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Use of CPAP to Reduce Arterial Stiffness in Moderate to Severe Obstructive Sleep Apnea, Without Excessive Daytime Sleepiness (STIFFSLEEP): an observational cohort study protocol.

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Title: Use of CPAP to Reduce Arterial Stiffness in Moderate to Severe Obstructive Sleep Apnea, Without Excessive Daytime Sleepiness (STIFFSLEEP): an observational cohort study protocol.

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ABSTRACT

Introduction. Obstructive sleep apnea (OSA) is associated with increased cardiovascular morbidity and mortality. Arterial stiffness, evaluated by pulse wave velocity (PWV), is related to atherosclerosis and cardiovascular (CV) risk. It has been reported that arterial stiffness is higher in patients with OSA than in healthy control groups, with improvement after treatment with Continuous Positive Airway Pressure (CPAP). It is still not known whether the same effect occurs in patients with OSA without daytime hypersomnolence.

Methods and analysis. This study aims to evaluate the effect of CPAP therapy in a cohort of patients with moderate to severe OSA; the effect on the subcohorts of sleepy and of non-sleepy patients will be compared. A systematic, consecutive, non-randomized sample of patients proposed to CPAP will be recruited from a single outpatient sleep clinic (Centro Hospitalar de Lisboa Central - CHLC, Portugal). Eligible patients are male, younger than 65 years and with confirmed moderate to severe (AHI>15/h) OSA. Other sleep disorders, diabetes or any CV disease beyond hypertension are exclusion criteria. At baseline, patients proposed to CPAP will have a clinical evaluation, a polygraphic study (cardiorespiratory, level 3), analytical blood evaluation count and the Epworth Sleepiness Scale (ESS) will be applied, score above 10 defines sleepiness. Participants will be proposed to undertake assessment of carotid-femoral pulse wave velocity (CF-PWV) and 24 hours evaluation of Ambulatory Blood Pressure Monitoring (ABPM), before and after 4 months of CPAP therapy. The compliance to CPAP and the effectiveness of CPAP will be assessed. We considered a main outcome changes on CF-PWV over time.

Ethics and dissemination. This protocol was approved by the Ethics Committees of both CHLC (ref. 84/2012) and NOVA Medical School (nr.36/2014/CEFCM), Lisbon. Informed, written consent will be obtained. Results will be presented at conferences and published in peer-reviewed journals.

Registration. ClinicalTrials.gov ID: NCT02273089.

BACKGROUND:

Obstructive sleep apnea (OSA), if left untreated, is associated with high cardio-vascular (CV) morbidity and mortality[1,2,3].

The diagnosis of OSA syndrome is based on the presence of symptoms suggestive of the disorder, with confirmation by polygraphic sleep study[4,5]. The Epworth Sleepiness Scale (ESS) is frequently used to evaluate the presence of excessive daytime somnolence[6]. Other symptoms besides sleepiness are nonspecific (decreased concentration, irritability)[4,5], hampering the diagnosis.

Continuous positive airway pressure (CPAP) is the standard therapy for patients with symptomatic OSA, but CPAP therapy for asymptomatic patients is consensual only when the apnea-hypopnea index (AHI) is above 30/hour[7].

In some studies, a greater severity in polysomnographic parameters has been reported in sleepy patients than in those who are not sleepy[8,9].

Considering the relationship between OSA and CV disease, namely arterial hypertension or CV risk, it is also less evident in patients with less symptoms. The effect of CPAP in lowering blood pressure (BP) levels seems greater in those patients with more severe OSA and sleepiness than in patients without it[10].

Changes in arterial distensibility (stiffness) are measurable and are one of the earliest manifestations of functional and structural damage to the vessel wall in the process of atherosclerosis. Arterial stiffness, evaluated by pulse wave velocity (PWV), is used as an early marker of organ damage in hypertensive patients[11]. It predicts cardiovascular events, beyond classical CV risk factors, and can be considered as an intermediate endpoint for CV events[11]. Carotid-femoral pulse wave velocity (CF-PWV), i.e. the speed of the pulse as it travels from the heart to the carotid and the femoral artery, is the most commonly used non-invasive method to assess arterial stiffness and is considered as the gold standard[12,13].

CF-PWV value is augmented in patients with OSA, even in those without high BP[15,16]. It is augmented even in the less symptomatic, severe patients. Improvement after treatment with CPAP occurs[17] but the effect in patients without sleepiness is less known and still debatable[18].

This research protocol aims to assess the progression of arterial stiffness in two cohorts of patients with OSA, either sleepy or non-sleepy, while undergoing treatment with CPAP. We hypothesize that a measurable improvement on the control of CF-PWV can be detected over time independently of sleepiness.

METHODS AND ANALYSIS

Study design

This project involves the prospective, observational study of a systematic, consecutive, non-randomized sample of adult male patients diagnosed with OSA and proposed to CPAP, recruited from a single outpatient sleep clinic (Centro Hospitalar de Lisboa Central - CHLC, Lisbon, Portugal).

Eligible patients are male, younger than 65 years and with confirmed moderate to severe (AHI>15/h) OSA. The sample is divided into two cohorts according to the Epworth sleepiness scale (ESS) and the effect of CPAP therapy on sleepy and of non-sleepy patients will be compared.

The primary outcome measure is the variation of CF-PWV after therapy with CPAP.

The secondary outcomes are:

- the decline of the severity of OSA assessed by the AHI and oxygen saturation,

- ambulatory blood pressure measurements (ABPM) variations

- the lipidic profile and metabolic changes after treatment with CPAP.

Each patient will be its own control, for ethical reasons. The effect of a three months trial of CPAP will be assessed.

The study is exploratory in nature and is based on a convenience sample, limited by the number of eligible patients consenting to participate. Recruitment started on October 2012 and data collection is expected to end by June 2016.

Inclusion criteria

Patients referred to the Outpatient Sleep Clinic of the Pneumology Department, CHLC, Lisbon, due to snoring or other complaints suggestive of OSA, are eligible for the study if they are male, younger than 65 years old and OSA of moderate to severe degree (AHI> 15 / h) is confirmed.

Exclusion criteria:

Patients are excluded if they have any established heart disease beyond arterial hypertension, peripheral vascular disease or a history of cardiovascular events; if they have other chronic diseases, excessive alcohol intake (> 80 g / day), chronic ingestion of hypnotics or other clinically identified sleep disorders. Some comorbidities are allowed, namely metabolic syndrome. Antihypertensive medication and treatment of dyslipidaemia are admitted.

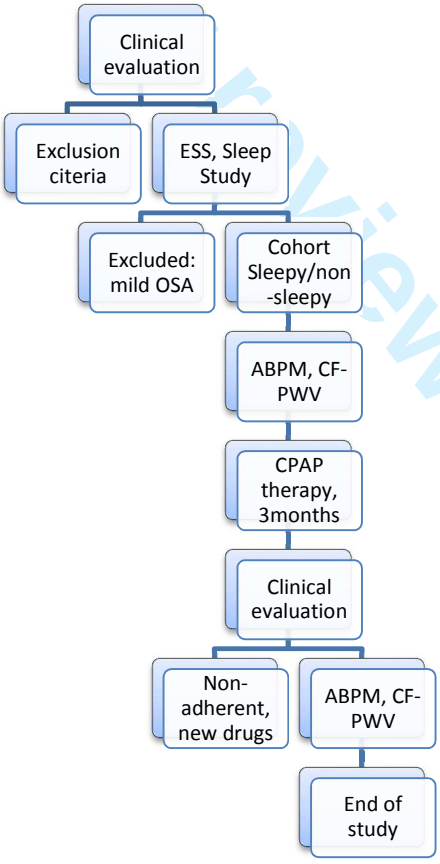

Fig.1 Design of the study.

Baseline evaluation

The protocol of the Outpatient Sleep Clinic requires that patients referred due to snoring or other complaints suggestive of OSA will be the proposed a clinical evaluation, in which the suspected OSA is confirmed. The severity of sleepiness is assessed using ESS. The presence of comorbidities and pharmacotherapy in progress is evaluated. Anthropometric data regarding adiposity and vital signs are collected.

For the diagnosis of OSA, every patient is submitted to a polygraphic sleep study type 3, cardiorespiratory (Embletta® system, Broomfield, USA), a technical outpatient registration, which includes the continuous recording from nasal cannulae (pressure and flow), thoracic-abdominal motion, pulse oxymetry, electrocardiogram and body position sensor. Results from the sleep studies are analyzed by two trained technicians, using standard criteria[19].

ESS is applied by the principal investigator, as part of the first clinical evaluation after referral.

An analytical screening evaluation will be done, including HbA1c, fasting glucose, total cholesterol, HDL-col, LDL-col and triglycerides.

Patients will be further proposed to undertake the following diagnostic techniques:

- body composition assessment, estimating the proportion of fat mass ref by tetrapolar bioimpedance, operated by the same technician (OMRON HBF- 510, Texas, USA);
- ECG (Page Writer TC 30, Philips), operated by the same technicians, as per institutional protocol;
- Ambulatory Blood Pressure Monitoring (ABPM), with Ambulatory Blood Pressure - SpaceLabs model 90207®, according current guidelines.
- noninvasive automatic assessment of CF-PWV (Complior®, Colson, Paris, France), performed by the same operator.

The assessment of pulse wave velocity is performed with the patient in the supine position, and the values take in account the blood pressure value, which in turn is measured first, at least twice at the upper arm and the average values obtained. CF-PWV is usually measured using the «foot-to-foot» velocity method from the pressure

waveforms, obtained using surface tonometry probes at the right common carotid artery and the right femoral artery. The time delay (Dt, or transit time) is measured between the «foot» of these two waveforms. The distance D covered by the waves is incorporated to the skin distance the two tonometry probes, i.e. the common carotid artery and the common femoral artery. PWV is estimated as PWV=D/Dt (m/s)[12]. Measurements will always be performed in the morning, without prior intake of tea or coffee.

Participants will be classified by the results of the ESS as Sleepy (ESS> 10) and Non-sleepy (ESS ≤ 10).

Patients will be classified as normotensive if the average systolic and diastolic blood pressure values obtained by ABPM are within normal limits. Patients will be classified as hypertensive if already on antihypertensive medication or if the average blood pressure values obtained by ABPM are above ≥130 mmHg of systolic and/or ≥80 mmHg diastolic blood pressure[11].

Dyslipidaemia, glucose intolerance and metabolic syndrome will be diagnosed according to the criteria of current guidelines[20].

Standard CPAP Intervention

According to current clinical recommendations,[4] patients with moderate or severe OSA, are proposed therapy with noninvasive ventilation by CPAP. Additionally, patients receive sleep hygiene advice.

CPAP titration is performed using an automatic auto-CPAP device (ResMed S9 AutoSet, California, USA)) for 3 nights. The definitive value of CPAP is the amount of pressure that eliminates events in about 95% of the total sleep time (95th percentile), once confirmed there is not significant leakage and nasal mask is used[21].

Patients will be evaluated at the end of the first, second and third months, for assessment of the compliance with and the effectiveness of the intervention. The compliance to the CPAP is measured by the automatic reading of the CPAP's card; a patient is considered as compliant when using CPAP at least 4h/day as the average of

at least 70% of nights. CPAP is considered as effective if the AHI falls below 10/h and the reduction is at least 50% of the baseline AHI[22].

The presence of nasal complaints or changes on the equipment will be considered.

Follow up assessments

After 4 months of standard CPAP intervention, the following evaluation will be proposed:

- Clinical evaluation (ESS),
- assessment of compliance to CPAP and evaluation of the effectiveness of therapy,
- body composition assessment,
- ABPM, CF-PWV and analytical evaluation (similar to baseline).

At this point, patients are excluded from follow up if significant weight loss (greater than 5%) occurs[4], if new drugs had been prescribed or new diseases are diagnosed.

Statistical analyses

Continuous variables are described with median and interquartile range (IQR, 25th percentile to 75th percentile) or extreme values, and were compared using the Mann-Whitney test. Categorical variables are described as percentage and were compared with Pearson's chi-square test or Fisher's exact test, and stratified Mantel-Haenszel analysis was performed as appropriate.

The primary outcome measure (CF-PWV) will be compared before and after therapy with CPAP for three months. Analysis will be performed as per intention to treat, considering the outcome measures both as continuous and categorical variables. Continuous variables will be compared as paired samples, using parametric or non-parametric tests as adequate. Categorized outcome measures will be used to estimate incidence rates of the outcomes and relative risk estimates, both with 95% confidence intervals, using www.openepi.com.

Univariable logistic regression analysis will be performed to explore associations between outcomes and exposures, in order to assess factors influencing the success of the intervention.

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Competing interests None

Patient consent Obtained

Provenance and peer review Not commissioned; externally peer reviewed

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Primary Subject Heading:	Respiratory medicine
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Keywords: obstructive sleep apnoea, sleepiness, arterial stiffness, pulse wave velocity

ABSTRACT

Introduction. Sleepiness is a cardinal symptom in obstructive sleep apnoea (OSA), but most patients have unspecific symptoms. Arterial stiffness, evaluated by pulse wave velocity (PWV), is related to atherosclerosis and cardiovascular (CV) risk. It has been reported that arterial stiffness is higher in patients with OSA, with improvement after treatment with Continuous Positive Airway Pressure (CPAP). It is still not known whether the same effect occurs in patients with OSA without sleepiness.

Methods and analysis. This study aims to evaluate the effect of CPAP therapy in a cohort of patients with moderate to severe OSA; the effect on the subcohorts of sleepy and of non-sleepy patients will be compared. A systematic, consecutive, non-randomized sample of patients proposed to CPAP will be recruited from a single outpatient sleep clinic (Centro Hospitalar de Lisboa Central - CHLC, Portugal). Eligible patients are male, younger than 65 years and with confirmed moderate to severe OSA, Apnea-Hypopnea Index (AHI) above 15/h. Other sleep disorders, diabetes or any CV disease beyond hypertension are exclusion criteria. At baseline, patients will have a clinical evaluation, including Epworth Sleepiness Scale (ESS). Sleepiness is defined as ESS above 10. OSA will be confirmed by polygraphic study (cardiorespiratory, level 3). Patients with will be proposed to undertake assessment of carotid-femoral pulse wave velocity (cfPWV) and 24 hours evaluation of Ambulatory Blood Pressure Monitoring (ABPM), before and after 4 months of CPAP therapy. The compliance to CPAP and the effectiveness of CPAP will be assessed. We considered a main outcome changes on cfPWV over time.

Ethics and dissemination. This protocol was approved by the Ethics Committees of both - CHLC (ref. 84/2012) and NOVA Medical School (nr.36/2014/CEFCM), Lisbon. Informed, written consent will be obtained. Results will be presented at conferences and published in peer-reviewed journals.

Registration. ClinicalTrials.gov ID: NCT02273089.

Strengths and limitations of this study protocol

- This study protocol allows obtaining new evidence about the effectiveness of CPAP to decrease cardio-vascular risk in sleepy and non-sleepy male patients with OSA.
- The strengths of this protocol are the controlled, clinic-based setting, the use of standardized instruments to characterize OSA and sleepiness, and the use of standardized quantitative early indicators of cardio-vascular risk (cf-PWV).
- This observational study protocol has the limitations of the convenience, non-randomized sample and the exclusion of patients with mild OSA or elder patients.

BACKGROUND:

Obstructive sleep apnoea (OSA), if left untreated, is associated with high cardio-vascular (CV) morbidity and mortality[1,2,3].

The diagnosis of OSA syndrome is based on the presence of symptoms suggestive of the disorder, with confirmation by polygraphic sleep study[4,5]. The Epworth Sleepiness Scale (ESS) is a validated questionnaire to evaluate the presence of excessive daytime somnolence[6]. Other symptoms besides sleepiness are nonspecific (decreased concentration, irritability)[4,5], hampering the diagnosis.

Continuous positive airway pressure (CPAP) is the standard therapy for patients with symptomatic OSA, but CPAP therapy for asymptomatic patients is consensual only when the apnea-hypopnea index (AHI) is above 30/hour[7].

In some studies, a greater severity in polysomnographic parameters has been reported in sleepy patients than in those who are not sleepy[8,9].

Considering the relationship between OSA and CV disease, namely arterial hypertension or CV risk, it is also less evident in patients with less symptoms. The effect of CPAP in lowering blood pressure (BP) levels seems greater in those patients with more severe OSA and sleepiness than in patients without it[10].

Changes in arterial stiffness are measurable and are one of the earliest manifestations of functional and structural damage to the vessel wall in the process of atherosclerosis. Arterial stiffness, evaluated by pulse wave velocity (PWV), is used as an early marker of

organ damage in hypertensive patients[11]. It predicts cardiovascular events, beyond classical CV risk factors, and can be considered as an intermediate endpoint for CV events[12]. Carotid-femoral pulse wave velocity (cf-PWV), i.e. the speed of the pulse as it travels from the heart to the carotid and the femoral artery, is the most commonly used non-invasive method to assess arterial stiffness and is considered as the gold standard[12,13].

Increased arterial stiffness depends on various conditions, of which blood pressure and ageing are dominant, but also other CV risk factors.

cf-PWV value is augmented in patients with OSA, even in those without high BP[15,16]. It is augmented even in the less symptomatic, severe patients. Improvement after treatment with CPAP occurs[17] but the effect in patients without sleepiness is less known and still debatable[18].

This research protocol aims to assess the progression of arterial stiffness in two cohorts of patients with OSA, either sleepy or non-sleepy, while undergoing treatment with CPAP. We hypothesize that a measurable improvement on the control of cf-PWV can be detected over time independently of sleepiness.

Secondarily, as obesity and metabolic syndrome are common findings in OSA, changes of metabolic parameters after CPAP will be investigated[19].

METHODS AND ANALYSIS

Study design

This project involves the prospective, observational study of a systematic, consecutive, non-randomized sample of adult male patients diagnosed with OSA and proposed to CPAP, recruited from a single outpatient sleep clinic (Centro Hospitalar de Lisboa Central - CHLC, Lisbon, Portugal).

Eligible patients are male, younger than 65 years and with confirmed moderate to severe OSA (AHI>15/h), who are residents in Greater Lisbon. The sample is divided into two cohorts according to the Epworth sleepiness scale (ESS) and the effect of CPAP

therapy on sleepy (ESS>10) and of non-sleepy (ESS≤10) patients will be compared (Fig.1).

We considered as a primary outcome measure the variation of cf-PWV after therapy with CPAP.

The secondary outcomes are:

- the decline of the severity of OSA assessed by the AHI

- ambulatory blood pressure measurements (ABPM) variations

- the lipid profile and metabolic changes after treatment with CPAP.

Each patient will be its own control, for ethical reasons. The effect of a four months trial of CPAP will be assessed.

The study is exploratory in nature and is based on a convenience sample, limited by the number of eligible patients consenting to participate. Sample size was estimated for the primary outcome measure as an effective sample of 70 patients to demonstrate a reduction on cfPWV from 12 m/s to 11 m/s (before and after 4 months on CPAP), with 95% confidence and power 80%. Based on the previous assessment of the institutional experience on compliance with CPAP and with outpatient clinic consultations, the recruited sample was increased in 15% to 80 patients.

Recruitment started on October 2012 and data collection is expected to end by June 2016.

Inclusion criteria

Patients referred to the Outpatient Sleep Clinic of the Pneumology Department, CHLC, Lisbon, due to snoring or other complaints suggestive of OSA, are eligible for the study if they are male, younger than 65 years old, residents in Greater Lisbon and OSA of moderate to severe degree (AHI> 15 / h) is confirmed. The Epworth questionnaire is to be completed during the first consultation, and data on anamnesis and clinical examination will be collected.

Exclusion criteria:

Patients are excluded if they have a history of cardiovascular events; established heart disease (beyond arterial hypertension); peripheral vascular disease. Smoking (over 10 units pack year) or alcohol intake (> 80 g / day) are not allowed. Other severe chronic illnesses (assessed by chronic use of medication), chronic ingestion of hypnotics or other sleep disorders (identified clinically) are exclusion criteria.

Patients with diabetes were excluded, as they might have vascular involvement. In patients with glucose intolerance, oral glucose tolerance test will be performed.

Some comorbidities, are allowed, namely hypertension and metabolic syndrome. Antihypertensive medication and treatment of dyslipidemia are allowed.

Baseline evaluation

The protocol of the Outpatient Sleep Clinic requires that patients referred due to snoring or other complaints suggestive of OSA will be the proposed a clinical evaluation, in which the suspected OSA is confirmed. The presence of comorbidities and pharmacotherapy in progress is evaluated. The duration of either comorbidity or pharmacotherapy was not considered to be collected as including them as accountable intervening factors would greatly increase the sample size. Anthropometric data regarding adiposity and vital signs are collected.

For the diagnosis of OSA, every patient is submitted to a polygraphic sleep study type 3, cardiorespiratory (Embletta® system, Broomfield, USA), a technical outpatient registration, which includes the continuous recording from nasal cannula (pressure and flow), thoracic-abdominal motion, pulse oximetry, electrocardiogram and body position sensor. Results from the sleep studies are analyzed by two trained technicians, using standard criteria[20].

ESS is applied by the principal investigator, as part of the first clinical evaluation after referral.

An analytical screening evaluation will be done, including HbA1c, fasting glucose, total cholesterol, HDL, LDL and triglycerides; and an ECG (Page Writer TC 30, Philips, Eindhoven, NL), operated by the same technicians, as per institutional protocol.

Patients will be further proposed to undertake the following diagnostic techniques:

- Ambulatory Blood Pressure Monitoring (ABPM), with Ambulatory Blood Pressure - SpaceLabs model 90207® (Issaquah, WZ, USA), according current guidelines.

Patients will be classified as normotensive if the average systolic and diastolic BP values obtained by ABPM are within normal limits; and will be classified as hypertensive if already on antihypertensive medication or if the average BP values obtained by ABPM are above ≥130 mmHg of systolic and/or ≥80 mmHg diastolic blood pressure[11]. Patients with successfully pharmacologically controlled hypertension are considered normotensive for this matter.

- noninvasive assessment of cf-PWV (Complior®, Colson, Paris, France), performed by the same operator.

At first, weight and height will be assessed. Body mass index (BMI) is calculated using the following formula – weight (kg) divided by height (m^2).

Office BP is measured using a semi-automatic device (Omron HEM-907XL, Omron Healthcare, Bannockburn, II, USA) with the use of an appropriate cuff in a quiet room in a seated position. The patient did not smoke or take any stimulant substance during the 3 h prior to the BP assessment. Two measurements are taken with a minimum of 3-min intervals and the average was considered. When large differences existed among them, additional measurements were performed. Pulse pressure is calculated as SBP minus DBP.

For the evaluation of cf-PWV is used the Complior device. User procedures and patient management were followed according to the recently published expert consensus document[14]. Briefly, measurement is done at the right common carotid and right common femoral arteries after 10 min of rest in supine position. Two pressure waveforms were transcutaneously recorded at the base of the neck for the right common carotid artery and over the right femoral artery. Pulse transit time was

determined as the average of 10 consecutive beats. The straight-line distance between the measurement sites is obtained by surface measurement, with the patient in the supine position. cf-PWV is calculated as the ratio of distance to transit time. The threshold for cf-PWV evaluated by the full direct carotid-to-femoral distance has been revised in a recent consensus to 10 m/s[14], in order to normalize cf-PWV values according to the arterial pathway.

Dyslipidemia, glucose intolerance and metabolic syndrome will be diagnosed according to the criteria of current guidelines[21].

Standard CPAP Intervention

According to current clinical recommendations,[4] patients with moderate or severe OSA, are proposed therapy with noninvasive ventilation by CPAP. Additionally, patients receive sleep hygiene advice.

CPAP titration will performed using an automatic auto-CPAP device (ResMed S9 AutoSet, California, USA) for 3 nights. The definitive value of CPAP is the amount of pressure that eliminates events in about 95% of the total sleep time (95th percentile), once confirmed there is not significant leakage and nasal mask is used[22].

Patients will be evaluated monthly, for assessment of compliance and effectiveness of the intervention. The compliance to the CPAP will be measured by downloading CPAP's card; a patient is considered as compliant when using CPAP at least 4h/day as the average of at least 70% of nights. CPAP is considered as effective if the AHI falls below 5/h[23].

The presence of nasal complaints or changes on the equipment will be considered.

Follow up assessments

After 4 months of standard CPAP intervention, the following evaluation will be proposed:

- Clinical evaluation (ESS)
- assessment of compliance to CPAP and evaluation of the effectiveness of therapy

- BMI, ABPM, cf-PWV and analytical evaluation (similar to baseline)

At this point, patients are excluded from follow up if they were not compliant to CPAP. Also, if significant weight loss (greater than 10%) occurs[4], if new drugs had been prescribed or new diseases are diagnosed.

Statistical analyses

Continuous variables are described with median and interquartile range (IQR, 25th percentile to 75th percentile) or extreme values, as previous evaluations have evidenced that a normal distribution of values was not to be expected. The comparisons between sleepy and non-sleepy subjects at each evaluation moment are made using the Mann-Whitney test. Categorical variables are described as percentage and were compared with Pearson's chi-square test or Fisher's exact test, and stratified Mantel-Haenszel analysis was performed as appropriate. Measurements on the cohort subjects before and after 4 months of CPAP will be analyzed using generalized linear mixed-effects models for repeated longitudinal data, to explore associations between outcomes and exposures, in order to assess factors influencing the success of the intervention.

The primary outcome measure (cf-PWV) will be compared before and after therapy with CPAP for four months. Analysis will be performed as per intention-to-treat.

Categorized outcome measures will be used to estimate incidence rates of the outcomes and relative risk estimates, both with 95% confidence intervals.

Discussion:

The results of this study will disclose differences in the CV risk and the response to CPAP therapy between patients with and without daytime sleepiness. In the subgroup of less symptomatic patients, the benefits of CPAP have to be identified.

This observational study protocol has the limitations of the convenience, non-randomized sample, and the exclusion of patients with mild OSA or elder patients. The strengths of this protocol are the controlled, clinic-based setting, the use of

standardized instruments to characterize OSA and sleepiness, and the use of standardized quantitative early indicators of cardio-vascular risk (cfPWV), a useful indicator of subclinical CV disease.

Ethics and dissemination

This protocol was approved by the Ethics Committees of both CHLC (ref. 84/2012) and NOVA Medical School (nr.36/2014/CEFCM), Lisbon. It has been registered as STIFFSLEEP at ClinicalTrials.gov (ID: NCT02273089). Informed, written consent will be obtained. Results will be presented at conferences and published in peer-reviewed journals.

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Author's contributors: Alexandra Mineiro developped the conceptual clinical background of the protocol; Pedro Marques-da-Silva provided clinical background and methodological input for the cardiovascular risk assessment; Marta Alves and Daniel Virella contributed to the epidemiologic design, causal framework and proposed epidemiologic and statistical analyses; Maria João Marques-Gomes and João Cardoso provided scientific overview. All authors provided intellectual input into the editing of the manuscript and preparation of publication.

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Competing interests None

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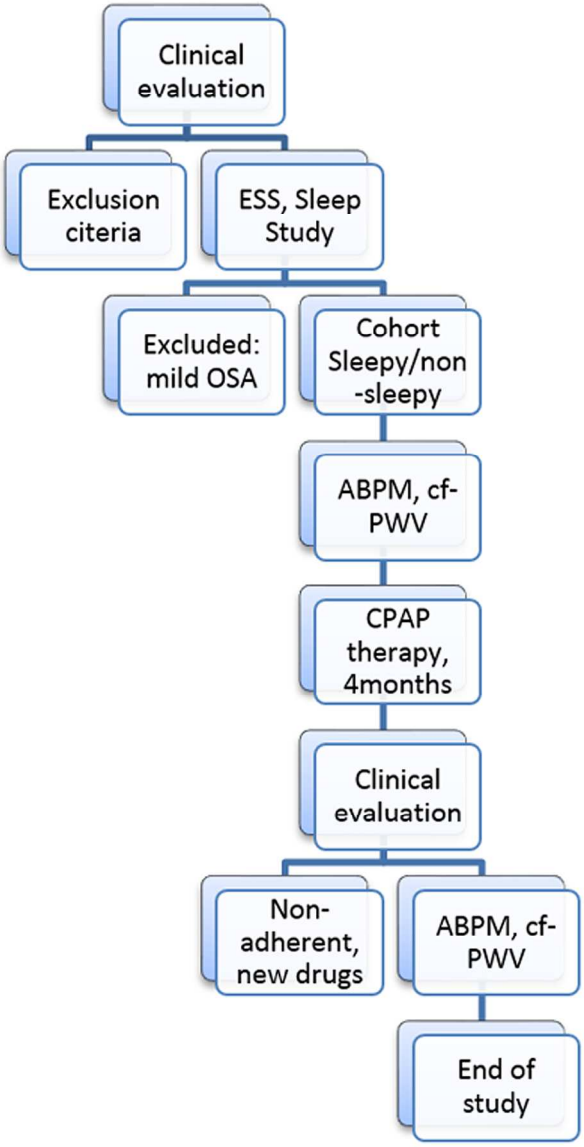

Fig.1 Study design
173x233mm (300 x 300 DPI)

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Use of CPAP to Reduce Arterial Stiffness in Moderate to Severe Obstructive Sleep Apnoea, Without Excessive Daytime Sleepiness (STIFFSLEEP): an observational cohort study protocol.

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Keywords:	Obstructive sleep apnea, Sleepiness, Pulse wave velocity, Arterial stiffness

Title: Use of CPAP to Reduce Arterial Stiffness in Moderate to Severe Obstructive Sleep Apnoea, Without Excessive Daytime Sleepiness (STIFFSLEEP): an observational cohort study protocol.

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Keywords: obstructive sleep apnoea, sleepiness, arterial stiffness, pulse wave velocity

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ABSTRACT

Introduction. Sleepiness is a cardinal symptom in obstructive sleep apnoea (OSA) but most patients have unspecific symptoms. Arterial stiffness, evaluated by pulse wave velocity (PWV), is related to atherosclerosis and cardiovascular (CV) risk. Arterial stiffness was reported to be higher in patients with OSA, improving after treatment with Continuous Positive Airway Pressure (CPAP). This study aims to assess whether the same effect occurs in patients with OSA and without sleepiness.

Methods and analysis. This observational study assesses the CV effect of CPAP therapy on a cohort of patients with moderate to severe OSA; the effect on the subcohorts of sleepy and of non-sleepy patients will be compared. A systematic, consecutive sample of patients proposed to CPAP will be recruited from a single outpatient sleep clinic (Centro Hospitalar de Lisboa Central - CHLC, Portugal). Eligible patients are male, younger than 65 years, with confirmed moderate to severe OSA, Apnoea-Hypopnea Index (AHI) above 15/h. Other sleep disorders, diabetes or any CV disease other than hypertension are exclusion criteria. Clinical evaluation at baseline includes Epworth Sleepiness Scale (ESS), and sleepiness is defined as ESS above 10. OSA will be confirmed by polygraphic study (cardiorespiratory, level 3). Participants are proposed to undertake assessment of carotid-femoral pulse wave velocity (cf-PWV) and 24 hours evaluation of Ambulatory Blood Pressure Monitoring (ABPM), at baseline and after 4 months of CPAP therapy. Compliance and effectiveness of CPAP will be assessed. The main outcome is variation of cf-PWV over time.

Ethics and dissemination. This protocol was approved by the Ethics Committees of CHLC (ref. 84/2012) and NOVA Medical School (nr.36/2014/CEFCM), Lisbon. Informed, written consent will be obtained. Its results will be presented at conferences and published in peer-reviewed journals.

Registration. ClinicalTrials.gov ID: NCT02273089.

Strengths and limitations of this study protocol

- This study protocol allows us to obtain new evidence on the effectiveness of CPAP in decreasing cardiovascular risk in sleepy and non-sleepy male patients with OSA.
- The strengths of this protocol are the controlled, clinic-based setting, the use of standardized instruments to characterize OSA and sleepiness, and the use of standardized quantitative early indicators of cardiovascular risk (cf-PWV).
- This observational study protocol has the limitations of the convenience, non-randomized sample and the exclusion of patients with mild OSA or older patients.

BACKGROUND

Obstructive sleep apnoea (OSA), if left untreated, is associated with high cardiovascular (CV) morbidity and mortality[1,2,3].

The diagnosis of OSA syndrome is based on the presence of symptoms suggestive of the disorder, with confirmation by polygraphic sleep study[4,5]. The Epworth Sleepiness Scale (ESS) is a validated questionnaire that evaluates the presence of excessive daytime somnolence[6]. Other symptoms besides sleepiness are nonspecific (decreased concentration, irritability) [4,5], hampering diagnosis.

Continuous positive airway pressure (CPAP) is the standard therapy for patients with symptomatic OSA, but CPAP therapy for asymptomatic patients is consensual only when the apnoea-hypopnea index (AHI) is above 30/hour[7].

In some studies, a greater severity in polysomnographic parameters has been reported in sleepy patients in comparison with those who are not sleepy[8,9].

Considering the relationship between OSA and CV disease, namely arterial hypertension or CV risk, it is also less evident in patients with fewer symptoms. The effect of CPAP in lowering blood pressure (BP) levels seems greater in patients with more severe OSA and sleepiness than in those without it[10].

Changes in arterial stiffness are measurable and are one of the earliest manifestations of functional and structural damage to the vessel wall in the process of atherosclerosis. Arterial stiffness, evaluated by pulse wave velocity (PWV), is used as an early marker of

organ damage in hypertensive patients[11]. It predicts cardiovascular events, beyond classical CV risk factors, and can be considered as an intermediate endpoint for CV events[12]. Carotid-femoral pulse wave velocity (cf-PWV), i.e. the speed of the pulse as it travels from the heart to the carotid and the femoral artery, is the most commonly used non-invasive method to assess arterial stiffness and is considered the gold standard[12,13].

Increased arterial stiffness depends on various conditions, of which blood pressure and ageing prevail, but also on other CV risk factors.

cf-PWV value is augmented in patients with OSA, even in those without high BP[14,15]. It is increased even in the less symptomatic, severe patients. There is an improvement after treatment with CPAP[16] but the effect in patients without sleepiness is less well known and still controversial[17].

This research protocol aims to assess the variation of arterial stiffness in two cohorts of patients with OSA, either sleepy or non-sleepy, while undergoing treatment with CPAP. We hypothesize that a measurable improvement on cf-PWV can be detected over time, independently of sleepiness.

METHODS AND ANALYSIS

Study design

This project involves the prospective, observational study of a systematic, consecutive, non-randomized sample of adult male patients diagnosed with OSA and proposed to CPAP, recruited from a single outpatient sleep clinic (Centro Hospitalar de Lisboa Central - CHLC, Lisbon, Portugal).

The sample is divided into two cohorts according to the Epworth sleepiness scale (ESS) and the effect of CPAP therapy on sleepy (ESS>10) and non-sleepy (ESS≤10) patients will be compared (Figure 1).

The primary outcome is the variation of cf-PWV after therapy with CPAP. The effect of a four months trial of CPAP is assessed. For ethical reasons, each patient will be its own control.

Obesity and metabolic syndrome are common findings in OSA, and possible changes of metabolic parameters after CPAP are relevant[18].

Secondary outcomes are ambulatory blood pressure measurements (ABPM) variations, as well as metabolic changes after treatment with CPAP.

The study is exploratory in nature and is based on a convenience sample, limited by the number of eligible patients consenting to participate. Sample size was estimated for the primary outcome as an effective sample of 70 patients to demonstrate a reduction on cf-PWV from 12 m/s to 11 m/s (from baseline to 4 months on CPAP), with 95% confidence and power 80%. Based on the previous assessment of the institutional experience on compliance with CPAP and with outpatient clinic consultations, the recruited sample was increased in 15% to 80 patients.

Recruitment started on October 2012 and data collection is expected to end by June 2016.

Inclusion criteria

Patients referred to the Outpatient Sleep Clinic of the Pneumology Department, CHLC, Lisbon, due to snoring or other complaints suggestive of OSA, are eligible for the study if they meet the following criteria: males, younger than 65 years old, living in Greater Lisbon and with confirmed moderate to severe OSA (AHI> 15/h). To confirm this, on the first consultation, data on anamnesis and clinical examination are collected and the Epworth questionnaire is applied.

Exclusion criteria

Patients are excluded if they have a history of cardiovascular events; established heart disease (beyond arterial hypertension); peripheral vascular disease. Smoking (over 10 units pack/year) or alcohol intake (> 80 g / day) are not allowed. Other severe chronic diseases (assessed by chronic use of medication), chronic ingestion of hypnotics or other sleep disorders (clinically identified) are exclusion criteria.

Patients with diabetes are excluded, as they might have vascular involvement.

Some comorbidities are allowed, namely hypertension and metabolic syndrome. Antihypertensive medication and treatment of dyslipidemia are allowed as well.

Baseline evaluation

The Outpatient Sleep Clinic protocol for patients referred due to snoring or other complaints suggestive of OSA requires a clinical evaluation, blood tests, electrocardiogram and a sleep study.

ESS will be applied by the principal investigator, and the presence of comorbidities and pharmacotherapy in progress is evaluated.

The collection of data regarding duration of comorbidity or pharmacotherapy has not been considered since including these two factors as accountable intervening factors would greatly increase the sample size.

Anthropometric data regarding adiposity and vital signs are collected. Body mass index (BMI) is calculated as weight (kg) divided by height (m) squared.

BP will be measured while the patient is seated in a quiet room, using a semi-automatic device (Omron HEM-907XL, Omron Healthcare, Bannockburn, II, USA) with an appropriate cuff. The patient could not have taken stimulant substances 3 hours prior to the BP assessment. Two measurements are taken with a minimum of 3-min intervals and the average considered. When large differences existed among them, additional measurements were performed.

The analytical screening evaluation includes HbA1c, fasting glucose, total cholesterol, HDL, LDL and triglycerides; in patients with high levels of fasting glucose, an oral glucose tolerance test will be performed. Dyslipidaemia, glucose intolerance and metabolic syndrome are diagnosed according to current guidelines[19].

An electrocardiogram (Page Writer TC 30, Philips, Eindhoven, NL) will be done by the same technicians, per institutional protocol.

Sleep study

To confirm the diagnosis of OSA, patients will submit to a polygraphic sleep study type 3, cardiorespiratory (Embletta® system, Broomfield, USA), a technical outpatient registration, which includes the continuous recording from nasal cannula (pressure and flow), thoracic-abdominal motion, pulse oximetry, electrocardiogram and body position sensor. Results from the sleep studies are analyzed by two trained technicians, using standard criteria[20].

Ambulatory Blood Pressure Monitoring

All participants will undertake 24 hours blood pressure monitoring with Ambulatory Blood Pressure - SpaceLabs model 90207® (Issaquah, WZ, USA), according to the current guidelines. Patients are classified as normotensive if the average systolic and diastolic BP values obtained by ABPM are within normal limits; and are classified as hypertensive if either already on antihypertensive medication or if the average BP values obtained by ABPM are above ≥130 mmHg of systolic and/or ≥80 mmHg diastolic BP[11].

Pulse wave velocity

Non-invasive assessment of cf-PWV (Complior®, Colson, Paris, France) will be performed by the same operator. For this, the investigators followed the recently published expert consensus document[21]. In a few words, measurement is done at the right common carotid and right common femoral arteries after 10 min rest in supine position. Two pressure waveforms were transcutaneously recorded at the base of the neck for the right common carotid artery and over the right femoral artery. Pulse transit time was determined as the average of 10 consecutive beats. The straight-line distance between the measurement sites is obtained by surface measurement, with the patient in the supine position. cf-PWV is calculated as the ratio of distance to transit time. In order to normalize cf-PWV values according to the arterial pathway, the threshold of 10 m/s for cf-PWV evaluated by the full direct carotid-to-femoral distance was considered[21].

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According to the current clinical recommendations[4], patients with moderate or severe OSA are offered therapy with non-invasive ventilation by CPAP. Additionally, patients receive counselling on sleep hygiene.

An automatic auto-CPAP device (ResMed S9 AutoSet, California, USA) is used. CPAP will be titrated for 3 nights. The definitive value of CPAP is the amount of pressure that eliminates events in approximately 95% of the total sleep time (95th percentile), once confirmed there is no significant leakage and use of nasal mask[22].

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The presence of nasal complaints or changes on the equipment will be taken into account.

Follow-up assessments

After 4 months of standard CPAP intervention, patients will undergo a clinical evaluation, including ESS, and analytical evaluation, similar to the one on baseline. BMI, ABPM and cf-PWV will be assessed, as well as compliance to CPAP and the effectiveness of CPAP therapy.

At any point of the study, patients will be excluded from follow-up if do not comply with CPAP, if significant weight loss (greater than 10%) occurs[4], if new drugs are prescribed or if new diseases are diagnosed.

Statistical analyses

The primary outcome measure (cf-PWV**)** is compared before and after therapy with CPAP for four months. Analysis is performed as per intention-to-treat.

Continuous variables are described with median and interquartile range (IQR, 25th percentile to 75th percentile) or extreme values, as previous evaluations have evidenced that a normal distribution of values was not to be expected. Data of sleepy and non-sleepy subjects at each evaluation are compared using the Mann-Whitney test. Categorical variables are described as percentage and are compared with Pearson's chi-square test or Fisher's exact test; stratified Mantel-Haenszel analysis is performed as appropriate**.**

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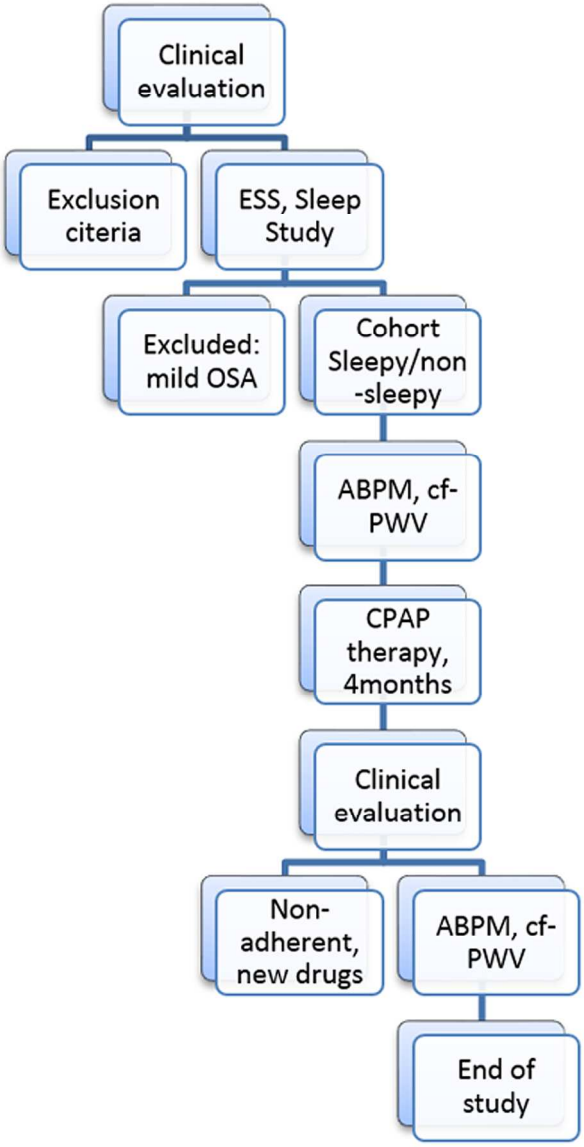

Fig.1 Study design
173x233mm (300 x 300 DPI)