BMJ Open

# Accuracy and reliability of the sensory test performed using the laryngopharyngeal endoscopic esthesiometer and rangefinder in patients with suspected obstructive sleep apnoea hypopnoea: protocol for a prospective double-blinded, randomised, exploratory study

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**To cite:** Giraldo-Cadavid LF, Bastidas AR, Padilla-Ortiz DM, *et al*. Accuracy and reliability of the sensory test performed using the laryngopharyngeal endoscopic esthesiometer and rangefinder in patients with suspected obstructive sleep apnoea hypopnoea: protocol for a prospective double-blinded, randomised, exploratory study. *BMJ Open* 2017;**7**:e015235. doi:10.1136/bmjopen-2016-015235

► Prepublication history and additional material are available. To view please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2016-015235).

Received 18 November 2016
Revised 8 June 2017
Accepted 16 June 2017

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## ABSTRACT

**Introduction** Patients with obstructive sleep apnoea hypopnoea syndrome (OSA) might have varying degrees of laryngopharyngeal mechanical hyposensitivity that might impair the brain's capacity to prevent airway collapse during sleep. However, this knowledge about sensory compromises in OSA comes from studies performed using methods with little evidence of their validity. Hence, the purpose of this study is to assess the reliability and accuracy of the measurement of laryngopharyngeal mechanosensitivity in patients with OSA using a recently developed laryngopharyngeal endoscopic esthesiometer and rangefinder (LPEER).

**Methods and analysis** The study will be prospective and double blinded, with a randomised crossover assignment of raters performing the sensory tests. Subjects will be recruited from patients with suspected OSA referred for baseline polysomnography to a university hospital sleep laboratory. Intra-rater and inter-rater reliability will be evaluated using the Bland–Altman's limits of agreement plot, the intraclass correlation coefficient, and the Pearson or Spearman correlation coefficient, depending on the distribution of the variables. Diagnostic accuracy will be evaluated plotting ROC curves using standard baseline polysomnography as a reference. The sensory threshold values for patients with mild, moderate and severe OSA will be determined and compared using ANOVA or the Kruskal–Wallis test, depending on the distribution of the variables. The LPEER could be a new tool for evaluating and monitoring laryngopharyngeal sensory impairment in patients with OSA. If it is shown to be valid, it could help to increase our understanding of the pathophysiological mechanisms of this condition and potentially help in finding new therapeutic interventions for OSA.

**Ethics and dissemination** The protocol has been approved by the Institutional Review Board of Fundacion Neumologica Colombiana. The results will be disseminated through conference presentations and peer-reviewed publication.

**Trial registration** This trial was registered at Clinical Trials Accuracy of the sensory test using the ILaryngopharyngeal endoscopic esthesiometer in obstructive sleep apnea. Protocol ID: 201611-22405. ClinicalTrials.gov ID: NCT03109171.

## Strengths and limitations of this study

- We will include various degrees of rater experience and different degrees of obstructive sleep apnoea severity (OSA) to reflect most clinical scenarios in which the test will be performed.
- Patients with OSA will be recruited from patients suspected to have the condition to reflect patients in which the test may be indicated.
- Sensory thresholds will be explored using air pulses of 10 different intensities (discrete variable), covering the full range of pressures necessary to explore such thresholds, which might decrease the statistical power.
- We do not have information about the SD of some sensory thresholds in OSA to calculate the sample size because mechanosensory thresholds like the cough and gag reflex thresholds have never been explored in OSA.
- Mechano-sensitivity measurements could help to better characterise OSA and to explore new therapeutic horizons for this condition.

## INTRODUCTION

Laryngopharyngeal mechanical sensitivity plays an important role in the regulation of various functions of the upper respiratory tract, including phonation, breathing, swallowing and protecting the lower respiratory tract from the entry of foreign materials.[1] Each one of these functions requires varying degrees of opening or closing of the laryngopharyngeal tract, which is achieved through the participation of different muscle groups regulated by the central nervous system (CNS) using the information provided by the mechanoreceptors of this tract.[1]

Reports on the states of hyposensitivity or hypersensitivity as pathophysiological mechanisms for the development of conditions associated with alterations in the function of the laryngopharyngeal tract are not rare. In obstructive sleep apnoea hypopnoea syndrome (OSA) and dysphagia, varying degrees of laryngopharyngeal hyposensitivity have been observed.[2–4]

OSA is a disorder for which the prevalence could reach 10% of the population in developed countries, with significant cardiovascular, metabolic and neurocognitive effects.[5] In OSA there is decreased laryngopharyngeal mechanosensitivity that may be related to a poor or delayed response to the airway collapse, which is characteristic of this condition.[2] A linear correlation between the degree of laryngopharyngeal hypoesthesia and the severity of OSA was found by Nguyen *et al*.[2] This sensory compromise of laryngopharyngeal reflexes may also affect the protective function of such reflexes during swallowing to prevent the passage of food to the airway. Alterations in these mechanisms might increase the risk of dysphagia and bronchial aspiration, with the subsequent impact on pulmonary and infectious complications.[6–8] The effects that OSA has on swallowing could be more dependent on sensory rather than motor functions; this is because no important compromise has been detected on motor force when evaluated through manometry.[8]

During sleep, the patient with OSA experiences repeated episodes of airway collapse in the pharynx, which can abruptly awaken the patient to restore patency.[9] In this condition, neurosensory alterations in the oropharynx, velopharynx, hypopharynx and larynx have been detected.[2] Patients with OSA have shown disturbances in the mechanoreceptors of the upper airway, possibly mediated by recurrent trauma, hypoxia or inflammation.[10]

In recent years various factors that may be involved in the pathophysiology of airway collapse in patients with OSA have been studied.[2 10] The pathophysiology of this neurological disorder is not clearly understood. Apparently, it is related to inflammatory and mechanical injuries at the oropharyngeal level secondary to airway traction as well as soft tissue trauma caused by low-frequency vibrations and snoring during sleep.[8 10] These vibrations occur chronically in OSA, which generates continuous and prolonged trauma in the oropharyngeal tissues, a situation that may lead to the loss of sensitivity, and secondarily to the decrease and loss of reflexes of the affected areas.[8 10] In addition, episodes of deoxygenation and reoxygenation, which are characteristic of OSA, favour the release of free radicals and oxidative stress, which are mechanisms that would also be involved in the alterations of the airway sensitivity.[9] The loss of reflexes and sensitivity leads to an increasing sensorimotor dysfunction that may favour the pharyngeal collapse and disruption of other pharyngeal functions, such as swallowing.[7 11]

In the study by Nguyen *et al*,[2] a 120 Hz vibration stimulation was applied to the tonsillar pillars level, velopharynx, soft palate and hypopharynx in healthy subjects and patients with OSA.[2] The results showed a sensory impairment at the velopharynx level and pharynx in patients with OSA compared with healthy controls.[2] This study was able to document that the higher the sensory impairment, the greater the severity of OSA.[2] These findings are consistent with tests performed with the administration of topical anaesthesia in the laryngopharyngeal tract in healthy subjects, which showed an increase in pharyngeal resistance to the air passage during sleep, which favoured apnoea and hypopnoea, the increased frequency of obstructive episodes of the airway, and snoring.[2 12] They are also consistent with studies that have documented disturbances in vibration and thermal sensation at the level of the upper airway in patients with OSA,[10] suggesting that these sensory disturbances play a role in the pathophysiology of this condition.

Recent studies have reported a direct relationship between snoring and impaired swallowing.[6 7] Swallowing depends on adequate pharyngeal anatomical configuration, a correct pharyngeal muscle function, and a sensitivity preserved.[6 7] It is believed that the prolongation of untreated OSA blocks the neuromuscular afferent stimulus of the upper airway and the central integration between the functions of swallowing and breathing.[6 7] In OSA, a loss of sensitivity in the upper airway can lead to an inappropriate reflex response, and a delayed swallowing reflex.[6]

Despite being a condition with multiple complications, OSA has limited treatments. The adherent use of continuous positive airway pressure (CPAP) has been shown to have beneficial effects preventing the airway's collapse, with various effects on neurocognitive, metabolic and cardiovascular complications.[5 13] CPAP adherence may be as low as 15% and could improve to 75% with increased practical support and encouragement,[14] which is not unlike other chronic respiratory diseases. In chronic obstructive pulmonary disease, half of the patients stop their treatment with inhaled corticosteroids within 6 months,[15] and only 9% continue with fluticasone propionate/salmeterol for more than 1 year.[16] In difficult asthma, the adherence to inhaled or oral steroids is less than 50%.[17] This adherence to CPAP may limit its effectiveness to prevent OSA complications. Added to the struggle experienced by patients with CPAP, it highlights the need for alternative treatments. Surgical procedures related to OSA treatment have varying results with high mortality,[13] and their effectiveness ranges anywhere from 20% to 60%.[13] A new device was developed that seeks

to treat OSA by stimulating the upper airway.[13] Several studies have shown that stimulating the hypoglossal nerve with such a device generates increased airspace in the oropharynx and hypopharynx, which decreases obstruction.[18] This device performs similar functions to a pacemaker, with a generator and an electrode that release an electrical stimulus synchronously with breathing.[13 18]

The findings on sensory impairment in OSA that were previously mentioned, which might affect the brain's capacity to prevent airway collapse during sleep, suggest a new therapeutic horizon that has not yet been explored in OSA: the use of sensory recovery interventions based on the concepts of neuroplasticity that are the basis of neurological rehabilitation.[19–22] Neuroplasticity is the property of neurons (recently documented) to establish new connections and synapses, as well as strengthen existing connections when they are stimulated.[19 20] The heightening in organised activity of a certain area of the brain leads the neurons to increase their number of dendrites, length and size, all of which creates an environment that improves communication between such neurons.[19 20] All of this strengthens synaptic connections, improving neuron efficiency and functional performance.[19 20 23] Because of this activity, the brain maps of the areas responsible for controlling the functions of the body part under stimulation change.[19 20 23] These discoveries have led to the development of effective therapeutic interventions to rehabilitate brain damage caused by stroke, trauma and other conditions.[19–24] These new interventions include functional electrical stimulation, sensory electrical stimulation, thermo-tactile stimulation and stimulation with flavours and chemicals, which have been applied to the rehabilitation of patients with motor and sensory impairments of the laryngopharyngeal tract.[19–24]

Functional electrical stimulation consists of applying electrical current of sufficient magnitude to elicit muscle contractions in the muscle groups over which it is applied.[21 22] In sensory electrical stimulation (also known as TENS), an electrical stimulation of lower intensity is applied, usually 75% of that required to contract the muscle, to activate the somatosensory system without inducing muscle contraction.[21 23–25] There is already prior knowledge on the effectiveness of such interventions in the sensory recovery of patients with oropharyngeal dysphagia.[23 24 26] Further development and evaluation of these new therapeutic interventions in OSA will require an objective method to quantify the mechanical laryngopharyngeal sensitivity.

The first device that explored the sensitivity of laryngopharyngeal mechanoreceptors was created by Aviv.[27 28] Unfortunately, there were no favourable results in terms of inter-rater reliability.[29] In subsequent studies, technical problems related to the pressure and duration of the pulses of air were corrected,[30] but no additional factors affecting stimulus intensity were considered. Recently, Giraldo-Cadavid *et al* developed the LPEER, a device with which it was possible to accurately measure the threshold of the laryngeal adductor, cough, and gag reflexes.[31–34] The initial study was conducted in patients with oropharyngeal dysphagia and high intra- and inter-rater reliability in these patients was found.[31 33] The laryngopharyngeal endoscopic esthesiometer and rangefinder (LPEER), if shown to be valid in OSA, could be very useful to quantify the laryngopharyngeal sensory alterations of these patients to better understand OSA pathophysiological mechanisms and evaluate new therapeutic interventions for this condition.

## Statement of the problem and justification

While there are results showing a relationship between altered sensitivity in the airway and OSA, these results come from research with methods that have poor inter-rater reliability, such as Aviv's method.[2 31 33] They can also have limited diagnostic validation studies for the laryngopharyngeal tract as methods to measure the vibration and thermal sensitivity.[11 35–38] Research studies on the relevance of laryngopharyngeal sensory impairment in OSA and the development of therapeutic interventions aimed to improve this impairment as primary or adjunctive therapy for OSA are needed. Such studies will require reliable and valid methods to measure the laryngopharyngeal sensitivity and the protective reflexes of the airway[11] that can also be used in clinical practice.

The LPEER[31–34] is a potential solution to the problem of the precise and accurate assessment of laryngopharyngeal sensitivity. This device had high reliability when used for the exploration of laryngopharyngeal reflexes in patients with dysphagia.[31] Therefore, it could be a new tool for evaluating and monitoring laryngopharyngeal sensory impairment in patients with OSA. However, the validity of sensory tests performed with this device has not yet been evaluated in patients with OSA; therefore, it's intra-rater and inter-rater reliability and accuracy in this population are unknown.

The high reliability obtained for the laryngopharyngeal sensory test using the LPEER on patients with dysphagia [31–34] cannot be generalised to patients with OSA, because these patients may have different reliability indexes. Any condition increasing or decreasing between-subject variance would affect the intraclass correlation coefficient (ICC) (which is the ratio of the between-subject variance over the total observed variance) of a clinical test. In other words, the reliability of a measure is tightly related to the population to which one wants to use the measure and it is not an independent property of the clinical test.[39–41] That is why it is highly recommended that reliability be calculated for each population and measurement situation with potentially different between-patient variance, such as patients with different conditions.[39–41] Therefore, a study of the reliability of the LPEER for the sensory evaluation of the upper airway in OSA is needed.

The purpose of this study is to determine the intra-rater and inter-rater reliability and accuracy of the measurement of mechanical laryngopharyngeal sensitivity in patients with OSA, using the LPEER developed by Giraldo-Cadavid and co-workers.[31–34]

### Research question

What is the reliability and accuracy of laryngopharyngeal sensory testing performed using the LPEER in patients under suspicion of OSA?

### Secondary research questions

What are the intra-rater and inter-rater Bland–Altman limits of agreement of the laryngopharyngeal sensory testing in a cohort of patients who were referred to baseline polysomnography for suspicion of OSA?

What are the intra-rater and inter-rater ICCs of the laryngopharyngeal sensory testing in a cohort of patients referred to baseline polysomnography under suspicion of OSA?

What are the intra-rater and inter-rater correlations of the laryngopharyngeal sensory testing in a cohort of patients referred to baseline polysomnography for suspicion of OSA?

What is the discriminative capacity of laryngopharyngeal sensory testing to detect severe OSA in a cohort of patients referred to baseline polysomnography under suspicion of OSA?

Is there any correlation between the severity of sensory compromise and the severity of OSA?

### Main objective

The primary objective is to evaluate the reliability and accuracy of the laryngopharyngeal sensory testing performed using the LPEER in patients referred to baseline polysomnography under suspicion of OSA.

### Specific objectives

1. To determine the intra-rater and inter-rater Bland–Altman limits of agreement of laryngopharyngeal sensory testing performed using the LPEER in patients referred to baseline polysomnography under suspicion of OSA.
2. To determine the intra-rater and inter-rater ICCs of laryngopharyngeal sensory testing performed using the LPEER in patients referred to baseline polysomnography under suspicion of OSA.
3. To calculate the intra-rater and inter-rater correlations of laryngopharyngeal sensory testing in a cohort of patients referred to baseline polysomnography under suspicion of OSA.
4. To establish the discriminative capacity of laryngopharyngeal sensory testing performed using the LPEER to detect patients with severe OSA using ROC curves and calculating the area under the ROC curve (AUC).
5. To investigate the presence of a correlation between the severity of laryngopharyngeal sensory impairment and the severity of OSA.
6. To calculate the mean/median thresholds for triggering the laryngopharyngeal reflexes and psychophysical sensitivity according to the severity of OSA and to compare them looking for significant differences between mild, moderate and severe OSA.

## METHODS AND ANALYSIS

### Type of study

This study will be prospective and double blinded, with a randomised crossover assignment of raters to determine the reliability and accuracy of the laryngopharyngeal mechanosensitivity quantification using the LPEER in patients with suspected OSA referred for a baseline polysomnography in a sleep laboratory of a tertiary care university hospital.

### Study setting

Tertiary Care University Hospital (Monocentre study) located at Bogota, Colombia.

### Population

Target population: patients over 18 years old with suspected OSA.

Accessible population: patients over 18 years old referred to the sleep laboratory of a tertiary care university hospital for baseline polysomnography for suspected OSA.

Eligible population: patients over 18 years old referred to the sleep laboratory of a tertiary care university hospital for a baseline polysomnography for suspected OSA, who meet the selection criteria and who agree to participate in the study.

### Selection criteria

#### Inclusion criteria

Patients over 18 years old referred to the sleep laboratory of a tertiary care university hospital for a baseline polysomnography for suspected OSA.

#### Exclusion criteria

To enter the study the patient must not have fulfil any of the following criteria:

1. Anticoagulation (though not a contraindication for the endoscopic laryngopharyngeal sensory test, anticoagulation is an exclusion criteria for this study to keep it a minimal-risk study).
2. Bleeding diathesis.
3. Basal awake oxygen saturation by pulse oximetry below 88%.
4. Not agreeing to participate in the study.
5. Glasgow Coma Scale below 15 (to avoid confusion with involvement of laryngopharyngeal reflexes due to a neurological disease accompanied by a decreased level of consciousness).
6. Baseline polysomnography that does not meet the validity criteria to be interpreted (according to the American Academy of Sleep Medicine).
7. Baseline polysomnography performed more than 15 days before the sensory testing. Ordinarily, the sensory testing will be performed on the same day or the day after baseline polysomnography.
8. More than 5% of total apnoea events being of central origin.
9. History of maxillofacial or pharyngeal surgery (to avoid confusion with the involvement of

laryngopharyngeal reflexes due to surgery in this region).
10. Laryngopharyngeal tract malignancies (to avoid confusion with the involvement of laryngopharyngeal reflexes due to tumours).
11. Central nervous system (CNS) surgery in the last 3 months or that has left neurological sequelae (to avoid confusion with the involvement of laryngopharyngeal reflexes due to the sequelae of CNS surgery).
12. Traumatic brain injury in the last 3 months or more than 3 months with neurological sequelae.
13. History of active neuromuscular disease that affects the muscles of the head and neck or with sequelae present at the time of the sensory testing (to avoid confusion with the involvement of laryngopharyngeal reflexes due to neuromuscular disease).
14. History of cerebrovascular disease (to avoid confusion with dysphagia or sensory compromise secondary to cerebrovascular disease).
15. Diabetes (to avoid confusion with diabetic neuropathy that compromises the laryngopharyngeal region).
16. Chronic use of systemic corticosteroids at a dose greater than or equal to 20 mg per day of prednisone or equivalent (to avoid confusion with steroid myopathy that compromises the laryngopharyngeal region).
17. Upper respiratory tract infection within 15 days prior to testing (to avoid confusion with neuropathy associated with respiratory viral disease that compromises the laryngopharyngeal region).
18. Inability to cooperate during the examination (to avoid a measurement error caused by a lack of patient cooperation).

### Enrolment of subjects

The subjects will be consecutively selected from among those who have undergone a baseline polysomnography under suspicion of OSA (figure 1).

Patients who meet the inclusion criteria and do not have exclusion criteria will be interviewed after the baseline polysomnography, to explain the study, its objectives, the tests that will be performed, and to obtain their informed consent before they enter the study (figure 1). The clinical evaluation will include questions about the conditions associated with OSA, including cardiovascular diseases, metabolic diseases, chronic respiratory diseases and dysphagia.[5–8] Subjects

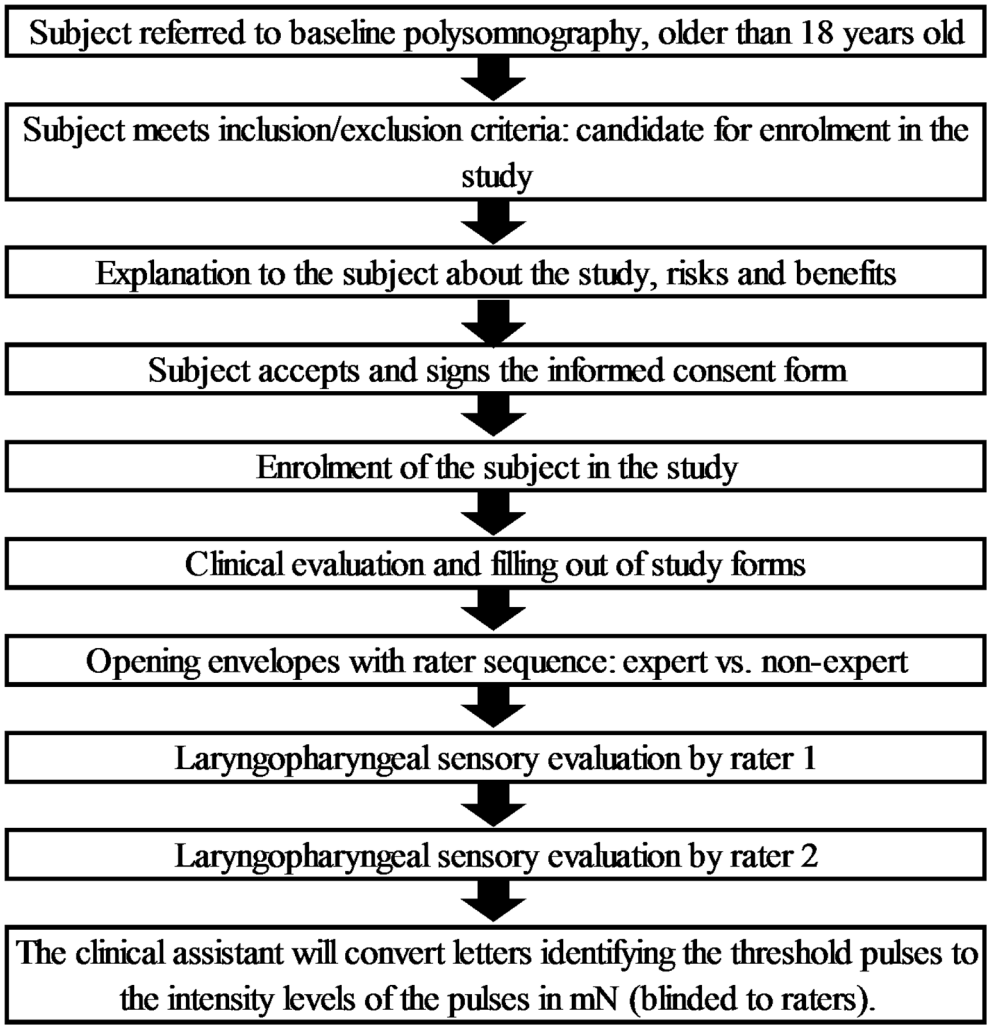


**Figure 1** Flowchart of the study.

will undergo a general physical exam including anthropometric measurements.

Patients will undergo the laryngopharyngeal sensory testing on the same day of baseline polysomnography or will be selected for the sensory testing later, at a maximum of 15 days after polysomnography (figure 1).

### Raters and sensory tests

The sensory testing will be performed according to the protocol published elsewhere[42] and attached as an appendix to this document (see online supplementary appendix 1). The sensory measurements will include thresholds for the laryngeal adductor reflex, cough reflex and gag reflex, as well as psychophysical sensory thresholds at the velopharynx and hypopharynx.

Subjects participating in the inter-rater reliability evaluation will be evaluated by two raters (an expert and a non-expert rater), each rater performing two measurements of laryngopharyngeal sensitivity two times at each side (right and left) of the corresponding laryngopharyngeal structure. All other subjects participating in the accuracy evaluation will be seen by only one expert rater who will perform three measurements of laryngopharyngeal sensitivity per subject. The varying degrees of rater experience aims to reproduce common scenarios when a new technique (like the measurement of laryngopharyngeal mechanosensitivity) is introduced to clinical practice. Raters may belong to one of the following groups:

1. Pulmonologist or otolaryngologist with experience in laryngopharyngeal sensory evaluation, who has completed more than 50 laryngopharyngeal sensory tests. He/she will be considered an expert rater.
2. Pulmonologist or otolaryngologist inexperienced in laryngopharyngeal sensory evaluation, who has completed a minimum of five and a maximum of 50 laryngopharyngeal sensory tests. He/she will be considered a non-expert rater.
3. Pulmonologist fellow who has completed the training provided to a Pulmonologist Fellow in bronchoscopy and who has performed a minimum of five and a maximum of 50 laryngopharyngeal sensory tests. He/she will be considered a non-expert rater.

The randomisation of the rater order will be generated by a computer, aiming to start sensory tests an equal number of times by an expert rater and non-expert rater to avoid bias induced by the order of examination. The two raters will perform the sensory tests sequentially and in a crossover design in each subject. The allocation sequence will be concealed using the SNOSE strategy (sequentially numbered opaque sealed envelopes). One of the authors (ARB), who does not have competing interests, will generate the allocation sequence and conceal it in envelopes. Before beginning the sensory testing an envelope containing the order of observers will be opened by the nurse assisting the procedure. Inside the envelope will be a paper stating who will be the first rater: the expert or the non-expert rater. While the first rater performs the sensory test the second rater will be in a different room for blinding purposes. There will be no communication about the testing results between the two raters or between the staff members who are helping the testing performance.

To mask the values of sensory thresholds, air pulses will be identified by a random combination of three letters instead of the intensity corresponding to the air pulse. Raters will not know the intensity corresponding to each letter's combinations. At the end of the test, an assistant will replace the letters corresponding to the threshold values by the intensity of the air pulses (figure 1) in units of force (millinewtons: mN). Sensory test raters will be masked to polysomnography results and polysomnography raters will be masked to sensory test results.

After the second rater finishes measuring, the subject will be asked about side effects presented during the test with questions specifically designed for this purpose (see online supplementary appendix 2). Adverse events (minor and serious) will be monitored during the trial. Any adverse events will be reported to the Institutional Review Board of Fundacion Neumologica Colombiana, which is independent from the investigator team and does not have competing interests. An adverse event is defined as an unwanted and harmful or potentially harmful outcome (eg, epistaxis, laryngospasm) while the subject is participating in the intervention, independently of whether the event is related or not related to the intervention. The management of any adverse event will be covered by the principal investigator of the study. The study will be audited by the Institutional Review Board of Fundacion Neumologica Colombiana.

### Statistical analysis

The data obtained will be written in an online form. Sensory thresholds will be registered by double typing, and access to the database will be secured by a password and limited to researchers. Subjects will be identified on the online database by a four-digit code. The principal investigator will be the only one with access to the information linking this code with the identity information of the subjects.

A description of the general population will be made initially. Qualitative variables will be summarised in frequencies and percentages, and the quantitative variables, if the distribution is normal, will be given as averages ± SD. If distribution is asymmetrical, they will be summarised in medians and interquartile ranges. Hypothesis tests will be two -sided, with statistical significance defined as having a p value of less than 0.05.

The intra-rater and inter-rater reliability will be assessed by the Bland–Altman limits of agreement plot and intra-rater and inter-rater ICCs. Consistency will be evaluated by the Pearson or Spearman correlation coefficient, depending on the normal or non-normal distribution of variables.

The following criteria will be used to interpret the results of ICC:[43 44]

1. ICC of 0.01 indicates 'poor' agreement.
2. ICC from 0.01 to 0.20 indicates 'slight' agreement.
3. ICC from 0.21 to 0.40 indicates 'fair' agreement.
4. ICC from 0.41 to 0.60 indicates 'moderate' agreement.
5. ICC from 0.61 to 0.80 indicates 'substantial' agreement.
6. ICC from 0.81 to 1.00 indicates 'almost prefect' agreement.

For the accuracy evaluation, the true threshold value for each reflex or psychophysical evaluation will be defined with the following criteria:

1. In subjects undergoing measurements by two raters the true threshold value will correspond to the median of all four measurements (including both raters' measurements) performed at the corresponding side of each subject.
2. In subjects undergoing measurements by only one rater the true threshold value will correspond to the median of all three measurements performed at the corresponding side of each subject.

The correlation between the sensory threshold values (true threshold values), the apnoea-hypopnoea indexes, and the desaturation indexes will be explored by the Pearson or Spearman correlation coefficient, depending on the symmetric or asymmetric distribution of the variables. The relationship between sensory thresholds and OSA severity indexes will also be explored by second-order and higher-order polynomial equations.[2]

The following criteria will be used to interpret the results of the correlation coefficients (CCs)[45]:

1. CC <0.3 weak correlation.
2. 0.3 ≥ CC <0.7 mild correlation.
3. CC ≥0.7 strong correlation.

Additionally, mean or median sensory threshold values (depending on the nature of the variable) will be determined for patients with mild, moderate or severe OSA. ANOVA or the Kruskal–Wallis test (depending on the distribution of the variable) will be used to assess significant differences between these OSA severity groups, the Bonferroni correction will be applied to the p values for post hoc contrasts within each family of tests.

The discriminative capacity of sensory thresholds to differentiate patients with and without severe OSA will be determined by plotting ROC curves using as a reference standard baseline polysomnography, which is the gold standard for OSA, and calculating the AUCs with their 95% confidence intervals (95% CIs). The 95% CI will be calculated using the binomial exact method and the AUC significance will be tested against the null hypothesis of an AUC=0.5. The discriminative capacity as well as correlations between laryngopharyngeal sensory thresholds and OSA severity indexes will be explored in subgroups of subjects with normal and abnormal mechanosensitivity.[2]

The AUC-ROC will be interpreted according to the following criteria[46]:

1. =0.5: no discrimination ability.
2. ≥0.6 to 0.69: weak discrimination ability.
3. ≥0.7 to 0.79: acceptable discrimination ability.
4. ≥0.8 to 0.89: excellent discrimination ability.
5. ≥0.9: outstanding discrimination ability.

## Sample size

To calculate the sample size, the data of the previous reliability laryngopharyngeal esthesiometer[31] study were applied to the formula for the sample size of an ICC: for an ICC of 0.86, with a 95% CI of ±0.1, two raters, an α error of 0.05 for 28 subjects, rounded to 30 to compensate for possible losses.[47 48] For the ROC curves we calculated that the required sample size was 117 subjects using the equation proposed by Machin *et al*,[49] with an estimated proportion of unwell subjects of 0.3, a sensitivity of 0.9, a specificity of 0.7, and a 95% CI width of 0.08 per side. Based on these considerations, the final sample size for the full study will be 117 subjects.

We will perform an interim analysis on the first 30 subjects to adjust the final sample size.

## Software

We will use the following software for the statistical analysis: Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA); MedCalc, version 16.8 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2016) and IBM-SPSS Statistics software, version 22 (Armonk, NY, USA).

## Ethics and dissemination

The study will follow the Declaration of Helsinki principles as well as the national (Colombian) legal regulations about research in human subjects. Scientific publications tracking more than 40 000 laryngopharyngeal sensory tests have not reported side effects requiring hospitalisation, emergency room referral or prolonged observation at the test site.[50–52] The reported side effects have occurred with a frequency of less than 1 in 1000 and consisted of mild to moderate discomfort and short-term epistaxis or dizziness.

To preserve the confidentiality of the participants, the identity of each subject will be anonymised in the study database by a four-digit ID code. Only the principal investigator will have the link between the identity of each subject and the database ID code.

This study was approved by the Institutional Review Board of Fundacion Neumologica Colombiana. It has an informed consent form, which must be completed and signed by each study subject after an interview, in which the risks and benefits of the test, as well as the purpose and justification of this study will be explained. Subjects will have the choice to not participate in the study or to withdraw voluntarily at any time, without affecting the medical care that is provided by the institution.

This study was registered at Clinical Trials (ClinicalTrials.gov ID: NCT03109171) before entering the first patient into the study.

The results of this work will be presented at local, national and international conferences. The final report of this research will be summarised in an original article that will be published in a peer-reviewed journal indexed in the ISI-Web of Knowledge and PubMed.

This research will serve as a degree project for two internal medicine residents.

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**Contributors** LFGC with input from the other investigators conceived this study. DMPO with DCCG, ARB and LFGC developed the protocol and study materials with input from all investigators. MAB and LV will help with the recruitment of patients and analysis of polysomnographies. ARB will perform the statistical analysis. All authors approved the final manuscript.

**Funding** This work is funded by Colciencias Colombia, grant contract: FP44842-225-2015, project number: 123064239652; and University of La Sabana grant: MED1642013.

**Competing interests** The laryngopharyngeal endoscopic esthesiometer and rangefinder (LPEER), which will be validated in this study, was granted a patent by the Superintendencia de Industria y Comercio de Colombia and a Patent Cooperation Treaty (PCT) by the World Intellectual Property Organization. The financial rights of the patent belong to University of La Sabana (one of this study's funders). LFGC is one of the inventors of LPEER and could potentially receive future royalties from this patent. The study funders will not participate in the conduction of the study, statistical analysis, writing of the manuscript or decision to submit the report for publication.

**Ethics approval** Comite de Etica en Investigacion, Fundacion Neumologica Colombiana.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** This is not an original research article but a study protocol.



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