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CLINICAL REVIEW

Peripheral biomarkers to diagnose obstructive sleep apnea in adults: A systematic review and meta-analysis

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SUMMARY

Background: Obstructive Sleep Apnea (OSA) has been recognized as a major health concern worldwide, given its increasing prevalence, difficulties in diagnosis and treatment, and impact on health, economy, and society. Clinical guidelines highlight the need of biomarkers to guide OSA clinical decision-making, but so far, without success. In this systematic review and meta-analysis, registered on the International Prospective Register of Systematic Reviews database (ID CRD42020132556), we proposed to gather and further explore candidates identified in the literature as potential OSA biomarkers.

Methods: Search strategies for eight different databases (PubMed/Medline, Cochrane Library, *Biblioteca Virtual da Saúde*, Web of Science, EMBASE, World Intellectual Property Organization database, and bioRxiV and medRxiV Preprint Servers) were developed. We identified studies exploring potential biomarkers of OSA, in peripheral samples of adults, with and without OSA, with no comorbidities defined in study inclusion criteria, published after the last systematic review and meta-analysis conducted on OSA biomarkers, until May 31st, 2020. Risk of bias was assessed through the 14-item Quality Assessment Tool for Diagnostic Accuracy Studies. Demographic, clinical, and candidate biomarkers' data were collected and analyzed via random effects meta-analyses.

Findings: Among the 1512 unique studies screened, 120 met the inclusion criteria and 16 studies with low risk of bias were selected for meta-analyses. The selected 16 studies enrolled a total of 2156 participants, from which 1369 were diagnosed with OSA and 787 were disease-free controls. The assessed variables showed high heterogeneity. From the 38 biomarker candidates evaluated, only two were evaluated in more than one study. Most studies pinpointed candidates with more potential for OSA prognosis. *ADAM29*, *FLRT2* and *SLC18A3* mRNA levels in PBMCs, Endocan and YKL-40 levels in serum, and IL-6 and Vimentin levels in plasma revealed the most promising candidates for OSA diagnosis.

Interpretation: Although the current systematic review and meta-analysis allowed us to identify candidates to further explore as potential biomarkers in future studies, it is evident that OSA biomarkers research is still at an early stage. Most findings derive from small-size single-center study cohorts and single-candidate studies. We point several gaps in current OSA biomarker research that may guide into new directions and approaches towards the identification of OSA biomarkers.

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Abbreviations

AHI	Apnea - hypopnea index
AUC	Area under the curve
BMI	Body mass index
BVS	*Biblioteca Virtual da Saúde*
CPAP	Continuous Positive Airway Pressure
DOR	Diagnostic Odds Ratio
EBC	Exhaled breath condensate
ESS	Epworth Sleepiness Scale
HDL	High-Density Lipoprotein
HSP90	heat shock protein 90
IL	Interleukin
IMA	Ischemia Modified Albumin
Lp-PLA2	Lipoprotein-associated phospholipase A2
LR+	Positive Likelihood Ratio
LR−	Negative Likelihood Ratio
mRNA	Messenger RNA
NPV	Negative Predictive Value
ODI	Oxygen Desaturation Index
OSA	Obstructive Sleep Apnea
PBMCs	Peripheral Blood Mononuclear Cells
PPV	Positive Predictive Value
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
PSG	Polysomnography
QUADAS	Quality Assessment Tool for Diagnostic Accuracy Studies
RDI	Respiratory Disturbance Index
RERAS	Respiratory Effort-Related Arousals
ROC	Receiver Operating Characteristics
SpO2	Peripheral Oxygen Saturation
SaO2	Arterial Oxygen Saturation
TG	Triglycerides
TnI	Troponin I
WIPO	World Intellectual Property Organization

Glossary of terms

Apnea - hypopnea index (AHI): Average number of apneas and hypopneas that occur *per* hour of sleep. This index has been used for OSA diagnosis and severity classification (AHI <5, no OSA; 5 $\leq$ AHI < 15, mild OSA; 15 $\leq$ AHI < 30, moderate OSA; AHI $\geq$ 30, severe OSA) and has been guiding treatment decisions.

Arousal Index: Average number of arousals *per* hour of sleep.

Epworth Sleepiness Scale (ESS): Scale widely used to evaluate daytime sleepiness based on eight questions.

Oxygen saturation: Percentage of hemoglobin molecules saturated with oxygen (O_2 bound to heme protein of hemoglobin) relative to total hemoglobin molecules in arterial blood, used as an indicator of blood oxygen levels (SaO_2). Blood oxygen levels can be measured directly through blood analysis (SaO_2) or indirectly through a pulse oximeter device (SpO_2). Normal readings in a healthy adult range from 94 % to 100 %.

Oxygen desaturation index (ODI): Average number of oxygen desaturation episodes per hour of sleep. An oxygen desaturation episode is defined as a decrease of 3 - 4 % or more below baseline SaO_2/SpO_2.

Polysomnography (PSG): Considered the gold standard diagnostic procedure in sleep medicine, especially to diagnose sleep-related breathing disorders as OSA. It allows to monitor sleep architecture, eye movements, heart rate, respiration parameters, oxygen saturation and body movements. It can be performed in-laboratory (type I) or out-of-center, unattended (type II). Type II studies can have as many monitoring sensors as in-laboratory type I studies, but generally lack monitoring of carbon dioxide levels (important for the diagnosis of previously undetected cardiovascular, pulmonary, or neuromuscular pathologies with hypoventilation) and video (helps the diagnosis of concomitant movement disorders and parasomnias).

Respiratory Disturbance index (RDI): Also known as respiratory distress index, RDI consists of the average number of apneas, hypopneas and respiratory-effort related arousals (RERAs) that occur per hour of sleep. Like AHI, RDI has also been used for OSA diagnosis and severity classification (RDI <5, no OSA; 5 $\leq$ RDI < 15, mild OSA; 15 $\leq$ RDI < 30, moderate OSA; RDI $\geq$ 30, severe OSA) and has been guiding treatment decisions.

Type III and IV sleep studies: Type III studies use devices that measure limited cardiopulmonary parameters, namely, respiratory variables (airflow and/or effort to breathe), oxygen saturation, and a cardiac variable (heart rate or electrocardiogram). Type IV studies utilize devices that measure only 1 or 2 parameters, typically oxygen saturation and heart rate, or in some cases, just air flow.

Introduction

Obstructive sleep apnea (OSA) has been recognized as one of the most prevalent sleep disorders worldwide. The most recent report on the global prevalence of this sleep-related breathing disorder has estimated that approximately 1 billion adults, with ages between 30 and 69 years, have OSA, a prevalence expected to continuously increase [1–3]. OSA is characterized by the repetitive occurrence of upper airway obstructions during sleep, while respiratory efforts continue. It emerges as a combination of several factors, including genetics, anatomical characteristics (narrow upper airway, abnormalities in craniofacial structure), functional traits (airway collapsibility, upper airway muscle responsiveness, arousability and breathing instability), age, sex, body mass index (BMI) and lifestyle [4,5]. Total (apnea) and partial (hypopnea) obstruction episodes promote transient breathing interruptions, frequently terminated by brief arousals or microarousals from sleep [6]. This intermittent hypoxia cycle and repetitive sleep fragmentation strongly impact on sleep quality, commonly causing high fatigue and excessive daytime sleepiness [6–8]. In addition, untreated OSA has also been associated with increased morbidity (especially

cardiopulmonary and metabolic diseases), increased risk of perioperative complications, and increased mortality [9–13].

Yet, the available data suggest that most cases of OSA remain undiagnosed and untreated, even in developed countries [2,3,14]. The gold standard diagnostic approach for OSA is an in-laboratory sleep study through polysomnography (PSG type I), which is technically demanding and time-consuming, expensive and has limited accessibility [4,6,7,15]. As an alternative, OSA questionnaire-based screenings have been developed with the goal of identifying individuals at higher risk or with more severe OSA [16], and domiciliary sleep studies (type II to type IV studies) have been more frequently opted [17,18]. However, while available questionnaires lack adequate specificity for OSA [16,19], unattended sleep studies are more susceptible to signal loss and study failure [17,20,21]. Technical limitations of home sleep studies have been associated with higher false-negative rates for OSA, particularly of mild-to-moderate OSA. In accordance, home sleep studies frequently imply repetition or confirmation in-laboratory, especially to rule out false negatives when there is high disease probability [17,20]. On the other hand, night-to-night variability further challenges OSA diagnosis both at home and in-laboratory [22,23]. All these difficulties result in long waiting periods and unnecessary delays in OSA diagnosis and treatment. In addition, OSA clinical management has been further challenged by the considerable variability in etiology (endotype), clinical manifestation (phenotype), risk levels for OSA-associated complications, and treatment response observed in patients with OSA [4,5,24].

There is thus an evident need to develop more effective approaches for OSA clinical management. Biomarkers have emerged as powerful tools in disease susceptibility/risk evaluation, diagnosis, prognosis, monitoring, treatment response prediction or evaluation [25,26]. These are measurable indicators of normal or pathogenic biological processes. Ideal biomarkers should be easily and objectively measured, consistent, specific (low false-positive rate), sensitive (low false-negative rate) and accessible [25,26]. Use cases for biomarkers in OSA clinical management include a more accurate identification of individuals with high risk for OSA and/or in need of a sleep study, classification, and differentiation of OSA endo- and phenotypes, evaluation of risk levels for OSA-associated complications, treatment triage and lifelong follow-up (treatment-response monitoring) [27]. However, despite numerous efforts to identify OSA biomarkers have been reported in the last two decades, the obtained results have not been consistently reproduced or candidates failed to meet the necessary requirements for routine use in a clinical setting [28,29]. In 2015, De Luca Canto et al. published two reviews of potential OSA biomarkers detectable in blood, exhaled breath condensate (EBC), salivary, or urinary biological samples [28,30]. Only one study showed potential to identify or exclude the presence of OSA, based on the high correlation of EBC interleukin (IL)-6 and IL-10 levels with Apnea - hypopnea index (AHI), in adults. Yet, no developments were observed in this direction so far.

In this context, in this systematic review and meta-analysis, we intended to gather and further explore peripheral candidate biomarkers (non-invasive/minimally invasive) for adult OSA diagnosis reported since the last reviews performed by De Luca Canto et al., 2015 [28,30]. Search strategies for eight different databases were developed. All studies assessing potential biomarkers of OSA, in peripheral biological samples, in adults with and without OSA (assessed by overnight PSG in a sleep unit), with no comorbidities defined in study inclusion criteria were considered. Risk of bias was evaluated using the 14-item Quality Assessment Tool for Diagnostic Accuracy Studies (QUADAS) and guided study selection for inclusion in the systematic review and meta-analyses. Demographic and clinical characteristics of study cohorts, candidate biomarkers and diagnostic accuracy measures were explored through meta-analyses. With this work, we expect to contribute to new directions on OSA biomarker research, paving the way to earlier, faster, and more personalized OSA diagnosis.

Methods

The study protocol of this systematic review and meta-analysis was registered at the International Prospective Register of Systematic Reviews database (PROSPERO, https://www.crd.york.ac.uk/prospero/), with the title *Peripheral Biomarkers to Diagnose Obstructive Sleep Apnea: A Systematic Review and Meta-Analysis*, on August 6th 2019 (ID: CRD42020132556). Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines were followed in the conduction of the current review [31].

Eligibility criteria

Inclusion criteria contemplated studies (1) published after 21st March 2014 (inclusively), following the last systematic review and meta-analysis performed in the context of OSA biomarkers research, performed by De Luca Canto et al. 2015 [28,30]; (2) in English, Portuguese, Spanish or French; (3) assessing potential peripheral biomarkers (in peripheral biological samples) for OSA diagnosis; (4) performed in adults ($\geq$18 years old); (5) including patients with OSA (AHI $\geq$ 5), and control subjects (without OSA, AHI < 5), as diagnosed by overnight in-laboratory PSG; (6) with no comorbidities defined in study inclusion criteria (e.g. hypertensive subjects with and without OSA).

Studies were excluded for the following reasons: (1) not focused on OSA; (2) published before March 21st 2014; (3) written in other languages besides English, Portuguese, Spanish or French; (4) not conducted in humans; (5) inclusion of individuals younger than 18 years old; (6) no full night PSG in-laboratory for OSA screening; (7) absence of a control group (no OSA, AHI <5); (8) no evaluation of peripheral markers (in peripheral biological samples); (9) inclusion of comorbidities in participants' inclusion criteria; (10) full-article not available; (11) insufficient data for analysis after several attempts to contact the author; (12) conference proceedings; (13) short commentary; (14) case-reports; (15) review article or (16) systematic reviews and meta-analyses.

Information sources and search strategies

Literature search was performed in five literature databases, PubMed/Medline, Cochrane Library, *Biblioteca Virtual da Saúde* (BVS), Web of Science and EMBASE literature databases, a patent database [World Intellectual Property Organization (WIPO) database] and in Preprint Servers (bioRxiV and medRxiV). The search strategy included pre-defined search terms related to OSA and biomarkers, namely, "sleep apnea" OR "OSA" OR "sleep apnoea" OR "sleep apnea" OR "obstructive" OR "obstructive sleep apnea" OR "obstructive sleep apnoea" AND "exhaled condensate biomarker" OR "salivary biomarker" OR "urinary biomarker" OR "blood biomarker" OR "serum biomarker" OR "biomarker", adjusted according to each database, as discriminated in Table S1. The search period was defined from March 21st, 2014 (following the last reviews performed by De Luca Canto et al., 2015 [28,30]), to May 31st, 2020. Duplicates between the different databases were removed.

Study selection process

Identified studies were screened according to the defined inclusion and exclusion criteria, based on the study title, in phase I,

and on the abstract, in phase II. Full manuscripts of selected studies were then retrieved and assessed for eligibility. All phases of study selection were performed by two independent review authors. Any disagreement between the two independent reviewers, at any of the study phases, was solved by discussion with involvement of a third reviewer.

Risk of bias assessment

The risk of bias of eligible studies was assessed using the QUADAS tool [32]. This tool comprises 14 items assessing risk of bias, sources of variation (applicability), and reporting quality. Before the assessment of eligible studies, authors discussed how to assess each item of QUADAS in the context of this specific review and how to use this information to judge the risk of bias, until consensus. For the target condition (OSA), we considered PSG type I as the reference standard, that discriminates patients with OSA from control subjects based on AHI (AHI < 5: no OSA; AHI ≥ 5: OSA), and the analysis of the candidate biomarker/s as the index test. Study evaluation was performed by two independent review authors. Reviewers scored each of the 14 items as "1" (item fulfilled = 1 point) or "0" (item not fulfilled or unclear = 0 points), as presented in Table S2. Authors of potentially eligible studies were contacted if necessary to provide further details about their studies. Any disagreement between the two independent reviewers was solved by discussion with involvement of a third reviewer.

Data collection

All studies that fulfilled the 14 items of QUADAS, or at least, the first 12 items, were included in this systematic review and meta-analysis. Uninterpretable/intermediate test results (item 13) and withdrawals (item 14) were rarely reported but these could be due to their inexistence, so, we did not consider these items for study selection. The remaining items are crucial to avoid erroneous conclusions, assure studies reproducibility and application in biomarkers research, so these were used as final criteria for study inclusion.

Information relative to the study aim, design, setting, country, recruitment process, sample size, study groups, reported weaknesses and main conclusions was collected from all included studies. For meta-analyses, information relative to participants (demographic and clinical characteristics) and primary outcome (potential biomarkers analyses) was further collected. Data collection was performed by one author. A second author cross-checked all data and confirmed its accuracy. Any disagreement was solved by discussion and involvement of a third author.

Data synthesis and analysis

Common reported variables between studies were compared. Each variable was evaluated globally (OSA group) and in subgroups according to disease severity, as frequently segmented in the included studies [Mild or Moderate OSA, and Severe OSA (based on AHI or Respiratory disturbance index¯—RDI)], in comparison with control subjects. Mild and moderate OSA patient groups were merged as some of the studies did not separate the two groups. Whenever studies presented values in sub-groups but not in the OSA global group, the mean and standard deviation of the total group were estimated using the weighted mean of the sub-groups and the joint standard deviation after assessing homoscedasticity by Levene's test. For the sex variable, we have considered its distribution as normal to compute mean and standard deviation.

Meta-analyses were performed using a random effects model in R software (version 4.0.3) and the metafor package to obtain a summary measure for each of the analyses. To assess the heterogeneity of the extracted variables, the I^2 value was determined (percentage of variation across studies that is due to heterogeneity rather than chance) using a meta-regression analysis [33]. Measures obtained for each of the described variables were converted into Z scores. The number of studies, total number of subjects, number of subjects per group (OSA and control), heterogeneity of studies (I^2), mean difference between OSA and control groups, Z scores and respective confidence intervals, p-value between OSA and control groups (considered statistically significant if less than 0.05, corresponding to an absolute Z score above 1.96), and Cohen's d (effect size index) are provided for each analyzed variable. Descriptive forest plots were created with the synthesis values of the different variables.

As most of the candidate biomarkers were explored only in one study, it was not possible to achieve a Z score. We proceeded with a descriptive comparison between candidate biomarkers, using the percentage difference between the patients' group or subgroup and the control group. We then plotted these results in a descriptive forest plot. Forest plots were obtained using bubble plots on Microsoft Excel, where the diameter of the bubble represents the sample size. The diagnostic capacity of candidate biomarkers was further explored using diagnostic accuracy measures [sensitivity, specificity, receiver operating characteristics (ROC) analysis, positive predictive value (PPV), negative predictive value (NPV)] provided in selected studies. Positive likelihood ratio (LR+), negative likelihood ratio (LR-), diagnostic odds ratio (DOR), and Youden's Index were calculated when possible.

Results

Search results and study selection

Our search strategy retrieved 2345 studies, 2255 from literature databases (PubMed/Medline: n = 684; Cochrane Library: n = 174; BVS: n = 565; Web of Science: n = 487; EMBASE: n = 345), 56 patents (WIPO), and 34 manuscripts from Preprint Servers (bioRxiV: n = 30; medRxiV: n = 4). Among these, 1512 were unique (no double entries for different databases), 699 studies were considered relevant based on their title, and 243 were further sought for retrieval based on their abstract. After full manuscript reading, 120 studies were considered eligible based on the defined inclusion and exclusion criteria. The 14-item QUADAS tool [32] was used to assess the risk of bias of all eligible studies. From the 120 studies, only 16 showed low risk of bias in all items or items 1–12, and were selected for inclusion in this systematic review and meta-analyses [34–49]. A flow-diagram summarizing each step of the review process is presented in Fig. 1.

Our search showed a considerable variability in the reference test being performed in research studies and in applied clinical criteria (AHI cut-off), a high number of studies with no control group, poor clinical characterization of participants, and frequent co-existence of other comorbidities, defined in inclusion criteria. Among the 120 eligible studies, major limitations were found in reporting and blinding. Most studies (78.3%) did not evaluate candidate biomarkers in blind conditions, meaning without knowledge of PSG results, or did not report it. Regarding reporting, many studies did not report the timeframe between the PSG diagnosis and biomarkers evaluation (30.8%) and/or the detailed methodology used either for candidate biomarkers evaluation (28.3%) or for PSG conduction and/or analysis (22.5%). Risk of bias assessment is summarized in Fig. 2 (see Table S2 for the detailed assessment of each study).

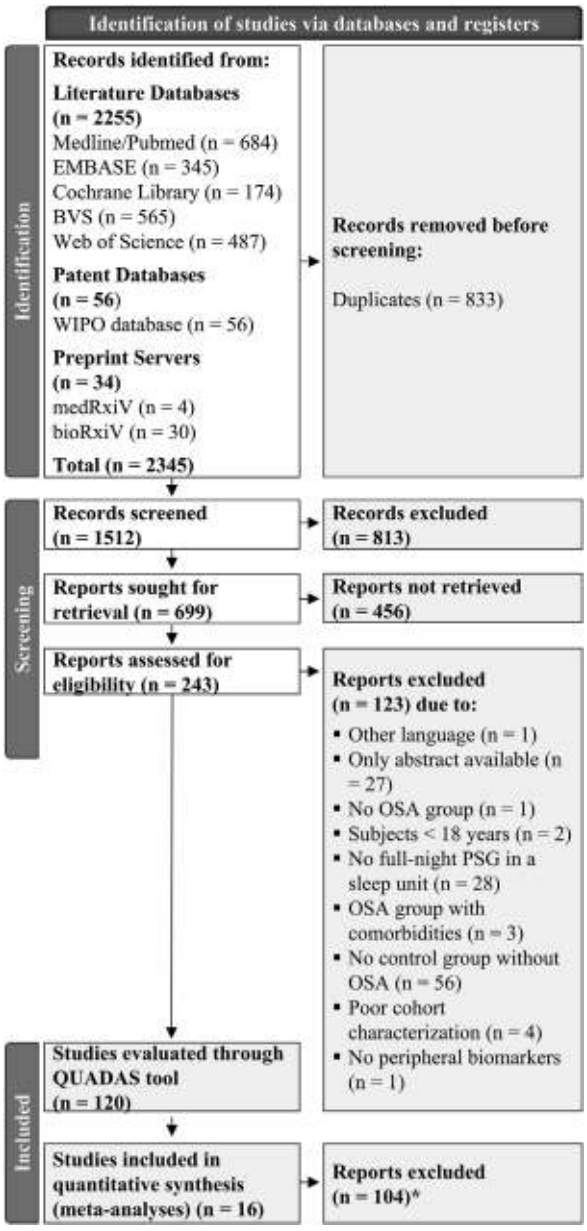

Fig. 1. PRISMA flow-diagram describing the process followed for study identification, screening, and selection. BVS: *Biblioteca Virtual da Saúde*; WIPO: World Intellectual Property Organization; PSG: Polysomnography; OSA: Obstructive Sleep Apnea; QUADAS: Quality Assessment Tool for Diagnostic Accuracy Studies; *See Table S2 for a detailed overview of study selection using QUADAS.

Overview of the included studies

The 16 studies selected for inclusion in this systematic review and meta-analyses were mostly single-centric (15/16) and observational (16/16), although only 5 studies reported the study type (4 cross sectional studies [34,38,39,45], and 1 case–control study [36]). The geographic location of these studies is dispersed over the globe, from Taiwan to Brazil, with increased prevalence in European countries (Bulgaria, Greece, Norway, Romania, Slovakia, Turkey, 14/16) [34,36,38–49], especially in Turkey (8/16) [40–44,46,48,49]. The 16 studies enrolled a total of 2156 participants, from which 1369 were diagnosed with OSA and 787 were disease-free/controls (AHI < 5). All studies were composed by two or more groups, one control group, and one or more OSA groups, more variable among studies. While some studies only included an OSA patient group (6/16) [34,35,37,45,47,48], others (7/16) further subdivided patients with OSA according to severity (mild, moderate and severe [40,41,43,49], or mild-moderate and severe [38,46], or moderate and severe [44]). Some studies further segmented the patient group according to the presence or absence of comorbidities or risk factors for comorbidities development, such as cardiovascular risk factors [42], obesity [46] or metabolic syndrome [36]. Inclusion and exclusion criteria varied widely between studies. Reported weaknesses were frequently a small sample size, oversampling of male sex, and absence of matched controls for sex or BMI. Even so, most studies were able to pinpoint candidates with potential application in OSA diagnosis and/or prognosis. Table S3 presents an overview of the 16 studies included in this review.

Cohort characteristics

The demographic and clinical characteristics of the cohorts of the final 16 studies are described in Table S4. The number of subjects evaluated in each study ranged from nine to 514 subjects, with an increased predominance of subjects in the disease group in comparison with the control group, in the majority of the studies (10/16, [35–39,42,44–47]). Although sex was balanced in both disease and control group in the majority of the studies (except in [34,43]), practically all studies included more male than female subjects, with three studies assessing only males [36,38,46]. Included participants were mainly in the middle-aged group, with the disease group (especially severe OSA subgroups) showing considerably older ages in comparison with the control group in some of the studies (>10 years difference, 4/16 [35,37,39,43]). Most of the included participants had a BMI superior to 25 kg/m^2 (>overweight), yet, many studies compared overweight–obese disease groups with normal–overweight control groups [34–38,41,42]. Only nine studies reported the presence or absence of comorbidities in their cohorts [34,36,38,42–45,47,49] and only three studies reported the medication being taken [38,42,47]. Arterial hypertension, metabolic conditions (diabetes mellitus; dyslipidemia) and cardiovascular complications were the most frequently reported comorbidities. The most common sleep-related parameters evaluated in the 16 studies were daytime sleepiness (evaluated according to the Epworth Sleepiness Scale), AHI and/or RDI, oxygen saturation [mean and minimum (min) percentage (%) of oxygen levels and % of time with oxygen saturation below 90% (T90), measured by pulse oximeter (SpO_2) or blood analysis (SaO_2)] and arousal index (number of arousals and microarousals per hour of sleep). Still, no single study provided information for all mentioned variables.

A meta-analysis was further performed using the common demographic and clinical data extracted from the 16 studies, namely, sex (defined as the percentage of males in the study), age, BMI, daytime sleepiness, AHI, oxygen saturation [mean and min %, and T90], oxygen desaturation index (ODI), and arousal index. All the assessed variables showed high heterogeneity between studies, as shown by the high percentage I^2 values, above 75% [33]. A forest plot is represented in Fig. 3, together with I^2 values, mean difference between each OSA group and the control group, Z scores, confidence intervals and p-values, and Cohen's d (effect size index), as calculated for each variable. Even so, collectively, the OSA group showed an increased percentage of males (11.91%; $p = 0.007$; medium–large effect), older ages (4.22 years; $p < 0.001$; large effect) and higher BMIs (3.41 kg/m^2; $p < 0.001$; large effect), in comparison with the control group. As expected, patients with OSA also showed statistically significant differences in all detailed clinical variables ($p < 0.001$), mostly with large effect sizes. Lower differences were observed in daytime sleepiness (3.50; $p = 0.010$;

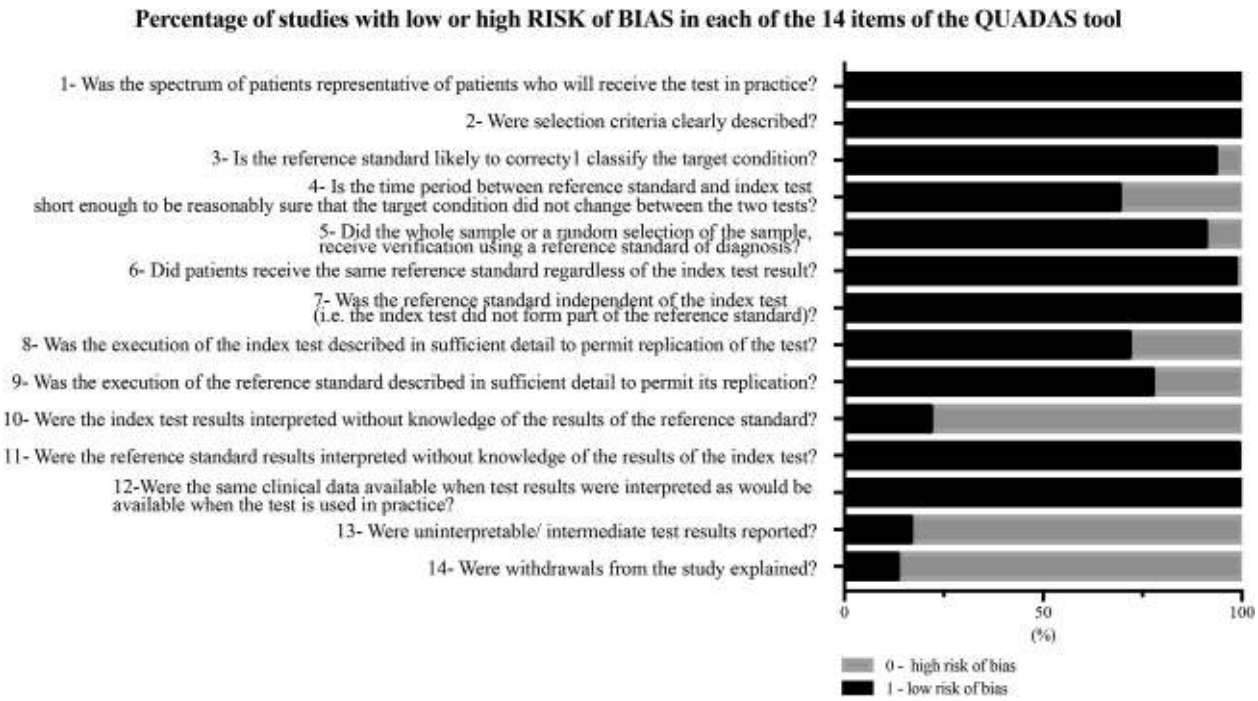

Fig. 2. Overview of the risk of bias assessment of the 120 eligible studies, using the 14-items of the QUADAS (Quality Assessment Tool for Diagnostic Accuracy Studies). The 14-item QUADAS was used to assess risk of bias, sources of variation (applicability), and reporting quality of eligible studies. For the target condition (OSA), we considered PSG type I as the reference standard, that currently discriminates patients with OSA from control subjects based on AHI (AHI < 5: no OSA; AHI ≥ 5: OSA), and the analysis of the candidate biomarker/s as the index test. Results are presented as the percentage of studies that fulfill (scored as 1) or do not fulfil (scored as 0) each of the 14 items specified by QUADAS. PSG: Polysomnography.

medium¯large effect). Severity subgroups were further analyzed. Severe patients showed older ages (3.78 years; $p = 0.037$; medium¯large effect), higher BMIs (2.93 kg/m^2; $p < 0.001$; large effect), more daytime sleepiness (ESS, 7.20; $p = 0.049$; large effect), superior AHIs (56.66; $p < 0.001$; large effect), decreased SpO_2/SaO_2 min (−15.86%; $p < 0.001$; large effect), increased T90 (77.45%; $p = 0.042$; small¯medium effect), ODI (54.40; $p < 0.001$; large effect) and Arousal Indexes (41.56; $p = 0.002$; large effect), in comparison with the control group. Although not statistically significant, severe patients also showed evidence of decreased mean SpO_2/SaO_2 levels (−1.80%; large effect) and increased percentage of males (16.33%; small¯medium effect). Such differences were less evident in the mild-moderate OSA subgroup that only showed statistically significant differences in BMI (1.95 kg/m^2; $p < 0.001$; large effect), AHI (14.57; $p < 0.001$; large effect), SpO_2/SaO_2 min (−5.61%; $p < 0.001$; large effect), ODI (14.66; $p < 0.001$; large effect) and Arousal Index (11.34; $p = 0.019$; large effect), in comparison with control subjects. Still, mild-moderate patients showed evidence of increased daytime sleepiness (3.39; medium¯large effect), higher T90 (13.03%; small¯medium effect) and increased age (3.39 years; small¯medium effect). Among all extracted variables, AHI, T90 and ODI were the variables that changed the most between patients' groups and the control group, as shown by their Z scores and Cohen's d values (Fig. 3). Effect size differences were interpreted based on the criteria proposed by Cohen [33,50].

Candidate biomarkers

From the included studies, data relative to 38 potential biomarkers was retrieved. All biological samples were collected in morning times and under fasting conditions. Most candidates were circulating factors evaluated in serum or plasma (30 candidates) [34,36–49], total blood (1 candidate) [49] or urine (1 candidate) [47]. In addition, one study validated changes in expression levels of six candidate genes, in peripheral blood mononuclear cells (PBMCs) [35]. From the 38 evaluated candidates, only two were evaluated in more than one study, using the same quantification methods, in the same type of biological samples. Dogan et al., 2016 [46] and Duger et al., 2020 [40] evaluated Ischemia modified albumin (IMA) levels in serum samples of patients with OSA, in comparison with non-OSA subjects. In both studies, IMA levels showed to be higher in patients with OSA in comparison with control subjects with higher differences reported by Dogan et al., 2016 [46]. The two studies also showed that IMA levels were positively correlated with OSA severity (AHI), but also with BMI, being higher in obese patients with OSA relative to non-obese patients. Still, when data from both studies were combined, IMA levels did not show to be statistically different between patient and control subjects, despite the associated large effect sizes in all groups of patients with OSA. Yet, Cohen's d values may be overestimated in variables analyzed in a low number of studies ($n \leq 3$) [50]. On the other hand, Archontogeorgis et al., 2016 [45] and Voulgaris et al., 2019 [39] assessed Cystatin C levels in serum samples of patients with OSA in comparison with control subjects (see Fig. 4A). In the two studies, higher Cystatin C levels were observed in patients with OSA and were shown to be positively correlated with T90 SpO_2 and negatively correlated with mean SpO_2 levels, during sleep. When data was combined (the only variable assessed that showed no considerable heterogeneity between studies, $I^2 = 0$), statistically significant differences in Cystatin C levels between patients with OSA and control subjects were still observed (mean difference of 205.01 ng/mL, $p < 0.001$), with large effect sizes (see Fig. 4A). Besides IMA and Cystatin C, only 17 other candidates showed statistically significant differences between patients with OSA and control groups (see Table S5). How much

	Group	Number of Studies	Subjects	OSA	Controls	I^2 (%) (95% CI)	Diff. to control Mean	95% CI	Z	95% CI	p	Cohen's d
% of Males	OSA	16	1911	1187	724	99.8 [99.8, 99.9]	11.91	[3.26, 20.56]	2.70	[0.74, 4.66]	0.007	0.60
	Sev	6	407	203	204	97.3 [95.3, 98.5]	16.33	[-3.91, 36.56]	1.58	[-0.38, 3.54]	0.114	0.42
	MM	7	551	312	239	94.8 [90.5, 97.1]	0.97	[-9.21, 11.14]	0.19	[-1.77, 2.15]	0.852	0.05
Age	OSA	16	2067	1309	758	77 [62.9, 85.7]	4.22	[2, 6.5]	3.70	[1.74, 5.66]	< 0.001	0.82
	Sev	7	507	253	254	67.4 [27.6, 85.3]	3.78	[0.2, 7.3]	2.09	[0.13, 4.05]	0.037	0.56
	MM	8	701	412	289	83.4 [68.8, 91.2]	3.39	[-0.6, 7.4]	1.66	[-0.30, 3.62]	0.096	0.44
BMI	OSA	17	2111	1337	774	93.7 [91.3, 95.4]	3.41	[1.8, 5]	4.13	[2.17, 6.09]	< 0.001	0.89
	Sev	7	507	253	254	71.5 [38.1, 86.9]	2.93	[1.2, 4.7]	3.30	[1.34, 5.26]	< 0.001	0.89
	MM	8	701	412	289	51.9 [0, 78.4]	1.95	[0.8, 3.1]	3.34	[1.38, 5.30]	< 0.001	0.89
ESS	OSA	8	788	537	251	92.6 [87.9, 95.5]	3.50	[0.8, 6.2]	2.57	[0.61, 4.53]	0.010	0.68
	Sev	3	186	87	99	95.8 [90.9, 98.1]	7.20	[0, 14.4]	1.97	[0.01, 3.93]	0.049	1.14
	MM	3	210	111	99	83.6 [50.4, 94.6]	3.39	[-2.3, 9.1]	1.16	[-0.80, 3.12]	0.245	0.67
AHI	OSA	15	2001	1252	749	99.6 [99.5, 99.6]	31.47	[24.1, 38.9]	8.32	[6.36, 10.28]	< 0.001	1.88
	Sev	4	319	173	146	84.6 [61.7, 93.8]	56.66	[48.4, 64.9]	13.49	[11.53, 15.45]	< 0.001	2.73
	MM	4	382	236	146	90.6 [79 , 95.8]	14.57	[10.7, 18.5]	7.32	[5.36, 9.28]	< 0.001	1.48
SpO_2/SaO_2 (mean)	OSA	8	1096	629	467	90.6 [83.8, 94.5]	-4.21	[-6.9, -1.5]	-3.03	[-4.99, -1.07]	0.002	-0.81
	Sev	1	86	40	46	NA	-1.80	[-4.5, 0.9]	-1.33	[-3.29, 0.63]	0.184	-1.33
	MM	1	118	72	46	NA	0.40	[-2.2, 3]	0.31	[-1.65, 2.26]	0.761	0.31
SpO_2/SaO_2 (min)	OSA	11	1503	936	567	97.4 [96.5, 98.1]	-14.65	[-18.6, -10.7]	-7.30	[-9.26, -5.34]	< 0.001	-1.82
	Sev	4	319	173	146	93.4 [86.3, 96.8]	-15.86	[-21.2, -10.5]	-5.82	[-7.78, -3.86]	< 0.001	-1.18
	MM	4	382	236	146	86.8 [68.3, 94.5]	-5.61	[-7.5, -3.7]	-5.76	[-7.72, -3.80]	< 0.001	-1.16
SpO_2/SaO_2 (T90)	OSA	9	1479	936	543	99.9 [99.9, 99.9]	28.92	[12.1, 45.8]	3.37	[1.41, 5.33]	< 0.001	0.88
	Sev	4	319	173	146	98.7 [98, 99.2]	77.45	[2.8, 152.1]	2.03	[0.07, 3.99]	0.042	0.41
	MM	4	382	236	146	99.4 [99.1, 99.6]	13.03	[-2.9, 28.9]	1.61	[-0.35, 3.57]	0.108	0.32
ODI	OSA	7	664	477	187	99.3 [99, 99.4]	29.52	[18.6, 40.5]	5.28	[3.32, 7.24]	< 0.001	1.42
	Sev	3	219	123	96	88.8 [69.2, 95.9]	54.50	[36.9, 72.1]	6.06	[0, 0]	< 0.001	3.50
	MM	3	318	222	96	92.4 [81.1, 97]	14.66	[6.8, 22.5]	3.67	[0, 0]	< 0.001	2.12
Arousal Index	OSA	7	767	560	207	99.7 [99.7, 99.8]	21.60	[10.2, 33]	3.72	[1.76, 5.68]	< 0.001	1.00
	Sev	3	233	133	100	98.4 [97.2, 99.1]	41.56	[15.7, 67.4]	3.16	[1.19, 5.11]	0.002	1.82
	MM	3	264	164	100	97.9 [96.1, 98.9]	11.34	[1.8, 20.8]	2.34	[0.38, 4.30]	0.019	1.35

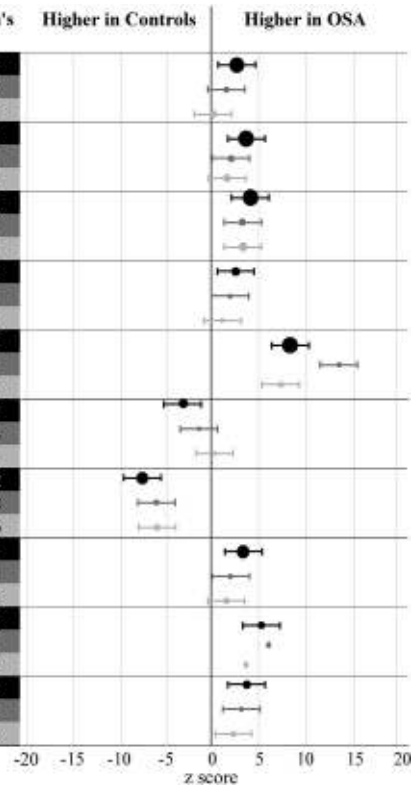

Fig. 3. Meta-analysis of the demographic and clinical data extracted from the 16 included studies, comparing OSA groups (further subdivided into mild-moderate, MM, and Severe, Sev, OSA when possible) with control groups (non-OSA). (A) Table presenting the number of studies, total number of subjects, number of subjects per group (OSA and control), heterogeneity of studies (I^2) and confidence intervals, mean difference between OSA and control groups and confidence intervals, Z scores, confidence intervals and p-value (considered statistically significant if less than 0.05, corresponding to an absolute Z score above 1.96) between OSA and control groups, and Cohen's d value (effect size index), for each variable analyzed [percentage of males (%), age (years), Body mass index (BMI, kg/m^2), daytime sleepiness (evaluated through the Epworth Sleepiness Scale—ESS), Apnea and Hypopnea Index (AHI), oxygen saturation [mean and minimum (min) percentage SpO_2 or SaO_2 (%) and percentage of time with SpO_2 or SaO_2 < 90% (T90, %)], Oxygen desaturation index (ODI) and Arousal Index]. (B) Descriptive forest plot with the synthesis values of the different variables.

each candidate is impacted in patients with OSA relative to control subjects is represented in Fig. 4B, by mean percentage difference (standard deviations are detailed in Table S5). *ADAM29*, *FLRT2* and *SLC18A3* mRNA levels in PBMCs, Endocan, Heat shock protein 90 (HSP90), IMA and Lipoprotein-associated phospholipase A2 (Lp-PLA2) levels in serum, and IL-6 and Vimentin levels in plasma showed the highest percentage mean differences in patients with OSA, relative to control subjects. Among the 17 candidates shown to be statistically different between the disease and control groups, 15 candidates were shown to correlate with clinically relevant parameters for OSA diagnosis. Yet, the majority of the evaluated candidates show more potential as OSA prognosis biomarkers, namely, of inflammation, oxidative stress, endothelial dysfunction, vascular damage, detrimental metabolic profiles, and cardiovascular or renal diseases, rather than diagnostic biomarkers (see Table S5). Endocan [44], YKL-40 [48], and IMA [40,46] levels in serum, IL-6 [46] and Vimentin [41] levels in plasma, and ADAM29, *SLC18A3, and FLRT2* gene expression in PBMCs [35], showed potential application for OSA diagnosis, according to the study authors, although in small size study cohorts (n = 49−¯200).

Among the included studies, only two proceeded further with measures of diagnostic accuracy [40,44] (see Table S6). Altintas et al., 2016 [44] showed that Endocan levels in serum have a specificity and sensitivity for OSA of 77.5 and 82.5%, respectively, demonstrating an acceptable diagnostic test accuracy (0.8 < AUC <0.9; LR+ > 3 and an LR- < 0.3; DOR = 16.2; Younden's index value = 0.6). Duger et al., 2020 [40] showed that IMA levels in serum have higher specificity for OSA, but the sensitivity is lower (39%), leading to lower diagnostic test accuracies (AUC = 0.62; LR+ > 3 and an LR- > 0.3; DOR = 5.2; Younden's index value = 0.28).

Among the included studies, Altintas et al., 2016 [44] was the only study that evaluated the levels of the same candidate after OSA treatment as well. The obtained results showed that the higher levels of endocan detected in serum samples from patients with OSA at baseline (before treatment), in comparison with control subjects, significantly decreased after 3 months of Continuous Positive Airway Pressure (CPAP) treatment. Yet, serum endocan levels in CPAP treated patients were still higher than in the control subjects.

Discussion

OSA has been increasingly recognized as a major public health concern worldwide given its increasing prevalence and impact on health, healthcare system, economy and society [51,52]. Yet, limitations in current diagnostic methods are evident in both developed and developing countries, which challenges OSA clinical management [3,7]. There is an evident need for alternative strategies to guide medical decision-making, such as biomarkers. In this systematic review and meta-analysis, we proposed to gather and further explore potential OSA biomarkers pinpointed in OSA biomarkers research. For this, we selected studies that performed in-laboratory PSG to avoid technical differences in OSA diagnosis and in participants characterization between studies, as well as the impact of undiagnosed comorbidities in the identification of

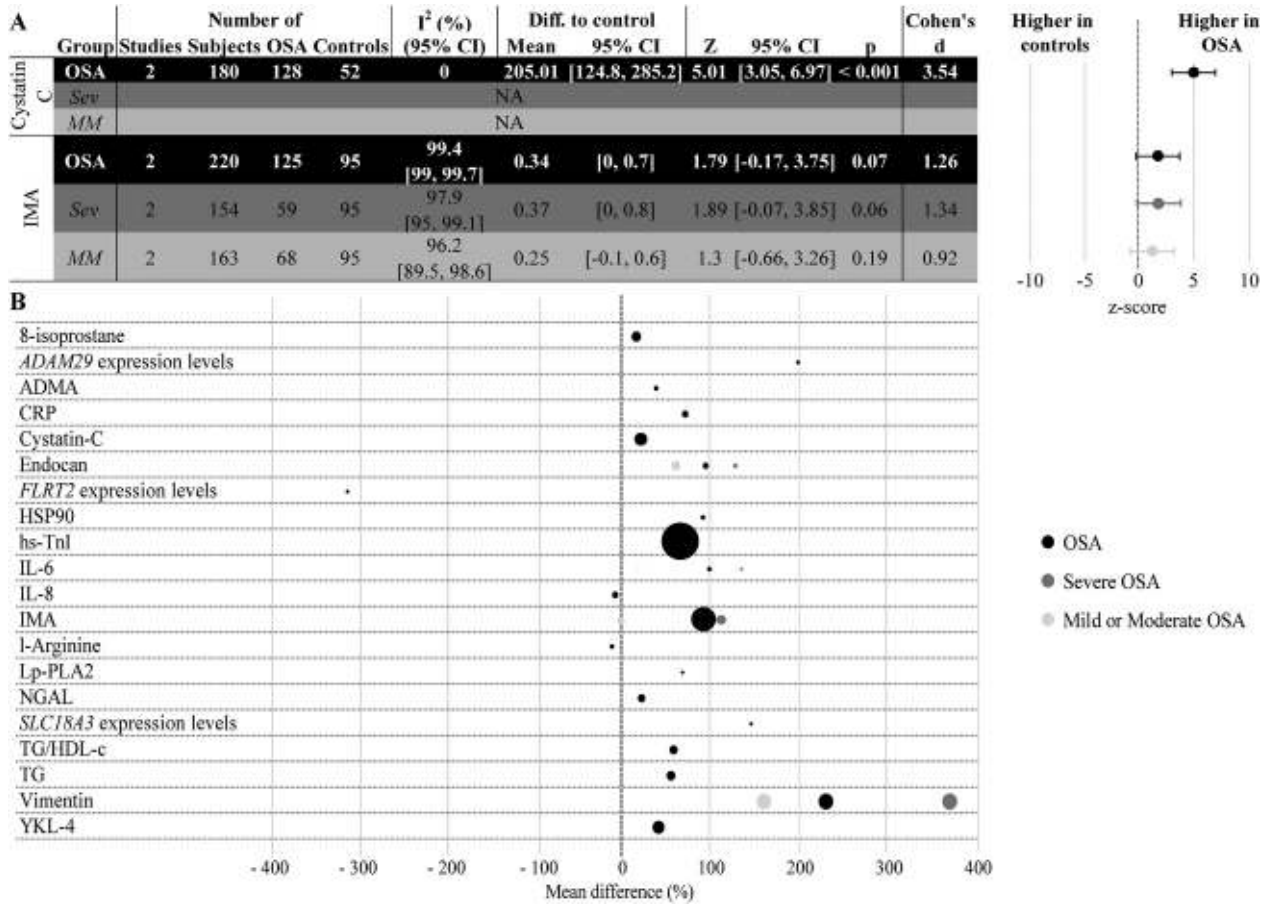

Fig. 4. Candidate biomarkers evaluated in the included studies. (A) Cystatin-C and Ischemia Modified Albumin (IMA) were explored in a meta-analysis, as the only candidate biomarkers evaluated in more than one study. Each candidate was evaluated globally (OSA group) and IMA was further explored in subgroups according to disease severity [mild-moderate OSA (MM), and severe (Sev) OSA] as segmented in the respective studies. The presented table describes the number of studies, total number of subjects, number of subjects per group (OSA and control), heterogeneity of studies (I^2) and confidence intervals, mean difference between OSA and control groups and confidence intervals, Z scores, confidence intervals and p-value (considered statistically significant if less than 0.05, corresponding to an absolute Z score above 1.96) between OSA and control groups, and Cohen's d value (effect size index), of each analysis, for each candidate biomarker. A descriptive forest plot with the synthesis values of the different variables is also shown. (B) Descriptive forest plot of the percentage difference between the OSA group or subgroup and the control group of the 17 candidate biomarkers with statistically significant differences between OSA and control groups, as retrieved from the included studies. The diameter of the bubble represents the sample size. As most of the candidate biomarkers were explored only in one study, it was not possible to achieve a Z score.

potential OSA biomarkers [17,18,53–55]. The incapacity to identify specific pathologies under type II (e.g., previously undetected cardiovascular, pulmonary, or neuromuscular pathologies with hypoventilation) and III (e.g., concomitant neurological disorders, movement disorders, parasomnias, or insomnia) studies may lead to confounding factors that can impact on OSA biomarkers research [17,18,53].

Risk of bias assessment showed that most studies conducted to identify potential biomarkers of OSA have high risk of bias and are predominantly exploratory. Poor reporting was evident in most studies. Methodological procedures were frequently not described in sufficient detail to allow for reproducibility, such as patients' biosampling conditions (e.g., collection hour, fasting conditions) and processing (e.g., references of used commercial kits). Both pre- and post-analytical variables strongly impact on the obtained results, being amongst the main causes of inconsistency in findings of independent groups [56]. There is thus an urgent need to adopt best practice guidelines and to improve reporting and/or standardize protocols, thus, enhancing reproducibility and comparability of studies. In addition, many studies also did not report demographic characteristics as race/ethnicity and clinical characteristics as PSG-derived or clinical history.

Included studies showed to be mostly single-centric, to have small sample sizes, and to be highly variable in assessed/reported demographic and clinical features. Most of these studies did not specifically examine sex differences or were based exclusively or predominantly on male cohorts. Since OSA is more prevalent men, this has led to a better identification and characterization of OSA in this sex [57,58]. However, a growing body of evidence has shown that differences in prevalence data of OSA in men and women are not reflected in clinical populations [57,58]. Women are most likely to be less diagnosed given the substantial differences in disease pathophysiology, clinical presentation, and comorbidities [57]. It is thus important to include more females in phenotyping studies to address OSA sex differences. Similarly, we also observed an oversampling of middle-aged patients with OSA and patients with excessive weight. Most studies lacked matched controls in accordance, especially for sex and BMI. Similarly, most studies did not control or did not report any control for frequent comorbid conditions, nor for associated medication, which may interfere with the obtained results. Future studies, with large sample sizes, may allow to cluster patients based on demographic and clinical features, as sex, age, BMI, sleepiness, comorbidities, and medication, and to compare with matched controls.

The demographic and clinical features of studies' participants showed high heterogeneity between studies, as shown by the high I^2 values. This heterogeneity may be mainly driven by differences in the study cohorts promoted by different inclusion and exclusion criteria and population-specific differences. The wide confidence intervals observed in each study may also contribute to the observed variability. Nevertheless, including and examining variability allows more representative conditions and may lead to stronger conclusions.

Links between clinical variables and biomarker candidates were explored mainly based on AHI or RDI in all studies, as also flagged in the last systematic reviews performed on OSA biomarkers [28,30], in 2015. Both AHI and RDI consider the number of apneas and hypopneas per hour of sleep, with RDI also counting respiratory effort-related arousals (RERAs). Using AHI or RDI as determinant factors for OSA diagnosis and severity classification has generated an enormous controversy among the scientific and medical community over the past years [59,60]. Within the same severity class of OSA, respiratory events can vary widely, from 10 s to more than 2 min, which differently impact on oxygen desaturation [61]. Longer obstruction episodes with deeper desaturations may have different consequences than shorter and shallower episodes or events that are followed by an arousal, for example. Thus, stratifying patients based on other PSG indicators, rather than only AHI/RDI, may allow to subgroup patients with OSA into more homogeneous cohorts and to better disentangle potential biomarkers of OSA.

Another major challenge in OSA biomarkers research is the selection of biomarkers that are specific for OSA, independently of the presence or absence of other comorbidities. OSA shares pathological mechanisms with many conditions, as chronic obstructive pulmonary disease, or heart failure. Indeed, most of the included studies pinpointed candidates with potential for OSA prognosis instead, as Troponin I (TnI), HSP90, L-Arginine, ADMA, Cystatin C, 8-isoprostane, Lp-PLA2, triglycerides (TG), TG to high-density lipoprotein (HDL) ratio, (repeated) and/or NEGAL. These candidates may be relevant to identify patients who are more likely to have clinical outcomes, allowing to stratify patients and tailor better treatment strategies, or to evaluate disease progression. On the other hand, it is important to note that, in combination with more specific OSA biomarkers, these may still reveal useful for the diagnosis of specific endotypes and phenotypes. For OSA diagnosis, *ADAM29*, *FLRT2* and *SLC18A3* mRNA levels in PBMCs, Endocan and YKL-40 levels in serum, and IL-6 and Vimentin levels in plasma revealed the most promising candidates, among the evaluated targets in the 16 studies. However, caution is needed in interpreting these findings, as these mostly derive from small-size and single-center study cohorts, as previously stated. Future studies should explore deeper the proposed candidates and evaluate their diagnostic accuracy in larger cohorts of independent and diverse populations to understand their potential individually or in combination with other diagnostic biomarkers. For that, data related to biomarker performance such as the area under the ROC curve, sensitivity, specificity, PPV, NPV and accuracy are crucial, which most of studies did not provide. It would also be relevant to explore both potential diagnostic and prognostic biomarkers after OSA treatment, to understand their capacity as treatment response biomarkers as well.

Among the proposed candidates, only IL-6 was also reported in the previous systematic reviews and meta-analysis [26,28]. Based on previous studies, IL-6 (and IL-10) were identified as a potential diagnostic biomarker in adults, in both serum and exhaled breath condensate samples, with high specificity and sensitivity, which encourages its further analysis, as a single biomarker and/or in combination with other promising candidates. Given the complex heterogeneity and comorbidity plethora associated with OSA, one would also expect heterogeneity in biomarker signatures. In this context, it is highly unlikely that a single biomarker will be sensitive and specific enough for the diagnosis of the different OSA endotypes and phenotypes. Approaches using omics (genomics, transcriptomics, epigenomics, proteomics, lipidomics, metabolomics) that generate "big data" may allow further insights into the different OSA signatures [21,56,62–65]. For example, the recent review of Pinilla and coworkers highlights the potential of the non-coding transcriptome for biomarker discovery, particularly, of miRNAs, in the context of OSA [56]. Integrative multi-domain approaches, such as machine learning, could then be used to link omics data with clinical endotypes and phenotypes, and guide the use and interpretation of OSA-sensitive and specific multi-marker panels. However, in the clinical setting, this would require significant improvement and validation of associated analytical platforms to allow simple, easily accessible, and inexpensive options applicable to high-volume routines. Similar limitations and difficulties were highlighted in the previous systematic reviews and meta-analyses on OSA biomarkers, which shows the evident need of a collaborative effort to overpass them and to develop new strategies and directions in OSA biomarkers research.

It is also important to recognize the limitations of this work. The fact that we only excluded studies in which comorbidities were stated in the inclusion criteria can be seen as a limitation. By opting for this criterion, we did not exclude studies in which participants have comorbidities. Yet, the contribution of the comorbidities that each participant may have to the study results will be diluted when compared with studies in which all the included individuals have a defined comorbidity (e.g., hypertensive individuals with and without OSA). We do agree with the importance of excluding comorbidities as confounding factors or to control for it, especially to explore OSA specific biomarkers, but we are also aware of the difficulties of conducting studies in which patients with OSA have no comorbidities. We also used the QUADAS tool to select only studies with low risk of bias for systematic review and meta-analyses. This criterion may have led us to leave out some studies assessing candidates with promising applications. It is often preferable to review all relevant evidence and then investigate possible reasons for heterogeneity, however, that is only applicable when there are enough studies evaluating the same variables. Given the lack of studies evaluating the same candidate biomarkers, we were not able to perform additional analyses. In this context, we found it more appropriate to restrict inclusion to the review based on risk of bias assessment, to avoid erroneous conclusions, increase studies reproducibility and application in biomarkers research. Nevertheless, the importance of issues as non-blinding conditions and poor reporting can be questioned at an exploratory research stage in comparison with focused research that evaluates candidates based on promising preliminary data. The absence of diagnostic accuracy measures or raw data in most of the studies did not allow further analysis of the proposed candidates, such as further subgroup analysis based on different demographic or clinical characteristics, which would be interesting to analyze.

Conclusion

Although the current systematic review and meta-analysis allowed us to pinpoint candidates to further explore as potential biomarkers in future studies, it mostly showed that OSA biomarkers research is still at an early stage. The field must move towards less biased and more transparent research, in larger multicentric

cohorts, repeated to disentangle biomarkers with application in the clinical management of such a complex multifactorial disorder.

Practice points

- OSA biomarkers research is still at an early stage. There are evident limitations in the design of most studies that frequently involve small sample sizes, mainly based on single centers, males oversampling, lack of appropriate controls and/or low reporting.
- Most of the included studies found candidates with potential for OSA prognosis. For OSA diagnosis, *ADAM29*, *FLRT2* and *SLC18A3* mRNA levels in PBMCs, Endocan and YKL-40 levels in serum, IL-6 and Vimentin levels in plasma revealed the most promising candidates to further explore in future studies, as single or clustered biomarkers.
- Future studies are needed to disentangle sensitive and specific OSA biomarkers with application in clinics.

Research agenda

- Development of guidelines for OSA biomarker research, to improve study design, avoid bias, and improve reporting;
- Create task forces to foster multicentric studies, with standardized pre- and post-analytical procedures;
- Move from single-target approaches towards omics and big data analysis for association of molecular, endotype and phenotype data and identification of sensitive and specific OSA biomarkers;
- Encourage publicly available data in publications and/or the development of OSA databases/repositories with demographic, clinical and experimental data.

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Conflicts of interest

The authors have no conflicts of interest to declare.

Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.smrv.2022.101659.

References

[1] Peppard PE, Young T, Barnet JH, Palta M, Hagen EW, Hla KM. Increased prevalence of sleep-disordered breathing in adults. Am J Epidemiol 2013;177:1006–14.
[2] Peppard PE, Hagen EW. The last 25 years of obstructive sleep apnea epidemiology and the next 25? Am J Respir Crit Care Med 2018;197:310–2.
[3] Benjafield AV, Ayas NT, Eastwood PR, Heinzer R, Ip MSM, Morrell MJ, et al. Estimation of the global prevalence and burden of obstructive sleep apnoea: a literature-based analysis. Lancet Respir Med 2019;7:687–98.
[4] Randerath W, Bassetti CL, Bonsignore MR, Farre R, Ferini-Strambi L, Grote L, et al. Challenges and perspectives in obstructive sleep apnoea. Eur Respir J 2018;52:1702616.
*[5] Malhotra A, Mesarwi O, Pepin J-L, Owens RL. Endotypes and phenotypes in obstructive sleep apnea. Curr Opin Pulm Med 2020;26:609.
*[6] Thorpy M. International classification of sleep disorders. Sleep Disord Med Basic Sci Tech Considerations Clin Asp Fourth Ed 2017:475–84.
[7] Gottlieb DJ, Punjabi NM. Diagnosis and management of obstructive sleep apnea: a review. JAMA 2020;323:1389–400.
[8] Léger D, Stepnowsky C. The economic and societal burden of excessive daytime sleepiness in patients with obstructive sleep apnea. Sleep Med Rev 2020;51:101275.
[9] Bonsignore MR, Baiamonte P, Mazzuca E, Castrogiovanni A, Marrone O. Obstructive sleep apnea and comorbidities: a dangerous liaison. Multidiscip Respir Med 2019;14:8.
[10] Kaw R, Chung F, Pasupuleti V, Mehta J, Gay P, Hernandez A. Meta-analysis of the association between obstructive sleep apnoea and postoperative outcome. Br J Anaesth 2012;109:897–906.
[11] Dodds S, Williams LJ, Roguski A, Vennelle M, Douglas NJ, Kotoulas S-C, et al. Mortality and morbidity in obstructive sleep apnoea–hypopnoea syndrome: results from a 30-year prospective cohort study. ERJ Open Res 2020;6:57–2020.
[12] Miller MA, Cappuccio FP. A systematic review of COVID-19 and obstructive sleep apnoea. Sleep Med Rev 2021;55:101382.
[13] Gaspar LS, Álvaro AR, Moita J, Cavadas C. Obstructive sleep apnea and hallmarks of aging. Trends Mol Med 2017;23:675–92.
[14] Singh M, Liao P, Kobah S, Wijeysundera DN, Shapiro C, Chung F. Proportion of surgical patients with undiagnosed obstructive sleep apnoea. Br J Anaesth 2013;110:629–36.
[15] McNicholas WT, Bassetti CL, Ferini-Strambi L, Pépin JL, Pevernagie D, Verbraecken J, et al. Challenges in obstructive sleep apnoea. Lancet Respir Med 2018;6:170–2.
[16] Amra B, Rahmati B, Soltaninejad F, Feizi A. Screening questionnaires for obstructive sleep apnea: an updated systematic review. Oman Med J 2018;33:184–92.
[17] Kapur VK, Auckley DH, Chowdhuri S, Kuhlmann DC, Mehra R, Ramar K, et al. Clinical practice guideline for diagnostic testing for adult obstructive sleep apnea: an American academy of sleep medicine clinical practice guideline. J Clin Sleep Med 2017;13:479–504.
[18] Ayas NT, Jen R, Baumann B. Revisiting level II sleep studies in the era of COVID-19: a theoretical economic decision model in patients with suspected obstructive sleep apnea. Sleep Sci Pract 2021;5:11.
[19] Bernhardt L, Brady EM, Freeman SC, Polmann H, Réus JC, Flores-Mir C, et al. Diagnostic accuracy of screening questionnaires for obstructive sleep apnoea in adults in different clinical cohorts: a systematic review and meta-analysis. Sleep Breath 2021:1–26 [Epub ahead of print].
[20] Kapoor M, Greenough G. Home sleep tests for obstructive sleep apnea (OSA). J Am Board Fam Med 2015;28:504–9.
[21] Lebkuchen A, Carvalho VM, Venturini G, Salgueiro JS, Freitas LS, Dellavance A, et al. Metabolomic and lipidomic profile in men with obstructive sleep apnoea: implications for diagnosis and biomarkers of cardiovascular risk. Sci Rep 2018;8:11270.
[22] Roeder M, Bradicich M, Schwarz EI, Thiel S, Gaisl T, Held U, et al. Night-to-night variability of respiratory events in obstructive sleep apnoea: a systematic review and meta-analysis. Thorax 2020;75:1095–102.
[23] Dzierzewski JM, Dautovich ND, Rybarczyk B, Taylor SA. Night-to-night fluctuations in sleep apnea severity: diagnostic and treatment implications. J Clin Sleep Med 2020;16:539–44.
*[24] Zinchuk A, Yaggi H. Phenotypic subtypes of OSA: a challenge and opportunity for precision medicine. Chest 2020;157:403–20.
[25] Selleck MJ, Senthil M, Wall NR. Making meaningful clinical use of biomarkers. Biomark Insights 2017;12:1177271917715236.
*[26] FDA-NIH Biomarker Working Group. BEST (biomarkers, EndpointS, and other tools). [Resource: Internet]. Silver spring (MD): food and drug administration (US). Bethesda (MD): National Institutes of Health (US); 2016. Available from: https://www.ncbi.nlm.nih.gov/books/NBK326791/%20Co-published.
[27] Mullington JM, Abbott SM, Carroll JE, Davis CJ, Dijk DJ, Dinges DF, et al. Developing biomarker arrays predicting sleep and circadian-coupled risks to health. Sleep 2016;39:727–36.

* The most important references are denoted by an asterisk.

*[28] De Luca Canto G, Pacheco-Pereira C, Aydinoz S, Major PW, Flores-Mir C, Gozal D. Diagnostic capability of biological markers in assessment of obstructive sleep apnea: a systematic review and meta-analysis. J Clin Sleep Med 2015;11:27–36.

*[29] Lebkuchen A, Freitas LS, Cardozo KHM, Drager LF. Advances and challenges in pursuing biomarkers for obstructive sleep apnea: implications for the cardiovascular risk. Trends Cardiovasc Med 2021;31:242–9.

*[30] De Luca Canto G, Pachêco-Pereira C, Aydinoz S, Major PW, Flores-Mir C, Gozal D. Biomarkers associated with obstructive sleep apnea: a scoping review. Sleep Med Rev 2015;23:28–45.

[31] Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71.

[32] Whiting P, Rutjes AWS, Reitsma JB, Bossuyt PMM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Med Res Methodol 2003;3:1–13.

[33] Higgins J, Thomas J, Chandler J, Cumpston M, Li T, Page M, et al., editors. Cochrane handbook for systematic reviews of interventions. 2nd ed. Chichester (UK): John Wiley & Sons; 2019.

[34] Einvik G, Rsøjø H, Randby A, Namtvedt SK, Hrubos-Strmø H, Brynildsen J, et al. Severity of obstructive sleep apnea is associated with cardiac Troponin I concentrations in a community-based sample: data from the akershus sleep apnea project. Sleep 2014;37:1111.

[35] Lin SW, Tsai CN, Lee YS, Chu SF, Chen NH. Gene expression profiles in peripheral blood mononuclear cells of Asian obstructive sleep apnea patients. Biomed J 2014;37:60–70.

[36] Moise LG, Marta DS, Raşcu A, Moldoveanu E. Serum lipoprotein-associated phospholipase A2 in males with metabolic syndrome and obstructive sleep apnea. Acta Endocrinol 2018;14:36.

[37] Silva LOE, Guimarães TM, Luz GP, Coelho G, Badke L, Almeida IR, et al. Metabolic profile in patients with mild obstructive sleep apnea. Metab Syndr Relat Disord 2018;16:6–12.

[38] Trojova I, Kozarova M, Petrasova D, Malachovska Z, Paranicova I, Joppa P, et al. Circulating lipopolysaccharide-binding protein and carotid intima-media thickness in obstructive sleep apnea. Physiol Res 2018;67:69–78.

[39] Voulgaris A, Archontogeorgis K, Nena E, Tsigalou C, Xanthoudaki M, Kouratzi M, et al. Serum levels of NGAL and cystatin C as markers of early kidney dysfunction in patients with obstructive sleep apnea syndrome. Sleep Breath 2019;23:161–9.

[40] Düger M, Seyhan EC, Günlüoğlu MZ, Bolatkale M, Özgül MA, Turan D, et al. Does ischemia-modified albumin level predict severity of obstructive sleep apnea? Sleep Breath 2021;25:65–73.

[41] Kerget B, Afşin DE, Kerget F, Aşkın S, Araz Ö, Akgün M. Is Vimentin the cause or effect of obstructive sleep apnea development? Lung 2020;198:275–82.

[42] İn E, Özdemir C, Kaman D, Sökücü SN. Heat shock proteins, L-arginine, and asymmetric dimethylarginine levels in patients with obstructive sleep apnea syndrome. Arch Bronconeumol 2015;51:544–50.

[43] Selcuk OT, Eyigor M, Renda L, Osma U, Eyigor H, Selcuk NT, et al. Can we use serum copeptin levels as a biomarker in obstructive sleep apnea syndrome? J Cranio-Maxillo-Fac Surg 2015;43:879–82.

[44] Altintas N, Mutlu LC, Akkoyun DC, Aydin M, Bilir B, Yilmaz A, et al. Effect of CPAP on new endothelial dysfunction marker, endocan, in people with obstructive sleep apnea. Angiology 2016;67:364–74.

[45] Archontogeorgis K, Nena E, Tsigalou C, Voulgaris A, Xanthoudaki M, Froudarakis M, et al. Cystatin C levels in middle-aged patients with obstructive sleep apnea syndrome. Pulm Med 2016;2016:8081723.

[46] Dogan D, Ocal N, Aydogan M, Tasci C, Arslan Y, Tapan S, et al. Assessment of the role of serum ischemia-modified albumin in obstructive sleep apnea in comparison with interleukin-6. Postgrad Med 2016;128:603–8.

[47] Cherneva RV, Cherneva ZV, Georgiev OB, Petrova DS, Petrova JI. 8-isoprostanes and resistin as markers of vascular damage in non-hypersomnolent obstructive sleep apnoea patients. Clin Physiol Funct Imag 2017;37:695–702.

[48] Mutlu LC, Tülübaş F, Alp R, Kaplan G, Yildiz ZD, Gürel A. Serum YKL-40 level is correlated with apnea hypopnea index in patients with obstructive sleep apnea syndrome. Eur Rev Med Pharmacol Sci 2017;21:4161–6.

[49] Sarioglu N, Demirpolat G, Erel F, Kose M. Which is the ideal marker for early atherosclerosis in obstructive sleep apnea (OSA)¯carotid intima-media thickness or mean platelet volume? Med Sci Mon Int Med J Exp Clin Res 2017;23:1674–81.

[50] Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale (NJ): Lawrence Erlbaum Associates, Inc.; 1988.

[51] Ding Q, Kryger M. Greater health care utilization and cost associated with untreated sleep apnea. J Clin Sleep Med 2020;16:5–6.

[52] Streatfeild J, Hillman D, Adams R, Mitchell S, Pezzullo L. Cost-effectiveness of continuous positive airway pressure therapy for obstructive sleep apnea: health care system and societal perspectives. Sleep 2019;42:zsz181.

[53] Rosenberg R, Hirshkowitz M, Rapoport DM, Kryger M. The role of home sleep testing for evaluation of patients with excessive daytime sleepiness: focus on obstructive sleep apnea and narcolepsy. Sleep Med 2019;56:80–9.

[54] Ghegan MD, Angelos PC, Stonebraker AC, Gillespie MB. Laboratory versus portable sleep studies: a meta-analysis. Laryngoscope 2006;116:859–64.

[55] Campbell AJ, Neill AM. Home set-up polysomnography in the assessment of suspected obstructive sleep apnea. J Sleep Res 2011;20:207–13.

[56] Pinilla L, Barbé F, de Gonzalo-Calvo D. MicroRNAs to guide medical decision-making in obstructive sleep apnea: a review. Sleep Med Rev 2021;59:101458.

*[57] Bonsignore MR, Saaresranta T, Riha RL, Riha R, Bonsignore M. Sex differences in obstructive sleep apnoea. Eur Respir Rev 2019;28:1–11.

[58] May AM, Wang L, Strohl KP, Walia H, Hazen SL, Mehra R. Sex-specific differential responses of circulating biomarkers in obstructive sleep apnea treatment A post hoc analysis of a randomized controlled trial. Ann Am Thorac Soc 2020;17:605–13.

[59] Punjabi NM. COUNTERPOINT: is the apnea-hypopnea index the best way to quantify the severity of sleep-disordered breathing? No. Chest 2016;149:16–9.

[60] Pevernagie DA, Gnidovec-Strazisar B, Grote L, Heinzer R, McNicholas WT, Penzel T, et al. On the rise and fall of the apnea–hypopnea index: a historical review and critical appraisal. J Sleep Res 2020;29:e13066.

*[61] Malhotra A, Force for ST, Ayappa I, Force for ST, Ayas N, Force for ST, et al. Metrics of sleep apnea severity: beyond the apnea-hypopnea index. Sleep 2021;44:zsab030.

[62] Leader BA, Koritala BSC, Moore CA, Dean EG, Kottyan LC, Smith DF. Epigenetics of obstructive sleep apnea syndrome: a systematic review. J Clin Sleep Med 2021;17:2533–41.

[63] Koritala BSC, Conroy Z, Smith DF. Circadian biology in obstructive sleep apnea. Diagnostics 2021;11:1082.

[64] Ambati A, Ju Y El, Lin L, Olesen AN, Koch H, Hedou JJ, et al. Proteomic biomarkers of sleep apnea. Sleep 2020;43:zsaa086.

*[65] Conte L, Greco M, Toraldo DM, Arigliani M, Maffia M, De Benedetto M. A review of the “OMICS” for management of patients with obstructive sleep apnoea. Acta Otorhinolaryngol Ital 2020;40:164–72.