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**REVIEW**

# A review of the "OMICS" for management of patients with obstructive sleep apnoea

## *Una review sulle scienze OMICHE nella gestione del paziente con sindrome dell'apnea ostruttiva del sonno*

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## SUMMARY

Obstructive sleep apnaea (OSA) syndrome is a condition characterised by the presence of complete or partial collapse of the upper airways during sleep, resulting in fragmentation of sleep associated with rapid episodes of intermittent hypoxia (IH), activation of the sympathetic nervous system and oxidative stress. OSA is associated with a broad spectrum of cardiovascular, metabolic and neurocognitive comorbidities that appear to be particularly evident in obese patients, while affecting both sexes in a different manner and varying in severity according to gender and age. In recent years, studies on OSA have increased considerably, but in clinical practice, it is still a highly underdiagnosed disease. To date, the gold standard for the diagnosis of OSA is nocturnal polysomnography (PSG). However, since it is not well suited for a large number of patients, the Home Sleep Test (HST) is also an accepted diagnostic method. Currently, the major aim of research is to identify non-invasive methods to achieve a highly predictive, non-invasive screening system for these subjects. The most recent reports indicate that research in this field has made significant progress in identifying possible biomarkers in OSA, using -OMIC approaches, particularly in the fields of proteomics and metabolomics. In this review, we analyse these OMIC biomarkers found in the literature.

KEY WORDS: OMICS, proteomics, metabolomics, OSA

## RIASSUNTO

*La sindrome da apnea ostruttiva nel sonno (OSA) è una condizione caratterizzata dalla presenza di completo o parziale collasso delle vie aeree superiori durante il sonno, con conseguente frammentazione del sonno associata a rapidi episodi di ipossia intermittente (IH) e attivazione del sistema nervoso simpatico e dello stress ossidativo. L'OSA è associata ad un ampio spettro di patologie cardiovascolari, metaboliche, neurocognitive e comorbidità che appaiono particolarmente evidenti nei pazienti obesi, interessando entrambi i sessi in modo diverso e variando la gravità a seconda del sesso e dell'età. Negli ultimi anni, gli studi sull'OSA sono aumentati considerevolmente, ma nella pratica clinica, si tratta ancora di una malattia altamente sottodiagnosticata. Ad oggi, il gold standard per la diagnosi di OSA è la polisonnografia notturna (PSG). Tuttavia, poiché non è adatto ad un gran numero di pazienti, anche l'Home Sleep Test (HST) è un metodo diagnostico accettato. Attualmente, l'obiettivo principale della ricerca è quello di identificare metodi non invasivi per ottenere un sistema di screening altamente predittivo e non invasivo per questa categoria di soggetti. I lavori più recenti indicano che la ricerca in questo campo ha compiuto progressi significativi nell'identificazione di possibili biomarcatori in OSA, utilizzando approcci OMICI, in particolare nel campo della proteomica e della metabolomica. In questa review, analizziamo una lista di questi biomarcatori presenti in letteratura.*

PAROLE CHIAVE: *OSA, scienze omiche, proteomica, metabolomica*

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## Introduction

Obstructive sleep apnoea (OSA) is considered by far the most important form of sleep disturbance in breathing. It is caused by increased collapsibility or insufficiency/loss of muscular dilation capacity of the upper airways, leading to repeated pharyngeal constriction (hypopnoea) or closure (apnoea), therefore resulting in decreasing oxyhaemoglobin saturation and with increasing partial pressure of carbon dioxide in arterial blood [1]. To restore pharyngeal patency, patients experience recurrent awakenings, resulting in fragmented sleep, followed by reduced cognitive performance and, in some cases, diurnal sleepiness episodes.

Despite its high prevalence and the high burden of morbidity, OSA remains a significantly underdiagnosed disease worldwide. The Hypnolaus study estimated that the prevalence of moderate-to-severe sleep-disordered breathing (≥ 15 events per h) was 23.4% (95% confidence interval (CI), with a range of 20.9-26.0) in women and 49.7% (with a range of 46.6-52.8) in men [2], whereas according to the American Academy of Sleep Medicine [3], only 20% of patients are diagnosed (about 6 million of a total of 24 million) in the US. The annual cost for an undiagnosed patient is estimated at around $ 5,500 (considering direct and indirect health costs), while it decreases to $ 2,100 per year for diagnosed patients [4]. On this basis, it is evident that OSA is not only a serious health problem, but also a socio-economic issue.

OSA is also becoming dangerously frequent in children, associated with adenotonsillar hypertrophy [5] as well as high rates of overweight and obesity in children in Western countries. These trends will have disastrous long-term consequences for global health and life expectancy if solutions are not taken to correct erroneous lifestyles from the earliest age [6]. These data also suggest that the only way to make the costs of OSA sustainable is through prevention.

To date, the gold standard for diagnosis of OSA is nocturnal polysomnography (PSG). This sleep examination utilises electroencephalography, electrooculography in both eyes, sub-mental electromyography, nasal airflow, snoring sounds, electrocardiography, thoracic/abdominal movements, pulse oxygen saturation and body position to measure various parameters. The PSG indices included are apnoea-hypopnoea index (AHI) and oxygen desaturation index. However, since it is not well suited for a large number of patients, the Home Sleep Test (HST) is also an accepted diagnostic method [7,8]. Given the difficulty of applying the HST to the population as a screening system due to high costs and examination timing, researchers are currently focusing on identifying new biomarkers for early diagnosis of OSA [9]. In the case of sleep disorders and lung diseases, traditional biomarker research techniques have proved to be not particularly well performing. Studies based on proteomics and metabolomics, however, are more sensitive, although, to date, the number of molecules potentially available for clinical application in the context of OSA is still limited. The development of new technologies is therefore necessary, also to provide a greater understanding of the biochemical mechanisms involved in OSA.

In Table I, the list of proteins and metabolites differently expressed in OSA subjects identified in the literature is reported.

## Proteomics approaches

The study of the proteome in OSA patients has been broadly assessed. Many studies have reported that OSA patients express increased levels of mediators of systemic inflammatory response. Zhang et al. [10] used, for the first time, a proteomic approach to detect protein profiles of serum extracellular microvescicle proteins in an intermittent hypoxia (IH) rodent model [11]. Extracellular microvescicles are vesicles released from cells into the extracellular fluid environment, including serum. Their potential utility in clinical diagnosis is well documented, since vesicles are reported to reflect the physiological or pathological status of the tissue from which they arise. They found 4 differentially expressed proteins in serum extracellular microvesicles compared to control: C-reactive protein (CRP), haptoglobin (HP), fibronectin (FN1) and platelet factor 4 (PF4). In addition, Nadeem et al., through meta-analysis of the literature [12], confirmed altered levels of CRP and other systemic inflammatory mediators, including intercellular adhesion molecules (ICAM), coagulation factors (factor VIII, tissue factor) and a significant increase in serum levels of tumour necrosis factor alpha (TNF-$\alpha$), interleukin 1$\beta$ (IL-1$\beta$) and interleukin 6 (IL-6) in patients with OSA. The excessive infiltration of inflammatory cells is also highlighted by the formation of subepithelial oedema in OSA patients as documented by histology. Among these proteins, circulating CRP is an important predictive factor of cardiovascular risk involved in the onset and progression of atherosclerosis [13,14]. Its pro-inflammatory and atherogenic properties have been found in endothelial cells, both smooth and striated muscle cells and macrophages. Its levels, as well as those of IL-6, are strongly associated with oxidative stress or anoxia [10,15] A similarly important role in the clinical picture of the OSA patient is the high level of TNF-$\alpha$ observed; it is, in fact, a pro-inflammatory cytokine with an important role in the host defence, which at the same time mediates the onset of a series of pathological processes including atherosclerosis, septic shock and autoimmune diseases. The release of TNF-$\alpha$ is mediated by IL-6, as well as by other pro-inflammatory cytokines such as IL-2, IFN-$\gamma$ and by TNF-$\alpha$ itself through a positive feedback process [16].

**Table I.** Metabolites and proteins found in OSA patients through OMICS approaches.

| Reference | Sample | Number of participants | Proteins/ metabolites | Differently expressed biomarkers |
|---|---|---|---|---|
| Chen et al. 2017 [69] | Peripheral blood mononuclear cells | 48 patients with sleep-disordered breathing | Proteins | Angiomotin (AMOT), pleckstrin homology, MyTH4 and FERM domain containing H3 (PLEKHH3), adenosine deaminase RNA specific (ADAR), baculoviral IAP repeat containing 3 (BIRC3), and galectin 3 (LGALS3) proteins |
| Krishna et al. 2006 [64] | Urine | 11 paediatrics OSA and 11 controls | Proteins | Gelsolin, Perlecan (a heparan sulfate proteoglycan), Albumin, Immunoglobulin |
| Shah et al. 2006 [65] | Serum | 20 paediatrics OSA and 20 controls | Proteins | 3 proteins with molecular masses of 5896, 3306 and 6068 Da |
| Gozal et al. 2009 [66] | Urine | 30 paediatrics OSA and 30 controls | Proteins | Uromodulin, Urocortin-3, Kallikrein, Bikunin, Tenascin, Human Tribbles homolog-2, Zinc finger protein-81, 36/1, Orosomucoid-2, a1-Microglobulin, PCAF histone acetylase, Prolyl hydroxylase domain |
| Becker et al. 2014 [70] | Urine | 14 paediatrics OSA and 13 controls | Proteins | 30-fold more candidate biomarkers |
| Jurado-Gamez et al. 2012 [33] | Serum | 30 OSA and 10 controls | Proteins | 30 proteins |
| Seetho et al. 2014 [67] | Urine | 27 OSA and 25 controls | Proteins | 15 peptides |
| Zheng et al. 2014 [30] | Saliva | 20 Non-CVD OSA and 18 CVD OSA | Proteins | Fibrinogen alpha chain (FGA), Alpha-2-HS-glycoprotein (AHSG), Tubulin alpha-4A chain (TUBA4A) and other 7 differentially expressed peptides still to be identified |
| Ferrarini et al. 2013 [34] | Plasma | 18 OSA severe and 15 OSA non severe | Metabolites | Phosphatidylcholine (PC), Phosphoserine (PS), Lysophosphatilysophosphatidylcholines (LPC), Lysophosphatidylethanolamine (LPE), LPA, PE methyl-hydroperoxy-octadecatrienoate, PGF2-alpha diethyl amide Pipecolic acid, Arg, Phe, His |
| Kawai et al. 2013 [3] | Saliva | 20 male OSA | Metabolites | Phosphatidylcholine (PC) |
| Engeli et al. 2012 [37] | Plasma | 29 OSA, 26 OSA type II diabetes, 21 controls | Metabolites | Anandamide; (AEA), Arachidonoylglycerols; (AG), Oleoyl ethanolamide; (OEA), Arachidonic acid (AA), increase in the total monounsaturated fatty acids (MUFA) |
| Ezzedini et al. 2013 [39] | Tonsillar tissue | 114 pediatrics OSA and 92 recurrent tonsillitis | Metabolites | Palmitoleic acid, Oleic acid, Stearic acid |
| Papandreu et al. 2013 [38] | Adipose tissue | 63 OSA | Metabolites | Myristic, Palmitic, Stearic, Oleic acid, n-6 fatty acids n-3 (precursors of prostaglandins and serotonin) and n-6 fatty acids |
| Fletcher et al. 1987 [60] | Urine | 8 severe OSA and 5 HTN and obese non OSA patients | Metabolites | Epinephrine (E), Norepinephrine (NE), Metanephrine (MN), Normetanephrine (NMN) |
| Paci et al. 2000 [45] | Plasma | 10 male OSA (8 normotensive and 2 untreated HTN) and 11 controls | Metabolites | Norepinephrine (NE), Epinephrine (E), Dopamine (DA), Endogenous digitalis-like factor (EDLF) |
| O'Driscoll et al. 2011 [62] | Urine | 70 snorers and 26 controls | Metabolites | Epinephrine (E), Norepinephrine (NE), Dopamine (DA), Noradrenaline, Adrenaline |
| Paik et al. 2014 [61] | Urine | 49 OSA (of which 23 with insomnia) | Metabolites | Homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) |
| Gislason et al. 1992 [63] | Cerebrospinal fluid | 15 OSA and 18 healthy controls, 12 patients with suspected neurological disease | Metabolites | 5-hydroxyindoleacetic acid (5-HIAA, serotonin metabolite), Homovanillic acid (HVA, DA metabolite), 3-methoxy-4-hydroxyphenyl glycol (MHPG) |
| Dikmenoglu et al. 2006 [47] | Plasma | 11 OSA and 11 controls | Metabolites/ proteins | Malondialdehyde (MDA), fibrinogen |
| Stanke-Labesque et al. 2009 [51] | Urine | 40 non obese OSA and 20 controls | Metabolites | Leukotriene E(4) (U-LTE (4)), 11-dehydroTXB2 |

*Continues*

**Table I.** *Follows.*

| | | | | |
|---|---|---|---|---|
| Barcelò et al. 2012 [31] | Saliva | 119 OSAS and 35 controls | Metabolites | Gamma glutamyltransferase (GGT), Fetuin-A, |
| Zhang et al. 2018 [10] | Serum extracellular microvescicles | 20 OSA and 20 controls | Proteins | Haptoglobin, C-reactive protein (CRP), Platelet factor 4 (PF4), Coagulation factor XIII (F13a1), Fibronectin (FN1) |
| Lebkuchen et al. 2018 [35] | Plasma | 37 OSA and 16 controls | Metabolites | Deoxy sugar; 2,6-diphenyl-1,7-dihydrodipyrrolo[2,3-b:3′,2′-e] pyridine; 9-hexadecenoic acid (Z), Arachidonic acid (AA), 5,5′-biphthalide, L-glutamine, Glycerophosphoethanolamines (PE), Monoacylglycerophosphocholines (lyso-phosphocholines) (LPC), sphingomyelin (SM), diacylglycerols (DAG), glycerophosphocholines (PC), glycerophosphates (PA), Glutamic acid, Methyl cysteine, Serine |
| Xu et al. 2016 [50] | Urine and plasma | 60 OSA, 30 simple snorers and 30 controls | Metabolites | 2-hydroxy-3-methylbutyric acid, 3,4-dihydrxoybutyric acid, 3-hydroxybutyric acid, 4-hydroxypentenoic acid, cytidine 5'-diphosphocholine, ethanolamine, myo-inositol, 2,3-dihydrxoypropanoic acid, arabinose, arabitol, cellobiose, maltose, threitol, alanine, isoleucine, serine, threoninyl-methionine, trimethylamine N-oxide, valine, 5-hydroxyindoleacetic acid, lactic acid, glycochenodeoxycholate-3-sulfate, putrescine, 4-hydroxybutyric acid, vanillic acid, hypoxanthine, inosine, xanthine |

An analysis of whole-genomic microarrays recently carried out by Yung-Che et al. found overexpression of angiomotin (AMOT), pleckstrin homology, MyTH4 and FERM domain containing H3 (PLEKHH3), adenosine deaminase RNA specific (ADAR), baculoviral IAP repeat containing 3 (BIRC3) and galectin 3 (LGALS3) proteins in treatment-naïve OSA patients [17]. LGALS3 has shown to be involved in cancer, inflammation and fibrosis, heart disease and stroke. Studies have also suggested that expression of galectin-3 is implicated in a variety of processes associated with heart failure, including myofibroblast proliferation, fibrogenesis, tissue repair, inflammation and ventricular remodelling [18].

Expression of AMOT in endothelial cells and its level is associated with proliferation and invasion of breast tumours [19]. ADAR are double chain RNA editing enzymes responsible for post-transcriptional modification of mRNA transcripts by changing the nucleotide content of the RNA. The conversion from A to I in the RNA disrupts the normal A:U pairing which makes the RNA unstable [20]. ADAR is considered to be involved in the insurgence of cancer. Studies in the sleep field also revealed that the ADA G22A polymorphism (c.22G > A, rs73598374) is associated with fewer awakenings throughout the night, and a higher duration of slow wave sleep (SWS), as compared to the normal ADA G22G genotype [21].

BIRC3 is a downstream effector of the ubiquitous hypoxia-inducible factor (HIF-1$\alpha$) that is involved in pro-survival and inflammatory responses induced by the docosahexaenoic acid/neuroprotectin D1 pathway under oxidative stress in an ischaemia-reperfusion stroke model. HIF-1$\alpha$ functions as a principle regulator activity of cellular and systemic homeostatic response to hypoxia. This heterodimer is composed of an alpha and a beta subunit that can activate the transcription of many genes, including those involved in energy metabolism, apoptosis and angiogenesis, as well as other genes whose protein products increase oxygen delivery and facilitate metabolic adaptation to hypoxia. Since many studies have shown that OSA is associated with an imbalance between oxidant production and antioxidant activity, this fact, combined with an overabundance of oxidants, can be linked to the multifactorial aetiology of metabolic disorders, including insulin resistance [22].

Almendros et al. [23] examined the correlation between HIF-1$\alpha$ factor and vascular endothelial growth factor (VEGF) expression in patients with cutaneous melanoma. Interestingly, they found in a large prospective study that the expression of HIF-1$\alpha$ was an independent factor associated with nocturnal IH measures of respiratory disturbance during sleep in patients affected by cutaneous melanoma [23], meaning that it has a significant contribution to the disease. Notably, the risk of melanoma was significantly higher in patients with OSA (HR = 1.14, 95% CI 1.10-1.18), along with pancreatic and kidney cancer [24]. In recent years, other potential associations between OSA and cancer have been reported, principally ascribed to an effect of IH on tumour biology [25-27].

A significant correlation between OSA and increased cardiovascular risk and hypertension (HTN) is strongly reported in the literature [28,29]. Mass spectrometry was performed on salivary samples of OSA patients with cardiovascular diseases (CVD) compared to non-CVD OSA patients [30]. A panel of 11 biomarkers were identified as differentially expressed between the two groups. It was found that the level of alpha-2-HS-glycoprotein (AHSG) peptide was significantly lower in the OSA-CVD group compared to the non-CVD group. A reduced level of AHSG had already been reported in severe OSA patients [31] at metabolic level [32]. AHSG protein is synthesised by hepatocytes and is involved in different process such as formation of brain and bone and endocytosis. Interestingly, lack of this protein is involved in leanness.

## Metabolomics approach

The field of metabolomics, and the consequent search for potential biomarkers in OSA patients, is beginning to be explored only in recent years. The lipidomic profile in OSA patients reported in the literature mainly reveals alterations in phospholipid biosynthesis and fatty acids. One of the major studies using mass spectrometry has allowed to identify, both at a serum and urinary level, as many as 103 proteins that are differently expressed in adult OSA patients compared to controls, all potentially associated with imbalances in lipid metabolism and alterations in the vascular system [33]. Among phospholipids, glycerophosphocholines (PC), lysophosphatidylcholines (LPE), glycerophosphoethanolamines (PE), lysophosphatidylethanolamine (LPA), phosphoserine (PS), and lysophosphatidic acids, along with glycerophosphates (PA), monoacylglycerophosphocholines, lyso-phosphocolyne (LPC) and sphingomyelin (SM) classes were found to be up-regulated in patients with OSA compared to controls [34,35]. Increased PC expression at the salivary level was also reported using LC-MS/MS methods [36,37].

Alterations in fatty acids have also been detected. Among those that are significantly increased in OSA compared to normal subjects, circulating anandamide (AEA), 2,4-dihydroxybutyric acid, 2-hydroxy-3-methylbutyric acid, 3,4-dihydrxoybutyric acid, 6-aminocaproic acid, pentanoic acid, and glyceraldehyde, 3-methyl-3-hydroxybutyric acid, and 4-hydroxypentenoic acid were up-regulated, whereas bile acid and glycochenodeoxycholate-3-sulphate (GCDCA-3-sulphate) were decreased [36-38]. Other groups, using GC-LC techniques, found that palmitoleic and oleic acid levels were lower, while stearic acid levels were higher in the tonsillitis tissue of infant control subjects compared to the hyperplastic tissue typical of the diseased counterpart [39].

Other research groups observed that in OSA patients levels of 1/2-arachidonoylglycerols (AG), and oleoyl ethanolamide (OEA) in plasma are higher compared to controls. It is interesting to note that arachidonic acid (AA) concentrations and eicosanoids[34,35] were also up-regulated in OSA patients, suggesting a role for the endocannabinoid system in regulating blood pressure in patients with high risk OSA for HTN and CVD [36,37,40].

The endocannabinoid system is, in fact, based on lipid molecules produced by the body in response to various stimuli that bind specific membrane receptors associated with the protein G, called cannabinoid receptors type 1 and 2 (CB1 and CB2) [41]. The endocannabinoid system represents a neuromodulation system, playing a role in the control of pain at the level of the central nervous system, in regulation of cell proliferation and in modulation of the immune response. Interestingly, it also seems to play a role in mechanisms that modulate appetite and therefore obesity [37]. The endocannabinoid system also plays an important role in the release of adipokines. Recent research has shown that the pharmacological blockade of CB1 by an antagonist, named *Rimonabant,* stimulates the release of adiponectin, which is normally inhibited. Adiponectin is a circulating hormone secreted by adipose tissue, with anti-atherogenic and antidiabetic properties that can reduce liver glucose production, as well as suppress lipogenesis and activate oxidation of fatty acids [42]. How endocannabinoids regulate metabolism are still only partially understood, despite the fact that their role in controlling hunger and satiety acts mainly in hypothalamic structures through activation of neurons capable of stimulating the action of neuropeptides [43].

Alterations in the endocannabinoid system therefore affect and alter energy metabolism of the body and homeostasis of lipids, as suggested by Di Marzo and Matias, who were the first to formulate the increasingly valid hypothesis that obesity can be associated with pathological hyperactivation of the endocannabinoid system [44]. All these conditions can be associated with an increased risk of cardiometabolic diseases such as type 2 diabetes, dyslipidaemia, arterial hypertension, myocardial infarction and stroke, conditions normally found in OSA patients.

Mediators involved in the systemic inflammatory response and oxidative stress have also been reported in OSA. Among the metabolites associated with oxidative stress, urinary 15-F2t- isoprostane, one of the most sensitive metabolites correlated with lipid peroxidation, is positively linked to thickness of the intima-media carotid tunic [45]. These molecules were shown to be a specific, chemically stable, quantitative marker of oxidative stress *in vivo*. In particular, F2t-isoprostanes are prostaglandin isomers synthesised *in vivo* through free radical catalysed peroxidation of AA in biological membranes, independently of the activity of cyclo-oxygenase. Increased urinary excretion or plasma concentrations of 15-F2t-isoprostane has been observed in many conditions including smoking, diabetes and cardiovascular diseases [46].

Another important biomarker of oxidative stress, malondialdehyde (MDA), is present at significantly higher concentrations in patients with OSA vs. control [47]. MDA is the result of lipid peroxidation of polyunsaturated fatty acids. It is an important product in the synthesis of thromboxane A2 in which cyclooxygenase 1 or cyclooxygenase 2 metabolises AA into prostaglandin H2 and ROS degrade polyunsaturated lipids to form MDA [48]. This compound is a reactive aldehyde and is one of many reactive electrophilic species that causes toxic stress in cells and reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts; it can thus be used as a biomarker to measure the level of oxidative stress in an organism [49].

Arguably, the tricarboxylic acid cycle (TCA) and its mediators tend to increase in OSA [50], suggesting augmentation of oxidative stress.

Among metabolites that are potential pro-inflammatory markers, Stanke-Labesque et al. [51] found leucotriene E4 (U-LTE4), an inflammatory molecule associated with cysteinyl leukotriene production, whose elevation in urinary concentration has been demonstrated in patients with OSA. Recently, Gautier-Veyret and his group have shown that activation of this pathway contributes to OSA-induced atherogenesis, and its blockade could therefore represent a new therapeutic target for reducing CVD [52]. It is also interesting to note that Continuous Positive Airway Pressure (CPAP), a respiratory ventilation method mainly used in the treatment of sleep apnoea, reduces the urinary concentration of U-LTE4 by up to 22%, but only if the treatment is carried out in patients with a normal body mass index (BMI) [51].

Arguably, CPAP treatment reduces also serum levels of homocysteine (Hcy) by almost 30%, which, along with plasma levels, were found to be significantly higher in patients with OSA compared to controls [38,39,53]. In addition, neural-like cell exposure to Hcy for a period of 5 days resulted in a 4.4-fold increase in production of reactive oxidative species (ROS) [54]. Hcy is known to mediate adverse effects on the cardiovascular endothelium and smooth muscle cells with resultant alterations in subclinical arterial structure and function [55], leading to CVD and its complications, such as heart attack and stroke [56]. Moreover, hyperhomocysteinaemia leads to enhancement of the adverse effects of risk factors like HTN, smoking, and lipid and lipoprotein metabolism, as well as promotion of inflammation [57]. Another study demonstrated that Hcy is capable of initiating an inflammatory response in vascular smooth muscle cells by stimulating CRP production, which is mediated through the NMDAr-ROS-ERK1/2/p38-NF-κB signal pathway [58]. CRP expression was also found to be altered in the proteome of OSA patients (see previous section).

Some studies also suggest that elevated Hcy levels may be associated with alterations in mental health such as cognitive impairment, dementia, depression, Alzheimer's and Parkinson's disease [59] through its capacity to act as a neurotransmitter. In particular, Hcy may act either as a partial agonist at glutamate receptors or as a partial antagonist of the glycine co-agonist site of the NMDA receptor. As such, in the presence of normal glycine levels and normal physiological conditions, Hcy does not cause toxicity but in case of head trauma or stroke, there is an elevation in glycine levels in which instance the neurotoxic effect of Hcy as an agonist outweighs its neuroprotective antagonist effect. This neuronal damage following a stroke has been attributed to the over stimulation of excitatory amino acids such as glutamate and aspartate through activation of NMDA receptors [55]. Ganguly et al. [55] have investigated how Hcy is able to selectively stimulate the release of these excitatory amino acids in stroke and concluded that they may trigger the release of catecholamine, resulting into detrimental effects in the brain and cardiovascular system. Interestingly, in OSA patients, glutamate metabolites were also found to be significantly altered [50].

The study of catecholamine metabolites and derivatives as potential predictors of the onset of the pathological process seems particularly promising. Fletcher et al. [60], for example, observed that norepinephrine (NE) and normetanephrine levels were significantly higher in the urine of patients with OSA than those in obese HTN controls, as well as epinephrine (E) levels, at the plasma level [45], who also found higher levels of dopamine (DA) in the comparison of 10 male patients with OSA and 11 controls. HPLC observations revealed a significant increase in all urinary catecholamines in OSA children, and the levels of NE and E during the night were strongly related to the severity with which patients manifest the altered phenotype. Paik et al. [61], after studies carried out using GC-MS to detect metabolites of urinary neurotransmitters, demonstrated that homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), both dopamine metabolites, were increased in sleepy patients with OSA, suggesting that excessive daytime sleepiness in these subjects is probably caused by an increase in night-time activity of the dopaminergic and sympathetic systems [62]. Although this theory seems intriguing, the results of several other studies question it. Paci et al. have reported that E and DA levels did not vary significantly between OSA patients and controls. In addition, the results of the studies of Gislason et al, found 5-hydroxyindo-lacetic acid (5-HIAA), HVA and 3-methoxy-4-hydroxyphe-glicolenyl glycol (MHPG) in the cerebrospinal fluid of 15 patients with OSA and 18 controls; however, even in this case, the levels of all these biomarkers were similar in patients with OSA and control subjects [45,63].

The inconsistency of the results obtained from the studies on catecholamine metabolites in patients with OSA may be due to various factors such as the heterogeneity of the analytical platforms used by the various research groups, the different biological matrices taken into account, small size of the cohorts and the different protocols used for sample collection. All these elements may also affect the reproducibility of studies.

The first studies aimed at finding differentially expressed metabolites at the urinary level in children with OSA was carried out by Krishna et al. [64]. They adopted a mass spectrometry technique on a cohort of 22 subjects, who demonstrated an alteration in glomerular and tubular filtration of the kidneys compared to healthy counterparts. High levels of proteins such as jasmine, perlecan (a heparan sulphate proteoglycan), albumin, and immunoglobulin were detected in urine. These results suggested increased catabolic activity of some proteins in OSA patients [64]. In the same period, Shah et al. also identified three proteins of 5,896, 3,306 and 6,068 kDa that were differently expressed in pathological children, which were capable of discriminating the latter from healthy patients with 90% specificity and 93% sensitivity [65]. Three years later, Gozal et al., using a method based on the use of 2-Dimensional DIfference Gel Electrophoresis and Mass Spectrometry (2D-DIGE-MS), were able to identify 16 metabolites differently expressed in the urine of OSA patients compared to controls. In particular, the analysis of concentrations of some of these, including uromodulin, urocortin-3, orosomucoid-1, and kallikrein, were able to identify the pathogenic phenotype with a sensitivity of 95% and a specificity of 100% [66].

The contribution of Seetho et al. and Zeng et al. in the field of research into potential OSA biomarkers is extremely interesting, with the former, focusing on polypeptides using urine of obese OSA patients as a biological matrix, and the second, looking for proteins differently expressed between OSA patients suffering from CVD in saliva. The work of the two groups allowed identification of 27 potential biomarkers, fibrinogen alpha chain (FGA), tubulin alpha-4A chain (TUBA4A) and AHSG. More specifically, AHSG has been shown to be expressed at lower levels in OSA frameworks associated with changes in cardiovascular function [30,67].

Alterations in amino acid biosynthesis were also reported in OSA using a metabolomics approach. Xu et al. identified 21 differentially expressed urinary metabolites among a simple snoring group and controls, including aspartyl-serine, isoleucine-threonine (Ile-Thr), and methionine, whereas levels of 3-hydroxyanthranilic acid and 5-hydroxytryptophan decreased. Hydroxyprolyl-methionine, hypoxanthine, Ile-Thr, indole-3-acetamide, isoleucine, lactic acid, myo-inositol, pentanoic acid, threitol, threoninyl-methionine, trimethylamine N-oxide (TMAO), uridine, and valine were consistently higher or lower [50]. Other groups have also reported that methylcysteine and serine decreased in OSA [36,37].

The metabolomics profiling of spermine biosynthesis, indoles and tryptophan metabolism, tyrosine metabolism as well as porphyrin metabolism were also altered significantly [38,50].

## Conclusions

OSA is characterised by recurrent episodes of collapse of the upper airways during sleep, which are reflected in a desaturation of haemoglobin that leads to the awakening of affected subjects. The chronic IH registered in this condition leads the body to enact molecular adaptations to the low-oxygen conditions to which it is subjected [68]. Despite this, sleep fragmentation results in a dangerous condition of excessive sleepiness during the rest of the day. In addition to the long-term problems mentioned, sleep fragmentation is a daily danger for the individual linked to the increased risk of road or work accidents. The body responds to chronic fatigue through compensatory mechanisms that evoke inflammatory responses, hyperactivation of the sympathetic system and alteration of endothelial function, such as regulation of tight junctions; these events have an important role in promoting the onset of atherosclerosis and, in the long term, cardiovascular and cerebrovascular diseases [12]. Recent studies also show a significant correlation between OSA and metabolic and neurocognitive risk as well as an association with cancer mortality.

In the literature, proteomics and metabolomics approaches were used to detect change in physiological or pathological status of OSA patients compared to controls, in order to discover new mediators that can be used as biomarkers of the disease. Notwithstanding, OSA and therapies related to this disease [71-73], are a somewhat 'new', and there are many proteins and metabolites that are associated with the disease, in particular those involved in inflammation and oxidative stress, in line with the clinical IH that patients undergo in OSA.

Lipid dysmetabolism in OSA reflects alterations in phospholipids biosynthesis, steroidogenesis and fatty acids. This may influence cell membrane formation, augmenting lipid uptake, atherogenesis and inflammation. In addition, alterations in amino acids, nucleic acids and some mediators that act as neurotransmitters, such as Hcy and the endocannabinoid system, have been seen in OSA patients, suggesting an increased risk of cardiometabolic diseases such as type 2 diabetes, dyslipidaemia, arterial HTN, myocardial infarction and stroke, conditions normally found in OSA patients.

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