Original Research Article

New Perspective in Obstructive Sleep Apnea Syndrome – The Use of Biomarkers as a Screening Tool

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Abstract

The sedentary lifestyle and extreme environmental changes led to a 3-fold increase in obesity prevalence since 1975. Although obesity can be prevented, once appeared it represents a major risk factor in developing/progression of obstructive sleep apnea syndrome (OSAS) and other comorbidities. Nowadays, the prevalence of OSAS is also continuously growing in an exponential mode, making it obvious that a new approach is needed. The association between obesity and OSAS has been described since ancient time and only recently has become of great interest worldwide because of its great impact on people’s quality of life. Obesity and OSAS represent a major health problem because of the increased risk of cardiovascular diseases (CVD), systemic inflammation, metabolic disorders, dyslipidemia, non-alcoholic fatty liver disease (NAFLD), hyperuricemia, gout, chronic kidney disease (CKD), depression, anxiety, cognitive impairment, neurological disorders, excessive daytime sleepiness, decreased vigilance leading to road traffic accidents. The polysomnography is considered to be the “gold standard method” to diagnose OSAS and its severity, but finding a new and non-invasive screening tool is becoming vital because of the worldwide disease impact upon the quality of life and also by judging the need of achieving a long-lasting therapeutic control.

Keywords: Biomarkers, Cardiovascular diseases, Non-alcoholic fatty liver disease, Obesity, Obstructive sleep apnea, Type 2 diabetes mellitus

INTRODUCTION

The sedentary lifestyle and extreme environmental changes led to a 3-fold increase in obesity prevalence since 1975: most of the world’s population lives in countries where overweight and obesity kills more people than underweight (World Health Organization, 2017).

In 2016, more than 1.9 billion adults, 18 years and older, were overweight worldwide. Of these over 650 million were obese (World Health Organization, 2017). 39% of adults aged 18 years and over were overweight in 2016, and 13% were obese (World Health Organization, 2017).

The PREDATOR study showed a high prevalence of obesity/overweight in Romanian population, 31% of adults aged 18 or more were obese and 35% were overweight (Popa et al., 2016).

The association between obesity and obstructive sleep apnea syndrome (OSAS), described since ancient time, only recently has become of great interest worldwide because of its significant impact on people’s quality of life.

The prevalence of OSAS worldwide is approximately 3-7% in adult men and 2-5% for adult women (Khurana et al., 2019).

As we see it, nowadays, the prevalence of OSAS is continuously growing in an exponential mode, making it obvious that a new approach is needed. Sleep is an important part of our lives and in the past years, along with lifestyle optimization, recent studies have been focusing on the quality of sleep as a novel missing puzzle-piece.

Obesity and OSAS represent a major health problem because of the increased risk of cardiovascular diseases...
can say that the relationship between obesity and OSAS et al., 2016; Aurora and Punjabi, 2013). Furthermore, we association between obesity and OSAS, T2DM and that the symptomatology of OSAS is nonspecific, using intensively and exhaustively worldwide showed the tight association between obesity and OSAS, T2DM and OSAS, respectively (Reutrakul and Mokhlesi, 2017; Protasiewicz et al., 2017; Jehan et al., 2018; Nagayoshi et al., 2016; Aurora and Punjabi, 2013). Furthermore, we can say that the relationship between obesity and OSAS is bidirectional and so does the one between OSAS and T2DM (Jehan et al., 2018; Nagayoshi et al., 2016; Aurora and Punjabi, 2013; Alterki et al., 2020; André et al., 2020).

The medical research field identified OSAS as an independent risk factor for stroke, arrhythmia, arterial hypertension and coronary heart disease (André et al., 2020).

The main pathological mechanisms by which OSAS leads to other comorbidities or interact with obesity and T2DM are shown in Figure 1 (Jehan et al., 2018; Protasiewicz et al., 2017; Nagayoshi et al., 2016; Aurora and Punjabi, 2013; Ryan, 2017; Ryan, 2018; May and Mehra, 2014; Kent et al., 2015; Murphy et al., 2017; Lam et al., 2012; Doumit and Prasad, 2016; Beaudin et al., 2019; Kohler and Stradling, 2010; Chopra et al., 2017; Bonsignore et al., 2019; Peres et al., 2020).

RESULT AND DISCUSSION

Understanding the mechanisms and following the physiopathological pathways by which OSAS leads to the well known comorbidities might help us to identify new screening tools for OSAS and also novel therapeutic approaches (Ryan, 2017; Ryan, 2018).

Although nowadays the polysomnography is considered to be “the gold standard method” in identifying new cases of OSAS (Conte et al., 2020), this current scoping review’s role is to present another “possible novel missing puzzle-piece” in the OSAS’ diagnose and/or management. This novel missing puzzle-piece is represented by a biological marker called biomarker (blood biomarkers - the majority, salivary biomarkers, urine biomarkers or exhaled breathing biomarkers). We intend to evaluate them according to their clinical applicability, resuming 21 newest medical articles published especially over the past few years.

These articles presented the results of hundreds of studies conducted intensively worldwide since 2004 on the adult population with confirmed OSAS and/or T2DM and/or obesity and/or cardiovascular diseases. Figure 1

Studying the latest comprehensive literature review of biomarkers associated with OSAS it is obvious that an ideal biomarker should have certain characteristics like sensitivity, specificity, affordability, ease of use, as well as being fast and able to be correlated with the severity of the disease (Lebkuchen et al., 2020; Fleming et al., 2016). Thus, a single blood test, or combination of two or more blood tests, could potentially meet these criteria (Fleming et al., 2016).

In 2015 Graziela De Luca Canto et al. published an interesting review of no less than 117 articles of studies conducted in humans without age restriction, evaluating the potential diagnostic value of biological markers (blood, exhaled breath condensate, salivary and urinary) in the OSAS diagnosis (Canto Gde L et al., 2015).
Between 2000 and 2014 there were conducted no less than 82 studies in adults, most of them assessed blood-based biomarkers, 2 focused on urinary biomarkers, a number of 2 explored for biomarkers in exhaled breath condensate and just one study examined saliva; just a few studies investigated a combination of 2 biomarkers, so 5 studies used both blood and urine and 4 studies used blood and exhaled breath condensate (Canto Gde L et al., 2015). The studies investigated blood biomarkers as C reactive protein (CRP), high sensitive C reactive protein (hsCRP), IL-6, IL-8, IL-10, TNF-α, MCP-1, S100 calcium-binding protein B (S100B), neuron-specific enolase (NSE), Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-kB), homocysteine, cysteine, leptin, adiponectin, intercellular adhesion molecule-1 (ICAM-1), plasminogen activator inhibitor type 1 (PAI-1), fibrinogen, endothelin-1, Vascular endothelial growth factor (VEGF), P-selectin, insulin, glucose, Cystatin C, uric acid; exhaled breath condensate biomarkers like nitric oxide, Leukotriene B4 (LTB4), IL-6; urinary biomarkers as norepinephrine, epinephrine, normetanephrine, metanephrine, urinary neurotransmitters, 8-isoprostan; salivary biomarkers like α-amylase, salivary cortisol, melatonin (Canto Gde L et al., 2015). In conclusion, the investigation revealed IL-6, TNF-α, and hsCRP as potentially promising biomarkers in adults (Canto Gde L et al., 2015).

In 2016 W.E. Fleming et al. published an article following a multicenter trial that was conducted on a number of 73 symptomatic male subjects suspected of OSAS that underwent polysomnography (Fleming et al., 2016). There were 11 biomarkers tested, as follows: HbA1c, CRP, erythropoietin (EPO), IL-6, uric acid, cortisol, insulin-like growth factor-1 (IGF-1), human growth hormone (hGH), prolactin, testosterone, dehydroepiandrosterona (DHEA) (Fleming et al., 2016). Out of 73 male subjects enrolled, 26 men had moderate/severe OSAS. Statistical analysis showed that using the HbA1c, CRP, EPO, IL-6, and uric acid as blood biomarkers was superior to the Epworth Sleepiness Scale (ESS) in diagnosing OSAS subjects. The combination of high levels of HbA1c and CRP provided a greater predictive power; a combination of elevated HbA1c, CRP, and EPO provided high sensitivity (85%) and specificity (79%) for moderate/severe OSA (Lebkuchen et al., 2020). In conclusion, the use of blood biomarkers alone or in combination seems to identify a greater percentage of moderate/severe OSAS patients and mild/no OSAS patients compared to the ESS questionnaire filling (Fleming et al., 2016).

In 2018 W.E. Fleming et al. return with a new article, this time following a multicenter prospective study that
enrolled 264 male subjects, OSA suspected that also underwent polysomnography (Fleming et al., 2018). Taking into consideration the last prospective study, Fleming et al. investigated only 5 blood biomarkers (HbA1c, CRP, EPO, IL-6, uric acid) (Fleming et al., 2018). The results were quite outstanding, showing that using a combination of 3 biomarkers (HbA1c+CRP+EPO) was superior to the ESS and STOP-Bang questionnaires filling (Fleming et al., 2018). Due to its high sensitivity (81%) and specificity (60%) results, this triple biomarker HbA1c+CRP+EPO can be used as a screening tool that correlates with severity of disease (Fleming et al., 2018).

In 2012 Y. Chihara et al. thought of a OSAS biomarker that could be extremely helpful, especially in identifying patients who have severe OSAS that would put them at risk for CVD (Chihara et al., 2013). Prostaglandin D2 (PGD2), widely spread in humans’ brain where is implicated in mechanisms like sleep induction, modulation of body temperature, hormone release, nociception and neuromodulation and in peripheral is involved in vasodilatation, inhibition of platelet aggregation, glycochenolysis, vasoconstriction (Chihara et al., 2013). Lipocalin-type prostaglandin D synthase (L-PGDS) is responsible for the biosynthesis of PGD2, and has a strong connection with CVD and sleep regulation. Regarding all this, Chihara et al. enrolled 64 subjects in a prospective study and measured the urinary concentrations of L-PGDS in the morning. The morning urinary L-PGDS concentrations seemed to have significant correlations with the apnea/hypopnea index and serum high-density lipoprotein cholesterol (HDL Col) (Chihara et al., 2013). In conclusion, urinary L-PGDS might be a moderately useful marker to identify patients with severe OSAS (Chihara et al., 2013).

Carolina V. R. D’Aurea et al. in 2017 published an article following a study conducted over a large number of participants (7115 subjects) that aimed to investigate the inter-relation between (hsCRP) and HbA1c in prediction of risk of OSAS (D’Aurea et al., 2017). The participants filled the Berlin questionnaire for risk of OSAS and hsCRP, Hba1c, HDL chol and triglycerides were evaluated (D’Aurea et al., 2017). The results showed that the HbA1c is independently associated with OSAS (D’Aurea et al., 2017). Due to the increasing prevalence of OSAS and considering the interrelationship between OSAS and obesity, Bozkuş F et al. in 2018 aimed to demonstrate the predictive power of the inflammatory markers upon the degree of systemic inflammation, which could represent a prognostic factor for metabolic risks (Bozkuş et al., 2018). So they started to explore a promising marker that could be used as an indicator of systemic inflammation. This new marker is neutrophil-to-lymphocyte ratio (NLR) (Bozkuş et al., 2018). In this study a number of 115 subjects were enrolled (58.7% male; 41.3% female) of which 38 were overweight and 39 obese. They underwent polysomnography and were divided into 2 groups considering the apnea hypopnea index (AHI) (<5 and >5) (Bozkuş et al., 2018). The OSAS group was divided considering the body mass index (BMI) value into normal weight, overweight and obese, respectively (Bozkuş et al., 2018). The results of the study showed that the lymphocyte counts of obese OSAS group were the lowest among all groups. NLR is an indicator of subclinical inflammation, it is simply measured from the complete blood count of peripheral blood. The neutrophil count reflects the inflammatory status, while the lymphocyte count is considered to be related to general stress and nutritional status of the body. Thus, the NLR could be used as a marker of inflammation, both in cardiac and non-cardiac disorders (Bozkuş et al., 2018). M. Traxdorf et al. in 2016 conducted a prospective study to determine whether serum or saliva S100B could be established as an invasive or non-invasive biomarker of cerebrovascular stress due to chronic intermittent hipoxemia in OSAS (Traxdorf et al., 2016). There were 40 subjects enrolled with OSAS (confirmed by polysomnography) and 20 healthy subjects in the control group (Traxdorf et al., 2016). Serum S100B was significantly higher in OSAS than in the healthy control group. Due to the fact that S100B was not related to the severity of OSAS and was independent of age, sex and subjective daytime symptoms it does not correlate with the severity of the disease, making it a not suitable biomarker to investigate (Traxdorf et al., 2016).

A Greek cross-sectional study conducted by Bouloukaki et al. (2017) enrolled 2983 subjects that underwent polysomnography for OSAS diagnosis. A number of 1053 subjects were grouped according to AHI as mild, moderate or severe form of OSAS. BMI, polysomnography data, levels of hs-CRP, fibrinogen, erythrocyte sedimentation rate(ESR), and uric acid were measured and compared between the groups. The results showed that all biomarkers were independently associated with OSAS severity and gender(p<0.05); females had increased levels of hs-CRP, fibrinogen, and ESR compared to men (p<0.001), but men had higher uric acid levels (p<0.001) (Bouloukaki et al., 2017). The Turkish research team led by professor B. Sertogullarindan started studying a novel protein that regulates fatty acid (FA) and triglyceride metabolism. This protein called betatrophin is related to obesity and metabolic abnormalities, including metabolic syndrome, T2DM, and dyslipidemia (Sertogullarindan et al., 2019). The researching team aimed to investigate the relationships among betatrophin, OSAS, and the serum lipid profile. So, they have enrolled 90 obese and middle aged subjects that underwent polysomnography (Sertogullarindan et al., 2019). After polysomnography was performed based on the AHI value the subjects were divided into 2 groups. Plasma betatrophin, adiponectin, leptin and the full lipid profile underwent serious statistical analysis. The results were very promising showing higher
levels of betatrophin, leptin, and adiponectin in patients with OSAS compared to the control group and betatrophin levels were correlated with the AHI (P=0.002), leptin (P=0.000) (Sertogullarindan et al., 2019).

According to the latest literature, Cystatin C (Cyst C) and neutrophil gelatinase-associated lipocalin (NGAL) are novel biomarkers for the earlier detection of latent kidney disease and OSAS (Voulgaris et al., 2019). A. Voulgaris, K. Archontogeorgis et al. published in 2019 a study conducted over 96 subjects (79.2% men) with no comorbidities and symptoms of OSAS that underwent PSG and blood examination for the measurement of serum Cyst C and NGAL levels (Voulgaris et al., 2019). Based on AHI value, the subjects were divided into 2 groups. The results showed that Serum Cyst C (p = 0.001) and NGAL (p = 0.035) mean levels were higher in OSAS patients compared to those in controls, making the 2 biomarkers trustworthy (Voulgaris et al., 2019).

Knowing the fact that platelets deliver specific enzymes and substrate molecules, it is quite astonishing how they are capable of synthesizing proteins in a stimuli-dependent manner despite their lack of nucleus. Atherosclerosis and cardiovascular complications result from disruption in homeostasis of blood coagulation and inflammatory processes modulation. Increased sympathetic activity promotes persistent platelet activation in OSAS patients. This persistent excessive platelet activation leads to myocardial infarction and other thromboembolic complications.

Available literature provides support to the fact that blood platelets in OSAS patients are a viable therapeutic target to decrease CVD risk (Gabryelska et al., 2018). Platelet parameters are routinely evaluated as a part of complete blood count test so they were proposed as markers of cardiovascular comorbidity in OSAS patients (Gabryelska et al., 2018)

In 2018 Kanbay A, Ceylan E et al. introduced a novel endothelial cell dysfunction marker called endocan, a proteoglycan secreted by vascular endothelium that can be detected in the blood. Endocan plays an important role in cell adhesion, migration and proliferation (Kanbay et al., 2018). OSAS is an independent risk factor for endothelial dysfunction and cardiometabolic diseases (Kanbay et al., 2018). Polysomnography was performed and blood tests were conducted (plasmatic endocan, serum levels of lipid profile, HbA1c and hsCRP) for all the 128 recruited subjects (113 OSAS subjects and 15 control subjects) of which 64 had hypertension, 41 had T2DM and 31 had CVD (Kanbay et al., 2018). With this study, the researchers showed that serum endocan levels are independently associated with presence of OSAS and OSAS severity (Kanbay et al., 2018).

The inflammatory profile – CRP, hsCRP, TNF-α, IL-6, IL-8, cell adhesion molecules (CAMs) is dysregulated in most of the infections and diseases including OSAS. So there is a need of a new perpetuum quest for revolutionary biomarkers in OSAS. This quest is rapidly achieving new heights in understanding the molecular mechanisms underlying the pathophysiology of OSA (miRNA and transcriptome profiling, DNA methylation and single nucleotide polymorphisms) (Khurana et al., 2019).

In 2019, Santamaria-Martos F. et al. published their evaluation upon the utility of miRNAs as biomarkers in the personalized management of OSAS (Santamaria-Martos et al., 2019). The study included 230 subjects, ages 18 to 60 years of old. They identified 3 major miRNAs that could contribute in the new medical approach. Mir-486 is associated with fasting levels of glucose and insulin and also with HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) (Santamaria-Martos et al., 2019). Mir-340 deregulates IGF 1R (insulin growth factor) gene expression and regulates MITF (melanocyte inducing transcription factor) gene expression (Santamaria-Martos et al., 2019). Mir-199b represses a key gene in the response to hypoxic stress, HIF 1A (hypoxia inducible factor 1 subunit alpha) (Santamaria-Martos et al., 2019). In this study, these 3 miRNAs expressions were downregulated in patients with OSA (Santamaria-Martos et al., 2019).

Based on the association of OSAS with the cardiovascular diseases and cancer, Lunara S Freitas et al. started the search for new molecular biomarkers using real-time quantitative polymerase chain reaction method to identify 2 circulating miRNAs associated with OSAS (Freitas et al., 2020). These 2 miRNAs are miR-1254 and miR-320e (Freitas et al., 2020). MiR-1254 is involved in heart failure, miR-320e in myocardial ischemia, the association between them both regards the cell proliferation (especially colorectal cancer) (Freitas et al., 2020). The article published in 2020 concluded that the severe form of OSAS is independently associated with these two miRNAs and thus, miR-1254 and miR-320e could represent promising biomarkers for OSAS (Freitas et al., 2020).

Matrix metalloproteinases (MMPs) are proteolytic enzymes and take part in remodeling of extracellular matrix. MMPs are synthesized as inactive zymogens by inflammatory cells, fibroblasts and endothelium (Franckczak et al., 2019). Recurrent episodes of apneas/hypopneas in OSAS cause a desaturation-reoxygenation cycle that resembles ischemia/reperfusion injury, causing which an excessive production of reactive oxygen species (ROS) and upregulation of oxidative stress markers and impaired antioxidant capacity in OSAS (Franckczak et al., 2019). A. Franczak, I. Bil-Lula, et al. launched the hypothesis that MMPs can be biomarkers of severity of OSAS and may “potentially be useful biomarkers of cardiovascular complications” in OSAS (Franczak et al., 2019).

Sunnetcioglu A. et al. in 2018 published the results of a retrospective study involving 600 subjects over 18 years of age, evaluated by polysomnography (Sunnetcioglu et al., 2018). According to the AHI value, patients
were grouped in mild OSAS, moderate OSAS, and severe OSAS groups (Sunnetcioglu et al., 2018). The hematological analysis measures hemoglobin and red cell distribution width (RDW), serum lipid status, including total cholesterol, low-density lipoprotein cholesterol, triglycerides and uric acid levels were also determined (Sunnetcioglu et al., 2018). The results showed that RDW was significantly higher in the severe OSAS group than in any of the other groups and so was the serum uric acid level (Sunnetcioglu et al., 2018).

A New Adipocytokine Involved in Obesity-Associated Insulin Resistance (NOV/CCN3), a multifunctional extracellular matrix protein, may play a mechanistic and/or prognostic role in OSAS associated with cardiovascular and metabolic abnormalities. It is believed to play a role in inflammation (Weingarten et al., 2017). The CCN family of proteins are multifunctional matricellular proteins involved in cell adhesion, migration, proliferation, survival in a cell-type specific manner (Weingarten et al., 2017). J. A. Weingarten, L. Bellner et al. in their 2017’s article “The association of NOV/CCN3 with OSAS: preliminary evidence of a novel biomarker in OSAS”, presented NOV/CCN3 as a novel biomarker of the presence and severity of OSAS and „a potential marker of future cardiovascular and metabolic disease in OSAS patients” (Weingarten et al., 2017).

P. Bielicki, R. Pływaczewski et al. considered a genetic background that involves coexistence of T2DM and OSAS (Bielicki et al., 2019). Their article presents the study’s aim to evaluate the „prevalence of polymorphisms of selected genes associated with diabetes or obesity in patients with OSAS and concomitant T2DM and to assess these polymorphisms in the context of OSAS severity” (Bielicki et al., 2019). The study enrolled 600 subjects with OSAS (of which 121 subjects had T2DM) underwent genotyping for single nucleotide polymorphisms (Bielicki et al., 2019). The results showed a possible link between the polymorphism of the gene encoding the apolipoprotein A5 (APOA5) and T2DM in patients with OSAS (Bielicki et al., 2019).

In 2019, B. U. Peres, Aj Hirsch Allen published a remarkable review out of ten studies that had primary focus on cardiovascular and metabolic disorder in OSAS (Peres et al., 2019). The authors conclude that in general, inflammatory markers (IL-1β, IL-6, TNF-α and CRP), vascular proteins (endothelin-1, soluble fms-like tyrosine kinase-1, soluble endoglin, resistin) and adhesion molecules (E-selectin, ICAM-1, vascular adhesion molecule-1 known as VCAM-1, soluble intercellular adhesion molecule or sICAM) seem to be associated with adverse cardiometabolic outcomes (Peres et al., 2019). In 2020, B.U. Peres, Aj Hirsch Allen et al. following the physiopathological pathways in OSAS-the oxidative stress pathway, conducted a study which included 402 subjects. At the end of it, they concluded that circulating levels of 8-isoprostane, marker of lipid peroxidation may be useful as a new biomarker in OSAS (Peres et al., 2020)

**CONCLUSION**

Resuming the latest findings, the novel biomarkers research enhances our understanding of the physiopathological mechanisms involved in OSAS and could allow a large-scale screening of at-risk populations. So, today we feel confident that we are closer and closer to a revolutionary discovery in OSAS diagnosing methods that will help patients worldwide, providing them early long-lasting therapeutic control, while minimising the global related health costs. Finding a new and non-invasive screening tool is becoming vital because of the worldwide disease impact upon the quality of life and also by judging the need of achieving a long-lasting therapeutic control. Although there are still gaps to fill in the research field, the latest results that came to our attention over the last decade makes the near future sounds promising.

**Conflict of Interest**

The authors declare that they do not have any conflict of interest.

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**REFERENCES**


