstrated that CPAP induced inflammation. Further investigation is important with OSA patients using CPAP therapy, therefore, this chapter will investigate the long-term effect of CPAP with OSA patients over six-month period.

1. This study aimed to investigate the long-term local (nasal) and systemic inflammatory effects of nasal CPAP therapy in nasal CPAP naïve OSA patients through examination of nasal wash and serum inflammatory markers, in addition to examining the changes in nasal symptoms, functional and structural parameters.
2. It aimed to investigate the changes in nasal symptoms, side-effects and impact on health-status associated with long-term nasal CPAP therapy in nasal CPAP-naïve OSA patients.

5.2 METHODS

5.2.1 OSA Patients

Twenty-two nasal CPAP treatment-naïve and somnogram test-confirmed obstructive sleep apnoea patients attending outpatient clinics at Royal Free NHS, London between November 2009 and May 2011 were included in this study. Patients received a standard nasal CPAP (Respironics REMstar®) at 7.5 cm H_2O (n=19) or 10 cm H_2O (n=3) fixed pressure without humidification, through a nasal mask. The protocol was approved by the Research Ethics Committee at Royal Free Hampstead NHS Trust (study reference 09/H0720/24) and was conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from all subjects prior to inclusion.

5.2.2 Study Device: CPAP and Patient Interface

The non-invasive respiratory support device used for this study was a standard non-humidified nasal CPAP (Respironics REMstar®), and the interface to deliver the supportive ventilation was nasal mask.

However, the mask that was intended to provide a subject interface for the application of non-invasive ventilation was nasal mask. The nasal mask is called Contour Deluxe^{TM,} which is designed to provide subjects or OSA patients with an enhanced comfort and leak control. The mask comes assembled with a unique one-size-fits-most head strap to enable quicker and easier set-up on a patient.

5.2.3 Measurements

OSA patients received nasal CPAP treatment for six months and assessments were performed before (baseline) and at one month, three months and at six months after the initiation of treatment. The following assessments were made: (i) nasal and systemic inflammation marker (interleukin (IL)-6, IL-8 and myeloperoxidase (MPO)) concentrations in serum and nasal wash samples, and nasal wash leukocyte count, and (ii) functional assessments (spirometry, acoustic rhinometry, and nasal mucociliary clearance). At each time point patients were asked to fill in a series of questionnaires regarding their nasal symptoms, side-effects of nasal CPAP, daytime sleepiness and compliance.

5.2.3.1 Nasal Wash Sampling

Nasal wash samples were obtained using a dilution method [327] modified from a technique described by Hilding [501] as discussed and validated for repeatability in chapter three. In brief, a paediatric tracheostomy tube (Bivona Fome-Cuf, size I.D 2.5 mm; Smiths Medical, Kent, UK) was used to collect the nasal lavage. The patient was asked to bend forward, sitting with an approximately 45^{o} angle between the chin and the chest. The paediatric tracheostomy tube was inserted into one of the nasal orifices, and the cuff was inflated to achieve a comfortable seal. Then, 7 ml 0.9% room-temperature saline solution was instilled slowly through the tracheostomy tube using a syringe and drawn back. This process was repeated once more; thus, the nasal cavity was washed in and out twice. A clean funnel tube was used to collect any leaked fluids during the removal of the tube from the nose, and this fluid was added to the sample. The technique was repeated for the other nasal cavity using another syringe, and the two recovered solutions were pooled for analysis. To ensure standard conditions, all sampling procedures were performed by the same investigator.

5.2.3.2 Nasal Wash Sampling Process for Analysis of Inflammatory Mediators

The total portion of the pooled nasal wash fluid from both nostrils was collected in a 15 ml centrifuge tube and processed for the analysis of inflammatory mediators and leukocyte count as below. The total pooled nasal wash sample was mixed for 15 seconds on the vortex mixer and then centrifuged for 10 minutes at 2000 rpm at 4°C for a supernatant analysis of inflammatory cytokines, and the remainder to yield a cell-pellet for leukocyte count. Aliquots of supernatant were collected and stored at -80°C for interleukins IL-(6), IL-8 and MPO assay. All samples in this thesis were processed within a maximum of two hours from collection.

5.2.3.3 Nasal Wash Sampling Process for Leukocyte Count

The cell pellet was re-suspended in 1 ml of phosphate-buffered saline (PBS) solution and gently mixed. A 50 ul aliquot was taken out into a 1.5ml Eppendorf tube and 50 ul of trypan blue stain was added and carefully mixed. This suspension was then used for the leukocyte count using a Neubauer haemocytometer.

5.2.3.4 Serum Sampling and Processing

Peripheral venous blood samples were obtained for serum measurements. A 5 ml of sample of venous blood was collected in to a sterile vacutainer, centrifuged at 2000 rpm for 10 minutes at 4°C, and the supernatant was stored at -80°C for later analysis of inflammatory mediators.

5.2.3.5 Samples Analysing and Reporting

Measurements of the inflammatory cytokines (IL-6, IL-8 and MPO) in nasal wash supernatants and serum were performed by a standard ELISA. The ELISA kits were obtained from R&D Systems, Abingdon, UK.

The limits of detection of the kit were 0.70 pg/ml for (IL-6), 3.5 pg/ml for (IL-8), and 1.5 ng/ml for MPO.

5.2.4 Physiological Assessments

For nasal airway obstruction, acoustic rhinometry was used and pulmonary airway obstruction, spirometry were used. For nasal mucociliary clearance, STT was used to assess the function of nasal clearance. All measurements were conducted on the same standardised protocols in the studies in this thesis.

5.2.4.1 Acoustic Rhinometry

Acoustic rhinometry measurements were performed in accordance with a previously published protocol [362], as discussed and validated in chapter three. Briefly, It allows measurement of the relationship between cross-sectional area and the distance of the nasal cavity by a sound-pulse propagating in the nasal cavity which reflected by local changes in acoustic impedance to calculate nasal cross-sectional area. All measurements were performed by the same operator in the same air-conditioned room to provide similar conditions with regard to temperature, humidity and ambient noise levels. The following five important acoustic rhinometry variables were assessed and examined separately: (i) outermost minimum cross-sectional area (MCA1), (ii) D-MCA1, (iii) innermost minimum cross-sectional area (MCA2), (iv) D-MCA2, and (v) V2-5. Area and volume values from the right and left nostrils were summed to obtain a combined value to account for variations with the nasal cycle.

5.2.4.2 Spirometry

Spirometry was measured and recorded using a Vitalograph 2160 (Maids Moreton, Buckingham, UK). A bronchodilator was not administered. The best of three attempts at spirometry was measured and recorded. We recorded forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio and peak expiratory flow rate (PEFR).

5.2.4.3 Mucociliary Clearance Time: *in vivo*

Via the aforementioned process we aimed to investigate *in vivo* the long-term effect of nasal CPAP on the mucociliary clearance time with OSA patients. The protocol of the study has been discussed in Chapter Four.

5.2.5 Questionnaires

At each evaluation visit, diary cards**,** nasal symptoms using a six-point nasal score, Epworth sleepiness scale (ESS), and compliance and periodicity questionnaires were collected for six-month periods.

5.2.5.1 Diary Cards

Patients also recorded daily, on diary cards, their hours of CPAP use and any change in respiratory symptoms and treatments over time. These diary cards were modified versions of diary cards that have been used successfully by the London COPD cohort [531-535] and others for years [427, 536-539].

This diary card data also enable calculation of an individual patient's compliance to CPAP therapy and any other changes in symptoms that may cause the interruption of CPAP compliance.

At recruitment, patients are taught how to record on diary cards their daily hours of CPAP use, and any changes in symptoms and treatments, using the detailed instructions at the back page of the dairy cards. Patients are reviewed after the use of CPAP therapy after one

month, three months and six months in the research clinic, to monitor compliance with data collection, and record changes in medication.

An example of a diary card and the instructions provided to the OSA patients are presented in Appendix Six.

5.2.6 Assessment of Nasal Symptoms

Subjects were asked to report any respiratory symptoms experienced on a regular basis during their visits to the research clinic. The six-point nasal score has been discussed in Chapter Four.

5.2.7 Epworth Sleepiness Scale

ESS is a simple, self-administered questionnaire which provides a measurement of the subject's general level of daytime sleepiness, as discussed in the introduction to this thesis.

In this study, ESS was administered to the OSA patients at recruitment visit before using CPAP therapy and during their follow up visits of using CPAP for six months. An example of a six-point nasal score is given in Appendix Five.

5.2.8 Compliance and Periodicity Questionnaire

The compliance and periodicity questionnaire was designed to study the adverse effects and the compliance with CPAP therapy in the OSA patients at baseline and during each visit made by patients.

The questionnaire asked about compliance and periodicity of the use of nasal CPAP. Side-effects examined in detail included adverse effects of the nasal mask, discomfort to the bridge of the nose, and air leaks near the eyes or over the face. The questionnaire also asked about dryness of the nose or mouth in the mornings on wakening, symptoms of sinusitis, nasal drip, sneezing, nasal congestion, nasal bleeding and air swallowing. The questionnaire is detailed in Appendix Eight.

5.2.9 Statistical Analysis

Data were analysed using GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA) and SPSS version 18.0 (SPSS Inc, Chicago, Ill).. The Kolmogorov–Smirnov test of normality was applied. Normally distributed data were expressed as means with standard deviations (SD), and skewed data for inflammatory indices were rendered normal by $\log_{10}$ transformation. Pearson (r) and Spearman (rho) correlations were conducted as appropriate to examine relationships between variables. To evaluate the change in inflammatory and physiological indices over time, one-way repeated measures ANOVA was run to examine differences between treatments, followed by post-hoc Bonferroni multiple comparison tests. To evaluate the changes in 6-point nasal score over time, a Friedman test was used and further post hoc tests were done using Wilcoxon Signed Rank test with Bonferroni correction. ESS scores were analysed with repeated measures ANOVA with Greenhouse-Geisser correction with further *post-hoc* tests conducted with a Bonferroni correction. A P value < 0.05 was considered statistically significant. A P value < 0.05 was considered statistically significant.

5.3 RESULTS

5.3.1 Patient Characteristics

The baseline demographics of the 22 patients enrolled into the study are shown in **Table 20**. The study cohort had a mean age of 59.5 ± 7.5; a mean BMI of 34.6 ± 8.6; 13 were male (59%) and 9 were female (61%). At the time of recruitment, 22.7% were current smokers. **Figure 21** shows the study sequence and the reurtiemnt of OSA patients in the study.

Table 20: Characteristics of the OSA patients*.

Details of subjects studied	**Mean**	**SD**
Age, years	59.5	7.5
BMI (kg/m^2)	34.6	8.6
AHI (events/h)	30.2	15.9
Neck circumference	37.2	1.5
CPAP (cm H_2O)	7.84	0.87

* at the time of recruitment; BMI, body mass index;
AHI, apnoea hypopnoea index; SD, standard deviation

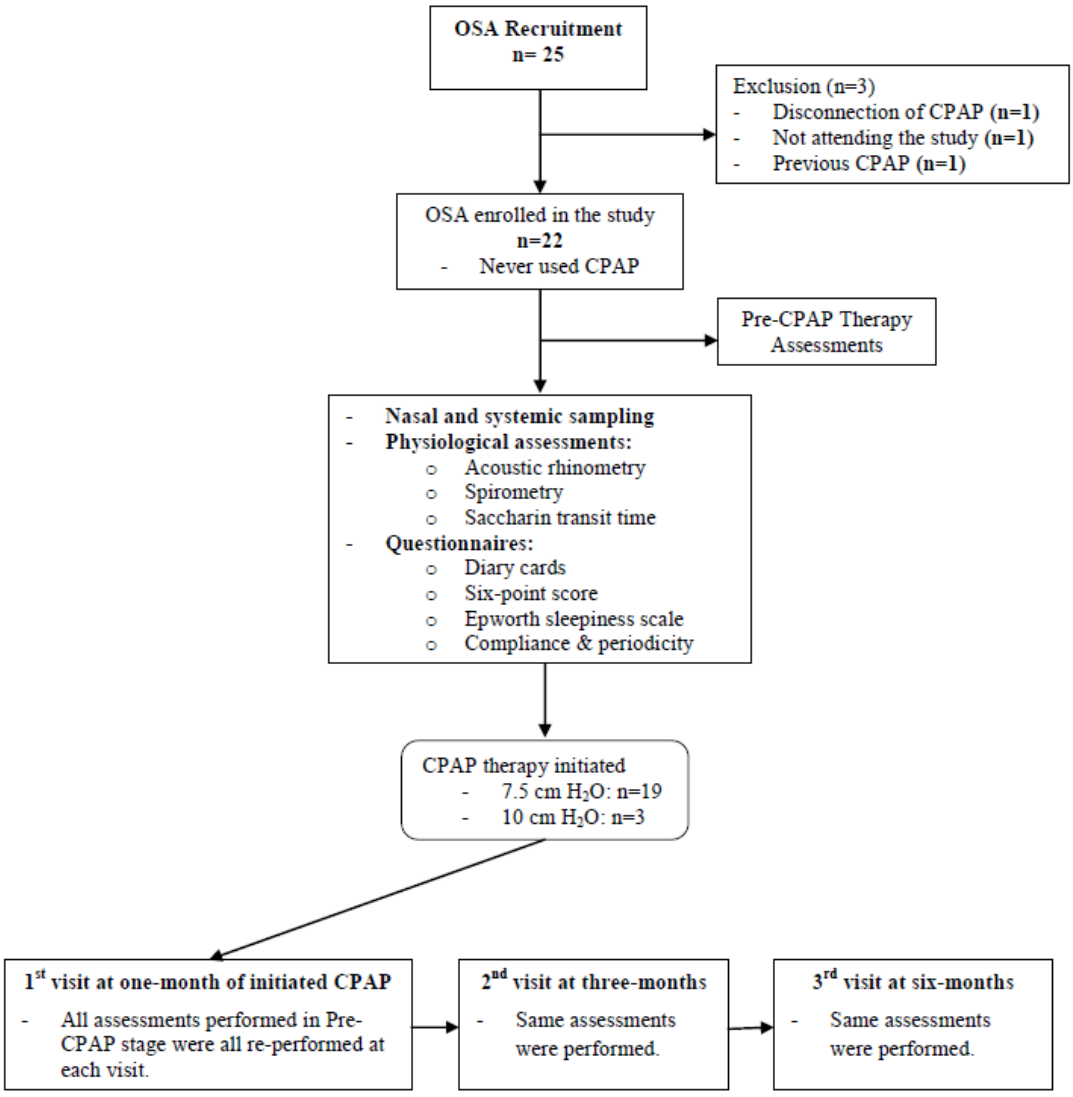


Figure 21: Flowchart of the OSA patients in the study.

5.3.2 Changes in Nasal and Systemic Inflammation with CPAP

Changes in systemic and nasal inflammatory parameters over time are shown in **Table 21**. **Figure 22-A** shows post-hoc analysis and **Figure 22-B** shows data plotted as tends within patients individually. Among systemic inflammatory parameters, only serum MPO level showed significant change at the end of study with significantly lower levels compared to baseline and 1 month. On the other hand, all inflammatory parameters measured in nasal

wash samples showed significant change throughout the study period. Nasal wash IL-6 levels showed an initial decrease at 1 month followed by a gradual increase thereafter. Nasal wash IL-8 and cell count started to increase after 1 month. Increased nasal wash MPO levels on the other hand was only evident at the end of the study period. Cell count showed gradual increase.

Table 21: Changes in inflammatory markers in serum and nasal wash fluid at baseline and after one month, three months and six months visits of nasal CPAP treatment.

Marker	**(n = 22)**	**After nasal CPAP**			**ANOVA**
	Baseline	**One month**	**Three months**	**Six months**	**P value**
Inflammatory markers in serum					
IL-6, log_{10} pg/ml	0.80 (0.38)	0.83 (0.41)	0.79 (0.38)	1.08 (0.29)	0.052
IL-8, log_{10} pg/ml	1.32 (0.14)	1.27 (0.11)	1.26 (0.22)	1.35 (0.21)	0.30
MPO, log_{10} ng/ml	2.84 (0.31)	2.87 (0.20)	2.73 (0.20)	2.59 (0.24)	0.002
Inflammatory markers in nasal wash sample					
Leukocyte count, log_{10} cells/ml	4.14 (0.14)	4.16 (0.13)	4.29 (0.14)	4.34 (0.17)	< 0.001
IL-6, log_{10} pg/ml	0.55 (0.29)	0.35 (0.26)	0.71 (0.22)	0.72 (0.23)	< 0.001
IL-8, log_{10} pg/ml	1.95 (0.41)	1.96 (0.35)	1.98 (0.37)	2.30 (0.21)	0.003
MPO, log_{10} ng/ml	2.05 (0.35)	1.90 (0.37)	1.94 (0.30)	2.20 (0.24)	0.019

Data are expressed as mean (SD). One-way ANOVA repeated measure. *Post hoc Bonferroni multiple comparison tests. ** P value < 0.05 was considered statistically significant. IL, interleukin; MPO, myeloperoxidase; CPAP, continuous positive airway pressure.

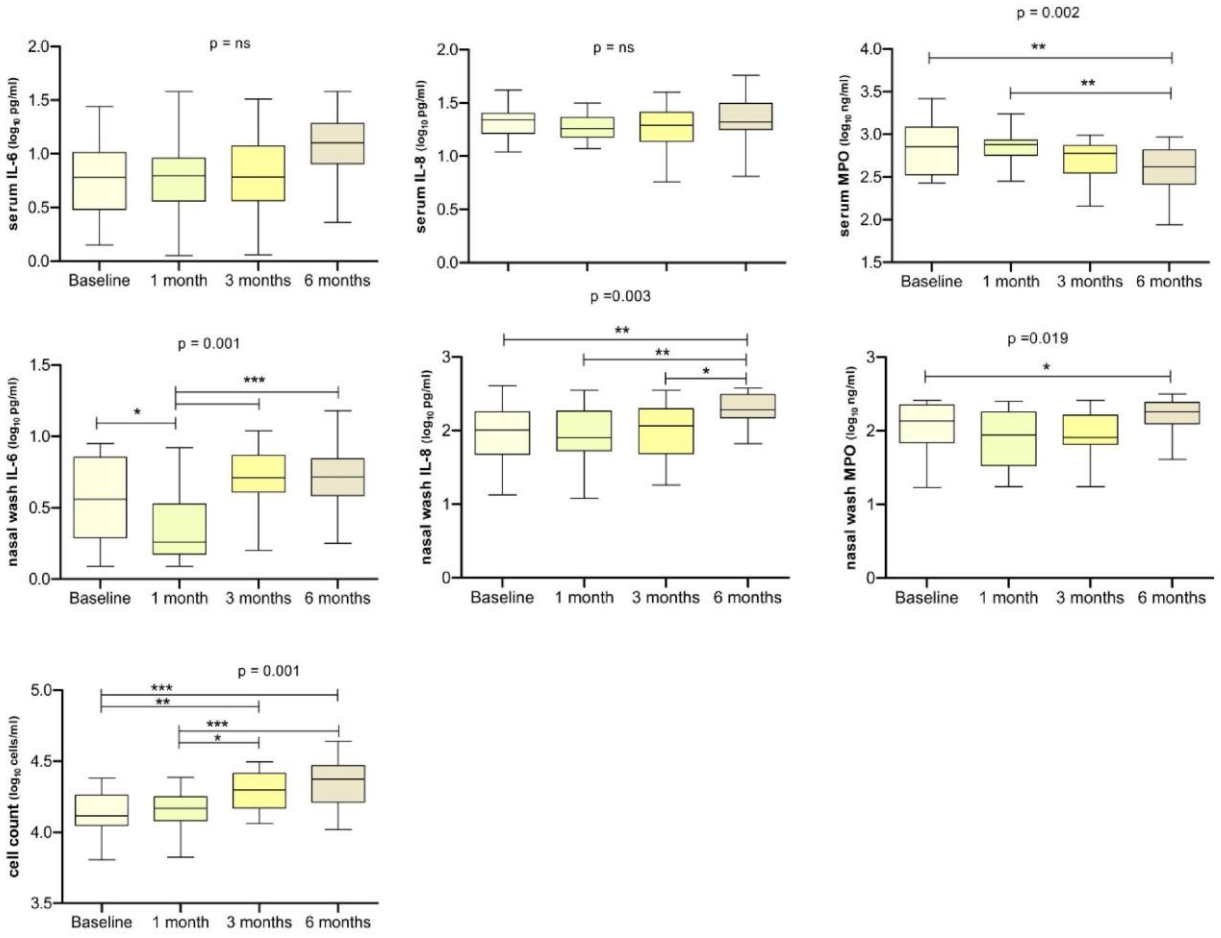


Figure 22-A: Effect of nasal CPAP in systemic and nasal inflammatory markers. Lines represent mean and standard error. Significant differences illustrated using ANOVA with post-hoc analysis.

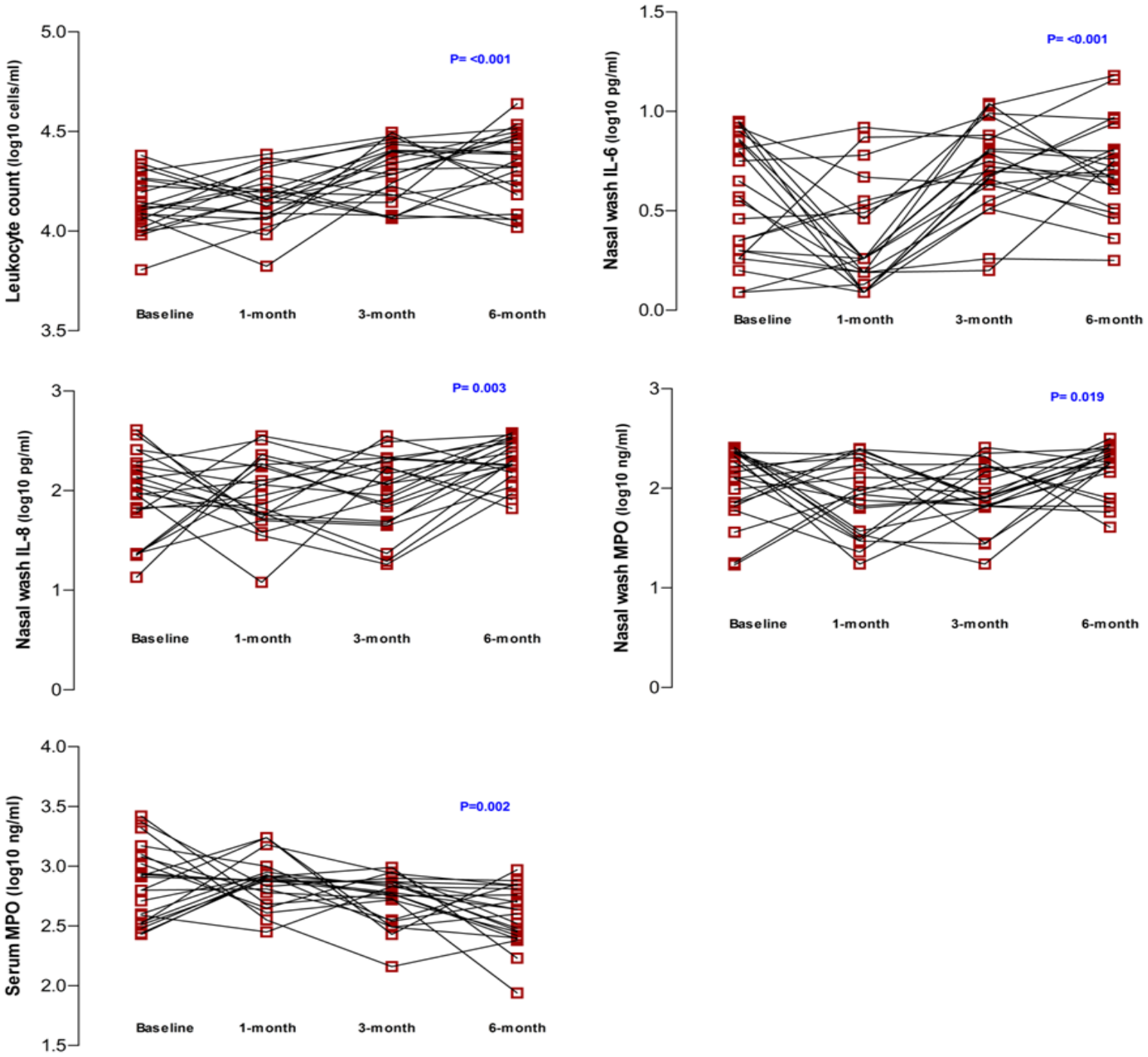


Figure 22-B: Effect of nasal CPAP in systemic and nasal inflammatory markers. Date plotted as trends within patients individually. Significant differences illustrated using ANOVA with post-hoc analysis.

5.3.3 Changes in Physiology with CPAP

Among physiological parameters studied, only FVC (l), MCA2 and nasal mucociliary clearance showed significant change (**Table 22, Figures 23 and 24**). Increase in FVC was evident after 3 months. An increased MAC2 value was detected only at 6 months when compared to baseline. On the other hand, saccharin transit time increased gradually starting from the first month. Saccharin transit time has shown to be slower with an increase in nasal inflammation as evident by nasal wash IL-6 concentration, **(Figure 25**).

Table 22: Changes in lung function, rhinometry findings and nasal mucociliary clearance at baseline and after one month, three months and six months visits of nasal CPAP treatment.

	(n = 22)	**After nasal CPAP**			**ANOVA**
Parameter	**Baseline**	**One month**	**Three months**	**Six months**	**P value**
Lung function test					
FEV (l)	2.93 (0.41)	2.93 (0.44)	3.17 (0.53)	2.94 (0.52)	NS
FEV1 %	84.01 (5.38)	86.96 (4.19)	85.99 (4.29)	84.72 (4.69)	NS
FVC (l)	3.22 (0.43)	3.42 (0.50)	3.78 (0.66)	3.71 (0.58)	0.001
FVC %	82.79 (4.42)	82.18 (4.08)	81.54 (5.67)	83.96 (4.93)	NS
FEV1/FVC ratio	84.93 (6.11)	84.72 (6.57)	85.63 (6.67)	85.13 (5.71)	NS
PEFR (l)	505.2 (59.81)	537.1 (60.42)	549.7 (50.10)	537.0 (56.96)	NS
Acoustic rhinometry					
MCA1	0.44 (0.12)	0.42 (0.12)	0.40 (0.11)	0.41 (0.11)	NS
DMCA1	2.15 (0.32)	2.13 (0.27)	2.24 (0.23)	2.20 (0.36)	NS
MCA2	1.67 (0.28)	1.79 (0.53)	1.97 (0.42)	1.98 (0.52)	0.021
DMCA2	4.32 (0.42)	4.27 (0.44)	4.49 (0.56)	4.35 (0.53)	NS
V2-5	5 (1.01)	4.87 (0.95)	5.12 (0.75)	5.18 (0.80)	NS
Nasal mucociliary clearance					
STT, seconds	16.82 (4.61)	20.14 (4.97)	21.06 (4.14)	21.95 (3.81)	< 0.001

Data are expressed as mean (SD). One-way ANOVA repeated measure. *Post hoc Bonferroni multiple comparison tests. ** P value < 0.05 was considered statistically significant. FEV, forced expiratory volume; FVC, forced vital capacity; PEFR, peak expiratory flow rate; MCA1, outermost minimum cross-sectional area; DMCA1, the distance of the MCA1 from the nasal orifice; MCA2, innermost minimum cross-sectional area; DMCA2, the distance of the MCA2.

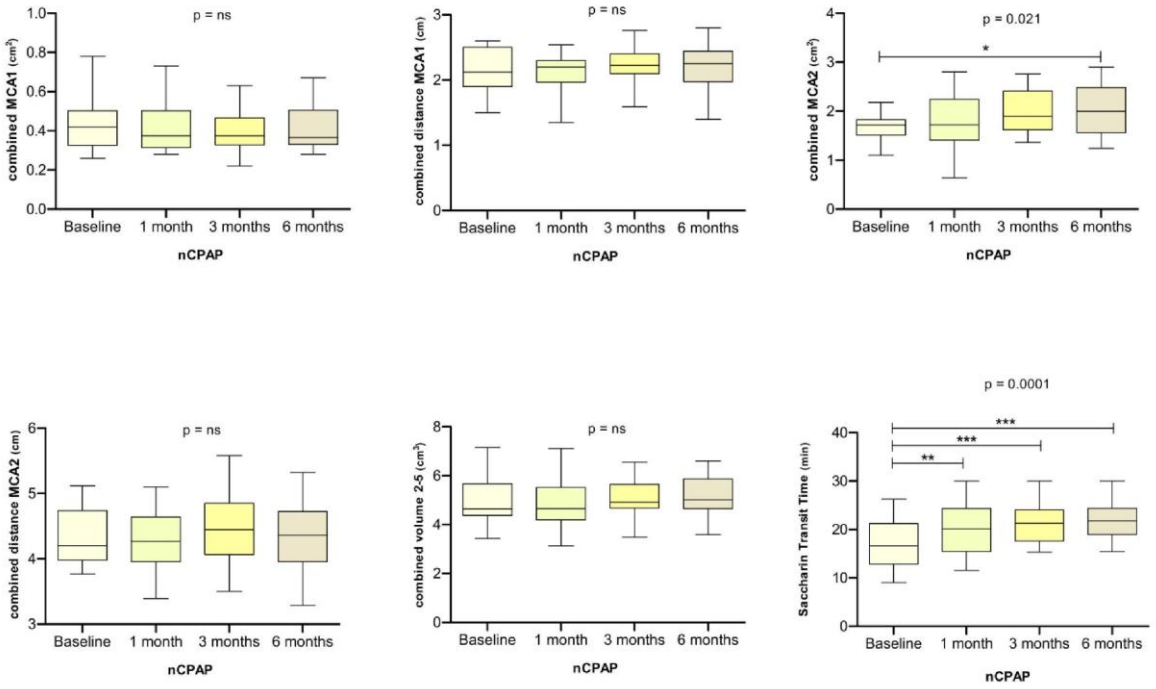


Figure 23: Effect of nasal CPAP in rhinometry findings. Lines represent mean and standard error. Significant differences illustrated using ANOVA with post-hoc analysis.

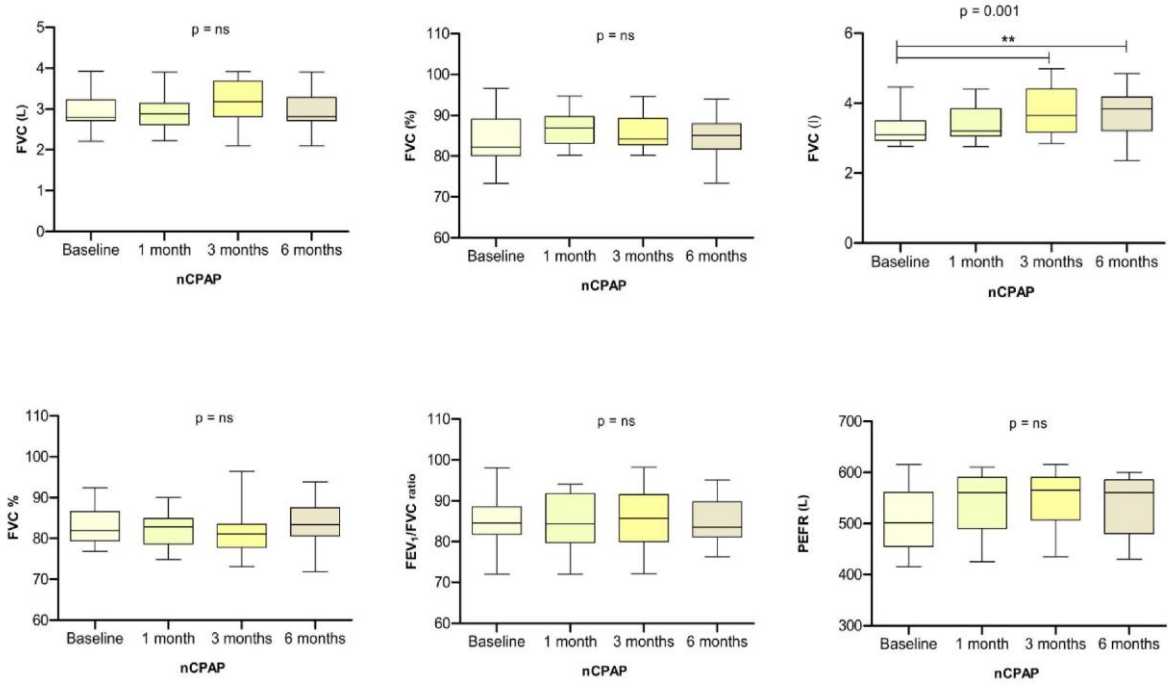


Figure 24: Effect of nasal CPAP in lung function. Lines represent mean and standard error. Significant differences illustrated using ANOVA with post-hoc analysis.

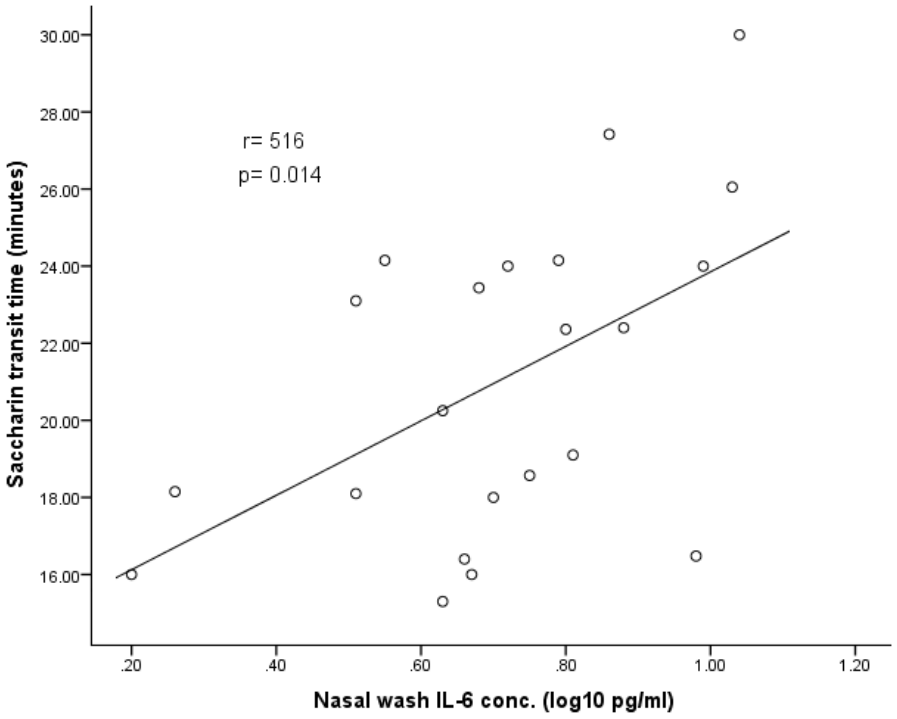


Figure 25: Correlation between nasal wash IL-6 levels and saccharin transit time.

5.3.4 Compliance with CPAP

According to patient diary card records there was no significant change in the average daily use of nasal CPAP during the six month study period (**Table 23)**. Recorded dairy card nasal CPAP usage was slightly higher than the average self-reported daily use of nasal CPAP (5.05 h ± 0.62) recorded at each study visit. 59% of patients reported to use nasal CPAP every night. The rate of lack of benefit of the machine decreased during the study period, with six patients (27%) reporting lack of benefit at one month, 4 (18%) at three months and only 2 patients (9%) reporting lack of benefit at six months (**Figure 26**).

Table 23: Daily hours of nasal CPAP use according to patient diary cards.

Daily average nasal CPAP use	Mean (SD)
At one month visit (hours)	5.28 (0.66)
At 3 months visit (hours)	5.02 (0.63)
At six months visit (hours)	5.21 (0.56)

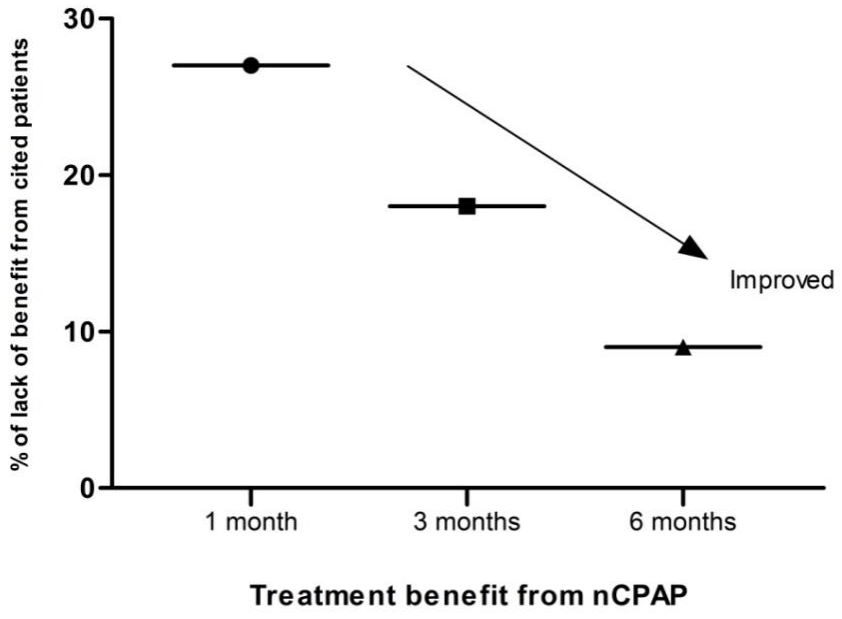


Figure 26: Variation in rate of lack of benefit from CPAP use with time.

5.3.5 Changes in Nasal Symptoms with CPAP

Table 24 shows the frequency of each condition of six-point nasal score at different time points. Six-Point nasal score increased significantly in relation to time throughout the study period (Friedman Test, $X^2(3) = 15.205$, p = 0.002; **Figure 27**). Whereas at the baseline and 1 month time points most patients recorded no conditions on the Six-Point Scale, at 6 months most patients were experiencing one or more of the conditions on the scale. Further *post-hoc* Wilcoxon Signed Rank tests with a Bonferroni correction ($p < 0.05/6 < 0.0083$) revealed a significant increase in the Six-Point Scale score between baseline and 6 months ($p = 0.006$).

The most commonly reported intra-nasal side-effects with nasal CPAP use were dry nose and dry mouth, reported by more than 50% of patients after nasal CPAP use, followed by rhinorrhoea and nasal blockage (**Figure 28.1**). A small number of patients reported sneezing, blood-stained mucus and epistaxis (**Figure 28.1**). Reported side–effects of the nasal mask included mask discomfort, air leak, sore eyes and nasal pain (**Figure 28.2**) whilst the most commonly reported machine-related side-effect was noise, **Figure 28.3**. A significant negative correlation was found between reduced compliance and increased report of nasal mask side-effects (r = -0.79, p=0.035; **Figure 29**).

Table 24: Frequency of the number of conditions for each time point for the Six-Point Nasal Scale. Conditions recorded on the scale rhinorrhoea, postnasal drip, nasal congestion, sneezing, reduced smell, and itchy nose.

No. of Conditions	Time Points			
	Baseline	1 month	3 months	6 months
0	14	12	11	5
1	6	6	4	8
2	2	1	4	5
3	0	1	2	2
4	0	1	0	0
5	0	1	1	2
6	0	0	1	0

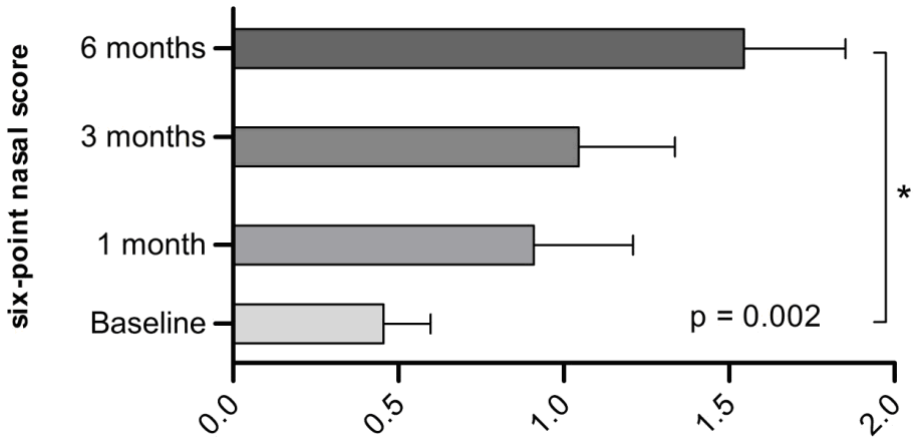


Figure 27: Six-Point Scale from baseline to 6 months. A Friedman Test showed that there was a statistically significant effect of time on Six Point Scale score ($x^2(3)= 15.205$, p=0.002). Further post-hoc Dunns tests revealed a significant increase in the Six-Point Scale score between baseline and 6 months (p=0.006).

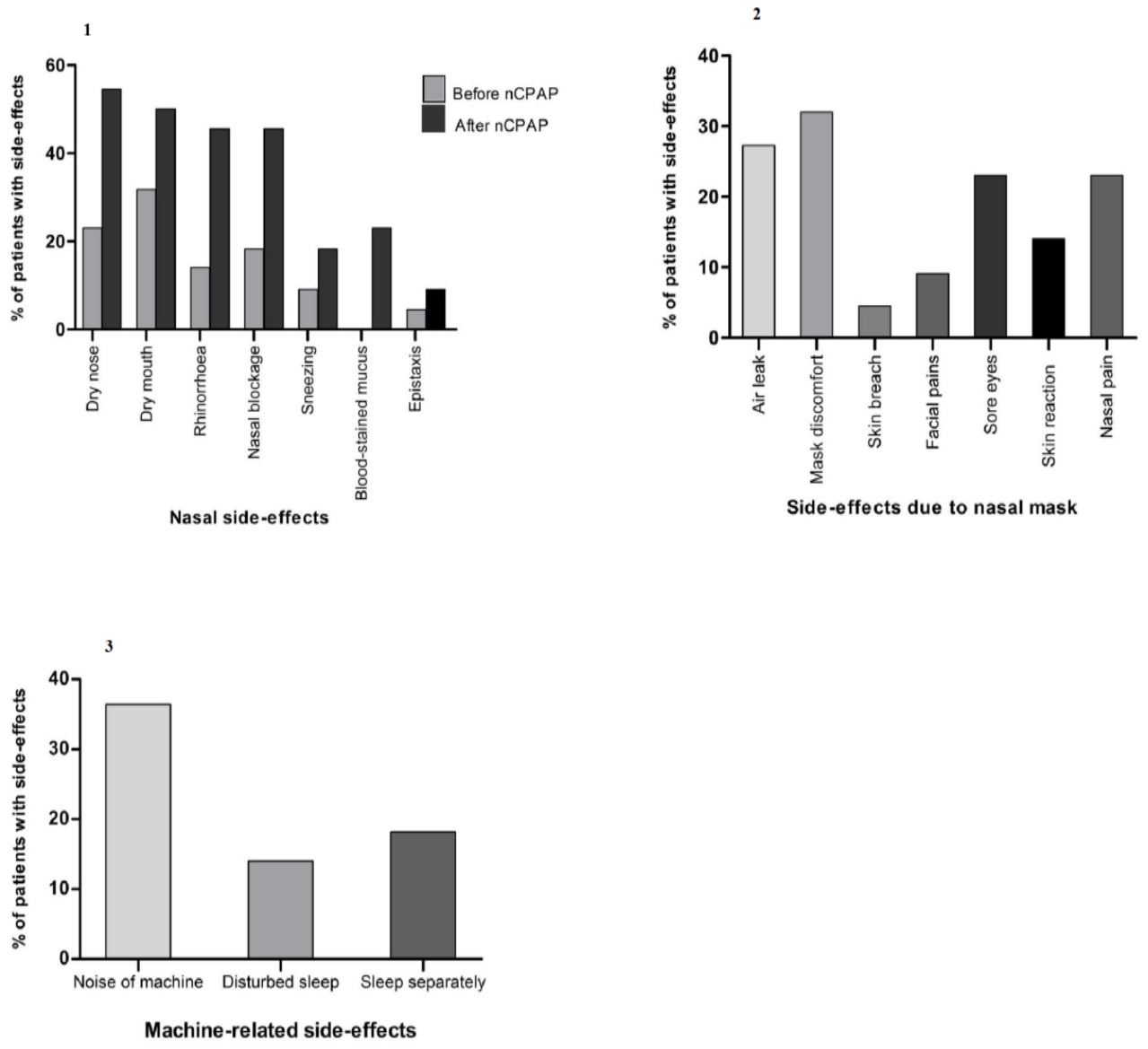


Figure 28: (1) Intra-nasal side effects by use of nasal CPAP. **(2)** Side-effects due to nasal mask. **(3)** Nasal CPAP-related side effects.

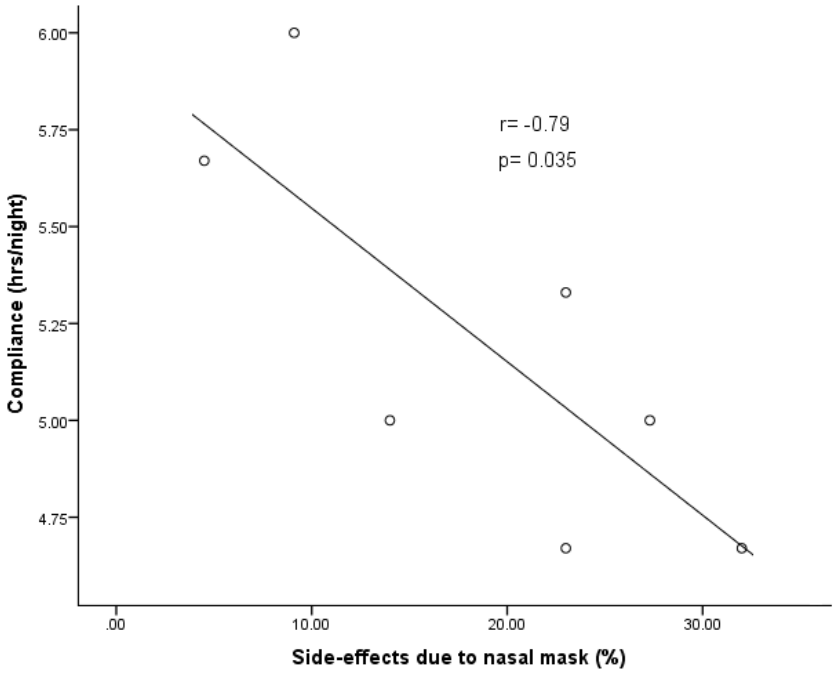


Figure 29: Correlation between compliance and side-effects due to the nasal mask.

5.3.6 Changes to Sleep Quality with CPAP

A statistically significant effect on time was observed in relation to the ESS score (F(2.187, 45.929) = 12.514, p < 0.0005; **Figure 30**). The ESS score progressively decreased throughout the study period from an initial mean score of 10.8 ± 1.1 at baseline. At one month the mean ESS score had decreased by 2.0 ± 0.9 (p = 0.221) compared to baseline, whilst at three and six months statistically significant decreases of 2.5 ± 0.8 (p = 0.041) and 4.7 ± 0.9 (p < 0.0001) were observed respectively. Significant decreases in the ESS scores were also observed between the one month and six month time points (2.7 ± 0.8, p = 0.016) and between three and six months (2.3 ± 0.7, p = .028). Analysis for potential correlations between inflammatory cytokine levels and sleep quality identified a significant positive correlation between increased IL-6 levels in nasal wash and reduced sleep quality according to the Epworth Sleepiness Scale (r = 0.492, p = 0.023; **Figure 31**).

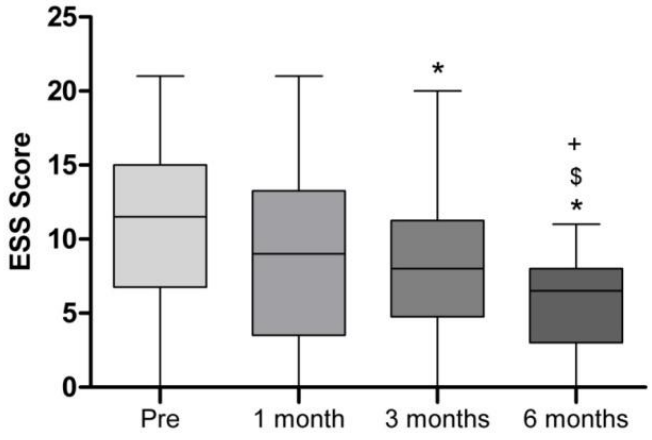


Figure 30: ESS score from baseline to 6 months. Data are mean ± standard error. * denotes statistically significantly different from baseline. $ denotes statistically significantly different from 1 month to 6 months. † denotes statistically significant difference from 3 months to 6 months.

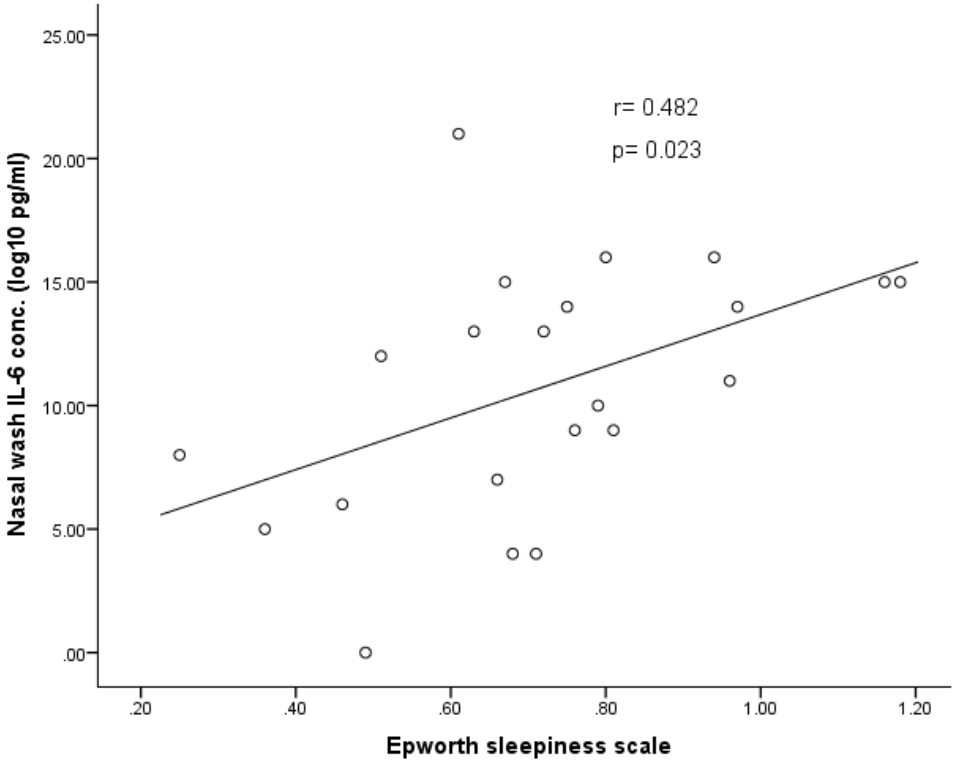


Figure 31: Correlation between nasal wash IL-6 concentrations with ESS.

5.4 DISCUSSION

We report that long-term use of nasal CPAP induces local nasal inflammation as evidenced by increased levels of all inflammatory markers in nasal wash fluid samples. Parallel to these changes, patients experienced increase of nasal symptoms with time at the end of the study compared to baseline, further confirming local inflammatory nature of nasal CPAP. Commonly reported side-effects were in line with previous studies [173, 98] and included dry nose and mouth, rhinorrhoea and nasal blockage. Patients also reported significant problems related to the mask, particularly discomfort and pain in the nose and eyes, and related to the machine itself, although only a small proportion found that the machine was preventing themselves or their partner from sleeping. Systemic inflammatory markers on the other hand did not change significantly, except for a significant improvement in serum MPO levels. Our findings provides insight on the mechanisms of CPAP intolerance in the crucial early phase of therapy and complements our previous study of short-term *in vivo* and *in vitro* effect of CPAP on healthy subjects and culture work [173] and other findings in patients with OSA, which suggests that CPAP increases nasal inflammation [512].

Several studies have tested the local inflammatory effects of nasal CPAP treatment. In an experimental study, the short-term mechanical stimulus triggered by nasal CPAP resulted in increase in neutrophils and overexpression of macrophage inflammatory protein-2 in tissue samples of rats [500]. Inflammatory effects of nasal CPAP treatment have also been shown in patients with OSA, both in the long- and short term [301, 373, 512, 524]. Skoczynski et al. [298] demonstrated significant increase in total cell count of nasal wash samples after short-term CPAP treatment in sleep apnoea syndrome patients qualifying for CPAP treatment, but not in patients with mild to moderate disease or in healthy controls. Constantinidis et al. [505] showed fundamental changes in nasal epithelium of subjects that underwent 3-10 months of nasal CPAP treatment on electron microscopic examination, including modifications in epithelial cell shape, conglutination and clumping of the microvilli and the appearance of immunocompetent cells. On the other hand, Lacedonia et al. [512] showed high levels of nasal neutrophilic inflammation among OSA patients before administration of nasal CPAP, when compared to controls. However, this nasal

inflammatory profile did not change in patients after 60 days of nasal CPAP treatment. This study evaluated several important inflammatory markers in nasal wash fluid and all of them increased after long-term nasal CPAP treatment, providing additional evidence supporting its local inflammatory nature.

In this study, long-term treatment did not change systemic inflammatory parameters measured in the serum, except for a decrease in MPO levels. In the long-term, Steiropoulos et al. [299] reported significant improvements in systemic inflammatory markers, including total lymphocyte counts, $CD4^+$ cells, TNF-α levels and uric acid levels after six months in patients with good compliance to CPAP therapy; however, no such improvements were identified in patients with poor compliance. In contrast, Kohler et al. [300] did not find any differences in systemic inflammatory markers, high-sensitive CRP, plasma IL-6, IFN-γ, and adiponectin levels between patients receiving therapeutic and sub-therapeutic CPAP treatments for four weeks. Thus, the relationships between CPAP, systemic inflammation, and the duration of therapy are complex. OSA itself may cause systemic inflammation and adequate nasal CPAP therapy may improve the condition.

The increase in nasal inflammatory markers was associated with clinical and functional consequences. We also demonstrated that CPAP reduced nasal clearance and was associated with an increase in 6-point nasal score. Our findings are contrary to those reported by de Oliviera et al. [382] in the short-term, who found significantly decreased STTs after 20 minutes of nasal CPAP in healthy individuals. This may be attributed to differences in the duration of nasal CPAP treatment, and suggests that nasal CPAP may provide an initial improvement in nasal clearance that is followed by impairment due to inflammation. Inflammatory and functional changes may contribute to the high incidence of symptoms and adverse effects associated with nasal CPAP treatment. Previous studies have reported high incidences of side effects during long-term therapy, which approached 97% in a large series [98].

In this study, despite apparent inflammatory response, nasal CPAP treatment did not result in any change in acoustic rhinometry parameters in OSA patients, except for an increased

MCA2 value after 6 months of treatment. Bossi et al. [515] have reported unaltered rhinomanometry results after long-term nasal CPAP therapy. Another study reported a reduction in airway resistance after an acute exposure to nasal nasal CPAP for 6 hours in healthy CPAP-naïve individuals [282]. Lack change in nasal geometry in the long term may be explained by adaptation. Additionally, a significant FVC improvement was identified in this study, indicating a demonstrable biological effect.

The mechanisms by which CPAP may be pro-inflammatory include airway drying (i.e., not using humidification) or direct distension. However, the possible benefits of humidification have been controversial. An experimental study in rats failed to demonstrate any beneficial effects of heated humidification on nasal inflammation [522], whereas clinical and experimental studies have reported conflicting results on the benefits of humidification [523-524].

The presence of side effects was significantly associated with reduced compliance, demonstrating the importance of identifying improved strategies for reducing nasal CPAP-related adverse effects. Interestingly however, long-term nasal CPAP use was also associated with a reduction in the reported lack of benefit and an improvement in sleep quality. These findings suggest that if patients are able to tolerate side effects into the longer term, the perceived benefit and improvement to sleep quality may shift the balance towards patients being more complaint with therapy. This is reflected in the compliance data, which show that overall hours of nasal CPAP use did not change during the study period suggesting that for many patients worsening side effects may be balanced by improvements to sleep quality. Alternative strategies to improve compliance may therefore involve improving patients' tolerance of side effects. Studies of treatment compliance in other diseases have found that patients who are prepared for side effects and educated on how to manage them are more likely to adhere [540]. Strategies to improve nasal CPAP adherence may therefore also focus on improved communication between patients' and their physicians, and encouragement of physicians to openly discuss potential side effects and how these can be managed.

Our previous studies, and those of other groups, have demonstrated significant associations between the long-term use of nasal CPAP and increased local (nasal) inflammation, including levels of the cytokines IL-6 and IL-8 [173, 298, 300, 373, 500, 512]. In this study we found a significant correlation between increased IL-6 levels in nasal wash samples and reduced sleep quality. These results demonstrate that local inflammation is not only associated with an increase in side effects and reduced compliance but also with reduced sleep quality. Hence, strategies to reduce nasal inflammation and associated side effects may also improve sleep quality, which in turn may encourage patients to continue nasal CPAP thereby further improving compliance.

5.5 CONCLUSION

The results of this study have important implications for clinical practice by demonstrating a relationship between the presence of side-effects and reduced compliance, and between increased inflammation and reduced quality of sleep. These findings underline the need for further investigation of strategies to reduce the nasal inflammation associated with CPAP treatment in an attempt to improve compliance. Such strategies may target the epithelial lining of the nose to address the origin of the inflammatory response. In addition, educational and support strategies to improve patients' tolerance of side effects may further increase compliance with nasal CPAP treatment for OSA patients.

6

IN VITRO STUDY:

EFFECT OF CPAP ON HUMAN CULTURED BRONCHIAL EPITHELIAL CELLS

This chapter presents an alternative approach to the investigation of the upper and lower airway through *in vitro* study of a human bronchial epithelial cell line, grown in a cell-culture system. It reports the analysis of the effect of short-term exposure of CPAP on the bronchial cell lines. The work has been published in the European Respiratory Journal [137] attached as Appendices One and Two, and has been presented at the American Thoracic Society in 2012.

6.1 INTRODUCTION

The upper airway wall, and more specifically its mucosa, is subjected to a continuous mechanical compression by nasal CPAP that triggers a biological response in the airways [496, 500], and induces an inflammatory process [291].

Several clinical and experimental studies have reported on the local and systemic inflammatory outcomes in association with CPAP treatment or mechanical ventilation in general with relatively less emphasis on the cascade of local events in response to continuous high pressure at the level of bronchial epithelium [298-299, 497-500]. Cell culture studies on the other hand are scarce and mainly focused on stretch injury rather than air pressure. However, we also sought to investigate the similarity or otherwise the effects on different level of mechanical pressures using CPAP on a bronchial epithelial cell line.

This chapter reports a novel study of a human bronchial epithelial cell line (BEAS-2B), grown in a cell-culture system, assessing release of the inflammatory cytokines IL-6 and IL-8 over 4 time intervals using CPAP therapy.

Therefore, we wished to determine whether CPAP is associated with pressure- and time-dependent release of inflammatory cytokines from cultured human bronchial epithelial cells, in an attempt to provide insight into mechanisms associated with early local inflammation taking place at the beginning of CPAP treatment.

This study aimed to examine *in vitro* the secretion of two key interleukins (IL)-6 and IL-8 by bronchial epithelial cell cultures (BEAS-2B) in response to continuous positive air pressure during several hours of application, and at two separate pressures (low-and high-response)

6.2 METHODS

6.2.1 Study Materials

All chemicals and reagents were of tissue culture grade and, unless otherwise stated, were obtained from the Sigma – Aldrich Chemical Co. (Poole, UK). Enzyme-linked immunosorbent assay (ELISA) kits used for the measurement of cytokines were purchased from R&D Systems Europe Ltd (Abingdon, UK).

6.2.2 Culture of Bronchial Epithelial Cells

6.2.2.1 Cell Culture: BEAS-2B Cells.

BEAS-2B cells, a virus-transformed human bronchial epithelial cell line, were obtained from American Type Culture Collection (Manassas, VA, USA) [541]. The cell-lines were cultured in 75 cm culture flasks in complete culture Medium 199 containing 1% (v/v) antibiotic / antimycotic solution composed of penicillin, streptomycin and amphotericin B (Sigma, UK). The antibiotic / antimycotic solution contained 10,000 units penicillin G, 10 mg streptomycin and 25 mcg amphotericin B per millilitre. When the cells became fully confluent the culture medium was removed and the cells were washed with 10 ml of sterile phosphate buffered saline (PBS). The PBS was then discarded after which 2-3 ml Trypsin/EDTA (Ethylenediaminetetraacetic acid)(0.25%, w/v) was added for 3 to 5 minutes to disperse the cells, to enable them to be transferred to 6 cm Falcon 'Primera' culture dishes (Becton Dickinson, Oxford, UK). Cultures were then incubated (Galaxy R;Wolf Laboratories, York, UK) in 2 ml fresh, sterile, complete culture medium containing 10% foetal calf serum (Sigma - Aldrich), 5 ml antibiotic / antimycotic solution, and 4 ml of each of the following: bovine pancreatic insulin (2.5 mcg/ml), human transferrin (2.5 mcg/ml), hydrocortisone (0.36 mcg/ml) and L-glutamine (0.02 mg/ml) made up to a final volume of 500 ml in Medium 199 and filter sterilised through a 0.22 μm syringe filter. Cells were then

incubated for one to three days at 37^{o}C in 95% air in 5% $CO_{2.}$ The appearance of the cells at this stage is illustrated as **Plate 12**.

Plate 12: Appearance of bronchial epithelial cells during culture.

6.2.3 Cell-Culture Exposure Experiments

6.2.3.1 Experimental Protocol

Cultured BEAS-2B was independently exposed *in vitro* with CPAP pressure in a chamber at 0, 4 and 7 cm H_2O for one, two, three, or four hours after which the release of cytokine concentration was measured in the culture medium. The protocol is summarized as **Figure 32**.

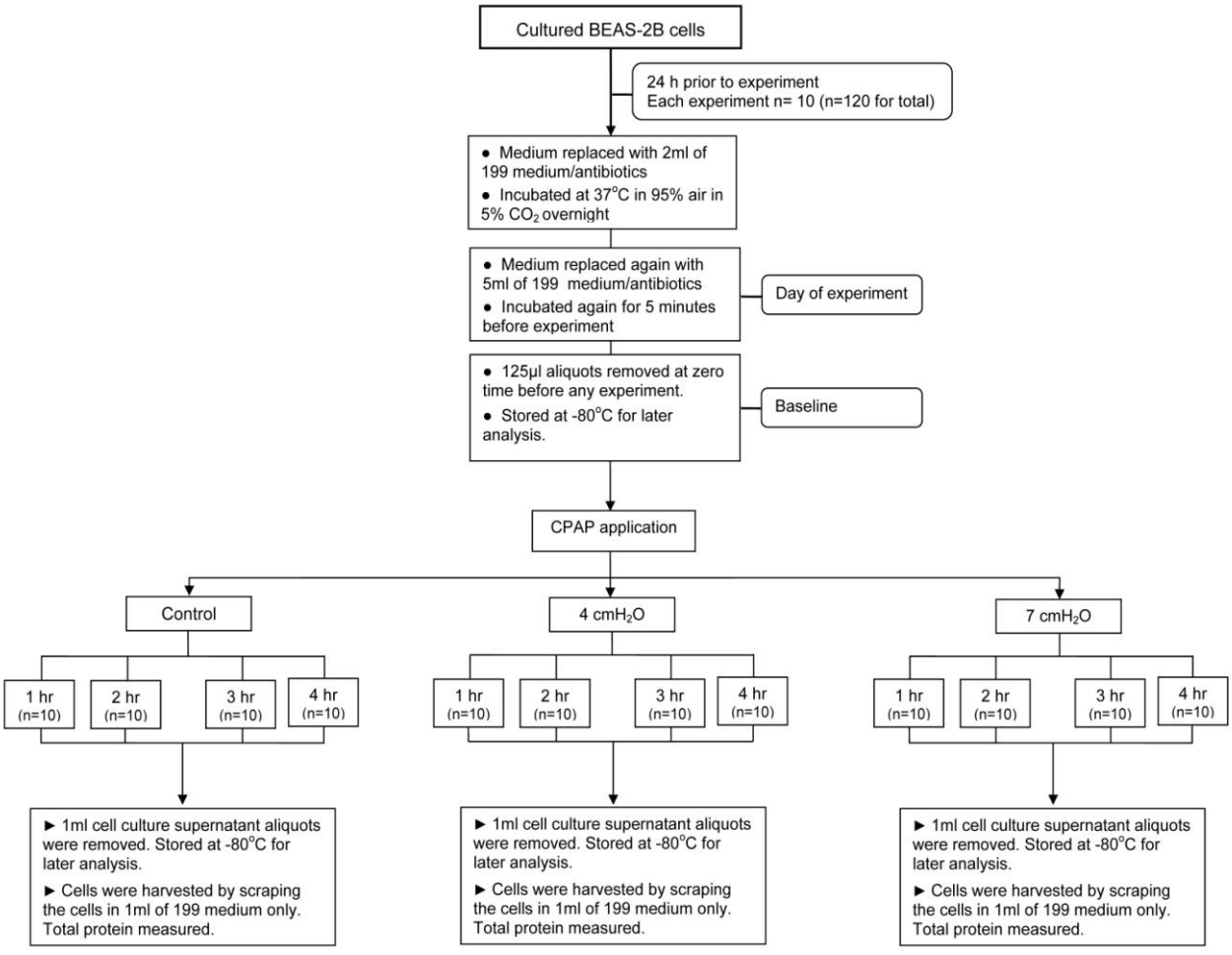


Figure 32: The experimental protocol of cell-culture exposure.

Ten tissue culture dishes size (60 x 15 mm) were used at each time-point. The day prior to the experiment, complete medium was removed and replaced with 2 ml of 199 medium/antibiotics and incubated at 37^{o}C in 95% air in 5% CO_2 overnight, and replaced the next morning before the experiment with fresh 5 ml 199 medium/antibiotics. The cells were then incubated for 5 minutes and 125 µl aliquots of cell culture supernatant were removed at zero time before any pressure application and stored at -80^{o}C for later analysis.

The experimental equipments are shown in diagrammatic form as **Figure 33**. CPAP (REMstar®), at a fixed pressure of 4 or 7 cm H_2O, was applied by incubating the cells in a air-tight chamber (4.6 l capacity) for the appropriate time, with 10 replicates per time point. The machine leak alarm was monitored to ensure that pressure was being delivered to the cells. The air-tight chamber was placed inside a 60-litre capacity acrylic SI.60 incubator (Stuart Scientific, Redhill, England, UK) to ensure conditions of controlled temperature reflecting that occurring in the nasal airway (31-33ºC), and close to 98% humidity (in contrast to our *in vivo* studies) by placing a tissue culture dish size (150 mm diameter) with 20 ml of sterile water in the chamber. Humidity was monitored using a Hygro-thermometer.

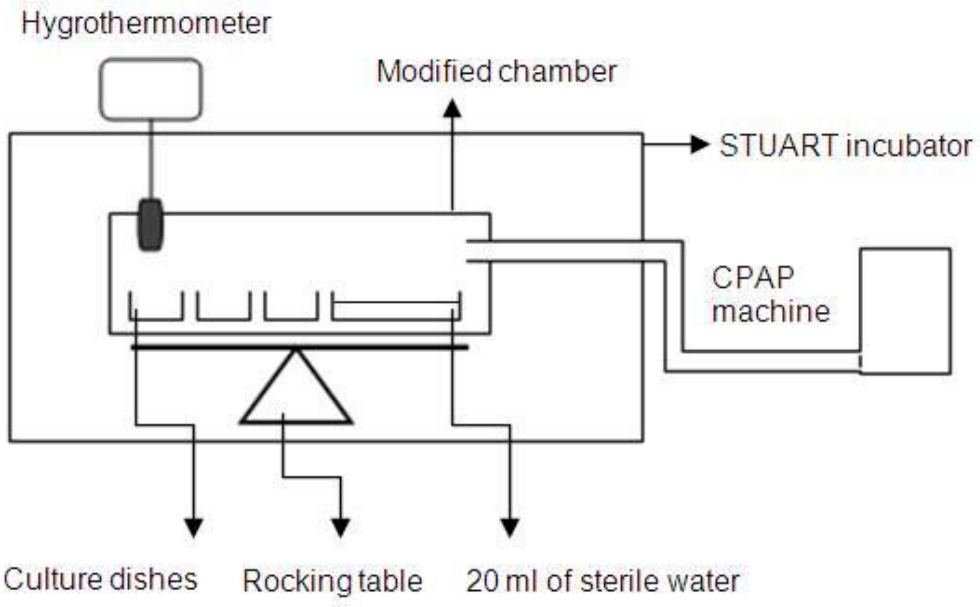


Figure 33: Diagrammatic form for the experimental equipments.

At the end of each time-interval for the allocated pressure, and at which point the experiment was terminated, 1 ml cell culture supernatant aliquots were removed. The remaining medium was then removed and the cells were harvested by scraping the cells in 1 ml of 199 medium only. All experimental medium and cell scrapes were stored at -80^{o}C for later analysis. Control experiments, not exposed to CPAP were also run for each of the four time points.

6.2.3.2 Physical Manipulations

Under standard conditions, the exposure polycarbonate chamber was tilted gently, at intervals of 2.5 s, to an angle of 10^{o} from the horizontal in each quarter of the horizontal plane on a Luckham 4RT rocking table (Luckham Ltd, Burgess Hill, England, UK), thereby momentarily displacing approximately half the medium covering the surface of the culture plate during each tilt to expose the cells to the pressurized gas air, humidified air. To ensure that the temperature was kept stable at 37^{o}C during exposure, the entire system was placed in a 60-litre capacity acrylic SI.60 incubator (Stuart Scientific, Redhill, England, UK), as in **Plate 13**.

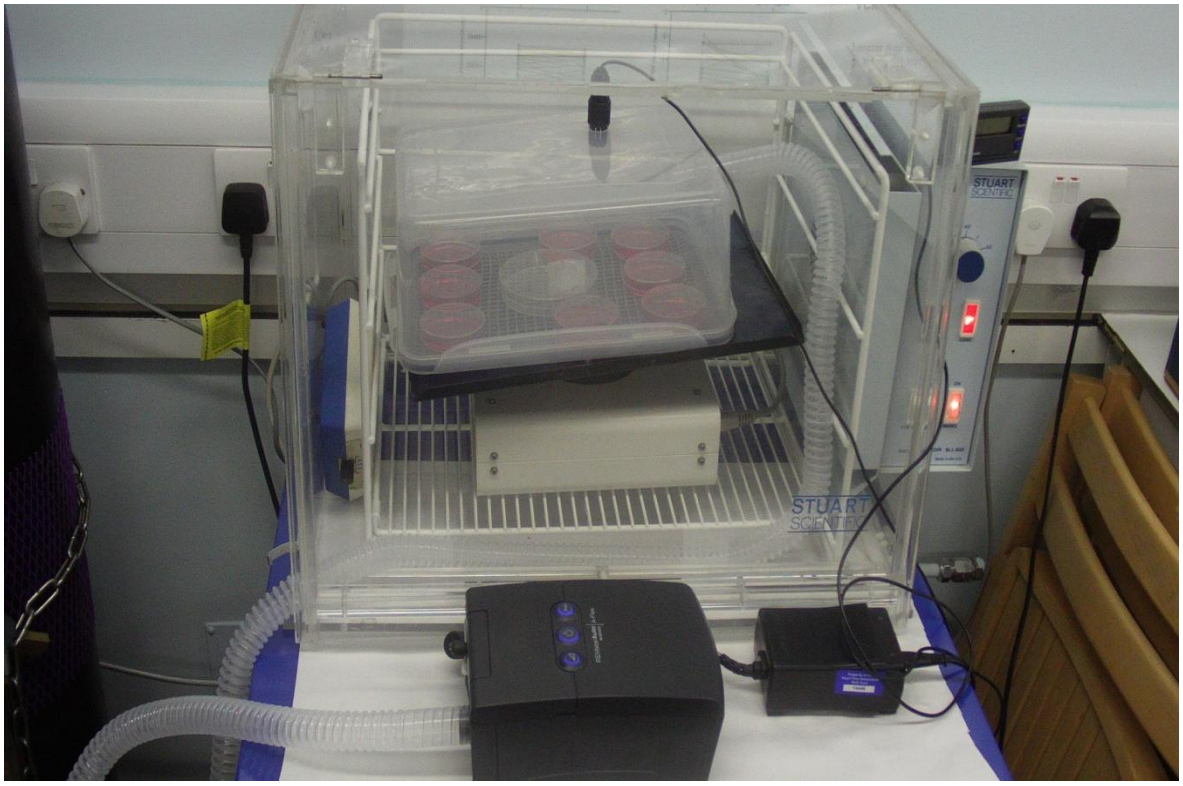


Plate 13: Set-up of cell-culture experimental equipments.

Pressure exposure of BEAS2B was homogenously distributed in the chamber. To the best of our knowledge, this had never been studied before and we designed and conducted our experiments carefully. According to the kinetic theory of gases, since the gas particles are always in motion the gas will diffuse homogenously over the cell chamber to occupy all available space. In addition, the cell chamber was sealed to keep the number of gas molecules, N, constant. The other experimental constants were volume, V, and temperature, T. This means that since N, V and T are constants, the gas law P V = N K T results in a constant pressure inside the chamber. To ensure this experimentally, the cell chamber was supplied with CPAP at a constant 4 or 7 cm H_2O. The platform will not produce any considerable turbulent flow since the gauge pressure (4 or 7 cm H_2O) is only slightly above atmospheric pressure. We therefore believe that the chamber pressure is uniformly distributed, transmitted to the cells and independent of the medium covering the cell surface. Finally, in an attempt to mimic the mechanical and physiological conditions of the

intranasal cavity, a rocking platform displacing approximately half of the medium covering the surface of the culture plate during each tilt was employed to directly expose the cells to pressurised, humidified air.

6.2.4 Measurements of Inflammatory Cytokines in Cell-Culture Supernatant

The cell-culture supernatant samples were analysed for IL-6 and IL-8 by ELISA kits. To account for differences in sizes of the culture, cytokine concentration were expressed accounting for total cellular protein. Total cell protein concentrations were measured by a modified Lowry assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA) [542]. This assay is based on the treatment of the protein content of a sample with a copper reagent which reacts with phenolic amino acids producing a measurable blue colour. Total protein content is determined spectrophotometrically. All results were expressed as pg cytokine/µg cellular protein, and **Figure 34** shows the best-fit line standard curve of our analysed kits.

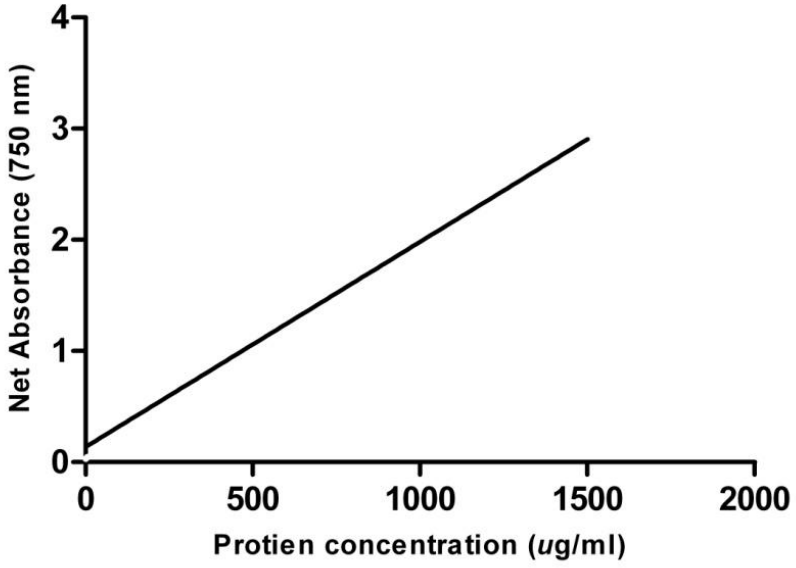


Figure 34: Standard curve graph involving the best-fit line.

6.2.5 Statistical Analysis

Data were analysed using GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA) and SPSS version 18.0 (SPSS Inc, Chicago, Ill). The Kolmogorov–Smirnov test of normality was applied. One-way ANOVAs were run to examine dose-response differences between cell culture responses to CPAP over several hours of application, followed by post-hoc Tukey's multiple comparison tests. Multiple linear regression analyses were performed to determine a set of independent variables (time and pressure) that predicted *in vitro* cytokine productions. A P value < 0.05 was considered statistically significant.

6.3 RESULTS

6.3.1 Time-Dependent Changes in IL-6 and IL-8 Concentrations

CPAP was associated with both time- and pressure (dose)-dependent release of the inflammatory cytokines IL-6 and IL-8 from cultured BEAS-2B cells *in vitro*. These data are reported in **Table 25** and illustrated in **Figure 35** and **36**.

Table 25 shows that *in vitro* data of CPAP was associated with both a time, and pressure (dose)-dependent release of inflammatory cytokines from cultured BEAS-2B cells *in vitro*. At 4 and 7 cm H_2O, IL-6 and IL-8 concentrations after 1, 2, 3 and 4 hours of CPAP were significantly raised ANOVA p=<0.001.

Table 25: CPAP is associated with time and pressure dependent release of IL-6 and IL-8 from cultured BEAS-2B cells *in vitro*.

IL-6 pg/μg of protein	**Mean (SD)**				**ANOVA**
	1 hour	**2 hours**	**3 hours**	**4 hours**	**P value***
Baseline	0.01 (0.001)	0.01 (0.002)	0.01 (0.015)	0.01 (0.002)	0.334
Control (no CPAP)	0.01 (0.002)	0.01 (0.003)	0.01 (0.004)	0.01 (0.002)	0.743
CPAP 4 cm H_2O	0.01 (0.005)	0.02 (0.005)	0.02 (0.005)	0.02 (0.008)	0.017
CPAP 7 cm H_2O	0.03 (0.005)	0.07 (0.014)	0.08 (0.016)	0.10 (0.051)	<0.001
ANOVA P=	<0.001	<0.001	<0.001	<0.001	
Posthoc 4 vs control†	0.012	0.016	0.09	0.649	
Posthoc 7 vs control†	<0.001	<0.001	<0.001	<0.001	
Posthoc 4 vs 7 control†	<0.001	<0.001	<0.001	<0.001	

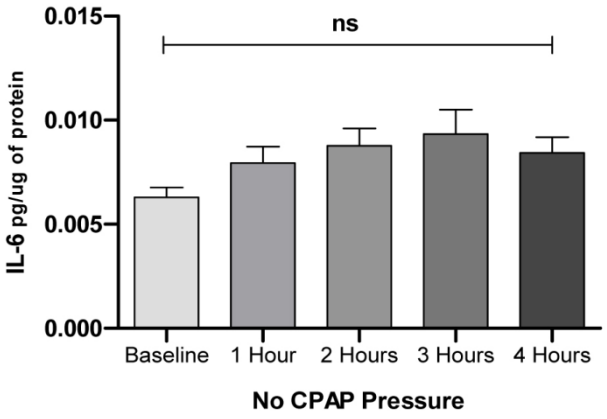
IL-8 pg/μg of protein					
Baseline	0.01 (0.003)	0.01 (0.004)	0.02 (0.02)	0.01 (0.004)	0.470
Control (no CPAP)	0.01 (0.011)	0.01 (0.004)	0.01 (0.006)	0.02 (0.009)	0.569
CPAP 4 cm H_2O	0.04 (0.03)	0.09 (0.022)	0.11 (0.014)	0.15 (0.040)	<0.001
CPAP 7 cm H_2O	0.06 (0.013)	0.09 (0.030)	0.11 (0.022)	0.14 (0.062)	<0.001
ANOVA P=	<0.001	<0.001	<0.001	<0.001	
Posthoc 4 vs control†	0.005	<0.001	<0.001	<0.001	
Posthoc 7 vs control†	<0.001	<0.001	<0.001	<0.001	
Posthoc 4 vs 7 control†	<0.001	<0.001	<0.001	<0.001	

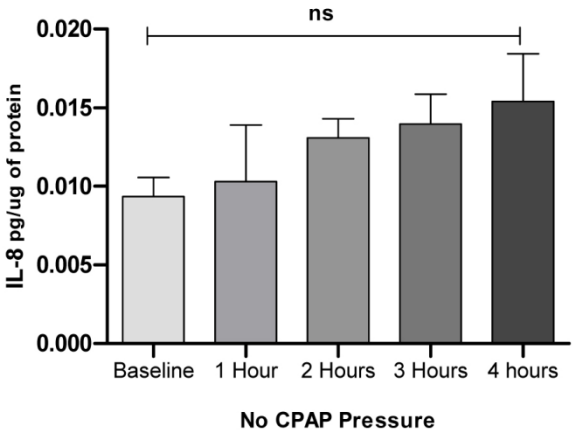
Data are expressed as the means (SD). One-way ANOVA test. † Post hoc Tukey's multiple comparison tests. * P value < 0.05 was considered statistically significant.

In linear regression analysis, both pressure and time independently contributed to release of IL-6 and IL-8 (IL-6 adjusted R^2=0.55, p<0.001;IL-8 adjusted R^2=0.60, p<0.001 time β 0.278 and 0.399, pressure β 0.694 and 0.671 respectively).

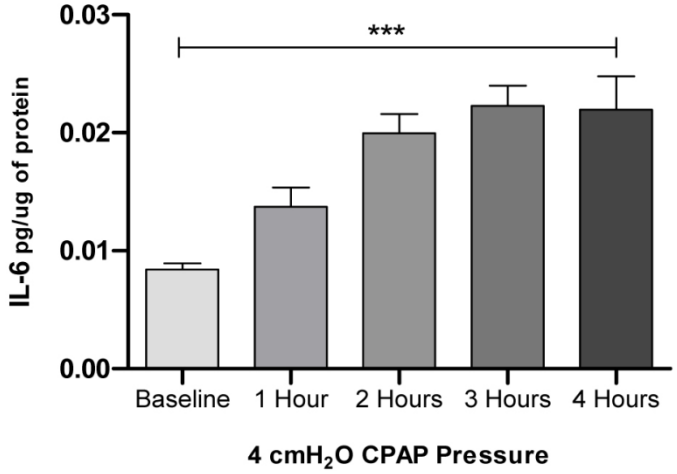
In the group that did not receive positive pressure, neither IL-6 (p=0.12) nor IL-8 (p=0.36), **Figure 35 (A)** concentrations changed significantly over time. At 4 cmH_2O pressure, IL-6 secretion significantly increased at 2 hours compared to baseline and high concentrations were maintained until the end of the experiment (p<0.0001 for overall difference), and the time course of IL-6 concentrations was similar at 7 cmH_2O (p=0.0009), **Figure 35 (B)**. Similarly, IL-8 secretion significantly increased at two hours compared to baseline and maintained thereafter, at both 4 and 7 cmH_2O pressure (overall p<0.0001 for both conditions as in **Figure 35 (C)**.

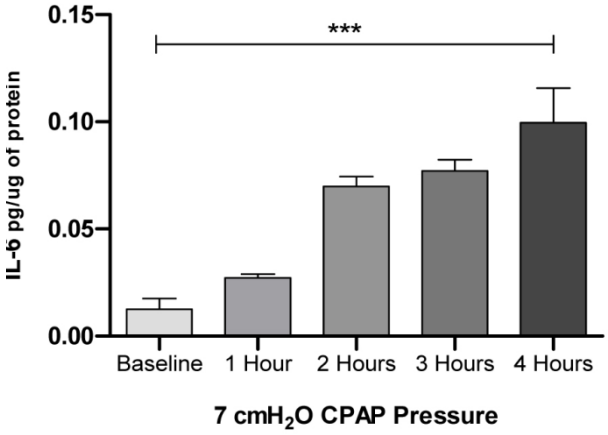
21 (A)


ns
0.015
0.010
0.005
0.000
IL-6 pg/ug of protein
Baseline
1 Hour
2 Hours
3 Hours
4 Hours
No CPAP Pressure


ns
0.020
0.015
0.010
0.005
0.000
IL-8 pg/ug of protein
Baseline
1 Hour
2 Hours
3 Hours
4 hours
No CPAP Pressure

21 (B)


***
0.03
0.02
0.01
0.00
IL-6 pg/ug of protein
Baseline
1 Hour
2 Hours
3 Hours
4 Hours
4 cmH2O CPAP Pressure


***
0.15
0.10
0.05
0.00
IL-6 pg/ug of protein
Baseline
1 Hour
2 Hours
3 Hours
4 Hours
7 cmH2O CPAP Pressure

21 (C)

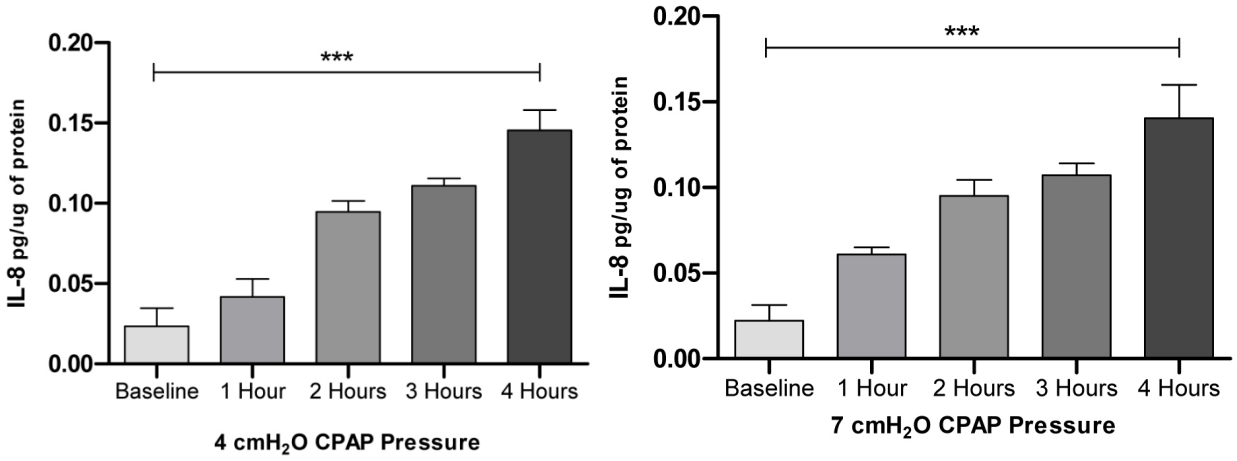
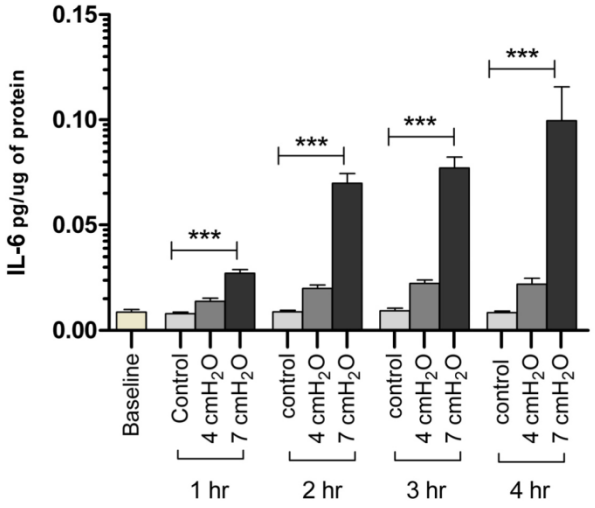


Figure 35: Time-dependent changes in IL-6 and IL-8 concentrations.

6.3.2 Effect of Pressure-Dependent Level on the Secretion of Inflammatory Mediators

When the means of all measurements done under each pressure level were compared, secretion of IL-6 by cell cultures subjected to 7 cm H_2O were significantly higher when compared to the cultures that received 4 cm H_2O pressure and the cultures received no pressure at all ($p<0.0001$ for overall difference) as in **Figure 36 (A)**.

22 (A)



22 (B)

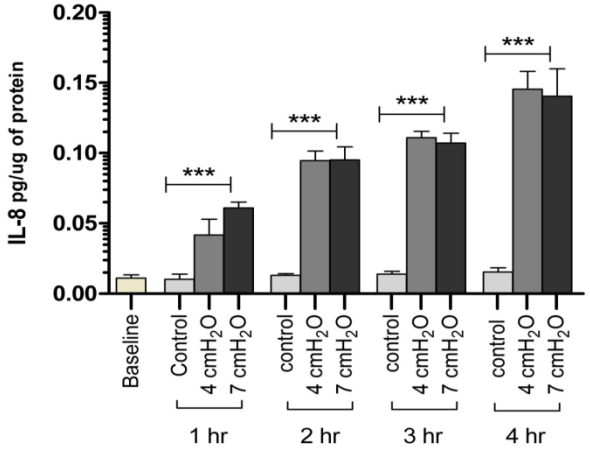


Figure 36: Effect of pressure-dependent level on the secretion of inflammatory mediators.

However, IL-8 concentrations did not change significantly between positive pressure groups, although both 4 and 7 cm H_2O groups had significantly higher secretion compared to the no pressure group as in **Figure 36 (B)**.

6.4 DISCUSSION

This study obtained direct evidence for the increased secretion of IL-6 and IL-8 by bronchial epithelial cells in response to continuous positive air pressure, providing a plausible explanation for the origin of the early inflammatory side effects of nasal CPAP observed in substantial number of patients at the onset of treatment. This study is unique in examining the early inflammatory consequences of continuous [low-to-high] pressure using bronchial epithelial cell culture. In contrast, most experimental studies so far have focused on systemic inflammatory markers or lung microvasculature. Previous cell culture studies on the other hand, used different models utilising mechanical stretch rather than applying direct air pressure [525-527]. To the best of our knowledge, this is the first report to examine *in vitro* effects of CPAP in this way.

Findings from the *in vitro* component of this study provided direct evidence for cytokine (IL-6 and IL-8) secretion by bronchial epithelial cells in response to CPAP in a pressure- and time-dependent manner. IL-6 is an important pro-inflammatory cytokine. IL-8 is a chemokine serving as a chemical signal that attracts neutrophils to the site of inflammation.

Most information on the inflammatory effects of CPAP comes from studies conducted with obstructive sleep apnoea patients. Nasal CPAP treatment seems to induce an inflammatory response at the early stages of treatment [298], partly explaining the high incidence of side effects at the onset of treatment [98]. However, in the long-term, these effects seem to be offset by the improvement of the obstructive sleep apnoea [299, 301], which itself is inflammatory in nature, and by tolerance of the patient, resulting in acceptable compliance rates in the long term [98, 298].

Early inflammatory effects of airway distention through lung hyperinflation have already been demonstrated in healthy individuals. Administration of up to 20 cm H_2O CPAP by a face mask for a short period of time resulted in increased serum concentrations of several cytokines including the ones investigated in the present study (TNF-α, IL-1β, IL-6, IL-8, IL-10, and IL-12), which returned to normal concentrations after 12 hours [264].

Most evidence on the potential inflammatory effects of mechanical ventilation in general comes from experimental studies using various animal models. In a mouse model, strain/injury induced by mechanical ventilation was associated with an increase in concentrations of a murine equivalent of IL-8 and its receptors [497]. In another animal study, all measured inflammatory mediators including plasma interleukin (IL)-1α, IL-1β, tumor necrosis factor- α, IL-6, and IL-10 were increased progressively in lung tissue with increasing duration of mechanical ventilation [498]. Even low mechanical ventilation pressures have been found to contribute increased lung cytokine response in another experimental study [499].

Lim et al. [496] examined the degree of leukocyte recruitment to the airway in the postcapillary venules of mechanically ventilated rats and found increased leukocyte recruitment after 1 hour of PEEP application, which was inhibited by the blockage of endothelin receptor and selectin, suggesting an ongoing pro-inflammatory process. In another animal study, PEEP induced changes in rat trachea were investigated with findings suggesting an important role for P-selectin and ICAM-I in the observed leukocyte recruitment in postcapillary endothelium after airway distention. The effects of nasal CPAP on nasal mucosa has been investigated in a rat model and five hours of 10 cm H_2O nasal CPAP resulted in early nasal inflammation mediated by macrophage inflammatory protein-2 that manifested itself by neutrophil extravasation [500]. Macrophage inflammatory protein-2 is a rodent chemokine homologous to human interleukin-8.

Studies on cytokine release from human lung cell cultures in response to conditions that resemble increased airway pressure are relatively scarce. Based on evidence from such studies, lung microvascular and alveolar endothelial cells appear to produce metalloproteins and IL-8 -a powerful chemokine for neutrophil activation-, respectively, upon stretch injury [525-527]. However, none of these studies has used positive pressure application as in the present study, which may better mimic the effects of continuous positive airway pressure.

When a distending force is applied to the airways, it is primarily withstood by the fibre system located in the basal membrane and all the cells that are attached to this extracellular

structure have to accommodate their shape. In the meantime, up-regulation of inflammatory cytokines may occur through interplay between signaling molecules. Taking this potential mechanism into account, the present study mainly focused on the molecular aspects of possible local inflammatory responses at the level of bronchial epithelium to increased pressure; we therefore utilised an isolated cell culture model. Although stress/strain relationship in cell culture may not be equivalent to that is observed *in vivo* due to the complex architecture of the bronchial wall as well as the absence of vascular and fibrous structures, we believe that the available data may be considered as offering a unique perspective to explain the distinct role of bronchial epithelium in the cascade of inflammatory events, since it represents the first structure to come into contact with high pressure.

Both interleukins tested in this study are markers of early inflammation. Interleukin-6 is an important pro-inflammatory cytokine that takes part in the induction of acute phase response of inflammation. IL-8 is a powerful chemokine for neutrophil activation. In this study, the secretion of both interleukins increased in response to pressure stimuli starting from the second hour of the experiment and the same concentrations of secretion were maintained throughout the whole duration of the study. On the other hand, IL-6 concentrations seem to be dependent on the amount of pressure with high IL-6 concentrations less than 7 cmH_2O compared to 4 cmH_2O. However, such a difference between pressure concentrations is not evident for IL-8 concentrations. These findings suggest that continuous pressure applied throughout the bronchial tree triggers an inflammatory response within the first few hours mainly originated from bronchial epithelial cells. Chemokine production on the other hand seem to be pressure independent with an early rise regardless of the level of pressure, whereas pro-inflammatory process appears to be more powerful under higher pressure concentrations.

The mechanisms by which CPAP may be pro-inflammatory include airway drying (i.e., not using humidification) or direct distension. The possible benefits of humidification have been controversial, and our *in vitro* work, in which cells were exposed to high-humidity, demonstrates that drying or the absence of humidification alone cannot be solely responsible

for the pro-inflammatory changes observed. An experimental study in rats failed to demonstrate any beneficial effects of heated humidification on nasal inflammation [522], whereas clinical and experimental studies have reported conflicting results on the benefits of humidification [523-524]. Stretch may affect inflammation via oxidative stress, as stretch-induced IL-6 and IL-8 production can be reduced by the use of anti-oxidants to increase intra-cellular glutathione; production can be increased with glutathione depletion [528]. Stretch induced IL-8 and IL-6 production were significantly inhibited when intracellular GSH was increased by antioxidants and productions of these interleukins were decreased when intracellular GSH was depleted, suggesting that oxidant release may play a role in lung cell stretch-induced cytokine release, and antioxidants, may protect lung cells against this injury. However, these experimental data need to be tested in clinical setting.

Our data add to the literature by reporting a direct effect of pressure rather than stretch. It is unlikely that the nasal epithelium is able to accommodate stretch given the confines of the nose within the bony structures of the skull. The pressures we selected for the *in vitro* work were necessarily different from the *in vivo* work, as higher pressures *in vitro* resulted in excessive evaporation of the cell culture fluid. Our results have important implications for clinical practice.

6.5 CONCLUSION

The findings of this study states that continuous pressure applied to the airway epithelial triggers early inflammatory reaction originated from bronchial cells as evidenced by the increased secretion of inflammatory markers IL-6 and IL-8 by epithelial cells. These findings have implications for the adherence of patients to CPAP therapy, especially during the important initiation phase. Future strategies to combat the initial side effects of this treatment modality and to improve compliance and treatment retention may targets epithelial lining of respiratory system, in an attempt to address the origin of inflammatory response.

7

PILOT STUDY: AIRWAY AND SYSTEMIC INFLAMMATION IN PATIENTS WITH STABLE COPD

This chapter presents an analysis of airway and systemic inflammatory indices in two matched groups of patients with stable COPD. The first group uses NIV whereas the other group never used NIV. This chapter is a pilot study.

7.1 INTRODUCTION

In previous chapters, we investigated the *in vivo* and *in vitro* effect of CPAP on inflammatory indices. The results revealed that the CPAP induces early airway and systemic inflammation [173]. COPD is a chronic lung inflammatory disease with significant extra-pulmonary effects which may contribute to disease severity in individual patients [231, 244], and greater lower airway inflammation in the stable state [543]. However, the effect of NIV on stable COPD patients has not been investigated with inflammatory markers.

Therefore, this chapter presents an investigation of the effect of NIV on inflammatory indices in patients, compared to a matched patient group which never received NIV therapy.

COPD affects approximately 5% of the adult population and is one of the leading causes of acute respiratory failure [544]. Bilevel positive pressure airway therapy has become a mainstay treatment for patients with an acute exacerbation of their COPD (AECOPD). Its use is supported by strong evidence from both observational and interventional studies which demonstrate the benefit of this therapy in reducing mortality, complications, length of hospital stay and need for intubation for patients with AECOPD [545-549]. For patients with severe, stable COPD, the role of nocturnal bilevel positive pressure airway therapy as a long-term management strategy is more controversial. However, there is a growing body of evidence suggesting that long-term use of bilevel positive pressure airway therapy is associated with improvements in physiological parameters, including blood gas data and pulmonary hyperinflation, as well as exercise endurance and subjective symptom scores [550-553]. Bilevel positive pressure airway therapy may therefore also favourably impact long-term survival in these patients.

Several clinical and experimental studies have demonstrated local and systemic inflammatory responses to the use of ventilator support [298, 264, 496-500]. In our own study we have demonstrated that CPAP use in healthy subjects and in cell-cultures is associated with dose-dependent up-regulation of nasal inflammatory markers [173]. This inflammatory response may be a result of stretch stress imposed on pulmonary parenchyma by hyperinflation, resulting in release of inflammatory cytokines by alveolar macrophages [265-266, 554-557]. To date, clinical studies have focused on the inflammatory response to NIV in healthy subjects without evidence of inflammatory activity. It is currently unknown whether use of bilevel positive pressure airway therapy in COPD, a disease that is associated with local airway and systemic inflammation, causes further up-regulation of the inflammatory response.

We hypothesised that in patients with COPD, bilevel positive pressure airway therapy therapy would be associated with an increased airway and systemic inflammatory response, compared with COPD patients who are not using bilevel positive pressure airway therapy.

This study aimed to compare the effect of bilevel positive pressure airway therapy on airway and systemic inflammation in COPD patients that had used bilevel positive pressure airway therapy for more than three months with a matched group that had never used bilevel positive pressure airway therapy.

7.2 METHODS

7.2.1 Study Subjects

Ten patients with moderate COPD were enrolled into the study from the London COPD cohort. Five of the 10 patients had been on bilevel positive pressure airway therapy for more than three months whilst the other five patients had never used this therapy. The protocol was approved by the Research Ethics Committee at Royal Free Hampstead NHS Trust (study reference 09/H0720/24) and was conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from all subjects prior to their inclusion in the study.

Samples of sputum (induced), nasal wash and serum were obtained at a single clinic visit. The following assessments were made: nasal and systemic inflammation (interleukin (IL)-6, IL-8 and myeloperoxidase (MPO) concentration in serum and nasal wash samples), and nasal wash leukocyte count.

7.2.2 Measurements

7.2.2.1 Nasal and Systemic Inflammation

Measurements of the inflammatory cytokines (interleukin (IL)-6, IL-8 and MPO) in nasal wash supernatants and serum were performed using a standard ELISA technique, as discussed in previous chapters.

7.2.3 Physiological Assessments

The best of three attempts at spirometry was recorded using a Vitalograph 2160 (Maids Moreton, Buckingham, UK). We recorded forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio and peak expiratory flow rate (PEFR).

7.2.4 Statistical Analysis

Data were analysed using GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA). The Kolmogorov–Smirnov test of normality was applied. Independent t-tests were conducted to compare the matched COPD groups. A P value less than 0.05 was considered statistically significant.

7.3 RESULTS

The baseline and clinical characteristics of the two groups (five subjects per group) enrolled in the study are described in **Table 26**. The majority of subjects were male. Both groups had similar mean ages, smoking history and spirometry assessment results.

Table 26: Clinical characteristics of the two groups of matched moderate COPD patients. Each matched group includes five COPD patients. Group one had used bilevel positive pressure airway therapy therapy for more than three months and the other group never used bilevel positive pressure airway therapy. Data is expressed as mean (SD).

	COPD BILEVEL POISITIVE THERAPY USED n =5	**COPD NO THERAPY USED n =5**
Age (years)	72.1 (7.1)	67.6 (6.9)
Male gender	3	4
FEV_1 (l)	1.19 (0.39)	1.34 (0.2)
FEV_1 (% predicted)	46.6 (12.1)	45.2 (11.2)
FVC (l)	2.42 (0.87)	2.9 (0.62)
FEV_1/FVC (%)	44.5 (14.2)	41.6 (13.9)
Smoking (pack years)	44.6 (24.1)	43.8 (23.3)

7.3.1 Relationship of NIV Therapy to Nasal and Systemic Inflammation in COPD Patients

Inflammatory markers in sputum, nasal and serum samples were compared between COPD patients who used bilevel positive pressure airway therapy and those who had never used this therapy. Results are presented in **Table 27**. No significant differences were observed between patients who used bilevel positive pressure airway therapy and those who had never used this therapy in the levels of inflammatory markers in the sputum, nasal wash or serum. Small, but statistically insignificant, increases in nasal wash leukocyte counts and IL-8 levels, and in sputum inflammatory markers were observed.

Table 27: Comparison of sputum, nasal wash and serum from two groups of stable COPD patients (five patients using bilevel positive pressure airway therapy and five who had never used this therapy). Data is reported as mean (SD). Comparisons use unpaired t-tests.

	COPD (BILEVEL POISITIVE THERAPY Group)	**COPD (NO THERAPY Group)**	**UNPAIRED**
	n=5	**n=5**	
	Mean (SD)	**Mean (SD)**	**p=**
SPUTUM			
Leukocyte Count (log_{10} cells/ml)	5.87 (0.44)	6.19 (0.39)	0.26
IL-6 (log_{10} pg/ml)	1.28 (0.67)	1.80 (0.52)	0.21
IL-8 (log_{10} pg/ml)	3.42 (0.14)	3.39 (0.17)	0.16
MPO (log_{10} ng/ml)	3.80 (0.10)	3.30 (0.66)	0.14

NASAL WASH			
Leukocyte Count (log_{10} cells/ml)	4.95 (0.74)	3.88 (0.86)	0.068
IL-6 (log_{10} pg/ml)	0.42 (0.38)	0.41 (0.18)	0.96
IL-8 (log_{10} pg/ml)	2.34 (0.39)	1.82 (0.54)	0.13
MPO (log_{10} ng/ml)	2.18 (0.21)	2.10 (0.30)	0.65
SERUM			
IL-6 (log_{10} pg/ml)	0.49 (0.16)	0.53 (0.24)	0.78
IL-8 (log_{10} pg/ml)	1.10 (0.23)	1.10 (0.17)	0.99
MPO (log_{10} ng/ml)	2.94 (0.06)	2.91 (0.08)	0.54

7.4 DISCUSSION

The main finding of this pilot study was that the use of bilevel positive pressure airway therapy in patients with stable COPD did not cause an increase in local or systemic inflammatory responses compared with patients who had never used this therapy. To the best of our knowledge, this is the first study to examine the inflammatory response to non-invasive supportive ventilation in COPD patients.

COPD is characterised by a progressive and not fully reversible limitation to airflow, accompanied by an abnormal inflammatory response mediated by macrophages, lymphocytes and neutrophils together with various chemokines, cytokines and proteinases. This inflammatory activity contributes directly to disease pathogenesis through the remodelling of pulmonary tissues and also plays an important role in the comorbidity often observed in COPD patients [558]. Notably, increased expression of inflammatory mediators, including IL-6, has been associated with reduced lung function and poorer prognosis in COPD [559]. Moreover, increased systematic IL-6 concentration is associated with increased cardiovascular risk [521]. Avoiding potential additional triggers of inflammation may therefore have an important impact on disease progression and outcome.

There is increasing evidence that the use of non-invasive ventilation methods may be beneficial to patients with stable COPD. However, such interventions may stretch the lung parenchyma beyond its physiological limits due to the application of high pressure to the airways. This can lead to modulation of the expression pattern of inflammatory and anti-inflammatory molecules in the lung and drive a lung-injury induced inflammatory response. A number of studies in healthy patients with no underlying inflammatory activity corroborate this theory, demonstrating increased leukocyte recruitment and cytokine infiltration both locally and systemically in response to mechanical ventilation [298, 265-266, 555]. As increased inflammatory activity has been associated with poorer long-term prognosis and increased comorbidities in COPD, these studies suggest the use of bilevel positive pressure airway therapy may amplifies COPD-specific inflammatory activity and worsen prognosis and systematic health. This would counteract the potential long-term benefit to lung function. Our study therefore examined whether the levels of key

inflammatory molecules involved in COPD pathogenesis were amplified in patients who had used bilevel positive pressure airway therapy for more than 3 months. However, in contrast to the results observed in patients with healthy lung-function, we were unable to detect any differences in the inflammatory responses of COPD patients who used ventilatory support when compared with those who did not. Notably, these results are in line with a recent study which examined the impact of bilevel positive pressure airway therapy on systemic inflammatory response and lung structure in patients undergoing a subarachnoid block for small or medium surgical procedures [560]. The researchers found no difference between the inflammatory response of patients receiving a single lung hyperinflation manoeuvre and a control group, suggesting lung hyperinflation did not further amplify the systemic inflammatory response triggered by the surgical procedure. Similarly, Puls and colleagues et al. [265] were unable to detect changes in the systemic levels of either inflammatory or anti-inflammatory cytokines in patients with atelactasis following lung hyperinflation.

In contrast, a recent study in patients with acute respiratory distress syndrome (ARDS) found an association between the use of mechanical ventilation and an increase in cytokine levels both systemically and locally in the lung [557]. The authors concluded that inflammation in response to ventilator therapy may partially explain the high rate of multiple organ failure and mortality observed in ARDS patients. These high rates are despite the apparent improvement in management provided through the use of mechanical ventilation support. Notably, a further study demonstrated a reduction in mortality rate with concommittant use of lung protection strategies to reduce the stress insult caused by hyperinflation on the lungs [561]. These studies suggest strategies to avoid lung injury whilst providing mechanical ventilation should be further explored in chronic lung disease.

This study has several limitations which must be considered. Most notably, the sample size was low. Whilst no statistical associations could be detected with this sample size, an increase in sample size may provide sufficient power to identify statistically significant differences in inflammatory activity between the two populations. In this study we have measured the activity of IL-6, IL-8 and MPO, important inflammatory molecules in COPD. However, there are additional markers which may have provided further insight into the

inflammatory effects of bilevel positive pressure airway therapy in COPD and associated comorbidity risk. Further work may therefore explore a wider range of inflammatory markers in a larger study population.

7.5 CONCLUSION

This study has found that the use of bilevel positive pressure airway therapy in patients with COPD does not have an additive effect on disease-specific inflammation. The ability to adjust inspiratory and expiratory pressures independently with bilevel positive pressure airway therapy may result in fewer symptomatic and hence inflammatory effects compared with the delivery of constant pressure as with those of CPAP. However, with the conflicting current literature in this field and given the limitations of this study, further investigation to draw a definitive conclusion on the potential inflammatory effects of bilevel positive pressure airway therapy in patients with stable COPD is warranted.

8

CONCLUSIONS AND SUGGESTIONS FOR FUTURE STUDIES

The upper and lower airways are anatomically distinct, but are interrelated in many ways. Similarities between them include common triggers, pathogenic mechanisms, many of the inflammatory cells and mediators, as well as treatment modalities.

This thesis was undertaken to test the hypothesis that the non-invasive respiratory support is a pro-inflammatory stimulus to the upper and lower airways. This hypothesis was tested on human bronchial epithelial cell-lines, healthy subjects, and OSA and COPD patients.

This chapter summarises the main findings of this thesis, with reference to the stated hypothesis and aims of chapter two, and concludes with suggestions for future studies based on the present results of this thesis.

8.1 CONCLUSIONS

The key findings of this thesis study may be summarised thus:

1. CPAP resulted in early induction of inflammation.

2. CPAP was associated with both time- and pressure (dose)-dependent release of the inflammatory cytokines IL-6 and IL-8 from cultured BEAS-2B cells *in vitro*.

3. In linear regression analysis, both CPAP pressure and time independently contributed to release of IL-6 and IL-8 from cultured BEAS-2B cells *in vitro*.

4. *In vitro* study demonstrated that even when cultured BEAS-2B cells were exposed to high-humidity, the drying or the absence of humidification alone cannot be solely responsible for the pro-inflammatory changes observed.

5. Acoustic rhinometry provides highly repeatable measurements of nasal patency, which is best for combined (mean) nasal parameters. This property makes it suitable for use in the diagnosis and follow-up of conditions associated with nasal obstruction, either structural or functional.

6. The newly developed nasal lavage technique utilizing a paediatric tracheostomy tube provides highly repeatable cytokine and cell-count measurements over successive sessions.

7. In healthy subjects, both 7.5 and 12.5 cm H_2O of nasal CPAP pressures over three hours resulted in significant increases in nasal inflammation as assessed by nasal wash leukocyte and MPO measurements.

8. Only a higher pressure, measured at 12.5 cm H_2O of nasal CPAP pressure, administered over three hours, and to healthy subjects, resulted in an increase in nasal IL-6 and IL-8 concentrations.

9. Further evidence shows that both pressures also resulted in changes in systemic inflammatory markers, with significant increases in serum IL-6 concentrations and decreases in serum IL-8 concentrations following three hours of nasal CPAP.

10. Physiologically, at both pressures (7.5 and 12.5 cm H_2O), three hours of nasal CPAP treatment resulted in a significant slowing of nasal clearance (i.e. increased saccharin transit time).

11. There was also association at both pressures (7.5 and 12.5 cm H_2O) of nasal CPAP with small but significant changes in FVC and PEFR.

12. There was an overall increase in the frequency of all of the symptoms during nasal CPAP treatment in healthy subjects. The higher the pressure, the more symptoms were recorded.

13. *In vivo* data demonstrated relationships between the development of nasal symptoms, nasal inflammation and impaired nasal function. The greater the nasal symptoms with CPAP, the slower the nasal mucociliary clearance and the greater the nasal inflammation.

14. Long-term use of nasal CPAP induces local nasal inflammation in OSA patients as made evident by the significant changes occurring within all inflammatory parameters used to measure nasal wash samples.

15. There was a relationship between physiological changes and inflammation in OSA patients. Saccharin transit time has been shown to be slower with an increase in nasal inflammation in OSA patients, as made evident by nasal wash IL-6 concentration.

16. The same significant improvement in lung function in healthy subjects is also seen in OSA patients after six months of CPAP therapy, as made evident by an increase in FVC.

17. OSA patients experienced an increase in nasal symptoms with time compared to baseline, further confirming the local, inflammatory nature of nasal CPAP.

18. The ESS score progressively decreased throughout the study period.

19. Patients also reported significant problems related to the mask, particularly discomfort and pain in the nose and eyes, as well as problems relating to the machine itself, although only a small proportion found that the machine was preventing themselves or their partner from sleeping.

20. There was a significant, positive correlation between inflammatory cytokine levels and daytime sleepiness: increased IL-6 levels in nasal wash was found to correlate with reduced sleep quality according to the Epworth Sleepiness Scale

21. Long-term nasal CPAP use was also associated with a reduction in the reported lack of benefit and an improvement in sleep quality.

22. Compliance data showed that overall hours of nasal CPAP use did not change during the six month period, suggesting that, for many patients, worsening side effects may undermine improvements to sleep quality.

23. These results demonstrated that local inflammation is not only associated with an increase in side effects and reduced compliance but also with reduced sleep quality which in turn impacts on quality of life for OSA patients.

24. In the pilot study of matched COPD groups, the results showed no changes in the inflammatory markers but further investigation may be needed in order to draw a definitive conclusion. This is due to the limitations stated in the study, e.g. sample size.

Therefore, with regard to the specific aims of the thesis, described in chapter 2:

- **Is CPAP a pro-inflammatory stimulus to human bronchial epithelial cell-lines in a culture model?**

  Yes, CPAP proved to be a pro-inflammatory stimulus to bronchial epithelial cells. The results of CPAP exposure to the cell-culture experiments demonstrated that there was both time- and pressure (dose)-dependent release of the inflammatory cytokines IL-6 and IL-8. This direct evidence of the effect of pressure, rather than stretch for the increased inflammatory cytokines, exerted by bronchial epithelial cells in response to CPAP, provided a plausible explanation for the origin of the early inflammatory side effects of nasal CPAP observed in a substantial number of patients at the onset of treatment as we have reported in our *in vivo* study [173].

- **Is CPAP a pro-inflammatory stimulus in healthy subjects?**

  In two healthy subjects groups with no prior history of nasal symptoms or disease, one exposed to higher and one to lower CPAP pressure, CPAP was associated with dose (pressure)-response changes in nasal and systemic inflammatory markers, reduced nasal function, and the development of nasal symptoms after receiving three hours of CPAP therapy. The development of nasal symptoms related to the degree of functional impairment and nasal inflammatory response. The greater the nasal symptoms with CPAP in those healthy subjects, the slower the nasal mucociliary clearance and the greater the nasal inflammation as assessed by nasal wash IL-6. Our *in vivo* findings are in line with the *in vitro* findings, particularly with regard to the neutrophilic nature of inflammation.

- **Is CPAP a pro-inflammatory stimulus in OSA patients?**

  We report that CPAP results in the release of inflammatory mediators from the *in vitro* study and in healthy control subjects. CPAP was also associated with changes in nasal and systemic inflammatory markers, reduced nasal function and the development of nasal symptoms [173]. All these findings are also in line with the OSA findings. Yes, long-term use of nasal CPAP induces local nasal inflammation with OSA patients, as evidenced by increased levels of all inflammatory markers in nasal wash fluid samples. Parallel to these changes, patients experienced an increase of nasal symptoms over time compared to the baseline, further confirming the local, inflammatory nature of nasal CPAP. Commonly reported side-effects were in-line with previous studies [173, 98] and included dry nose and mouth, rhinorrhoea and nasal blockage. Patients also reported significant problems related to the mask, particularly discomfort and pain in the nose and eyes, and related to the machine itself, although only a small proportion found that the machine was preventing themselves or their partner from sleeping. Our findings provide insight into the mechanisms of CPAP intolerance in the crucial, early phase of therapy and complement our previous study of the short-term *in vivo* and *in vitro* effect of CPAP on healthy subjects and culture work [173] and other findings in patients with OSA, which suggests that CPAP increases nasal inflammation [299].

- **Is NIV a pro-inflammatory stimulus in a matched group of COPD patients?**

  As in stable COPD, there is a relationship between the degree of upper and lower airway inflammation at exacerbation. This pilot matched study of ten COPD patients (5 patients with no bilevel positive pressure airway therapy and the other five on bilevel positive pressure airway therapy) has shown no changes in local or systemic inflammatory indices in both groups. Notably, this finding is in line with a recent study which examined the impact of this therapy on systemic inflammatory response and lung structure and showed no changes in inflammatory markers in patients undergoing a subarachnoid block for small or medium surgical procedures [560].

However, more research is needed in order to draw a definitive conclusion regarding the potential inflammatory effects of bilevel positive pressure airway therapy in patients with stable COPD. We draw attention to the fact that the sample size in the study was low and whilst no statistical associations could be detected with this sample size, an increase in sample size may suffice to identify statistically significant differences in inflammatory activity between the two groups.

- **Subsidiary aims to validate acoustic rhinometry and a novel upper airway sampling methodological techniques used in this thesis:**

3. To examine the validity of intersession repeatability of acoustic rhinometry measurements of unilateral and combined nasal parameters in a group of healthy subjects.

   We examined the intersession repeatability of acoustic rhinometry using ICC on averaged combined and separate nostril minimum cross-sectional areas and nasal volumes in healthy subjects. We showed for the first time that acoustic rhinometry provides excellent reproducible results, best over different sessions when combined nasal parameters values are used, as opposed to right or left nostrils separately. This finding advocates use of such an approach for the follow-up of conditions associated with structural or functional obstructions.

4. To establish the repeatability and acceptability of a novel upper airway sampling technique we have developed, employing a paediatric tracheostomy tube (TT).

   We examined the intersession repeatability of a newly developed nasal lavage technique utilizing a paediatric tracheostomy tube. To our knowledge, few studies have examined the repeatability of different nasal lavage techniques for clinically relevant parameters and varying results have been obtained. The main findings of this study suggest that the newly developed nasal lavage technique utilizing a paediatric tracheostomy tube provides highly repeatable cytokine and

cell count measurements over successive sessions, with a better sealing of the nasal opening and comfort.

8.2 SUGGESTIONS FOR FUTURE STUDIES

The following is a set of suggestions for further research in the field..

- To perform a comprehensive evaluation of sham-CPAP effects in healthy subjects with and without sham-CPAP. This would give a better understanding of the mechanism and origin of the inflammatory effects. The use of sham-CPAP is currently the placebo intervention of choice in RCTs evaluating the effectiveness of CPAP treatment [562-563].

- A thorough assessment and evaluation is needed of recovery from symptoms and changes in mediators after CPAP therapy in healthy subjects.

- Further work is required to explore the role of humidification. Although we showed that in *in vitro* work, exposure of cell-cultures to high-humidity demonstrated that drying or the absence of humidification alone cannot be solely responsible for the pro-inflammatory changes observed. Also an experimental study in rats failed to demonstrate any beneficial effects of heated humidification on nasal inflammation [522], whereas clinical and experimental studies have reported conflicting results on the benefits of humidification. Therefore, this needs to be investigated carefully in two controlled groups with and without humidification.

- In addition to the acute inflammatory markers we selected for our study which provided us with the answers to the hypothesis and aims of this thesis, it is also important to further investigate other cardiovascular risk biomarkers such as hsCRP and TNF-alpha**.** It is important to mention that the major stimulus to CRP release is IL-6 and this is the reason we selected IL-6 rather

than CRP, together with the fact that it is also produced by epithelial cells (unlike CRP) making direct comparison between our *in vitro* and *in vivo* data possible using this marker

- A nasal steroids trial study, we believe, is needed to investigate whether nasal steroids would help to alleviate the nasal symptoms and thus reduce nasal inflammation, which in turn improves compliance. A study by Strobel showed no effect of nasal steroids on nasal symptoms in CPAP. This was disappointing from a clinical perspective, but the study did not assess nasal inflammatory markers and it remains possible that a different anti-inflammatory compound that is effective in reducing nasal inflammation may reduce troublesome nasal symptoms [529].

- Further studies should be carried out which compare the different forms of CPAP delivery interface with OSA patients, compared to the effect of CPAP on symptoms and inflammation, and which establishes whether the response to therapy differs between subgroups in terms of sleep daytime sleepiness, symptoms and adherence with CPAP usage based on the severity of OSA.

- Further work is required to explore the role of patient education on the compliance by sleep/respiratory specialist at the point of initial CPAP equipment delivery and subsequent necessary follow up care.

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APPENDICES

Appendix 1: Abstracts from this thesis

Appendix 2: Articles published from this thesis

Appendix 3: Binova Reprocessing Instructions

Appendix 4: Nasal Mucociliary Clearance Protocol

Appendix 5: Six-Point Nasal Score

Appendix 6: Diary Card and instruction, OSA Version

Appendix 7: Epworth Sleep Score

Appendix 8: Compliance & Periodicity Questionnaire

APPENDIX 1

Abstracts from this thesis:

Nasal inflammation and sleep quality with continuous positive airway pressure (CPAP) therapy in obstructive sleep apnoea. **Mohammed D. AlAhmari**, Christine Mikelsons, Raymond J. Sapsford, JadwigaA. Wedzicha and John R. Hurst. "Abstract" European Respiratory Society September 2013, Barcelona, Spain.

Effect of nasal CPAP on nasal symptoms in obstructive sleep apnoea (OSA) patients. **Mohammed D. AlAhmari**, JadwigaA. Wedzicha and John R. Hurst. "Abstract" European Respiratory Society September 2013, Barcelona, Spain.

Nasal inflammation and compliance with nasal CPAP therapy in obstructive sleep apnoea (OSA). **Mohammed D. AlAhmari**, Christine Mikelsons, Raymond J. Sapsford, JadwigaA. Wedzicha and John R. Hurst. "Poster Discussion" Vienna, Austria, 1-5, 2012

Dose Response of Continuous Positive Airway Pressure (CPAP) On Inflammatory Responses In Human Bronchial Epithelial Cells *in vitro*. **AlAhmari M,** Sapsford RJ, Wedzicha JA, Hurst. "Abstract" American Thoracic Society ATS 2012 San Franscico, USA.

Dose Response Of Nasal Continuous Positive Airway Pressure (nCPAP) On Nasal Mucociliary Clearance And Upper Airway Symptoms In Healthy Subjects. **AlAhmari M,** Sapsford RJ, Wedzicha JA, Hurst. "Abstract" American Thoracic Society ATS 2012 San Franscico, USA.

Dose Response Of Nasal Continuous Positive Airway Pressure (nCPAP) On Airway Inflammatory Responses In Healthy Subjects. **AlAhmari M,** Sapsford RJ, Wedzicha JA, Hurst. "Abstract" American Thoracic Society ATS 2012 San Franscico, USA.

Nasal and Systemic Inflammation, and Nasal Mucociliary Clearance in Obstructive Sleep Apnoea Patients Using Nasal Continuous Positive Airway Pressure (nCPAP). **AlAhmari M,** Sapsford RJ, Wedzicha JA, Hurst. "Abstract" American Thoracic Society ATS 2012 San Franscico, USA.

Effect Of Nasal Continuous Positive Airway Pressure (nCPAP) On Airway And Systemic Inflammation In Normal Subjects. **AlAhmari M,** Sapsford RJ, Wedzicha JA, Hurst. "Abstract" American Thoracic Society ATS 2011, Denver, USA.

Effect Of Nasal Continuous Positive Airway Pressure (nCPAP) On Nasal Mucociliary Clearance And Upper Airway Symptoms In Healthy Subjects. **AlAhmari M,** Sapsford RJ, Wedzicha JA, Hurst. "Poster Presentation" American Thoracic Society ATS 2011, Denver, USA.

Acoustic Rhinometry: Repeatability of Multiple Measurements in Healthy Volunteers. **AlAhmari M,** Wedzicha JA, Hurst. "Abstract" American Thoracic Society ATS 2010, New Orleans, USA

Repeatability of a Novel Nasal Wash Collection Technique Using a Paediatric Tracheostomy Tub. **AlAhmari M,** Sapsford RJ, Wedzicha JA, Hurst. "Abstract" European Respiratory Society September 2010, Barcelona, Spain.

APPENDIX 2

Articles published from this thesis

1. Mohammed D. AlAhmari, Christine Mikelsons, Raymond J. Sapsford, Jadwiga A. Wedzicha and John R. Hurst. Inflammatory, physiological and clinical consequences of CPAP therapy in patients with obstructive sleep apnoea (OSA) over six month follow-up. Under review.

2. Alahmari MD, Sapsford RJ, Wedzicha JA, Hurst JR. Dose response of CPAP on nasal symptoms, obstruction and inflammation *in vivo* and *in vitro*. **Eur Respir J**. 2012 Mar 9.

3. Al Ahmari MD, Wedzicha JA, Hurst JR. Intersession repeatability of acoustic rhinometry measurements in healthy volunteers. **Clin Exp Otorhinolaryngol.** 2012 Sep;5(3):156-60.

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APPENDIX 3

Binova Reprocessing Instructions

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PRODUCT INFORMATION NOTICE

ALTERNATIVE RE-PROCESSING OF PORTEX® BIVONA® TRACHEOSTOMY TUBES

This Product Information Notice is intended to advise users that they can apply the alternative re-processing instructions below to all Portex® Bivona® Tracheostomy Tubes that are recommended for re-processing. These alternative re-processing instructions have been validated to ensure that there will be no adverse effect to the product and they will be included in future IFUs accompanying the product.

This Product Information Notice only affects the re-processing portion of the IFU. All other aspects of the IFU that accompanies the product must be complied with.

ALTERNATIVE RE-PROCESSING INSTRUCTIONS

WARNINGS:

1. During re-processing, removal of all encrustations and other biological material is vital to removing bacterial contamination from the product. This must be carried out before disinfection is attempted.
2. During re-processing, aggressive scrubbing of the tube or use of hard bristled brushes or sharp wire-mounted swabs may damage the surface of the tube.
3. Sanitization should be undertaken within the two hour period before tube re-insertion. Once removed from the above process there is a risk that the tubes, particularly if left in a warm moist environment, will re-colonize with bacteria.

CAUTIONS:

1. Bivona® Silicone tracheostomy tubes are designed for single patient use and these instructions for re-processing assume that the product will only be re-used on the same patient.
2. These products have been validated up to 121 ^{o}C. Do not expose the product to temperatures in excess of this temperature or product integrity may be compromised. Do not use deep vacuum "flash" or pulse vacuum cycles.
3. Following cleaning, handle the product only by the neck flange or connector to avoid subsequent contamination of the part of the tube that will enter the patient.
4. Do not re-process decannulation cap to prevent compromise of cap.

Hospital or sterilization service re-processing:

Step 1 Cleaning:

a. Following removal from the patient, the device should be placed in a suitable container and marked clearly with the patient's reference/identifier. Tracking of the device should be conducted in accordance with local hospital procedures for the re-processing of **single patient** use devices.
b. Following the instructions for use for an enzymatic solution (preferably using a neutral non-coloured and non-scented cleaner e.g. Ruhof 345APANS Endozyme AW Plus No Scent), remove all biological material from the tracheostomy tube, by soaking and gentle manual cleaning.
c. Inspect for any residual contamination and, if necessary, remove it by repeat soaking in enzymatic solution and then light rubbing with a soft cleaning implement.
d. Inspect the product for any signs of damage. Discard the product if there is any sign of damage.

Step 2: Method 1 Sanitization Instructions:

a. Place the product and its obturator separately in an HTM 2030/ISO 15883-2 compliant washer/disinfector/dryer. Follow the manufacturer's instructions for a thermal disinfection cycle giving a minimum A_0 value of 600 (ref. ISO 15883-2). Ensure that the wash reaches all parts of the product to be cleaned. This will mean flushing through the tube bore and talk attachment tube (if fitted).
b. Using aseptic technique, inspect the dry/disinfected product for any signs of damage. Discard the product if there is any sign of damage.
c. Store product in sealed plastic bag or container, marked with the patient's reference/identifier.

Alternate Step 2: Method 2 Sterilization Instructions:

a. Insert the obturator into the tube and wrap in protective lint-free cloth or place them in a sterilization pouch.
b. Sterilize in a gravity displacement steam autoclave at 121° C (250° F) for 40 minutes.
c. Using aseptic technique, inspect the dry/disinfected product for any signs of damage. Discard the product if there is any sign of damage.

Store product in sealed plastic bag or container, marked with the patient's reference/identifier.

HOSPITAL WARD OR HOME-CARE RE-PROCESSING

Step 1 Cleaning:

a. Following removal, place the tube in a sealed plastic bag or container until ready to clean it. This will avoid drying out and hardening of secretions.
b. Soak the tracheostomy tube and its obturator, separately, in a container of warm water containing a mild soap solution for a period of 60 minutes. Ensure that the wash reaches all parts of the product to be cleaned. This may mean using a syringe to flush through the talk attachment tube (if fitted) and manipulating small tubes to ensure that the liquid does fully fill the bore.
c. Remove any contamination with a lint-free swab. Small tracheostomy tube bores can be cleaned by pulling a small portion of a lint-free swab through the tube.
d. Inspect for any residual contamination and, if necessary, repeat the soak and clean operations.
e. Rinse the tube inside and outside with clean warm water, flushing thoroughly with water and then air dry.
f. Store in a clean sealed plastic bag or container.

Step 2 Sanitization:

a. Remove the tube and the obturator from its container and place in a pan of rapidly boiling clean water.
b. Cover the pan and REMOVE IT FROM THE HEAT. Allow the water to cool to “hand hot” before removing the parts.
c. Handle the obturator by its handle and the tube by its neck flange.

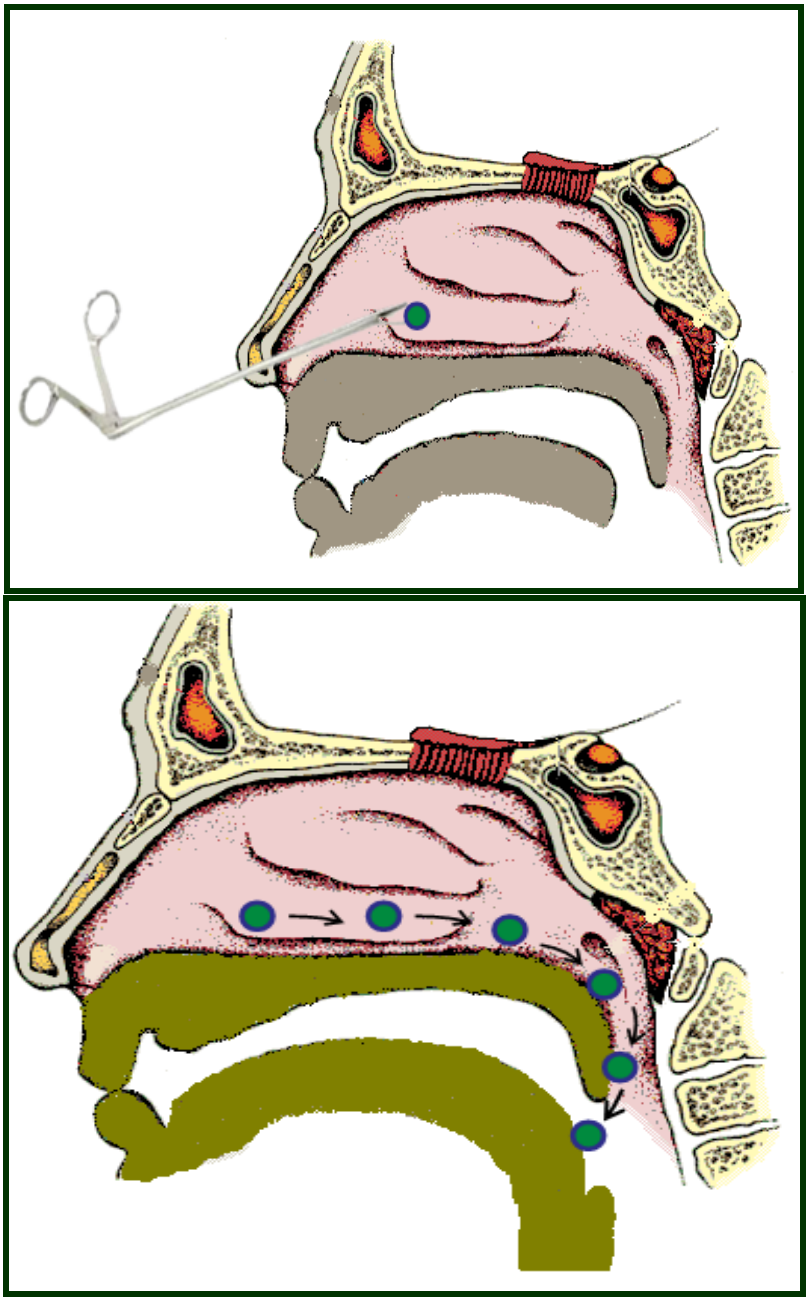
C.S. Turnbull
Senior Development Manager
Smiths Medical International

APPENDIX 4

SACCHARIN TRANIST TIME PROTOCOL*

- Maintaining sterile technique.
- Explain the procedure to the patient.
- Prepare procedure equipments: pure saccharin tables; forceps for particle placement; stopwatch for calculating the time (minutes & seconds); power handle rhinoscope for direct visualisation.
- Maintain a stable environment (temperature 21-24°C) for all tests.
- Have the subject blew his/her nose gently to remove any excess secretions before the test.
- The most patent nostril verified by inspection not to be obstructed will be chosen for the test.
- A standardised measure of saccharin (a 5 mg particle; ~ 0.5 mm in diameter) is gently placed, using a forceps, at the front of the subject's inferior nasal turbinate of a nostril.
- The participants remained seated with their head tipped slightly forward (about 15^{o} forward, to prevent the saccharin from falling to the back of their noses) while breathing normally (not forced), without sneezing, coughing, eating or blowing their nose during the test.
- After successful placement of saccharin particle, at this time, the stopwatch was started. Subjects were requested to stop the stopwatch when they first detected a sweet taste.
- Saccharin clearance time is recoded.

Note: saccharin test will not be collected at a time of acute exacerbation of nasal symptoms or upper airway infection.



APPENDIX 5

SIX-POINT NASAL SCORE

Study group & number:	
Patient number:	
Visit number:	
Date:	

On most days of the week are you troubled by

PROBLEM	Score
Rhinorrhoea (runny nose)	
Post-nasal drip (The excess mucus accumulates in the throat or back of the nose.)	
Sneezing	
Congestion (blocked nose)	
Anosmia (reduced smell)	
Itchy nose	
Total score	

APPENDIX 6

DIARY CARD AND INSTRUCTION, OSA VERSION

NAME								Month:_____ 2009	NEXT APPOINTMENT
Study Number/Group								WORSENING SYMPTOMS? CALL US	/ / 09 . am

Non-invasive respiratory support: OSA study											
DATE	1mon	2 tue	3 wed	4 thu	5 fri	6 sat	7 sun	8 mon	9 tue	10 wed	11 thu
Peak Flow											
CHANGE in Symptoms											
CHANGE in Treatment											
Hours out of the home											
Hours of Machine use	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:
DATE	12 fri	13 sat	14 sun	15 mon	16 tue	17 wed	18 thu	19 fri	20 sat	21sun	22 mon
Peak Flow											
CHANGE in Symptoms											
CHANGE in Treatment											
Hours out of the home											
Hours of Machine use	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:
DATE	23 tue	24 wed	25 thu	26 fri	27sat	28 sun	29 mon	30 tue	31 wed		
Peak Flow											
CHANGE in Symptoms											
CHANGE in Treatment											
Hours out of the home											
Hours of Machine use	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:	D: L:		

Instructions for filling in the DIARY CARDS

EVERY DAY…

1. **After taking morning medications record the best of 3 attempts at the PEAK FLOW blowing test in the box on the sheet.**

2. **Please record any WORSENING of chest and nose symptoms from your usual daily level. The symptoms we are interested in are listed below, just put the appropriate letter in the box on the sheet. Continue recording until the symptom has gone away or got back to the level you consider 'normal'.**

Letter	Symptom
A	Increased BREATHLESSNESS.
B1	Increased SPUTUM COLOUR.
B2	Increased SPUTUM AMOUNT.
D	Increased WHEEZE or CHEST TIGHTNESS.
E1	SORE THROAT.
E2	Increased COUGH.
F	FEVER.
Z	Sneezing
V	Runny nose
C	Congestion (Blocked nose)
M	Mucus from nose running from back of the nose and throat
T	Increased itchy nose

3. **Please record any CHANGE to your usual treatment for as many days as it applies. Again, just put the appropriate letter in the box on the sheet.**

Letter	Treatment
H	I am in Hospital.
I	I am taking more than usual INHALED STEROID (red / brown). HOW MANY PUFFS? eg. I2 for 2 puffs more than usual.
R	I needed to take extra RELIEVER (blue / green / grey / nebuliser). HOW MANY PUFFS? Write, eg 'R3' for 3 puffs, 'R2' for 2 etc
S	I am taking STEROID (Prednisolone) TABLETS. HOW MANY TABLETS? Write, eg 'S6' for 6 tablets, 'S5' for 5 etc
G	I am taking ANTIBIOTIC TABLETS. PLEASE RECORD WHICH (write the name on the diary card).

4. **Please estimate the time that you were out of your own home on the previous day.**

5. **Finally, please estimate the hours for the machine use during night when gets dark (use letter D from dark) and day when still light (use the letter L from light).**

The secretary will answer the phone and we can usually arrange to see you later the same day or the following morning. It is best to phone first-thing in the morning.

APPENDIX 7

EPWORTH SLEEP SCORE

ROYAL FREE HAMPSTEAD
NHS Trust

Study group/number: _____
Today's date: _____
Visit number: _____
Patient number: _____

The following scale is called Epworth sleep score (ESS). This test is an effective instrument used to measure excessive sleepiness or excessive daytime sleepiness. By filling your Epworth Score, you get an index for whether you may be suffering from sleep apnea, based on your quality of life.

It is important that you answer each question as best you can.

Situation	Score Your Daytime Sleepiness in situation			
	0 = would never fall asleep	1 = slight chance of falling asleep	2 = moderate chance of falling asleep	3 = high chance of falling asleep
Sitting and reading				
Watching television				
Sitting inactive in a public place				
As a passenger in a car for an hour without a break				
Lying down to rest in the afternoon when circumstances permit				
Sitting and talking to someone				
Sitting quietly after a lunch without alcohol				
In a car, while stopped for a few minutes in the traffic				
TOTAL SCORE				

APPENDIX 8

COMPLIANCE & PERIODICITY QUESTIONNAIRE

Study group & number:	
Patient number:	
Visit number:	
Date:	

The questionnaire will ask about the *compliance* and *periodicity* of the use of nasal CPAP. It also examines side-effects and symptoms in detail.

Please answer the following questions:

When did you start using your NIV/CPAP machine?

_____

Do you use your NIV/CPAP machine every night?

_____

No. of hours used each night _____

A - Do you currently suffer from any of the following?

Nasal blockage	Yes ☐	No ☐
Dryness in nose	Yes ☐	No ☐
	☐	☐

Nose bleeds	Yes	No
Pain over bridge of nose	Yes ☐	No ☐
Excessive sneezing	Yes ☐	No ☐
Blood stained mucus from nose	Yes ☐	No ☐
Runny nose	Yes ☐	No ☐
Dry mouth/throat in the mornings	Yes ☐	No ☐
Mucus in throat	Yes ☐	No ☐
Dizziness	Yes ☐	No ☐
Sore eyes	Yes ☐	No ☐
Facial pains	Yes ☐	No ☐
Eczema	Yes ☐	No ☐
Hayfever	Yes ☐	No ☐
Asthma	Yes ☐	No ☐
Blocked ears	Yes ☐	No ☐

B - Have you had any of the above problems in the past? (before starting the NIV/CPAP treatment)

What is your experience of the following?

Noise of machine (Noisy)	Yes ☐	No ☐
Comfort of mask (Comfortable)	Yes ☐	No ☐
Air leaking from mask	Yes ☐	No ☐
Air leaking from your mouth	Yes ☐	No ☐
Skin reaction to mask	Yes ☐	No ☐

Breakdown of skin over nose (i.e. Pressure sore)	Yes ☐	No ☐
Have you taken any inhalers/spray for nasal stuffiness?	Yes ☐	No ☐
Do you sleep in a separate room to your spouse/partner?	Yes ☐	No ☐
Do you have sleep disturbance because of the machine?	Yes ☐	No ☐
Treatment benefit from the machine use	Yes ☐	No ☐

Any other experiences/problems?

_____

_____

_____

_____