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SCIENTIFIC ARTICLE

Adverse respiratory events after general anesthesia in patients at high risk of obstructive sleep apnea syndrome

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KEYWORDS

Obstructive sleep
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Abstract

Introduction: Patients with STOP-BANG score >3 have a high risk of Obstructive sleep apnea. The aim of this study was to evaluate early postoperative respiratory complications in adults with STOP-BANG score >3 after general anesthesia.

Methods: This is a prospective double cohort study matching 59 pairs of adult patients with STOP-BANG score >3 (high risk of obstructive sleep apnea) and patients with STOP-BANG score <3 (low risk of obstructive sleep apnea), similar with respect to gender, age and type of surgery, admitted after elective surgery in the Post-Anaesthesia Care Unit in May 2011. Primary outcome was the development of adverse respiratory events. Demographics data, perioperative variables, and postoperative length of stay in the Post-Anesthesia Care Unit and in hospital were recorded. The Mann–Whitney test, the chi-square test and the Fisher exact test were used for comparisons.

Results: Subjects in both pairs of study subjects had a median age of 56 years, including 25% males, and 59% were submitted to intra-abdominal surgery. High risk of obstructive sleep apnea patients had a higher median body mass index (31 versus 24 kg/m², $p < 0.001$) and had more frequently co-morbidities, including hypertension (58% versus 24%, $p < 0.001$), dyslipidemia (46% versus 17%, $p < 0.001$) and insulin-treated diabetes mellitus (17% versus 2%, $p = 0.004$). These patients were submitted more frequently to bariatric surgery (20% versus 2%, $p = 0.002$). Patients with high risk of obstructive sleep apnea had more frequently adverse respiratory events (39% versus 10%, $p < 0.001$), mild to moderate desaturation (15% versus 0%, $p = 0.001$) and inability to breathe deeply (34% versus 9%, $p = 0.001$).

Conclusion: After general anesthesia high risk of obstructive sleep apnea patients had an increased incidence of postoperative respiratory complications.

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PALAVRAS-CHAVE

Apneia obstrutiva do sono;
Eventos respiratórios;
Desfecho no pós-operatório

Eventos respiratórios adversos após anestesia geral em pacientes com alto risco de síndrome da apneia obstrutiva do sono

Resumo

Justificativa e objetivo: Os pacientes com escore STOP-BANG > 3 possuem alto risco de desenvolver apneia obstrutiva do sono. O objetivo deste estudo foi avaliar as complicações respiratórias no pós-operatório imediato em adultos com escore STOP-BANG > 3 após anestesia geral.

Métodos: Estudo prospectivo de dupla-coorte, comparando 59 pares de pacientes adultos com escore STOP-BANG > 3 (alto risco de apneia obstrutiva do sono) e pacientes com escore STOP-BANG < 3 (baixo risco de apneia obstrutiva do sono), similares no que diz respeito ao gênero, idade e tipo de cirurgia, admitidos após a cirurgia eletiva em sala de recuperação pós-anestésica (SRPA) em maio de 2011. O desfecho primário foi o desenvolvimento de eventos respiratórios adversos. Dados demográficos, variáveis no perioperatório e tempos de permanência na SRPA e no hospital após a cirurgia foram registrados. Os testes de Mann-Whitney, qui-quadrado e exato de Fisher foram usados para comparação.

Resultados: Os indivíduos de ambos os grupos de pacientes do estudo tinham uma média de idade de 56 anos, 25% eram do sexo masculino e 59% foram submetidos à cirurgia intra-abdominal. Os pacientes com alto risco de apneia obstrutiva do sono apresentavam uma mediana maior do índice de massa corporal (31 versus 24 kg/m², $p < 0,001$) e comorbidades mais frequentes, como hipertensão (58% vs. 24%, $p < 0,001$), dislipidemia (46% vs. 17%, $p < 0,001$) e diabetes melito dependente de insulina (17% vs. 2%, $p = 0,004$). Esses pacientes foram submetidos com mais frequência à cirurgia bariátrica (20% vs. 2%, $p = 0,002$). Os pacientes com alto risco de apneia obstrutiva do sono apresentaram mais eventos respiratórios adversos (39% vs. 10%, $p < 0,001$), dessaturação de leve a moderada (15% vs. 0%, $p = 0,001$) e incapacidade de respirar profundamente (34% vs. 9%, $p = 0,001$).

Conclusões: Após a anestesia geral, os pacientes com alto risco de apneia obstrutiva do sono apresentaram um aumento da incidência de complicações respiratórias no período pós-operatório.

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Introduction

Obstructive sleep apnea (OSA) can occur in all age groups¹ and is a common form of sleep-disorder breathing affecting 2–26% of the general population.² Studies have shown that patients with OSA have an associated increase in morbidity and mortality.^{3,4} These patients also have higher rates of postoperative complications.^{5–9} Since many patients with OSA have not been formally diagnosed at the time of surgery,¹⁰ preoperative management and the adoption of measures to reduce postoperative risk are difficult to apply. It is estimated that a great number of men or women with moderate-to-severe sleep apnea have not been diagnosed.¹¹ Overnight polysomnography (PSG) is still the “gold standard” for diagnosis of OSA, but it may be unfeasible to perform during the preoperative evaluation.

The routine performance of preoperative screening instruments is important to identify patients with undiagnosed OSA.^{12–14} Many tools for screening patients for OSA have been proposed – such as the Berlin questionnaire, the STOP questionnaire and the American Society of Anesthesiologists (ASA) checklist – and their use improves the likelihood of identifying OSA preoperatively.^{1,9,13,15} The STOP-BANG questionnaire (Table 1), which was validated for surgical

population by F. Chung et al., is a scoring model consisting of eight easily administered questions, referred to by the acronym STOP-BANG (Snoring, Tiredness during daytime, Observed apnea, high blood pressure, body mass index, age, neck circumference, gender). This questionnaire is scored based on Yes/No answers (score: 1/0), and scores range from a value of 0 to 8. A score of ≥ 3 has shown a high sensitivity for detecting OSA: 93% and 100% for moderate and severe OSA, respectively.¹² Owing to its high sensitivity and being an easy-to-use and a screening tool, the STOP-BANG questionnaire is considered very useful to identify patients having moderate and severe OSA.¹²

In surgical patients the prevalence of OSA is even higher than in the general population and it can vary broadly according to the presence of medical comorbidities.¹⁶ In particular, as many as 70% of patients undergoing bariatric surgery were found to have OSA.¹⁷ OSA has been recognized as a potential independent risk factor for adverse perioperative outcome.¹⁸ OSA patients undergoing surgical procedures are vulnerable to postoperative airway obstruction,¹⁸ myocardial ischemia, congestive heart failure, stroke and oxygen desaturation.^{18–20} Patients with OSA may be more susceptible to respiratory complications during the perioperative period because drugs used during general

Table 1 STOP-BANG questionnaire. High risk of OSA: Yes to ≥ 3 questions. Low risk of OSA: Yes to < 3 questions.

SNORING: do you snore loudly (loud enough to be heard through closed doors)?	YES	NO
TIRED: do you often feel tired, fatigued, or sleepy during daytime?	YES	NO
OBSERVED: has anyone observed you stop breathing during your sleep?	YES	NO
BLOOD PRESSURE: do you have or are you being treated for high blood pressure?	YES	NO
BMI: BMI more than 35 kg m ⁻² ?	YES	NO
AGE: age over 50 years old?	YES	NO
NECK circumference: neck circumference >40 cm?	YES	NO
GENDER: male?	YES	NO

anesthesia may increase the risk for prolonged periods of apnea.¹⁴

The objective of this study was to evaluate the early postoperative respiratory complications in patients with STOP-BANG score ≥ 3 , after general anesthesia.

Methods

The Centro Hospitalar São João Ethics Committee approved this study and written informed consent was obtained from all participants. Centro Hospitalar São João, in Porto, is an 1124-bed tertiary hospital in a metropolitan area serving 3,000,000 people. This prospective double-cohort study was conducted in a 13-bed Post-Anesthesia Care Unit (PACU) over a three-week period (from May 9 to May 27, 2011). Every patient able to provide written informed consent and admitted to the PACU after general anesthesia was included in the study. Exclusion criteria were patient refusal, incapacity of providing informed consent, a score of < 25 in the Mini-Mental State Examination (MMSE), age below 18 years, foreign nationality, known neuromuscular disease, urgent/emergent surgery and cardiac surgery, neurosurgery or other procedures that required therapeutic hypothermia.

All patients were interviewed either in the eve of the surgery or on the day of the surgery, at least three hours before surgery, in the surgical ward. During this interview the consent was obtained, MMSE test and the STOP-BANG questionnaire were completed and the medical history was collected.

Anesthesiologists were blinded to patient involvement in the study. Anesthesia was provided and monitored according to the criteria of the anesthesiologist in charge, but this conduct followed minimum departmental standards. In accordance to our standard procedures, general anesthesia was induced with an intravenous anesthetic in combination with an opioid, followed when needed by neuromuscular blockade (NMB). Anesthesia was maintained by total intravenous anesthesia (TIVA) or with inhalation anesthetics. The anesthesiologist was free to choose to use nitrous oxide. Fluid management was completely guided by the anesthesiologist.

Neuromuscular blocking drugs (NMBD) were used for tracheal intubation, and additional boluses were provided, if needed. No written policy exists concerning the use of neuromuscular monitoring, so this was performed at the discretion of the anesthesiologist.

To ensure that the anesthesiologist remained blinded to the patients' participation in the study, we did not

attempt to observe the intraoperative use or interpretation of Train-of-Four (TOF). The anesthesiologist was free to decide whether to reverse the NMB with neostigmine at the conclusion of the surgical procedure. Usually, the patient was extubated in the operating room and transferred to the PACU. All subjects were administered 100% oxygen by a facemask after tracheal extubation. The anesthesiologist in charge decided whether to administer oxygen during the time between transfer to the cart and admission to the PACU.

Upon arrival at the PACU oxygen was provided to all subjects by a nasal cannula or face mask, with the decision of the type and oxygen concentration being taken by the anesthesiologist scheduled to the PACU.

Residual neuromuscular blockade (RNMB) was defined as TOF < 0.9 and it was quantified at admission to the PACU using acceleromyography of the adductor pollicis muscle (TOF-Watch®). Three TOF measurements (separated by 15 s) were obtained, and the average of the three values was recorded. If a value differed from the others by more than 10%, an additional TOF measurement was obtained and the closest three ratios were averaged. Neuromuscular block was re-assessed hourly while patients maintained TOF < 0.9 . When patients had a TOF below 0.9, then the attending anesthesiologist was contacted and informed.

Patients were classified as being at high risk for OSA (HR-OSA) if their STOP-BANG score was 3 or more and were classified as being at low risk of OSA (LR-OSA) if their score was less than 3.

A double-cohort study design with prospectively defined cases was performed. All cases during the study period with HR-OSA were identified and then matched with selected control patients for comparison. Cases and controls were identified by collecting data on all consecutive patients arriving in the PACU during the study period. The cases consisted of all HR-OSA patients and were matched with similar in respect to gender, age and type of defined as intra-abdominal, musculoskeletal or head and neck, admitted in the PACU after general anesthesia for elective surgery.

The LR-OSA patients were classified based on a one-to-one match with the HR-OSA patients and were selected from the consecutive patients without STOP-BANG ≥ 3 according to the matching characteristics.

Variables registered on admission in PACU were age, gender, type of surgery (intra-abdominal, skeletal muscle, bariatric, head and neck), body mass index, ASA physical status and pre-hospitalization comorbidities. Using the classification developed by Lee et al. for predicting cardiac risk, we calculated the revised cardiac risk index (RCRI)

for each patient, signaling a point for each of the following risk factors: high risk surgery, history of ischemic heart disease, history of congestive heart failure, history of cerebrovascular disease, pre-operative treatment with insulin for diabetes mellitus and pre-operative serum creatinine > 2.0 mg/dl.²¹ Surgical risks were evaluated according to the Cardiac Risk Stratification for Noncardiac Surgical Procedures of the 2007 guidelines of Perioperative Cardiovascular Evaluation and Care for Noncardiac Surgery of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines.²²

Premedication with benzodiazepines and its chronic use were recorded. Intraoperative details were also recorded and included type and duration of anesthesia, and the use of NMBD and neostigmine.

Patients' tympanic temperature and mean train-of-four ratio were recorded on admission to the PACU. All patients had continuous monitoring of blood pressure, cardiac frequency, electrocardiogram (ECG) and peripheral oxygen saturation, and mean train-of-four ratio recorded on admission to the PACU.

The postoperative data registered included mortality and length of hospital and PACU stay.

Early postoperative adverse respiratory events

Each postoperative adverse respiratory events (ARE) were defined on the data collection sheet using the following criteria using a classification described by Murphy et al.²³:

1. *Upper airway obstruction* requiring an intervention (jaw thrust, oral airway, or nasal airway);
2. *Mild-moderate hypoxemia* (O₂ saturations (SpO₂) of 93%–90%) on 3L nasal cannula O₂ that was not improved after active interventions (increasing O₂ flows to >3 L/min, application of high-flow facemask O₂, verbal requests to breathe deeply, tactile stimulation);
3. *Severe hypoxemia* (SpO₂ <90%) on 3L nasal cannula O₂ that was not improved after active interventions (increasing O₂ flows to >3 L/min, application of high-flow facemask O₂, verbal requests to breathe deeply, tactile stimulation);
4. Signs of *respiratory distress* or impending ventilatory failure (respiratory rate >20 breaths per minute, accessory muscle use, tracheal tug);
5. *Inability to breathe deeply* when requested to by the PACU nurse;
6. Patient complaining of symptoms of respiratory or upper airway *muscle weakness* (difficulty breathing, swallowing, or speaking);
7. Patient requiring *reintubation* in the PACU;
8. Clinical evidence or suspicion of *pulmonary aspiration* after tracheal extubation (gastric contents observed in the oropharynx and hypoxemia).

During PACU stay the patients were observed continuously by the PACU nurses who contacted a study investigator without delay if an ARE was observed. The inability to breathe deeply and assessment of symptoms of respiratory or upper airway muscle weakness were done at intervals of 10 min. One other investigator of the study then observed

the patient to verify that the patient met at least one of the criteria for an ARE.

Statistical analysis

Variable descriptive analysis was used to summarize the data and Mann–Whitney *U* test was used for comparison of continuous variables between groups of individuals; Chi square test and Fisher's exact test were used for comparison of proportions between groups of individuals. All variables were considered significant when $p < 0.05$.

The statistical software package SPSS for Windows version 19.0 (SPSS, Chicago, IL) was used to analyze the data.

Results

A total of 59 pairs of study subjects were admitted in PACU during the period of the study. Table 2 presents the characteristics of patients admitted in the PACU, the surgical data, anesthetic management, the postoperative data and a comparison between the patients with HR-OSA and patients with LR-OSA. Both pairs of study subjects had a median age of 56 years, included 25% males, and 59% were submitted to intra-abdominal surgery. Combined anesthesia was used in 13 of the 118 patients studied.

Patients with HR-OSA had a higher body mass index (median 31 versus 24 kg/m², $p < 0.001$) and had more frequently co-morbidities, including hypertension (58% versus 24%, $p < 0.001$), dyslipidemia (46% versus 17%, $p < 0.001$) and insulin-treated diabetes mellitus (17% versus 2%, $p = 0.004$). These patients were submitted more frequently to bariatric surgery (20% versus 2%, $p = 0.002$).

Twenty-nine patients of the entire population presented ARE (24.6%; 95% confidence interval: 16.7, 32.5) (Table 3); 25 were unable to breathe deeply when requested (21.2%; 95% confidence interval: 13.7, 28.7), 9 had symptoms of respiratory or upper airway muscle weakness (7.6%; 95% confidence interval: 2.8, 12.5), 9 developed mild-moderate hypoxemia (7.6%; 95% confidence interval: 2.8, 12.5), 6 had upper airway obstruction (5.1%; 95% confidence interval: 1.1, 9.1), 5 had severe hypoxemia (4.2%; 95% confidence interval: 0.1, 7.9) and 5 presented signs of respiratory distress (4.2%; 95% confidence interval: 0.1, 7.9). No patient required re-intubation or had clinical evidence or suspicion of pulmonary aspiration.

Patients with HR-OSA developed more respiratory complications in the PACU (39% versus 10%, $p < 0.001$). Only HR-OSA patients had mild-moderate hypoxemia and these patients also showed high inability to breathe deeply (34% versus 9%, $p = 0.001$).

HR-OSA patients had a median longer stay in the PACU (120 min versus 99 min, $p = 0.035$). Length of stay in the hospital was similar in both groups of patients.

Discussion

The majority of OSA patients scheduled to surgery remain without a formal diagnosis. This entity may be considered a prevalent condition among surgical patients and may cause significant adverse effects in the perioperative period.¹⁰ During preoperative assessment, it is essential to screen for

Table 2 Characteristics of patients.

Variables, median (IQR) or n (%)	STOP-BANG \geq 3 (HR-OSA) n = 59	STOP-BANG < 3 (LR-OSA) n = 59	p-Value
Age in years	56 (41–67)	56 (44–67)	0.859 ^a
Gender			1 ^b
Male	15 (25)	15 (25)	
Female	44 (75)	44 (75)	
Body Mass Index (Kg/m ²)	31 (26–38)	24 (22–28)	<0.001 ^a
ASA-PS			0.167 ^b
I/II	49 (83)	54 (91)	
III/IV	10 (17)	5 (9)	
High-risk surgery	19 (32)	23 (39)	0.442 ^b
Ischemic heart disease	1 (2)	0	0.500 ^c
Congestive heart disease	2 (3)	1 (2)	0.500 ^c
Cerebrovascular disease	2 (3)	0	0.248 ^c
Insulin therapy for diabetes	10 (17)	1 (2)	0.004 ^c
Renal insufficiency	2 (3)	4 (7)	0.340 ^c
RCRI			0.752 ^c
<2	58 (98)	58 (98)	
>2	1 (2)	1 (2)	
Hypertension	34 (58)	14 (24)	<0.001 ^b
Hyperlipidemia	27 (46)	10 (17)	<0.001 ^b
COPD	3(5)	0	0.122 ^c
Site of surgery			1 ^b
Intra-abdominal	35 (59)	35 (59)	
Musculoskeletal	18 (31)	18 (31)	
Head and neck	6 (10)	6 (10)	
Bariatric surgery	12 (20)	1 (2)	0.002 ^c
Premedication with BZD	18 (31)	22 (37)	0.437 ^b
Chronic BZD medication	10 (17)	4(7)	0.076 ^c
Hypothermia	19 (32)	23 (39)	0.442 ^b
Type of anesthesia			0.378 ^c
General	51 (86)	54 (92)	
Combined	8 (14)	5 (9)	
Neostigmine use	46 (78)	41 (70)	0.480 ^b
NMB use	49 (83)	47 (80)	0.407 ^b
RNMB	23 (37)	13 (22)	0.070 ^b
Duration of anesthesia (minutes)	110 (80–190)	120 (85–180)	0.897 ^a
Respiratory events in PACU	23 (39)	6 (10)	<0.001 ^b
Length of PACU stay (minutes)	120 (80–155)	99 (75–120)	0.035 ^a
Length of hospital stay (days)	3 (2–6)	3 (2–6)	0.482 ^a

HR-OSA, high-risk of obstructive sleep apnea; LR-OSA, low-risk of obstructive sleep apnea; ASA-PS, American Society of anesthesiologists' physical status; RCRI, revised cardiac risk index; COPD, chronic obstructive pulmonary disease; BZD, benzodiazepine; NMB, neuromuscular blockade; RNMB, residual neuromuscular blockade; PACU, Post-Anesthesia Care Unit.

^a Mann–Whitney *U* test.

^b Pearson's chi-squared test.

^c Fisher's exact test.

OSA to promote the implementation of strategies to minimize the perioperative risk of adverse events. As the clinical history is an unreliable indicator of the presence of OSA, and PSG is not available for all surgical patients, it is necessarily an effective screening modality.²⁴

The STOP questionnaire was validated in surgical patients at preoperative clinics as a screening tool and it has

demonstrated a high sensitivity and Negative Predictive Value, especially for patients with moderate to severe OSA.^{12,15}

The reported incidence of adverse respiratory events in the PACU varies widely, with observational studies describing an incidence of 1.3%–34%^{25–27} depending on ARE studied and on patient comorbidities.

Table 3 Postoperative complications.

	All (n = 118)	HR-OSA	(LR-OSA) p	
<i>Respiratory complications</i>	29 (25)	23 (39)	6 (10)	<0.001
Obstruction airway	6 (5)	4 (7)	2 (3)	0.340
Mild-moderate hypoxemia	9 (8)	9 (15)	0	0.001
Severe hypoxemia	5 (4)	3 (5)	2 (3)	0.500
Respiratory failure	5 (4)	3 (5)	2 (3)	0.500
Inability to breathe deeply	25 (21)	20 (34)	5 (9)	0.001
Muscle weakness	9 (8)	7 (12)	2 (3)	0.081

HR-OSA, high-risk of obstructive sleep apnea syndrome; LR-OSA, low-risk of obstructive sleep apnea syndrome.

Different studies had already documented the association of OSA with comorbidities^{8,28,29} and in our study HR-OSA patients had more frequently hypertension, dyslipidemia and renal failure, but we could not show the association between OSA and ischemic or congestive heart disease that was established by others.^{28,29} One reason for this might be related to the relative low median age of our population.

OSA has been described as an independent risk factor for perioperative pulmonary complications,^{30,31} but there is little evidence describing respiratory complications of OSA patients at PACU. Liao et al.⁸ documented that the majority of the postoperative complications occurred after patients were transferred to the ward and that the major contributor to the high occurrence of postoperative complications in OSA patients was the increased incidence of respiratory complications.

Our study shows that after surgery HR-OSA patients may have a high risk of ARE and they had a nearly 4-fold increase in developing ARE in the PACU compared with LR-OSA patients. This is according to the study of Liao et al. that has shown a higher incidence of pulmonary complications in patients with OSA (33% versus 22%).

Respiratory complications in the immediate postoperative period can lead to increased morbidity and mortality.³² Pulmonary and pharyngeal physiology may be affected by perioperative factors that may have detrimental effects in patients with OSA.^{33,34} Impairment of the activity of pharyngeal muscles promoted by pharmacologic and mechanical related factors may be viewed as aggravating factors increasing the risk for ARE in the postoperative period in patients with OSA.²³ The study of impact of the anesthetic management was not the aim of the present study, so the authors decided to perform an observational work allowing different anesthetic protocols. Actually, only 11% of the patients were submitted to combined anesthesia, so its impact on the occurrence of ARE could not be studied.

Patients with HR-OSA presented more RNMB and were more frequently submitted to bariatric surgery and these two factors may be responsible for the higher rates of ARE.

The most common ARE occurring in HR-OSA group was the inability to breathe deeply, recorded in 34% of patients. Mild-moderate hypoxemia was the second most common ARE in the HR-OSA group and occurred in 15%. Other studies have demonstrated similar results regarding higher risk of hypoxia,^{13,35,36} but a recent study in morbidly obese patients did not find a difference between OSA and non-OSA patients in the number of hypoxemic episodes after bariatric surgery.³⁷

The consequences of the ARE delaying PACU discharge are difficult to calculate and diverge based on singular institutional factors including staffing models, PACU size and readiness for ward beds. In this study, HR-OSA patients had a longer length of PACU stay, which is in concordance with the majority of the studies.⁸

Our study has a number of limitations that must be acknowledged. First, the sample is small and was completed at a single center, making it difficult to generalize results beyond our study site. Second, we rely only on STOP-BANG score to make the OSA diagnosis because there were no polysomnographic data available for all the patients; thus, we could not quantify the severity of considered HR-OSA patients. Third, the definitions of ARE had some subjective criteria which may have influenced the diagnosis. Forth, the respiratory events were only registered in the PACU and complications that could have occurred after PACU discharge are not considered.

And lastly, although the authors attempted to have similarity concerning the type of surgery that was performed in the two groups studied, HR-OSA patients were more often submitted to bariatric surgery and this could have affected the incidence of ARE among these patients.

The principal findings of this study were that patients with STOP-BANG score ≥ 3 had a higher body mass index and were submitted more frequently to bariatric surgery; HR-OSA patients had more frequently co-morbidities, including hypertension, dyslipidemia and insulin-treated diabetes mellitus; patients with STOP-BANG score ≥ 3 had a higher incidence of postoperative respiratory complications; inability to breathe deeply and mild/moderate hypoxia were the most frequent adverse respiratory events in the immediate postoperative period and they were more frequent in patients with high risk of OSA.

Conclusion

In conclusion ARE was a common occurrence in the PACU and were more frequently observed in HR-OSA patients.

Authorship

All people listed as authors contributed to the preparation of the manuscript and no one other than the authors listed have contributed significantly to its preparation. Each listed author participated in the work to the extent that they could all publicly defend its content. They all read the manuscript

before its submission for publication and are prepared to sign a statement stating they had read the manuscript and agree to its publication.

Fernando Abelha made all coordination of the study, performed statistical analyses and wrote the draft and the manuscript. Daniela Xará participated in the design of the study and made the critical revision of the final manuscript. Julia Mendonça participated in the design of the study and coordinated the data acquisition and helped in databases construction. Helder Pereira participated in the design of the study, coordinated databases construction and had substantial contribution in acquisition of data. Alice Santos made substantial contributions in the analyses and interpretation of data, wrote the draft and the manuscript and coordinated revision of the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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