Short Communication

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Biomarkers in Medicine

Circulating levels of clusterin and complement factor H in patients with obstructive sleep apnea

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Aim: Obstructive sleep apnea (OSA) activates the complement system; however, the levels of membrane attack complex (MAC) are unaltered suggesting regulatory mechanisms. Our aim was to investigate complement factor H (CFH) and clusterin, two important complement regulators in OSA. **Materials & methods:** We analyzed clusterin and CFH levels in plasma of 86 patients with OSA and 33 control subjects. **Results:** There was no difference in CFH levels between patients (1099.4/784.6–1570.5/μg/ml) and controls (1051.4/652.0–1615.1/μg/ml, p = 0.72). Clusterin levels were higher in patients with OSA (309.7/217.2–763.2/μg/ml vs 276.1/131.0–424.3/μg/ml, p = 0.048) with a trend for a positive correlation with disease severity (p = 0.073). **Conclusion:** Increase in clusterin levels may be protective in OSA by blocking the MAC formation.

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Keywords: complement system • inflammation • OSA • sleep apnea

Obstructive sleep apnea (OSA) is a common disease, which is characterized by repetitive collapse of the upper airways during sleep resulting intermittent hypoxemia and frequent arousals. These lead to enhanced systemic inflammation that is reflected by increased concentration of several circulating pro-inflammatory molecules [1–3] and decreased level of anti-inflammatory molecules [4,5]. Understanding inflammation in OSA is important as it could link OSA to its cardiometabolic comorbidities, such as cardiovascular disease, hypertension, diabetes or dyslipidemia.

The complement system is part of the humoral innate immunity which is involved in the anti-microbial defense, augmentation of the antibody response, immunoregulation and clearance of immune complexes and apoptotic cells [6]. It has also a role in the pathophysiology of OSA [7–10]. More particularly, higher levels of early elements of the complement cascade, such as C3 [9,10] and C3a [8] were reported in sleep apnea. However, despite the activation of the complement system, molecules present at further steps of the pathway, such as C5a and SC5b-9 were unaltered in OSA [8] suggesting regulatory mechanisms.

Regulatory molecules of the complement cascade have not been widely investigated in OSA before [7,11,12]. The levels of thrombomodulin, which enhances complement factor I and complement factor H (CFH) function thus inhibiting the C5 convertase, and facilitates the degradation of C3a and C5a, were reported unaltered in OSA [11]. Reduced expression of complement component 4-binding α has been shown in OSA [7]. Component 4-binding α is an inhibitor of the classical and lectin pathway, but also blocks the formation of C3b with C4b2b [6]. Emin *et al.* reported that the internalization of CD59, an inhibitor of C5b-9 (membrane attack complex), is augmented in OSA [12] suggesting enhanced formation of C5b-9. However, this has not been confirmed by our previous study reporting unaltered SC5b-9 levels [8]. There was a tendency for increased CD35 following sleep deprivation [13]. CD35 destabilises C3 convertase and induces cleavage and inactivation C3b and C4b [6].

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CFH is the main regulator of the alternative pathway [6], but it also inhibits the C5 convertase [6]. In addition, it has a direct anti-inflammatory effect by decreasing the levels of pro-inflammatory cytokines such as IL-6, IL-8, IL-12, IFN-γ, TNF-α and facilitating the production of the immunoregulatory cytokines, including IL-10 and TGF-β [14]. Clusterin or apolipoprotein J is a 75–80 kDa disulphide-linked heterodimeric protein [15]. It is involved in lipid transport forming high-density lipoprotein particles, has an anti-apoptotic, anti-atherogenic and anti-inflammatory role [15]. More importantly, it inhibits the formation of the cytolytic active membrane attack complex resulting prevention from cell lysis, thus clusterin is thought to play a protective role in several pathological circumstances [6]. Previous studies have shown that the deficiency of clusterin causes altered complement response and aggravates inflammation. According to our knowledge, CFH has not been investigated in OSA before and only one study measured clusterin in sleep apnea. Although higher levels of clusterin have been reported in OSA, there was no relationship between clusterin concentration and disease severity following adjustment on age and BMI [16].

In this study we analyzed plasma samples for clusterin and CFH in patients with OSA and non-OSA controls and correlated their levels with markers of disease severity.

Materials & methods

Study design & subjects

We included 119 volunteers (55 ± 13 years, 62 men) who were referred for a diagnostic sleep study due to suspected OSA (i.e., sleep difficulties, snoring, witnessed apnea, daytime tiredness, obesity, uncontrolled comorbidities, etc.). We took a detailed medical history and patients filled out the Epworth Sleepiness Scale (ESS). This was followed by a full night inpatient polysomnography (n = 66) or cardiorespiratory polygraphy (n = 53). Blood samples were collected for clusterin, CFH, fasting glucose, C-reactive protein (CRP), glomerular filtration rate (GFR) and lipid profile (total cholesterol, high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C] and triglyceride).

The study was conducted in accordance with the amended Declaration of Helsinki. The study protocol has been approved by the Semmelweis University Scientific Research Ethics Committee (TUKEB, 30/2014 and RKEB 172/2018). Written informed consent was obtained from each patient included in the study.

Sleep studies

Inpatient polysomnography and cardiorespiratory polygraphy were performed using the Somnoscreen Plus Tele PSG and Somnoscreen RC devices, respectively (Somnomedics GmbH Germany) as described previously [8]. Sleep stages, movements and cardiopulmonary events were scored manually according to the American Academy of Sleep Medicine (AASM) guideline [17]. Apnea was defined by >90% drop in nasal airflow lasting for ≥10 s. Hypopnea was defined by >30% reduction in the nasal airflow lasting for ≥10 s, resulting in ≥3% drop in oxygen saturation or arousal. Total sleep time (TST), sleep period time (SPT) and minimal oxygen saturation (MinSatO2) were recorded, apnea–hypopnea index (AHI), oxygen desaturation index (ODI), percentage of total sleep time spent with saturation below 90% (TST90%) and arousal index (AI) were calculated to evaluate the severity of OSA. OSA was defined with an AHI >5/h.

Biomarker measurements

EDTA-treated venous blood samples were processed within 30 min and centrifuged at 1500 rpm for 10 min at 4°C. After centrifugation plasma samples were separated and stored immediately at -80°C until further analysis. Biomarker levels were measured using commercially available ELISA kits (Human Complement Factor H ELISA kit from Hycult Biotech, Uden, The Netherlands and Human Clusterin Quantikine ELISA Kit from R&D Systems, MN, USA). The measurements were performed in duplicates according to the manufacturers' instructions and their mean concentrations were used. The detection limits were 3.9 ng/ml and 0.189 ng/ml for CFH and clusterin. The intra-assay coefficient variabilities were 12 ± 17% and 19 ± 23% CFH and clusterin, respectively. All concentrations were above the detection limit.

Statistical analysis

Statistica 12 (StatSoft, Inc., OK, USA) and JASP 0.11.1 (University of Amsterdam, Amsterdam, The Netherlands) were used for statistical analysis. The normality of the data was assessed with Shapiro–Wilk test which showed non-parametric distribution for CFH and clusterin. Demographics and clinical characteristics were compared between the OSA and control groups using *t*-test, Mann–Whitney test and Chi-square test. The levels of clusterin and CFH

Table 1. Patient characteristics.

Characteristics	Total (n = 119)	OSA (n = 86)	Control (n = 33)	p-value
Age (years)	58.0/47.0–64.0	59.5/49.2–64.8	56.0/45.0–60.0	0.04
Gender, n (%)	52	59	33	0.01
BMI (kg/m^2)	30.21 ± 6.28	31.97 ± 6.04	25.61 ± 4.29	<0.01
Hypertension (%)	66	72	48	0.02
Diabetes (%)	24	29	12	0.05
Cardio/cerebrovascular disease (%)	15	20	3	0.23
Dyslipidemia (%)	51	58	33	0.02
Arrhythmia (%)	27	30	18	0.18
Glucose (mmol/l)	5.2/4.7–6.3	5.34/4.9–6.5	4.7/4.3–5.1	<0.01
Total cholesterol (mmol/l)	5.39 ± 1.18	5.30 ± 1.23	5.60 ± 1.04	0.21
HDL-C (mmol/l)	1.37/1.16–1.71	1.29/1.06–1.56	1.72/1.54–1	<0.01
LDL-C (mmol/l)	3.14 ± 1.07	3.13 ± 1.09	3.19 ± 1.01	0.77
Triglycerides (mmol/l)	1.40/1.06–2.09	1.56/1.13–2.29	1.16/0.86–1.65	<0.01
CRP (mg/l)	3.0/1.3–5.1	3.49/1.71–5.80	1.72/0.89–3.42	<0.01
GFR (ml/min/1.73 m^2)	86.76 ± 15.89	85.97 ± 15.08	88.27 ± 17.49	0.51
ESS	5/3–7	5/3–7	4/2–6	0.19
TST (min)	407.25/368.25–443.86	421.0/385.0–452.5	388.5/331.5–415.5	<0.01
SPT (min)	445.8/405.0–478.5	465.0/412.5–485.8	419.0/396.8–452.0	0.09
AHI (1/h)	17.3/4.2–32.3	23.7/15.8–39.6	1.9/1.1–3.0	<0.01
ODI (1/h)	14.1/2.2–27.6	20.2/11.9–35.5	1.1/0.6–1.7	<0.01
TST90% (%)	2.25/0.10–11.13	6.2/1.2–17.7	0.0/0.0–0.1	<0.01
MinSatO_2 (%)	84.5/78.0–89.0	82.0/76.0–85.0	90.0/88.0–92.0	<0.01
AI (1/h)	41.4/29.13–53.35	39.7/29.6–53.8	48.3/25.6–53.2	0.82

Subjects' characteristics. Data are presented as mean ± standard deviation or median/25–75%.
AHI: Apnea–hypopnea index; AI: Arousal index; CRP: C-reactive protein; ESS: Epworth Sleepiness Scale; GFR: Glomerular filtration rate; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; ODI: Oxygen desaturation index; SPT: Sleep period time; TST: Total sleep time; TST90%: Percentage of total sleep time spent with oxygen saturation below 90%.

were compared with clinical variables and the parameters of sleep architecture using nonparametric Spearman test. The biomarker levels were compared between patients with and without a specific comorbidity (i.e., hypertension, diabetes, etc.) with the Mann–Whitney test. Clusterin and CFH concentrations were compared between OSA and control groups as well as along disease severities with nonparametric analysis of covariance (ANCOVA) adjusted for age, gender and BMI. Data are expressed as mean ± standard deviation for parametric and as median/interquartile range/for nonparametric variables. A p-value <0.05 was considered significant.

The sample size was calculated to detect significant differences in CFH and clusterin levels between the OSA and control groups with an effect size of 0.40, statistical power of 0.80 and α error probability of 0.05. This sample size also allowed us to detect differences with an effect size of 0.40 among different groups of disease severity with a power of 0.80 [18].

Results

Comparison of the OSA & control groups

Eighty-six patients were diagnosed with OSA. Twenty-one had mild (AHI 5–14.9/h), 33 had moderate (AHI 15–29.9/h) and 32 had severe (AHI ≥30/h) disease. Patients with OSA were older, had higher prevalence of males, patients with hypertension, diabetes and dyslipidemia, they had higher CRP, fasting glucose and triglyceride levels and lower HDL-C levels (all $p < 0.05$). As expected, patients with OSA had higher AHI, ODI and TST90% and lower minSatO_2 (all $p < 0.05$, Table 1).

Plasma CFH results

Plasma CFH levels did not relate to age, BMI or any of the clinical or sleep parameters (all $p > 0.05$). However, CFH tended to be higher in men (1256.1/852.2–1628.3 μg/ml vs 963.2/576.2–1543.1 μg/ml, $p = 0.06$). Comparing patients with OSA (1099.4/784.6–1570.5 μg/ml) and controls (1051.4/652.0–1615.1 μg/ml, Figure 1A), no

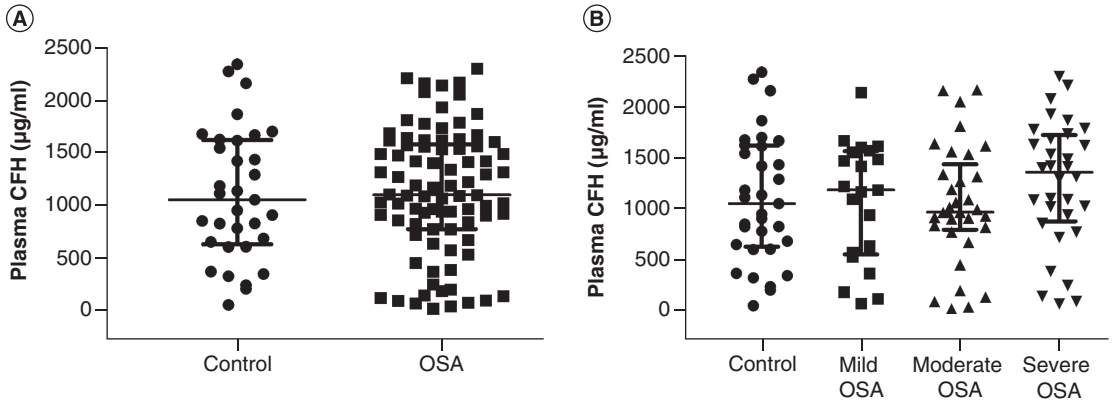

Figure 1. Plasma complement factor H levels. (A) Plasma complement factor H levels between the obstructive sleep apnea and control groups. **(B)** Plasma complement factor H levels between the severity groups.
CFH: Complement factor H; OSA: Obstructive sleep apnea.

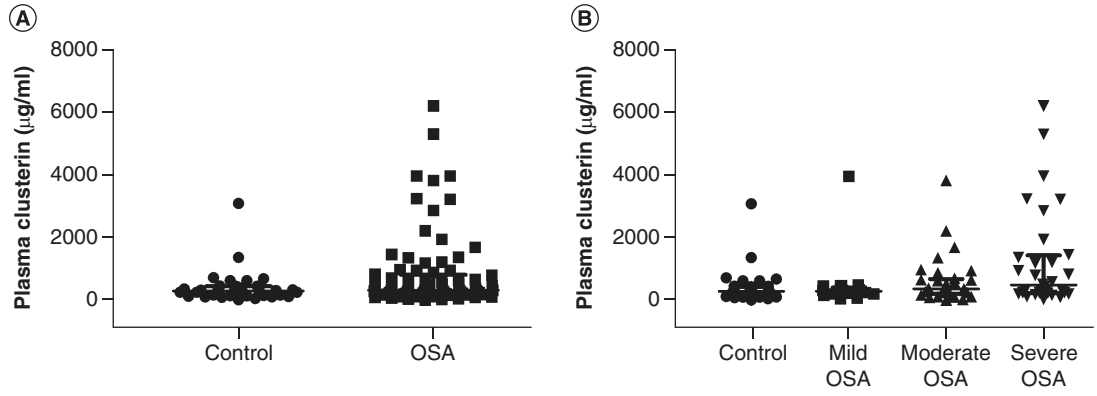

Figure 2. Plasma clusterin levels. (A) Plasma clusterin levels between the obstructive sleep apnea and control groups. **(B)** Plasma clusterin levels between the severity groups.
OSA: Obstructive sleep apnea.

difference was found (p = 0.72). Similarly, comparing CFH levels along groups of OSA severity, no difference was observed (p = 0.41, Figure 1B).

Plasma clusterin results

Plasma clusterin levels were higher in patients with OSA (309.7/217.2–763.2 µg/ml vs 276.1/131.0–424.3 µg/ml, p = 0.048, Figure 2A). There was also a trend for a positive correlation between increasing clusterin concentrations and disease severity (p = 0.073, Figure 2B). In line with this, clusterin levels related to AHI (ρ = 0.22, p = 0.02), ODI (ρ = 0.23, p = 0.01), TST90% (ρ = 0.18, p = 0.04). A significant, but weak relationship between clusterin and glucose levels (ρ = 0.25, p = 0.01) was observed together with a tendency for a correlation with CRP (ρ = 0.19, p = 0.05) and triglyceride levels (ρ = 0.16, p = 0.09) and for an inverse correlation with HDL-C levels (ρ = −0.17, p = 0.06). Plasma clusterin levels were higher in patients with hypertension (309.7/221.6–812.4 µg/ml vs 281.8/111.5–377.1 µg/ml, p = 0.02), and arrhythmia (595.2/264.6–1026.3 µg/ml vs 279.6/161.6–450.9 µg/ml, p < 0.01). Due to these significant relationships, comparison analyses between OSA and control groups were adjusted for glucose levels, and the presence of hypertension and arrhythmia as further covariates. Following adjustment, the difference between OSA and control groups was still significant (p = 0.03) and the trend between OSA severity and increasing clusterin concentrations became significant (p = 0.02).

Discussion

In this study we analyzed the circulating levels of CFH and clusterin in patients admitted for a diagnostic sleep test for suspected OSA. We found significantly higher levels of clusterin in OSA suggesting that this molecule could be responsible for the blunted complement response reported in our previous study [8]. In contrast, the levels of CFH were unaltered in OSA.

CFH has widely been investigated and altered levels were reported in renal disease, age-related macular degeneration, infections and various malignancies [19]. However, a fivefold variation in plasma CFH levels were noticed in the general population [20] and it is believed that its levels are primarily determined by genetic factors [20]. This variation in healthy subjects overlaps with values reported in various diseases limiting the diagnostic utility of CFH in clinical practice. Mechanisms inducing and regulating CFH expression are not fully known [21]. It has been shown that CFH secretion is induced by IFN-γ and glucocorticoids [22], which are elevated in OSA [1,23]. On the other hand, hypoxemia blunts CFH expression [24]. These contradictory mechanisms could explain why there was no overall change in CFH in OSA. Of note, CRP may potentiate the complement-inhibitory function of CFH [25] and we reported higher CRP levels in the current study. Thus, despite there was no difference in the quantity of CFH, this molecule could still contribute to the blunted complement response.

In line with the study by Peng *et al.* [16], we reported elevated plasma levels of clusterin in OSA. Interestingly, that study did not find a relationship between clusterin and AHI following adjustment on age and BMI. In our report, the association was significant following adjustment on further confounders, strengthening the relationship between clusterin and OSA. Elevated clusterin levels in OSA can be explained by multiple mechanisms. It is known that hypoxia may induce nuclear clusterin transcription via the *HIF-1α* [26]. In line with this, the correlation between markers of chronic intermittent hypoxemia and clusterin was significant. Upregulated clusterin expression facilitates autophagy resulting cytoprotective effects in hypoxic conditions [27]. Under oxidative stress, clusterin modulates the expression of several oxidative stress-related genes. In clusterin knock-out mice, increased expression of inducible nitric oxide synthase was detected and there was a decrease in expression of antioxidant superoxide dismutase [28]. In addition, glucose may also induce clusterin expression [29]. Supporting this, a significant correlation has been observed between glucose and clusterin in our study. Of note, the difference between OSA and control groups was still significant following adjustment on glucose levels. Furthermore, increased concentrations of clusterin in OSA could be due to elevated thrombin [30] and pro-inflammatory cytokine [1] levels, as thrombin and IL-1β [31] may facilitate clusterin expression [32]. Moreover, clusterin attenuate inflammation via the inhibition of NF-κB activity [33], which has an important role in systemic inflammatory processes in OSA [34]. In patients with OSA lower airway inflammation is characterized by neutrophil dominancy [35]. High clusterin expression has been shown in bronchial epithelial cells during inflammation [36], thus clusterin is proposed to have a protective effect via decreasing neutrophil infiltration and promoting phagocytosis [33]. According to the previous study, elevated clusterin levels were related to cognitive decline in OSA [16]. This not necessarily mean a causal relationship, as elevated clusterin levels in Alzheimer's disease represent a compensatory mechanism [37]. Apart from blunting the complement response, clusterin is involved in the clearance of amyloid-β peptides [37] and apoptotic cells [38], and has an anti-atherogenic role [15].

Manzar *et al.* previously reported that altered levels of complements are related to impaired sleep quality [39]. However, in line with our previous report [8], CFH and clusterin did not relate to Epworth Sleepiness Scale. This discrepancy could be due to the fact that Manzar *et al.* investigated a healthy and young population. Further studies, clarifying daytime consequences of complement system in OSA are warranted.

As expected, patients with OSA had a higher BMI. Obesity itself is associated with heightened systemic inflammation and fat tissue may be a source for pro-inflammatory molecules in OSA [40]. However, the observed differences were unlikely due to obesity. First, neither CFH nor clusterin levels correlated with BMI. Secondly, the comparisons were adjusted for BMI.

The study has some limitations. Our study included subjects who were referred to our Sleep Unit due to suspected OSA (i.e., snoring, witnessed apnea, fatigue, daytime sleepiness, obesity or cardiometabolic comorbidities). Snoring itself causes upper airway inflammation due to the vibrational trauma and inflammation of the upper airways affect the systemic inflammatory processes. Moreover, OSA-related cardiovascular or metabolic comorbidities are associated with oxidative stress and low-grade systemic inflammation. Previous studies showed evidence that altered circulating clusterin levels were associated with metabolic and cardiovascular diseases [41], which disorders were frequent in our control group (48% hypertension, 18% arrhythmia, 33% dyslipidemia). In line with this, the levels

circulating clusterin were higher in the current study than in a previous report analyzing healthy, young women [42], but comparable to those reported in a middle-aged general populations [43,44], although in the latter two studies comorbidities were not evaluated systematically. To reduce the level of bias due to comorbidities, our analyses were adjusted for these in addition to the demographics and BMI; however, further studies balancing OSA and control groups on demographics and clinical characteristics are warranted to eliminate this bias. Another limitation of the study is the relatively small number of control participants. We believe that our data will serve basis for study design and sample size calculations. Sixty-six volunteers had a full-night polysomnography, therefore the values of AI, TST and SPT need to be interpreted with caution. The study had a cross-sectional and case control design, patients with OSA did not have follow-up measurements after treatment with continuous positive airway pressure (CPAP). Further studies should address how CPAP therapy influences plasma clusterin levels.

Conclusion

In summary, we reported increased concentrations of plasma clusterin in OSA. This suggests a potential immunoregulatory mechanism and could be an explanation for the previously reported blunted complement response in OSA. However, to understand the role of clusterin in OSA in detail, studies evaluating control groups balanced for demographic characteristics and comorbid profile, as well as CPAP trials are warranted. Moreover, further studies analyzing other regulatory molecules of the C5, such as Factor I or decay-accelerating factor [6] are needed to understand the role of complement cascade in OSA.

Summary points

- Results of this article:
  - Circulating clusterin levels are higher in obstructive sleep apnea (OSA) and relate to disease severity;
  - The plasma levels of complement factor H are unaltered in OSA.
- Conclusion: increased concentrations of plasma clusterin suggest a potential immunoregulatory mechanism and could be an explanation for the previously reported blunted complement response in OSA.

Author contributions

A Bikov, AD Tarnoki, DL Tarnoki, Z Lazar and L Kunos conceived the study and participated in study design. A Bikov, A Kis and L Kunos performed sleep studies and analysed data. M Meszaros and P Horvath performed complement measurements. A Bikov and M Meszaros performed statistical analyses. The manuscript was drafted by M Meszaros and A Bikov and was critically reviewed and approved by all authors.

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the local Ethics Committee (Semmelweis University, TUKEB 30/2014). Informed consent was obtained from all individual participants included in the study.

References

1. Unnikrishnan D, Jun J, Polotsky V. Inflammation in sleep apnea: an update. *Rev. Endocrin. Metab. Disord.* 16(1), 25–34 (2015).
2. Kerget B, Afşin DE, Kerget F, Aşkın S, Araz Ö, Akgün M. Is vimentin the cause or effect of obstructive sleep apnea development? *Lung* doi:10.1007/s00408-020-00341-6 (2020).
3. Horvath P, Lazar Z, Galffy G *et al.* Circulating P-selectin glycoprotein ligand 1 and P-selectin levels in obstructive sleep apnea patients. *Lung* 198(1), 173–179 (2020).
4. Kunos L, Horvath P, Kis A *et al.* Circulating survivin levels in obstructive sleep apnoea. *Lung* doi:10.1007/s00408-018-0120-z (2018).
5. Pako J, Kunos L, Meszaros M *et al.* Decreased levels of anti-aging klotho in obstructive sleep apnea. *Rejuv. Res.* doi:10.1089/rej.2019.2183 (2019).
6. Noris M, Remuzzi G. Overview of complement activation and regulation. *Semin. Nephrol.* 33(6), 479–492 (2013).
7. Jurado-Gamez B, Gomez-Chaparro JL, Munoz-Calero M *et al.* Serum proteomic changes in adults with obstructive sleep apnoea. *J. Sleep Res.* 21(2), 139–146 (2012).
8. Horvath P, Tarnoki DL, Tarnoki AD *et al.* Complement system activation in obstructive sleep apnea. *J. Sleep Res.* 27(6), e12674 (2018).
9. Lu X, Wang X, Xu T *et al.* Circulating C3 and glucose metabolism abnormalities in patients with OSAHS. *Sleep Breathing* 22(2), 345–351 (2018).
10. Wang X, Wang Y, Cai Z *et al.* Alterations of IGF-1, complement C3 and superoxide dismutase in patients with moderate-to-severe obstructive sleep apnea hypopnea syndrome. *Biomark. Med.* 12(3), 217–228 (2018).
11. Takagi T, Morser J, Gabazza EC *et al.* The coagulation and protein C pathways in patients with sleep apnea. *Lung* 187(4), 209–213 (2009).
12. Emin M, Wang G, Castagna F *et al.* Increased internalization of complement inhibitor CD59 may contribute to endothelial inflammation in obstructive sleep apnea. *Sci. Transl. Med.* 8(320), 320ra321 (2016).
13. Christoffersson G, Vagesjo E, Pettersson US *et al.* Acute sleep deprivation in healthy young men: impact on population diversity and function of circulating neutrophils. *Brain Behav. Immun.* 41, 162–172 (2014).
14. Olivar R, Luque A, Cardenas-Brito S *et al.* The complement inhibitor factor H generates an anti-inflammatory and tolerogenic state in monocyte-derived dendritic cells. *J. Immunol.* 196(10), 4274–4290 (2016).
15. Pereira RM, Mekary RA, Da Cruz Rodrigues KC *et al.* Protective molecular mechanisms of clusterin against apoptosis in cardiomyocytes. *Heart Failure Rev.* 23(1), 123–129 (2018).
16. Peng Y, Zhou L, Cao Y *et al.* Relation between serum leptin levels, lipid profiles and neurocognitive deficits in Chinese OSAHS patients. *Int. J. Neurosci.* 127(11), 981–987 (2017).
17. Berry RB, Budhiraja R, Gottlieb DJ *et al.* Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. *J. Clin. Sleep Med.* 8(5), 597–619 (2012).
18. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav. Res. Meth.* 41(4), 1149–1160 (2009).
19. Parente R, Clark SJ, Inforzato A, Day AJ. Complement factor H in host defense and immune evasion. *Cell. Mol. Life Sci.* 74(9), 1605–1624 (2017).
20. Esparza-Gordillo J, Soria JM, Buil A *et al.* Genetic and environmental factors influencing the human factor H plasma levels. *Immunogenetics* 56(2), 77–82 (2004).
21. Parente R, Clark SJ, Inforzato A, Day AJ. Complement factor H in host defense and immune evasion. *Cell. Mol. Life Sci.* 74(9), 1605–1624 (2017).
22. Dauchel H, Julen N, Lemercier C *et al.* Expression of complement alternative pathway proteins by endothelial cells. Differential regulation by interleukin 1 and glucocorticoids. *Eur. J. Immunol.* 20(8), 1669–1675 (1990).
23. Tomfohr LM, Edwards KM, Dimsdale JE. Is obstructive sleep apnea associated with cortisol levels? A systematic review of the research evidence. *Sleep Med. Rev.* 16(3), 243–249 (2012).
24. Okroj M, Corrales L, Stokowska A, Pio R, Blom AM. Hypoxia increases susceptibility of non-small cell lung cancer cells to complement attack. *Cancer Immunol. Immunother.* 58(11), 1771–1780 (2009).
25. Mold C, Gewurz H, Du Clos TW. Regulation of complement activation by C-reactive protein. *Immunopharmacology* 42(1–3), 23–30 (1999).
26. Park J, Park SY, Shin E *et al.* Hypoxia inducible factor-1α directly regulates nuclear clusterin transcription by interacting with hypoxia response elements in the clusterin promoter. *Mol. Cells* 37(2), 178–186 (2014).
27 . Alnasser HA, Guan Q, Zhang F, Gleave ME, Nguan CY, Du C. Requirement of clusterin expression for prosurvival autophagy in hypoxic kidney tubular epithelial cells. *Am. J. Physiol. Renal Physiol.* 310(2), F160–F173 (2016).

28. Kwon MJ, Ju TJ, Heo JY *et al.* Deficiency of clusterin exacerbates high-fat diet-induced insulin resistance in male mice. *Endocrinology* 155(6), 2089–2101 (2014).
29. Kim G, Kim GH, Oh GS *et al.* SREBP-1c regulates glucose-stimulated hepatic clusterin expression. *Biochem. Biophys. Res. Commun.* 408(4), 720–725 (2011).
30. Robinson GV, Pepperell JC, Segal HC, Davies RJ, Stradling JR. Circulating cardiovascular risk factors in obstructive sleep apnoea: data from randomised controlled trials. *Thorax* 59(9), 777–782 (2004).
31. Zwain IH, Grima J, Cheng CY. Regulation of clusterin secretion and mRNA expression in astrocytes by cytokines. *Mol. Cell. Neurosci.* 5(3), 229–237 (1994).
32. Laping NJ, Olson BA, Short B, Albrightson CR. Thrombin increases clusterin mRNA in glomerular epithelial and mesangial cells. *J. Am. Soc. Nephrol.* 8(6), 906–914 (1997).
33. Du J, Liu L, Yu B *et al.* Clusterin (apolipoprotein J) facilitates NF-κB and Bax degradation and prevents I/R injury in heart transplantation. *Int. J. Clin. Exp. Pathol.* 11(3), 1186–1196 (2018).
34. Wei Q, Bian Y, Yu F *et al.* Chronic intermittent hypoxia induces cardiac inflammation and dysfunction in a rat obstructive sleep apnea model. *J. Biomed. Res.* 30(6), 490–495 (2016).
35. Bikov A, Losonczy G, Kunos L. Role of lung volume and airway inflammation in obstructive sleep apnea. *Respir. Invest.* 55(6), 326–333 (2017).
36 . Zheng D, Limmon GV, Yin L *et al.* A cellular pathway involved in Clara cell to alveolar type II cell differentiation after severe lung injury. *PLoS ONE* 8(8), e71028 (2013).
37. Nuutinen T, Suuronen T, Kauppinen A, Salminen A. Clusterin: a forgotten player in Alzheimer's disease. *Brain Res. Rev.* 61(2), 89–104 (2009).
38. Cunin P, Beauvillain C, Miot C *et al.* Clusterin facilitates apoptotic cell clearance and prevents apoptotic cell-induced autoimmune responses. *Cell Death Dis.* 7(5), e2215 (2016).
39. Manzar MD, Rajput MM, Zannat W *et al.* Association between sleep quality and inflammatory complement components in collegiate males. *Sleep Breathing* 20(2), 867–872 (2016).
40. Ryan S, Arnaud C, Fitzpatrick SF, Gaucher J, Tamisier R, Pépin JL. Adipose tissue as a key player in obstructive sleep apnoea. *Eur. Respir. Rev.* 28(152), 190006 (2019).
41 . Baralla A, Sotgiu E, Deiana M *et al.* Plasma clusterin and lipid profile: a link with aging and cardiovascular diseases in a population with a consistent number of centenarians. *PLoS ONE* 10(6), e0128029–e0128029 (2015).
42. Dombai B, Ivancsó I, Bikov A *et al.* Circulating clusterin and osteopontin levels in asthma and asthmatic pregnancy. *Can. Respir. J.* 2017, 1602039 (2017).
43. Mayado A, Teodosio C, Garcia-Montero AC *et al.* Increased IL6 plasma levels in indolent systemic mastocytosis patients are associated with high risk of disease progression. *Leukemia* 30(1), 124–130 (2016).
44. Hsu JL, Lee WJ, Liao YC, Lirng JF, Wang SJ, Fuh JL. Plasma biomarkers are associated with agitation and regional brain atrophy in Alzheimer's disease. *Sci. Rep.* 7(1), 5035 (2017).