Physical properties of blood in patients with differing severities of obstructive sleep apnoea and the effect of continuous positive airway pressure treatment

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Physical properties of blood in patients with differing severities of obstructive sleep apnoea and the effect of continuous positive airway pressure treatment

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DECLARATION

This work has not been submitted previously for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

……

Zavier Bent

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ABSTRACT

Background: Obstructive sleep apnoea (OSA) is a common sleep breathing disorder that results from the repetitive collapse of the upper airway. Clinical research has demonstrated complex multifaceted relationships between OSA and adverse cardiovascular events, such as coronary artery disease and stroke. Changes in blood rheology parameters represent a mechanistic link between aberrant coagulation and risk for cardiovascular events.

Objectives: The main objective of the present thesis was to investigate the association between different OSA severity patterns and blood rheology-related parameters. The effects of acute OSA treatment with continuous positive airway pressure (CPAP) on blood rheology and rheology-related parameters were evaluated.

Methods: The study involved a prospective evaluation of diagnosed OSA patients who were referred to perform a polysomnograph in a sleep laboratory of a tertiary teaching hospital. The design consisted of two research studies. First, the association between rheological and OSA severity was studied in three patient groups (mild, moderate, and severe) as well as polysomnographic variables (including apnoea-hypopnoea index (AHI), oxygen desaturation index (ODI), and arousal index (AI)). Second, the effect of acute CPAP therapy on rheological parameters was examined in the different OSA patient groups. Rheological parameters included whole blood viscosity (WBV), red blood cell (RBC) deformability, and RBC aggregation. Measurements of aggregation and WBV were performed at native and standardised (i.e., 0.4 L/L) haematocrit. Both the native and standardised viscosities were measured at the following shear rates: 75, 150, 300, 750, and 1500 s^{-1}. RBC aggregation measured by the relevant magnitude parameters (after 10 s of stasis (M_0), after 10 s at 3 sec^{-1} (M_1), and after 120 s at stasis (AI_{120})) as well as aggregation half time (T½). Rheology-related parameters were plasma fibrinogen concentration and routine haematology measurements. CPAP was performed for one night.

Results: Patients (n=31) volunteered to participate in the study. Mean WBV was significantly higher in severe OSA patients (4.15 ± 0.58 mPa·s) than that in the moderate OSA patients at the highest shear rate (3.13 ± 0.42 mPa·s, $p<0.05$). There was no correlation between WBV at any shear rate and AHI, ODI, or AI. For both native and standardised

haematocrit, a significant shear thinning effect was observed, whereby WBV decreased with increasing shear rates ($p<0.05$). There was no significant difference in RBC aggregation parameters between the different severity groups of OSA ($p > 0.05$). RBC aggregation at M_0 was positively correlated with AHI ($p = 0.036$) and ODI ($p = 0.026$), whereas T½ was negatively correlated with AHI ($p=0.032$) and ODI ($p=0.018$). RBC deformability increased consistently in a linear pattern with increased shear stress among all OSA severity groups. Plasma fibrinogen concentrations were significantly elevated in severe OSA patients (3.35±0.62 g/L) when compared to mild (2.97±0.90 g/L, $p < 0.05$) and moderate patients (2.89±0.92 g/L, $p < 0.05$). Insulin and glycated haemoglobin levels differed significantly among OSA severity groups ($p<0.05$). Platelet count was positively correlated with ODI ($p=0.023$). When compared to baseline values, acute CPAP therapy caused a significant reduction of platelet count (259.9±46.9 vs 238.5±55.7 10^9/L, respectively, $p < 0.05$), haematocrit (0.44±0.47 vs 0.41±0.56 %, respectively, $P<0.05$), and WBV at the moderate shear rate of 300 sec^{-1} in both native (5.0±0.9 vs 3.4±0.6 mPa·s, respectively, $p < 0.05$) and standardised (4.8 ± 0.41 vs 3.8 ± 0.78 mPa·s, respectively, $p < 0.05$) haematocrit samples. There was no significant effect of acute CPAP therapy on RBC aggregation, RBC deformability, and plasma fibrinogen concentrations.

Conclusion: Patients with severe OSA had marked increases in high WBV and increased RBC aggregation. Therefore, blood rheology parameters may be related to the heightened cardiovascular risk associated with OSA. These blood rheology markers could therefore provide a meaningful insight into early disease processes. A single night of CPAP therapy improved blood viscosity and thus may be effective for reducing the risk of cardiovascular events in OSA patients. Future blood rheology-based studies should employ larger cohorts along with the recruitment of a control group. Long-term CPAP therapy should be prospectively evaluated for its impact on blood rheology in OSA patients.

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LIST OF ABBREVIATIONS

AHI	Apnoea hypopnoea index
ANOVA	Analysis of variance
APAP	Auto-titrating positive airway pressure
BMI	Body mass index
EDS	Excessive daytime sleepiness
ESS	Epworth Sleepiness Scale
HDL	High-density lipoproteins
LDL	Low-density lipoproteins
LORCA	Laser-assisted optical rotational cell analyser
MMA	Mandible, namely maxillomandibular advancement
MPV	Mean platelet volume
NREM	Non-rapid eye movement
ODI	Oxygen desaturation index
PAP	Positive airway pressure
PLMD	Periodic limb movement disorder
RDI	Respiratory disturbance index
RDW	Red cell distribution width
REM	Rapid eye movement
ROS	Reactive oxygen species
SDB	Sleep-disordered breathing
SST	Serum separation tubes
AASM	American Academy of Sleep Medicine
CPAP	Continuous positive airway pressure
ECG	Electrocardiogram
EEG	Electroencephalogram
OSA	Obstructive sleep aponea
PSG	Polysomnogram
RBC	Red blood cell

1. INTRODUCTION

Obstructive sleep apnoea (OSA) is a condition caused by recurrent and frequent interruptions of breathing during sleep due to upper airway collapse; it is thus considered a severe form of "sleep-disordered breathing". Affecting at least 5% of the adult population [1], OSA is a significant health issue considering that it increases the risk of developing conditions such as coronary heart disease, atrial and ventricular arrhythmias, and hypertension [2, 3]. It is plausible that some of these complications may be due to impaired oxygen delivery to organs, given that OSA is associated with impairments in blood rheology, such as elevated morning plasma fibrinogen concentration and increased plasma viscosity. These observations are reported to occur independently of common risk factors for cardiovascular disease [4]. Although there are several treatment options for OSA, efficacy is limited due to patients typically showing poor response and/or compliance [5].

The best diagnostic test for OSA is a polysomnography which is performed in a sleep laboratory. This test entails simultaneous recordings of several signals during sleep, such as the electroencephalogram, electrooculogram, and electromyogram. Oxyhaemoglobin saturation and oronasal airflow are also included in the monitoring [6].

Haemorheology includes the analysis of aggregation and deformability of erythrocytes and are determinants of blood viscosity [4]. For instance, an increase in plasma viscosity may increase the resistance for the free flow of blood and can thus lead to impaired tissue perfusion. Aggregation of erythrocytes also has a significant role in the circulation of blood and other useful materials in the body system, particularly at low shear rates [7].

The consequence of blood hyperviscosity is impaired oxygen supply to distal tissues that may induce hypoxia and ischaemic tissue damage. This may lead to a vicious cycle that further promotes hyperviscosity. CPAP is the first line of treatment in patients with moderate to severe obstructive sleep apnoea; however, evidence is limited for the effects of CPAP improving blood rheology and other CVD risk factors in those with severe OSA [8]. Some studies have demonstrated that CPAP may be effective in reversing haemorheological impairment; for example, Gopalakrishnan & Tak (2011) reported that there is significant impact on blood rheology in patients with OSA that are treated post CPAP. Mainly due to the lack of subjects in this group being referred and ultimately treated post CPAP [9]. The

long-term implementation of CPAP in the treatment of OSA has been observed to accompany risk reduction when compared with other methods such as mandibular advancement splints and ENT (ear nose thorat) surgery. The risks associated with cardiovascular disease and metabolic syndrome can be reduced using CPAP, and thus CPAP has been regarded as the gold standard method of OSA management [10].

2. LITERATURE REVIEW

2.1 History

Although OSA is thought to be have become more prevalent over the past few decades due to increased prevalence of its associated risk factor, obesity [11], symptoms of OSA were formally identified since the nineteenth century. The first description of sleep-disordered breathing in the medical literature was in 1818 by John Cheyne when he observed a patient with irregular breathing and paralysis who was dying from heart disease [12]. The most notable description of sleep-disordered breathing was in the Pickwick Papers, by Charles Dickens [13], through the fat boy character "Joe" who falls asleep against his will. Subsequently, the physician William Osler used the term "Pickwickian syndrome" to describe obese and sleepy individuals [14]. The observations of George Catlin were also considered when he noted that the prevalence of mouth breathing was higher among European-Americans than American Indians and those patients snored while asleep with the feeling of tiredness and tendency to sleep during the day [15].

It was not until the middle of the 20th century that the actual aspects of sleep physiology were correctly perceived as a passive disconnection of the brain from other body parts [16]. The first recordings of a Pickwickian patient were reported by Gerardy, Herberg [17] in an obese patient suffering from reduced work capacity and headache on awakening. An electroencephalogram was performed and the results revealed cessation of breathing during sleep associated with bradycardia and dramatic heart rate acceleration upon renewal of breathing. Later studies led to further understanding of disturbed oxygen desaturation during apnoea events [18] and the association between daytime sleepiness and recurrent interrupted sleep [19]. During the first major international conference of sleep specialists in California, Elliot Weitzman [20] showed that the potential cause of upper airway blockade is the collapse of pharyngeal walls and the movement of the tongue posteriorly.

The most impactful contemporary studies concerned with OSA were the Wisconsin Sleep Cohort Study [21] and the Sleep Heart Health Study [22]. Overall, according to these studies, obesity was found to have a major effect on the evolution of OSA. The incidence of OSA in men was 11.1% and in women was 4.9%. OSA is a long-standing disease which imposes a considerable economic burden in all societies, affecting the developed and developing as well. Therefore, there has been an increasing interest regarding OSA and its cardiometabolic sequelae.

2.2 Pathophysiology

The complicated task of the upper airway includes maintaining adequate ventilation with the simultaneous facilitation of phonation and speech [19]. Airflow enters from the environment into the lungs for subsequent gas exchange. A patent airway, therefore, is critical for bulk flow of oxygen-containing gas. Patency of the upper airways requires activation (i.e., tone) of local muscles, given that no rigid supports (e.g., bone) are present in this region. Additionally, the collapsible soft tissues in this region may impede airflow.

The tongue plays a critical role in supporting the ventilation. Its relationship with the skull base contributes to the stability of the airway independent of muscular tone. Upper airway muscles, including the genioglossus, the tensor palatine, and the sternohyoid have an essential function to maintain patent airways through dilatation and stiffness the extrathoracic airways [20]. Multiple mechanisms have been involved to maintain upper airway stability, such as the anatomical structure of the tongue and pharyngeal muscles, neuromuscular tone, level of consciousness, mechanisms of ventilator control, body position, and craniofacial structures.

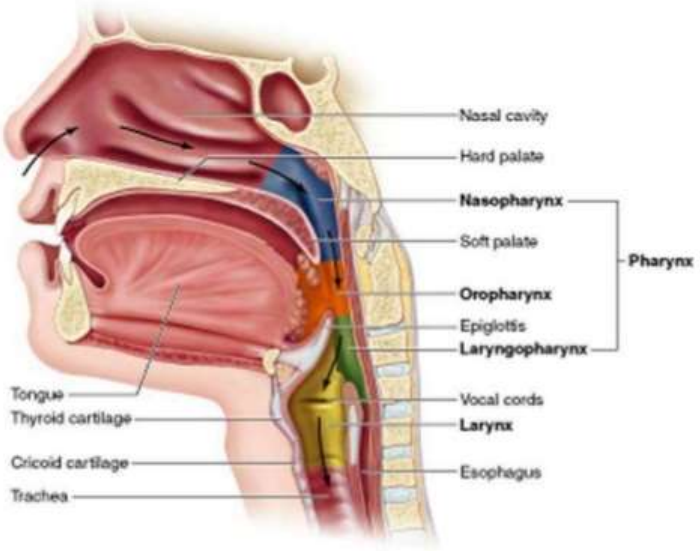


Figure 1: Upper Airway Anatomy. Adapted from Cleary and Clarke [23]

Apnoeic airways have thicker lateral walls as well as smaller minimal airway area as demonstrated by MRI in the cross-sectional area of the upper airway [24, 25]. There is a threshold pressure needed for maintaining a patent airway, and this is called the "pharyngeal closing pressure" (Pcrit). Pcrit is found to be correlated with the severity of OSA [26].

During wakefulness, the patency of the airway could be maintained by a higher activity of airway dilator muscles (mainly the genioglossus and tensor palatine muscles). With sleep onset, a small and consistent decrement of their activity takes place in normal individuals as demonstrated by Mezzanotte and colleagues [27] using EMG. On the other hand, they concluded that there were significantly greater decrements in the activity of both muscles in OSA patients, a matter which potentiates further apnoeas. There is a possible interaction between the decreased ability of such muscles to maintain a patent airway during sleep and the pharyngeal anatomy in OSA patients.

Changes in lung volume are possibly integrated in modulating upper way patency in patients with OSA [28]. Indeed, the reduction of lung volume causes displacement of the diaphragm and thorax toward the head, leading to loss of traction on the upper airway and subsequent increased airway collapse [29, 30].

2.3 Causes

OSA is a multifactorial condition. There are multiple environmental, genetic, and developmental factors related to the disease. Several genetic risk factors are emphasized for OSA including the obesity, craniofacial dysmorphism, and the central control of ventilation [31]. The risk of OSA appears to be increased to 10-14-fold with obesity, considering the occurrence of marked effects in middle-aged subjects [32]. It is noteworthy that the susceptibility of OSA may be augmented through fat deposition in the upper airway tissues leading to a reduction in the nasopharyngeal calibre. Surprisingly, obesity-related genes could also exert their impact on OSA patients through multiple aspects. The same gene or set of genes may influence ponderosity, ventilator control and/or craniofacial morphology. Genetic structural abnormalities in craniofacial morphology can cause reduction in the size of upper airway [33]. For example, reduction of the anteroposterior dimension of the cranial base, reduction of the size of the posterior and superior airway spaces, macroglossia [34], and elongation of the soft palate [35] may all contribute in developing the symptoms of OSA. The last possible genetic risk factor of OSA is the neuromuscular control of both chest wall and upper airway that may influence the ventilation during sleep [31].

Deposition of adipose tissue may be age-related. Studies have shown that approximately 70% of older men and 56% of older women aged between 65 and 99 years

have suffered from OSA [36]. On the other hand, the prevalence of OSA among postmenopausal women was found to be higher than those premenopausal [37].

The role of gender difference in OSA has been extensively studied. Women are generally less likely to develop OSA symptoms due to their different anatomical and functional properties of the airway as well as their existing differences in breathing control. For example, men exhibit a greater upper airway resistance from wakefulness to sleep than females [38]. Pharyngeal collapsibility is increased in men than women due to their increased neck obesity, increased soft tissue mass, and enlarged tonsils [39]. Further, men show greater reductions in pharyngeal calibres with a significant difference in the retrusion of the mandible, revealing a remarkable difference in the dynamic and geometrical characteristics of the upper airways between both genders [40]. As such, women tend to have less classical symptoms of OSA during the daytime, which means that they usually report fatigue and lack of energy rather than daytime sleepiness [41].

Generally, there are two hypothesised mechanisms contributing to the maintenance of pharyngeal airway in OSA. First, the loss of wakefulness stimulation and reduced activity of the pharyngeal dilator muscles represent the main pillars of pharyngeal narrowing [42]. Second, it has been suggested that there are mechanical pharyngeal factors that affect the mechanism of airway collapse. Since there was no difference between males and females in the response to negative intraluminal pressure following activation of pharyngeal dilator muscles, it has been suggested that the second mechanical hypothesis, rather than the neural hypothesis, constitutes the most suitable mechanism of increased OSA prevalence in men [40].

Cigarette smoking is thought to be an independent modifiable risk factor for OSA [43, 44]. The possible reason is associated inflammation of upper airway which results in snoring. In addition, alcohol ingestion can lead to exacerbation of OSA by reducing the activity of genioglossus muscle and subsequently upper airway collapse [45]. Nasal congestion and respiratory allergies can be considered as possible contributing factors to OSA [46].

2.4 Consequences

Excessive daytime sleepiness (EDS) is a common complaint of patients with OSA. EDS results from sleep fragmentation during night due to repeated arousal from the associated hypopnoeas and apnoeas. Even the patients with mild OSA symptoms are unable to effectively carry out their daily activities due to inability to perform them tirelessly [47, 48]. People suffering from OSA are more likely to have motor vehicle accidents up to seven times more than patients without OSA [49]. Moreover, the risk of having multiple crashes in patients with OSA is increased compared to normal participants [50].

Cognitive function was demonstrated to be declined in OSA patients with some unique patterns of cognitive deficits [51]. The effect of OSA on attention or vigilance was strongly affected by sleep fragmentation rather than hypoxaemia [52]. Nonetheless, it remains unclear whether the most significant factor leading to cognitive impairment is the oxygen saturation or sleep fragmentation. A unique syndrome has been identified in association with OSA, where it has been characterized by impairment in attention memory and executive functioning [53]. Additionally, Aloia and colleagues [52] had concluded that the language and global cognitive functioning were relatively impaired with OSA.

Accumulating evidence supports that OSA is linked to metabolic disorders, including IGT and diabetes. Insulin resistance, and impaired glycaemic control, for example, have been associated with the frequency of apnoeic events and severe oxygen desaturation during REM sleep [54, 55]. Longitudinal cohort studies revealed that patients with OSA with moderate to severe symptoms have increased risks of incident diabetes [56, 57]. The nocturnal hypoxia of OSA was found to be linked to impaired glucose tolerance independent of obesity [58, 59]. About 87% of obese patients with type 2 diabetes showed the clinical symptoms of OSA as per study of Foster and co-workers [60]. It was also observed that the most potent parameter associated with impaired glucose tolerance in OSA patients is the time spent at <90% oxygen saturation [61]. This may provide a strong indication of a robust link between OSA and type 2 diabetes, considering obesity as a common risk factor. Nonetheless, no major improvement occurred in glycaemic control in patients during OSA treatment [62]. From another diabetes-related perspective, there is a link between OSA severity and glycosylated haemoglobin (HbA1C) levels in patients with and without diabetes. The frequencies of abnormal HbA1C levels were significantly different between subjects having sleep-disordered breathing and patients without SDB [63]. Moreover, the

levels of HbA1C and fasting glucose were significantly correlated with OSA severity among non-diabetic men [64]. In patients with type 2 diabetes, Aronsohn and co-authors [65] have showed that poor glucose control and HbA1C levels are associated with increased OSA severity. It is imperative to observe that two major parameters of hypoxaemia have been associated with HbA1C levels, namely the percent time spent with SpO2<90% and the average SpO2 [66].

2.5 Cardiovascular Consequences of OSA

Accumulating evidence over the past decades indicates an association between OSA and cardiovascular morbidity. The repetitive episodes of apnoea and hypopnoeas of OSA are accompanied by hypoxia, sleep arousal and hemodynamic changes [67-69]. In addition, sympathetic activation takes place possibly due to the arterial desaturation and hypercapnia [70] and subsequently leads to vasoconstriction. This would eventually predispose to increases in both the blood pressure and heart rate [68, 71].

In animal experiments, there is a direct correlation between OSA and hypertension as shown in the causal effect of OSA on the acute transient elevation of nocturnal blood pressure in dogs [72]. Normalization trials of blood pressure in rates led to a significant reduction of apnoea events in rat [73]. Multiple studies conducted on human showed a reduction of systemic hypertension in OSA patients with treatment [21, 74, 75]. The effect of treatment was more evident among patients with resistant hypertension and those having uncontrolled blood pressure.

The involvement of OSA in the aetiology of coronary artery disease is multifaceted. The associated enhancement of oxidative stress in OSA patients results in oxidative modification of lipoproteins and other molecules **[76]** which perpetuate the damage of coronary endothelium and accelerate cholesterol accumulation in the atherosclerotic plaque [77]. Increased inflammatory substances and adhesion molecules in OSA due to hypoxaemia may also contribute to the facilitation of the atherosclerotic process [78, 79]. The OSA-accompanied airway obstruction can result in increasing the intrathoracic negative pressure and thus increasing the transmural gradients across the ventricles. Myocardium is then put under stress with high oxygen demand during apnoeic events. In some patients with preexisting coronary artery disease, the increased myocardial oxygen demand together with the diminished supply would lead to myocardial ischaemia [9].

Several complicated mechanisms have been involved in the metabolic worsening in OSA patients. Chronic intermittent hypoxia may be a causative factor for neurohumoral changes, sympathetic activation, disruption of glucose homoeostasis, and oxidative stress – all would finally cause significant deteriorative impacts on the metabolic functions [80, 81]. Furthermore, OSA may be a risk factor for dyslipidaemia. Newman, Nieto [82] found an inverse relationship between the AHI in OSA patients and the levels of HDL-cholesterol and triglyceride. Karkinski, Georgievski [83] reported significant increases in the levels of triglycerides and total cholesterol as well as significant reduction in HDL levels in OSA subjects with BMI< 30 versus OSA negative patients with BMI ≤ 30. Based on this finding, OSA may have a significant role in lipid metabolism deterioration in non-obese patients [83]. For confirmation, all these findings were consistent with other older studies [84, 85]. In fact, a higher prevalence of dyslipidaemia in OSA patients was reported in several studies of sleep cohort clinics when compared to patients without OSA [86-88]. There has been an association between the number of apolipoprotein E4 alleles (result in higher levels of LDL-cholesterol) where the OSA severity was related to increased LDL-cholesterol levels [89]. In contrast to the previously mentioned findings, Lam and co-workers have not found an association between OSA and HDL or triglycerides levels in their middle-aged Chinese cohort [90].

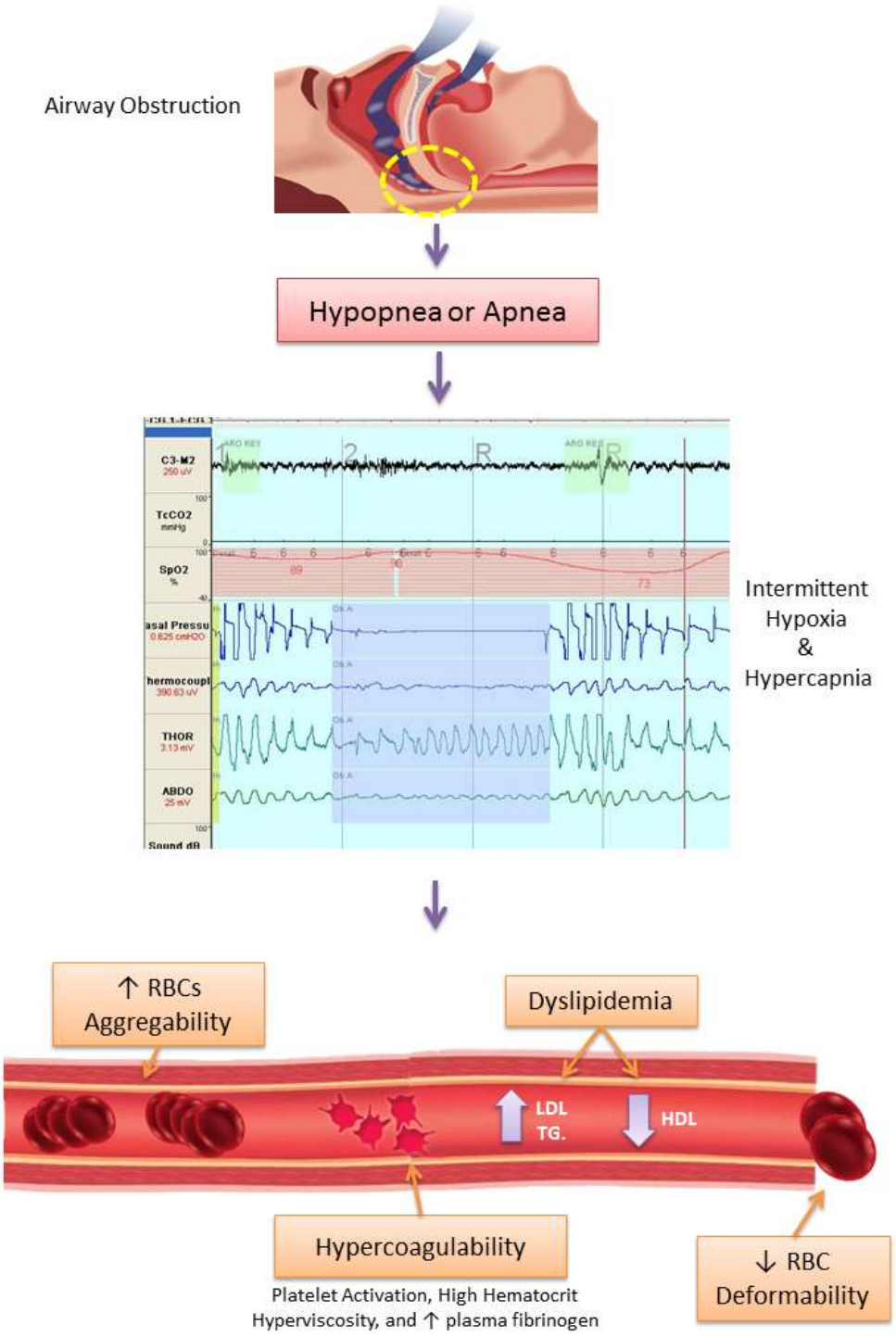


Figure 2: (Original diagram)Schematic representation of the hypoxic effects on blood rheology during a polysomnogram

2.6 OSA and Blood Rheology

Investigators have posited that coagulation disorders, endothelial damage, an increase in inflammatory mediators, and platelet activation are associated with OSA [91-93]. OSA patients usually have significantly higher levels of endothelin which contribute to impaired blood pressure regulation [92, 94]. Moreover, nitric oxide, which is a potent endothelium-derived relaxing substance, was lowered in OSA patients than healthy controls as stated by Ip and colleagues [94]. It is noteworthy that the OSA-related hypoxia would activate the redox-sensitive transcription factors which elicit the inflammatory pathways leading to promoting the activation of platelets, endothelial cells, and leukocytes [92]. Subsequently, those cells begin to express proinflammatory cytokines and adhesion molecules leading to endothelial injury and dysfunction.

Another possible pathophysiological mechanism has been observed in regards to the relationship between OSA and cardiovascular disease. Referring to the inflammatory factors once again, there would be a subsequent alteration in the micromilieu of the blood resulting in increased blood coagulability [95, 96]. Several factors might be involved in hypercoagulability induction in OSA patients. Increased haematocrit levels had been demonstrated in OSA patients and were correlated positively with disease severity [97, 98]. However, on the other hand, only Reinhart et al [99] found no difference in haematocrit values between patients treated with CPAP, untreated patients, and controls. In spite of the significant elevations in haematocrit among OSA patients, these levels didn't reach the threshold of polycythaemia [97]. Despite the potential clot formation as a result of elevated haematocrit values, to our knowledge, no studies have been established in order to assess the related clotting cardiovascular outcomes in OSA patients.

Hyperviscosity may be involved in the pathophysiological mechanism of hypercoagulability [87, 100]. Increased blood viscosity in OSA patients may contribute to slowing of blood flow, clot formation, blood stasis, and vascular occlusion. Untreated OSA patients usually have higher morning plasma viscosity and this is associated with the decreased oxygen saturation [101]. Increased levels of some clotting factors, such as FXlla, FVlla and thromhin-antithromhin (TAT), have been demonstrated in patients with OSA [87]. Since the increased levels of both of FXlla and FVlla factors have been associated with increased mortality due to cardiovascular events [102, 103], it is imperative to conclude the potential mechanism by which OSA may predispose to their risk.

Fibrinogen, a coagulation mediator, is considered an independent risk factor for cardiovascular disease [104, 105]. Increased fibrinogen concentration poses a risk for stroke, heart attack and mortality in stroke survivors [105]. There are inconsistent results regarding the levels of fibrinogen in OSA patients. Some studies have found a significant increase in fibrinogen levels [100, 106, 107] and, conversely, other trials [87, 108] revealed detection of normal fibrinogen levels in patients with OSA. Sleep fragmentation and hypoxaemia during apnoeic events are the major attributable factors of increased fibrinogen in OSA patients [109]. The relationship between OSA hypoxemic events and fibrinogen levels needs to be further studied.

Red cell distribution width (RDW), another RBC property, can be considered as an independent predictor for cardiovascular diseases. It has been associated with chronic heart failure and coronary artery disease [110, 111]. In OSA patients, it can be elevated due to either ineffective erythropoiesis as a result of chronic inflammation [112] or enhanced erythropoiesis caused by increased erythropoietin production as described in patients with coronary artery disease [113]. Chronic inflammatory processes and subsequent endothelial dysfunction possibly play a vital role in the progress of cardiovascular complications in OSA patients. Intermittent hypoxia in OSA leads to activation of pro-inflammatory transcription factors and, subsequently, activation of several inflammatory cells such as monocytes and lymphocytes [114]. There has been a positive association between RDW and AHI and more deeply the RDW is related to the severity of OSA that is dependent on intermittent hypoxia and inflammation despite its independence of anaemia [115].

RBCs, having a diameter of 8 μm, should be highly deformable to pass through blood capillaries with a diameter 2–3 μm. Several factors may be responsible to maintaining such deformability, including cellular geometry, membrane composition, and cytoplasmic viscosity [116]. The microcirculatory profile of OSA patients is altered due to the disease and this has been associated with decreased RBCs deformability [117]. The possible reason for this finding may be related to the osmotic fragility and the cellular metabolism of RBCs. Moreover, the OSA-associated intermittent hypoxia can increase the levels of reactive oxygen species (ROS) as demonstrated in rats [118]. A robust link between loss of deformability and ROS could be clearly observed when an in vitro experiment showed that human RBCs lose their deformability after exposure to H_2O_2 [119]. In addition, RBC deformability was prevented in rats treated with the ROS scavenger α-tocopherol [120]. On

the other hand, erythrocyte deformability was not significantly affected in OSA patients [101, 121] although the filtration period of RBCs has been reversibly affected after exposure to high altitude (relatively resembles intermittent hypoxia). Both chronic intermittent hypoxia and continuous intermittent hypoxia have no effects on the deformability and the rigidity index of RBCs as reported by two recent studies [122, 123]. Another possible mechanism affecting erythrocyte deformability in OSA patients is the reduction in nitric oxide concentration. In fact, the intentional inhibition of nitric oxide by specific or non-specific inhibitors results in significant impairment of RBC mechanical properties [124].

Another important factor contributing to the comorbid cardiovascular disease in OSA patients is red blood cell aggregation. It has been demonstrated that RBC aggregation/adhesion has been increased in OSA patients when compared to normal subjects [125]. The fact that 20% of patients with polycythaemia of unknown cause have expressed nocturnal disturbed breathing or apnoea [126] may provide a probability of higher erythrocytic mass in OSA patients, a matter which requires further investigations. Increased morning levels of 2,3 diphosphoglycerate in OSA patients [127, 128] will potentially provide an evidence of possible alterations in the red blood cells. Once again, chronic inflammation would potentially be involved in the sequential mechanism of increased RBC aggregation [125]. Additionally, both the oxidative stress and the generated inflammatory markers in patients having metabolic syndrome can provide an explanation for the altered haemorheological state, particularly erythrocytic adhesiveness. Indeed, red blood cell aggregation can be a useful biomarker for evaluation of the severity and progression of metabolic syndrome [129] in OSA patients.

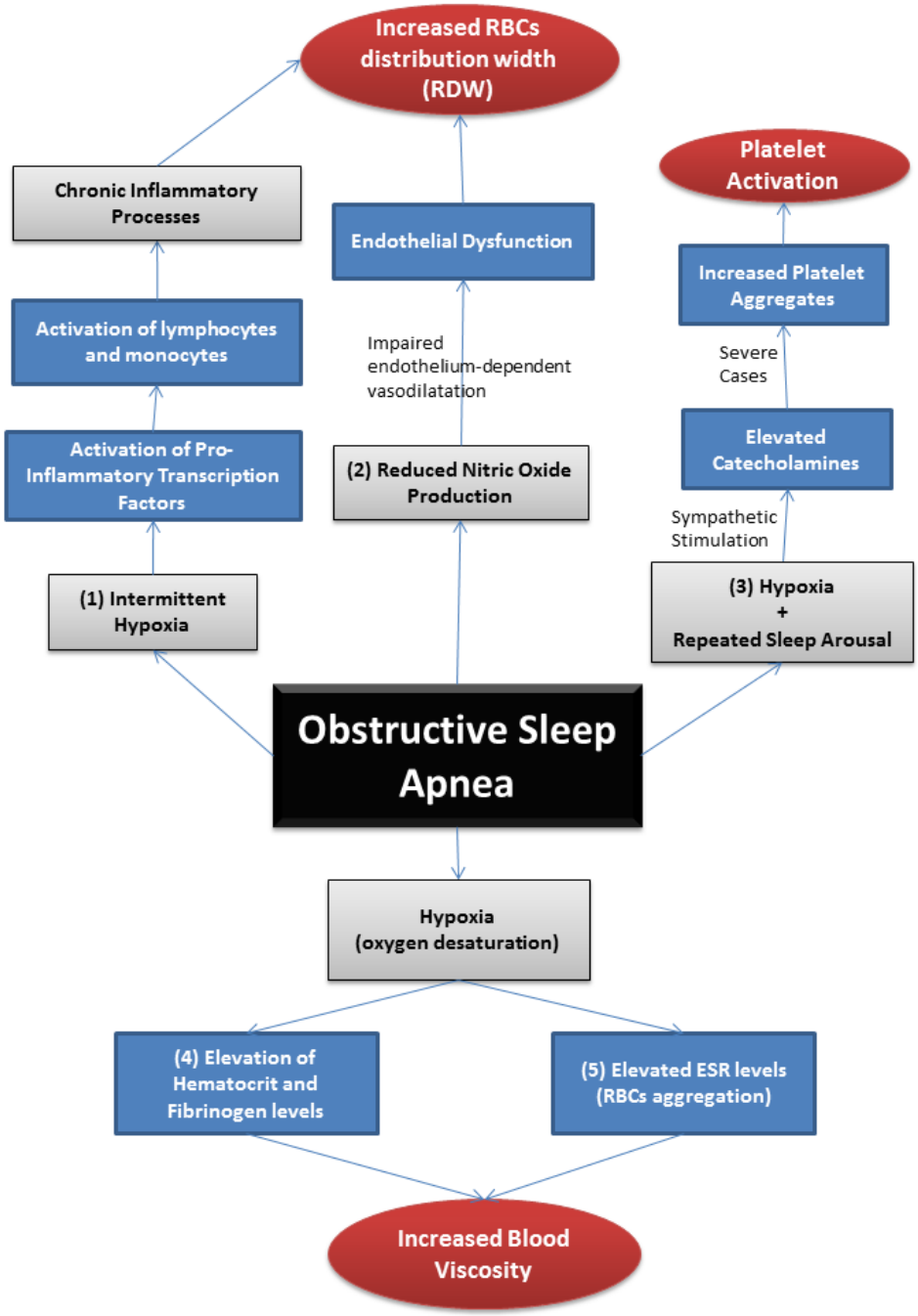


Figure 3: A diagram showing the potential haemorheological changes in OSA patients

(1) [115]

(2) [130]

(3) [131]

(4) [132]

(5) [133]

2.7 Treatment

2.7.1 Conservative

Since 30% of OSA patients experience their hypoapneic/Apnoeic events due to sleeping in supine position [134], they are generally advised to avoid such position to reduce the effect of gravity on tongue and soft palate position [135]. Accordingly, several positional therapy techniques are available, including simple 'tennis ball technique', where a tennis ball is applied to the back to keep the patient away from supine position, supine alarm devices and a number of positional pillows [134, 136]. However, treating OSA by using corrective positional techniques can be difficult to be monitored at home and its clinical outcomes cannot be assessed. Therefore, the combination of positional therapy with other treatment such as surgery and oral devices is encouraged.

Other conservative therapeutic approaches include minimising alcohol intake and weight reduction. Although there have been no systematic reviews referring to the feasibility of reducing alcohol intake for OSA patients, data from studies had provided an assessment of the effect of additional alcohol intake on sleep-disordered breathing and suggested that the advice is appropriate [137]. Weight loss through exercise and diet can be also helpful for OSA patients [60]. Since some epidemiological data [138] have indicated that the treatment with CPAP may cause an increase in body weight, the need for diet and exercise would then be preferred. Weight loss was found to influence OSA severity where the longitudinal data of Wisconsin Sleep Cohort Study showed that 10% weight loss was accompanied with 26% AHI fall and, on the other hand, 10% weight gain led to 32% increase in AHI [139].

2.7.2 Non-invasive

Continuous positive airway pressure (CPAP)

CPAP is considered the gold standard treatment of choice for adult OSA patients [140]. It involves maintaining a positive transmural pressure in the pharynx by using an oronasal or a complete face mask connected to a flow generator. The transmitted positive airway pressure overcomes the tendency of the airway to collapse at sleep onset. Overall, CPAP can act as a pneumatic splint. CPAP treatment can also increase end-expiratory lung volume leading to stabilisation of airway during the stage of caudal traction [141].

Despite the simplicity of CPAP therapeutic concept, it is imperative for patients to adhere to treatment through wearing the mask each night. In addition, several aspects should be considered for CPAP-adhering patients, such as patient's support and education regarding understanding the treatment benefits and focusing on the importance of adherence [142], the use of nasal decongestants and heated humidification for patients with nasal difficulties, and the use of hypnotherapy in some patients who develop insomnia or frequent waking during the treatment [143].

2.7.3 CPAP Alternatives

When CPAP treatment is unsuccessful, several alternative treatment approaches may be used. Auto-titrating positive airway pressure (APAP) can be useful to maintain stable ventilation through applying varied pressure levels according to body posture or sleep stage. Patients may get benefit from lowering the applied pressure when appropriate but, however, several trials have revealed no improvement in APAP-adhering patients compared with those adhering to CPAP therapy [144]. Conversely, some data have shown worse outcomes where the patients experienced changes in intrathoracic pressure and subsequent arousal from sleep and instability in their hemodynamic system [145].

Although some patients who have expiratory pressure discomfort may get benefit from applying bi-level positive airway pressure, a randomized clinical trial conducted by Reeves-Hoche and colleagues [146] have concluded that the benefit was not significant as much as CPAP treatment. Other strategies may be helpful for relief of expiratory pressure, such as C-Flex or EPR "expiratory pressure relief", but similar to the former approach, a study had suggested that there had been no major improvement using these therapies in comparison to the standard CPAP [147].

Oral Appliances

For patients with mild to moderate OSA, several oral devices may be preferable to CPAP treatment [148, 149]. Mandibular advancement splints are the most commonly used and safest oral appliances working by retaining the mandible in a forward position leading to widening of the upper airway, especially in its lateral dimensions, as well as improving the function of upper airway dilator muscles [150].

2.7.4 Surgery

Since the airway obstruction can take place at variable sites, several surgical approaches can be available whether being minimally invasive (local anaesthesia) or more invasive procedures. They are performed on either the nasal, oropharyngeal tract, lingual or craniofacial levels. Some OSA patients may have excessive tissue in the oropharynx and this could be treated by performing conventional or laser-assisted uvulo-palatopharyngoplasty (UPPP). In such procedures, surgeons perform a resection of uvula as well as part of the excessive tissue [151]. Tonsillectomy is usually accompanied by performing UPPP where the overall success rate is raised from 30% (if UPPP alone) to 60% (if UPPP with tonsillectomy) [152]. The efficacy of UPPP procedures is generally decreasing over years after the operation and long-term complications, including velopharyngeal insufficiency, swallowing difficulty, and dry throat may develop. Therefore, all patients should be informed about the possible complications particularly the velopharyngeal insufficiency (occurs in 30% of patients) as it may interfere with the patient response to CPAP if applied in the future [153].

Another surgical approach for treating OSA is by stiffening of the soft palate. This can be obtained by either radiofrequency ablation of the palate (RFA) [154] or by insertion of pillar palatal implants [155]. In the study of Carroll and co-workers, of 130 patients underwent RFA, 42% of patients showed complete snoring resolution and the remainders had residual improved snoring. Generally, both procedures have significant effects on reducing snoring and apnoeas but there has been a lack of evidence regarding improving OSA.

Another site of obstruction causing OSA is the retroglossal region. Tongue suspension can result in improving OSA symptoms, AHI, and quality of life as per the study of Handler and colleagues [156]. Indeed, the overall success rate of such procedure was 36.6% and it should be limited to be performed in a multilevel approach for the selected OSA patients.

Electrical stimulation of upper airway dilator muscles is useful for alleviating OSA symptoms. In animal models, hypoglossal nerve stimulation led to an increase in the size of pharyngeal airway by ventral displacement of lateral and ventral pharyngeal walls [157]. Oliven et al. [158] have conducted their study on human cases by which the OSA patients

underwent electrical stimulation of the genioglossus and they showed a reduction in both Pcrit and airflow limitation.

Tracheostomy is the most effective surgical treatment for OSA patients and should be performed exclusively for severe cases. Nonetheless, Tracheostomy can substantially reduce patients' life quality. Finally, there are more aggressive surgeries which can be considered more effective although they are avoidable by the patients. These surgeries usually obviate the difficulties of adherence to the other approaches. Osteotomy of maxilla and mandible, namely maxillomandibular advancement (MMA), induces anterior displacement of the soft palate together with pharyngeal space widening [159]. The overall reported mean reduction in AHI after MMA is 87%. However, it can be associated with other complications and is should be approached only when all other options have failed or patients have presented with craniofacial malformations [160].

2.8 Treatment Efficacy of CPAP

In spite of good adherence to CPAP treatment, some patients may experience persistence of EDS [161]. However, the exact mechanism of this finding is still unclear although this may be an irreversible consequence of Apnoeic events. Supportive management of such patients could be beneficial through improving therapeutic adherence and sleep duration as well [5]. Several studies have been conducted to evaluate the usefulness of stimulants, such as modafinil, for alleviating the symptoms of EDS in CPAP-adhering patients. Although most studies had revealed good effects of stimulants, it had been suggested that patient education regarding the adherence to CPAP is important to be accompanied by the use of stimulants for treating sleepiness [162].

CPAP resulted in significant improvements in several measures of life quality, such as the social function, activity level, and vigilance, as well as depression in OSA patients [163]. Moreover, the objective and subjective sleepiness had improved markedly. Greater improvements in life quality were observed in patients with more disease severity [164]. Surprisingly, life quality improvements also included the bed partners of OSA patients after CPAP treatment [165].

When the effect of short-term CPAP on patients with severe OSA was studied, the subjects showed improvement in some of the neuropsychological assessments when

compared to placebo subjects [166]. In contrast, other conducted studies on mild cases had revealed no improvements in the neurocognitive functions after CPAP therapy performed on mild OSA patients [167, 168].

2.8.1 CPAP effects on Metabolic Health

Insulin resistance has been shown to be improved within two days of CPAP therapy [169]. Moreover, there have been two studies which revealed glucose level improvements in OSA patients having type 2 diabetes and treated with CPAP [170, 171]. The metabolic effects of CPAP therapy for 2 weeks were evaluated in patients with prediabetes versus oral placebo subjects [172]. The therapy resulted in improved glucose metabolism with subsequent metabolic risk reduction. A meta-analysis of randomized controlled trials which have investigated the impact of CPAP on the homeostasis model assessment of insulin resistance (HOMA-IR) showed significant improvement in the HOMA index with CPAP therapy compared with placebo [173]. Overall, it has been observed that the adherence to CPAP therapy is more significant in moderate and severe cases of OSA, poorly controlled diabetes and obesity [62, 170, 174].

In regards to HbA1C, some authors have found that CPAP therapy for 3 months may yield a positive effect on glucose levels in a diabetic group of patients and HbA1C in a subset of these patients [170] and others, in contrast, could not demonstrate a similar effect of CPAP treatment on glycaemic control as well as HbA1C levels [175]. Indeed, Shpirer and co-workers [66] have concluded that there has been a significant reduction in HbA1C levels in patients with severe OSA, and without a previous diagnosis of diabetes, after adherence to CPAP therapy for 3-5 months. Furthermore, the improvement in HbA1C levels in such patients could be augmented through establishing lifestyle changes or addition of metformin.

2.8.2 CPAP effects on All-Cause Mortality

CPAP therapy was associated with significantly lower mortality rates in male patients having OSA over a study period of 10 years but, however, this relation disappeared for female subjects [176]. Further evaluation in the female population may be required in the future to confirm these results. Similarly, using 27 cohort studies, all-cause mortality and cardiovascular mortality were significantly reduced in CPAP treated subjects than control ones [177]. As per a recent meta-analysis, in contrast, CPAP therapy had no significant

effects on mortality, stroke and cardiovascular consequences when compared to control groups [178].

2.8.3 CPAP effects on Cardiovascular Risk factors

CPAP treatment has significant impacts in reducing the morbidity of cardiovascular conditions that may be associated with OSA. Utilizing placebo control subjects which underwent sham (ineffective) CPAP treatment, the study of Dimsdale and colleagues [179] had showed a significant reduction in the levels of blood pressure in CPAP treated OSA patients compared with sham CPAP controls. It is noteworthy that sham therapy had also impacted blood pressure levels, a matter which adds an importance to using such subjects. Significant falls in blood pressure levels were also reported among CPAP-treated OSA patients with normal blood pressure versus a tablet placebo, with more significance in severe cases [180]. On the other hand, there had been no reduction in blood pressure levels post CPAP treatment in patients with severe OSA in the report of Robinson and co-authors [181]. CPAP therapy was also associated with a reduction in nocturnal myocardial ischaemic events in a group of patients having comorbid ischaemic heart disease [182].

Several evidence aspects refer also to the long-term outcomes of CPAP therapy on cardiovascular conditions. Incompletely CPAP treated patients reported an increased incidence of cardiovascular disease than those underwent complete treatment over 7 years follow-up period [183].

Endothelial dysfunction was found to be improved in patients treated with CPAP as reported by Ohike and colleagues [184]. They have found that the concentration of Asymmetric NG, NG-dimethylarginine (ADMA), an inhibitor of nitric oxide synthase, was reduced in nasal CPAP-treated patients and thus nitric oxide production was potentiated affecting the endothelial function. The importance of endothelial function is related to vascular health and may be important for some related conditions such as arteriosclerosis and ischaemic heart disease [185]. Adherence to CPAP treatment for 4 weeks led to significant increases in endothelial nitric oxide and thus improved flow-mediated dilation [78]. Additionally, Oyama *et al.* [186] concluded an overall increase in nitric oxide levels after CPAP therapy for 3 months. Both AMDA and nitric oxide concentration may be useful for evaluating the vascular conditions associated with OSA.

2.8.4 CPAP Effects on Blood Rheology

Several observations confirm the relationship between sleep-disordered breathing and blood rheological properties. Therefore, it is imperative to review the effect of CPAP treatment on the blood rheology of OSA patients and further deep studying of its correlation to the development of cardiovascular disease in such patients. On a macro level, blood viscosity was significantly reduced after 30-day CPAP treatment as reported by Zhang et al [187], and this was associated with a prolonged prothrombin time and activated partial thromboplastin time compared to healthy control subjects. Similarly, blood viscosity fell rapidly after 5 days of adherence to effective CPAP therapy from 18.6% to 10.5% [121]. Specifically, the same researchers observed a reduction in plasma viscosity by 4.2% among OSA patients after CPAP treatment within the same period. On the other hand, Reinhart and co-authors concluded that blood viscosity together with platelet activity was not changed in OSA patients compared to the control group after a 6-months treatment with nasal CPAP therapy [99]. Overall, recent studies should be focusing on the effect of CPAP on whole blood and plasma viscosity considering larger samples and effective matching to control subjects.

A possible mechanism of increased plasma viscosity in OSA patients can be indicated by elevated levels of some inflammatory biochemical markers. Increased levels of CRP and IL-6 in OSA patients, as stated above, could be a possible confirmation of this hypothesis. Treatment post CPAP has resulted in decreasing the levels of both CRP and IL-6 and production of IL-6 by monocytes in the OSA patients compared to obese controls in the study of Yokoe and colleagues [188]. Furthermore, adherence to CPAP treatment for six months was associated with significant reduction in CRP levels and this was confirmed in two different studies [189, 190]. Conversely, other studies reported that the short-term CPAP therapy did not improve CRP, IL-6, and TNF-receptor [191] but this might require 3 months of treatment adhesion to reduce CRP levels [192]. Dorkova and co-authors [193] have reported unchanged serum CRP, IL-6, or leptin levels post CPAP therapy. However, they have observed reductions in the proinflammatory cytokine TNF-α post CPAP-adherence in OSA patients. Their findings were consistent with other reports although they suggested that elevated CRP, IL-6, or leptin levels were ascribed to obesity rather than OSA [194, 195].

Significant increases in several clotting factors have been found in OSA cases and this may contribute in increasing blood coagulability. The increased levels of thrombin-antithrombin (TAT), a marker of thrombin turnover, together with FVIIa levels were demonstrated to be reduced to as much as 30% after long-term CPAP treatment [103]. The actual role of CPAP therapy on negating the effects of clotting factors remains unclear. On the other hand, although patients showed increased levels of these clotting factors, Robinson and co-workers [87] observed no effect on post-CPAP therapy levels after one month.

Fibrinogen levels were also extensively studied in OSA patients despite the inconsistent results obtained in regard to their levels after CPAP therapies. On their 11 OSA patients, Chin and colleagues [106] have demonstrated a significant reduction in fibrinogen levels after CPAP therapy, and this was consistent with the results of Zhang and co-workers [187] performed on elderly Chinese OSA patients. Other investigators had found converse results. After 5 nights of CPAP treatment, it has been found that the levels of fibrinogen remained unchanged after five nights [121], four weeks [196], or six months [87] of CPAP therapy. Such variance in results may be ascribed to varied OSA severity, hypoxaemia, differences in sample sizes, and inconsistent adherence to the therapy among those studies.

CPAP treatment can also exert an effect on haematocrit values which may be increased in OSA patients. Short-term CPAP therapy for one night [197, 198] and one month [187] resulted in significant decrease in haematocrit levels. In addition, all patients investigated in Saarelainen and co-worker's study (1996) had shown the same results after 3 months of CPAP treatment. Long-term therapy for one year at home had also yielded significant haematocrit reductions [198].

On the cellular level, platelet aggregation was decreased significantly after both one night and 3 months of CPAP treatment when compared to the control subjects as reported by [199]. According to other studies, only one month of adherence to the therapy may be sufficient to reduce platelet aggregation [187, 200]. However, another study revealed that it might require 90 days of CPAP therapy to produce a significant reduction of such aggregation [201]. Overall, a significant reduction will take place in platelet aggregation after CPAP treatment although the duration of therapy may vary.

Platelet size, as measured by mean platelet volume (MPV) is related to platelet reactivation and can be considered a monitoring tool for platelet activity. Two studies have

shown a significant decrease in MPV after effective CPAP therapy for 6 months in OSA patients when compared to control groups [115, 202]. This might be due to decreased hypoxia and inflammation. However, there was no difference in platelet number between both groups after the same period of treatment [203].

The increased ability of erythrocytes to aggregate is significant in OSA patients. The RBCs aggregation index was found to be significantly decreased (7% lower with $p<0.05$) and aggregation half-time was increased (25.4% higher with $p<0.05$) following CPAP treatment for five consecutive nights when compared to a control group [121]. These results were included as of several other rheologic and polysomnographic variables in OSA patients. Since the RDW is positively related to several markers such as CRP, ESR, IL-6 [204], it is important to study the effects of CPAP therapy on their values. Although RDW was significantly increased in OSA patients, CPAP-adherence for one year has no effect on RDW in all studied subjects [205].

Based on the aforementioned evidence, the present study aimed at investigating the association between blood rheology-related parameters and the different severity patterns of OSA. We hypothesized that there might be a possible association between different OSA severity patterns and blood rheology parameters, particularly those measured at the morning. Another objective of this thesis was to study the effects of acute OSA treatment with continuous positive airway pressure (CPAP) on blood rheology and rheology-related parameters were evaluated. As such, we postulated that acute CPAP therapy might improve blood rheology despite the paucity of relevant data.

3. METHODS

3.1 Experimental Design

This study was designed as a prospective study and was approved by the review board of the Gold Coast University Hospital (ref number – HREC/16/QGC/241) and Griffith University.

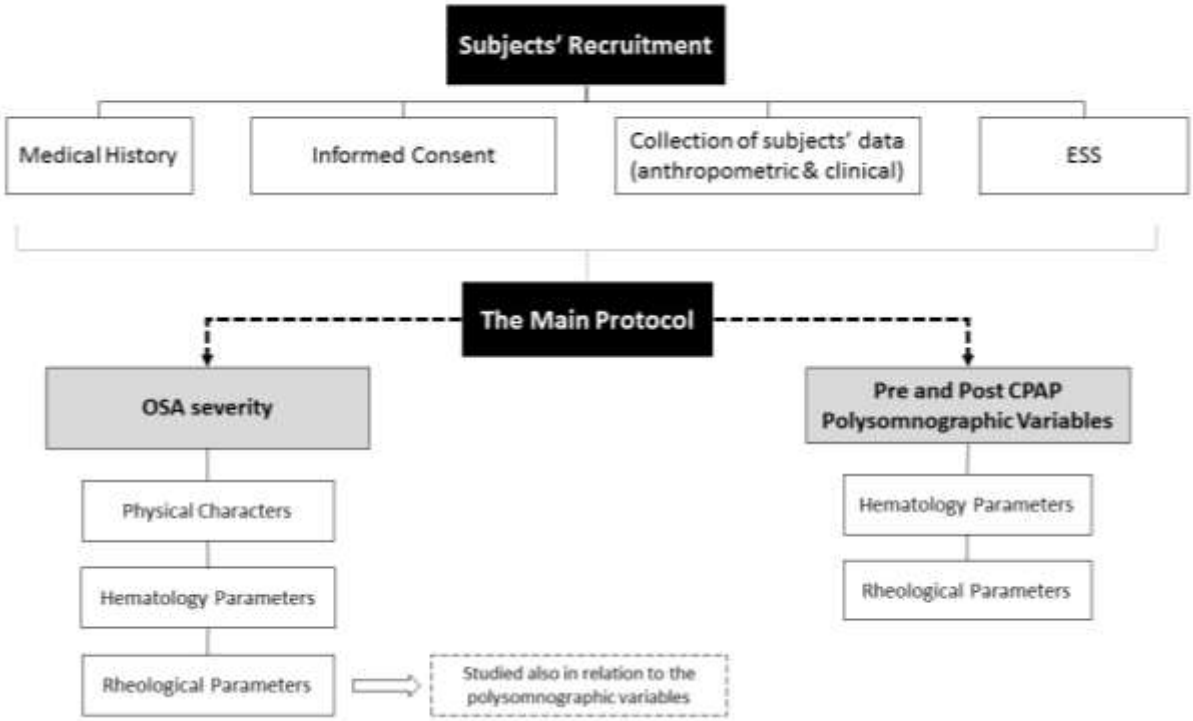


Figure 4: Schematic representation of study design. ESS: Epworth Sleepiness Scale; CPAP: Continuous Positive Airway Pressure therapy

3.2 Patient Recruitment

3.2.1 Referral of Patients

Patients referred by their General Practitioner were assessed by a respiratory physician that is accredited in sleep medicine at the Gold Coast University Hospital, Australia. For each referred patient, he/she attended the sleep physician clinic for evaluation of potential sleep-disordered breathing (SDB). The patient was assessed thoroughly for obstructive sleep apnoea (OSA) risks, including family history of sleep apneoa, smoking, nasal congestion, snoring, history of sleep fragmentation or apneoas, myocardial infarction, heart failure, hypertension, gastroesophageal reflux disease, angina or stroke. If the patient met criteria

or had a high suspicion of SDB they were referred to the sleep laboratory for a polysomnograph. Therefore, the study group consisted of those individuals who attended the sleep laboratory.

3.2.2 Attending the Sleep Laboratory

1. *Before patient attendance*

The sleep technician was responsible for all patient related care prior to and during the PSG. He/she works in a planned manner with a sequence of steps prior patient attendance, on arrival, and during the study. The technician reviewed the records and the chart of the patient. The chart contains a summary of medical history, results of previous physical examinations, the demographic data, and the special needs that may be of interest. Therefore, the technician considers all this data to provide the appropriate care.

Importantly, the lab technician should be aware of the physician order to perform the suitable study type. If there was a difference between the physician order and that of the lab manager, when one of them required a diagnostic polysomnography and the other called for a CPAP titration, the technician should discuss that matter with both of them. Other considerations associated with the physician order should be also emphasized, such as providing supplemental oxygen.

2. *Upon patient arrival*

Thirty - one patients attending the sleep laboratory were recruited into the study. Every patient was asked to sign an informed consent including an explanation of the study and the included procedures, risks and follow - up. For assessment of subjective daytime sleepiness, patients were asked to answer the Epworth Sleepiness Scale (ESS) questionnaire (M. W. Johns, 1993) consisting of eight questions with a total score of 0 (minimum) to 24 (maximum).

Patients' anthropometric data were collected, including their height (Seca 217 Stable stadiometer for mobile height measurement, Seca, USA), weight (Seca 813 High capacity digital flat scale, Seca, USA), and neck size. Body mass index (BMI) was calculated by dividing patient's weight (kg) by squared height (m2). Seated blood pressure, body temperature, oxygen saturation, respiratory rate at rest, and heart rate were also recorded for

each patient. A complete history of any pathological conditions, medications taken, as well as alcohol and caffeine consumption during the previous 24 hours.

3.3 Polysomnograph Set up (diagnostic)

3.3.1 Device

Patients were recorded overnight via type I full polysomnogram (PSG) using Compumedics Grael system (Compumedic Neuroscan, Australia),. Polysomnography (PSG) which is a comprehensive recording of the biophysiological changes that occur during sleep. The PSG monitors many physiological parameters, including brain waves (EEG), eye movements (EOG), chin and leg muscle activity (EMG) and heart rhythm (ECG) during sleep. Other sensors, including pulse oximetry, nasal pressure, and microphone to record snoring/breathing, digital camera to record whole-body movement.

3.3.2 Set up

Once the patient has arrived at the laboratory, it takes approximately 1 hour to set a patient up to record the multiple channels of data when they fall asleep. A sleep technician who works at the sleep laboratory must be continuously monitoring the patient and is accountable for attaching the electrodes to the patient and checking the patient during the study. The technicians usually have to monitor 2 or 3 patients per night. The technician usually starts with the patient who retires earliest. A polysomnogram will record at least 12 channels demanding at least 22 leads to the patient.

3.3.3 EEG leads

There are multiple leads that are attached to various points around the patient's head and face. The international 10/20 electrode placement system was used as it entails employing the anatomical features of the ears and nose as landmarks for measurements. Electrode placement was applied using percentages of the distances between such landmarks rather than the distinct lengths. For most of the used percentages, 10% or 20% of the distances are considered between the anatomical features.

The electroencephalogram (EEG) in general would utilize six "exploring" electrodes and three to four "reference" electrodes depending on the kind of used equipment. If seizure activity is suspected, additional electrodes will be attached to record the presence of seizure

activity. The recommended placement of EEG electrodes by the American Academy of Sleep Medicine (AASM) is as follows: Mastoid 1 (M1), Mastoid 2 (M2), Central 3 (C3) Central 4 (C4), Frontal 3 (F3) Frontal 4 (F4), Occipital 1 (O1), and Occipital 2 (O2). These electrodes will offer information of the brain's activity that can be "scored" into different stages of sleep (N1, N2, N3 which combined are referred to as non-rapid eye movement (NREM) sleep and Stage R which is rapid eye movement sleep or REM, and Wakefulness). The EEG electrodes are positioned in accordance with the International 10-20 system by accurate determination of particular landmarks on the head: the nasion (the bridge of the nose), inion (the most apparent bony ridge at the back of the skull), and right and left preauricular points, which are located in front of the notch in the middle of the ear (Figure 5).

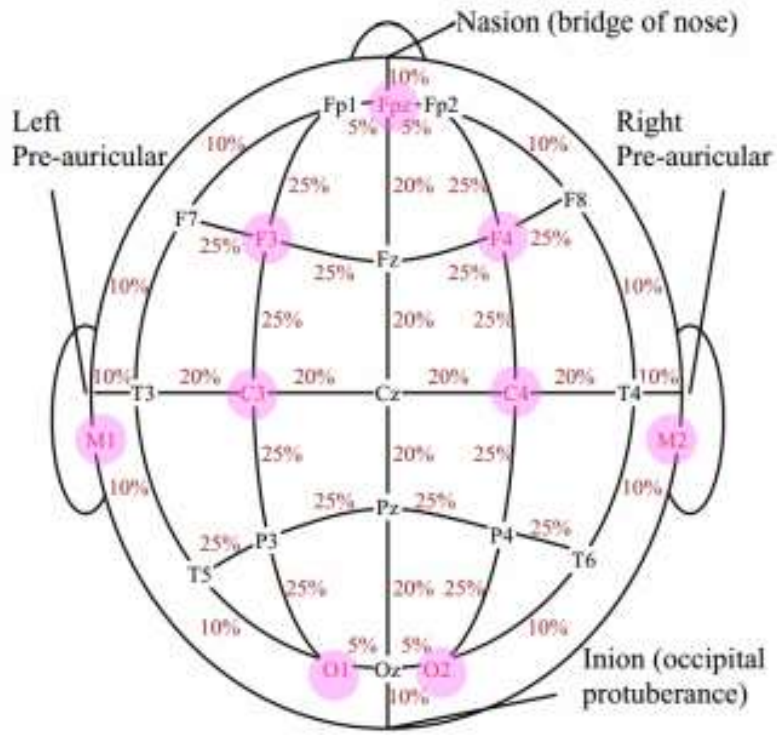


Figure 5: Electrode Placement according to the International 10-20 system. Fp: frontal pole F: Frontal; T: Temporal; C: Central; P: Parietal; O: Occipital; M: Mastoid

First, the measurements between the nasion and inion were established. The total distance between the two points was measured (assume it was 40 cm for a given patient). Then, the first mark was placed at 10% (4cm) from the nasion, namely Fpz. The four subsequent points were marked at distances 20% (8cm) from the previous point; Fz at 20%

(8cm) from Fpz, Cz at 20% (8cm) from Fz, Pz at 20% (8cm) from Cz, and Oz at 20% (8cm) from Pz (Figure 5). Finally, the distance from Oz to the inion should be 10% (4cm).

For the preauricular points, the total distance between the right and left points was measured (provided that it was 34 cm for a given patient) with the tape passing on the previously marked central point (Cz). Again, 10% distances from the left and right auricular points were counted (3.4cm) and marked as T3 and T4 respectively (Figure 5). Starting from the T3 point, count 20% of the distance (6.8cm) and mark it as C3, the following 20% point would be Cz, and, finally, the next point was marked at 20% of the distance to be named C4.

The points O1 and O2 were marked on 5% on the head circumference on each side of Oz. The same was applied when marking Fp1 and Fp2 at each side of Fpz. F3 point was marked at the half way from Fp1 to C3, while F4 was place at the half way from Fp2 to C4.

3.3.4 Chin EMG

The electromyogram (EMG) of the chin utilises three leads. The first one was located above the anterior edge of the mandible. The other two were placed below the chin, one of them was 2 cm down and 2 cm to the left of the middle, while the last electrode was placed 2 cm down and 2 cm to the right of the middle. In general, two of these electrodes were used to provide the primary signal and the third one was considered as a backup to work if any of the electrodes were unattached during sleep.

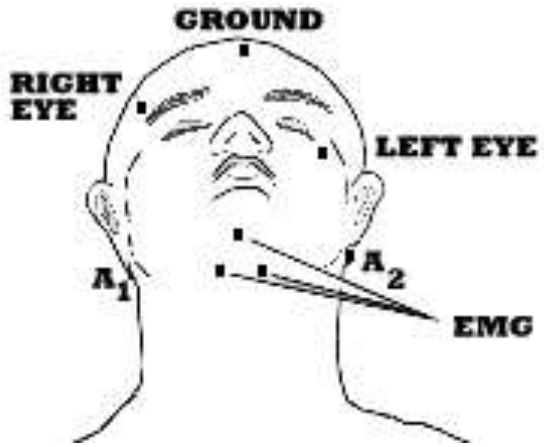


Figure 6: Electrode placement for chin EMG - source

3.3.5 Leg EMG

Two EMG electrodes are attached to each leg to measure the amount they move during sleep. Excessive movement during sleep may be indicative of periodic limb movement disorder (PLMD). They are placed on the tibialis anterior muscle of each leg to quantify leg movements.

3.3.6 Eye Leads

The electrooculogram (EOG) uses two electrodes; one that is placed 1 cm above the outer canthus of the right eye and one that is placed 1 cm below the outer canthus of the left eye. The activity of the eyes is picked up by these electrodes by the electropotential difference between the positively charged cornea and the relatively positive retina. This helps to determine when rapid eye movements (REM) sleep occurs, and fundamentally, as well, helps in determining when sleep occurs.

3.3.7 ECG Electrodes

Cardiac monitoring during polysomnography involves the placement of 3 electrocardiograms (ECG leads). They are placed just beneath the right clavicle, slightly lower than this at the midclavicular line on the left, and at the fifth intercostal space at the axilla line.

These electrodes measure the electrical activity of the heart as it contracts and expands, recording such features as the "P" wave, "QRS" complex, and "T" wave as well as rate and regularity. These can be studied for any variations from the norm that may be indicative of an underlying arrhythmia.

3.3.8 Ground Electrode

An electrode or pair of electrodes can be directly attached to earth grounds from the polysomnograph for electrical safety.

3.3.9 Respiratory Bands (thoracic and abdomen bands)

There are two respiratory bands (Compumedics Grael Thoracic/Abdominal Band and cable, Compumedics Neuromedical Supplies, Australia) attached around the chest and abdomen to measure how the patient breathes in terms of rate, rhythm and effort during

sleep. The movement of the bands caused by the movements of chest and upper abdominal walls are measured with respiratory inductance plethysmography. This movement is linked to exertion and produces a low-frequency sinusoidal waveform as the patient breathes in and breathes out.

3.3.10 Microphone (snore detector)

A snore microphone (Compumedics Grael Tracheal microphone, Compumedics Neuromedical supplies, Australia) is placed on the laryngeal prominence on the neck to the corresponding side where the Grael headbox is located. This microphone is used to record snoring and other abnormal vocalizations.

3.3.11 Thermistor

Thermistors are used to detect airflow from the nose and mouth, this is achieved by detecting the difference in temperature between room air and expired air. They are placed at the base of the nose, with a sensor in each nostril.

3.3.12 Pulse oximeter

An oximeter works by estimating the amount of haemoglobin attached to the oxygen molecules, displayed as a percentage the higher the number the better. The oximeter is attached to a patient's finger and oxygen saturation is determined by the amount of red light that passes through the finger.

3.3.13 Position sensor

The role of this sensor is to record the position of the patient during sleep, either as lateral right or left, supine, or prone. It is placed around the thoracic band at the centre of the chest.

3.3.14 HD camera monitoring

The HD camera located at the ceiling of the room captures the activity of each patient during sleep.

3.3.15 Electrode Preparation and Placement

Since a wrong electrode placement would eventually lead to misdiagnosis and treatment provided to the patient, it was important to emphasize an appropriate localization of EEG, EOG, EMG, or other channel kinds.

Upon determining the exact location, the technician began to prepare the site for placement in order to reduce the level of impedance and, therefore, improve signal quality. It is well known that dead skin and debris at the bridge between the skin and electrode may contribute in high electrode imprudence. The technician wore latex gloves as personal protective measures because the process of skin preparation entails abrasion of the top layer of the skin revealing an intact layer and carry a risk of transmitting blood-borne micro-organisms.

Combs or hairclips were used to separate the hair in case of working in a hairy area. Other sites, including the legs and the chest, might need shaving to accomplish firm sticking of the electrodes. Site preparation included removing dead skin and debris, particularly while preparing skin on the scalp, using a cotton swab and NuPrep solution. After cleaning, the technician would apply a paste or gel which is electrically conductive. This paste or gel is important to facilitate signal transmission through the cup electrode with minimal resistance. The electrode was then placed at its exact location where it was measured. To confirm the appropriateness of paste filling, there should be no squeezing out from the sides and the top of cup electrode.

It was necessary for each technician to confirm firm electrode placement to render electrode impedance minimal. Upon using a clip or snap leads, the lead was applied to the button-like electrode to place it on the face or the recommended parts on the body, considering the aforementioned considerations. It has been ensured that the centre of the electrode was in direct contact with the skin.

3.3.16 Electrode Application

A piece of gauze was used (square shape) when applying the cup electrodes in a diagonal position over the cup, confirming that the centre of the gauze was directed over the centre of cup. Also, the lower portion of the gauze was extending downward with the wire. Additionally, the lower part of the gauze should contain a small amount of the paste. The technician exerted gentle pressure using a fingernail in the form of repeated circular movements and including the central part of electrode placement site.

A sticky gauze could be used when applying the electrode to the face or the body. Generally, a firm placement of the electrodes on body parts should be ensured and secured

with a small piece of tape with the sticky part of the electrode extended against the skin to minimize the movement during the night. This was done by placing the tape across two sides to the skin and the lead wire was then connected to the electrode with placing another strip of tape over the lead wire head.

3.4 Polysomnograph set up (CPAP)

First, the technician confirmed that the patient's referral involved both diagnosis and treatment of OSA. After proper scheduling, patients were hooked-up with the same previously mentioned methods of electrode placement. Furthermore, the technician informed the patient about the benefits, possible side effects, and how to reduce them. This would ultimately enhance the outcome of positive airway pressure (PAP) treatment and further enabled the patient to strictly comply with treatment.

For the process of titration, each patient was educated about its importance and that there might be a likelihood of changing the pressure on a periodical basis during the night. Such pressure adjustment was performed according to breathing disturbances. The patients were informed also about the possibility of changing the maximum pressure through PAP titration rather than that recommended by the treating physician. This was considered to determine the most suitable level of pressure, which might be more or less than the recommended pressure, for each sole patient.

On the other hand, the technicians were aware that a patient might experience a degree of difficulty falling asleep. Therefore, the needs of every patient should be exclusively provided in an appropriate manner. To get familiar with PAP titration, the patient is asked to try desensitization by sitting up on the side of the bed. It is necessary to start PAP titration with a low pressure in accordance with the laboratory protocol. To accomplish a successful PAP titration, a PAP mask was applied by the responsible technician.

3.4.1 The PAP masks

Both nasal and full-face masks were available. For the nasal masks, it should be fitted from the middle of the nasal bridge down to just above the upper lip. If it was properly fit, this type of mask provides a good seal. The patients who were unable to close their mouth during sleep, a chin strip was applied and placed under the chin up above the head to render a closed mouth while the patient was on PAP.

Full-face masks were used for patients who were unable to close their mouth and were uncomfortable with nasal masks with chin strips. These large masks covered both the mouth and nose and might be considered a necessary choice for some patients having nasal congestion or a deviated septum. Since the patients with untreated OSA had their mouth open frequently, full-face masks can be helpful to keep a patent airway. Oral masks, another type of mask, were used in patients who cannot accommodate with any other type of masks. An oral mask can be fitted on the end of the mouth with a small device which helps to keep it in place.

3.4.2 The PAP Machine

We used highly specialized sleep lab machines in this study (VPAP Tx, ResMed, US). Continuous positive air pressure (CPAP) machines work by filtering air and passing it to a blower, which sends it by increasing its pressure through the mask to keep the airway open during sleep. The technicians set the mask type, therapy mode, and other settings from the bedside or from the monitoring room. By turning on a device, pressure began to apply to the mask and the technician checked it for possible leaks.

The process of gradually adjusting air pressure through CPAP machine is called CPAP titration. Technicians calibrate CPAP therapy to keep the airway of a given patient open and to maintain it corrected to a suitable degree. The overall montages of CPAP titration were identical to that of the diagnostic sleep studies with the addition of a CPAP pressure channel and a CPAP flow channel. At each PAP level, important data were recorded to facilitate reaching an optimal therapeutic level for each patient. Such data included the respiratory disturbance index (RDI), the minimum oxygen concentration (SaO_2), EEG arousal indices, and the duration to which the patient reached REM sleep. An optimal CPAP titration included reducing RDI to less than 5 events per hour for at least 15 minutes, minimum SaO_2 was more than 90%, nonfrequent interruption of REM sleep by less than 5 arousals per hour.

In addition to increasing airway pressure, CPAP was increased in certain conditions according to lab protocols. All technicians were aware of them since any deviation from normal procedures could result in several adverse outcomes, such as central aponeas if CPAP was increased without an acclimatization phase. At low pressures, CPAP therapy could be increased at a rate of 2 cm, while it becomes only a 1 cm increase at higher

pressures. In general, the interval between each increase was 20 minutes to enable the patient to acclimate with such changes.

3.5 PSG Analysis and reports

For fast and accurate analysis, the PSG profusion manager software was used to analyse sleep staging and respiratory events. It entails using hotkeys, waveform measuring tools, digital displays and other properties.

3.5.1 AASM 2012 scoring rules

The scoring of sleep events was completed in 5-minute epochs from the start to the end of the study. For aponea scoring, a drop in the peak thermistor ≥ 90% for 10 seconds or more for at least 90% of the event can be considered an aponea event. An obstructive event is usually associated with an increased respiratory effort during the event. Central aponea is defined when there is no inspiratory effort throughout the event. Finally, a mixed aponea is that when there is a lack of inspiratory effort during the first part of the event, while the effort is resumed in the last part.

For hypopnoea scoring, hypopnoea is scored when there is a drop in the nasal pressure ≥ 30% of the baseline, which lasts for 10 seconds or more for equal to or more than 90% of the event, and this is associated with 3% drop in oxygen saturation and/or an arousal (Berry et al., 2012).

Arousals are the abrupt shifts in EEG frequency when they reach to greater than 16Hz for more than 3 seconds when this period is preceded by at least 10 seconds of stable sleep. This shift in EEG must be associated with an increase in chin EMG during REM sleep.

The scoring of periodic leg movement was established when there have been leg movements lasted between 0.5 to 10 seconds with more than 8 micro-volts increase in the baseline amplitude of leg EMG. To be included, there must be 4 events in a row with 5-90 seconds between each event.

Finally, the scoring of bruxism was achieved if there were brief periods elevated chin EMG of at least twice baseline chin EMG between 0.25 to 2 seconds in duration for a minimum of 3 elevations. Audio recording could be helpful to listen for the presence of tooth grinding sounds.

3.5.2 Establishment of OSA severity

According to the AHI (aponea hypopnoea index), OSA can be scored by calculating the average number of times where the aponea or hypopnoea events can occur per hour of sleep (Kryger, 2009). The classifications of AHI values is as follow: no OSA when AHI <5; mild OSA when AHI=5-14.9; moderate OSA when AHI=15-29.9; and severe OSA when AHI>30 (Ruehland, Rochford, O'donoghue, Pierce, Singh, & Thornton, 2009).

3.5.3 Establishment of daytime sleepiness severity

The self-administered ESS questionnaire was used to establish the severity of everyday situations. Patients respond from 0 "would never doze" to 3 "high probability for dozing" for 8 questions. The scores for the eight questions were added together to get a single number. The excessive sleepiness was suggested when the total score exceeded 10 (M. Johns & Hocking, 1997).

The scoring of the PSG also comprises the following information:

• The onset of sleep from the time the lights were switched off; this is called "sleep onset latency" and normally is less than 20 minutes. Sleep efficiency: the number of minutes of sleep divided by the number of minutes in bed. Normal is approximately 85 to 90% or higher.

• Sleep stages; these depend on data originating from 7 channels: EEG (4 channels usually), EOG (2) and chin EMG (1). From this data every 30-second era is scored as "awake" or one of 4 sleep stages: 1, 2, 3, and REM or Rapid Eye Movement sleep. Stages 1–3 are called non-REM sleep. Non-REM sleep is recognized from REM sleep, which is overall dissimilar. Within non-REM sleep, stage 3 is called "slow wave" sleep because of the comparatively wide cerebral waves in comparison to other stages; another name for stage 3 is "deep sleep". On contrary, stage 1 and 2 are "light sleep".

(The percentage of each sleep stage varies by age, with diminishing measures of REM and deep sleep in elderly. The greater part of sleep at all ages (with the exception of infancy) is Stage 2. REM ordinarily comprises around 20-25% of sleep time. Many factors other than age can influence the sum and percentage of each sleep stage, including drugs (especially anti-depressants and pain medication), alcohol taken before sleep time and lack of sleep.

• "Arousals" are sudden moves in brain wave activity. They might be produced by various influences, including breathing anomalies, leg movements, etc. an unusual number of arousals designates "interrupted sleep" and may clarify an individual's daytime symptoms of tiredness and/or somnolence.

• Arousal index is the total number of arousals during sleep divided by total sleep time. This index is used to assist in classifying sleep fragmentation.

Once scored, the test recording and the scoring information are sent to the sleep physician for interpretation. Preferably, interpretation is done in combination with the therapeutic history, a full list of medications the patient is receiving, and any other pertinent data that may affect the study, for example napping done before the test.

Once interpreted, the sleep physician composes a report which is sent to the referring provider, usually with particular suggestions in relating to the test outcomes.

3.6 Blood Rheology

3.6.1 Blood collection

Venous blood samples were obtained from all patients within 30 minutes of waking via venepuncture between 0500 – 0600 immediately after both the diagnostic and CPAP titration polysomnography and kept at the appropriate temperature until analysis which was performed within 4 hours post collection.

All equipment, such as tourniquets, alcohol swabs, needles, etc. were collected in a safe and easy to reach the place and close to the principal investigator. Hands were washed thoroughly with soap and water and were dried using a single-use towel. Then, the investigator wore well-fitting, non-sterile gloves to follow the optimal hygienic practices.

Every patient was asked whether he/she has allergies, phobias, or other similar syncope events during previous injections or blood draws. A clean towel or paper was placed under the patient’s arm prior to collection. The patient’s arm was extended to check the forearm or the antecubital fossa. The most visible, clear, and straight vein was located, mostly the median cubital vein. The needle size was determined according to the visible vein. Then, a tourniquet (Tourniquet Quick Release, Medshop Australia) was placed 4-5 finger width’s above the venipuncture site and the vein is rechecked.

The entry site was disinfected using a 70% alcohol swab applied for 30 seconds and then the site is allowed to dry for another 30 seconds. A firm and gentle pressure were applied at the entry site and at the surrounding area of 2 cm or more. The area should be left for sufficient time to dry to avoid possible contamination. The vein was anchored by holding the arm with thumb placement below (not above) the entry site. The investigator asked the patient to form a fist so that the vein gets more prominent. A sterile needle (Vacutainer Needle 21G x 38mm, 360213, BD Medical Supplies & Equipment, Australia) was uncovered and was inserted and fixed into a vacutainer holder (BD Vacutainer Holder Reusable-Yellow, 364879, BD Medical Supplies & Equipment, Australia). The needle cap was removed and the needle was inserted swiftly at nearly 30 degrees into the vein. For each patient, blood samples were evacuated into two vacutainers (4ml) with ethylenediaminetetraacetic acid (EDTA) for haematological evaluation, two serum separation tubes (SST) which contain a special gel to separate blood cells from serum (6ml), and one tube (2.7ml) containing sodium citrate for measurement of coagulation parameters. The vacutainer system allows the tubes to filled directly with minimal discomfort to the patient.

Once sufficient amount of blood has been collected into the vacutainers, the tourniquet was released, a ball of cotton was placed above entry site, and then the needle was withdrawn. The cotton piece can be placed directly on the entry site at this time. All blood samples were stored at appropriate temperature before analysis.

After sample collection, the haematological analysis (full blood count), as well as fluorescence flow cytometry, was performed using Sysmex XT 1800i automated haematology analyzer (Kobe, Japan). The Sysmex CA-660 system was used for clotting testing (plasma fibrinogen) while blood biochemical parameters, including triglycerides, HDL, LDL, cholesterol, insulin, glucose and glycated haemoglobin (HB1Ac) were measured using an autoanalyzer (Beckman Coulter AU680, Japan).

3.6.2 Preparing solutions

For the experimental protocol to be completed polyvinylpyrrolidone (PVP) solution was prepared by adding and dissolving the following components into 1 L of deionised water: 50 g of powdered polyvinylpyrrolidone, 8.00 g NaCl, 0.20 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4. In addition to the osmolarity and PH, the viscosity of the solution was

adjusted to 30.0±1.5 cP, while the PH and osmolarity were 7.4 and 292 mOsmol/L respectively.

3.6.3 Haematological analysis

The haematological analytic procedures of OSA patients in this study were performed using an automated haematology Analyser (UniCell DxH600, Coulter Cellular Analysis System, Beckman Coulter, Brea, CA). For optimal accuracy, the haematology analyser was calibrated using samples of blood of known haematocrit values and white cell count (Coulter cell control) and a material-based solution of known size and volume (Coulter latron control). The measurements performed for all patients were as follow: red blood cell count (RBC, $x10^{12}$/L); haematocrit (Hct, %); Platelet count ($x10^{9}$/L); Plasma Fibrinogen concentration (g/L); haemoglobin concentration (HB, g/L); Mean cell volume (fL); Mean cell haemoglobin (pg); white blood cell count (WBC, $x10^{9}$/L); Neutrophils count ($x10^{9}$/L); Lymphocytes count ($x10^{9}$/L); Monocytes count ($x10^{9}$/L); Eosinophils count ($x10^{9}$/L); Basophils count ($x10^{9}$/L). In addition, blood sugar parameters were measured and they included the levels of insulin (mU/L) and glucose (mmol/L). Finally, the following lipid profile parameters were also investigated for the patients: low density lipoproteins (LDL, mmol/L); high density lipoproteins (HDL, mmol/L); glycated haemoglobin (HbA1c, mmol/L); cholesterol (Chol, mmol/L); Chol/HDL (mmol/L); triglycerides (Trig, mmol/L).

3.6.4 Adjustment of haematocrit values

First, haematocrit values were measured using the mean packed cell volume method (the ratio of packed of RBC and plasma to whole blood). For such purpose, 75 µL of the whole blood sample of each patient were added into two microcapillary tubes and then placed into a centrifuge at 1500 × g for 5 minutes. This would allow separation of blood and plasma components. Finally, the haematocrit ratio was measured by expressing the packed blood cells relative to the total blood/plasma column.

In order to easily compare haematocrit values between the subjects in the study, blood haematocrit was adjusted to a standardised value (0.4 L/L). This adjustment was also performed since RBC aggregation and blood viscosity are sensitive to haematocrit. To obtain an adjusted haematocrit sample, 1000 µL and 500 µL of whole blood were placed in two 1.5 mL microtubes, where they were centrifuged at 1500 × g for 10 minutes to separate

plasma from other components. In order to adjust the sample to the target 0.4 L/L haematocrit, plasma volume was calculated using the following equation:

$$Plasma\ adjustment\ (\mu L) = \left(\frac{1000\ \mu L\ \times Haematocrit}{0.4}\right) -\ 1000\ \mu L$$

If the resultant haematocrit was less than 0.4 L/L, the investigator aspirated the suitable volume of plasma out of the 1000 µL microtube and discarded it. If it was more than 0.4 L/L, autologous plasma was aspirated out of the 500 µL microtube and transferred into the 1000 µL microtube. Before starting any rheological investigation, the adjusted samples should be thoroughly mixed.

3.6.5 Measurement of blood viscosity

For accurate measurements of viscosity, the viscometer (Cone Plate Wells Brookfield Viscometer, Brookfield Ametek, Middleboro, MA) was calibrated so that the gap between the rotating cone and stationary plate was maintained at a 1 mm. In addition, the viscometer was connected to a water bath at 37°C±0.2 to maintain a constant temperature in the device. This is due to blood viscosity being sensitive to temperature. The viscometer produces torque operating at distinct rotational speeds. It consists of a calibrated beryllium-copper spring between the drive and the rotating cone. This spring senses the resistance to rotation implied by the blood sample which produces a torque proportional to the shear stress in the fluid. To convert the torque into a shear stress, the torque value was multiplied by 126 (calibration coefficient; this was adjusted annually using precision calibration oil). For all OSA patients, blood viscosity was determined for native blood samples and blood adjusted to a standardised haematocrit (0.4 L/L).

Measurement process included placing 1000 µL of blood into the central part of the stationary plate. This plate was then locked onto the cone. Torque values were taken upon reaching the correct temperature at shear rates of 75, 150, 300, 750 and 1500 s^{-1}, and then recorded. The resultant shear stress (from the torque) was subsequently divided by the corresponding shear rate to finally calculate the viscosity. After obtaining the measurements of each sample, deionised water was applied to wash the plate and cone in order to ensure optimal cleanliness for the following samples. The same processes was repeated for measuring the standardised haematocrit at 0.4 L/L samples and plasma.

3.6.6 Measurement of red cell aggregation

A cone-plate shearing aggregometer (Myrenne GmbH, Roetgen, Germany) was used to measure red cell aggregation. The shearing segment of the Myrenne aggregometer comprises of a 2-degree cone that is pivoted by a DC motor and a plate (i.e., a glass microscope slide) situated over it; a small volume of blood (50 µl) is transferred into the gap between the cone and plate. An infrared LED is utilized as the light source and the light beam travels through the specimen at a distance 1.5 mm from the peak of the cone. The measurement process begins with shearing of the specimen at a settled, high shear rate (600 s^{-1}) by revolving the cone for 10 s. The light transmission signal is then recorded, either following the total immobilisation of the cone (i.e., zero shear rate) or when decreasing the rotational speed to a very low-level equivalent to a shear rate of 3 s^{-1}.

In the present thesis, 50 µL of the whole blood was aspirated from an EDTA tube and was added on the cone of the aggregometer (centred). Then, a glass plate was placed above the cone with manual agitation several times in order to render the RBC disaggregated. RBC aggregation measurements were performed using the following values: 1) M0: RBC aggregation after 10 s at stasis after stopping the applied disaggregating shear at 600 s^{-1}, 2) M1: RBC aggregation after 10 s at a low shear rate "3 s^{-1}" after stopping the applied disaggregating shear at 600 s-1, 3) AI120: aggregation index over 120 s, and 4) T1/2: the time required to achieve half of the AI120 value. M0 and M1 were performed twice and the average values were reported. AI120 and T1/2 were calculated using Labview ((LabView 2009, National Instruments) software by making a monoexponential curve to the light transmission-time data.

For optimal measurement conditions, cone and plate agitation was performed following each measurement condition of M0 and M1. In addition, both the cone and plate were washed with deionized water. Finally, RBC aggregation values were measured at native and standardised (0.4 L/L) haematocrit conditions.

3.6.7 Assessment of RBC deformability

RBC deformability was assessed using the rotational ektacytometry technique. It is a technique for the appraisal of RBC deformability in view of laser diffraction and thus the elongation index could be produced over varying shear stresses (0.1 – 100 Pa). In this procedure, a suspension of erythrocytes in a high viscosity medium is exposed to flow stress,

and the laser diffraction arrangement coming from a laser beam crossing the suspension was analyzed to give an estimate of cell deformability. The applied shear stress can be computed from the known values of rotational speed of the moving component, the gap width between the two cylinders and the viscosity of the suspension medium

A laser-assisted optical rotational cell analyser (LORCA, Lorrca MaxSis, RR Mechatronics) was used and operated at 37±1°C. In order to obtain the elongation index, the following equation was used:

$$\text{Elongation index (EI)} = (\text{A} - \text{B}) \div (\text{A} + \text{B})$$

(where A is the major axis of RBC and B is the minor axis of the cell).

Before deformability measurements, the investigators aspirated 25 µL of whole blood from an EDTA tube into a glass tube containing 5000 µL of the PVP solution. The solution was inverted several times in the tube after placing a parafilm over the tube in order to mix both components. After that, 900 µL of the mixture was aspirated from the tube and placed between the cylinders of the LORCA system. First, a control baseline measurement was set from the unsheared deformability assessed between 0.1 to 100 Pa. Then, the system was washed with deionised water and a fresh 900 µL of the mixture was used and added to the preconditioning shear. After such exposure, RBC deformability was measured over the range of 0.1 to 100 Pa.

Raw data of the elongation index were analysed using Prism software ((Prism 7, Graphpad Software Inc, La Jolla, CA). Lineweaver-Burk equation was modified in a non-linear manner so that it could be applied to the raw data within the range between 0.1 to 100 Pa [206]. The following indices were calculated (Figure 7): *i)* The maximum value of elongation index at a given shear stress (EI_{max}), *ii)* the shear stress required to attain the half value of EI_{max} ($SS_{1/2}$), and *iii)* $SS_{1/2}$: EI_{max} for accurate standardization of $SS_{1/2}$ for EI_{max} alterations.

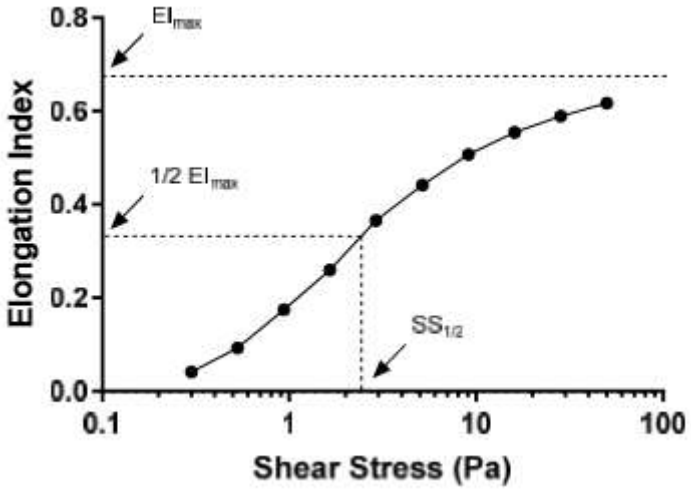


Figure 7: Demonstration of the elongation index of RBC at various shear stresses.

3.7 Statistical Analysis

Shapiro-Wilk normality test was used to assess the normality of the experimental data. Data were presented as mean values. Differences in mean aggregation and haematological parameters between different OSA severities were analysed using independent samples t-test. Paired samples t-test was used to assess the differences in RBC aggregation and unsheared RBC deformability curve parameters. For part 1a, the analysis of variance (ANOVA) was used when comparing three or more means and Dunnetts test was used for post hoc analysis. For part 1b, Pearson correlation was used to investigate the association between the polysomnographic variables and blood rheological and haematological parameters.

4. RESULTS

The primary objective of the present thesis was to study the changes in blood rheological parameters and their relationship to the severity of obstructive sleep apnoea (OSA). In addition, the efficacy of continuous positive airway pressure (CPAP) treatment to influence biomarkers associated with cardiovascular disease was investigated in OSA patients. The study was conducted in two parts; the first demonstrated the severity of OSA and its relation to the different values of the parameters related to the polysomnographic data, anthropometric data (physical characters), blood rheological parameters, and haematological parameters. The relationship between several polysomnographic variables and important blood rheological parameters were also investigated. For the second part of the study, the blood rheological and haematological parameters, following a single night of CPAP therapy were compared with baseline (pre-therapy) values to explore the acute effects of CPAP on these dependent variables.

4.1 Part 1

4.1.1 OSA Severity and demographic, clinical, and polysomnographic parameters:

1. OSA severity and physical characteristics:

The general anthropometric characters of the patients under study are presented in Table 1. There were no differences in the mean ages, heights, and weights among the different groups. The body mass index increased significantly in severe OSA patients when compared to the moderate group (p<0.05). Neck circumference was significantly increased in patients with severe OSA when compared with mild patients (p<0.05, Table 1).

Table 1: Physical characteristics of patients with mild, moderate and severe obstructive sleep apneoa (OSA)

	APNOEA HYPOPNOEA INDEX		
Physical Characteristics	**MILD (*N=6)***	**MODERATE*(N=4)***	**SEVERE *N=21)***
Gender			
Male	2	2	13
Female	4	2	8
Age (years)	58 ± 12	59 ± 11	62 ± 13
Height(cm)	168.0 ± 9.8	167.2 ± 7.6	169.4 ± 14.6
Weight(kg's)	85.6 ± 14.5	76.7 ± 11.4	109.2 ± 29.3
BMI(kg/m²)	30.5 ± 5.3	27.5 ± 4.3	37.8 ± 8.5[b]
Neck(cm)	39.3 ± 2.4	39.8 ± 2.9	43.7± 6.1[a]

Data are expressed as mean values ± standard deviation. [a], significant difference between severe OSA and mild OSA at p<0.05, [b], significant difference between severe OSA and moderate OSA at p<0.05.

2. OSA severity and polysomnographic data

Selected polysomnographic variables collected during initial diagnostic tests are listed in **Table 2**. A qualitative measure of sleep quality, the Epworth Sleepiness Scale, was not significantly different between groups with mild, moderate, or severe OSA ($p > 0.05$). Quantitative measures of general sleep quality, which included total sleep time, sleep latency, and sleep efficiency were also not significantly different between mild, moderate, or severe OSA ($p > 0.05$). Significant differences were detected however, in polysomnography measurements that interrogate EEG-derived sleep quality; the number of brain arousals per hour (arousal index) was significantly increased in severe OSA when compared with both mild and moderate cases ($p < 0.05$). Moreover, a measure that explores the number of substantial oxygen desaturations per hour (i.e., oxygen desaturation index) was significantly increased in severe cases when compared with mild and moderate OSA groups ($p < 0.05$, **Table 2**).

Table 2: Polysomnographic variables of patients with mild, moderate and severe obstructive sleep apneoa (OSA)

	APNOEA HYPOPNOEA INDEX		
SLEEP DATA	**MILD (*N=6*)**	**MODERATE *(N=4)***	**SEVERE *(N=21)***
ESS	12 ± 6	17 ± 4	10 ± 6
TST(min)	366 ± 82	328 ± 89	279 ± 89
Sleep Latency(min)	19 ± 28	26 ± 38	22 ± 23
Sleep Efficiency (%)	82 ± 13	79 ± 11	66 ± 19
Arousal Index(/hr)	19 ± 5	26 ± 5	46 ± 19[a, b]
ODI(/Hr)	2.0 ± 1	8 ± 6	45 ± 25[a, b]

Data are expressed as mean values ± standard deviation. ESS, Epworth Sleepiness Score; TST, Total sleep time; ODI, Oxygen desaturation index – the number of times per hour oxygen saturation dropped 3% or greater; [a], significant difference between severe OSA and mild OSA at $p<0.05$, [b], significant difference between severe OSA and moderate OSA at $p<0.05$.

3. OSA severity and blood rheology parameters

a. Red Blood Cell Aggregation

Measurement of aggregation parameters was performed at native and standardised (i.e., 0.4 L/L) haematocrit (**Table 3**). Parameters that describe the magnitude of RBC aggregation (i.e., M0, M1, AI120) were not significantly different between the three groups when measured at native or standardised haematocrit ($p > 0.05$). Additionally, there was no difference in the half **.**

Table 3: Aggregation of red blood cells at native and standardised haematocrit.

Aggregation	APNOEA HYPOPNOEA INDEX		
	MILD *(N=6)*	**MODERATE** *(N=4)*	**SEVERE** *(N=21)*
Native haematocrit			
M_0	11.8 ± 5.5	14.8 ± 4.5	14.6 ± 5.1
M_1	20.6 ± 6.2	24.0 ± 8.1	24.2 ± 7.2
T½	10.6 ± 2.9	8.3 ± 3.9	7.5 ± 2.6
AI_{120}	81.4 ± 2.7	82.7 ± 4.0	83.5 ± 2.6
Standardised haematocrit			
M_0	12.4 ± 4.6	15.0 ± 2.9	15.9 ± 4.6
M_1	20.9 ± 6.3	24.5 ± 5.1	26.3 ± 6.9
T½	10.1 ± 2.8	8.1 ± 3.4	7.3 ± 2.9
AI_{120}	81.5 ± 2.4	83.4 ± 2.4	84.3 ± 2.6

Data are expressed as mean values ± standard deviation. M_0: red blood cell (RBC) aggregation after 10 s at stasis. M_1: RBC aggregation after 10 s at 3 sec⁻¹. T½: Half the time it takes for RBC aggregation to occur. AI_{120}: RBC aggregation after 120 seconds at stasis.

b. Blood viscosity

Table 4 depicts the mean values of blood viscosity at native and standardised haematocrit to 0.4 L/L. Both the native and standardised viscosities were assessed at the following shear rates: 75, 150, 300, 750, 1500. All disease severities showed decreases in the suspension viscosities with increased shear rate for both the native and standardised viscosities. Regarding the standardised viscosity at 0.4 L/L, again, patients with moderate OSA showed a marked reduction from the rate 150 (5.81 ± 2.07 mPa·s) to 300 (4.03 ± 1.62 mPa·s). Decline patterns were interchangeable between different disease severities. Nonetheless, there was a significant difference between the mean value of blood viscosity in severe OSA patients (4.15 ± 0.58 mPa·s) and that in the moderate OSA patients at the shear rate 1500 (3.13 ± 0.42 mPa·s, **Table 4**, $p<0.05$).

Table 4: Viscosity of RBC suspensions at native haematocrit and standardised to 0.4 L/L haematocrit of patients with mild, moderate and severe obstructive sleep apneoa (OSA).

Shear Rate	**MILD** ***(N=5)***	**MODERATE** ***(N=4)***	**SEVERE** ***(N=18)***
Native haematocrit			
75(sec^{-1})	6.6 ± 2.1	6.8 ± 2.0	6.7 ± 1.2
150(sec^{-1})	5.5 ± 1.4	4.6 ± 0.5	5.6 ± 1.0
300(sec^{-1})	4.6 ± 0.8	4.3 ± 1.0	4.9 ± 0.6
750(sec^{-1})	3.9 ± 0.8	3.6 ± 0.3	4.2 ± 0.6
1500(sec^{-1})	3.9 ± 0.3	4.0 ± 0.3	4.3 ± 0.7
Standardised haematocrit			
75(sec^{-1})	6.9 ± 2.0	6.3 ± 2.4	6.3 ± 1.3
150(sec^{-1})	5.1 ± 0.5	5.8 ± 1.2	5.2 ± 0.8
300(sec^{-1})	4.2 ± 0.2	4.0 ± 1.6	4.8 ± 0.6
750(sec^{-1})	3.8 ± 0.2	3.6 ± 0.8	4.1 ± 0.8
1500(sec^{-1})	3.7 ± 0.5	3.1 ± 0.4	4.2 ± 0.6[a]

Data are expressed as mean values ± standard deviation. [a], significant difference between severe OSA and moderate OSA at $p<0.05$.

c. Red blood cell deformability

RBC deformability (as Elongation index) measured via laser ektacytometry for mild, moderate, and severe OSA is presented in Figure 8. In general, the deformability increased strongly and approximately in an identical linear pattern with increased shear stresses until reaching 10 Pa, where the strong increases were declined slightly (Figure 8). There were no significant differences in RBC deformability of the three OSA groups ($p > 0.05$).

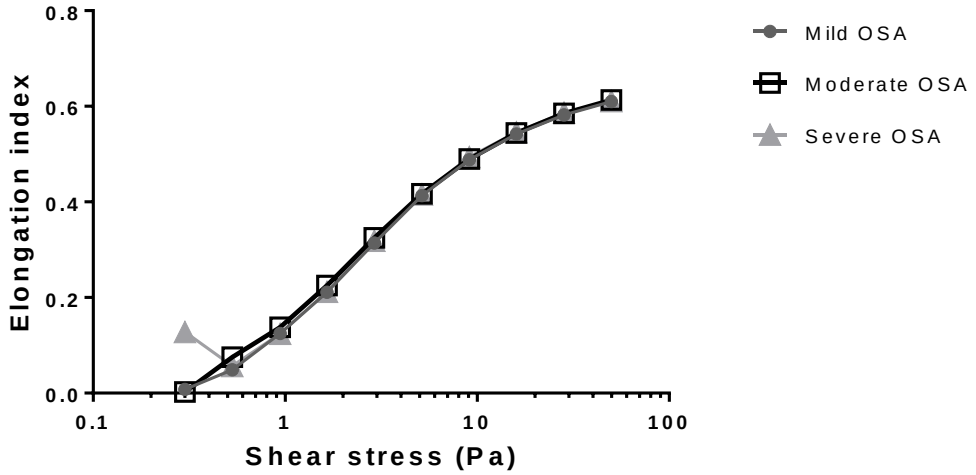


Figure 8: The elongation index (RBCs deformability) determined at different shear stresses in patients with mild, moderate, and severe obstructive sleep apnoea (OSA).

4. OSA severity and haematological parameters

Haematological parameters of the patients are presented in **Table 5**. The mean values of plasma fibrinogen increased significantly ($p<0.05$) in severe OSA patients (3.35 ± 0.62 g/L) when compared with mild (2.97 ± 0.90 g/L) and moderate patients (2.89 ± 0.92 g/L). Indices related to glycaemic control indicated that while there was no significant difference between insulin levels in severe and moderate OSA, there was a significant increase in the severe group when compared to the mild group (21.94 ± 12.77 mU/L vs 7.50 ± 5.20 mU/L respectively, $p<0.05$, **Table 5**). Blood glucose concentration in patients with severe OSA was significantly increased when compared to patients with moderate OSA, although no significant difference was observed relative to mild OSA. Glycated haemoglobin (HbA1c) were significantly increased ($p<0.05$) in patients with severe OSA (42.94 ± 12.22 mmol/L) compared with moderate OSA (34.25 ± 2.06 mmol/L). Despite the lack of significant difference in LDL levels between moderate and mild OSA patients, severe OSA group had significantly decreased LDL when compared to moderate OSA patients (2.91 ± 1.00 vs 4.34 ± 0.41 mmol/L respectively, **Table 5**, $p<0.05$). Other lipid profile parameters, including HDL, cholesterol, and triglycerides, were not statistically different among patients with different OSA severities. Red blood cell count, haematocrit, haemoglobin, mean cell volume and mean cell haemoglobin were not significantly different between the three severity groups of OSA patients. Total white blood cells count, neutrophils, lymphocytes, monocytes, eosinophils basophils and platelet counts were also not different between OSA groups (**Table 5**).

Table 5: Haematology results of patients with mild, moderate and severe OSA.

	MILD (*N*=5)	**MODERATE (*N*=4)**	**SEVERE (*N*=16)**	**F**
Plasma Fibrinogen(g/L)	2.97 ± 0.90	2.89 ± 0.92	3.35 ± 0.62[a,b]	1.14
Insulin(mU/L)	7.50 ± 5.20	11.00 ± 5.48	21.94 ± 12.77[a]	3.49
Glucose(mmol/L)	5.05 ± 0.10	4.33 ± 0.54	6.01 ± 1.42[b]	3.7
LDL(mmol/L)	3.07 ± 1.14	4.34 ± 0.41	2.91 ± 1.00[b]	3.66
HDL(mmol/L)	1.77 ± 0.52	1.50 ± 0.39	1.21 ± 0.38	3.47
HbA1c SI(mmol/L)	36.75 ± 2.06	34.25 ± 2.06	42.94 ± 12.22[b]	1.43
Chol(mmol/L)	5.90 ± 0.52	6.23 ± 0.74	5.05 ± 1.09	2.9
Chol/HDL (mmol/L)	3.52 ± 0.90	4.53 ± 1.82	4.41 ± 1.21	1.05
Trig(mmol/L)	1.40 ± 0.76	1.73 ± 0.88	1.61 ± 1.00	0.34
HB(g/L)	140.25 ± 7.37	148.75 ± 11.41	146.25 ± 16.45	0.85
RBC($x10^{12}$/L)	4.68 ± 0.40	4.73 ± 0.21	5.00 ± 0.59	1.43
HCT/Haematocrit (%)	0.42 ± 0.29	0.45 ± 0.40	0.45 ± 0.48	0.59
Mean cell volume (fL)	92.40 ± 5.68	94.25 ± 5.74	89.25 ± 3.98	2.33
Mean cell haemoglobin (pg)	30.20 ± 1.10	31.50 ± 1.29	29.38 ± 1.50	3.81
Platelet($x10^{9}$/L)	195.86 ± 77.39	249.25 ± 38.59	247.13 ± 53.69	0.16
WBC($x10^{9}$/L)	7.10 ± 2.41	7.45 ± 1.37	7.32 ± 1.44	0.69
Neutrophils($x10^{9}$/L)	3.63 ± 1.22	3.60 ± 1.19	4.14 ± 1.25	0.45
Lymphocytes($x10^{9}$/L)	2.43 ± 0.94	2.78 ± 1.04	2.22 ± 0.60	0.42
Monocytes($x10^{9}$/L)	0.53 ± 0.15	0.68 ± 0.15	0.62 ± 0.15	0.27
Eosinophils($x10^{9}$/L)	0.41 ± 0.51	0.38 ± 0.27	0.29 ± 0.24	0.76
Basophils($x10^{9}$/L)	0.06 ± 0.05	0.03 ± 0.38	0.05 ± 0.04	0.67

Data are expressed as mean values ± standard deviation. [a], a significant difference between severe OSA and mild OSA at $p<0.05$, [b], significant difference between severe OSA and moderate OSA at $p<0.05$.

Relationship between the polysomnographic variables and blood rheological and haematological parameters:

4.1.2 Apnoea hypopnoea index (AHI)

The relationships between sleep quality (AHI) and several blood rheological and haematological parameters are listed in **Table 6**.

Table 6: Pearson's Correlation between apnoea-hypopnoea index (AHI) and various rheological and haematological parameters.

		Pearson's r	Significance (2-tailed)
RBC Aggregation			
Native haematocrit			
	M_0	0.378	0.036*
	M_1	0.300	0.101
	T½	-0.386	0.032*
	AI_{120}	0.316	0.083
Standardised haematocrit (40%)			
	M_0	0.415	0.020*
	M_1	0.376	0.037*
	T½	-0.361	0.050*
	AI_{120}	0.406	0.026*
Whole Blood Viscosity			
Native haematocrit			
	75(sec^{-1})	-0.146	0.497
	150(sec^{-1})	0.017	0.938
	300(sec^{-1})	0.017	0.938
	750(sec^{-1})	0.000	0.998
	1500(sec^{-1})	0.051	0.811
Standardised haematocrit (40%)			
	75(sec^{-1})	-0.124	0.572
	150(sec^{-1})	-0.204	0.351
	300(sec^{-1})	0.108	0.623
	750(sec^{-1})	-0.155	0.48
	1500(sec^{-1})	0.016	0.944
RBCs Deformability	SS½:EI	0.028	0.89
Other	Haematocrit	0.271	0.19
	Hct	0.271	0.19
	Platelets	-0.381	0.06
	Plasma fibrinogen	0.197	0.344

**. Correlation is significant at the 0.05 level (2-tailed). SS½:EI : Elongation index ratio; M0: red blood cell (RBC) aggregation after 10 s at stasis. M1: RBC aggregation after 10 s at 3 sec ⁻¹. T½: Half the time it takes for RBC aggregation to occur. AI120: RBC aggregation after 120 s at stasis.*

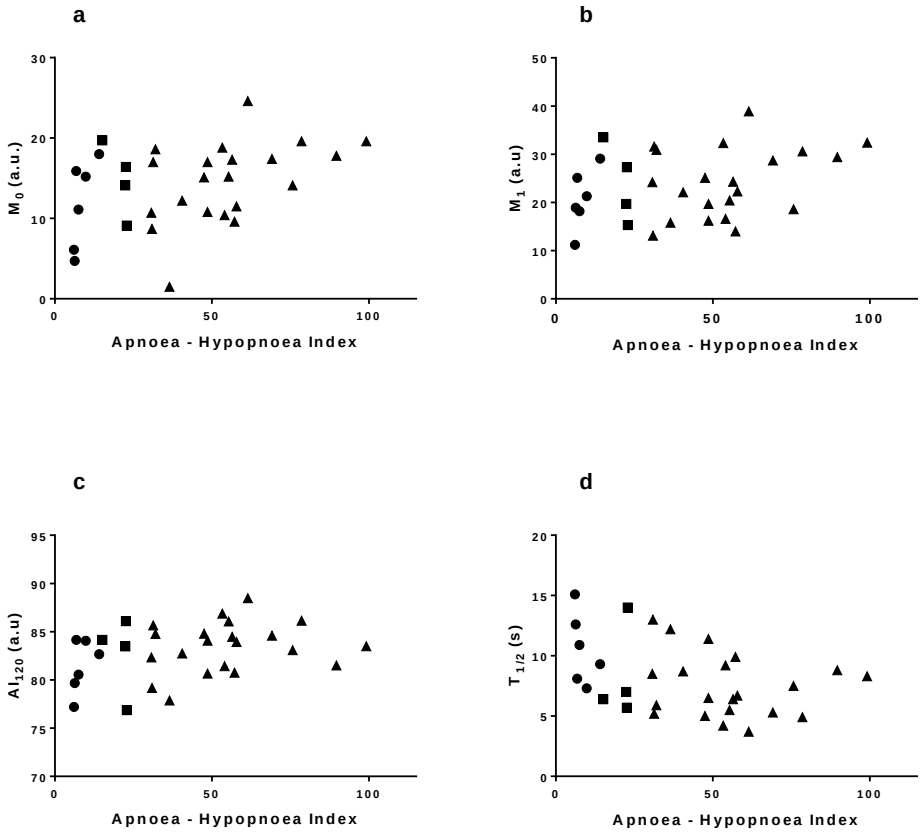
1. Aggregation

The relationship between AHI and parameters of red cell aggregation in OSA patients is demonstrated in Figure 9. There was a statistically significant correlation between AHI and RBCs aggregation measured at stasis (i.e., M0) (P<0.05, Figure 9a, Table 6). Similar observations were made in the severe OSA patients when RBC aggregation was assessed at

low shear (Figure 9b, Table 6). There was a significant negative correlation between the aggregation half time (T½) and AHI with disease severity (Figure 9d, Table 6).

Figure 10 illustrates the relationship between AHI and red cell aggregation standardised to 0.4 L/L haematocrit. The severity of OSA yielded marked increases in the AHI index.

A strong positive correlation between AHI and RBC aggregation (when standardised at 40% haematocrit) after 10 seconds at stasis in patients with severe OSA despite the presence of an outlier (Figure 10a, Table 6). The relationship was also strong in severe cases between AHI and RBC aggregation after 10 seconds at a shear rate 3 sec^{-1} (Figure 10b, Table 6), and after 120 seconds at stasis (Figure 10c). Aggregation half-time appeared to be significantly shorter with increased AHI in severe OSA patients as shown in Figure 10d and Table 6.



● = mild OSA, ■ = moderate OSA, ▲ = severe OSA

Figure 9: The relationship between Apnoea hypopnoea index (AHI) and red blood cell aggregation after 10 s at stasis(a), after 10 s at 3 secs ⁻¹ (b), after 120 s at stasis (c), and aggregation halftime (d) in patients with different severities of obstructive sleep apnoea (OSA).

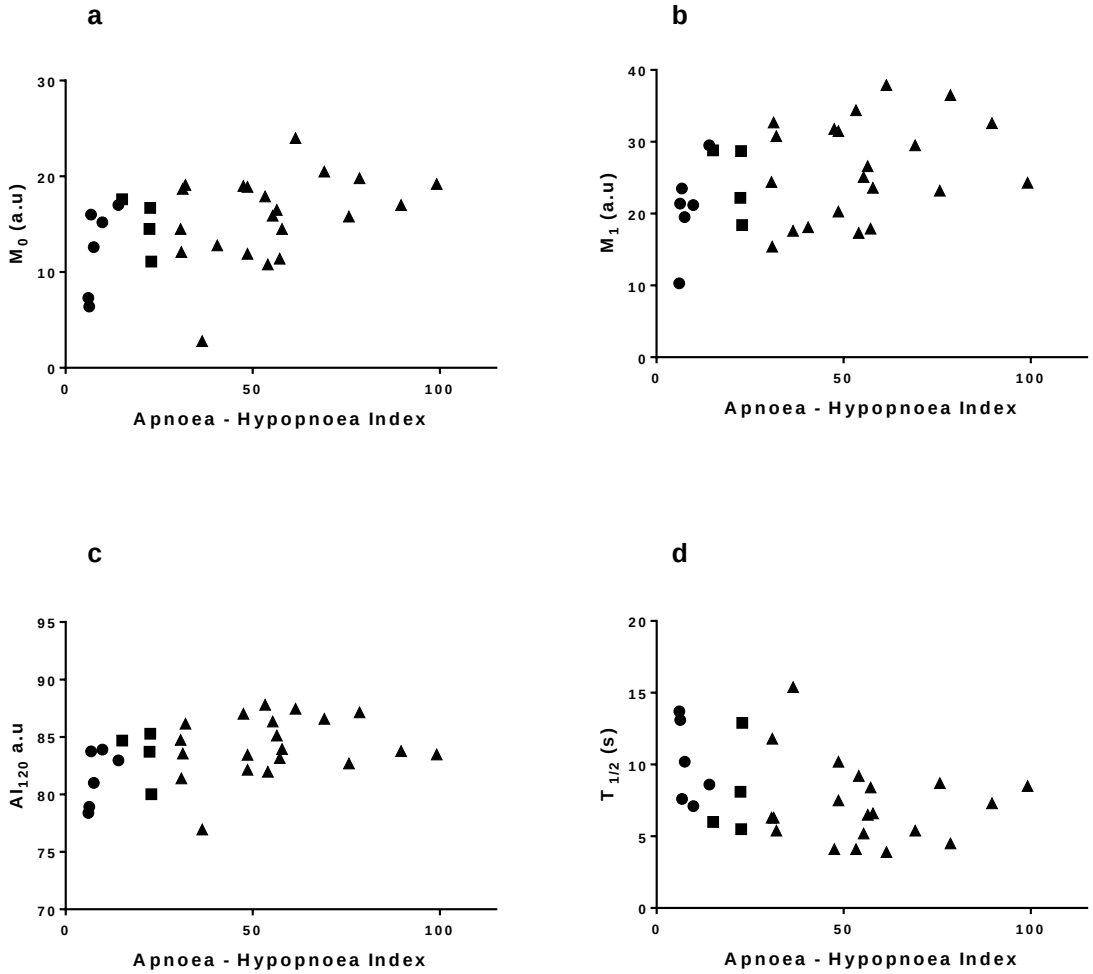
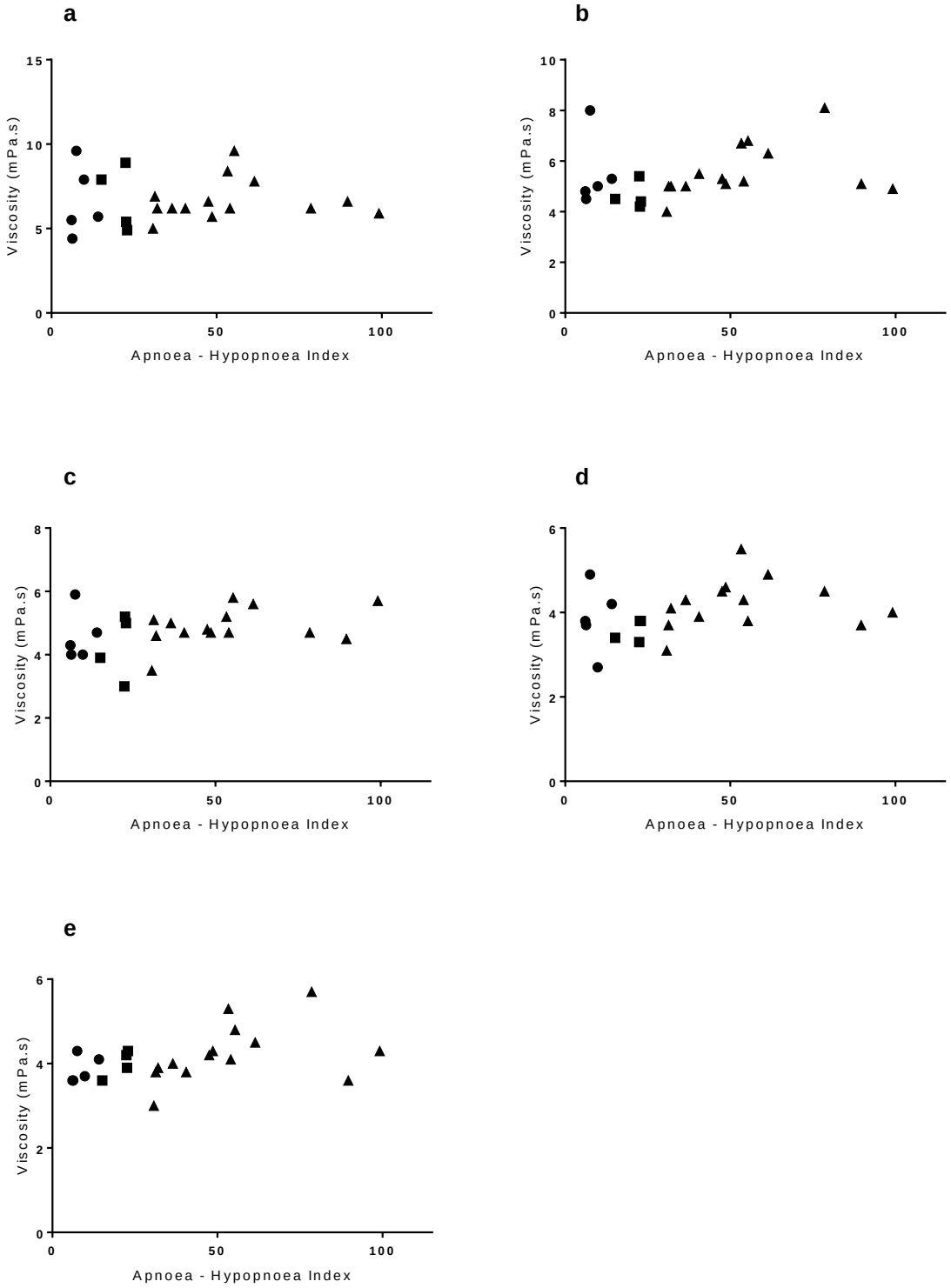


Figure 10: The relationship between Apnoea-hypopnoea index (AHI) and the standardised measurement of red blood cell aggregation at 40% haematocrit after 10 s at stasis(a), after 10 s at 3 secs ⁻¹ (b), after 120 s at stasis (c), and aggregation halftime (d) in patients with different severities of obstructive sleep apnoea (OSA).

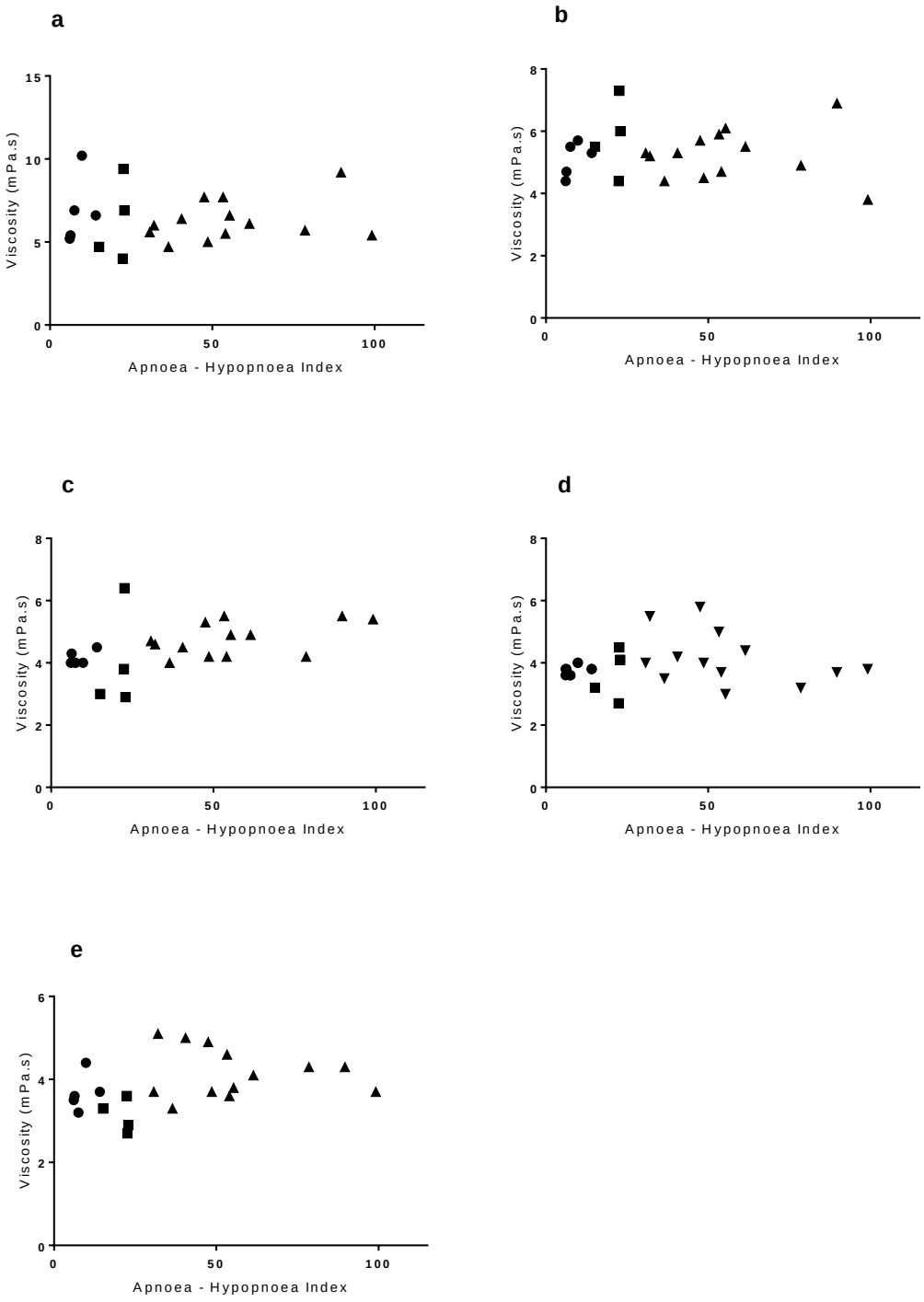
2. Viscosity

The correlation between AHI and plasma viscosity is shown in Figure 11. Generally, there was no significant correlation between AHI and plasma viscosity at different shear rates (Figure 11, Table 6). Similarly, the correlation was not evident between AHI and different shear rates of standardised plasma viscosity (Figure 12, Table 6).



● = mild OSA, ■ = moderate OSA, ▲ = severe OSA

Figure 11: The relationship between Apnoea hypopnoea index (AHI) and blood viscosity of red blood cell in patients with mild, moderate and severe obstructive sleep apnoea (OSA) at the following shear rates: 75 (a), 150 (b), 300 (c), 750 (d), 1500 (e).

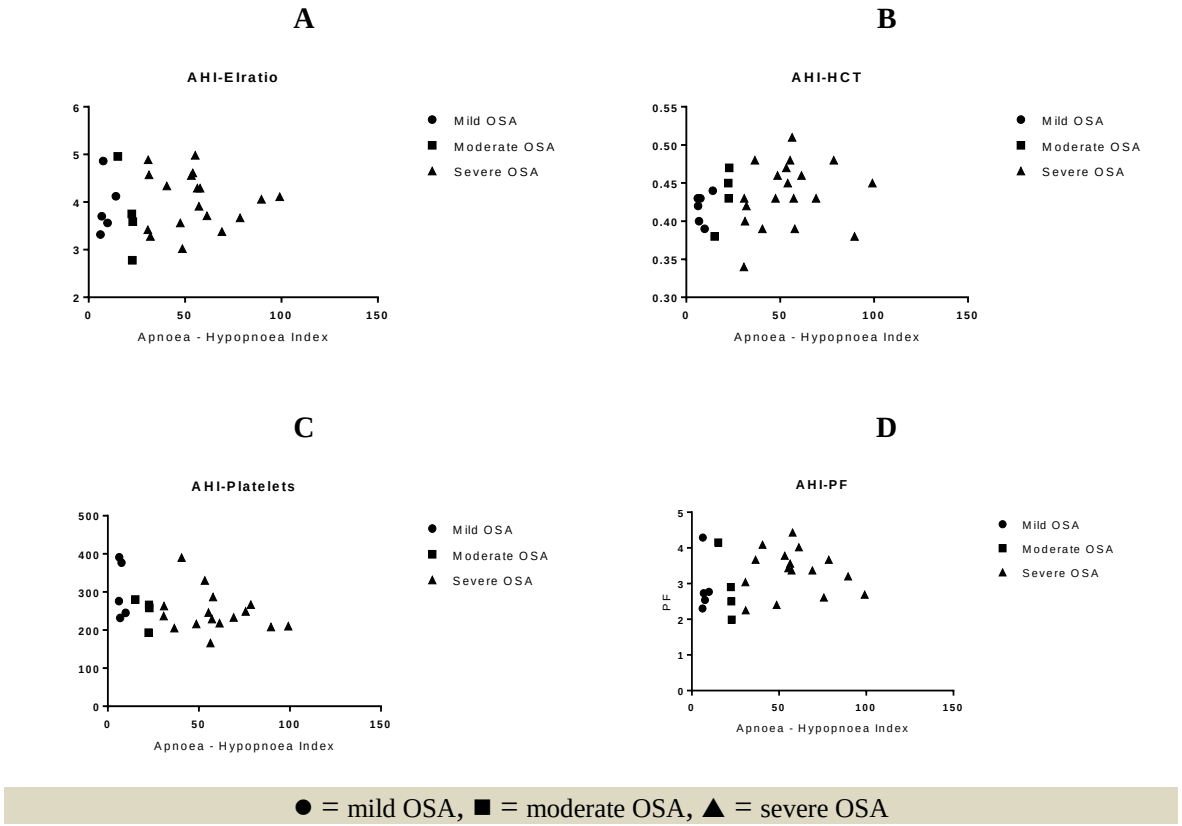


● = mild OSA, ■ = moderate OSA, ▲ = severe OSA

Figure 12: The relationship between apnoea hypopnoea index (AHI) and blood viscosity of red blood cell at standardised 40% haematocrit in patients with mild, moderate and severe obstructive sleep apnoea (OSA) at the following shear rates: 75 (a), 150 (b), 300 (c), 750 (d), 1500 (e).

3. Other Haemorheological parameters

Figure 13 presents the relationship between AHI and elongation index, haematocrit, platelets, and plasma fibrinogen. There was no correlation between AHI and red cell deformability, represented as the elongation index, as shown in Figure 13A. Patients with both mild and moderate symptoms had AHI below 25, while severely affected patients showed irregular patterns in their deformability values (Figure 13A). There was no correlation between haematocrit values and AHI index in mild, moderate, and severe groups.



● = mild OSA, ■ = moderate OSA, ▲ = severe OSA

Figure 13: The relationship between apnoea hypopnoea index (AHI) and elongation index (A), haematocrit (B), platelets (C), and plasma fibrinogen (D) in patients with mild, moderate and severe obstructive sleep apnoea (OSA).

Patients with mild and moderate OSA had no changes in their platelet values with increasing AHI (Figure 13C). On the other hand, there was a negative correlation between AHI and blood platelets in severely affected patients (Figure 13C, Table 6). Plasma fibrinogen demonstrated no significant correlation with AHI in severe OSA patients as illustrated in (Figure 13D).

4.1.3 Oxygen desaturation index (ODI)

Table 7: Pearson's Correlation between oxygen desaturation index and various rheological and haematological parameters.

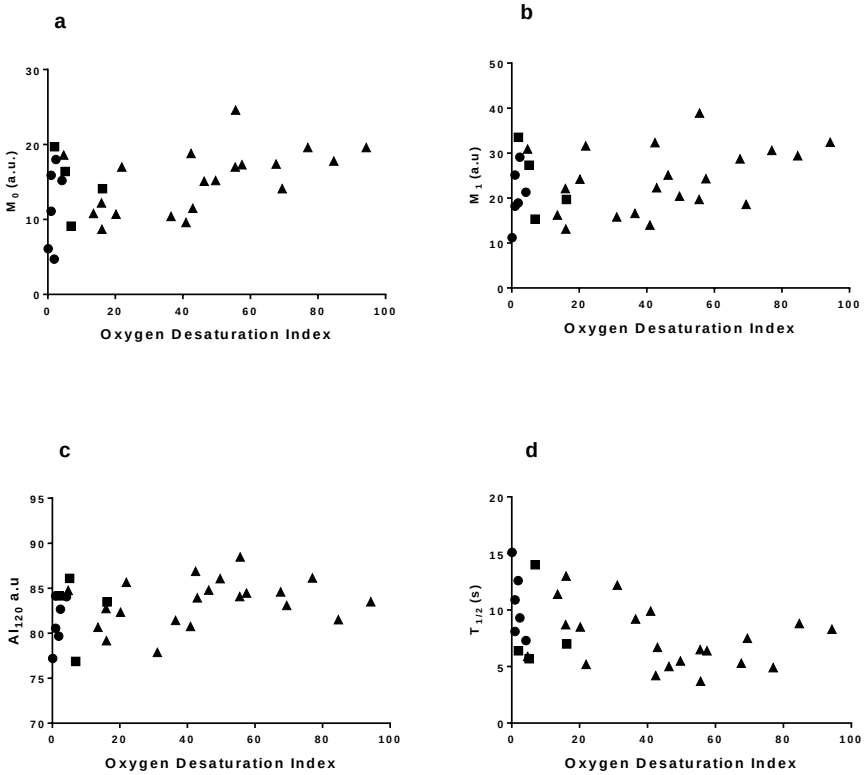
		Pearson's r	Significance (2-tailed)
RBCs Aggregation			
Native haematocrit	M_0	0.399	0.026*
	M_1	0.313	0.086
	T½	-0.421	0.018*
	AI_{120}	0.345	0.058
Standardised haematocrit(40%)	M_0	0.442	0.013*
	M_1	0.430	0.016*
	T½	-0.363	0.049*
	AI_{120}	0.396	0.030*
Whole Blood Viscosity			
Native haematocrit	75	-0.124	0.565
	150	0.023	0.914
	300	-0.032	0.881
	750	-0.067	0.757
	1500	0.013	0.951
Standardised haematocrit(40%)	75	-0.132	0.547
	150	-0.241	0.268
	300	0.064	0.77
	750	-0.240	0.269
	1500	-0.050	0.819
RBCs Deformability	SS½:EI	0.069	0.734
Other	Haematocrit	0.303	0.141
	Hct	0.292	0.125
	Platelets	-0.452	0.023*
	Plasma fibrinogen	0.157	0.453

**. Correlation is significant at the 0.05 level (2-tailed). SS½:EI : Elongation index ratio; M0: red blood cell (RBC) aggregation after 10 s at stasis. M1: RBC aggregation after 10 s at 3 sec ⁻¹. T½: Half the time it takes for RBC aggregation to occur. AI120: RBC aggregation after 120 s at stasis.*

1. Aggregation

Figure 14 shows the correlation between ODI and red cell aggregation in OSA patients. Pearson's correlation and significance for several haematological and rheological parameters are shown in Table 7.There was a significant positive correlation between ODI and red cell aggregation after 10 seconds at stasis ($P<0.05$, Table 7) in severe OSA cases, while the correlation was not apparent in mild and moderate OSA patients. Other After RBC aggregation parameters (AI120, M1) was were not influenced by ODI (Figure 14B, Figure 14C, Table 7). In severe OSA patients, the aggregation half time was decreased

significantly with increasing ODI (P<0.05, Figure 14D, Table 7). Patients with mild and moderate OSA showed varied aggregation half times at low ODI values (Figure 14D, Table 7).



● = mild OSA, ■ = moderate OSA, ▲ = severe OSA

Figure 14: The relationship between oxygen desaturation index (ODI) and red blood cell aggregation after 10 s at stasis (A), after 10 s at 3 secs ⁻¹ (B), after 120 s at stasis (C), and aggregation half time (D) in patients with different severities of obstructive sleep apnoea (OSA).

The relationship between ODI and red cell aggregation in OSA patients is illustrated in (Figure 15). In severe OSA patients, there was a significant correlation between ODI and standardised RBC aggregation after 10 s at stasis (P<0.05, Figure 15A, Table 7). The correlations between standardised aggregation after 10 s at 3 sec^{-1} and ODI and after 120 s at stasis were significantly positive in the severe group (P<0.05 Figure 15B and Figure 15C respectively, Table 7). Aggregation half time was significantly decreased in severe OSA patients with a clear correlation to ODI (P<0.05, Figure 15D, Table 7). The relationships between the standardised aggregation at all measurements, as well as the standardised

aggregation half time and ODI in mild and moderate OSA patients, were absent although the aggregation values were restricted between 0-20 ODI (Figure 15A, B, C, and D).

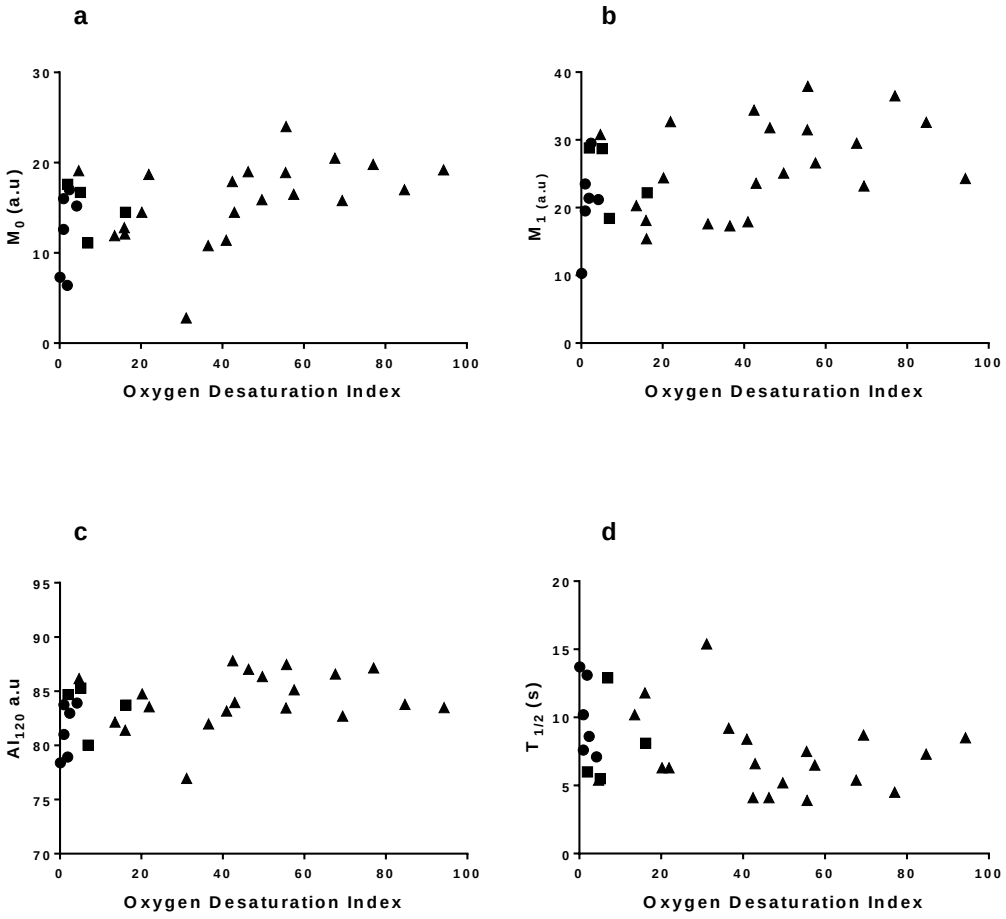
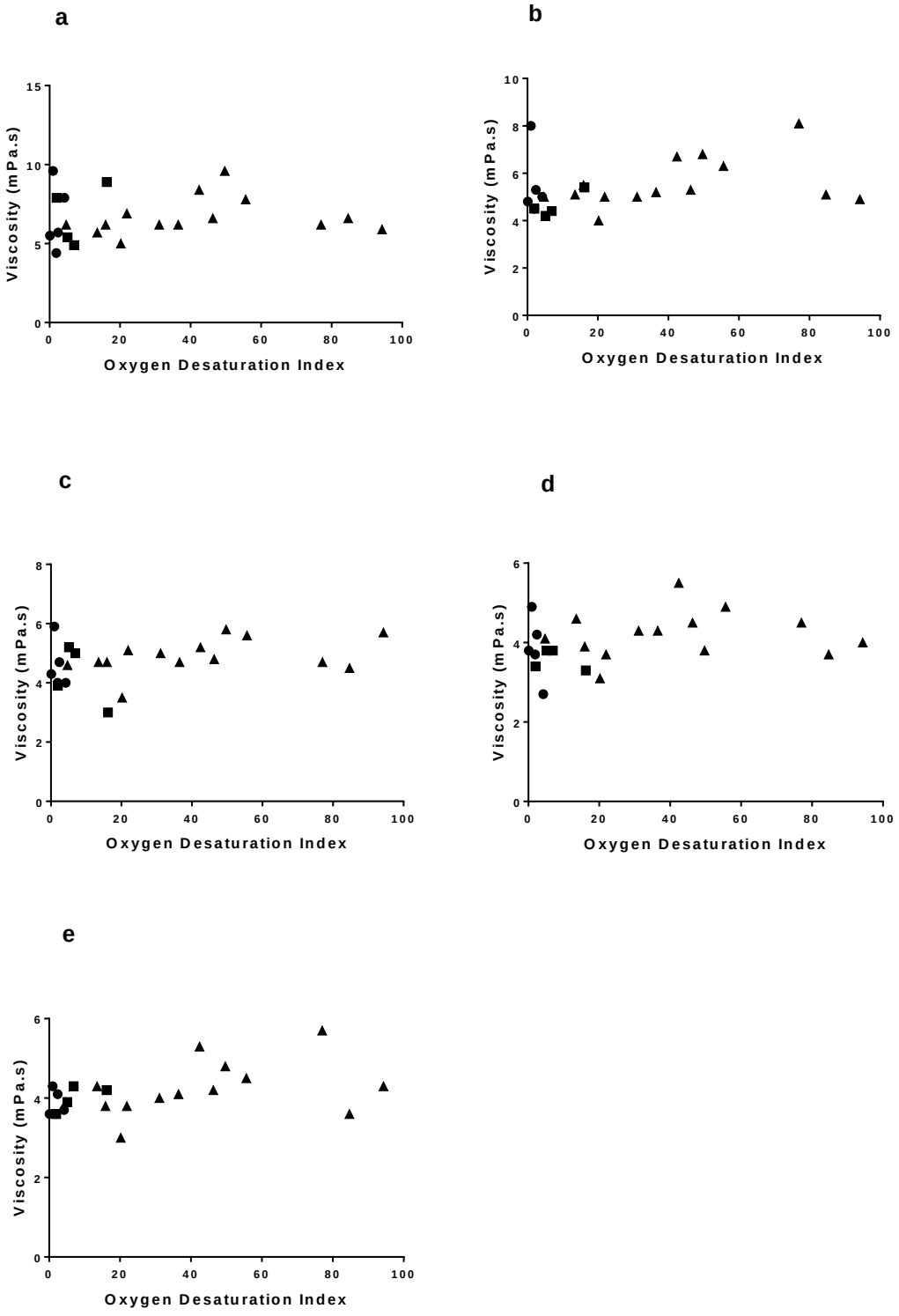


Figure 15: The relationship between oxygen desaturation index (ODI) and the standardised measurement of red blood cell aggregation at 40% haematocrit after 10 s at stasis(A), after 10 s at 3 secs ⁻¹ (B), after 120 s at stasis (C), and aggregation half time (D) in patients with different severities of obstructive sleep apnoea (OSA).

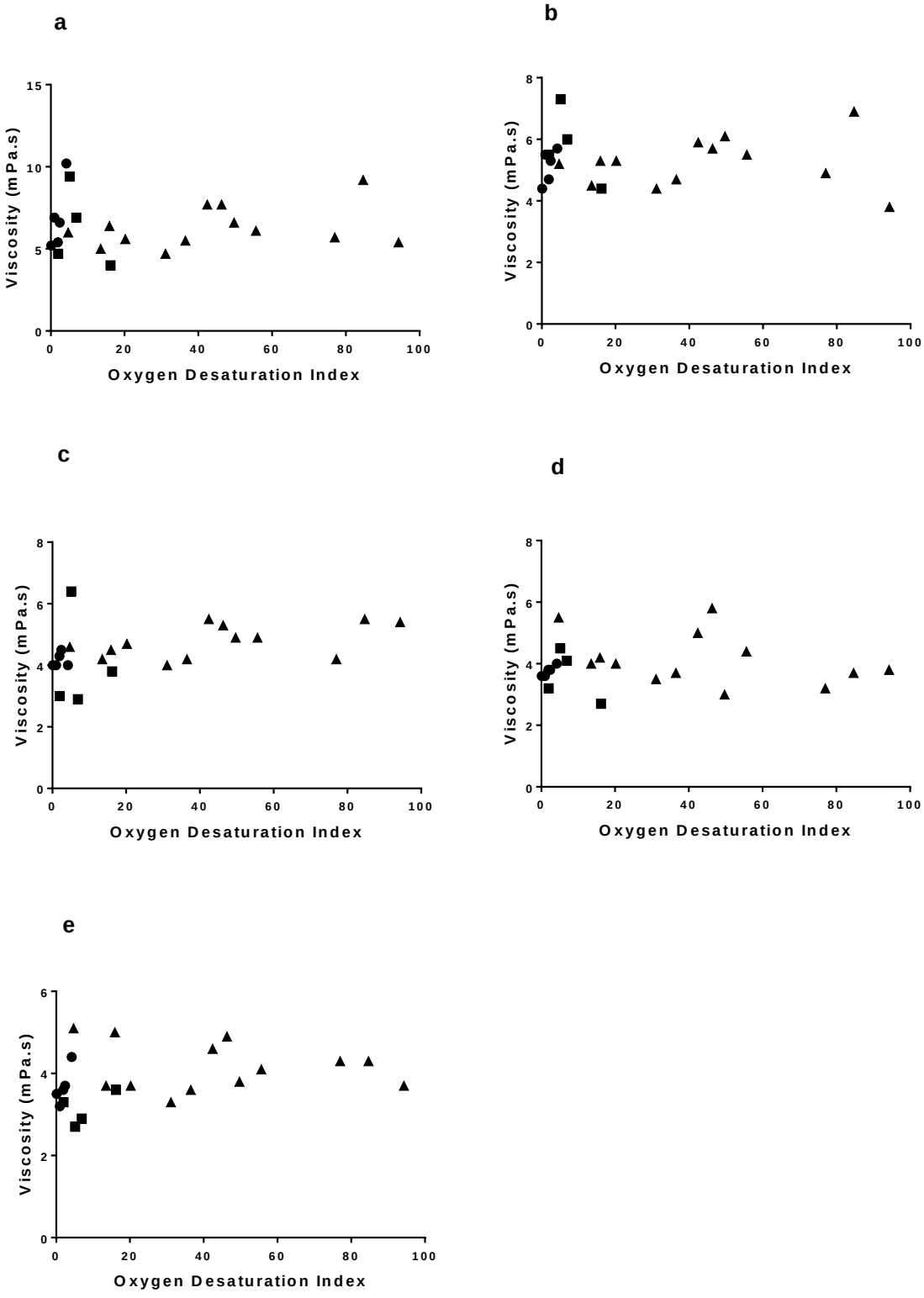
2. Viscosity

The relationship between ODI and plasma viscosity is demonstrated at a shear rate of 750 in Figure 16. The patients with mild, moderate, and severe OSA showed no correlation between those parameters (Figure 16). The relationship between ODI index and standardised plasma viscosity is shown in Figure 17. A general observation in this category is that there was no correlation between plasma viscosity and ODI among the different degrees of OSA severity as shown in Figure 17.



● = mild OSA, ■ = moderate OSA, ▲ = severe OSA

Figure 16: The relationship between oxygen desaturation index (ODI) and blood viscosity of red blood cells in patients with mild, moderate and severe obstructive sleep apnoea (OSA) at the following shear rates: 150 (a), 300 (b), 750 (c) 750 (d), 1500 (e).

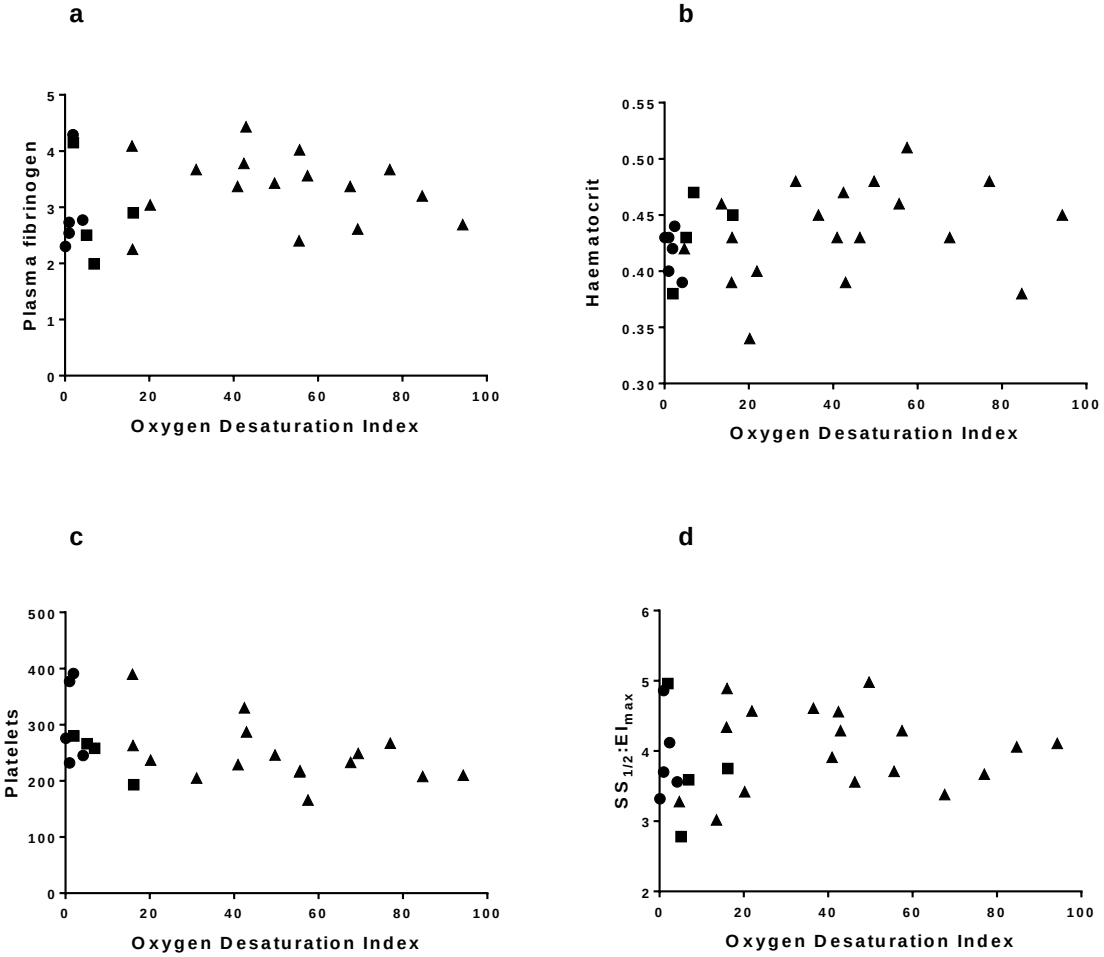


● = mild OSA, ■ = moderate OSA, ▲ = severe OSA

Figure 17: The relationship between oxygen desaturation index (ODI) and blood viscosity of red blood cells at standardised 40% haematocrit in patients with mild, moderate and severe obstructive sleep apnoea (OSA) at the following shear rates: 75 (A), 150 (B), 300 (C), 750 (D), 1500 (E).

3. Other Haemorheological parameters

Figure 18 presents the relationships between ODI and some blood parameters in OSA patients. No correlation was shown between RBC deformability and ODI in different OSA severities (Figure 18d). There was a slight tendency for haematocrit values, which ranged from 32-52%, to be increased with increasing ODI indices (Figure 18b).



● = mild OSA, ■ = moderate OSA, ▲ = severe OSA

Figure 18: The relationship between oxygen desaturation index (ODI) and plasma fibrinogen (a), haematocrit (b), platelets (c), and SS1/2: EImax ratio (d) in patients with mild, moderate and severe obstructive sleep apnoea (OSA).

4.1.4 Arousal index (AI)

Table 8: Pearson's Correlation between oxygen desaturation index and various rheological and haematological parameters.

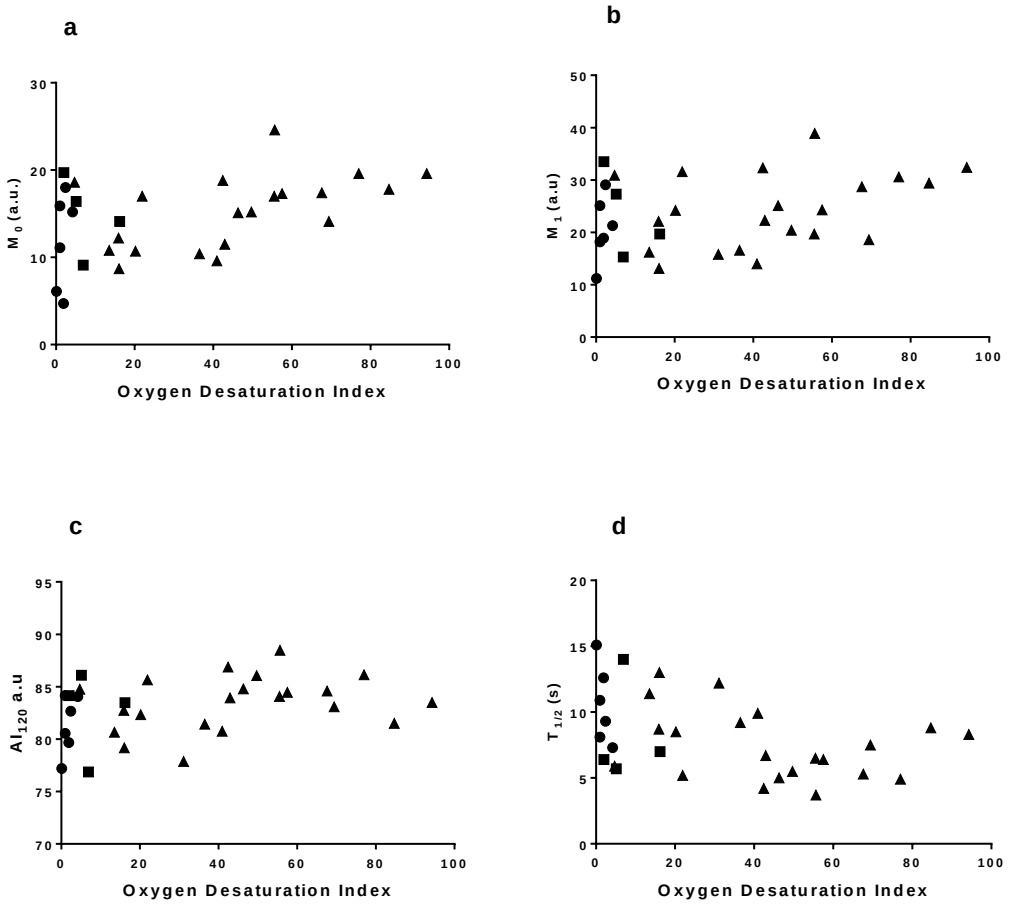
	Parameter	Pearson's r	Significance (2-tailed)
RBCs Aggregation	**M_0**	0.339	0.062
	M_1	0.231	0.211
	T½	-0.257	0.163
	AI_{120}	0.200	0.281
	M_0f	0.381	0.035*
	M_1 f	0.282	0.124
	T½ f	-0.277	0.138
	AI_{120}f	0.316	0.089
Whole Blood Viscosity	**75**	-0.201	0.346
	150	-0.064	0.766
	300	-0.084	0.697
	750	-0.120	0.577
	1500	-0.078	0.719
	75f	-0.126	0.567
	150f	-0.224	0.303
	300f	0.062	0.778
	750f	-0.240	0.270
	1500f	-0.081	0.713
RBCs Deformability	**EI-Ratio**	-0.016	0.938
Other	**Haematocrit**	0.133	0.527
	Hct	0.108	0.575
	Platelets	-0.309	0.133
	Plasma fibrinogen	0.029	0.892

**. Correlation is significant at the 0.05 level (2-tailed). EI-Ratio: Elongation index ratio; f means a standardised haematocrit value. M0: red blood cell (RBC) aggregation after 10 s at stasis. M1: RBC aggregation after 10 s at 3 sec⁻¹. T½: Half the time it takes for RBC aggregation to occur. AI120: RBC aggregation after 120 s at stasis.*

1. Aggregation

The correlation between AI and red cell aggregation is demonstrated in Figure 19. Pearson's correlation and significance for several haematological and rheological parameters are shown in Table 8. No clear relationships were detected between AI and red cell aggregation in mild and moderate OSA patients after 10 seconds at stasis or when

measured at a shear rate of 3 sec^{-1} (Figure 19A and B). There was a negative correlation between red cell aggregation after 120 seconds at stasis and AI index in mild OSA patients until the aggregation reached AI 30, while there was no correlation in moderate and severe patients (Figure 19C). Aggregation half time was longer with increasing AI in mild OSA, while it was shorter with increasing AI in severe OSA until AI 70, where the relationship began to disappear (Figure 19D).

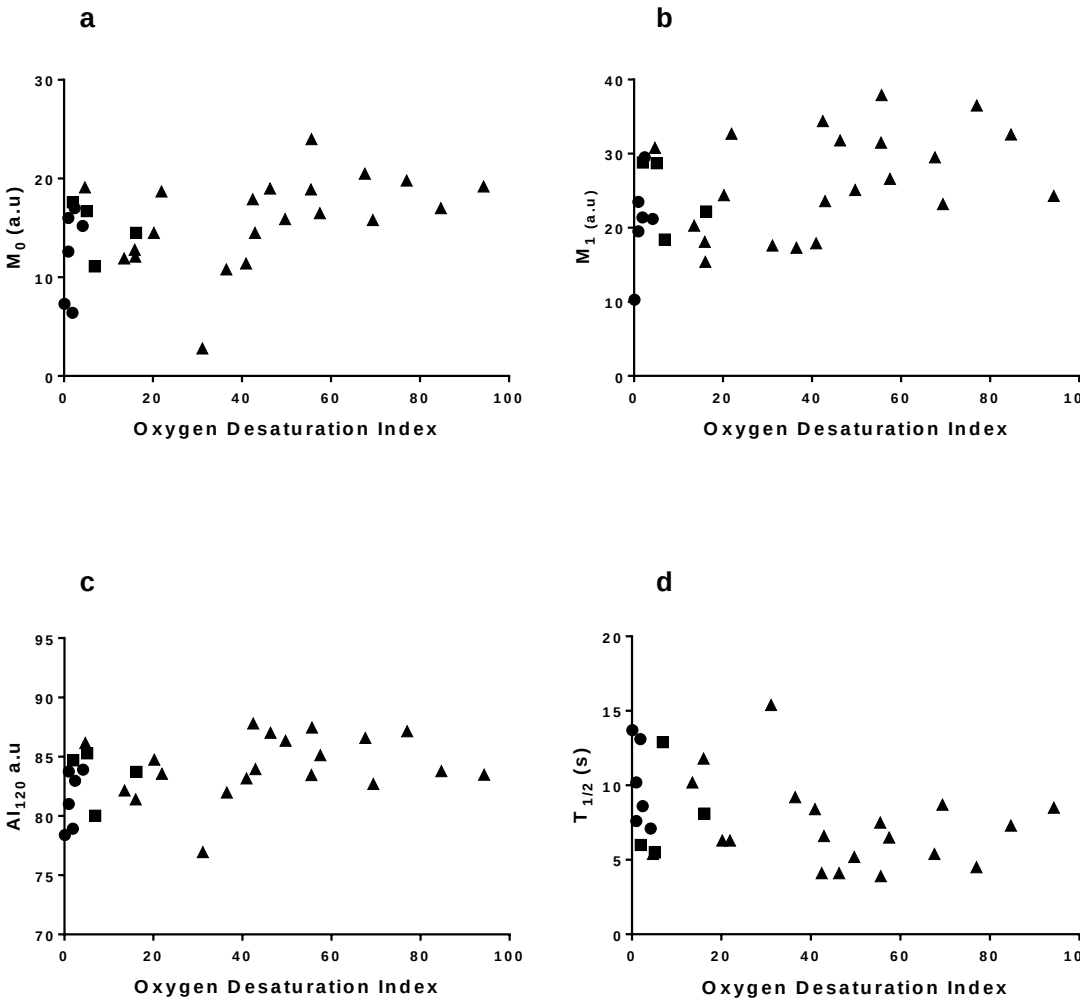


● = mild OSA, ■ = moderate OSA, ▲ = severe OSA

Figure 19: The relationship between arousal index (AI) and red blood cell aggregation after 10 s at stasis (A), after 10 s at 3 sec⁻¹ (B), after 120 s at stasis (C), and aggregation half time (D) in patients with different severities of obstructive sleep apnoea (OSA).

Figure 20 shows the relationship between standardised red cell aggregation at 40% haematocrit and AI. There was a significant positive correlation between both parameters in mild and moderate, and severe OSA cases for aggregation after 10 seconds at stasis ($P<0.05$, Figure 20A, Table 8). Aggregation half time was longer in mild OSA patients with increasing AI index and, on the other hand, it was shorter with increasing AI in moderate

and severe OSA patients (Figure 20D) with an overall negative correlation with AI (Table 8).



● = mild OSA, ■ = moderate OSA, ▲ = severe OSA

Figure 20: The relationship between oxygen desaturation index (ODI) and the standardized measurement of red blood cell aggregation at 40% haematocrit after 10 s at stasis(A), after 10 s at 3 secs⁻¹ (B), after 120 s at stasis (C), and aggregation half time (D) in patients with different severities of obstructive sleep apnoea (OSA).

2. Viscosity

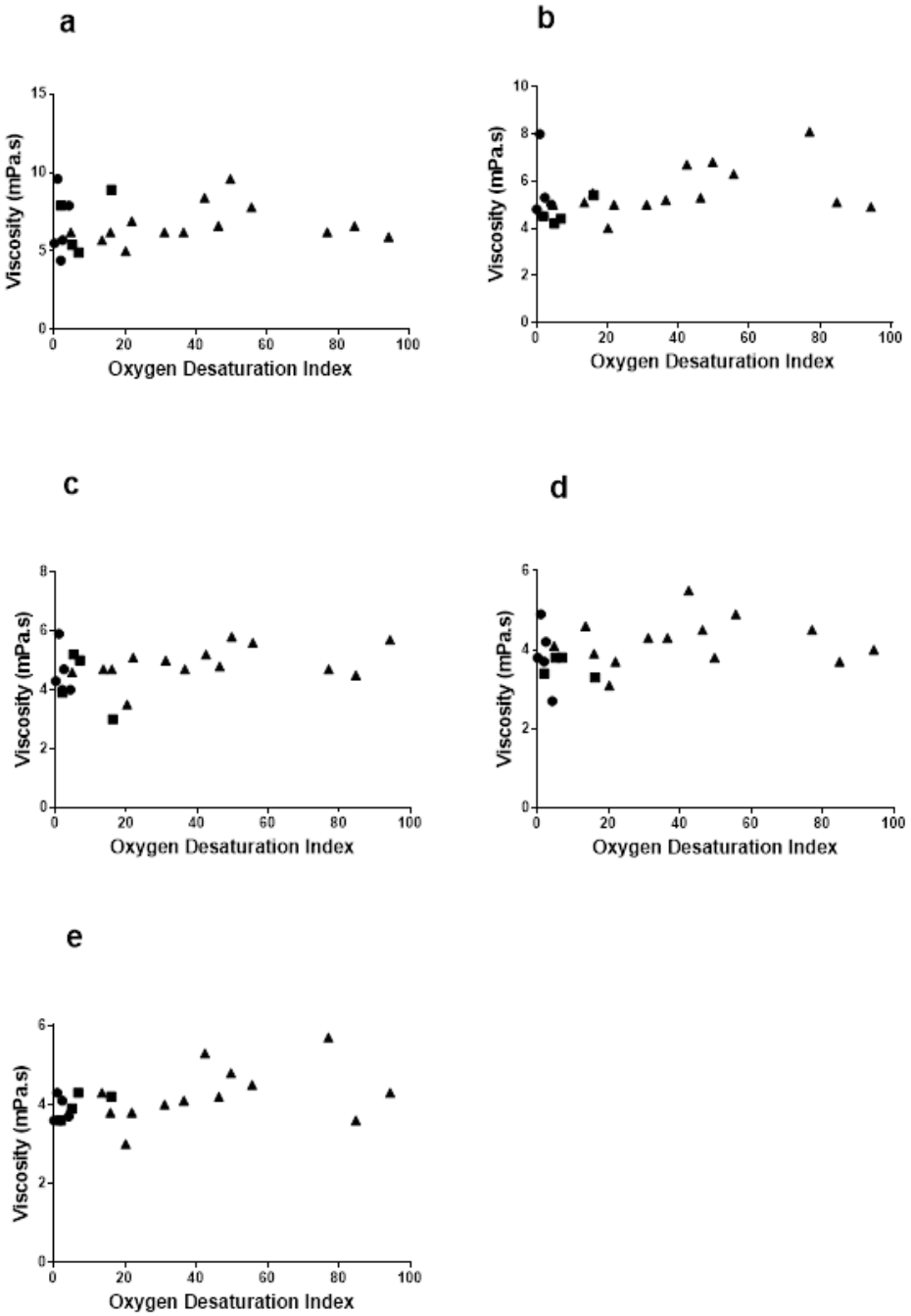


Figure 21 shows the relationship between AI and whole blood viscosity in OSA patients. At lower shear rates (75, 150, and 300), there were no correlations between such parameters in all disease severities (Figure 21A, B, C). However, at higher shear rates (750 and 1500), weak positive relationships were observed between AI and whole blood viscosity until AI 60 (Figure 21D and E). The relationship was generally absent in all mild and moderate OSA patients.

Generally, there was no correlation between AI and plasm viscosity at the standardised 40% haematocrit in patients having mild, moderate, severe OSA at all shear rates as shown in all of the panels in Figure 22. However, there has been a slight tendency to a positive correlation in severe patients. At the shear rate 150, the measurements of standardised plasma viscosity are higher than other shear rates, i.e. starting from 4 mPas (Figure 22B).

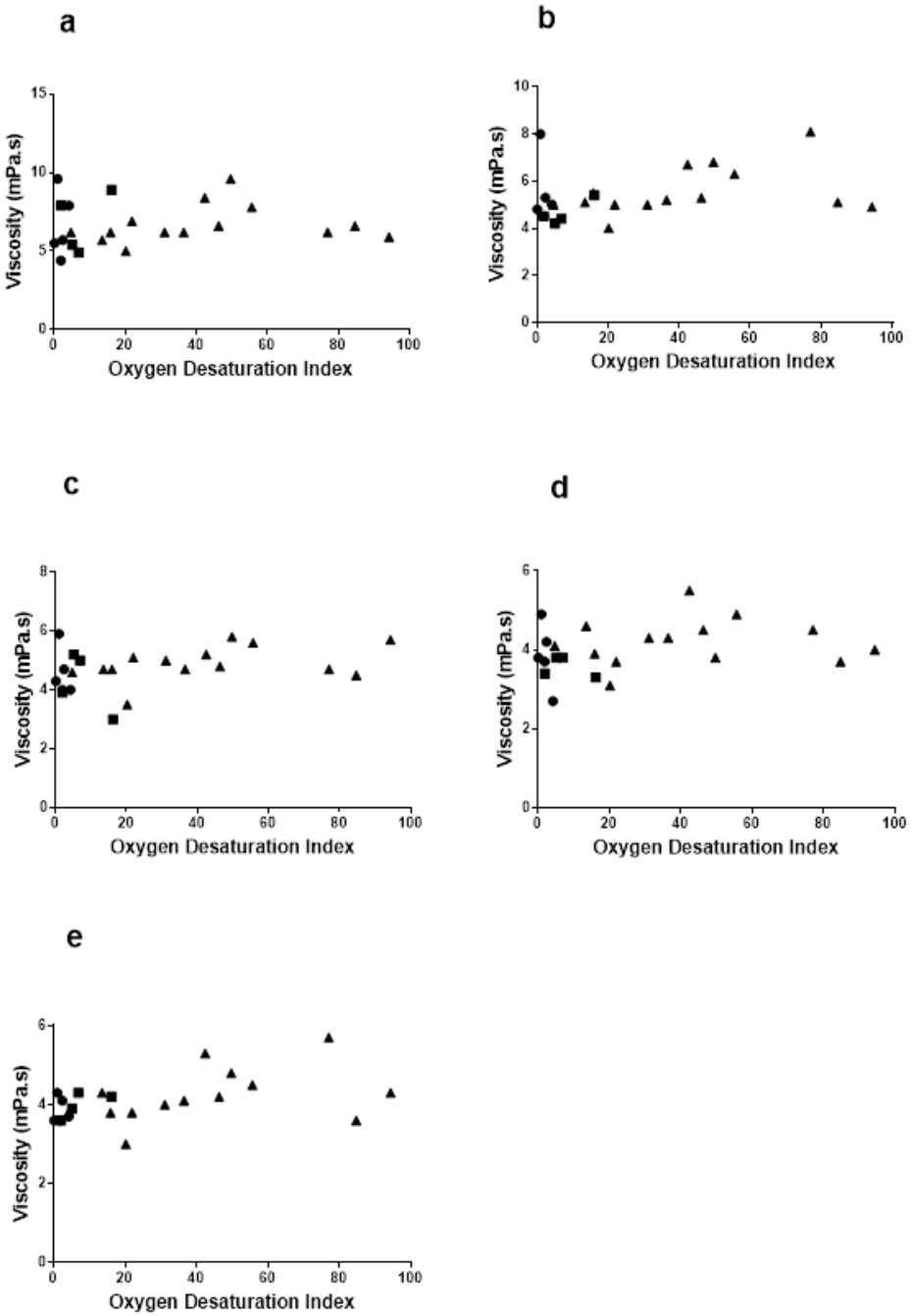


Figure 21: The relationship between oxygen desaturation index (ODI) and blood viscosity of red blood cells in patients with mild, moderate and severe obstructive sleep apnoea (OSA) at the following shear rates: 150 (a), 300 (b), 750 (c) 750 (d), 1500 (e).

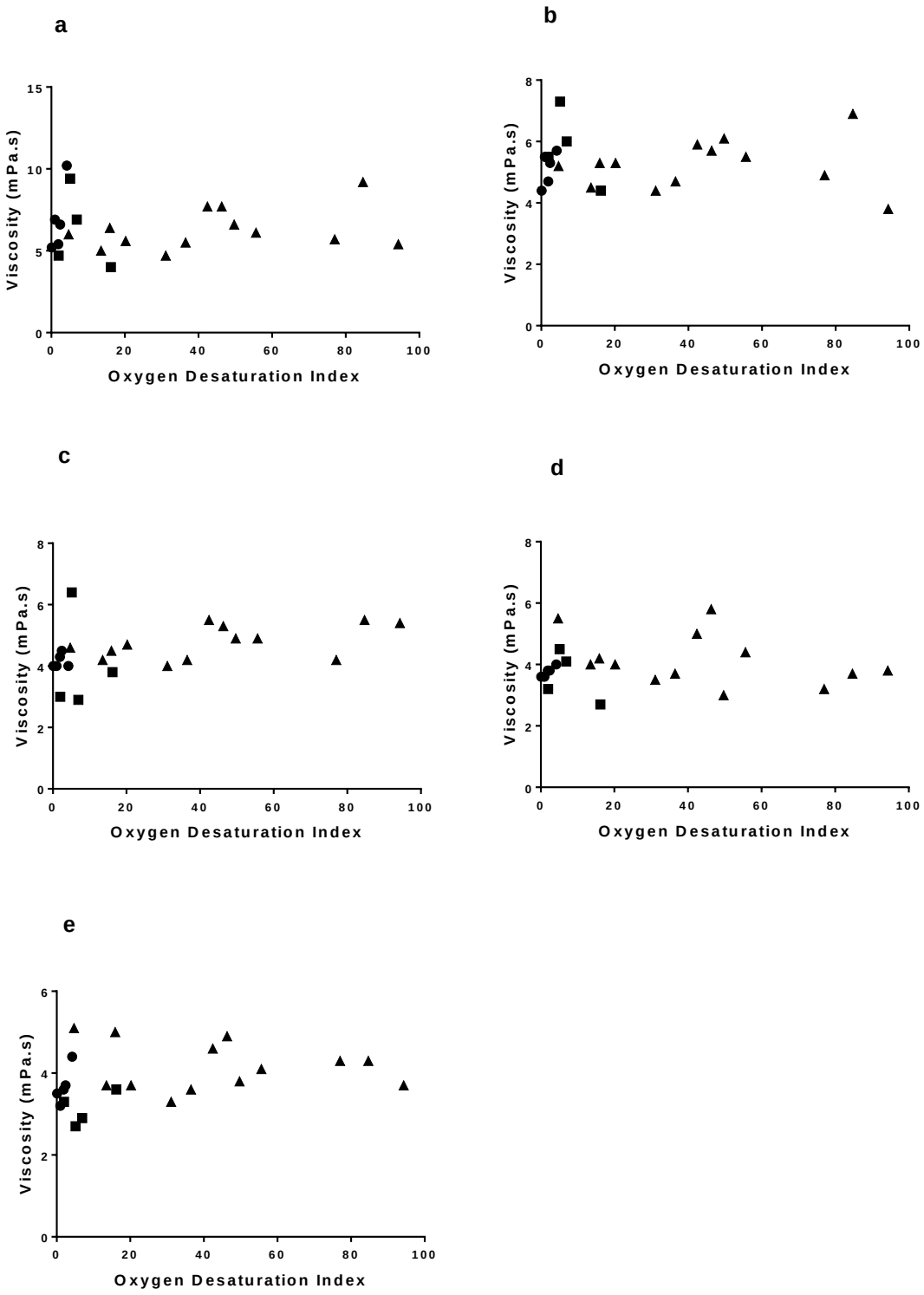
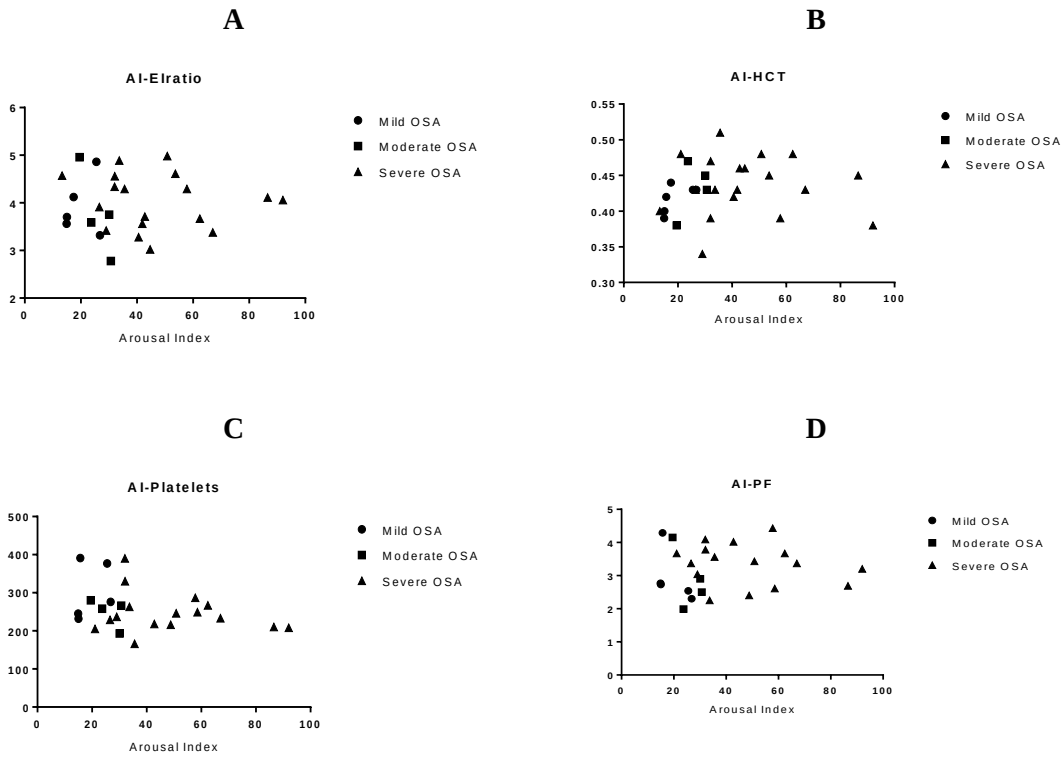


Figure 22: The relationship between oxygen desaturation index (ODI) and blood viscosity of red blood cells at standardized 40% haematocrit in patients with mild, moderate and severe obstructive sleep apnoea (OSA) at the following shear rates: 75 (A), 150 (B), 300 (C), 750 (D), 1500 (E).

3. Other Haemorheological Parameters

The correlation between some haemorheological parameters and AI is shown in Figure 23. Both the elongation index, indicating RBC deformability, and haematocrit values showed no correlation with AI among different disease severities (Figure 23A and B). Platelet count decreased slightly with increasing AI in moderate and severe OSA patients as demonstrated in Figure 23C, ranging between 400 and down to 150×100^{9}/L. Finally, plasma fibrinogen showed a slight positive relationship with AI in severe OSA patients.



● = mild OSA, ■ = moderate OSA, ▲ = severe OSA

Figure 23: The relationship between arousal index (AI) and elongation index (A), haematocrit (B), platelets (C), and plasma fibrinogen (D) in patients with mild, moderate and severe obstructive sleep apnoea (OSA)

4.2 Part 2

4.2.1 Polysomnographic data before and after CPAP treatment

The mean values of select polysomnographic variables recorded for the patients (n=16) at the time of diagnosis and after acute treatment with continuous positive airway pressure (CPAP) are listed in Table 9. Sleep efficiency at the time of diagnosis was significantly increased after CPAP treatment (69.99 ± 17.69% vs 76.96 ± 15.89% Table 9). In addition, the most important indices indicated for sleep disorders were significantly altered. OSA patients showed significant decreases in their arousal indices after receiving CPAP therapy (18.09 ± 12.08/hr) when compared to pre-treatment values (45.04 ± 19.67/hr, Table 9). Additionally, there was a significant difference between the mean values of oxygen desaturation index before treatment (36.84 ± 29.25/hr) and after treatment (7.87 ± 10.37/hr, Table 9). For the apnoea, hypopnoea index, there was a significant decrease in the mean values before and after CPAP treatment in OSA patients ($P<0.05$, Table 9).

Table 9: Polysomnographic variables of patients before and after continuous positive airway pressure treatment(CPAP) for obstructive sleep apneoa (OSA).

SLEEP DATA	Post CPAP	Pre CPAP
TST(min)	305 ± 94	336 ± 81
Sleep Latency(min)	24 ± 25	16 ± 18
Sleep Efficiency (%)	70 ± 18	77 ± 16†
Arousal Index(/hr)	45 ± 20	18 ± 12†
ODI(/Hr)	37 ± 29	8 ± 10†
AHI(/hr)	48 ± 25	8 ± 13†

Data are expressed as mean values ± standard deviation. TST, Total sleep time; ODI, Oxygen desaturation index – the number of times per hour oxygen saturation dropped 3% or greater; †, significant difference after CPAP treatment in patients diagnosed with OSA.

4.2.2 Haematological parameters before and after CPAP treatment

Table 10: Haematology results of patients before and after continuous positive airway pressure treatment(CPAP) for obstructive sleep apneoa (OSA).

Haematology	Post CPAP	Pre CPAP
Plasma Fibrinogen(g/L)	3.1 ± 0.7	3.1 ± 0.7
Insulin(mU/L)	22 ± 13	18 ± 14
Glucose(mmol/L)	6.1 ± 1.5	6.1 ± 1.6
LDL(mmol/L)	2.6 ± 2.6	2.6 ± 0.9
HDL(mmol/L)	1.3 ± 0.4	1.3 ± 0.4
Chol/HDL (mmol/L)	4.2 ± 1.4	3.9 ± 1.4
Chol(mmol/L)	5.0 ± 1.0	4.7 ± 1.1
Trig(mmol/L)	1.6 ± 1.2	1.3 ± 0.6
HB(g/L)	142 ± 16	136 ± 20
RBC($x10^{12}$/L)	4.9 ± 0.7	4.7 ± 0.7
Haematocrit (%)	0.44 ± 0.47	0.41 ± 0.56†
Platelet($x10^9$/L)	260 ± 47	239 ± 56†
WBC($x10^9$/L)	6.68 ± 1.35	6.04 ± 1.78
Neutrophils($x10^9$/L)	3.66 ± 1.24	3.1 ± 1.20
Lymphocytes($x10^9$/L)	2.13 ± 0.52	2.08 ± 0.75
Monocytes($x10^9$/L)	0.59 ± 0.12	0.57 ± 0.15
Eosinophils($x10^9$/L)	0.25 ± 0.13	0.27 ± 0.13
Basophils($x10^9$/L)	0.44 ± 0.35	0.03 ± 0.31

Data are expressed as mean values ± standard deviation. †, significant difference after CPAP treatment in patients diagnosed with OSA.

Table 10 illustrates the results of the haematological parameters tested in OSA patients (n=12) before and after receiving CPAP treatment. The mean values of both the insulin and glucose levels were not statistically different, although there was a slight decrease in insulin levels. Lipid profile parameters, including LDL, HDL, cholesterol, triglycerides, were not statistically different at the time of diagnosis and after CPAP treatment. Additionally, following CPAP treatment, there was no significant difference for haemoglobin, nor RBC count ($P>0.05$, Table 10). In addition, there was no significant difference after CPAP therapy in the count of neutrophils, lymphocytes, monocytes, eosinophils, and basophils as well as white cell count following CPAP treatment.

Haematocrit decreased significantly after CPAP treatment (0.41 ± 0.56%) when compared to at the time of diagnosis (0.44 ± 0.47%, Table 10). Regarding blood coagulation parameters, there was no significant change in plasma fibrinogen levels after receiving treatment. However, when platelet counts were compared before and after CPAP therapy, a

statistically significant decrease was observed (259.92 ± 46.88×109/L vs 238.5 ± 55.71×109/L, Table 10).

4.2.3 Blood rheological parameters Pre and Post CPAP treatment

1. Red cell Aggregation

The mean values of RBC aggregation at native and standardised haematocrit in patients with OSA before and after CPAP treatment are shown in Table 11.

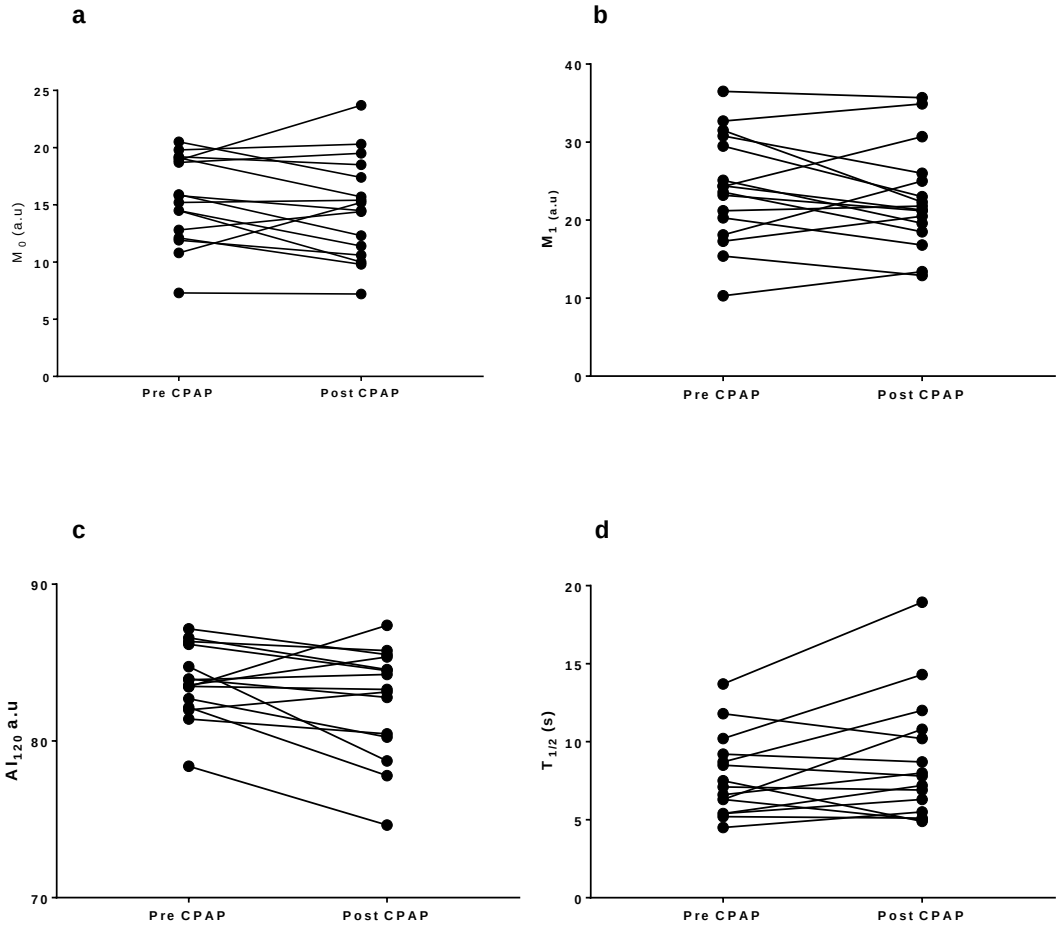


Figure 24 demonstrates the differences in the mean values of RBCs aggregation at both the native and standardised haematocrit after 120 seconds at stasis at the time of diagnosis and after CPAP treatment. Aggregation half time showed no significant changes pre- and post-treatment Table 11.

Table 11: Aggregation of red blood cells at native and standardised haematocrit of patients before and after continuous positive airway pressure treatment(CPAP) for obstructive sleep apneoa (OSA).

Aggregation	Post CPAP	Pre CPAP

Native haematocrit		
M_0	14.0 ± 4.0	14.3 ± 4.3
M_1	22.5 ± 6.7	22.7 ± 6.4
T½	8.1 ± 2.9	8.5 ± 3.8
AI_{120}	83.1 ± 2.5	82.4 ± 3.6
Standardised haematocrit (40%)		
M_0	15.4 ± 3.8	14.7 ± 4.4
M_1	24.0 ± 7.0	22.7 ± 6.6
T½	7.8 ± 2.6	8.8 ± 3.9
AI_{120}	83.7 ± 2.3	82.6 ± 3.5

Data are expressed as mean values ± standard deviation. M_0: red blood cell (RBC) aggregation after 10 s at stasis. M_1: RBC aggregation after 10 s at 3 sec⁻¹. T½: Half the time it takes for RBC aggregation to occur. AI_{120}: RBC aggregation after 120 s at stasis.

For aggregation measurements at the standardised haematocrit, there were slight decreases in aggregation after 10 seconds at stasis and at 3 sec^{-1}, and after 120 seconds at stasis after CPAP treatment if compared to the time of diagnosis, however, the differences were not statistically significant. No marked differences were observed between RBCs aggregation after 120 seconds in both groups at the native and standardised haematocrit (Figure 24).

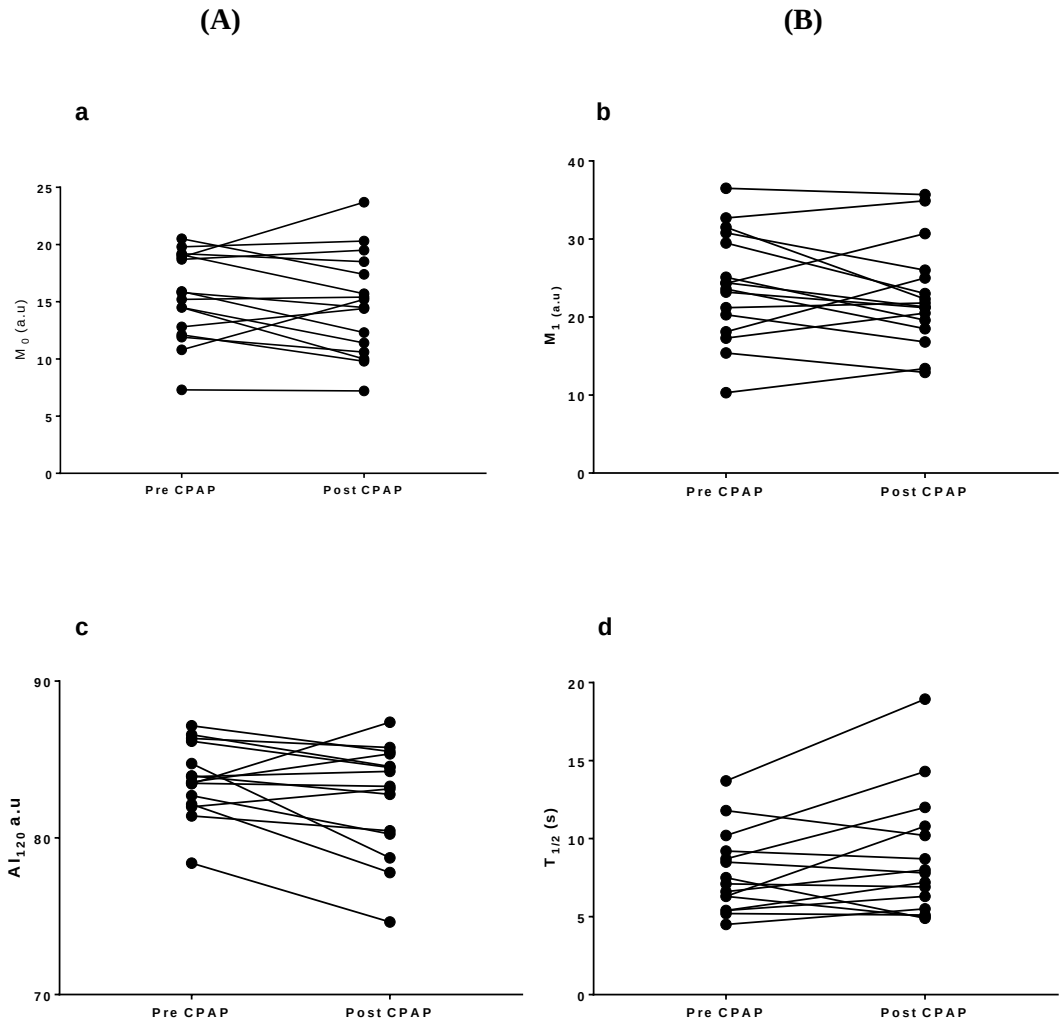


Figure 24: Standardised measurement of red blood cell aggregation at 40% haematocrit after 10 s at stasis(a), after 10 s at 3 secs ⁻¹ (b), after 120 s at stasis (c), and aggregation half time (D) in patients before and after CPAP therapy.

2. Whole blood viscosity

Table 12: Viscosity of blood at native haematocrit and standardised to 0.4 L/L haematocrit of patients before and after continuous positive airway pressure treatment (CPAP) for obstructive sleep apnoea (OSA).

Shear Rate (sec $^{-1}$)	**Post CPAP**	**Pre CPAP**
Native haematocrit		
75	6.7 ± 1.68	5.8 ± 2.01
150	5.4 ± 1.02	4.7 ± 0.85
300	5.0 ± 0.89	3.4 ± 0.57†
750	4.1 ± 0.61	3.4 ± 0.44
1500	4.2 ± 0.63	3.6 ± 0.52
Standardised haematocrit		
75	6.0 ± 0.55	6.8 ± 2.90
150	5.1 ± 0.82	5.2 ± 0.95
300	4.8 ± 0.41	3.8 ± 0.78†
750	4.1 ± 0.82	3.5 ± 0.56
1500	4.0 ± 0.54	3.6 ± 0.53

Data are expressed as mean values ± standard deviation. †, significant difference after CPAP treatment in patients diagnosed with OSA.

The viscosity of blood was measured in OSA patients (n=6) at both native and 0.4 L/L standardised haematocrit at the time of diagnosis and after CPAP treatment. The mean values of viscosities at different shear rates (75, 150, 300, 750, and 1500 sec^{-1}) are listed in Table 12. For both native and standardised haematocrits, a significant shear thinning effect was observed, whereby viscosity decreased with increasing shear rates ($P<0.05$). Additionally, all of the mean values of the viscosities at different shear rates after treatment were insignificantly less than their peers before receiving treatment at the native haematocrit (Table 12).

No significant changes were detected in the mean values of suspension viscosities at shear rates 75, 150, 750, and 1500 sec^{-1} at both native and standardised haematocrits. For the native haematocrit, however, the mean values of RBCs suspension viscosity at the shear rate 300 sec^{-1} decreased significantly after CPAP treatment (3.4 ± 0.57 mPa·s) when compared to that at the time of diagnosis (5.0 ± 0.89 mPa·s, Table 12). Similarly, at the standardised haematocrit, the mean values of viscosity at the shear rate 300 sec^{-1} at the time of diagnosis were decreased significantly post CPAP therapy (4.8 ± 0.41 mPa·s vs 3.8 ± 0.78 mPa·s respectively, Table 12).

3. RBC deformability

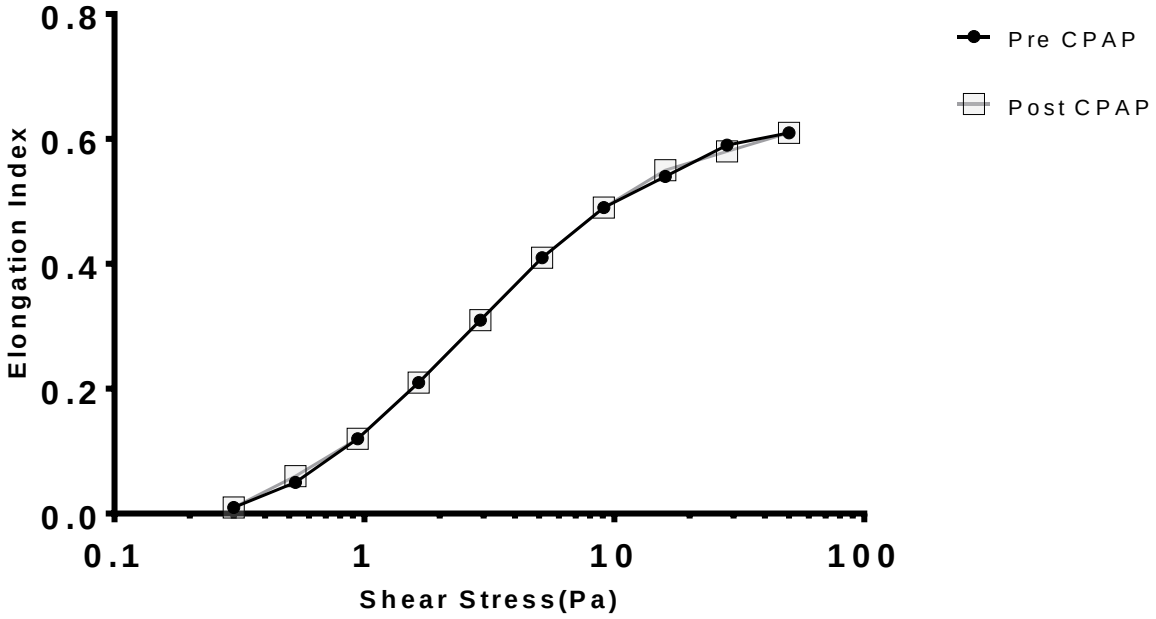


Figure 25: The elongation index (RBCs deformability) determined at different shear stresses in OSA patients at the time of diagnosis and after CPAP treatment.

The changes of elongation index, representing red blood cell deformability, at different shear stresses at the time of OSA diagnosis and after CPAP treatment are demonstrated in Figure 25. The relationship between both parameters was typically sigmoidal for both groups and approximately identical in their values.

5. DISCUSSION

The present thesis explored how blood rheological parameters may differ according to the severity of obstructive sleep apnoea (OSA). An additional aim was to explore the effect of acute treatment with continuous positive airway pressure (CPAP) on blood rheological parameters in OSA patients. Blood rheological properties examined included whole blood viscosity, red blood cell (RBC) deformability, and RBC aggregation; related clinical measures included plasma fibrinogen concentration and routine haematology.

5.3 Physical Characters and OSA severity

There were no significant effects of age on the severity of OSA in our study. Indeed, this is inconsistent with other previous reports investigating the possible linkage between age and OSA using AHI indices in patients of different ethnic origins [207, 208] although the effect of age on OSA tends to be complicated with disease severity. [209]. Gabbay and Lavie [207] showed that the prevalence of OSA tends to steeply increase with age in patients aged from 20 to 40 years. Another study has demonstrated significant relationships between age, waist-hip ratio and BMI and the severity of OSA in a large population [210]. Therefore, the lack of age-related effect in the present study might be related to the small number of recruited patients and the overall similarity of their age ranges. That is, it seems that most of them appear to be in the 6^{th} or 7^{th} decade of life.

The severity of OSA is correlated with the degree of obesity, as shown in increased pharyngeal fat in men than women [40], which may be causative for the increased OSA prevalence in men [211, 212]. There were no significant differences in absolute body mass between the OSA groups, although BMI mean values were significantly increased in the severest form of OSA. The relation between BMI and the severity of OSA is not straightforward, given studies have demonstrated a linear relationship between BMI and increasing OSA severity [213] and a less robust association was found in the sleep heart health study [214]. On the other hand, other population-based studies [215] and a hospital-based case-study control [216] revealed insignificant correlations between BMI and disease severity. There was a tendency in the literature for significant correlations to be detected only when sample sizes were far larger than the current. For example, the recruited samples of studies revealing significant relations consisted of 658 [213] and 5615 [214] patients. This may explain the lack of detecting statistically-significant relations between BMI and

OSA severity in the present study. Moreover, it is likely that the relatively small sample size of the present thesis may also explain the incongruence between our lack of finding relationships between OSA severity with age and body mass, and those reported in the literature [207, 213].

The results of the present study showed marked increases in neck circumference of severe OSA patients in relation to mildly affected patients. Indeed, neck circumference has been a useful predictor of OSA, although its apparent role in OSA pathogenesis has not yet been fully clarified [217]. In addition, as a result of the increased neck circumference in males, a greater risk of OSA may be associated when compared to females [218]. The possible explanation of this finding is that the resistance of upper airways was found to be increased in men than women especially during slow-wave sleep [38]. The response to hypoxia and hypercapnia is relatively decreased in obese men when compared to their female counterparts, which make them more liable to develop sleep breathing disorders [219]. Pillar and co-authors [220] found that upper airway collapse has been observed more frequently in men despite the lack of difference in the central drive and ventilatory response between men and women.

For polysomnographic variables, oxygen desaturation index (ODI) has been the strongest indicator of OSA severity in adults [221] and children [222]. Furthermore, ODI has a robust association with the apnoea-hypopnoea index and even can be considered a reliable indicator of OSA in general. This is consistent with our findings which revealed increased ODI values with greater OSA severity. Epworth Sleepiness Score (ESS), which has been classically used to assess excessive daytime sleepiness [223], was not significantly altered in relation to OSA severity in the present thesis. The present finding is in agreement with previous studies which indicated no significant correlations between the ESS and apnoea-hypopnoea index (AHI) [224] and that the ESS may be only significantly associated with depression-associated OSA [225].

5.4 RBC Aggregation

Increased RBC aggregation is comorbid with cardiovascular risk factors such as hypertension, atherosclerosis, and obesity [226]. In addition, RBC aggregation was reported to be increased in OSA patients, a matter which may be related to the elevated inflammatory marker CRP [125]. CRP has been associated with obesity and OSA severity [109]. In addition, the elevated CRP levels are reduced with nasal CPAP treatment [188]. In the present thesis, we focused on the potential alterations of red cell aggregation measured at native and standardised haematocrit and the resultant relationship with OSA severity. There was a significant correlation detected between AHI and several RBC aggregation measurements in our study at both the native and standardised haematocrit. This is in agreement with another study which employed a larger cohort with OSA [227] where there were gradual significant increments in RBC aggregation with increasing AHI indices, indicating an increase with disease severity. In addition, the present data revealed a strong association between ODI and various aggregation measurements at different haematocrit.

It is well-known that the RBC aggregation occurs within the vascular compartments having low shear stresses such as in the veins and post-capillary venules [228]. RBC aggregation may also impact vascular resistance of arteries as a result of a decrease in the expression of nitric oxide synthase [229]. Since RBC must enter the capillary network single-file for adequate tissue perfusion, it has been found that hyperaggregation may augment vascular resistance [230]. As a consequence, RBCs aggregation may have a strong contributing role as an agent to increase the cardiovascular risk in patients with OSA.

Additionally, it has been demonstrated that there is a strong correlation between BMI and RBC aggregation [226]. In the present thesis, although BMI was significantly associated with OSA severity, RBC aggregation showed no correlation with disease severity. In contrast to our findings, Sinnapah et al. [227] found that the strength of RBC aggregation was significantly higher in obese OSA patients by the reference to the patients with normal weight. This may be explained by the fact that the RBCs aggregates of obese patients are more difficult to dissociate than RBCs aggregates in normal people.

Tazbirek and co-authors [121] found that OSA patients had elevated RBC aggregation when compared to controls. Such patients had a significantly increased baseline RBC aggregation and a reduced aggregation halftime. The present data revealed, however,

the similarity in the aggregation half time between different disease severity groups. RBC aggregation is affected by multiple factors. First, fibrinogen, a2-globulins, immunoglobulins and other plasma factors are involved in promoting the aggregation of RBCs. Second, the lipid composition of RBC cell membrane plays also an important role in cell aggregation [231, 232]. Finally, there is a potential interaction between RBC glycocalyx and charged or neutral polymers or proteins, in a model identified as polymer model [233].

Results of the present study demonstrated weak positive correlations between the AHI and RBC aggregation at normal and standardised haematocrits which is supported by the observation of a weak negative correlation between AHI and aggregation half time. Tazbirek and colleagues [121] recruited approximately the same number of patients as our sample, and demonstrated similar findings, where the aggregation index was positively correlated to AHI (r= 0.45) and the aggregation half time was negatively related to AHI (*r*= -0.42). These findings provide an evidence of the changes in the aggregation properties of RBCs in OSA patients. After acute treatment post CPAP, RBCs aggregation decreased when compared to values recorded prior to CPAP. Tazbirek and co-authors [121] found significant changes in RBCs aggregation index (lowered by 7%) as well as aggregation half time (increased by 25.4%) in OSA patients after CPAP treatment for five consecutive nights. The observed weak correlation between RBC aggregation and OSA severity parameters after one day of CPAP treatment in the present findings and the significant correlation after longer period of treatment (5 days) emphasize the importance of adherence to long-term CPAP therapy to achieve favourable therapeutic outcomes. However, to the best of our knowledge, there is no scientific evidence of the exact mechanism by which the CPAP treatment impacts RBC aggregation. Future research should be based on studying the mechanistic changes in aggregation parameters post CPAP treatment after short- and long-term therapy.

5.5 Blood viscosity, fibrinogen, and haematocrit with OSA severity

Elevated morning blood viscosity may have a strong impact on the cardiovascular morbidity in OSA patients due to the associated hemodynamic disturbances, and elevations in morning fibrinogen and haematocrit [106]. Patients with severe OSA in this study had significant increases in blood viscosity (standardised to 0.4 L/L haematocrit) when compared to those that had moderate OSA. However, Sinnapah, Cadelis [227] did not show

increased blood viscosity with increased OSA severity in their patients. The authors claimed that the lack of difference in blood viscosity was attributable to measuring blood viscosity at high shear rates (225 sec^{-1}), where RBC aggregates could not form due to unfavourable flow dynamics.

Elevated fibrinogen concentration may be the potential reasons for elevated blood viscosity. This can be clearly concluded from our results which showed marked increases in the fibrinogen levels with advanced disease severity. The continuous rise in fibrinogen concentration may be related to its production during the associated inflammatory processes observed in OSA patients [234]. A second possible explanation is a protein-protein interaction in the plasma. High plasma malondialdehyde levels may be involved in fibrinogen elevation in severe OSA patients [235]. It is noteworthy that malondialdehyde is an end product of lipid peroxidation and is known to induce secondary oxidative damage to the plasma proteins [236]. Another reason for the elevated fibrinogen levels in severe OSA patients is the transient elevation during the night. This can be understood from other studies which have reported that the levels of plasma fibrinogen in the morning were significantly elevated than on the previous afternoon in OSA patients [106, 237] resulting in increases in the whole blood viscosity at low and relatively moderate shear rates (0.47-118 s^{-1}). The transient fibrinogen elevation may be due to hemoconcentration as the OSA patients experience excessive nocturnal diuresis. Further, partial improvements in the viscosity during the day may emphasize such assumption. After partial improvement of blood viscosity, it was surprisingly found that blood viscosity may return to its elevation in the evening in OSA patients when compared to healthy control subjects [235]. However, the real mechanism of the way by which the viscosity was elevated during the night is still unclear. We could not confirm such hypothesis as we have collected patients' blood samples only once in the morning.

An additional theory that explains the reason for increased blood viscosity in OSA patients is the excessive activity of the sympathetic nervous system leading to enhanced platelet activation [238]. Geiser and co-investigators [239] determined platelet activation and the formation of platelet-derived microparticles in untreated OSA patients at two time intervals (4 and 7 a.m). The results revealed a significant increase in platelets positive for CD63 and CD62P epitopes in OSA patients when compared to control subjects. Enhanced platelet activation during sleep might eventually contribute to the incidence of

cardiovascular consequences in patients with OSA. Platelet count in the patients of the present thesis showed no change from mild to moderate OSA severity. However, the results showed a significant correlation between ODI and platelet count with OSA severity.

CPAP treatment for one night did not impact platelet count in OSA patients in the present study. This is consistent with the findings of after 2 nights of CPAP treatment [239]. Although Feliciano and co-authors [240] have observed a lack of remarkable alterations of platelet count with OSA severity in their 73 patients, CPAP treatment for 6 months improved their platelet count significantly for all diseases severities. Therefore, more prolonged CPAP therapy may be required to yield a considerable impact on platelet counts in OSA patients.

5.6 The Effect Of CPAP Treatment On Blood Viscosity, Fibrinogen, And Haematocrit

Blood viscosity at moderate shear rates (300 sec^{-1}) was significantly reduced after CPAP treatment. There was a trend for blood viscosity to be lower at all measured shear rates following acute CPAP therapy although the changes did not reach significance. Chin, Ohi [106] recruited 11 patients and explored other haemorheological parameters (haematocrit and whole blood viscosity at a shear rate of 208 sec^{-1}) before and after a single night of CPAP treatment. Without nasal CPAP, the authors reported significant differences in fibrinogen concentrations in the morning than the previous afternoon. CPAP therapy significantly improved fibrinogen concentrations in the morning when the values were compared to those without treatment. CPAP treatment decreased the established increase in whole blood viscosity at a shear rate 208 sec^{-1}, which is consistent with the present CPAP effects on blood viscosity at a shear rate of 300 sec^{-1}. However, the results of Chin, Ohi [106] are extended by the present, as those authors used indirect, rather than direct measures of blood viscosity; that is, they predicted viscosity values based on the haematocrit and total plasma protein levels.

Tazbirek, Slowinska [121] evaluated blood rheological properties in 31 OSA patients (mean age, 49.4±8.6 years) before and after CPAP therapy for 5 days and compared their results with a control group of 19 individuals without OSA (mean age, 47.1±6.9 years). When compared to the control group, the mean values of whole blood viscosity were significantly higher in OSA patients after CPAP treatment. However, when compared to

their own pre-CPAP values, OSA patients had significant reductions in the mean values of whole blood viscosity, plasma viscosity, and corrected blood viscosity.

Zhang and co-authors [241] tested 41 OSA patients (mean age: 63.4 ± 4 years) with moderate and severe symptoms before and after 30 days of CPAP treatment and found that whole blood viscosity and plasma fibrinogen concentration relative to healthy controls were significantly reduced after 30 days. Reinhart and colleagues [99] measured whole blood viscosity at high (94.5 s^{-1}) and low (0.1 s^{-1}) shear rates and plasma fibrinogen concentration in OSA patients following 6 months of nasal CPAP. The authors observed that both parameters were similar in OSA patients and healthy subjects, indicating their normal levels at the time of measurements. However, the authors did not measure those parameters at the baseline to detect the possibility of improvement. Nonetheless, it has been suggested that it is necessary to provide chronic CPAP treatment to effectively reduce plasma fibrinogen concentration. This may explain why our acute intervention did not influence plasma fibrinogen concentration.

It has been shown that the cyclical intermittent deoxygenation and re-oxygenation of blood that occurs in OSA patients secondary to the cessation of breathing may promote coagulation [242]. In the present thesis, there were weak positive correlations between some baseline polysomnographic parameters, such as AHI, ODI, and AI, and blood viscosity, haematocrit, and plasma fibrinogen. Besides, these weak relationships were consistent with other studies where the altered abnormal values were corrected with short-term CPAP therapy [121]. Overall, OSA is usually associated with complex changes in the whole blood and plasma viscosity and these parameters would be slightly improved with treatment and lifestyle modifications over short periods, a matter which requires more long-term studies employing CPAP therapy.

Haematocrit is a parameter that strongly influences blood viscosity in a positive relation; no significant difference was observed between different OSA severities in the present thesis. This is consistent with the findings of a recent study [240] which compared some haematological parameters in males with different OSA severities and also Reinhart, Oswald [99] found no differences between these parameters in OSA patients and controls. Although the results of a study conducted by Yousef and Alkhiary [243] no increase in haematocrit percentage among OSA patients with different severities, they found that the

haematocrit was positively correlated with AHI and ODI. Our data showed slight, and insignificant, correlations between the latter parameters and haematocrit.

Acute CPAP therapy in the present study significantly reduced haematocrit of OSA patients compared with the values obtained during the initial diagnostic sleep study (i.e. Pre CPAP). Similar results have been observed for short-term and long-term CPAP treatment. Tazbirek and co-authors [121] found significant differences between haematocrit values in the untreated OSA patients and CPAP treated patients after a 5-days-therapy ($p<0.01$), while Krieger and colleagues [198] found significantly decreased haematocrit values after one night post CPAP treatment, similar to our results, and significant reduction after one year when compared to the baseline untreated night.

5.7 Red Blood Cell Deformability

Another determinant of blood viscosity is the capacity of the red blood cell to deform. This property enables the erythrocytes to properly adapt to flow conditions throughout the circulation and is affected by three essential determinants: membrane viscoelasticity, cellular biconcavity, and the intracellular viscosity represented mainly as haemoglobin concentration. In the present study, post-CPAP red cell deformability, expressed as elongation index, was compared to diagnostic measurements. A recent study evaluated RBCs deformability and nitric oxide metabolites in two groups of OSA patients (L group had AHI <30 and H group had AHI >30) and compared the results to another group of healthy people [244]. RBCs deformability was measured using Myrenne diffractometer at shear stress 30 and 60 Pa and the authors found that, at each shear stress, the elongation index was significantly decreased in both OSA groups when compared to the normal subjects. No significant correlations were detected between elongation index and nitric oxide, AHI, or mean nocturnal oxygen saturation (SO_2). On the other hand, Dikmenoğlu, Çiftçi [235] used a filtration technique to measure RBC deformability and they found no significant difference between OSA patients and healthy controls. Using a laser optical rotational cell analyzer, Tazbirek, Slowinska [121] also did not find significant differences in the elongation index between OSA patients and a control group, and even between the CPAP-treated group for five days when compared to the control group or an untreated group of OSA patients. This is consistent with our findings which showed that there was no difference in the elongation indices before and after one night of CPAP therapy.

Nonetheless, the duration of CPAP therapy may influence its overall efficacy to alter RBC deformability.

In our study, no clear relationship was detected between the elongation index and the parameters of OSA severity, including AHI, ODI, and AI. These observations supported those of Tazbirek and co-authors [121] that found no relation between elongation index and AHI (r= -0.04) and oxygen saturation (r = 0.05). The mechanism by which erythrocyte deformability can be different among different OSA severities is not fully elucidated. For example, no changes have been reported in the osmotic fragility of RBCs [235, 245], cellular metabolism [235], or RBCs membrane peroxidation. Erythrocyte deformability is possibly altered by exogenous factors, such as nitric oxide or intermittent desaturation although there were no obtainable confirmed statistical associations [121]. If we assumed that the internal viscosity of RBCs is normal in OSA patients [128, 235, 245], then the potential alteration in deformability may be attributed to the abnormality of membrane dynamic properties, which essentially depends on the cholesterol/phospholipid ratio, the saturated/unsaturated fatty acid ration, and on membrane proteins [117].

5.8 Other haematological parameters

Since the OSA and type 2 diabetes mellitus are commonly associated [246, 247], it was important to study the potential correlation of glycaemic control with OSA severity. There has been a strong correlation between the impaired glucose metabolism and the severity of OSA as demonstrated in a study of Japanese patients [248]. Both the insulin and glucose levels were significantly increased in those with severe OSA [248]. Based on fasting serum insulin concentration and insulin resistance index, Ip et al [249] investigated whether a relationship exists between OSA and insulin resistance in 270 subjects. They confirmed an independent association between OSA and insulin resistance, as indicated by higher levels of fasting serum insulin in those with the markers of severe OSA, such as increased AHI and the level of desaturation during sleep. In addition, detailed analysis in the latter study revealed a robust relationship between insulin resistance and hypertension in OSA patients, indicating that insulin may be a significant factor for hypertension in those patients.

Although the cross-sectional study of the Sleep Heart Health Study [58] and the Wisconsin Sleep Cohort [57] found that the OSA severity markers are associated with insulin resistance, the longitudinal results of Wisconsin Cohort did not show such

relationship. In general, the correlations in OSA patients were independent of obesity despite the fact that obesity enhances the risk for impaired glucose tolerance.

HbA_{1c}, a marker of elevated blood glucose, was significantly increased in severe OSA patients when compared to mild OSA patients, indicating that a significant number of patients with severe OSA might be diabetic or pre-diabetic. This is in agreement with the study of Papanas and co-authors [250] which showed that both the HbA_{1c} and fasting glucose levels were increased with OSA severity among nondiabetic men with. HbA_{1c} levels were also increased in 30 nondiabetic OSA patients and this increase was correlated with the disease severity and the AHI index as per results of Shpirer and other researchers [66].

Several studies have shown that CPAP treatment has a beneficial impact on both glucose and insulin metabolism. Insulin levels in our cohort were insignificantly decreased, while glucose levels remained unchanged after CPAP therapy, which might support the above-mentioned presumption of being diabetic or prediabetic. CPAP evaluated for its effects on the glucose and insulin metabolism by a hyperinsulinemic-euglycaemic clamp in OSA patients having diabetes [251]. In that study, insulin sensitivity increased significantly by 28% after CPAP therapy. Nonetheless, after 4 months of CPAP treatment, there were no significant changes in fasting insulin, glucose, and HbA_{1c} levels. Conversely, Shpirer, Rapoport [66] found that the levels of HbA_{1c} improved significantly in patients with severe OSA after 3-5 months of CPAP treatment. The patients in this study were ranging from normal to prediabetic to diabetic. Another study has shown a significant reduction in insulin sensitivity after 2 days of CPAP therapy in 40 non-diabetic OSA patients [169].

The effects of OSA severity on serum lipid levels were also studied in our patients. We did not find any remarkable changes in the levels of HDL, cholesterol, and triglycerides, with disease severity as well as any significant differences between those levels pre- and post-CPAP treatment. Surprisingly, although LDL concentrations have been increased in moderately affected patients than mild OSA patients, the results of patients with severe OSA have shown a significant reduction in LDL levels when compared to moderate OSA patients. This can be possibly attributed to the fact that the patients with severe OSA probably have more co-morbidities and therefore are more likely medicated for hypercholesterolaemia and dyslipidaemia.

Hyperlipidemia has been involved in the pathogenesis of coronary artery pathological events in OSA patients. This is corroborated by several findings which have elicited a critical link between increased LDL levels and OSA severity [252, 253]. Moreover, in a clinical trial, CPAP treatment resulted in significant reduction in serum cholesterol levels in OSA patients when compared to those treated with a sham device for 1 month [87].

In addition, it has been suggested that the severity of OSA was independently associated with LDL/HDL ratio and this ratio was significantly decreased after CPAP treatment over 6 months [254]. It can be assumed that OSA-mediated chronic activation of the sympathetic nervous system would eventually increase LDL synthesis in the liver and reduce its catabolism through the stimulation of α_1 receptors [255, 256]. Indeed, increased blood viscosity may also contribute as a possible theory for increased serum lipids as it affects the frictional forces and was found to be positively correlated with total cholesterol [257], LDL, and triglycerides [258]. In the study of Kawano et al. (2012), the results showed a significant decrease in serum HDL with OSA severity and an increase in LDL, which was correlated positively with AHI. CPAP therapy was found to be effective for improving HDL levels when employing large sample sizes [259].

Conversely, Alexopoulos and co-authors [260] did not find a relationship between serum lipids concentrations and OSA severity in obese children, while HDL levels were negatively correlated with OSA severity in non-obese OSA children.

6. CONCLUSION

OSA is associated with elevated risk of developing cardiovascular complications, possibly due to increased comorbid risk factors such as hypertension and dyslipidemia. Blood rheology is known to be an important determinant of blood coagulation which subsequently contributes to the increased risk of cardiovascular events in OSA patients. Particularly, the degree of OSA-associated nocturnal hypoxaemia may play a major role in impairing blood rheology in OSA patients. In the present thesis, we hypothesised that morning blood rheological properties would be altered in OSA patients and there would be possible associations between different OSA severity patterns and blood rheology parameters. Additionally, it was predicted that CPAP therapy may improve blood rheology although it was unknown whether one night of treatment would be sufficient to significantly improve these indices.

Blood rheology parameters were markedly associated with OSA severity. OSA patients showed significant increases in whole blood viscosity although they were not correlated with the indices of OSA severity. RBC aggregation increased consistently with OSA severity, which supports an increased risk of cardiovascular disease with advanced OSA severity. We observed marked increases in morning plasma fibrinogen concentration and RBC aggregation in severe OSA patients. Such findings support previous observations of peak incidence of thrombosis and cardiovascular events occurring in the morning with increased disease severity. Overall, these data point towards OSA rightfully being considered a major cardiovascular risk factor. Blood rheological properties can be useful to determine which patients with OSA are particularly at high-risk of adverse cardiovascular. Acute CPAP therapy was effective in OSA management as demonstrated through normalisation of sleep quality indices. These improvements were concurrent with a significant reduction in altered blood viscosity following a single night of CPAP treatment emphasise the effectiveness of even short-term OSA therapy to improving markers known to be associated with cardiovascular risk.

Future research studies should be based on larger cohorts along with the recruitment of a control group for significant matching and reducing the possible effects of confounding factors, such as sex, age, body weight, and alcohol consumption since they may impact blood rheology parameters. Moreover, the effects of CPAP therapy should be studied over long periods with regular follow-up of the rheological parameters to detect the rate of

possible improvement in each stage and the exact period wherein a maximal benefit could be attained. During such periods, adherence to CPAP therapy should be ensured to avoid misinterpretation. Long-term studies will be also beneficial to compare fatality rates among patient groups.

7. REFERENCES

1. Young, T., et al., *The occurrence of sleep-disordered breathing among middle-aged adults.* N Engl J Med, 1993. **328**(17): p. 1230-5.
2. Marin, J.M., et al., *Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure: an observational study.* Lancet, 2005. **365**(9464): p. 1046-53.
3. Mehra, R., et al., *Association of nocturnal arrhythmias with sleep-disordered breathing: The Sleep Heart Health Study.* Am J Respir Crit Care Med, 2006. **173**(8): p. 910-6.
4. Steiner, S., et al., *Altered blood rheology in obstructive sleep apnea as a mediator of cardiovascular risk.* Cardiology, 2005. **104**(2): p. 92-6.
5. Jordan, A.S., D.G. McSharry, and A. Malhotra, *Adult obstructive sleep apnoea.* Lancet, 2014. **383**(9918): p. 736-47.
6. Lam, J.C., S.K. Sharma, and B. Lam, *Obstructive sleep apnoea: definitions, epidemiology & natural history.* Indian J Med Res, 2010. **131**: p. 165-70.
7. Ramar, K. and S.M. Caples, *Cardiovascular Consequences of Obese and Nonobese Obstructive Sleep Apnea.* The Medical clinics of North America, 2010. **94**(3): p. 465-478.
8. Zhao, Q., et al., *Effects of obstructive sleep apnea and its treatment on cardiovascular risk in CAD patients.* Respir Med, 2011. **105**(10): p. 1557-64.
9. Bradley, T.D. and J.S. Floras, *Obstructive sleep apnoea and its cardiovascular consequences.* Lancet, 2009. **373**(9657): p. 82-93.
10. Celec, P., et al., *Oxidative and carbonyl stress in patients with obstructive sleep apnea treated with continuous positive airway pressure.* Sleep Breath, 2012. **16**(2): p. 393-8.
11. World Health Organization. *Obesity and overweight*. 2016 [cited 2018 Mar 02]; Available from: http://www.who.int/mediacentre/factsheets/fs311/en/.
12. Cheyne, J., *A case of apoplexy in which the fleshy part of the heart was converted into fat.* Dublin Hosp Rep, 1818. **2**: p. 216-223.
13. Dickens, C., *The Posthumous Papers of the Pickwickian Club*. 1837, London: Chapman and Hall.
14. Osler, W., *The Principles and Practice of Medicine: Designed for the Use of Practitioners and Students of Medicine/by William Osler.* New York: D. Appleton and Co, 1894: p. c1892.
15. Catlin, G., *The Breath of Life*. 1861, New York: Wiley.
16. Kushida, C.A., *Obstructive Sleep Apnea: Pathophysiology, Comorbidities and Consequences: Pathophysiology, Comorbidities, and Consequences*. 2007: Informa Health Care.
17. Gerardy, W., D. Herberg, and H. Kuhn, *Comparative studies on pulmonary function and the electroencephalogram in 2 patients with Pickwick's syndrome.* Zeitschrift Fur Klinische Medizin, 1960. **156**: p. 362–380.
18. Drachman, D.B. and R.J. Gumnit, *Periodic alteration of consciousness in the Pickwickian syndrome.* Archives of neurology, 1962. **6**(6): p. 471-477.
19. Jung, R. and W. Kuhlo, *Neurophysiological studies of abnormal night sleep and the Pickwickian syndrome*, in *Progress in brain research*. 1965, Elsevier. p. 140-159.
20. Weitzman, E.D., et al., *The hypersomnia-sleep apnea syndrome: site and mechanism of upper airway obstruction.* Sleep apnea syndromes, 1978. **15**: p. 235-248.

21. Peppard, P.E., et al., *Prospective Study of the Association between Sleep-Disordered Breathing and Hypertension.* New England Journal of Medicine, 2000. **342**(19): p. 1378-1384.
22. Newman, A.B., et al., *Progression and regression of sleep-disordered breathing with changes in weight: the Sleep Heart Health Study.* Arch Intern Med, 2005. **165**(20): p. 2408-13.
23. Cleary, D.W. and S.C. Clarke, *The nasopharyngeal microbiome.* Emerging Topics in Life Sciences, 2017. **1**(4): p. 297-312.
24. Schwab, R.J., et al., *Upper airway and soft tissue anatomy in normal subjects and patients with sleep-disordered breathing. Significance of the lateral pharyngeal walls.* Am J Respir Crit Care Med, 1995. **152**(5 Pt 1): p. 1673-89.
25. Burger, C.D., et al., *Fast-computed tomography evaluation of age-related changes in upper airway structure and function in normal men.* Am Rev Respir Dis, 1992. **145**(4 Pt 1): p. 846-52.
26. Issa, F.G. and C.E. Sullivan, *Upper airway closing pressures in obstructive sleep apnea.* J Appl Physiol Respir Environ Exerc Physiol, 1984. **57**(2): p. 520-7.
27. Mezzanotte, W.S., D.J. Tangel, and D.P. White, *Waking genioglossal electromyogram in sleep apnea patients versus normal controls (a neuromuscular compensatory mechanism).* J Clin Invest, 1992. **89**(5): p. 1571-9.
28. Heinzer, R.C., et al., *Effect of increased lung volume on sleep disordered breathing in patients with sleep apnoea.* Thorax, 2006. **61**(5): p. 435-439.
29. Kairaitis, K., et al., *Tracheal traction effects on upper airway patency in rabbits: the role of tissue pressure.* Sleep, 2007. **30**(2): p. 179-86.
30. Van de Graaff, W.B., *Thoracic traction on the trachea: mechanisms and magnitude.* J Appl Physiol (1985), 1991. **70**(3): p. 1328-36.
31. Redline, S. and P.V. Tishler, *The genetics of sleep apnea.* Sleep Med Rev, 2000. **4**(6): p. 583-602.
32. Smith, P.L., et al., *Weight loss in mildly to moderately obese patients with obstructive sleep apnea.* Ann Intern Med, 1985. **103**(6 (Pt 1)): p. 850-5.
33. Lowe, A.A., et al., *Cephalometric and demographic characteristics of obstructive sleep apnea: an evaluation with partial least squares analysis.* Angle Orthod, 1997. **67**(2): p. 143-53.
34. Bacon, W.H., et al., *Craniofacial characteristics in patients with obstructive sleep apneas syndrome.* Cleft Palate J, 1988. **25**(4): p. 374-8.
35. Lowe, A.A., et al., *Facial morphology and obstructive sleep apnea.* Am J Orthod Dentofacial Orthop, 1986. **90**(6): p. 484-91.
36. Ancoli-Israel, S., et al., *Sleep-disordered breathing in community-dwelling elderly.* Sleep, 1991. **14**(6): p. 486-95.
37. Punjabi, N.M., *The epidemiology of adult obstructive sleep apnea.* Proc Am Thorac Soc, 2008. **5**(2): p. 136-43.
38. Trinder, J., et al., *Gender differences in airway resistance during sleep.* Journal of applied physiology, 1997. **83**(6): p. 1986-1997.
39. Ryan, C.F. and L.L. Love, *Mechanical properties of the velopharynx in obese patients with obstructive sleep apnea.* American journal of respiratory and critical care medicine, 1996. **154**(3): p. 806-812.
40. Mohsenin, V., *Effects of gender on upper airway collapsibility and severity of obstructive sleep apnea.* Sleep medicine, 2003. **4**(6): p. 523-529.
41. Badr, M.S., *Essentials of Sleep Medicine: An Approach for Clinical Pulmonology*. 2002: Totowa, NJ: Springer Science+Business Media, LLC.

42. Horner, R.L., *Motor control of the pharyngeal musculature and implications for the pathogenesis of obstructive sleep apnea.* Sleep, 1996. **19**(10): p. 827-853.
43. Hoffstein, V., *Relationship Between Smoking and Sleep Apnea in Clinic Population.* Sleep, 2002. **25**(5): p. 517-522.
44. Kashyap, R., L.M. Hock, and T.J. Bowman, *Higher prevalence of smoking in patients diagnosed as having obstructive sleep apnea.* Sleep Breath, 2001. **5**(4): p. 167-72.
45. Scanlan, M.F., et al., *Effect of moderate alcohol upon obstructive sleep apnoea.* Eur Respir J, 2000. **16**(5): p. 909-13.
46. Lavie, P., et al., *The effects of partial and complete mechanical occlusion of the nasal passages on sleep structure and breathing in sleep.* Acta Otolaryngol, 1983. **95**(1-2): p. 161-6.
47. Young, T., P.E. Peppard, and D.J. Gottlieb, *Epidemiology of obstructive sleep apnea: a population health perspective.* Am J Respir Crit Care Med, 2002. **165**(9): p. 1217-39.
48. Baldwin, C.M., et al., *The association of sleep-disordered breathing and sleep symptoms with quality of life in the Sleep Heart Health Study.* Sleep, 2001. **24**(1): p. 96-105.
49. Teran-Santos, J., A. Jimenez-Gomez, and J. Cordero-Guevara, *The association between sleep apnea and the risk of traffic accidents. Cooperative Group Burgos-Santander.* N Engl J Med, 1999. **340**(11): p. 847-51.
50. BarbÉ, F., et al., *Automobile Accidents in Patients with Sleep Apnea Syndrome.* American Journal of Respiratory and Critical Care Medicine, 1998. **158**(1): p. 18-22.
51. Lal, C., C. Strange, and D. Bachman, *Neurocognitive impairment in obstructive sleep apnea.* Chest, 2012. **141**(6): p. 1601-1610.
52. Aloia, M.S., et al., *Neuropsychological sequelae of obstructive sleep apnea-hypopnoea syndrome: a critical review.* J Int Neuropsychol Soc, 2004. **10**(5): p. 772-85.
53. Naismith, S., et al., *Neurobehavioral functioning in obstructive sleep apnea: differential effects of sleep quality, hypoxemia and subjective sleepiness.* J Clin Exp Neuropsychol, 2004. **26**(1): p. 43-54.
54. Chami, H.A., et al., *Association between Glucose Metabolism and Sleep-disordered Breathing during REM Sleep.* Am J Respir Crit Care Med, 2015. **192**(9): p. 1118-26.
55. Grimaldi, D., et al., *Association of obstructive sleep apnea in rapid eye movement sleep with reduced glycemic control in type 2 diabetes: therapeutic implications.* Diabetes Care, 2014. **37**(2): p. 355-63.
56. Rice, T.B., et al., *The Relationship Between Obstructive Sleep Apnea and Self-Reported Stroke or Coronary Heart Disease in Overweight and Obese Adults with Type 2 Diabetes Mellitus.* Sleep, 2012. **35**(9): p. 1293-1298.
57. Reichmuth, K.J., et al., *Association of sleep apnea and type II diabetes: a population-based study.* Am J Respir Crit Care Med, 2005. **172**(12): p. 1590-5.
58. Punjabi, N.M., et al., *Sleep-disordered breathing, glucose intolerance, and insulin resistance: the Sleep Heart Health Study.* Am J Epidemiol, 2004. **160**(6): p. 521-30.
59. Seicean, S., et al., *Sleep-disordered breathing and impaired glucose metabolism in normal-weight and overweight/obese individuals: the Sleep Heart Health Study.* Diabetes Care, 2008. **31**(5): p. 1001-6.

60. Foster, G.D., et al., *A randomized study on the effect of weight loss on obstructive sleep apnea among obese patients with type 2 diabetes: the Sleep AHEAD study.* Arch Intern Med, 2009. **169**(17): p. 1619-26.
61. Sulit, L., et al., *Differences in polysomnography predictors for hypertension and impaired glucose tolerance.* Sleep, 2006. **29**(6): p. 777-83.
62. Weinstock, T.G., et al., *A controlled trial of CPAP therapy on metabolic control in individuals with impaired glucose tolerance and sleep apnea.* Sleep, 2012. **35**(5): p. 617-625b.
63. Okada, M., et al., *Relationship between sleep-disordered breathing and lifestyle-related illnesses in subjects who have undergone health-screening.* Intern Med, 2006. **45**(15): p. 891-6.
64. Papanas, N., et al., *HbA1c is associated with severity of obstructive sleep apnea hypopnoea syndrome in nondiabetic men.* Vasc Health Risk Manag, 2009. **5**: p. 751-6.
65. Aronsohn, R.S., et al., *Impact of untreated obstructive sleep apnea on glucose control in type 2 diabetes.* Am J Respir Crit Care Med, 2010. **181**(5): p. 507-13.
66. Shpirer, I., et al., *Normal and elevated HbA1C levels correlate with severity of hypoxemia in patients with obstructive sleep apnea and decrease following CPAP treatment.* Sleep Breath, 2012. **16**(2): p. 461-6.
67. Pinto, J.M., et al., *Hemodynamic changes associated with obstructive sleep apnea followed by arousal in a porcine model.* J Appl Physiol (1985), 1993. **75**(4): p. 1439-43.
68. Somers, V.K., et al., *Sympathetic neural mechanisms in obstructive sleep apnea.* J Clin Invest, 1995. **96**(4): p. 1897-904.
69. Patil, S.P., et al., *Adult obstructive sleep apnea: pathophysiology and diagnosis.* Chest, 2007. **132**(1): p. 325-37.
70. Fletcher, E.C., *Cardiovascular disease associated with obstructive sleep apnea.* Monaldi Arch Chest Dis, 2003. **59**(3): p. 254-61.
71. Narkiewicz, K., et al., *Altered cardiovascular variability in obstructive sleep apnea.* Circulation, 1998. **98**(11): p. 1071-7.
72. Brooks, D., et al., *Obstructive sleep apnea as a cause of systemic hypertension. Evidence from a canine model.* Journal of Clinical Investigation, 1997. **99**(1): p. 106-109.
73. Carley, D.W., S.M. Trbovic, and M. Radulovacki, *Hydralazine reduces elevated sleep apnea index in spontaneously hypertensive (SHR) rats to equivalence with normotensive Wistar-Kyoto rats.* Sleep, 1996. **19**(5): p. 363-6.
74. Lavie, P., P. Herer, and V. Hoffstein, *Obstructive sleep apnoea syndrome as a risk factor for hypertension: population study.* Bmj, 2000. **320**(7233): p. 479-82.
75. Montesi, S.B., et al., *The effect of continuous positive airway pressure treatment on blood pressure: a systematic review and meta-analysis of randomized controlled trials.* J Clin Sleep Med, 2012. **8**(5): p. 587-96.
76. Singh, U. and I. Jialal, *Oxidative stress and atherosclerosis.* Pathophysiology, 2006. **13**(3): p. 129-42.
77. Davignon, J. and P. Ganz, *Role of endothelial dysfunction in atherosclerosis.* Circulation, 2004. **109**(23 Suppl 1): p. Iii27-32.
78. Jelic, S., et al., *Inflammation, oxidative stress, and repair capacity of the vascular endothelium in obstructive sleep apnea.* Circulation, 2008. **117**(17): p. 2270-8.
79. Minoguchi, K., et al., *Increased carotid intima-media thickness and serum inflammatory markers in obstructive sleep apnea.* Am J Respir Crit Care Med, 2005. **172**(5): p. 625-30.

80. Levy, P., M.R. Bonsignore, and J. Eckel, *Sleep, sleep-disordered breathing and metabolic consequences.* Eur Respir J, 2009. **34**(1): p. 243-60.
81. McNicholas, W.T. and M.R. Bonsigore, *Sleep apnoea as an independent risk factor for cardiovascular disease: current evidence, basic mechanisms and research priorities.* Eur Respir J, 2007. **29**(1): p. 156-78.
82. Newman, A.B., et al., *Relation of sleep-disordered breathing to cardiovascular disease risk factors: the Sleep Heart Health Study.* Am J Epidemiol, 2001. **154**(1): p. 50-9.
83. Karkinski, D., et al., *Obstructive Sleep Apnea and Lipid Abnormalities.* Open Access Macedonian Journal of Medical Sciences, 2017. **5**(1): p. 19-22.
84. Togeiro, S.M., et al., *Consequences of obstructive sleep apnea on metabolic profile: a Population-Based Survey.* Obesity (Silver Spring), 2013. **21**(4): p. 847-51.
85. Roche, F., et al., *Obstructive sleep apnoea/hypopnoea influences high-density lipoprotein cholesterol in the elderly.* Sleep Med, 2009. **10**(8): p. 882-6.
86. Ip, M.S., et al., *Serum leptin and vascular risk factors in obstructive sleep apnea.* Chest, 2000. **118**(3): p. 580-6.
87. Robinson, G., et al., *Circulating cardiovascular risk factors in obstructive sleep apnoea: data from randomised controlled trials.* Thorax, 2004. **59**(9): p. 777-782.
88. Schafer, H., et al., *Body fat distribution, serum leptin, and cardiovascular risk factors in men with obstructive sleep apnea.* Chest, 2002. **122**(3): p. 829-39.
89. Kadotani, H., et al., *Association between apolipoprotein E epsilon4 and sleep-disordered breathing in adults.* Jama, 2001. **285**(22): p. 2888-90.
90. Lam, J.C., et al., *Obstructive sleep apnea and the metabolic syndrome in community-based Chinese adults in Hong Kong.* Respir Med, 2006. **100**(6): p. 980-7.
91. Dyken, M.E., et al., *Investigating the relationship between stroke and obstructive sleep apnea.* Stroke, 1996. **27**(3): p. 401-7.
92. Lavie, L., *Obstructive sleep apnoea syndrome--an oxidative stress disorder.* Sleep Med Rev, 2003. **7**(1): p. 35-51.
93. Kasasbeh, E., D.S. Chi, and G. Krishnaswamy, *Inflammatory aspects of sleep apnea and their cardiovascular consequences.* South Med J, 2006. **99**(1): p. 58-67; quiz 68-9, 81.
94. Ip, M.S., et al., *Circulating nitric oxide is suppressed in obstructive sleep apnea and is reversed by nasal continuous positive airway pressure.* Am J Respir Crit Care Med, 2000. **162**(6): p. 2166-71.
95. Ryan, S., C.T. Taylor, and W.T. McNicholas, *Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome.* Circulation, 2005. **112**(17): p. 2660-7.
96. Schulz, R., *The vascular micromilieu in obstructive sleep apnoea.* European Respiratory Journal, 2005. **25**(5): p. 780.
97. Choi, J.B., et al., *Does obstructive sleep apnea increase hematocrit?* Sleep Breath, 2006. **10**(3): p. 155-60.
98. Hoffstein, V., et al., *Hematocrit levels in sleep apnea.* Chest, 1994. **106**(3): p. 787-91.
99. Reinhart, W.H., et al., *Blood viscosity and platelet function in patients with obstructive sleep apnea syndrome treated with nasal continuous positive airway pressure.* Clin Hemorheol Microcirc, 2002. **27**(3-4): p. 201-7.
100. Thompson, S.G., et al., *Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group.* N Engl J Med, 1995. **332**(10): p. 635-41.

101. Dikmenoglu, N., et al., *Erythrocyte deformability, plasma viscosity and oxidative status in patients with severe obstructive sleep apnea syndrome.* Sleep Med, 2006. **7**(3): p. 255-61.
102. Junker, R., et al., *Coagulation factor VII and the risk of coronary heart disease in healthy men.* Arterioscler Thromb Vasc Biol, 1997. **17**(8): p. 1539-44.
103. Chin, K., et al., *Improvement of factor VII clotting activity following long-term NCPAP treatment in obstructive sleep apnoea syndrome.* Qjm, 1998. **91**(9): p. 627-33.
104. Kannel, W.B., et al., *Fibrinogen and risk of cardiovascular disease. The Framingham Study.* Jama, 1987. **258**(9): p. 1183-6.
105. Ernst, E., *The role of fibrinogen as a cardiovascular risk factor.* Atherosclerosis, 1993. **100**(1): p. 1-12.
106. Chin, K., et al., *Effects of NCPAP therapy on fibrinogen levels in obstructive sleep apnea syndrome.* Am J Respir Crit Care Med, 1996. **153**(6 Pt 1): p. 1972-6.
107. Wessendorf, T.E., et al., *Fibrinogen levels and obstructive sleep apnea in ischemic stroke.* Am J Respir Crit Care Med, 2000. **162**(6): p. 2039-42.
108. Saletu, M., et al., *Structural and serum surrogate markers ofcerebrovascular disease in obstructive sleepapnea (OSA).* Journal of Neurology, 2006. **253**(6): p. 746-752.
109. Shamsuzzaman, A.S., B.J. Gersh, and V.K. Somers, *Obstructive sleep apnea: implications for cardiac and vascular disease.* Jama, 2003. **290**(14): p. 1906-14.
110. Tonelli, M., et al., *Relation Between Red Blood Cell Distribution Width and Cardiovascular Event Rate in People With Coronary Disease.* Circulation, 2008. **117**(2): p. 163-168.
111. Perlstein, T.S., et al., *Red blood cell distribution width and mortality risk in a community-based prospective cohort.* Arch Intern Med, 2009. **169**(6): p. 588-94.
112. Macdougall, I.C. and A. Cooper, *The inflammatory response and epoetin sensitivity.* Nephrol Dial Transplant, 2002. **17 Suppl 1**: p. 48-52.
113. Fukuta, H., et al., *Elevated plasma levels of B-type natriuretic Peptide but not C-reactive protein are associated with higher red cell distribution width in patients with coronary artery disease.* Int Heart J, 2009. **50**(3): p. 301-12.
114. Ryan, S., C.T. Taylor, and W.T. McNicholas, *Systemic inflammation: a key factor in the pathogenesis of cardiovascular complications in obstructive sleep apnoea syndrome?* Thorax, 2009. **64**(7): p. 631-6.
115. Sökücü, S.N., et al., *Can Red Blood Cell Distribution Width Predict Severity of Obstructive Sleep Apnea Syndrome?* Journal of Clinical Sleep Medicine : JCSM : Official Publication of the American Academy of Sleep Medicine, 2012. **8**(5): p. 521-525.
116. Piagnerelli, M., et al., *Modifications in erythrocyte membrane protein content are not responsible for the alterations in rheology seen in sepsis.* Shock, 2012. **37**(1): p. 17-21.
117. Caimi, G., et al., *Erythrocyte deformability, plasma lipid peroxidation and plasma protein oxidation in a group of OSAS subjects.* Clin Hemorheol Microcirc, 2016. **64**(1): p. 7-14.
118. Wang, Y., S.X. Zhang, and D. Gozal, *Reactive oxygen species and the brain in sleep apnea.* Respir Physiol Neurobiol, 2010. **174**(3): p. 307-16.
119. Srour, et al., *Exposure of human erythrocytes to oxygen radicals causes loss of deformability, increased osmotic fragility, lipid peroxidation and protein degradation.* Clin Hemorheol Microcirc, 2000. **23**(1): p. 13-21.
120. Powell, R.J., et al., *Oxygen free radicals: effect on red cell deformability in sepsis.* Crit Care Med, 1991. **19**(5): p. 732-5.

121. Tazbirek, M., et al., *Short-term continuous positive airway pressure therapy reverses the pathological influence of obstructive sleep apnea on blood rheology parameters.* Clin Hemorheol Microcirc, 2009. **41**(4): p. 241-9.
122. Kang, J., et al., *Chronic intermittent hypoxia versus continuous hypoxia: Same effects on hemorheology?* Clin Hemorheol Microcirc, 2016. **63**(3): p. 245-55.
123. Yelmen, N., et al., *The effects of chronic long-term intermittent hypobaric hypoxia on blood rheology parameters.* Gen Physiol Biophys, 2011. **30**(4): p. 389-95.
124. Bor-Kucukatay, M., et al., *Effects of nitric oxide on red blood cell deformability.* Am J Physiol Heart Circ Physiol, 2003. **284**(5): p. H1577-84.
125. Peled, N., et al., *Increased erythrocyte adhesiveness and aggregation in obstructive sleep apnea syndrome.* Thromb Res, 2008. **121**(5): p. 631-6.
126. Moore-Gillon, J.C., et al., *Intermittent hypoxia in patients with unexplained polycythaemia.* British Medical Journal (Clinical research ed.), 1986. **293**(6547): p. 588-590.
127. McKeon, J.L., et al., *Urinary uric acid:creatinine ratio, serum erythropoietin, and blood 2,3-diphosphoglycerate in patients with obstructive sleep apnea.* Am Rev Respir Dis, 1990. **142**(1): p. 8-13.
128. Maillard, D., et al., *Decreased oxyhemoglobin affinity in patients with sleep apnea syndrome.* Am Rev Respir Dis, 1991. **143**(3): p. 486-9.
129. Gyawali, P., et al., *Erythrocyte aggregation and metabolic syndrome.* Clin Hemorheol Microcirc, 2014. **57**(1): p. 73-83.
130. Kato, M., et al., *Impairment of endothelium-dependent vasodilation of resistance vessels in patients with obstructive sleep apnea.* Circulation, 2000. **102**(21): p. 2607-10.
131. Kondo, Y., et al., *Significant relationship between platelet activation and apnea-hypopnoea index in patients with obstructive sleep apnea syndrome.* Tokai J Exp Clin Med, 2011. **36**(3): p. 79-83.
132. Liak, C. and M. Fitzpatrick, *Coagulability in obstructive sleep apnea.* Canadian Respiratory Journal : Journal of the Canadian Thoracic Society, 2011. **18**(6): p. 338-348.
133. Min, J.-Y., et al., *A Relationship between the Obstructive Sleep Apnea Syndrome and the Erythrocyte Sedimentation Rate.* Clinical and Experimental Otorhinolaryngology, 2009. **2**(3): p. 126-130.
134. Frank, M.H., et al., *Positional OSA part 1: Towards a clinical classification system for position-dependent obstructive sleep apnoea.* Sleep Breath, 2015. **19**(2): p. 473-80.
135. Bidarian-Moniri, A., et al., *The effect of the prone sleeping position on obstructive sleep apnoea.* Acta Otolaryngol, 2015. **135**(1): p. 79-84.
136. Oksenberg, A., *Positional and non-positional obstructive sleep apnea patients.* Sleep Med, 2005. **6**(4): p. 377-8.
137. Hukins, C.A., *Obstructive sleep apnea – management update.* Neuropsychiatric Disease and Treatment, 2006. **2**(3): p. 309-326.
138. Awad, K.M., et al., *Exercise is associated with a reduced incidence of sleep-disordered breathing.* The American Journal of Medicine, 2012. **125**(5): p. 485-490.
139. Peppard, P., et al., *Longitudinal study of moderate weight change and sleep-disordered breathing.* Jama, 2000. **284**(23): p. 3015-21.
140. Weaver, T.E., et al., *Continuous positive airway pressure treatment of sleepy patients with milder obstructive sleep apnea: results of the CPAP Apnea Trial North American Program (CATNAP) randomized clinical trial.* Am J Respir Crit Care Med, 2012. **186**(7): p. 677-83.

141. Squier, S.B., et al., *Effect of end-expiratory lung volume on upper airway collapsibility in sleeping men and women.* J Appl Physiol (1985), 2010. **109**(4): p. 977-85.
142. Hoy, C.J., et al., *Can intensive support improve continuous positive airway pressure use in patients with the sleep apnea/hypopnoea syndrome?* Am J Respir Crit Care Med, 1999. **159**(4 Pt 1): p. 1096-100.
143. Lettieri, C.J., et al., *Effects of a short course of eszopiclone on continuous positive airway pressure adherence: a randomized trial.* Ann Intern Med, 2009. **151**(10): p. 696-702.
144. Ayas, N.T., et al., *Auto-titrating versus standard continuous positive airway pressure for the treatment of obstructive sleep apnea: results of a meta-analysis.* Sleep, 2004. **27**(2): p. 249-53.
145. Ip, S., et al., *Auto-titrating versus fixed continuous positive airway pressure for the treatment of obstructive sleep apnea: a systematic review with meta-analyses.* Syst Rev, 2012. **1**: p. 20.
146. Reeves-Hoche, M.K., et al., *Continuous versus bilevel positive airway pressure for obstructive sleep apnea.* Am J Respir Crit Care Med, 1995. **151**(2 Pt 1): p. 443-9.
147. Vennelle, M., et al., *Randomized Controlled Trial of Variable-Pressure Versus Fixed-Pressure Continuous Positive Airway Pressure (CPAP) Treatment for Patients with Obstructive Sleep Apnea/Hypopnoea Syndrome (OSAHS).* Sleep, 2010. **33**(2): p. 267-271.
148. Cistulli, P.A. and R.R. Grunstein, *Medical devices for the diagnosis and treatment of obstructive sleep apnea.* Expert Rev Med Devices, 2005. **2**(6): p. 749-63.
149. Almeida, F.R., et al., *Patient preferences and experiences of CPAP and oral appliances for the treatment of obstructive sleep apnea: a qualitative analysis.* Sleep Breath, 2013. **17**(2): p. 659-66.
150. Chan, A.S., et al., *The effect of mandibular advancement on upper airway structure in obstructive sleep apnoea.* Thorax, 2010. **65**(8): p. 726-32.
151. Aurora, R.N., et al., *Practice parameters for the surgical modifications of the upper airway for obstructive sleep apnea in adults.* Sleep, 2010. **33**(10): p. 1408-13.
152. Verse, T. and K. Hörmann, *The Surgical Treatment of Sleep-Related Upper Airway Obstruction.* Deutsches Ärzteblatt International, 2011. **108**(13): p. 216-221.
153. Bloch, K.E., *Alternatives to CPAP in the treatment of the obstructive sleep apnea syndrome.* Swiss Med Wkly, 2006. **136**(17-18): p. 261-7.
154. Carroll, W., et al., *Snoring management with nasal surgery and upper airway radiofrequency ablation.* Otolaryngol Head Neck Surg, 2012. **146**(6): p. 1023-7.
155. Gillespie, M.B., et al., *Effect of palatal implants on continuous positive airway pressure and compliance.* Otolaryngol Head Neck Surg, 2011. **144**(2): p. 230-6.
156. Handler, E., et al., *Tongue suspension: an evidence-based review and comparison to hypopharyngeal surgery for OSA.* Laryngoscope, 2014. **124**(1): p. 329-36.
157. Brennick, M.J., et al., *Pharyngeal airway wall mechanics using tagged magnetic resonance imaging during medial hypoglossal nerve stimulation in rats.* J Physiol, 2004. **561**(Pt 2): p. 597-610.
158. Oliven, A., et al., *Upper airway response to electrical stimulation of the genioglossus in obstructive sleep apnea.* J Appl Physiol (1985), 2003. **95**(5): p. 2023-9.
159. Prinsell, J.R., *Maxillomandibular advancement surgery for obstructive sleep apnea syndrome.* J Am Dent Assoc, 2002. **133**(11): p. 1489-97; quiz 1539-40.

160. Epstein, L.J., et al., *Clinical guideline for the evaluation, management and long-term care of obstructive sleep apnea in adults.* J Clin Sleep Med, 2009. **5**(3): p. 263-76.
161. Pepin, J.L., et al., *Prevalence of residual excessive sleepiness in CPAP-treated sleep apnoea patients: the French multicentre study.* Eur Respir J, 2009. **33**(5): p. 1062-7.
162. Kingshott, R.N., et al., *Randomized, double-blind, placebo-controlled crossover trial of modafinil in the treatment of residual excessive daytime sleepiness in the sleep apnea/hypopnoea syndrome.* Am J Respir Crit Care Med, 2001. **163**(4): p. 918-23.
163. Giles, T.L., et al., *Continuous positive airways pressure for obstructive sleep apnoea in adults.* The Cochrane Database of Systematic Reviews, 2006(1).
164. Sin, D.D., et al., *Can continuous positive airway pressure therapy improve the general health status of patients with obstructive sleep apnea?: a clinical effectiveness study.* Chest, 2002. **122**(5): p. 1679-85.
165. Parish, J.M. and P.J. Lyng, *Quality of life in bed partners of patients with obstructive sleep apnea or hypopnoea after treatment with continuous positive airway pressure.* Chest, 2003. **124**(3): p. 942-7.
166. Henke, K.G., J.J. Grady, and S.T. Kuna, *Effect of nasal continuous positive airway pressure on neuropsychological function in sleep apnea-hypopnoea syndrome. A randomized, placebo-controlled trial.* Am J Respir Crit Care Med, 2001. **163**(4): p. 911-7.
167. Barnes, M., et al., *A randomized controlled trial of continuous positive airway pressure in mild obstructive sleep apnea.* Am J Respir Crit Care Med, 2002. **165**(6): p. 773-80.
168. Monasterio, C., et al., *Effectiveness of continuous positive airway pressure in mild sleep apnea-hypopnoea syndrome.* Am J Respir Crit Care Med, 2001. **164**(6): p. 939-43.
169. Harsch, I.A., et al., *Continuous positive airway pressure treatment rapidly improves insulin sensitivity in patients with obstructive sleep apnea syndrome.* Am J Respir Crit Care Med, 2004. **169**(2): p. 156-62.
170. Babu, A.R., et al., *Type 2 diabetes, glycemic control, and continuous positive airway pressure in obstructive sleep apnea.* Arch Intern Med, 2005. **165**(4): p. 447-52.
171. Hassaballa, H.A., et al., *The effect of continuous positive airway pressure on glucose control in diabetic patients with severe obstructive sleep apnea.* Sleep Breath, 2005. **9**(4): p. 176-80.
172. Pamidi, S., et al., *Eight Hours of Nightly Continuous Positive Airway Pressure Treatment of Obstructive Sleep Apnea Improves Glucose Metabolism in Patients with Prediabetes. A Randomized Controlled Trial.* Am J Respir Crit Care Med, 2015. **192**(1): p. 96-105.
173. Iftikhar, I.H., et al., *Meta-analysis: continuous positive airway pressure improves insulin resistance in patients with sleep apnea without diabetes.* Ann Am Thorac Soc, 2013. **10**(2): p. 115-20.
174. Guest, J.F., et al., *Clinical outcomes and cost-effectiveness of continuous positive airway pressure to manage obstructive sleep apnea in patients with type 2 diabetes in the U.K.* Diabetes Care, 2014. **37**(5): p. 1263-71.
175. West, S.D., et al., *Effect of CPAP on insulin resistance and HbA1c in men with obstructive sleep apnoea and type 2 diabetes.* Thorax, 2007. **62**(11): p. 969-74.
176. Jennum, P., et al., *All-cause mortality from obstructive sleep apnea in male and female patients with and without continuous positive airway pressure treatment: a*

registry study with 10 years of follow-up. Nature and Science of Sleep, 2015. **7**: p. 43-50.
177. Fu, Y., et al., *Meta-analysis of all-cause and cardiovascular mortality in obstructive sleep apnea with or without continuous positive airway pressure treatment.* Sleep and Breathing, 2017. **21**(1): p. 181-189.
178. Guo, J., et al., *Effect of CPAP therapy on cardiovascular events and mortality in patients with obstructive sleep apnea: a meta-analysis.* Sleep Breath, 2016. **20**(3): p. 965-74.
179. Dimsdale, J.E., J.S. Loredo, and J. Profant, *Effect of Continuous Positive Airway Pressure on Blood Pressure.* A Placebo Trial, 2000. **35**(1): p. 144-147.
180. Faccenda, J.F., et al., *Randomized placebo-controlled trial of continuous positive airway pressure on blood pressure in the sleep apnea-hypopnoea syndrome.* Am J Respir Crit Care Med, 2001. **163**(2): p. 344-8.
181. Robinson, G.V., et al., *Continuous positive airway pressure does not reduce blood pressure in nonsleepy hypertensive OSA patients.* Eur Respir J, 2006. **27**(6): p. 1229-35.
182. Peled, N., et al., *Nocturnal ischemic events in patients with obstructive sleep apnea syndrome and ischemic heart disease: effects of continuous positive air pressure treatment.* J Am Coll Cardiol, 1999. **34**(6): p. 1744-9.
183. Peker, Y., et al., *Increased incidence of cardiovascular disease in middle-aged men with obstructive sleep apnea: a 7-year follow-up.* Am J Respir Crit Care Med, 2002. **166**(2): p. 159-65.
184. Ohike, Y., et al., *Amelioration of vascular endothelial dysfunction in obstructive sleep apnea syndrome by nasal continuous positive airway pressure--possible involvement of nitric oxide and asymmetric NG, NG-dimethylarginine.* Circ J, 2005. **69**(2): p. 221-6.
185. Egashira, K., *Clinical importance of endothelial function in arteriosclerosis and ischemic heart disease.* Circ J, 2002. **66**(6): p. 529-33.
186. Oyama, J., et al., *Continuous positive airway pressure therapy improves vascular dysfunction and decreases oxidative stress in patients with the metabolic syndrome and obstructive sleep apnea syndrome.* Clin Cardiol, 2012. **35**(4): p. 231-6.
187. Zhang, X., et al., *Effect of continuous positive airway pressure treatment on elderly Chinese patients with obstructive sleep apnea in the prothrombotic state.* Chin Med J (Eng), 2003. **116**: p. 1426-1428.
188. Yokoe, T., et al., *Elevated levels of C-reactive protein and interleukin-6 in patients with obstructive sleep apnea syndrome are decreased by nasal continuous positive airway pressure.* Circulation, 2003. **107**(8): p. 1129-34.
189. Steiropoulos, P., et al., *Effect of Continuous Positive Airway Pressure Treatment on Serum Cardiovascular Risk Factors in Patients With Obstructive Sleep Apnea-Hypopnoea Syndrome.* CHEST, 2007. **132**(3): p. 843-851.
190. Ishida, K., et al., *Appropriate use of nasal continuous positive airway pressure decreases elevated C-reactive protein in patients with obstructive sleep apnea.* Chest, 2009. **136**(1): p. 125-129.
191. Kritikou, I., et al., *Sleep apnoea, sleepiness, inflammation and insulin resistance in middle-aged males and females.* Eur Respir J, 2014. **43**(1): p. 145-55.
192. Bouloukaki, I., et al., *Obstructive sleep apnea syndrome and cardiovascular disease: The influence of C-reactive protein.* World Journal of Experimental Medicine, 2015. **5**(2): p. 77-83.

193. Dorkova, Z., et al., *Effects of continuous positive airway pressure on cardiovascular risk profile in patients with severe obstructive sleep apnea and metabolic syndrome.* Chest, 2008. **134**(4): p. 686-92.
194. Harsch, I.A., et al., *Leptin and ghrelin levels in patients with obstructive sleep apnoea: effect of CPAP treatment.* Eur Respir J, 2003. **22**(2): p. 251-7.
195. Barcelo, A., et al., *Effects of obesity on C-reactive protein level and metabolic disturbances in male patients with obstructive sleep apnea.* Am J Med, 2004. **117**(2): p. 118-21.
196. Comondore, V.R., et al., *The impact of CPAP on cardiovascular biomarkers in minimally symptomatic patients with obstructive sleep apnea: a pilot feasibility randomized crossover trial.* Lung, 2009. **187**(1): p. 17-22.
197. Krieger, J., et al., *Overnight decrease in hematocrit after nasal CPAP treatment in patients with OSA.* Chest, 1990. **97**(3): p. 729-30.
198. Krieger, J., et al., *Decrease in haematocrit with continuous positive airway pressure treatment in obstructive sleep apnoea patients.* Eur Respir J, 1992. **5**(2): p. 228-33.
199. Hui, D.S., et al., *The Effects of Nasal Continuous Positive Airway Pressure on Platelet Activation in Obstructive Sleep Apnea Syndrome.* Chest, 2004. **125**(5): p. 1768-1775.
200. Shimizu, M., et al., *Platelet activation in patients with obstructive sleep apnea syndrome and effects of nasal-continuous positive airway pressure.* Tokai J Exp Clin Med, 2002. **27**(4): p. 107-12.
201. Oga, T., et al., *Effects of obstructive sleep apnea with intermittent hypoxia on platelet aggregability.* J Atheroscler Thromb, 2009. **16**(6): p. 862-9.
202. Varol, E., et al., *The effects of continuous positive airway pressure therapy on mean platelet volume in patients with obstructive sleep apnea.* Platelets, 2011. **22**(7): p. 552-6.
203. Sokucu, S.N., et al., *Complete blood count alterations after six months of continuous positive airway pressure treatment in patients with severe obstructive sleep apnea.* J Clin Sleep Med, 2014. **10**(8): p. 873-8.
204. Montagnana, M., et al., *The role of red blood cell distribution width in cardiovascular and thrombotic disorders.* Clin Chem Lab Med, 2011. **50**(4): p. 635-41.
205. Leon, S.E., D.S. Gomara, and J.M. Marin, *Red Cell Distribution Width in Obstructive Sleep Apnea.* Arch Bronconeumol, 2017. **53**(3): p. 114-119.
206. McNamee, A.P., et al., *Biphasic impairment of erythrocyte deformability in response to repeated, short duration exposures of supraphysiological, subhaemolytic shear stress.* Biorheology, 2016. **53**(3-4): p. 137-149.
207. Gabbay, I.E. and P. Lavie, *Age- and gender-related characteristics of obstructive sleep apnea.* Sleep Breath, 2012. **16**(2): p. 453-60.
208. Ip, M.S., et al., *A community study of sleep-disordered breathing in middle-aged Chinese women in Hong Kong: prevalence and gender differences.* Chest, 2004. **125**(1): p. 127-34.
209. Subramanian, S., et al., *Gender and ethnic differences in prevalence of self-reported insomnia among patients with obstructive sleep apnea.* Sleep Breath, 2011. **15**(4): p. 711-5.
210. Deng, X., et al., *Age-Group-Specific Associations between the Severity of Obstructive Sleep Apnea and Relevant Risk Factors in Male and Female Patients.* PLoS ONE, 2014. **9**(9): p. e107380.
211. Millman, R.P., et al., *Body fat distribution and sleep apnea severity in women.* Chest, 1995. **107**(2): p. 362-6.

212. Fogel, R.B., et al., *Anatomic and physiologic predictors of apnea severity in morbidly obese subjects.* Sleep, 2003. **26**(2): p. 150-155.
213. Udwadia, Z.F., et al., *Prevalence of sleep-disordered breathing and sleep apnea in middle-aged urban Indian men.* Am J Respir Crit Care Med, 2004. **169**(2): p. 168-73.
214. Young, T., et al., *Predictors of sleep-disordered breathing in community-dwelling adults: the Sleep Heart Health Study.* Arch Intern Med, 2002. **162**(8): p. 893-900.
215. Sreedharan, S.E., et al., *Clinical and polysomnographic predictors of severe obstructive sleep apnea in the South Indian population.* Annals of Indian Academy of Neurology, 2016. **19**(2): p. 216-220.
216. Pradeep Kumar, V.G., et al., *Obstructive sleep apnoea: a case-control study.* Neurol India, 2003. **51**(4): p. 497-9.
217. Onat, A., et al., *Neck circumference as a measure of central obesity: associations with metabolic syndrome and obstructive sleep apnea syndrome beyond waist circumference.* Clin Nutr, 2009. **28**(1): p. 46-51.
218. Young, T., J. Skatrud, and P.E. Peppard, *Risk factors for obstructive sleep apnea in adults.* Jama, 2004. **291**(16): p. 2013-6.
219. Kunitomo, F., et al., *Sex differences in awake ventilatory drive and abnormal breathing during sleep in eucapnic obesity.* Chest, 1988. **93**(5): p. 968-976.
220. Pillar, G., et al., *Airway mechanics and ventilation in response to resistive loading during sleep: influence of gender.* American Journal of Respiratory and Critical Care Medicine, 2000. **162**(5): p. 1627-1632.
221. Xiao, Y., et al., *The evaluation of the severity of nocturnal oxygen desaturation of patients with obstructive sleep apnea hypopnoea syndrome.* Zhonghua Nei Ke Za Zhi, 2007. **46**(6): p. 458-61.
222. Tsai, C.M., et al., *Usefulness of desaturation index for the assessment of obstructive sleep apnea syndrome in children.* Int J Pediatr Otorhinolaryngol, 2013. **77**(8): p. 1286-90.
223. Johns, M.W., *A New Method for Measuring Daytime Sleepiness: The Epworth Sleepiness Scale.* Sleep, 1991. **14**(6): p. 540-545.
224. Macey, P.M., et al., *Relationship between Obstructive Sleep Apnea Severity and Sleep, Depression and Anxiety Symptoms in Newly-Diagnosed Patients.* PLoS ONE, 2010. **5**(4): p. e10211.
225. Ishman, S.L., et al., *Depression, sleepiness, and disease severity in patients with obstructive sleep apnea.* Laryngoscope, 2010. **120**(11): p. 2331-5.
226. Samocha-Bonet, D., et al., *Flow-resistant red blood cell aggregation in morbid obesity.* Int J Obes Relat Metab Disord, 2004. **28**(12): p. 1528-34.
227. Sinnapah, S., et al., *Overweight explains the increased red blood cell aggregation in patients with obstructive sleep apnea.* Clin Hemorheol Microcirc, 2015. **59**(1): p. 17-26.
228. Baskurt, O.K. and H.J. Meiselman, *Blood rheology and hemodynamics.* Semin Thromb Hemost, 2003. **29**(5): p. 435-50.
229. Baskurt, O.K., et al., *Modulation of endothelial nitric oxide synthase expression by red blood cell aggregation.* Am J Physiol Heart Circ Physiol, 2004. **286**(1): p. H222-9.
230. Baskurt, O.K. and H.J. Meiselman, *RBC aggregation: more important than RBC adhesion to endothelial cells as a determinant of in vivo blood flow in health and disease.* Microcirculation, 2008. **15**(7): p. 585-90.
231. Keddad, K., et al., *Blood lipids and rheological modifications in glycogen storage disease.* Clin Biochem, 1996. **29**(1): p. 73-8.

232. Zilberman-Kravits, D., et al., *Increased red cell aggregation is correlated with HbA1C and lipid levels in type 1 but not type 2 diabetes.* Clin Hemorheol Microcirc, 2006. **35**(4): p. 463-71.
233. Neu, B. and H.J. Meiselman, *Depletion-mediated red blood cell aggregation in polymer solutions.* Biophysical journal, 2002. **83**(5): p. 2482-2490.
234. Hatipoglu, U. and I. Rubinstein, *Inflammation and obstructive sleep apnea syndrome pathogenesis: a working hypothesis.* Respiration, 2003. **70**(6): p. 665-71.
235. Dikmenoğlu, N., et al., *Erythrocyte deformability, plasma viscosity and oxidative status in patients with severe obstructive sleep apnea syndrome.* Sleep medicine, 2006. **7**(3): p. 255-261.
236. Traverso, N., et al., *Malondialdehyde, a lipoperoxidation-derived aldehyde, can bring about secondary oxidative damage to proteins.* J Gerontol A Biol Sci Med Sci, 2004. **59**(9): p. B890-5.
237. Nobili, L., et al., *Morning increase of whole blood viscosity in obstructive sleep apnea syndrome.* Clin Hemorheol Microcirc, 2000. **22**(1): p. 21-7.
238. Toraldo, D.M., et al., *Obstructive sleep apnea syndrome: blood viscosity, blood coagulation abnormalities, and early atherosclerosis.* Lung, 2013. **191**(1): p. 1-7.
239. Geiser, T., et al., *In vivo platelet activation is increased during sleep in patients with obstructive sleep apnea syndrome.* Respiration, 2002. **69**(3): p. 229-234.
240. Feliciano, A., et al., *Hematological evaluation in males with obstructive sleep apnea before and after positive airway pressure.* Revista Portuguesa de Pneumologia (English Edition), 2017. **23**(2): p. 71-78.
241. Zhang, X., et al., *Effect of continuous positive airway pressure treatment on elderly Chinese patients with obstructive sleep apnea in the prethrombotic state.* Chin Med J (Engl), 2003. **116**(9): p. 1426-8.
242. Dean, R.T. and I. Wilcox, *Possible atherogenic effects of hypoxia during obstructive sleep apnea.* Sleep, 1993. **16**(8 Suppl): p. S15-21; discussion S21-2.
243. Yousef, A. and W. Alkhiary, *The severity of obstructive sleep apnea syndrome is related to red cell distribution width and hematocrit values.* J Sleep Disord Ther, 2015. **4**(192): p. 2167-0277.1000192.
244. Canino, B., et al., *Nitric oxide metabolites and erythrocyte deformability in a group of subjects with obstructive sleep apnea syndrome.* Clinical hemorheology and microcirculation, 2015. **59**(1): p. 45-52.
245. Ozturk, L., et al., *Lipid peroxidation and osmotic fragility of red blood cells in sleep-apnea patients.* Clin Chim Acta, 2003. **332**(1-2): p. 83-8.
246. West, S.D., D.J. Nicoll, and J.R. Stradling, *Prevalence of obstructive sleep apnoea in men with type 2 diabetes.* Thorax, 2006. **61**(11): p. 945-50.
247. Einhorn, D., et al., *Prevalence of sleep apnea in a population of adults with type 2 diabetes mellitus.* Endocr Pract, 2007. **13**(4): p. 355-62.
248. Tamura, A., et al., *Relationship between the severity of obstructive sleep apnea and impaired glucose metabolism in patients with obstructive sleep apnea.* Respiratory Medicine, 2008. **102**(10): p. 1412-1416.
249. Ip, M.S., et al., *Obstructive sleep apnea is independently associated with insulin resistance.* Am J Respir Crit Care Med, 2002. **165**(5): p. 670-6.
250. Papanas, N., et al., *HbA(1c) is associated with severity of obstructive sleep apnea hypopnoea syndrome in nondiabetic men.* Vascular Health and Risk Management, 2009. **5**: p. 751-756.
251. Brooks, B., et al., *Obstructive sleep apnea in obese noninsulin-dependent diabetic patients: effect of continuous positive airway pressure treatment on insulin responsiveness.* J Clin Endocrinol Metab, 1994. **79**(6): p. 1681-5.

252. Xu, H., et al., *Elevated low-density lipoprotein cholesterol is independently associated with obstructive sleep apnea: evidence from a large-scale cross-sectional study.* Sleep Breath, 2016. **20**(2): p. 627-34.
253. Nadeem, R., et al., *Effect of obstructive sleep apnea hypopnoea syndrome on lipid profile: a meta-regression analysis.* J Clin Sleep Med, 2014. **10**(5): p. 475-89.
254. Kawano, Y., A. Tamura, and J. Kadota, *Association between the severity of obstructive sleep apnea and the ratio of low-density lipoprotein cholesterol to high-density lipoprotein cholesterol.* Metabolism, 2012. **61**(2): p. 186-92.
255. Seals, D.R., N.O. Suwarno, and J.A. Dempsey, *Influence of lung volume on sympathetic nerve discharge in normal humans.* Circ Res, 1990. **67**(1): p. 130-41.
256. Morgan, B.J., T. Denahan, and T.J. Ebert, *Neurocirculatory consequences of negative intrathoracic pressure vs. asphyxia during voluntary apnea.* J Appl Physiol (1985), 1993. **74**(6): p. 2969-75.
257. Lowe, G.D., *Blood viscosity, lipoproteins, and cardiovascular risk.* Circulation, 1992. **85**(6): p. 2329-2331.
258. de Simone, G., et al., *Relation of blood viscosity to demographic and physiologic variables and to cardiovascular risk factors in apparently normal adults.* Circulation, 1990. **81**(1): p. 107-17.
259. Borgel, J., et al., *Obstructive sleep apnoea and its therapy influence high-density lipoprotein cholesterol serum levels.* Eur Respir J, 2006. **27**(1): p. 121-7.
260. Alexopoulos, E.I., et al., *Effects of obstructive sleep apnea severity on serum lipid levels in Greek children with snoring.* Sleep Breath, 2011. **15**(4): p. 625-31.