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# Diagnostic Capability of Biological Markers in Assessment of Obstructive Sleep Apnea: A Systematic Review and Meta-Analysis

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**Objective:** The purpose of this systematic review is to evaluate the diagnostic value of biological markers (exhaled breath condensate, blood, salivary and urinary) in the diagnosis of OSA in comparison to the gold standard of nocturnal PSG.

**Methods:** Studies that differentiated OSA from controls based on PSG results, without age restriction, were eligible for inclusion. The sample of selected studies could include studies in obese patients and with known cardiac disease. A detailed individual search strategy for each of the following bibliographic databases was developed: Cochrane, EMBASE, MEDLINE, PubMed, and LILACS. The references cited in these articles were also crosschecked and a partial grey literature search was undertaken using Google Scholar. The methodology of selected studies was evaluated using the 14-item Quality Assessment Tool for Diagnostic Accuracy Studies.

**Results:** After a two-step selection process, nine articles were identified and subjected to qualitative and quantitative analyses. Among them, only one study conducted in children and one in adults found biomarkers that exhibit sufficiently satisfactory diagnostic accuracy that enables application as a diagnostic method for OSA.

**Conclusion:** Kallikrein-1, uromodulin, urocotin-3, and orosomucoid-1 when combined have enough accuracy to be an OSA diagnostic test in children. IL-6 and IL-10 plasma levels have potential to be good biomarkers in identifying or excluding the presence of OSA in adults.

**Keywords:** biological markers, diagnosis, sleep apnea syndromes, review

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Obstructive sleep apnea (OSA) has become widely recognized as a potential cause of significant morbidity in both children and adults.[1,2] OSA symptoms include habitual snoring and reporting of disturbed unrefreshing sleep, frequently accompanied by excessive daytime sleepiness, and daytime neurobehavioral problems.[3] The increasing understanding, awareness and familiarity with OSA has resulted in an ever expanding spectrum of OSA-associated morbidities that encompasses not only the central nervous system (cognitive, mood disturbances, and behavioral deficits), but affects also many other organ systems, ultimately imposing substantial increases in healthcare costs, as well as adverse outcomes.[4–7]

Among the prototypic risk factors associated with OSA, adenotonsillar hypertrophy, obesity, craniofacial and anatomical anomalies, and neuromuscular disorders, seemingly interact to a greater or lesser extent among patients, leading to the putative assumption that multiple clinical phenotypes exist and potentially merit divergent therapeutic approaches better tailored at the constellation of pathophysiological mechanisms leading to OSA in these clinical clusters.[3] The prevalence of OSA is markedly variable both during childhood (1% to 5%) and during adulthood (4% to 15%), with major contributions of age, gender, and ethnicity.[1,8–11] However, it is clear that independently of whether we consider the lowest or the highest estimated prevalence reported for any population, OSA is a frequent condition that imposes a high degree of disease burden, thereby requiring timely diagnosis and effective treatment.

**BRIEF SUMMARY**

**Current Knowledge/Study Rationale:** The purpose of this systematic review was to evaluate the diagnostic properties of markers in biological samples, such as in exhaled breath condensate, blood, saliva, and urine, and compare their predictive characteristics to the gold standard in the diagnosis of OSA—nocturnal PSG.

**Study Impact:** A substantial number of studies have been published in the literature in the quest for diagnostic biomarkers of OSA in both children and adults; however, most of the explored approaches do not identify definitive biomarkers, and only a small number of candidates appears promising and merits further research.

An overnight in-laboratory polysomnographic evaluation (PSG) remains the gold standard diagnostic method for OSA at any age.[3,12] Unfortunately, overnight PSGs are onerous, labor-intensive, may impose substantial inconvenience to the child and caretakers, and are variably accessible around the world. Waiting time between referral for evaluation to diagnosis may commonly take 3–6 months across the United States and even longer elsewhere.[13] Although the PSG is employed as the gold standard for diagnosing the vast majority of sleep disorders, the relative complexity of PSG application and the inherent costs associated with PSG has spurred the quest for alternative diagnostic methods.[13] Among these, simple approaches such as questionnaires with or without medical history and physical examination, audiotaping, videotaping, pulse oximetry, abbreviated polysomnography (aPSG), home-based polygraphy, or multichannel recordings have all been assessed, albeit with variable success.[12,14–18] However, among the alternative diagnostic tools, special interest has recently centered on the identification of biomarkers.

A biomarker is a "biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal processes, or of a condition or disease."[13] Gene expression arrays have revealed significant and reproducible changes in a restricted number of genes that could enable discriminatory ability in the recognition of OSA. Similarly, a number of serum and urinary proteins have been identified that display favorable significant receiver-operator properties towards the diagnosis of OSA.[13] Provided that acceptable sensitivity and specificity are achieved, a unique set of disease biomarkers would enable greatly simplified, user-friendly, and context-relevant approaches to the diagnosis of OSA in the future.[19] Over the last 14 years, a substantial number of studies have tackled the identification of an ideal biomarker for OSA, and although, there is still no simple and useful disease marker panel for OSA available, considerable progress has been accomplished and merits critical review and scrutiny.[19] Therefore the purpose of this systematic review was to critically evaluate the diagnostic properties of markers in biological samples, such as in exhaled breath condensate (EBC), blood, saliva, and urine, and compare their predictive characteristics to the gold standard—nocturnal PSG. We further aimed to formulate potential future exploratory research directions aiming at advancing this promising area of clinical translation in sleep medicine.

## METHODS

This systematic review was done adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses PRISMA Checklist.[20]

### Diagnostic Terminology

All terms that mean obstructive sleep apnea (OSA), including sleep disordered breathing (SDB), sleep-related breathing disorder (SRBD) and obstructive sleep apnea syndrome (OSAS) were standardized as OSA.

### Protocol and Registration

The systematic review protocol was registered at the international prospective register of systematic reviews (PROSPERO). The number of register is CRD42014007427.

### Study Design

A systematic review of human studies that evaluated the diagnostic value of biological markers (blood, EBC, salivary, and urinary) in the diagnosis of OSA was undertaken.

### Eligibility Criteria

#### *Inclusion Criteria*

Studies that differentiated the OSA group from controls based on full PSG results, without age restriction, were eligible for inclusion. The sample of selected studies could include studies in obese patients and in those with known cardiac disease.

Retained articles included only those studies whose primary objective was to identify biomarkers in subjects with OSA confirmed by overnight PSG. Only studies in English, Portuguese and Spanish languages were considered.

#### *Exclusion Criteria*

Reviews, letters, conference abstracts, and personal opinions were not considered.

Studies using daytime PSG, home-based PSG or multichannel polygraphic recordings were also excluded. Studies using biomarkers to detect the presence of OSA-associated morbidities (cognitive or behavioral deficits, excessive daytime sleepiness, cardiovascular or metabolic end-organ dysfunction) were excluded. In addition, studies in which the clinical cohort included craniofacial, genetic syndromes, neuromuscular diseases, or patients with a primary disease for which OSA prevalence is being investigated, such as patients with kidney disease or rheumatologic conditions were also discarded. In phase 2, we excluded studies that did not report sensitivity and specificity or in which the data presented did not enable these assessments to be extrapolated.

### Information Sources

Detailed individual search strategies for each of the following bibliographic databases were developed: Cochrane, EMBASE, MEDLINE, PubMed, and LILACS. A partial grey literature search was taken using Google Scholar. The end search date was January 3, 2014, and an updated search was completed on March 20, 2014, across all databases. The references cited in the selected articles were also checked for any incremental references that could have been inadvertently omitted during the electronic database searches.

### Search

Appropriate truncation and word combinations were selected and adapted for each database search (see **Appendix 1**). All references were managed by reference manager software (RefWorks-COS, ProQuest, Bethesda, MD), and duplicate hits were removed.

### Study Selection

The selection was completed in 2 phases.

In phase 1, two reviewers independently reviewed the titles and abstracts of all identified electronic database citations (GDL and CPP). The following criteria were applied to select studies in phase 1: studies with an objective of identifying biomarkers in subjects with OSA confirmed by full overnight PSG. A third author (SA) was involved when disagreements emerged among the 2 initial evaluators. Any studies that did not fulfill the inclusion criteria were discarded.

In phase 2, the following selection criteria were applied to the full articles to confirm their eligibility: only studies that reported sensitivity and specificity or in which the data presented enabled these diagnostic assessments to be extrapolated were selected. The same 2 reviewers (GDL and CPP) independently participated in phase 2. The reference list of all included articles was critically assessed by one examiner (GDL). The articles that were selected were then read by both examiners (GDL and CPP). Any disagreement in either phase was resolved by discussion and mutual agreement among the 3 reviewers (GDL, CPP, SA). A fourth author with extensive experience in sleep medicine and biomarker discovery (DG) was involved when controversy arose in the process of reaching a final decision. Final selection was always based on the full-text of the publication.

### Data Collection Process

One author (GDL) collected the required information from the selected articles. A second author (CPP) crosschecked all the collected information and confirmed its accuracy. Again, any disagreement in either phase was resolved by discussion and mutual agreement among the 3 reviewers (GDL, CPP, SA). The fourth author was involved as required, to enable formulation of the final decision (DG).

### Data Items

For all of the included studies the following information was recorded: author(s), year of publication, country, sample size, age, type of biomarkers, apnea hypopnea index used to define OSA from the PSG, name of biomarkers, and results (including sensitivity and specificity). If the required data were not complete, attempts were made to contact the authors to retrieve the missing information.

### Risk of Bias in Individual Studies

The methodology of selected studies was evaluated using the 14-item Quality Assessment Tool for Diagnostic Accuracy Studies (QUADAS).[21] Two reviewers (GDL and CPP) scored each item as "yes," "no," or "unclear" and assessed independently the quality of each included study. Disagreement between the 2 reviewers was resolved by a third reviewer (CFM).

### Summary Measures

Sensitivity and specificity of biomarkers as diagnostic tests against PSG were considered as the main outcomes.

### Synthesis of Results

The diagnostic capability of the identified biomarkers against PSG was combined through a meta-analysis following the appropriate Cochrane guidelines.[22] Review Manager 5.2 (RevMan 5.2, The Nordic Cochrane Centre, Copenhagen, Denmark) was used to constructed receiver operating characteristic (ROC) graphs and forest plots as part of the meta-analysis. Some of the required data were calculated by the authors.

### Risk of Bias across Studies

To decrease the heterogeneity, the studies were separated in 3 groups according to age (children or adults) and biomarker characteristics (single or combined biomarkers).

### Additional Analyses

Additional analysis was performed using positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood Ratio (LR-), diagnostic odds ratio (DOR), and Youden's Index. The cutoff values used to interpret these data are presented in **Appendix 2**.

## RESULTS

### Study Selection

A flowchart describing the process of identification, inclusion, and exclusion of studies is shown in **Figure 1**. A total of 141 articles were retrieved during phase 1 selection. Thereafter, 132 studies were excluded due to different reasons (see **Appendix 3**). Only 9 articles were finally included in the qualitative and quantitative synthesis. Eight of those[23–31] were initially identified from the main electronic search; only one[31] was directly received from expert sources.

### Study Characteristics

From the 9 selected studies, 4 were conducted in children[23,24,30,31] and 5 in adults.[25–29] The studies in children were conducted in 2 different countries: Hungary[23] and United States.[24,30,31] The following PSG-based criteria were used for OSA: AHI ≥ 1/h TST,[23] AHI ≥ 2/h TST,[31] AHI > 2/h TST,[24] AHI > 5/h TST.[30] Two of these studies tested urinary biomarkers against PSG,[24,31] one tested blood-based biomarkers,[30] and one evaluated EBC.[23] The sample size ranged from 28 to 120 subjects.[23,24] A summary of the study descriptive characteristics can be found in **Table 1**.

The studies conducted in adults were conducted in Brazil,[25] China,[28] Germany,[29] Thailand,[26] and Turkey.[27] The following definitions for OSA were used: AHI > 5/h TST,[29] AHI ≥ 5/h TST,[26–28] and AHI ≥ 15/h TST.[25] All of the studies[25,27–29] evaluated blood biomarkers. One of the studies appraised both blood and EBC.[26] The sample size ranged from 63[28] to 1,021[25] participants. A summary of the study descriptive characteristics can be found in **Table 2**.

### Risk of Bias within Studies

The studies were very homogeneous—all had high methodological quality, even though none of the studies fulfilled all methodological quality criteria. In 8 studies, the QUADAS[21] criteria were fulfilled in 78.6%. In another study,[25] QUADAS criteria were met in 85.7% (**Appendix 4**).

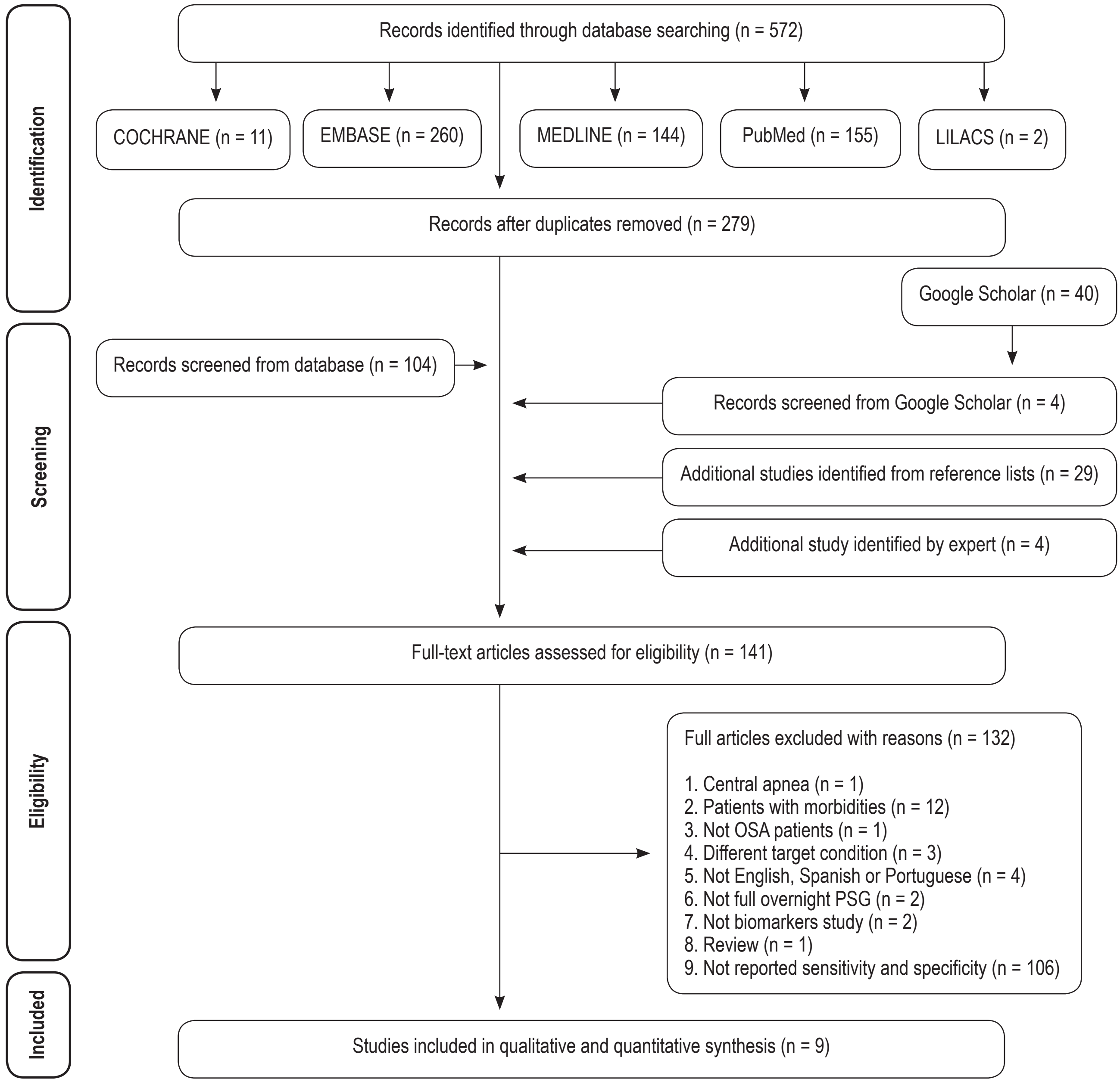
### Results of Individual Studies

Although the studies used different types of biomarkers and reported different sensitivity and specificity all 9 articles concluded that biomarkers had the capacity to correctly classify OSA and non-OSA subjects.

### Synthesis of Results

To improve our interpretation of results, the studies were clustered in 3 groups, according the sample and the index test: using only one biomarker in children or adults, and using combined biomarkers in children. Diagnostic tables were constructed using the data extracted from each article (**Tables 3**, **4**, **5**). In these tables, all accuracy measurements (sensitivity, specificity, PPV, NPV, LR+, LR-, DOR, and Youden's Index) are presented. Some studies[25–27] provided more than one accuracy measurement. Therefore those findings are reported twice in the same table. From the 4 studies

**Figure 1**—Flow diagram of literature search and selection criteria.




Adapted from PRISMA.

conducted in children, a total of 258 subjects were assessed. From the 5 studies conducted in adults, 1,458 subjects were evaluated. The total sample for this meta-analysis was 1,716 subjects.

The diagnostic accuracy (sensitivity, specificity, and 95% confidence interval) of the studies included in a meta-analysis is shown in **Figures 2** and **3**. The sensitivity and specificity for different selected studies varied substantially from 43% to 100%, and from 45% to 100%, respectively. Only 5 studies reported excellent sensitivity: Li et al. (100%),[26] Gozal et al. (95%),[24] Shah et al. (93%),[30] Guo et al. (91%),[28] and Kheirandish-Gozal et al. (82%).[31] From these 5 studies, only Gozal et al.[24] and Li et al.[26] also reported excellent specificity (both 97%).

### Risk of Bias across Studies

The main methodological limitations of the studies were related to poor reporting for items 1 (Was the spectrum of patients representative of the patients who will receive the test in clinical practice?), and for items 10 and 11 (blind interpretation of the reference and index test). The complete item list analyzed is presented in **Appendix 3**.

### Additional Analysis

Only one pediatric[24] and one adult study[26] reported LR values considered excellent DTA. Thioredoxin (TRX),[28] kallikrein-1,[24] uromodulin,[24] urocortin-3,[24] orosomucoid-1,[24]

**Table 1**—Summary of study descriptive characteristics of included studies (children).

| Year | Author | Country | Cases | Controls | Age range, years | Type of Biomarker | OSA* diagnostic criteria at PSG | Biomarker | Main Conclusion |
|---|---|---|---|---|---|---|---|---|---|
| 2006 | Shah et al.[30] | United States | OSA (n = 20) | Non-OSA with HS (n = 20) | 3–12 | Blood | AHI > 5 | Proteomic patterns | Proteomic profiling of serum samples in OSA children revealed differential expression of circulating proteins that may provide useful future diagnostic approaches. |
| 2009 | Gozal et al.[24] | United States | Children with HS and suspected OSA: (n = 60/32 male) | Children from community (n = 30/16 male) + Children with HS and suspected OSA: PS (n = 30/16 male) | 2–9 | Urine | OAI > and/ or OAHI > 2 | Kallikrein-1 Uromodulin Urocortin-3 Orosomucoid-1 | Kallikrein-1, uromodulin, urocortin-3, and orosomucoid-1 may be potential biomarkers to identify OSA and non-OSA children. |
| 2013 | Benedek et al.[23] | Hungary | OSA (n = 18) | Non-OSA with HS (n = 10) | 8.5 | EBC | AHI ≥ 1 | VOCs mixtures | VOCs mixtures can be distinguished in OSA children from controls. |
| 2013 | Kheirandish-Gozal et al.[31] | United States | OSA (n = 50) Matched with controls for age, sex, BMI and ethnicity | Non-OSA (n = 20) | 3–12 | Urine | AHI ≥ 2 | Urinary Neurotransmitters | Combinatorial approaches of selected neurotransmitters in urine may predict OSA in children. |

*All terms that mean obstructive sleep apnea (SDB, SRDB, OSAS) were standardized as OSA. AHI, apnea-hypopnea index; BMI, body mass index; EBC, exhaled breath condensate; GABA, γ–aminobutyric acid; HS, habitual snoring; OAHI, obstructive apnea-hypopnea index; OAI, obstructive apnea index; OSA, obstructive sleep apnea; PS, primary snoring; PSG, polysomnography; ROC, receiver operating characteristic; VOCs, complex volatile organic compounds.

proteomic patters,[30] and urinary neurotransmitters[31] had accuracy enough to be an acceptable diagnostic test.

Regarding PPV values, taurine,[31] the combined biomarkers tested by Gozal et al.[24] had the highest PPV values among pediatric studies (100% and 97%). In studies conducted in adults interleukin-6 (IL-6),[26] creatine phosphokinase (CK),[29] interleukin-10 (IL-10),[26] and TRX[28] had the highest PPV values.

The combined biomarkers tested by Gozal et al.[24], y-amino-butyric acid,[32] and phenylethylamine (PEA)[31] had the highest NPV (100%) in pediatric studies, while IL-6 and IL-10[26] had the highest NPV in adults ones (100%).

The combined biomarkers tested by Gozal et al.[24] and the 2 biomarkers tested by Li et al.[26] reported excellent Youden's Index (0.97, 1.00, 0.97).

Five studies[24,26,28,30,31] reported the highest diagnostic odds ratio (DOR). The results reported when the biomarkers were combined in Gozal et al.[24] and Kheirandish-Gozal et al.[31] showed better accuracy results than when they were tested individually (DTA measurements are presented in **Tables 3** and **4**).

In summary, only the biomarkers tested by Gozal et al.[24] satisfied the criteria required for an excellent diagnostic test in children. Kheirandish-Gozal et al.[31] and Shah et al.[30] satisfied the criteria for acceptable diagnostic test in children.

In adults, the study conducted by Li et al.[26] that tested IL-6 and IL-10 could be considered an excellent diagnostic test.

## DISCUSSION

### Summary of Evidence

This systematic review investigated the available evidence on the diagnostic capability of biomarkers for the diagnosis of OSA. The actual gold standard for OSA diagnosis, i.e., the overnight PSG, has several important limitations: (a) it is potentially stressful, (b) requires sleep outside the home environment, (c)

**Table 2**—Summary of study descriptive characteristics of included studies (adults).

| Year | Author | Country | Cases | Controls | Mean age, years | Type of Biomarker | OSA* diagnostic criteria at PSG | Biomarker | Main Conclusion |
|---|---|---|---|---|---|---|---|---|---|
| 2006 | Lentini et al.[29] | Germany | OSA suspected: Mild to Moderate OSA (n = 93/17 male) Severe OSA (n = 89/71 male) | OSA suspected: (n = 19/8 male) | 54.9 | Blood | AHI > 5 | CK levels | Mild-to-moderate elevation in CK level was predictive of OSA. The application of CPAP therapy in OSA patients resulted in a significant decrease in CK level. |
| 2007 | Ursavas et al.[27] | Turkey | Moderate-to-severe OSA (n = 39/30 male) Matched for age, gender, BMI, smoking history, and CVD with controls. | Non-OSA (n = 34/23 male) | 50.5 # | Blood | AHI ≥ 5 | ICAM-1 VCAM-1 | ICAM-1 and VCAM-1 may be potential biomarkers to identify OSA and non-OSA adults. |
| 2009 | Li et al.[26] | Thailand | Patients with EDS and loud snoring: Mild OSA (n = 22) Moderate OSA (n = 22) Severe OSA (n = 24) | Patients with EDS and loud snoring: Smoker control group (n = 10) Non-OSA (n = 22) | 44 # | Blood EBC | AHI ≥ 5 | 8-isoprostane IL-6 TNF-α IL-10 | EBC IL-6 and IL-10 have potential to predict severity of OSA in non-smoker OSA suspects. |
| 2013 | Guo et al.[28] | China | OSA suspected: Mild OSA (n = 14) Moderate OSA (n = 11) Severe OSA (n = 29) | OSA suspected (n = 9) | 47.8 # | Blood | AHI ≥ 5 | TRX | Plasma TRX level is associated with OSA severity and may be used as a severity indicator of OSA. |
| 2013 | Hirotsu et al.[25] | Brazil | Volunteers: OSA adults (n = 339/18.8% male) | Volunteers: (n = 682/25.9% male) | 44.6 # | Blood | AHI ≥ 15 | Uric acid | A strong association was found between uric acid levels and OSA, although acid uric levels do not qualify for a biomarker alone. |

*All terms that mean obstructive sleep apnea (SDB, SRDB, OSAS) were standardized as OSA. # Mean calculated by author. AHI, apnea-hypopnea index; BMI, body mass index; CK, creatine phosphokinase; CPAP, continuous positive airway pressure; CVD, cardiovascular disease; EBC, exhaled breath condensate; EDS, excessive daytime sleepiness; ICAM-1, intercellular adhesion molecule 1; IL-6, interleukin-6; IL-10, interleukin-10; OAHI, obstructive apnea-hypopnea index; OSA, obstructive sleep apnea; PS, primary snoring; PSG, polysomnography; ROC, receiver operating characteristic; TNF-α, tumor necrosis factor α; TRX, thioredoxin; VCAM-1, vascular cell adhesion molecule-1.

may not be widely available; and (d) is expensive.[33] Therefore, development of simple, cheap, and reliable diagnostic tools that would at least permit large scale screening of at-risk populations, and enable accurate identification of the subjects with definitive disease or with definitive absence of disease would potentially revolutionize the field.[13] This urgent need to find an ideal biomarker for OSA could explain the large number of studies about this topic published since 2000. We found a large number of studies in phase 1 screening process. Unfortunately, 106 studies were excluded because they did not report sensitivity and specificity. Without these values it is impossible to properly assess the real diagnostic capability of any alternative test. Brockman et al.[33] emphasizes that the lack of important information in DTA publications is sobering, as clear guidelines for reporting validity measures of alternative exploratory diagnostic methods were published in 2003, and encourage future authors of DTA studies to follow these recommendations.

Before we analyze our results, it is important emphasize that there was wide variation in the OSA diagnostic criteria employed by the pediatric studies. The AHI was the most frequently used diagnostic PSG measure of OSA severity. However, the use of AHI was associated with two major limitations. Firstly, the clinically accepted consensus for the cutoff AHI value for either the presence or absence of OSA remains unresolved. Secondly, no widely accepted agreement has been reached regarding whether children with PSG-based AHI values between the "normal cutoff" and 5/h TST should undergo surgical adenotonsillectomy.[19] Based on these considerations, it becomes apparent that the definitive diagnosis of OSA solely based on the low-end spectrum of the PSG-based measures (e.g., AHI, RDI, OAHI) is difficult if not

**Table 3**—Diagnostic test accuracy (children).

| Author | Age (mean or range in years) | Sample Size (N) | OSA/ NON-OSA | Biomarker | Sensitivity/ Specificity (%) | PPV/NPV (%)# | LR+/LR-# | DOR# | Younden's Index Value# |
|---|---|---|---|---|---|---|---|---|---|
| Benedek et al.[23] | 8.5 | 28 | 18/10 | VOCs mixtures | 78/70 | 82/64 | 2.59/0.31 | 8.35 | 0.48 |
| Gozal et al.[24] | 2–9 | 120 | 60/60 | Kallikrein-1 | 97/77 | 81/96 | 4.22/0.04 | 105.50 | 0.74 |
| Gozal et al.[24] | 2–9 | 120 | 60/60 | Uromodulin | 88/80 | 81/87 | 4.40/0.15 | 29.33 | 0.63 |
| Gozal et al.[24] | 2–9 | 120 | 60/60 | Urocortin-3 | 94/79 | 82/93 | 4.48/0.08 | 56.00 | 0.73 |
| Gozal et al.[24] | 2–9 | 120 | 60/60 | Orosomucoid-1 | 90/83 | 84/89 | 5.29/0.12 | 44.08 | 0.73 |
| Kheirandish-Gozal et al.[31] | 6.3 | 70 | 50/20 | GABA | 100/45 | 82/100 | 1.82/zero | ∞ | 0.45 |
| Kheirandish-Gozal et al.[31] | 6.3 | 70 | 50/20 | PEA | 100/65 | 88/100 | 2.86/zero | ∞ | 0.65 |
| Kheirandish-Gozal et al.[31] | 6.3 | 70 | 50/20 | Taurine | 88/100 | 100/77 | ∞/0.12 | ∞ | 0.88 |
| Shah et al.[30] | 3–12 | 40 | 20/20 | Proteomic Patterns | 93/90 | 90/93 | 9.30/0.08 | 116.25 | 0.83 |

# Data not available in the original article. The authors calculated data from information available in the article. OSA, obstructive sleep apnea; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR-, negative likelihood ratio; DOR, diagnostic odds ratio; VOCs, complex volatile organic compounds; GABA, γ-aminobutyric acid; PEA, β-phenylethylamine.

**Table 4**—Measurements for combined biomarkers (children).

| Author | Age (range in years) | Sample Size (N) | OSA/ NON-OSA | Biomarker | Sensitivity/ Specificity (%) | PPV/NPV (%)# | LR+/LR-# | DOR# | Younden's Index Value# |
|---|---|---|---|---|---|---|---|---|---|
| Gozal et al.[24] | 2–9 | 120 | 60/60 | Kallikrein-1 Uromodulin Urocortin-3 Orosomucoid-1 | 100/97 | 97/100 | 28.60/zero | ∞ | 0.97 |
| Kheirandish-Gozal et al.[31] | 6.3 | 70 | 50/20 | Urinary Neurotransmitters | 82/90 | 95/67 | 8.20/0.20 | 41.00 | 0.72 |

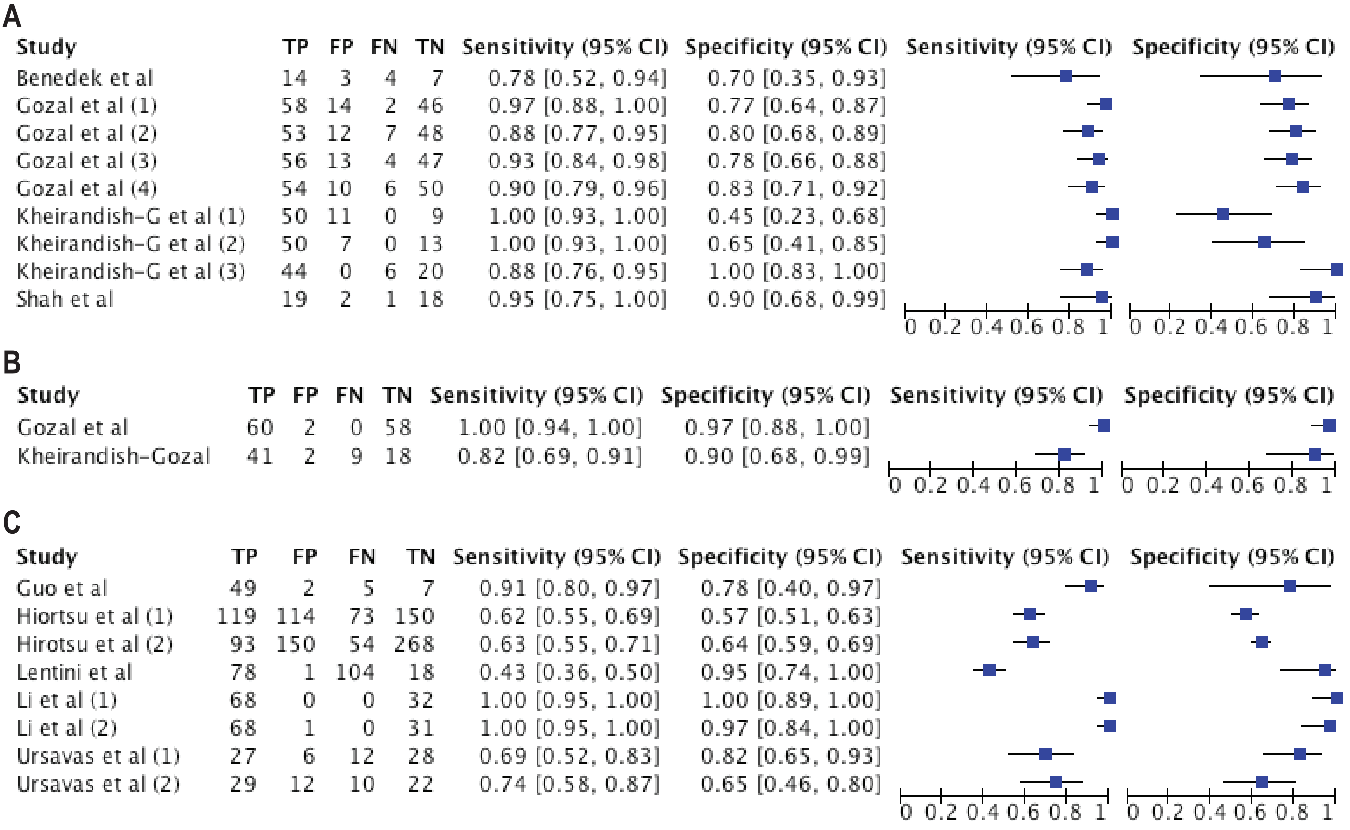
# Data not available in the original article. The authors calculated data from information available in the article. OSA, obstructive sleep apnea; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR-, negative likelihood ratio; DOR, diagnostic odds ratio.

**Table 5**—Diagnostic test accuracy (adults).

| Author | Mean age, years | Sample Size (N) | OSA/ NON-OSA | Biomarker | Sensitivity/ Specificity (%) | PPV/NPV (%)# | LR+/LR-# | DOR# | Younden's Index Value# |
|---|---|---|---|---|---|---|---|---|---|
| Guo et al.[28] | 47.8# | 63 | 54/9 | TRX | 91/78 | 96/58 | 4.08 | 34.00 | 0.69 |
| Hirotsu et al.[25] | 44.6# | 456 (male) | 192/264 | Uric acid | 62/57 | 51/67 | 1.44 | 0.02 | 0.26 |
| Hirotsu et al.[25] | 44.6# | 565 (female) | 147/418 | Uric acid | 63/64 | 38/64 | 1.75 | 3.01 | 0.27 |
| Lentini et al.[29] | 54.9 | 201 | 182/19 | CK | 43/95 | 99/14 | 8.14 | 13.57 | 0.38 |
| Li et al.[26] | 44.0# | 100 | 68/32 | IL-6 | 100/100 | 100/100 | ∞ | ∞ | 1.00 |
| Li et al.[26] | 44.0# | 100 | 68/32 | IL-10 | 100/97 | 99/100 | 32.00 | ∞ | 0.97 |
| Ursavas et al.[27] | 50.5# | 73 | 39/34 | ICAM-1 | 69/82 | 81/70 | 3.92 | 10.59 | 0.51 |
| Ursavas et al.[27] | 50.5# | 73 | 39/34 | VCAM-1 | 74/65 | 71/69 | 2.11 | 5.41 | 0.39 |

# Data not available in the original article. The authors calculated data from information available in the article. OSA, obstructive sleep apnea; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR-, negative likelihood ratio; DOR, diagnostic odds ratio; CK, creatine phosphokinase; TRX, thioredoxin; IL-6, interleukin-6; IL-10, interleukin-10; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule-1.

**Figure 2**—Forest plot with diagnostic test accuracy (sensitivity, specificity, and 95% confidence interval) of each study.



**A**

| Study | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|---|---|---|---|
| Benedek et al | 14 | 3 | 4 | 7 | 0.78 [0.52, 0.94] | 0.70 [0.35, 0.93] |
| Gozal et al (1) | 58 | 14 | 2 | 46 | 0.97 [0.88, 1.00] | 0.77 [0.64, 0.87] |
| Gozal et al (2) | 53 | 12 | 7 | 48 | 0.88 [0.77, 0.95] | 0.80 [0.68, 0.89] |
| Gozal et al (3) | 56 | 13 | 4 | 47 | 0.93 [0.84, 0.98] | 0.78 [0.66, 0.88] |
| Gozal et al (4) | 54 | 10 | 6 | 50 | 0.90 [0.79, 0.96] | 0.83 [0.71, 0.92] |
| Kheirandish-G et al (1) | 50 | 11 | 0 | 9 | 1.00 [0.93, 1.00] | 0.45 [0.23, 0.68] |
| Kheirandish-G et al (2) | 50 | 7 | 0 | 13 | 1.00 [0.93, 1.00] | 0.65 [0.41, 0.85] |
| Kheirandish-G et al (3) | 44 | 0 | 6 | 20 | 0.88 [0.76, 0.95] | 1.00 [0.83, 1.00] |
| Shah et al | 19 | 2 | 1 | 18 | 0.95 [0.75, 1.00] | 0.90 [0.68, 0.99] |

**B**

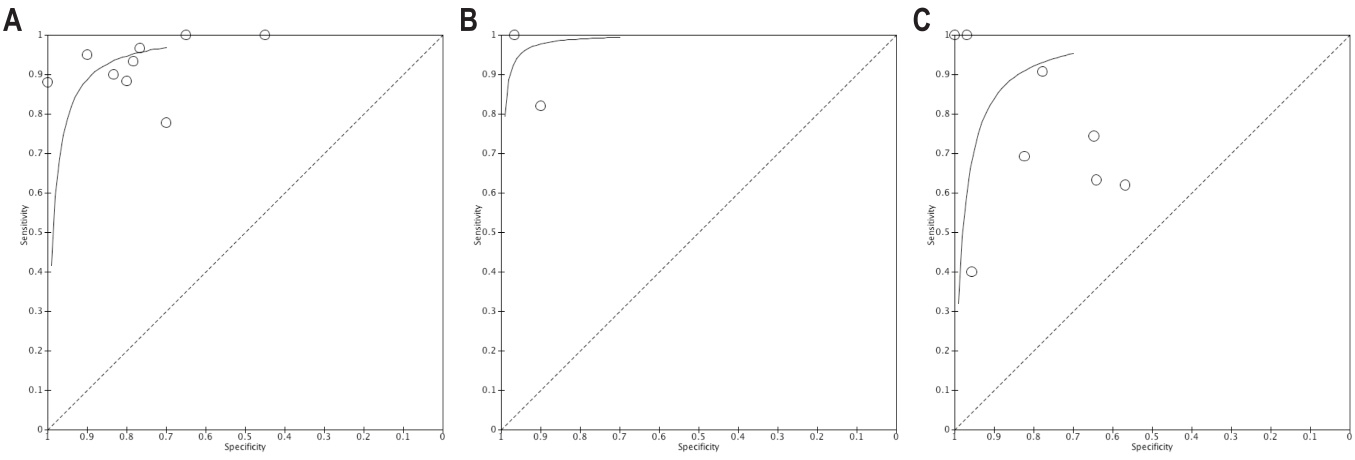
| Study | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|---|---|---|---|
| Gozal et al | 60 | 2 | 0 | 58 | 1.00 [0.94, 1.00] | 0.97 [0.88, 1.00] |
| Kheirandish-Gozal | 41 | 2 | 9 | 18 | 0.82 [0.69, 0.91] | 0.90 [0.68, 0.99] |

**C**

| Study | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|---|---|---|---|
| Guo et al | 49 | 2 | 5 | 7 | 0.91 [0.80, 0.97] | 0.78 [0.40, 0.97] |
| Hiortsu et al (1) | 119 | 114 | 73 | 150 | 0.62 [0.55, 0.69] | 0.57 [0.51, 0.63] |
| Hirotsu et al (2) | 93 | 150 | 54 | 268 | 0.63 [0.55, 0.71] | 0.64 [0.59, 0.69] |
| Lentini et al | 78 | 1 | 104 | 18 | 0.43 [0.36, 0.50] | 0.95 [0.74, 1.00] |
| Li et al (1) | 68 | 0 | 0 | 32 | 1.00 [0.95, 1.00] | 1.00 [0.89, 1.00] |
| Li et al (2) | 68 | 1 | 0 | 31 | 1.00 [0.95, 1.00] | 0.97 [0.84, 1.00] |
| Ursavas et al (1) | 27 | 6 | 12 | 28 | 0.69 [0.52, 0.83] | 0.82 [0.65, 0.93] |
| Ursavas et al (2) | 29 | 12 | 10 | 22 | 0.74 [0.58, 0.87] | 0.65 [0.46, 0.80] |

**(A)** Studies in children that analyzed each biomarker individually. **(B)** Studies in children that combined three or four biomarkers in one analysis. **(C)** Studies in adults. TP, true positive; FP, false positive; FN, false negative; TN, true negative.

**Figure 3**—Receiver operating characteristic (ROC) curves for each group.




**(A)** Studies in children that analyzed each biomarker individually. **(B)** Studies in children that combined three or four biomarkers in one analysis. **(C)** Studies in adults.

impossible. Similar, albeit less vague overlap exists among adult patients, even if the PSG criteria for the presence of OSA have been more firmly established and accepted around the world.[34]

Although we found only nine eligible studies, this meta-analysis is informative, because by combining the available data it increases the sample size to 258 children and 1,458 adults. The results of meta-analysis showed that five studies[24,26,28–31] provide acceptable metrics enabling identification of those who really suffered from OSA (true positive), while two of them[24,26] performed well for identification of those who did not have OSA (true-negative). The LR values confirm that both had excellent DTA. Similarly, PPV and NPV values showed that five studies[24,26,28,29,31] performed acceptably in identifying OSA subjects. Two studies[24,26] were also good in identifying OSA and non-OSA subjects, thereby concurring with sensitivity and specificity numbers.

Also, the combined biomarker approaches tested by Gozal et al.[24] and the two biomarkers tested by Li et al.[26] reported excellent Youden's Index (0.97, 1.00, 0.97), the latter indicative of high accuracy.[22]

The DOR for three pediatric[24,30,31] and two adult studies[26,28] indicate that the biomarkers tested in children (combined kallikrein-1, uromodulin, urocotin-3, orosomucoid-1, proteomic patterns, and urinary neurotransmitters) and in adults (IL-6, IL-10, TRX) had better discriminatory test performance. Is it important to emphasize that the results reported when the biomarkers were combined in Gozal et al.[24] and Kheirandish-Gozal et al.[31] showed better accuracy measurements than when the biomarkers tested in these studies were analyzed individually (**Table 3**).

In summary, only the putative biomarkers tested in Gozal et al.[24] satisfied the required criteria for an excellent diagnostic test in children. Gozal et al.[24] investigated urinary biomarkers in 60 OSA patients, 30 primary snorers, and 30 healthy controls in order to identify urinary protein clusters that were highly sensitive and specific for OSA. They found that unique sets of proteins were either increased or decreased in the urine of OSA children, and that their combined ROC curve analysis using four candidate proteins simultaneously provided a near-perfect DTA (close to 100%). Another useful set of different biomarkers was subsequently identified by Kheirandish-Gozal et al.,[31] who examined urinary neurotransmitters in 50 OSA and 20 controls. They reported an overnight increase in epinephrine and norepinephrine levels in children with OSA, while taurine levels were decreased. Using combinatorial approaches and cutoff values for overnight changes of these four neurotransmitters enabled a good prediction of OSA. Also, Shah et al.[30] evaluated the proteomic patterns of 20 children with OSA and of 20 children with habitual primary snoring but no evidence of OSA using surface-enhanced laser desorption/ionization time of flight mass spectrometry. The proteomic patterns were capable of diagnosing OSA with 93% sensitivity and 90% specificity. However, their methodological approaches did not allow for identification of the actual candidates, such that this work remains a proof of principle rather than provide yet other defined biomarker candidates.

In adults, the study conducted by Li et al.,[26] which tested IL-6 and IL-10, could be considered an excellent diagnostic test. The study aimed to identify the best biomarker, either single or in combination, with best cost-effectiveness ratio. The authors analyzed 8-isoprostane, IL-6, TNF-α, and IL-10 in the EBC and serum of OSA, non-OSA, and healthy smoking subjects. These investigators reported that levels, in both EBC and serum, differed significantly across the four biomarkers tested.

### Overall Assessment

A previous review focused on different pediatric OSA diagnostic tests[33] have identified several approaches that putatively provide either acceptable or excellent DTA in the prediction of OSA. These tests have included sleep lab-based polygraphy, anterior rhinomanometry, and urinary biomarkers. However, the authors[33] stated that there was still insufficient evidence to recommend any of these alternative tests to PSG for diagnosis of pediatric OSA.

The current systematic meta-analysis indicates that although all selected articles concluded that biomarkers could be useful to reliably diagnose OSA, not all approaches can actually be used as viable or definitive biomarkers. Only the combination of kallikrein-1, uromodulin, urocortin-3, and orosomucoid-1 displayed sufficient accuracy to be considered an OSA diagnostic test in children. In contrast, the combination of urinary neurotransmitters[31] and of the serum proteomic patterns[30] displayed acceptable accuracy to serve as a screening test in children. In adults, IL-6 and IL-10 show favorable potential to become a good biomarker to identify OSA and non-OSA subjects.

### Limitations

Except for one study,[25] the other studies used a sample from sleep center or subjects with OSA symptoms. This can affect the prevalence, which can bias the sensitivity and specificity of the biomarker-based test. Thus, we do not know if the tests would respond similarly when applied to the general population. Other identified limitations in the published studies were: lack of a masked interpretation of the reference and index test and no clear information regarding how many investigators analyzed the test data or if their techniques were calibrated. Finally, 106 potential biomarkers studies had to be excluded due to lack of DTA values, suggesting that if such DTA assessments were available, the present conclusions could be markedly affected, further reinforcing the need for standardized reporting of predictive DTA values. Notwithstanding such considerations, the current findings are encouraging toward the implementation of biomarkers in the diagnosis of OSA, and prompted us to perform a relatively simplistic financial cost analysis of potential savings embedded in such a clinically based approach. For example, assuming that a combinatorial multiple biomarker-based assay would be required, and estimating that the global cost of such assay would amount to one-fourth of the cost of a PSG, then application of the biomarker-based approach would be economically advantageous if < 25% of the biomarker test results would be equivocal, thereby necessitating a PSG. Similar models can be implemented using various cost estimates, with obviously, more favorable "equivocal result" rates still being financially viable if the assay costs are lower.

## CONCLUSIONS

Kallikrein-1, uromodulin, urocortin-3, and orosomucoid-1 have enough accuracy to be used as an OSA diagnostic test in children when used in combination.

Plasma IL-6 and IL-10 levels are potentially promising to become a good biomarker aiming to identify adult individuals with and without OSA.

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## SUBMISSION & CORRESPONDENCE INFORMATION

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## DISCLOSURE STATEMENT

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## Appendix 1—Search.

| Database | Search |
|---|---|
| Cochrane (March 20, 2014)<br>EMBASE (1974-March 20, 2014)<br>MEDLINE (1948-March 20, 2014)<br>PubMed (March 20, 2014) | (sleep apnea or sleep apnoea or sleep apnea, 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*) |
| LILACS (March 20, 2014) | "apnea" or "apneia" and "marcadores biologicos" |
| Google Scholar (March 20, 2014) | "biomarkers and sleep apnea" (without patents or citations) |

## Appendix 2

| Test indicators | Data analysis | References |
|---|---|---|
| DOR | The value of a DOR ranges from 0 to infinity, with higher values indicating better discriminatory test performance. A value of 1 means that a test does not discriminate between patients with the disorder and those without it. Values lower than 1 point to improper test interpretation (more negative tests among the diseased). | Glas et al.[1] |
| LR | LR+ >3 and an LR- <0.3 – acceptable diagnostic test accuracy (DTA)<br>LR+ >10 and LR- <0.1 – excellent DTA. | Brockmann et al.[2] |
| Sensitivity | >80% excellent, 70-80% good, 60-69% fair, <60% poor | No consensus in this regard exists in the literature. |
| Specificity | >90% excellent, 80-90% good, 70-79% fair, <70% poor | No consensus in this regard exists in the literature. |
| Youden's Index | Youden's Index values close to 1 indicate high accuracy; a value of zero is equivalent to uninformed guessing and indicates that a test has no diagnostic value. | Deeks et al.[3] |


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**Appendix 3—**Excluded articles and reasons for exclusion.

| Author/Year | Reason for exclusion* |
|---|---|
| Aihara et al. 2013[1] | 9 |
| Akinnusi et al. 2011[2] | 9 |
| Alzoghaibi et al. 2005[3] | 9 |
| Antonopoulou et al. 2008[4] | 9 |
| Arias et al. 2008[5] | 9 |
| Bhushan et al. 2011[6] | 9 |
| Braga et al. 2006[7] | 9 |
| Bratel et al. 1999[8] | 6 |
| Burioka et al. 2008[9] | 9 |
| Calvin et al. 2010[10] | 1 |
| Carpagnano et al. 2002[11] | 9 |
| Carpagnano et al. 2003[12] | 9 |
| Chin et al. 2000[13] | 9 |
| Cholidou et al. 2013[14] | 2 |
| Christou et al. 2002[15] | 9 |
| Chung et al. 2013[16] | 9 |
| Cintra et al. 2011[17] | 9 |
| Cofta et al. 2013[18] | 9 |
| Constantinidis et al. 2008[19] | 9 |
| Culla et al. 2010[20] | 2 |
| DeBoer 2012 [21] | 9 |
| Duru et al. 2012[22] | 9 |
| El-Solh 2002 [23] | 2 |
| Feng et al. 2012[24] | 9 |
| Ferrarini et al. 2013[25] | 9 |
| Goldbart et al. 2006[26] | 9 |
| Gozal et al. 2002[27] | 9 |
| Gozal et al. 2007[28] | 2 |
| Gozal et al. 2008[29] | 9 |
| Gozal et al. 2010[30] | 2 |
| Gozal et al. 2013[31] | 9 |
| Guilleminault et al. 2004[32] | 9 |
| Guven et al. 2012[33] | 9 |
| Hira et al. 2012[34] | 9 |
| Htoo et al. 2006[35] | 9 |
| Imagawa et al. 2004[36] | 9 |
| Jurado-Gamez et al. 2011[37] | 9 |
| Jurado-Gamez et al. 2012[38] | 9 |
| Kaditis et al. 2005[39] | 9 |
| Kaditis et al. 2007[40] | 9 |
| Kaditis et al. 2009[41] | 9 |
| Kaditis et al. 2009[42] | 9 |
| Kanbay et al. 2008[43] | 9 |
| Khalyfa et al. 2011[44] | 3 |

| Author/Year | Reason for exclusion* |
|---|---|
| Khalyfa et al. 2012[45] | 9 |
| Kheirandish-Gozal 2014[46] | 9 |
| Kheirandish-Gozal et al. 2006[47] | 9 |
| Kim et al. 2009[48] | 9 |
| Kim et al. 2010[49] | 9 |
| Kim et al. 2012[50] | 2 |
| Kim et al. 2013[51] | 9 |
| Kishida et al. 2014 [52] | 8 |
| Kohler et al. 2011[53] | 9 |
| Krishna et al. 2006[54] | 9 |
| Kuramoto et al. 2009[55] | 9 |
| Kurt et al. 2013[56] | 9 |
| Ladesich et al. 2011[57] | 9 |
| Lam et al. 2009[58] | 9 |
| Larkin et al. 2005[59] | 9 |
| Lavie et al. 2002[60] | 9 |
| Lederer et al. 2009[61] | 9 |
| Lee et al. 2010[62] | 9 |
| Lee et al. 2012[63] | 9 |
| Li et al. 2006[64] | 9 |
| Li et al. 2008[65] | 9 |
| Li et al. 2008[66] | 9 |
| Li et al. 2010[67] | 9 |
| Lin et al. 2013 [68] | 5 |
| Loubaki et al. 2008[69] | 7 |
| Lui et al. 2009[70] | 9 |
| Makino et al.[71] | 7 |
| Malakasioti et al. 2012[72] | 9 |
| Mancuso et al. 2012[73] | 9 |
| Mehra et al. 2006[74] | 9 |
| Montgomery-Downs et al. 2006[75] | 9 |
| Murase et al. 2013[76] | 9 |
| Norman et al. 2008[77] | 9 |
| Ntalapascha et al. 2013[78] | 9 |
| O'Brien et al. 2006[79] | 9 |
| Ohga et al. 2003 [80] | 9 |
| Osorio 2014[81] | 2 |
| Oyama et al. 2012[82] | 2 |
| Ozben et al. 2013[83] | 9 |
| Pallayova et al. 2010[84] | 9 |
| Pallayova et al. 2011[85] | 9 |
| Papaioannou et al. 2012[86] | 9 |
| Park et al. 2013[87] | 9 |
| Patacchioli et al. 2014[88] | 9 |

| Author/Year | Reason for exclusion* |
|---|---|
| Patel et al. 2009[89] | 4 |
| Peled et al. 2007[90] | 9 |
| Petrosyan et al. 2008[91] | 9 |
| Phillips et al. 2007[92] | 9 |
| Pinto et al. 2013[93] | 9 |
| Przybylowski et al. 2006[94] | 5 |
| Punjabi et al. 2007[95] | 9 |
| Roche et al. 2009[96] | 2 |
| Rubinsztajn et al. 2006[97] | 5 |
| Ryan et al. 2007[98] | 9 |
| Salord 2014[99] | 2 |
| Schulz et al. 2002[100] | 9 |
| Shamsuzzaman et al. 2002[101] | 9 |
| Shi et al. 2013[102] | 9 |
| Simiakakis et al. 2012[103] | 9 |
| Sokucu et al. 2012[104] | 9 |
| Stats et al.[105] | 4 |
| Stefanini et al. 2012[106] | 9 |
| Steiropulos et al. 2010[107] | 9 |
| Sukegawa et al. 2005[108] | 9 |
| Svensson et al. 2012[109] | 9 |
| Takahashi et al. 2008[110] | 9 |
| Tauman et al. 2004[111] | 9 |
| Tauman et al. 2007[112] | 9 |
| Ting et al. 2009[113] | 9 |
| Tual-Chalot et al. 2014[114] | 9 |
| Ucar et al. 2009[115] | 9 |
| Uysal et al. 2014[116] | 2 |
| Van Hoorenback [117] | 6 |
| Vavougios et al. 2014[118] | 9 |
| Villa et al. 2014[119] | 9 |
| Wang et al. 2005 [120] | 5 |
| Wang et al. 2010[121] | 2 |
| Wang et al. 2012[122] | 4 |
| Wang et al. 2013 [123] | 9 |
| Wang et al. 2013[124] | 9 |
| Wang et al. 2014[125] | 9 |
| Yamauchi et al. 2005[126] | 9 |
| Ye et al. 2007[127] | 9 |
| Ye et al. 2010[128] | 9 |
| Yokoe et al. 2003[129] | 9 |
| Zamarron et al. 2008[130] | 9 |
| Zamarron et al. 2011[131] | 9 |
| Zhang et al. 2013[132] | 9 |

**Appendix 3** (*continued*)**—**Excluded articles and reasons for exclusion.

*** Reasons for exclusion: (1)** Central apnea (n = 1), **(2)** OSA with morbidities (n = 12), **(3)** Not OSA patients (n = 1), **(4)** Different target condition (n = 3), **(5)** Not English, Spanish or Portuguese (n = 4), **(6)** Not full overnight PSG (n = 2), **(7)** Not biomarkers study (n = 2), **(8)** Review (n = 1), **(9)** Not reported sensitivity and specificity.

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**Appendix 3** (*continued*)—Excluded articles and reasons for exclusion.


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**Appendix 3** (*continued*)—Excluded articles and reasons for exclusion.

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**Appendix 4**—QUADAS criteria fulfilled.

| Item | Benedek et al. [23]* | Gozal et al. [24] | Guo et al. [28] | Hirotsu et al. [25] | Kheirandish-Gozal et al. [31] | Lentini et al. [29] | Li et a.l [26] | Shah et al. [30] | Ursavas et al. [27] |
|---|---|---|---|---|---|---|---|---|---|
| 1. Was the spectrum of patients representative of the patients who will receive the test in the practice? | N | N | N | N | N | N | N | N | N |
| 2. Were selection criteria clearly described? | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| 3. Is the reference standard likely to correctly classify the target condition? | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| 4. Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests? | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| 5. Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis? | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| 6. Did patients receive the same reference standard regardless of the index test results? | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| 7. Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)? | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| 8. Was the execution of the index test described in sufficient detail to permit replication of the test? | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| 9. Was the execution of the reference standard describes in sufficient detail to permit its replication? | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| 10. Were the index test results interpreted without knowledge of the results of the reference standard? | U | U | U | U | U | U | U | U | U |
| 11. Were the reference standard results interpreted without knowledge of the results of the index test? | U | U | U | U | U | U | U | U | U |
| 12. Were the same clinical data available when test results were interpreted as would be available when the test used in practice? | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| 13. Were uninterpretable/ intermediate test results reported? | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| 14. Were withdrawals from the study explained? | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| QUADAS CRITERIA FULFILLED (%) | 78.6 | 78.6 | 78.6 | 85.7 | 78.6 | 78.6 | 78.6 | 78.6 | 78.6 |

Y, yes; N, no; U, unclear. *Numbers in brackets refer to reference number in original manuscript.