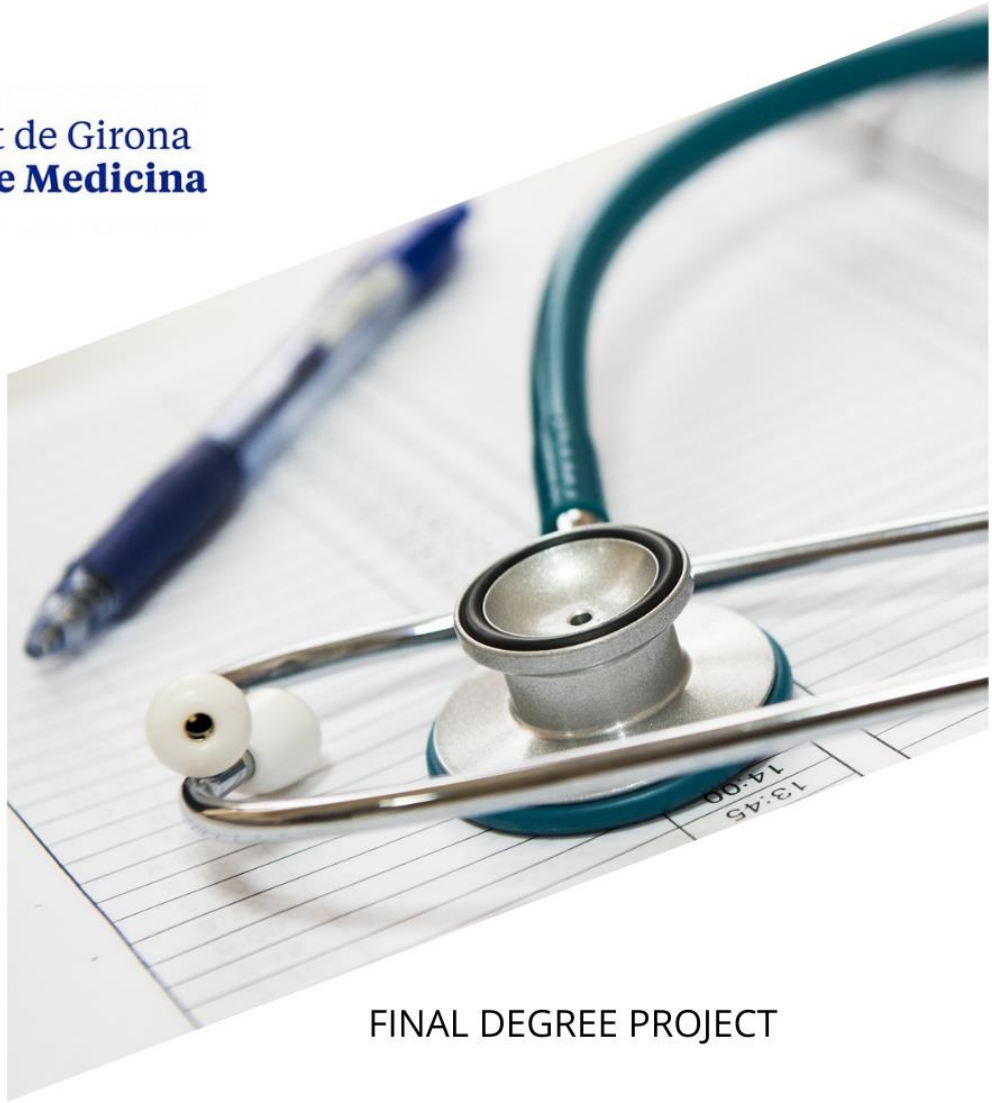
Universitat de Girona
Facultat de Medicina



FINAL DEGREE PROJECT

The future in OSAS diagnosis: analysis of exhaled breath by an eNose

PROSPECTIVE DESCRIPTIVE AND OBSERVATIONAL STUDY

Author: Francisco Javier Soto Tugores
Clinical tutor: Jose A. Peña Zarza
Methodological tutor: Teresa Puig Miquel

Hospital Universitari Son Espases
Facultat de Medicina, Universitat de Girona
January 2021

First of all, I would like to show my gratitude to my tutor, Jose Peña, for the opportunity to do this project in my hometown. He is a doctor I truly admire from long ago.

I would like to thank the department of paediatrics of Hospital Universitari Son Espases. Concretely, to the emergencies department, and specifically, to Catián and Inés, I enjoyed and learned every hour with you.

I appreciate the guiding Teresa Puig has given to me in the methodologic development of this project. Also, special thanks to Marc Saez for helping with the statistical analysis.

Finally, thank you to all my family, friends and Clara who have supported and loved me not only through this project, but through the whole degree.

"... I would keep myself
I would find a way"

Trent Reznor

INDEX

CONTENT OF TABLES, FIGURES AND IMAGES

1 ABBREVIATIONS:

OSAS	Obstructive Sleep apnoea Syndrome
JIA	Juvenile Idiopathic Arthritis
VOC	Volatile Organic Compound
ROS	Reactive Oxygen Species
GERD	Gastroesophageal Reflux Disease
ADHD	Attention-Deficit Hyperactivity Disorder
PSG	Polysomnography
$SatO_2$	Oxygen saturation
REM	Rapid Eye Movement
AASM	American Academy of Sleep Medicine
AEPED	Asociación Española de Pediatría
OAI	Obstructive Apnoea Index
CAI	Central Apnoea Index
AHI	Apnoea Hypopnea Index
ODI	Hourly desaturation Index
HBP	High Blood Pressure
HF	Heart Failure
BP	Blood Pressure
BNP	Brain Natriuretic Peptide
RCP	Reactive C-Protein
IL	Interleukin
CPAP	Continuous Positive Airway Pressure
BiPAP	Bi Positive Airway Pressure
PPI	Proton Pump Inhibitors
COPD	Chronic Obstructive Pulmonary Disease
eNose	Electronic Nose
HUSE	Hospital Universitari Son Espases
HEPA	High Efficiency Particle Arresting

2 ABSTRACT:

Background: respiratory sleep disorders are common in paediatric age, encompassing a spectrum of pathologies ranging from simple snoring to obstructive sleep apnoea-hypopnea syndrome (OSAS). This illness is associated with systemic inflammation and other important comorbidities. This inflammation could be monitored by the analysis of volatile organic compounds in exhaled breath.

Objective: This study aimed to compare the exhaled biomarker profile, with an eNose, in children with OSAS and habitual snorers

Design: This study will be prospective, descriptive and observational, carried out in a single hospital (Son Espases University Hospital) for a year and a half.

Methods: Up to 180 children between 3 and 14 ages, who must undergo a polysomnography (PSG), for OSAS or simple snorer diagnosis will be asked to fill some questions and to give an exhaled breath sample. Exhaled breath will be collected from the airway, processed using an electronic nose (eNose) and analysed off-line using means and standard deviations. Then we will compare OSAS and simple snorer samples using paired Student's test and Wilcoxon contrast. Finally, linear regression model will assess the difference between exhaled patterns.

Keywords: OSAS in children; exhaled breath analysis; eNose; Volatile organic compounds; systemic inflammation; polysomnography.

3 INTRODUCTION:

3.1 Infant Obstructive Sleep Apnoea-Hypopnea Syndrome (OSAS):

OSAS is defined as a partial (hypopnea) or complete (apnoea) intermittent blockage (obstructive apnoea) of the upper airways during sleep that causes disturbances in ventilation and a destructiveness of normal airway patterns (1–4).

3.1.1 Prevalence:

The prevalence in paediatric age varies according to the series due to an underdiagnosis because of the unknowingness of pathology by health professionals and heterogeneity in diagnostic explorations between different studies. However, the prevalence could be above 2% (4,5), according to a systematic review carried out by Marcus CL et *al*. which includes articles from several countries, including Spain. In the Mediterranean population it is estimated that the prevalence is similar, 3%, although it is suspected that the value may be higher (6).

The main symptom of paediatric OSAS is snoring (2) that is caused by the vibration which inhaled air generates when going through a narrow pathway. Snoring is, in our case, of particular interest in the study because it allows us to recognize patients at risk of OSAS. Internationally, the prevalence is between 3 and 12% (7); while nationally it is accepted that between the first months of life and adolescence it exists between 7 and 16.7% of snoring population (8,9). Studies show that prevalence is unrelated to sex or age (10). However, an increase in prevalence has been found in patients with obesity, African-American and sinus problems (10).

3.1.2 Physiopathology:

Physio pathologically, OSAS is characterized by the collapse or pharynx due to different mechanisms. Physiologically, the pharynx collapses when swallowing or talking. In inspiration the pharynx suffers negative pressures that could collapse it, but thanks to the dilating muscles of the pharynx (palate tensioner, genihioid, esternohioid and, above all, the genioglose), it remains open allowing air to pass through, allowing proper ventilation (11).

However, pharynx can collapse pathologically for the following reasons reflected in Table 1:

Table 1. Factors predisposing to pathological collapse of the pharynx (2,3)	
Congenital malformations	Down S., Prader Willi, Alport, Crouzon, Ehlers-Danlos.
Otorhinolaryngological factors	Tonsil and adenoid hypertrophy, allergic rhinitis, laryngomalacia.
Anatomical factors	Retrognathia, elevated hard palate, ogival palate, macroglossia, mandibular hypoplasia.
Other	Obesity, gastroesophageal reflux, neuromuscular diseases (Duchenne, Becker...), JIA, epilepsy, prematurity.

JIA: juvenile idiopathic arthritis

In normal ventilation, exhaled air has a set of volatile organic compounds (VOCs). They are useful for creating a pattern if analysed with the right technology. In OSAS that pattern may change due to the physiopathology of the disease itself (12). The collapse of the pharynx in the context of OSAS causes abnormal ventilation resulting in intermittent hypoxia and hypoxemia (13). Hypoxia is a lack of oxygen in the body that causes cellular stress that can affect organs. Cells try to adapt by changing protein expression and enzymatic activity. The organelle that suffers the most from the lack of oxygen is the mitochondria, which changes its metabolism to allow the correct transport of electrons. This change results in the creation of reactive oxygen species (ROS) at the end of the metabolic pathway, if these occur in too much concentration they create cytokines related to inflammation and cell death (14). Inflammation causes changes in normal metabolic pathways. These pathways send out a specific pattern of volatile organic compounds (VOCs), and these can create a different pattern if affected by the disease's physiopathology (12). This topic will be assessed further on (see Volatile organic compounds).

3.1.3 Phenotypes:

Paediatric patients suffering from OSAS can be divided into three different phenotypes, even if they share symptoms (15). These are:

1. *Classic:* adenoid facies, characterized by an elongated face, dark circles, loss of lip closure, hypotonic lip, narrow and arched palate, its main involvement is adenotonsillar hypertrophy. Thin constitution, stunting. It mostly affects preschool patients, and is the phenotype that presents most of the paediatric population (2,15,16).
2. *Adult:* obesity, short and wide neck, reduced face in its lower third. It is the phenotype that characterizes obese adults and adolescents (2,15,17).
3. *Congenital:* micrognathia, mandibular hypoplasia, macroglossia and other craniofacial malformations associated with genetic syndromes (2,15,16).

In this way we can observe that, although the presentation of patients is different, the physiopathology of obstruction is found in all phenotypes, allowing the development of the disease, and showing similar symptoms.

3.1.4 Symptoms:

Unfortunately, although the main symptoms of OSAS (Table 2) are agreed and described, the diagnosis certainty they provide is very low to be able to consider that good anamnesis and physical examination could serve to establish the diagnosis. (2,17–20). Clinical assessment has been shown to be insufficient for proper diagnosis and screening of the snoring patient, as snoring is poorly investigated by professionals and poorly explained by parents/guardians (7,16). Some symptoms have good specificity, but as has been reflected before their analysis does not serve for diagnosis, although it is useful for initiating clinical suspicion and could serve as indicators for further study with other tests. (7,21)

Table 2. Symptoms of OSAS (2,7,16–22)	
During sleep	Snoring, apnoea, oral breathing, increased breathing effort, abnormal postures, profuse sweating, anxious awakening, enuresis, micro awakenings, nightmares, bruxism, night terrors.
Digestive	Nausea and vomiting, GERD.
During the day	Oral breathing, daytime hypersomnolence, morning headache.
Behavioural disturbances	Aggressiveness, hyperactivity, antisocial behaviours, ADHD, poor school performance.
Other	Feeling of dyspnoea, increased seizures crisis, frequent upper respiratory infections, speech disturbances.

GERD: gastroesophageal reflux disease; ADHD: attention-deficit hyperactive disorder.

3.1.5 Diagnosis:

To diagnose this pathology, the *gold-standard* is video polysomnography (PSG). This test is recommended in patients with symptoms of OSAS, prior to adenotonsillectomy especially if there is associated obesity, craniofacial malformations, neuromuscular diseases or syndromes such as Chiari, Down or Prader-Willi (4,23). It can also be performed in patients where there are doubts on the need for treatment (23). PSG is an expensive test, usually performed in sleep units. These units are not available in all hospitals and in cases where PSG is not available, the following diagnosis tests are used (2,16):

- **Cardiorespiratory polygraphy**: it measures variables such as airflow, breathing effort and oxygen saturation together with an electrocardiography. Unlike PSG it lacks neurophysiological variables, but its realization is simpler and allows home studies. Compared to hospital PSG it has an acceptable sensitivity of almost 90%(2,7,17,21,24).
- **Night oximetry:** nocturnal O_2 saturation ($SatO_2$) and heart rate are recorded. Patients with OSAS have a characteristic pattern of acute $SatO_2$ falls in clusters defined by McGill University. This allows physicians to classify patients by a score that receives the same name (Figure 1). It is a simple test both in its realization and interpretation and it is used as screening in patients without

comorbidities. A negative result does not exclude the possibility of having OSAS, and if symptoms are persistent, more tests should be performed. However, it's used as a low-risk predictor for suffer OSAS having a negative oximetry after undergo an adenotonsillectomy (2,7,17,21).

Oximetry Score	Comment	Criteria			
		No. of Drops in SaO_2 <90%	No. of Drops in SaO_2 <85%	No. of Drops in SaO_2 <80%	Other
1	Normal study/ inconclusive for OSA	<3	0	0	Baseline: stable (<3 clusters of desaturation) and >95%
2	OSA, mild	≥3	≤3	0	Three or more clusters of desaturation events[14]
3	OSA, moderate	≥3	>3	≤3	Three or more clusters of desaturation events[14]
4	OSA, severe	≥3	>3	>3	Three or more clusters of desaturation events[14]

Figure 1 McGill Oximetry Scoring System (25). *OSA: obstructive sleep apnoea.*

- **Sleep questionnaires**: are used to detect the risk of OSAS and are therefore used as screening. The combination of questionnaires, with complete anamnesis and scanning and oximetry, have been shown to have greater reliability in diagnosing primary snoring, but does not differ well between OSAS severity, and especially in greater severity (2,17,26).
- **Endoscopy during induced sleep:** Endoscopy and dynamic evaluation of the upper airway during pharmacologically induced sleep is performed to assess obstruction points and guide therapeutic attitudes. Studies suggest that it is useful in cases of complex airway abnormalities (Pierre Robin, laryngomalacia...) (2,3,22,27).
- **Sleep video recording and Sivan score:** the recording of the patient during sleep at home by his parents. The recording should include from the head to the naked trunk of the patient for thirty minutes in which symptoms of risk of OSAS such as snoring, difficulty in breathing etc. are observed. The patient's posture should not be corrected. It is best to try recording in the last third of the night as it is during REM sleep that obstructive events occur most frequently. The findings are assigned a score (Figure 2) and the recording is reviewed by a professional to determine if the recording seems suggestive of normality or abnormality compatible with OSAS (28).

Vídeo-score de Sivan
(30 minutos de grabación de vídeo)

1 **Ruido inspiratorio**
0- Ausente
1- Débil
2- Intenso

2 **Tipo de ruido inspiratorio**
1- Episódico
2- Continuo

3 **Movimientos durante el sueño**
0- Sin movimientos
1- Pocos movimientos (≤3)
2- Frecuentes movimientos (≥3), todo el cuerpo

4 **Número de episodios de despertar**
Un punto por cada episodio

5 **Número de apneas**
0- Ninguna
1- Una o dos
2- Frecuentes (≥3)

6 **Retracciones torácicas**
0- Ausentes
1- Intermitente (periódicas)
2- Continuas

7 **Respiración bucal**
0- Ausente
1- Intermitente (periódica)
2- Continua

Interpretación de la puntuación:
Menor o igual a 5: Normal
Mayor o igual a 11: altamente sugestiva de SAHS
Entre 6 y 10: Dudosa

Figure 2 SIVAN score system

In the event that these tests are inconclusive, PSG is performed in the hospital (3,17). This test is responsible for continuously measuring neurophysiological and cardiorespiratory variables during sleep (29,30). In this way you can see what alteration occurs and in what stage of sleep it happens. During the test, sleep should be achieved without drugs, as these alter their normal structure. This test requires a laboratory and specifically trained nursing and medical personnel (29). The parameters that are measured are:

- **Neurophysiological:** by electroencephalography, electrooculography and electromyography (submental).
- **Cardiorespiratory:** electrocardiography (measures heart rate and rhythm), oral and nasal airflow (using a thermistor or nasal cannula), breathing movements (chest bands), pulsioximetry, microphone (intensity and frequency of snoring).
- **Others:** body position detector, electrodes in anterior tibial, capnography.

Both the American (AASM) and Spanish (AEPED) guides define with the PSG the following concepts (2,17,25):

- **Obstructive apnoea:** stop airflow for more than two breathing cycles with preserved breathing movements.
- **Central apnoea:** cessation of airflow and respiratory movements for more than two cycles.
- **Mixed apnoea:** is an apnoea in which the previous two apnoea occur alternately.
- **Hypopnea:** reduction of airflow >50% (30% according to ≥AASM) that is accompanied by a saturation drop of >4% (≥3% according to AASM) and/or a micro-waste.
- **Obstructive or central apnoea index (OAI or CAI):** No apnoea per hour of sleep.
- **Apnoea hypopnea index (AHI):** nº of apnoea and hypopnea per hour of sleep.
- **Desaturation:** Decrease in oxygen saturation of 3 or 4%. At the clinical level, an hourly desaturation index (ODI) is calculated, and one is defined for each desaturation value, ODI_3 or ODI_4.

According to American publications, there are no clinical differences between hypopnea and obstructive apnoea (2). What they have observed is an increase in heart rate and blood pressure in central apnoea with induced movement (2,22).
With all this data you can classify the severity of the OSAS according to the IAH as seen in the following table (Table 3):

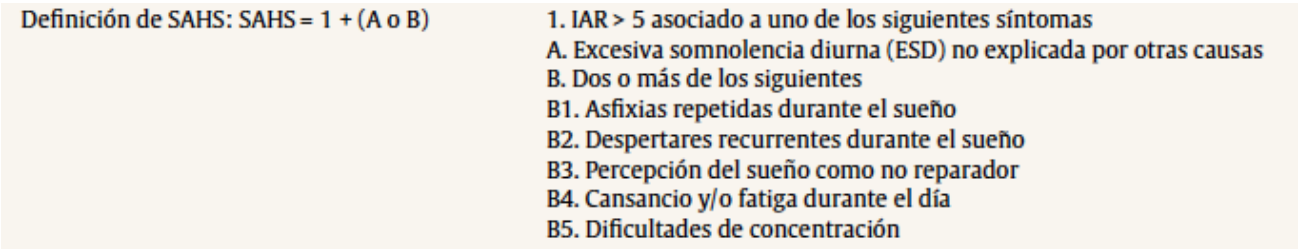
Table 3. OSAS Severity (31)

AHI	Classification
<1	Primary snorer
1-5	OSAS Mild
5-10	OSAS Moderate
>10	OSAS Severe

AHI: apnea hypopnea index; OSAS: obstructive sleep apnoea syndrome.

OSAS severity criteria depend on IAH, values have been inherited from OSAS guidelines in adults but new American studies have modified the criteria for diagnosing infant

OSAS by 1 AHI or more, and a <92% desaturation (2,7). According to a Spanish study that collects data from AEPED and AASM, OSAS is defined as (Figure 3)(17):


Definición de SAHS: SAHS = 1 + (A o B)

1. IAR > 5 asociado a uno de los siguientes síntomas
A. Excesiva somnolencia diurna (ESD) no explicada por otras causas
B. Dos o más de los siguientes
B1. Asfixias repetidas durante el sueño
B2. Despertares recurrentes durante el sueño
B3. Percepción del sueño como no reparador
B4. Cansancio y/o fatiga durante el día
B5. Dificultades de concentración

Figure 3Criteria for defining OSAS (17). *IAR equates to AHI (apnoea hypopnea index)*

OSAS is associated with several, very important comorbidities because they affect the patient at important developmental ages at both the physical and cognitive level (Table 4). The impact on the health of the patient affection goes beyond non repairing sleep, as fragmentation of the normal sleep pattern and intermittent hypoxia have repercussions on the patient's metabolism causing a predisposition to high blood pressure, dyslipidemia and alterations in glucose metabolism. This metabolic impact is reflected in blood, urine, endothelial tissue and exhaled air (1,3,7,17,32).

Table 4. OSAS morbidity

Neurobehavioral	Poor school performance, behavioural problems, psycho-motor developmental delay, hyperactivity, aggressiveness, depressive symptoms, memory impairment, visuo-spatial orientation and analytical and mathematical capabilities.
Cardiovascular	Diastolic HBP, HF, ventricular hypertrophy, ventricular dysfunction, endothelial dysfunction, decreased baroreceptor reflex, peripheral vasoconstriction, exaggerated BP variations, decreased ejection fraction, increased BNP, increased lung pressure
Development	Growth delay in both weight and size, nocturnal enuresis,
Inflammation	Systemic inflammation with alteration of PCR, platelet activation, increase in IL-6 and decrease in IL-10, increased glucocorticoid receptors and leukotriene
ANS	sympathetic activation, increased catecholamines in the urine
Other	Daytime sleepiness, worse quality of life, metabolic syndrome

HBP: high blood pressure; HF: heart failure; BP: blood pressure; BNP: brain natriuretic peptide; RCP: reactive C-protein; IL: interleukin.

As described above the classic phenotype is the largest paediatric population, its main treatment will therefore be adenotonsillectomy that requires a few indications. The literature recommends treating patients in the following cases (3,23,32):

1. When PSG of an AHI result >5/h even if there is no associated morbidity should be initiated treatment as symptoms are not usually resolved alone, AHI normalizes in many cases post-surgery and heart rate is reduced in parallel with AHI.
2. If in night oximetry we obtain a McGill >1 or more than 3 desaturations greater than 3% or 1 greater than 4%. In this situation the McGill >1 has a positive predictive value for presenting AHI <5/h of 90%, plus post-surgery desaturations improve in 95% of cases.
3. When there is an AHI 1-5/h and there are comorbidities. Although it is necessary to associate affectation or higher apnoea rate with AHI of 1 for treatment. In these cases, comorbidity can be solved with surgery and, as we said above, we can normalize AHI. In obese patients it has lower resolution as the phenotype of OSAS may be different.
4. If AHI >1/h and is associated with genetic diseases, neuromuscular diseases or anatomical abnormalities. In these cases, if no treatment is performed the patient may develop pulmonary hypertension. In addition, it can further compromise cognitive function in children with Down syndrome if left untreated.
5. In the primary snorer treatment is called into question since the existing literature is limited in these fields leaving treatment often at clinical discretion. In this case the patient should be evaluated annually for follow-up (23).

3.1.6 Treatment:

OSAS should be treated in a multidisciplinary manner due to the different anatomical and functional factors that cause it.

The vast majority of OSAS diagnosed in paediatric ages is due to adenotonsillar hypertrophy, therefore, the first-line treatment that arises in these cases is adenotonsillectomy (17). This procedure has been shown to be effective in treating respiratory problems and night-time symptomatology around 80% of cases (23,33,34). However, there are other procedures that should be considered individually in each patient such as turbinectomy, septoplasty, glossopexy... It should be noted that this data

is collected in children without craniofacial involvement. There exists up to 27% risk of postoperative complications, therefore it is important to have in place preventative measures.

Post-surgical risk is mainly related to the age and severity of OSAS although other factors influence (Table 5 extracted from the AEPED (35)), so it is important to set it to schedule subsequent monitoring ranging from major outpatient surgery to intensive care unit (36).

Table 5. Risk factors to develop post-operative complications in children with OSAS. Extracted from Asensi et al (35)

Tabla VI.Factores de riesgo para desarrollar complicaciones post-operatorias en niños con SAHS
• Edad menor de 3 años
• SAHS severo
• Complicaciones cardiacas (hipertrofia ventricular derecha)
• Retraso póndero-estatural
• Obesidad
• Prematuridad
• Infección respiratoria reciente
• Anomalías craneofaciales
• Alteraciones neuromusculares

Once discharged, clinical follow-up is carried out and PSG is repeated in case of showing OSAS symptomatology again. In cases where adenotonsillar hypertrophy is not the main problem, other procedures, whether surgical or other measures, should be performed:

1. **Surgical:**
    - Uvulo-pharyngo-palatoplasty: indicated in OSAS with psychomotor delay, these patients suffer obstruction at the soft palate level and hypertrophy is not adenoid or tonsillar, but is from the uvula and soft palate (37).
    - Turbinate radiofrequency**:** is indicated in patients where the obstruction is mainly nasal, useful in patients with chronic rhinitis resistant to medical treatment (38).
    - Orthodontics: correction of maxillomandibular anomalies that do not allow the correct airflow. It is an effective intervention on ogival palates and nasal occlusions. There should be no adenotonsillar hypertrophy or obesity in patients to consider this treatment (39)**.**

- Maxillofacial surgery: the elective surgery in case of malformations as in the syndromes described above, hemifacial microsomia, choanae atresia, retrognathia and alterations of the nasal septum (40).
- Tracheostomy: this is the last procedure that arises, it is only used in cases where anatomical alterations cannot be corrected, apnoea were very severe and less aggressive treatments could not be carried out.

2. **Other measures:**
    - Continuous positive airway pressure (CPAP): This is the second line of treatment if adenotonsillectomy fails or cannot be performed. A mask with a compressor is used to introduce pressured air into the patient and maintain good flow in the airways without them collapsing. The pressure is individualized according to the characteristics of the patient detected in the PSG. The ideal pressure is the one that prevents apnoea and reduces snoring and must also be tolerated by the patient to allow sleep. In adults it has been proven to improve sleep quality and daytime symptomatology, in children the experience is more limited, but the results are similar. Close follow-up is needed the first few weeks to see the side effects it causes on the patient and correct them. The use of BiPAP is reserved for patients with other chronic pathologies such as neuromuscular pathology for example (17,41,42).
    - Sleep hygiene: sleep and bedtime routines, suitable ambience for sleep with adequate lighting, calm and moderate temperature. Stipulated hours to go to sleep and get up, no television in the bedroom, daily exercise and exposure to daylight. Avoid naps too late or too long. Exposure to news with violent content has also been seen to worsen sleep quality (43).
    - Fix nasal congestion: it should be used as a bridge to surgery as nasal obstruction accounts for half of the air resistance to passage through the airways. Anticongestives, montelukast or topical corticosteroids may be used in the case of allergic rhinitis. The use of topical corticosteroids also helps to have CPAP tolerance in case of nasal obstruction (17,23,44,45).
    - Night oxygen therapy: only in patients with hypoxemic respiratory failure associated with OSAS and once the ventilation problem caused by the disease has been corrected.

- Proton pump inhibitors (PPIs): occur in cases where the root of the problem is gastroesophageal reflux, during sleep it has been shown that the passage of acid to the distal part of the oesophagus is more common so it should be treated with PPIs (46,47).

3. **Specific situations:**
    - Central hypoventilation syndrome: either congenital or acquired these patients will need some form of ventilation (whether invasive or non-invasive) due to decreased respiratory rate. This ventilation must be maintained for a lifetime.
    - Obesity syndrome - hypoventilation: This problem is more common in adolescents and adults. Obesity causes fat infiltration of the soft palate and hypopharynx, decreasing upper airway diameter. In addition, the abdominal fatty belt makes it difficult to move the diaphragm by worsening ventilation in the hypotonic phase of sleep. In this case the most effective treatment is weight loss (4,48,49).
    - Arnold Chiari II malformation associated with myelomeningocele: more than half of children suffer from respiratory disorders during sleep (50). There are four main mechanisms that produce it and should be treated differently and individually, these are central apnoea, obstructive apnoea, central hypoventilation and restrictive lung disease. In general, ventriculoperitoneal derivation or posterior pit decompression function as measures to improve symptomatology.

3.2 Organic volatile compounds (VOCs)

Once the complexity of the diagnosis and treatment of childhood OSAS has been described, the need to improve and speeding up diagnosis is evident to differentiate primary snoring population from pathological snorers suffering from OSAS. Recent studies claim that the detection of volatile organic compounds (VOCs) could be useful in diagnosing certain pathologies including OSAS (51).

VOCs are compounds that derive from various metabolic pathways, the body produces them normally and expels them through various pathways such as skin, faeces, urine or

breath (12). These exhaled VOCs are the result of several biochemical processes that occur in the body and are the result of a homeostatic balance, these components are also exchanged between breath, blood and adipose tissue constantly (12). We can conclude then that healthy people have a pattern of VOCs that derives from normal body homeostasis. Similarly, sick people may have a pattern of VOCs that differs from normal due to pathological changes that cause some diseases at the cellular level (12,52–54). In the case of OSAS, widespread increase in inflammation may cause changes in the normal VOCs pattern. Its use has already been described above for diseases such as diabetes, metabolic diseases of sodium, calcium and potassium malabsorption, lactase deficiency etc (54,55). In the field of lung diseases, its detection has been applied in diseases such as asthma, COPD, cystic fibrosis and lung cancer with good results (53,54,56–58). Nonetheless, most publications focus on adult population, so research on the paediatric population needs to be continued.

However, a problem arises for its perception. Historically, Aristotle taught his disciples to use the sense of smell to detect diseases (12). Nowadays, there are patients who emit a characteristic smell such as diabetics, people with pseudomonas infection etc. Although humans are able to differentiate thousands of odours, we cannot perceive such fine odours, which is why a technology has been developed, being capable of detecting different patterns of VOCs more accurately such as gas chromatography and mass spectrometry that allow to identify the molecules that make up these VOCs (12). Its application in clinical practice is complicated because it requires trained personnel, rigorous analysis and therefore it would be an expensive technique (12).

3.3 eNose:

Due to the difficulty of detecting individual chemical compounds, another technology has been developed that works such as mammalian olfactory systems, eNose (12) (Image No. 1). This device features sensors that react to VOCs by creating odour patterns in the same way as mammalian smell (12,59). This device is capable of generating VOCs patterns from organic samples such as exhaled air, urine or faeces (60–62). The device has 32 sensors that may or may not react in different ways to the different VOCs, depending on the affinity between them, changes in the electrical resistance of the sensors will be recorded (12,63). In this way multiple sensors react with the same VOC differently,

resulting in sample-related patterns. These patterns are processed and compared to others using pattern recognition algorithms with the eNose software. Then the patterns are incorporated again to the eNose as a recognition profile (12). When this recognition profile is transferred to the eNose, it acquires the ability to differentiate between the patterns that interest researchers, in our case healthy primary snorers and OSAS patients. In the respiratory disease field, the eNose has been used for the diagnosis of diseases such as lung cancer, asthma, COPD and OSAS obtaining good results in various studies (53–57,64). However, there are few studies that have used this technology in patients of paediatric age. On one hand, that is why it is interesting to apply this technology to try to differentiate pathological respiratory patterns of OSAS and healthy primary snorers, on the other hand its non-invasive nature helps to apply this new technique on paediatric patients.

Image 1 eNose

4 JUSTIFICATION:

As described above, OSAS is a pathology with comorbidities that affects health and quality of life of patients suffering from it (3,4,17,32). To prevent these comorbidities from developing, a diagnosis is needed to initiate the most indicated treatment; today it depends on the tests described above, especially PSG in case of wanting a certain diagnosis (4,23).

PSG is an expensive, poorly accessible test due to the need for a sleep unit. It is also invasive as it forces the patient to sleep in a hospital being highly monitored (2,16,65).
In addition, PSG reflects the parameters collected on a specific night and, in paediatric age, nocturnal symptoms may vary throughout the months of the year due to their relationship to tonsil hypertrophy and seasonal infectious tables.

Given the disadvantages of PSG and the lower reliability of the tests that have been suggested to replace it in case of not having access to a sleep unit, it is necessary to consider other methods that are effective, less invasive and faster than PSG (65).

In this context, the emergence of VOCs can be a major step towards rapid, effective and non-invasive diagnosis in respiratory diseases such as OSAS (12,65). Detection of VOCs patterns using eNose has already been used in other respiratory pathologies and may result in a diagnostic improvement of OSAS (53–55,65). Another added advantage is that metabolic changes reflecting VOCs are more durable and have less variability than single-night parameters, which would allow to be more accurate in the assessment of the disease on the patient's metabolism.

Thanks to this new technology we hope to differentiate between healthy primary snorers and OSAS affected patients without resorting to more invasive tests, such as PSG, and in this way, ensure a more comfortable and faster diagnosis for the patient.

5 GENERAL HYPOTHESIS:

The analysis of volatile organic components of exhaled air (VOC's) using the electronic nose (eNose) allows us to differentiate between paediatric patients affected by OSAS and simple snorers.

6 OBJECTIVES:

- **Main objective**

Identify a VOC pattern for snoring patients with OSAS different from the pattern of simple snoring patients.

- **Side objective**

1. Identify patterns that stratify snoring patients based on the severity of OSAS.
2. Identify a pattern for preschool phenotype OSAS.
3. Identify a pattern for adolescent-obese phenotype OSAS.

7 METHODOLOGY:

7.1 Study Design:

The study will be prospective, descriptive and observational, carried out in a single hospital (Son Espases university hospital) for a year and a half.

7.2 Study population:

The population for the study will be made up of children of paediatric age aged 3 to 14 who go to HUSE to perform PSG on suspicion of OSAS.

7.3 Selecting subjects:

The study members will be these children who go to HUSE to perform a PSG for the diagnosis of OSAS. Once the test has been completed and with due informed consent (Annex 2 and 3) signed by the parents/guardians, a series of anthropometric and clinical data will be collected (Annex 4), and an exhaled air sample will be obtained from the patient.

- Inclusion criteria: Any patient of paediatric age (up to age 14) referred to the HUSE sleep unit to perform a PSG on suspicion of OSAS.
- Exclusion criteria: catarrhal state 2 weeks prior to the study and age too low to collaborate in sampling (< 3years).

7.4 Exhaled air collection:

The sample will be picked up from the patient in a fasting state, after PSG. Once the informed consent has been delivered and signed, the patient will be prepared for the collection of exhaled air. Before collection, the patient shall be informed that he/she must breathe at tidal volume through the mouth and through the nozzle. The subject must put a clamp on his nose to prevent air from entering outside from the sample collection circuit. In this way the air will follow in the direction described below with Image 2:

1) The clamp (Image 2 No. 1) will be placed on the nose to prevent unfiltered air from entering the circuit.
2) Holding the circuit, the patient should breathe at tidal volume for 30 seconds. In inspiration the air will enter through the humidity filter (Image 2 No. 3). It will pass through a one-way valve (Image 2 No. 4) and then through the HEPA

filter (Image 2 No. 5) to pass through the nozzle (Image 2 No. 2) and enter the patient's respiratory system.

3) Once the inspiration is over, the exhaled air collection will begin. The patient will exhale through the nozzle and the air will pass through the HEPA filter and the one-way valve will allow the air's passage to the Tedlar bag (Image 2 No. 6) where it will be stored.
4) This process will be repeated for 30 seconds in which the exhaled air will be stored in the Tedlar bag.
5) At the end of time the bag will be sealed until analysis by the eNose, the nozzle and clamp will be discarded, and new ones will be used for the next patient.

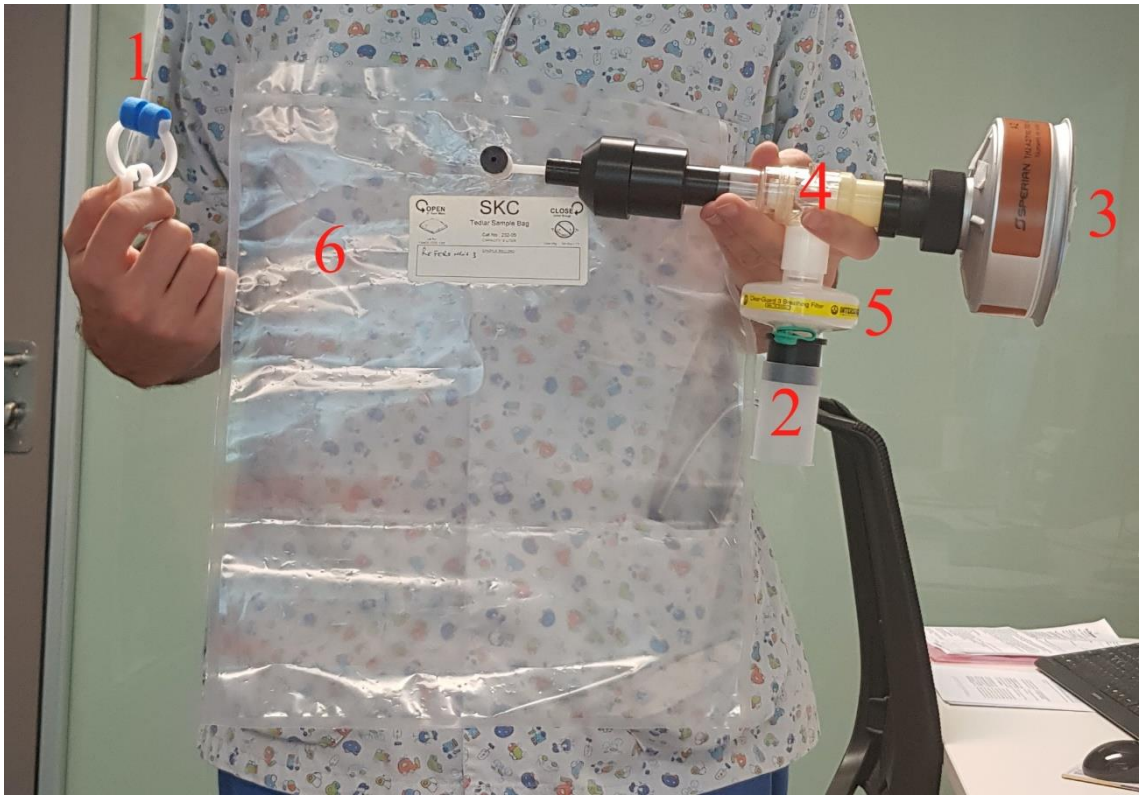


Image 2. Exhaled air collection circuit. 1 Nasal clamps. 2 Plastic nozzle. 3 Sperian A2 humidity filter. 4 Unidirectional valve. 5 HEPA air filter (Clear-Guard™ 3). 6 Tedlar bag.

Image 3 Patient ready for exhaled air collection

7.5 Sample:

Due to the small number of studies in the paediatric population and the lack of consensus in standardization for the collection of samples at such early ages this protocol is proposed as a pilot study. Therefore, the sample size will not be calculated. In the few existing studies, samples are very small, between 15 and 40 subjects. Taking all the above into account and that the diagnosis will be made previously by PSG in the sleep unit (gold standard), it is decided that the sample will consist of all patients who come to the sleep unit for the diagnosis of OSAS in a year. In the HUSE between 10-15 PSG per month are done therefore the sample size would be of between 120 and 180 subjects.

7.6 Variables:

Dependent: is a continuous and intervallic quantitative variable consisting of the exhaled air pattern measured 3 times (repeating the analysis on the same sample), in a maximum of 32 sensors (of which some will be selected to discard in case they do not generate a pattern that provides information).

Independent: nominal dichotomous qualitative. It is expressed as yes or no, and it is defined as the presence of OSAS disease in study subjects. The test that will define the presence or absence of the disease will be a hospital PSG in the sleep unit, this being the gold standard for the diagnosis of this pathology.

Covariates:

- Sex: nominal dichotomous qualitative variable. It will be divided into female and male sex.
- Age: quantitative discrete, expressed in years.
- Weight: quantitative continuous, it shall be expressed in kg.
- BMI: continuous quantitative, it shall be expressed in kg/m^2.
- Tonsil hypertrophy: ordinal qualitative, the value is reflected according to the Brodsky scale (Annex 1).
- Facies adenoid: Nominal dichotomous qualitative, it will be reflected as yes or no, to the presence of elongated face and poor dental occlusion.
- Ogival palate: nominal dichotomous qualitative palate, will be valued as yes or no, to the presence of a compressed upper jaw, resulting in a narrow and high palate.
- Retrognathia: nominal dichotomous qualitative, will be assessed as yes or no, to the lack of growth of the jawbone, thus decreasing the airway.
- Nocturnal enuresis: nominal dichotomous qualitative, expressed as yes or no.
- Difficult awakening: nominal dichotomous qualitative, expressed as yes or no.
- Parent/Guardian study level: Nominal qualitative, will be collected to establish the family socioeconomic level.
- Parent/Guardian occupancy: Nominal qualitative, will be collected to establish the family socioeconomic level.

8 STATISTICAL ANALYSIS:

8.1 Descriptive analysis:

For the analytical description, the dependent variables will be summarized, these being the different exhaled air patterns defined by each sensor, using means or medians (if there are extreme values) as a measure of the central trend, with the standard deviation or interquartile ranges (if there are extreme values), as a dispersion measure.

This descriptive shall be stratified whether the child is healthy or not and according to the repetitions of the exhaled breath sample analysis.

All these analyses will be stratified by the different covariates. When the variables are quantitative, they will be categorized.

8.2 Bivariate inference:

To assess the difference in the exhaled air pattern between healthy and sick children, in each of the repetitions, the paired Student's test contrast as well as the Wilcoxon contrast will be used.

All these analyses will be stratified by the different covariates. When the variables are quantitative, they will be categorized.

8.3 Multivariate analysis:

For each of the dependent variables (each repetition and each sensor) we will assess the difference in the exhaled breath pattern using a linear regression model with independent variable healthy/sick child adjusting by the covariates.

For each sensor and repetition, we will get an estimator. If there is no difference in the pattern, that estimator will not be statistically significant. With so many coefficients (sensors x repetitions), a meta-analysis will be done using a random effects model, to obtain the combined effect. The significance of this combined effect will tell if there is a difference between patterns.

We accept that a P value <0.05 is statistically significant.

8.4 Predictive model

To introduce the necessary data into the eNose for giving it the capacity to diagnose between different patterns, we are going to use a predictive model based in automatic supervised learning algorithms.

Also, we will use a support vector machine (SVM) to find a lineal decision surface (hyperplane) which will divide sample categories with a maximum distance between the frontier samples.

The eNose will be trained and optimized using a cross validation strategy.

9 LIMITATIONS:

The following limitations should be taken into account in trying to reduce their impact on the study:

1. The collection of the exhaled air sample would be more correct and easier to standardize if it could be collected with airflow control since a very high airflow does not contain as many VOCs as a smaller and more controlled flow, as other studies refer (51). In paediatric patients it is difficult to apply these measures, therefore, other attempts have been made to come up with a standardization. This consists in breathing at a tidal level through the nozzle for 30 seconds through the air collection system.
2. By including all patients who attend PSG we include all possible OSAS phenotypes described above. Therefore, we should be careful when interpreting the results as the different phenotypes may have different exhaled breath patterns. That is why the secondary objectives have reflected the possibility of studying patterns that also differ between the different phenotypes of the disease.
3. OSAS may have some seasonality during the winter months due to colds. This could be a possible artifact in the study, so as an exclusion criterion it has been stipulated not to include patients who have had a catarrhal state two weeks prior to the study.
4. Due to the global pandemic situation and the certainty that SARS-CoV-2 transmission is through exhaled air droplets from patients with the disease, it is decided to interleave a HEPA filter on the circuit after contacting Nicole Bouvy, a research physician at Maastrich University in the Netherlands, who conducts research with an eNose for SARS-CoV-2 screening (66). Installing the filter raises doubts about whether it can interact with VOCs or not. This limitation is controlled by analysing VOC samples from the study physicians with and without HEPA filter, the results are sent to the eNose technician with the following conclusions:

   "Comparing the response of the sensors selected in the analysis of the two samples, it seems that the use of the HEPA filter at the input of the exhaled collection circuit is feasible."

10 LEGAL AND ETHICAL CONSIDERATIONS:

The study complies with the basic principles set out in the *Declaration of Helsinki of Ethical Principles for Medical Research Involving Human* Subjects signed by the Global Health Organization in October 2013:

- Principle of autonomy: the study ensures the autonomy of the patient by providing informed consent and the patient information sheet (Annex 2 and 3) which must be read carefully and signed once the professional has explained the implications of the study and the parents/guardians have asked the necessary questions. It is an indispensable requirement to participate in the study.
- Charitable principle: the patient will be diagnosed with the test considered gold standard for the pathology suspected and subsequently an exhaled air sample will be collected in an innocuous manner. This allows all patients to have the same access to a safe and scientifically accepted diagnosis. To complete the study, it is justified (see justification) and the design is appropriate (see methodology).
- Non-maleficence: Patients are offered the best diagnostic technique and after performing it they are offered to participate by giving a sample in an innocuous and non-invasive manner, on the other hand, if they prefer, they may not participate in the study having undergone already a PSG.
- Justice: all patients referred to the sleep unit may enter the study if they meet the criteria of inclusion and exclusion. Even if they do not meet the criteria to enter the study, they will be cared by the same professionals and have access to the PSG.

Since we are going to take samples of the exhaled air of patients we must respect and follow *Ley 14/2007* y el *Real Decreto 1716/2011* for research in biological samples.

On the other hand, patient confidentiality and privacy is ensured in accordance with *Regulation (EU) 2016/679 of parliament and the European Council, April 27, 2016* on the protection of individuals with respect to the processing of personal data and *Ley*

Orgánica 3/ 2018, de 5 de diciembre, de Protecciόn de Datos Personales y Garantía de los Derechos Digitales (LOPD-GDD).

The research protocol will be submitted and sent for approval to the CIS-IB (Ethical Research Committee of the Balearic Islands) of the Balearic Islands Health Service (IB-SALUT) to which the Son Espases University Hospital belongs before the start of the study.

There is no conflict of interests in this study.

11 WORK PLAN AND TIMELINE:

11.1 Work plan:

The working group will include the sleep unit of son Espases University Hospital, consisting of one paediatric pneumologist and a nurse who will pick up the participants and collect the samples. A technician from the Universitat de les Illes Balears (UIB) will oversee the maintenance of the eNose and the data process. The last participant of the group will be a statistician in charge of the analysis of the data and the necessary statistical calculations.

Phase 0: Study design

From January to March 2021:

- The bibliographic search will be carried out.
- The protocol will be developed
- Study subjects will be defined and chosen.

Phase 1: Ethical evaluation of the protocol

In March 2021:

- The protocol will be submitted to the Ethics Research Committee (CIE) to the HUSE partner university.
- The protocol will be submitted to the hospital administration for approval.

Phase 2: Data collection

From April 2021 to April 2022:

- Patients referred to HUSE who are required to perform PSG on suspicion of OSAS will be offered the opportunity to participate in the study once the inclusion and exclusion criteria have been reviewed.
- Consent signatures will be collected for participation in the study from families wishing to collaborate.
- Data from PSG, an exhaled air sample for immediate analysis by the eNose as well as anthropometric and clinical variables for each patient will be collected.
- For each patient, the data described above will be collected individually through a series of questionnaires (Anexo 4) after the PSG and exhaled air collection.
- The above data will be processed in an electronic database and paper questionnaires will be kept in a folder.

- In parallel to the questionnaire collection the technician will process the exhaled air data and create the patterns associated with each patient, these patterns will be stored along with the rest of the information collected.

Phase 3: Statistical analysis, interpretation and discussion of data

During May 2022:

- The statistician will analyse the recorded data and calculate the statistical value obtained.
- The pneumology team will meet to present and interpret the data obtained.

Phase 4: Publication

Between June and July 2022.

- The results and their interpretation, as well as their discussion and conclusion of the investigation, will be developed.
- Once elaborated and agreed, the study will be published and presented in congress.

11.2 Timeline:

Steps	2021				2022			
	J	F	M	A-D	J-A	M	J	J
Phase 0: design								
Choice of study subjects								
Bibliographic search								
Protocol elaboration								
Phase 1: Ethical assessment								
Protocol approval by an CIS								
Phase 2: data collection								
Sample collection								
Data collection								
Data logging								
Phase 3: Analysis								
Statistical analysis								
Phase 4: Publication								
Preparation of the article								
Publication								

12 BUDGET:

	Amount	Price/unit	Total
Personnel			
Technician	1,920 h	13.7 €/h	15,000€
Statistician	50 h	50 €/h	2,500€
Materials			
Cyranose 320	1	15,000€	15,000€
Tedlar 5L bag	10	20.5€	205€
Disposable nozzles	50	23.3€	23.3€
Humidity filter (Sperian A2)	1	30€	30€
HEPA Filter (Clear-Guard™ 3)	3	99€	99€
Disposable nose clamp	50	19.5€	19.5€
Other materials/ need to rebuy the above	-	-	623.2€
Maintenance			
eNose	once/year	3,500€	3,500€
Publication			
Scientific review and publication			3000€
Attendance at national or international congresses			3000€
TOTAL COST			43,000€

The budget needed to be able to develop the project is based on:

- The need to hire a technician capable of calibrating and maintaining the eNose.
- Statistician for the analysis of the data obtained.
- The need for an eNose (Cyranose 320; Smiths Detection Pasadena, CA, USA) for the detection of exhaled air patterns.
- Consumable material necessary for safety and correct collection of data (nozzles, tedlar bags, HEPA filter...).
- Calibration and maintenance of the electronic nose after the year of use.

The advantage of this investment is that the eNose, once acquired, can be used for different studies. In addition, the bibliographic research is carried out by PubMed free of

charge, the collection of samples will be carried out by the sleep unit team itself at no cost and families will not receive financial compensation for participating in the study as the collection of samples is totally harmless to the subjects of the study and will be carried out when leaving the PSG preventing them from having to come back to the hospital.

13 IMPACT:

OSAS in paediatric ages is a pathology that is interesting to address due to its multiple morbidities and the affectation on health and normal development of children (3,7,17,32,51).

The diagnostic methods that exist today are too laborious, like PSG, or do not have the best sensitivity or specificity (2,16). That is why attempts are being made to introduce new diagnostic methods. The eNose could be a new, faster and more accessible diagnostic method than PSG; in addition, it has been shown to be valid in lung diseases such as cancer, asthma and COPD (53–55,65). That's why this study can be helpful in gaining more information about eNose's diagnostic ability over other respiratory pathologies such as OSAS.

Although the electronic device may seem expensive, it is not limited to the recognition of a pathology, does not need a room/laboratory as a sleep unit, is able to generate results in 15 minutes and is not an invasive test.
All this makes the eNose a state-of-the-art technology capable of allowing the diagnosis of OSAS (if effective), among other pathologies, in hospitals that do not have the capacity to afford having a sleep unit, facilitating the diagnosis of this pathology and allowing patients not to have to rely on a high-level hospital to be diagnosed and treated or have to resort to private medicine in order to have access to other diagnostic tests.

14 FEASIBILITY:

The protocol serves as a tool for the organization of a study that will be carried out in a simple and feasible way by the following aspects:

- Conducting the study will not alter the functioning of the sleep unit, as after the test the same staff will provide the information for participation in the study and collect the necessary data, as well as the exhaled air sample.
- The exhaled air test is non-invasive so it will not cause any discomfort for patients.
- It will not alter the agenda of the HUSE sleep unit as programmed polysomnography will continue to be performed.
- The study does not require follow-up of patients, so no losses of the subjects of the study are estimated.
- The planning, defined in the schedule, gives sufficient time to recruit patients and perform the process and data analysis tasks so that the study can be properly developed.
- The team that collects the data does not need any type of training; they only need to know the protocol for sample collection since the process of these is handled by the eNose technician.

15 REFERENCES:

1. Peña-Zarza JA, De la Peña M, Yañez A, Bauça JM, Morell-Garcia D, Caimari M, et al. Glycated hemoglobin and sleep apnea syndrome in children: beyond the apnea–hypopnea index. Sleep Breath. 2018;22(1):205–10.
2. Joosten KF, Larramona H, Miano S, Van Waardenburg D, Kaditis AG, Vandenbussche N, et al. How do we recognize the child with OSAS? Pediatr Pulmonol. 2017;52(2):260–71.
3. Jackowska J, Piersiala K, Klimza H, Wierzbicka M. Obstructive sleep disordered breathing in children – an important problem in the light of current European guidelines. Otolaryngol Pol. 2018;72(4):1–4.
4. Marcus CL, Brooks LJ, Draper KA, Gozal D, Halbower AC, Jones J, et al. Diagnosis and management of childhood obstructive sleep apnea syndrome. Pediatrics. 2012;130(3):576–84.
5. Brunetti L, Rana S, Lospalluti ML, Pietrafesa A, Francavilla R, Fanelli M, et al. Prevalence of obstructive sleep apnea syndrome in a cohort of 1,207 children of Southern Italy. Chest [Internet]. 2001 [cited 2021 Jan 12];120(6):1930–5. Available from: https://pubmed.ncbi.nlm.nih.gov/11742924/
6. Castronovo V, Zucconi M, Nosetti L, Marazzini C, Hensley M, Veglia F, et al. Prevalence of habitual snoring and sleep-disordered breathing in preschool-aged children in an Italian community. J Pediatr [Internet]. 2003 Apr 1 [cited 2020 Dec 30];142(4):377–82. Available from: https://pubmed.ncbi.nlm.nih.gov/12712054/
7. Marcus CL, Brooks LJ, Ward SD, Draper KA, Gozal D, Halbower AC, et al. Diagnosis and management of childhood obstructive sleep apnea syndrome. Pediatrics. 2012;130(3).
8. Teculescu DB, Caillier I, Perrin P, Rebstock E, Rauch A. Snoring in French preschool children. Pediatr Pulmonol [Internet]. 1992 Aug 1 [cited 2020 Dec 30];13(4):239–44. Available from: http://doi.wiley.com/10.1002/ppul.1950130412
9. Gislason T, Benediktsdottir B. Snoring, apneic episodes, and nocturnal hypoxemia among children 6 months to 6 years old: An epidemiologic study of lower limit of prevalence. Chest [Internet]. 1995 [cited 2020 Dec 30];107(4):963–6. Available from: https://pubmed.ncbi.nlm.nih.gov/7705162/

10. Redline S, Tishler P V., Schluchter M, Aylor J, Clark K, Graham G. Risk factors for sleep-disordered breathing in children: Associations with obesity, race, and respiratory problems. Am J Respir Crit Care Med [Internet]. 1999 [cited 2020 Dec 17];159(5 I):1527–32. Available from: https://pubmed.ncbi.nlm.nih.gov/10228121/
11. P. de Lucas Ramos. J.M. Rodríguez González-Moro J de MD. La vía aérea superior durante el sueño: fisiología y fisiopatología. Trastor Respir del sueño. 2004;45–57.
12. Van Der Schee MP, Paff T, Brinkman P, Van Aalderen WMC, Haarman EG, Sterk PJ. Breathomics in lung disease. Chest. 2015;147(1):224–31.
13. Urschitz MS, Eitner S, Wolff J, Guenther A, Urschitz-Duprat PM, Schlaud M, et al. Risk factors for sleep-related hypoxia in primary school children. Pediatr Pulmonol. 2007;42(9):805–12.
14. Fuhrmann DC, Brüne B. Mitochondrial composition and function under the control of hypoxia. Redox Biol [Internet]. 2017;12(February):208–15. Available from: http://dx.doi.org/10.1016/j.redox.2017.02.012
15. Piumetto E, Sammartano AM, Meinardi G, Dagna F, Gervasio FC, Albera R. Diagnostic and therapeutic iter in paediatric OSAS: Personal experience. Acta Otorhinolaryngol Ital (Testo Stamp. 2011;31(3):149–53.
16. Chan J, Edman J, Koltai P. Obstructive sleep apnea in children with syndromic craniosynostosis. Am Fam Physician. 2010;613–20.
17. Lloberes P, Durán-Cantolla J, Martínez-García MÁ, Marín JM, Ferrer A, Corral J, et al. Diagnóstico y tratamiento del síndrome de apneas-hipopneas del sueño. Arch Bronconeumol. 2011;47(3):143–56.
18. CROFT CB, BROCKBANK MJ, WRIGHT A, SWANSTON AR. Obstructive sleep apnoea in children undergoing routine tonsillectomy and adenoidectomy. Clin Otolaryngol Allied Sci [Internet]. 1990 [cited 2020 Dec 31];15(4):307–14. Available from: https://pubmed.ncbi.nlm.nih.gov/2225498/
19. Hoffstein V, Szalai JP. Predictive value of clinical features in diagnosing obstructive sleep apnea. Sleep. 1993;16(2):118–22.
20. Brietzke SE, Katz ES, Roberson DW. Can history and physical examination reliably diagnose pediatric obstructive sleep apnea/hypopnea syndrome? A systematic review of the literature. Otolaryngol - Head Neck Surg [Internet]. 2004 [cited 2020 Dec 31];131(6):827–32. Available from:

https://pubmed.ncbi.nlm.nih.gov/15577775/

21. Marcus CL, Chapman D, Ward SD, McColley SA, Herrerias CT, Stillwell PC, et al. Clinical practice guideline: Diagnosis and management of childhood obstructive sleep apnea syndrome. Pediatrics. 2002;109(4):704–12.
22. Guilleminault C, Pelayo R. Sleep-disordered breathing in children. Ann Med. 1998;30(4):350–6.
23. Kaditis AG, Alvarez MLA, Boudewyns A, Alexopoulos EI, Ersu R, Joosten K, et al. Obstructive sleep disordered breathing in 2- to 18-year-old children: Diagnosis and management. Eur Respir J. 2016;47(1):69–94.
24. Ikizoglu NB, Kiyan E, Polat B, Ay P, Karadag B, Ersu R. Are home sleep studies useful in diagnosing obstructive sleep apnea in children with down syndrome? Pediatr Pulmonol. 2019;54(10):1541–6.
25. Nixon GM, Kermack AS, Davis GM, Manoukian JJ, Brown KA, Brouillette RT. Planning adenotonsillectomy in children with obstructive sleep apnea: the role of overnight oximetry. Pediatrics. 2004;113(1 Pt 1).
26. Brouilette R, Hanson D, David R, Klemka L, Anna S, Fernbach S, et al. A diagnostic approach to suspected obstructive sleep apnea in children. J Pediatr [Internet]. 1984 [cited 2020 Dec 31];105(1):10–4. Available from: https://pubmed.ncbi.nlm.nih.gov/6737123/
27. Brockbank JC. Update on pathophysiology and treatment of childhood obstructive sleep apnea syndrome. Paediatr Respir Rev [Internet]. 2017;24:21–3. Available from: http://dx.doi.org/10.1016/j.prrv.2017.06.003
28. Sivan Y, Kornecki A, Schonfeld T. Screening obstructive sleep apnoea syndrome by home videotape recording in children. Eur Respir J. 1996;9(10):2127–31.
29. Loughlin GM, Brouillette RT, Brooke LJ, Carroll JL, Chipps BE, England SJ, et al. Standards and indications for cardiopulmonary sleep studies in children [Internet]. Vol. 153, American Journal of Respiratory and Critical Care Medicine. American Thoracic Society; 1996 [cited 2020 Dec 31]. p. 866–78. Available from: https://pubmed.ncbi.nlm.nih.gov/8564147/
30. Michael S. Schechter. Technical Report: Diagnosis and Management of Childhood Obstructive Sleep Apnea Syndrome. Am Acad Pediatr [Internet]. 2002;109(4). Available from: www.aappublications.org/news
31. Tan HL, Gozal D, Ramirez HM, Bandla HPR, Kheirandish-Gozal L. Overnight polysomnography versus Respiratory polygraphy in the diagnosis of pediatric

obstructive sleep apnea. Sleep. 2014;37(2):255–60.

32. Tan HL, Alonso Alvarez ML, Tsaoussoglou M, Weber S, Kaditis AG. When and why to treat the child who snores? Pediatr Pulmonol. 2017;52(3):399–412.
33. Suen JS, Arnold JE, Brooks LJ. Adenotonsillectomy for Treatment of Obstructive Sleep Apnea in Children. Arch Otolaryngol Neck Surg [Internet]. 1995 [cited 2021 Jan 12];121(5):525–30. Available from: https://pubmed.ncbi.nlm.nih.gov/7727086/
34. Darrow DH, Siemens C. Indications for tonsillectomy and adenoidectomy. Laryngoscope [Internet]. 2002 [cited 2021 Jan 12];112(8):6–10. Available from: https://pubmed.ncbi.nlm.nih.gov/12172229/
35. Asensi V, Carrasco M, Perez P, Anzar C, Gil G, Alvarez P, et al. Sindrome de Apnea - hiponeas del sueño. Asoc Española Pediatría [Internet]. 2008;7:111–32. Available from: https://www.aeped.es/sites/default/files/documentos/7_4.pdf
36. McColley SA, April MM, Carroll JL, Naclerio RM, Loughlin GM. Respiratory Compromise After Adenotonsillectomy in Children With Obstructive Sleep Apnea. Arch Otolaryngol Neck Surg [Internet]. 1992 [cited 2021 Jan 12];118(9):940–3. Available from: https://pubmed.ncbi.nlm.nih.gov/1503720/
37. Kerschner JE, Lynch JB, Kleiner H, Flanary VA, Rice TB. Uvulopalatopharyngoplasty with tonsillectomy and adenoidectomy as a treatment for obstructive sleep apnea in neurologically impaired children. Int J Pediatr Otorhinolaryngol [Internet]. 2002 Feb 28 [cited 2021 Jan 12];62(3):229–35. Available from: https://pubmed.ncbi.nlm.nih.gov/11852126/
38. De Corso E, Bastanza G, Di Donfrancesco V, Guidi ML, Morelli Sbarra G, Passali GC, et al. Riduzione volumetrica dei turbinati inferiori con radiofrequenze: Risultati clinici a lungo termine. Acta Otorhinolaryngol Ital [Internet]. 2016 Jun 1 [cited 2021 Jan 18];36(3):199–205. Available from: https://pubmed.ncbi.nlm.nih.gov/27214831/
39. Pirelli P, Saponara M, Guilleminault C. Rapid maxillary expansion in children with obstructive sleep apnea syndrome. Sleep [Internet]. 2004 Jun 15 [cited 2021 Jan 12];27(4):761–6. Available from: https://pubmed.ncbi.nlm.nih.gov/15283012/
40. Cohen SR, Holmes RE, Machado L, Magit A. Surgical strategies in the treatment of complex obstructive sleep apnoea in children. Paediatr Respir Rev [Internet]. 2002 [cited 2021 Jan 12];3(1):25–35. Available from:

https://pubmed.ncbi.nlm.nih.gov/12065179/

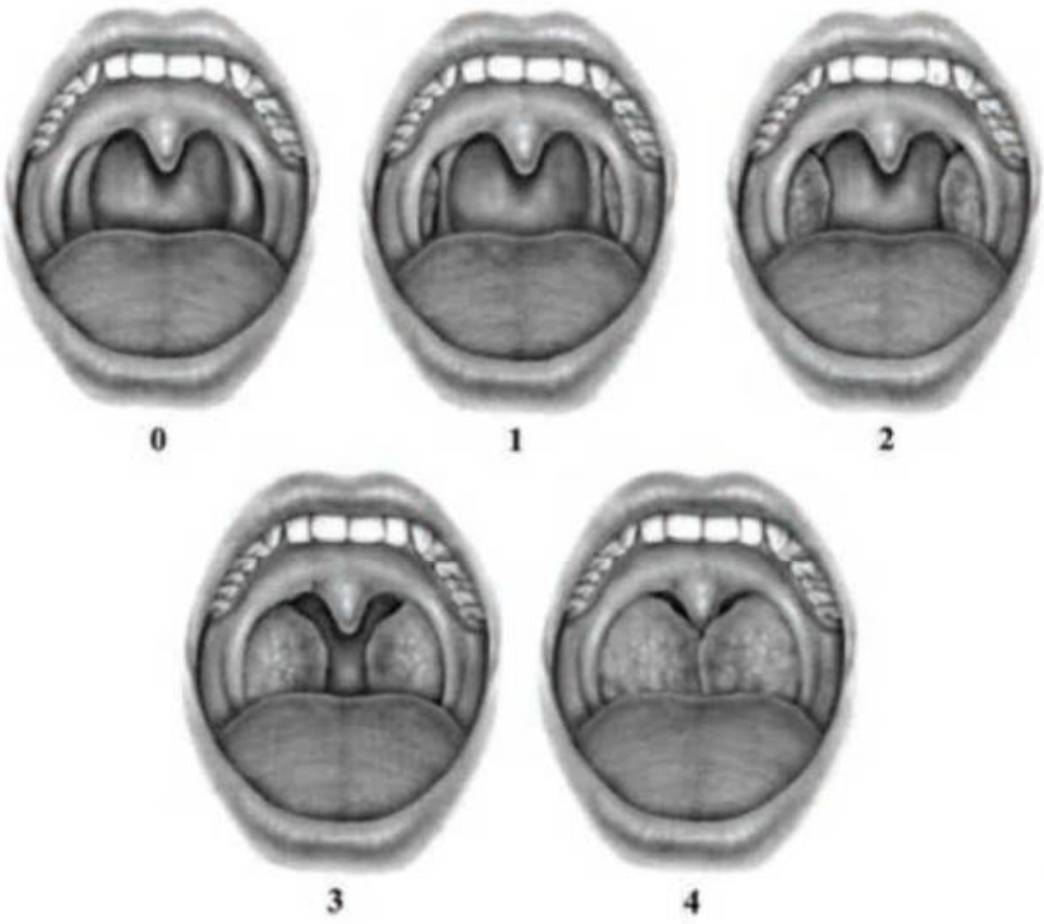
41. McNamara F, Sullivan CE. Obstructive sleep apnea in infants and its management with nasal continuous positive airway pressure. Chest [Internet]. 1999 [cited 2021 Jan 12];116(1):10–6. Available from: https://pubmed.ncbi.nlm.nih.gov/10424497/
42. Smith PL, Hudgel DW, Olson LG, Partinen M, Rapoport DM, Rosen CL, et al. Indications and standards for use of nasal continuous positive airway pressure (CPAP) in sleep apnea syndromes [Internet]. Vol. 150, American Journal of Respiratory and Critical Care Medicine. American Thoracic Society; 1994 [cited 2021 Jan 12]. p. 1738–45. Available from: https://pubmed.ncbi.nlm.nih.gov/7952642/
43. Bathory E, Tomopoulos S. Sleep Regulation, Physiology and Development, Sleep Duration and Patterns, and Sleep Hygiene in Infants, Toddlers, and Preschool-Age Children. Curr Probl Pediatr Adolesc Health Care [Internet]. 2017;47(2):29–42. Available from: http://dx.doi.org/10.1016/j.cppeds.2016.12.001
44. Alexopoulos EI, Kaditis AG, Kalampouka E, Kostadima E, Angelopoulos N V., Mikraki V, et al. Nasal corticosteroids for children with snoring. Pediatr Pulmonol [Internet]. 2004 Aug [cited 2021 Jan 12];38(2):161–7. Available from: https://pubmed.ncbi.nlm.nih.gov/15211701/
45. Kiely JL, Nolan P, McNicholas WT. Intranasal corticosteroid therapy for obstructive sleep apnoea in patients with co-existing rhinitis. Thorax. 2004;59(1):50–5.
46. Orr WC. Sleep-related breathing disorders: Is it all about apnea? [Internet]. Vol. 121, Chest. American College of Chest Physicians; 2002 [cited 2021 Jan 12]. p. 8–11. Available from: https://pubmed.ncbi.nlm.nih.gov/11796424/
47. Senior BA, Khan M, Schwimmer C, Rosenthal L, Benninger M. Gastroesophageal reflux and obstructive sleep apnea. Laryngoscope [Internet]. 2001 [cited 2021 Jan 12];111(12):2144–6. Available from: https://pubmed.ncbi.nlm.nih.gov/11802013/
48. Kalra M, Inge T, Garcia V, Daniels S, Lawson L, Curti R, et al. Obstructive sleep apnea in extremely overweight adolescents undergoing bariatric surgery. Obes Res [Internet]. 2005 [cited 2021 Jan 12];13(7):1175–9. Available from: https://pubmed.ncbi.nlm.nih.gov/16076986/

49. Verhulst SL, Franckx H, Van Gaal L, De Backer W, Desager K. The effect of weight loss on sleep-disordered breathing in obese teenagers. Obesity [Internet]. 2009 Jun [cited 2021 Jan 12];17(6):1178–83. Available from: https://pubmed.ncbi.nlm.nih.gov/19265797/
50. Guilleminault C, Ji HL, Chan A. Pediatric obstructive sleep apnea syndrome [Internet]. Vol. 159, Archives of Pediatrics and Adolescent Medicine. Arch Pediatr Adolesc Med; 2005 [cited 2021 Jan 12]. p. 775–85. Available from: https://pubmed.ncbi.nlm.nih.gov/16061787/
51. Benedek P, Lázár Z, Bikov A, Kunos L, Katona G, Horváth I. Exhaled biomarker pattern is altered in children with obstructive sleep apnoea syndrome. Int J Pediatr Otorhinolaryngol. 2013;77(8):1244–7.
52. Zetola NM, Modongo C, Matsiri O, Tamuhla T, Mbongwe B, Matlhagela K, et al. Diagnosis of pulmonary tuberculosis and assessment of treatment response through analyses of volatile compound patterns in exhaled breath samples. J Infect. 2017 Apr 1;74(4):367–76.
53. Klaassen EMM, Van De Kant KDG, Jöbsis Q, Van Schayck OCP, Smolinska A, Dallinga JW, et al. Exhaled biomarkers and gene expression at preschool age improve asthma prediction at 6 years of age. Am J Respir Crit Care Med. 2015;191(2):201–7.
54. Boots AW, Bos LD, van der Schee MP, van Schooten FJ, Sterk PJ. Exhaled Molecular Fingerprinting in Diagnosis and Monitoring: Validating Volatile Promises [Internet]. Vol. 21, Trends in Molecular Medicine. Elsevier Ltd; 2015 [cited 2020 Dec 2]. p. 633–44. Available from: https://0-pubmed-ncbi-nlm-nih-gov.cataleg.udg.edu/26432020/
55. Mazzatenta A, Di Giulio C, Pokorski M. Pathologies currently identified by exhaled biomarkers. Respir Physiol Neurobiol [Internet]. 2013;187(1):128–34. Available from: http://dx.doi.org/10.1016/j.resp.2013.02.016
56. Robroeks CM, Van Berkel JJ, Jöbsis Q, Van Schooten FJ, Dallinga JW, Wouters EF, et al. Exhaled volatile organic compounds predict exacerbations of childhood asthma in a 1-year prospective study. Eur Respir J. 2013;42(1):98–106.
57. Fens N, Zwinderman AH, Van Der Schee MP, De Nijs SB, Dijkers E, Roldaan AC, et al. Exhaled breath profiling enables discrimination of chronic obstructive pulmonary disease and asthma. Am J Respir Crit Care Med. 2009;180(11):1076–82.

58. Mazzatenta A, Di Giulio C, Pokorski M. Pathologies currently identified by exhaled biomarkers. Vol. 187, Respiratory Physiology and Neurobiology. Elsevier; 2013. p. 128–34.
59. Bushdid C, Magnasco MO, Vosshall LB, Keller A. Humans can discriminate more than 1 trillion olfactory stimuli. Science (80-) [Internet]. 2014 [cited 2021 Jan 13];343(6177):1370–2. Available from: https://pubmed.ncbi.nlm.nih.gov/24653035/
60. Shafiek H, Fiorentino F, Merino JL, López C, Oliver A, Segura J, et al. Using the electronic nose to identify airway infection during COPD exacerbations. PLoS One. 2015;10(9):1–16.
61. Arasaradnam RP, Quraishi N, Kyrou I, Nwokolo CU, Joseph M, Kumar S, et al. Insights into "fermentonomics": Evaluation of volatile organic compounds (VOCs) in human disease using an electronic "e-nose." J Med Eng Technol [Internet]. 2011 Feb [cited 2021 Jan 13];35(2):87–91. Available from: https://pubmed.ncbi.nlm.nih.gov/21204611/
62. Farraia MV, Cavaleiro Rufo J, Paciência I, Mendes F, Delgado L, Moreira A. The electronic nose technology in clinical diagnosis. Porto Biomed J [Internet]. 2019 Jul [cited 2021 Jan 13];4(4):e42. Available from: https://pubmed.ncbi.nlm.nih.gov/31930178/
63. Dragonieri S, Schot R, Mertens BJA, Le Cessie S, Gauw SA, Spanevello A, et al. An electronic nose in the discrimination of patients with asthma and controls. J Allergy Clin Immunol. 2007;120(4):856–62.
64. van Bragt JJMH, Brinkman P, de Vries R, Vijverberg SJH, Weersink EJM, Haarman EG, et al. Identification of recent exacerbations in COPD patients by electronic nose. ERJ Open Res [Internet]. 2020;6(4):00307–2020. Available from: http://dx.doi.org/10.1183/23120541.00307-2020
65. Greulich T, Fischer H, Lubbe D, Nell C, Ingo Baumbach J, Koehler U, et al. Obstructive sleep apnea patients can be identified by ion mobility spectrometry-derived smell prints of different biological materials. J Breath Res [Internet]. 2018 Apr 1 [cited 2021 Jan 13];12(2). Available from: https://pubmed.ncbi.nlm.nih.gov/29083318/
66. Wintjens AGWE, Hintzen KFH, Engelen SME, Lubbers T, Savelkoul PHM, Wesseling G, et al. Applying the electronic nose for pre-operative SARS-CoV-2 screening. Surg Endosc. 2020;

16 ANNEX:

16.1 ANNEX 1 Brodsky scale:



16.2 ANNEX 2 Informative sheet for the patient:

HOJA INFORMATIVA PARA EL PACIENTE

TÍTULO DEL ESTUDIO:
INVESTIGADOR PRINCIPAL:
CENTRO: Hospital universitario Son Espases.

Nos dirigimos a Ud. para informarle sobre un estudio de investigación, aprobado por el Comité Ético Asistencial, en el que se le invita a participar. Nuestra intención es que Ud. reciba la información correcta y suficiente para que pueda evaluar y juzgar, si quiere o no participar en este estudio. Para ello le rogamos lea esta hoja informativa con atención, pudiendo consultar con las personas del servicio que considere oportuno. También le aclararemos las dudas que le puedan surgir. Debe saber que su participación en este estudio es voluntaria, y que puede decidir no participar, o cambiar su decisión y retirar su consentimiento en cualquier momento, sin que por ello se altere la relación con su médico ni produzca perjuicio alguno en su tratamiento.

Sospechamos que su hijo sufre una enfermedad llamada síndrome de apneas- hipopneas obstructivas del sueño (SAHS), debido a esa sospecha se le ha realizado una polisomnografía (PSG) en la Unidad de Sueño del hospital Son Espases. Queremos solicitarle su consentimiento para incluir a su hijo/a en un estudio que recoge datos sobre pacientes como el/ella.
Nuestro estudio quiere intentar realizar este diagnóstico con una prueba no invasiva, de menor duración y más cómoda para el paciente para la cual necesitamos que respire 30 segundos por una boquilla conectada a un circuito que recogerá su aire exhalado. Esta recogida de datos es totalmente inocua y no comporta ningún riesgo para su hijo/a. El aire que recojamos, junto con los datos que nos aporten los cuestionarios asociados, serán investigados para intentar validar esta nueva prueba para el diagnóstico de SAHS.

La inclusión de estos datos no supondrá para usted ni su hijo/a ninguna molestia añadida, pero tampoco ningún beneficio económico, sin embargo, ayudará a conocer y diagnosticar mejor la enfermedad que puede padecer su hijo en un futuro. El diagnóstico, la comunicación y la cesión de los datos de carácter personal de todos los sujetos participantes se ajustará a lo dispuesto en la Ley Orgánica 3/ 2018, de 5 de diciembre, de Protecciǿn de Datos Personales y Garantía de los Derechos Digitales (LOPD-GDD).
De acuerdo con la legislación mencionada, usted puede ejercer los derechos de acceso, modificación, oposición y cancelación de datos, para lo cual se deberá dirigir a su médico del estudio. Los datos recogidos para el estudio estarán identificados mediante un código y sólo su médico del estudio/colaboradores podrán relacionar dichos datos con su hijo/a y con su historia clínica. Por lo tanto, su identidad no será revelada a persona alguna salvo en caso de urgencia médica o requerimiento legal. El acceso a su información personal quedará restringido al médico del estudio / colaboradores, autoridades sanitarias (Agencia Española del Medicamento y Productos Sanitarios), al Comité Ético de Investigación Clínica y personal autorizado por el promotor, cuando lo

precisen para comprobar los datos y procedimientos del estudio, pero siempre manteniendo la confidencialidad de estos de acuerdo con la legislación vigente en nuestro país.

De la misma manera le ofrecemos los siguientes datos de contacto en caso de querer aclarar dudas u otros aspectos que vayan surgiendo a lo largo del tiempo:

- Teléfono de contacto: 971010110
- Email de contacto: eNose@sonespases.com

Muchas gracias por su atención, ante cualquier duda restamos a su disposición.
Equipo de neumología pediátrica del hospital universitario Son Espases.

16.3 ANNEX 3 Informed consent:

CONSENTIMIENTO INFORMADO

PADRES

Yo,....., en calidad de (relación con el participante) .....

He leído la hoja de información que se me ha entregado.
He podido hacer preguntas sobre el estudio.
He recibido suficiente información sobre el estudio.
He hablado con:.....
Comprendo que mi participación es voluntaria. Comprendo que puedo retirarme del estudio:

- Cuando quiera.
- Sin tener que dar explicaciones.
- Sin que esto repercuta en mis cuidados médicos.

Presto libremente mi conformidad para participar en el estudio.

Fecha: .....

Firma del padre/madre: .....

TUTORES LEGALES

Yo,....., en calidad de (relación con el participante) .....

He leído la hoja de información que se me ha entregado.
He podido hacer preguntas sobre el estudio.
He recibido suficiente información sobre el estudio.
He hablado con:(nombre del investigador) .....

Comprendo que su participación es voluntaria.
Comprendo que puede retirarse del estudio:

- Cuando quiera.
- Sin tener que dar explicaciones.
- Sin que esto repercuta en sus cuidados médicos.

En mi presencia se ha dado a (nombre del participante) ..... toda la información pertinente adaptada a su nivel de entendimiento y está de acuerdo en participar.

Y presto mi conformidad para que (nombre del participante)…….. .....……..... participe en el estudio.

Fecha: ..... Firma del representante: .....

16.4 ANNEX 4 Data collecting sheet:

HOJA DE RECOGIDA DE DATOS

En la siguiente hoja debe marcar con una X o con los valores que correspondan los datos que se refieren a su hijo/a de los ítems de color **NEGRO.** Los ítems de color **ROJO** serán rellenados por el profesional sanitario.

Sexo:

Hombre	
Mujer	

Edad:

3		9	
4		10	
5		11	
6		12	
7		13	
8		14	

Peso:

	kg

Talla:

	m

IMC:

	Kg/m^2

Hipertrofia amigdalar (Brodsky):

0		3	
1		4	
2			

Facies Adenoidea:

Si	
No	

Paladar ojival:

Si	
No	

Retrognatia:

Si	
No	

Enúresis nocturna:

Si	
No	

Despertar difícil:

Si	
No	

Nivel estudios padre/madre/tutor/a 1:

Analfabeto por problemas físicos o psíquicos	
Analfabeto por otras razones	
Sin estudios	
Estudios primarios o equivalentes	
Enseñanza general secundaria, 1er ciclo	
Enseñanza profesional de 2º grado, 2º ciclo	
Enseñanza general secundaria 2º ciclo	
Enseñanzas profesionales superiores	
Estudios universitarios equivalentes	

Nivel estudios padre/madre/tutor/a 2:

Analfabeto por problemas físicos o psíquicos	
Analfabeto por otras razones	
Sin estudios	
Estudios primarios o equivalentes	
Enseñanza general secundaria, 1er ciclo	
Enseñanza profesional de 2º grado, 2º ciclo	
Enseñanza general secundaria 2º ciclo	
Enseñanzas profesionales superiores	
Estudios universitarios equivalentes	

Ocupación padre/madre/tutor/a 1:

Ocupación padre/madre/tutor/a 2: