



Early View

Original article

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Increased liver stiffness in patients with severe sleep apnoea and metabolic comorbidities

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"take home" message: In patients with metabolic comorbidities, severe OSA is independently linked with an increased risk of liver disease as assessed by liver stiffness measurement.

Abstract

The goal of this study was to assess the relationship between the severity of obstructive sleep apnoea (OSA) and liver stiffness measurement (LSM), one of the most accurate non-invasive screening tools for liver fibrosis in nonalcoholic fatty liver disease.

The study included 147 patients with at least one criterion for the metabolic syndrome (MS), assessed by polysomnography for suspected OSA. LSM was performed using FibroScan. Significant liver disease and advanced liver fibrosis were defined as $LSM \geq 7.3$ kiloPascal (kPa) and $LSM \geq 9.6$ kPa, respectively.

Twenty-three patients were excluded because of unreliable LSM. Among 124 patients, 34 (27.4%) had mild OSA, 38 (30.6%) had moderate OSA and 52 (42.0%) had severe OSA. LSM values were between 7.3 and <9.6 kPa in 18 (14.5%) patients and ≥ 9.6 kPa in 15 (12.1%) patients. A dose-response relationship was observed between OSA severity and LSM values ($p=0.004$). After adjustment for age, gender, MS and insulin resistance, severe OSA was associated with an increased risk of $LSM \geq 7.3$ kPa (odds ratios [95% confidence interval]): 7.17 [2.51-20.50]) and $LSM \geq 9.6$ kPa (4.73 [1.25-17.88]).

In patients with metabolic comorbidities, severe OSA is independently associated with increased liver stiffness, which may predispose to a higher risk of significant liver disease and poorer prognosis.

Keywords: Obstructive sleep apnoea; Non-alcoholic fatty liver disease; Liver stiffness

Introduction

The growing worldwide obesity epidemic is resulting in an increased prevalence of obesity-related chronic diseases, including metabolic syndrome (MS), type 2 diabetes, obstructive sleep apnoea (OSA) and nonalcoholic fatty liver disease (NAFLD) [1, 2]. The risk factors, pathophysiology and consequences of these chronic diseases may share common pathways and could impact one each other. Experimental and clinical data have suggested that OSA and its hypoxia-related consequences may contribute to the development and exacerbation of NAFLD, the hepatic manifestation of obesity and MS [3]. In morbidly obese patients undergoing bariatric surgery during which a liver needle biopsy was obtained, a dose-response relationship has been demonstrated between intermittent hypoxia severity and the severity of liver injury [4].

NAFLD includes a wide spectrum of histological lesions, ranging from simple steatosis, steatohepatitis, and fibrosis, potentially leading to end-stage cirrhosis. Convergent findings from longitudinal studies indicate that the amount of liver fibrosis is the main determinant of the liver-related prognosis in patients with NAFLD [5]. The reference procedure for liver fibrosis evaluation is histological examination of a liver biopsy. However, liver biopsy is an invasive procedure and is associated with considerable cost and a significant complication rate of around 1% [6]. Most importantly, in view of the magnitude of the current obesity epidemic and the subsequent number of patients at risk for liver alterations, liver biopsy cannot be considered to be a practical, efficient, and large-scale tool to identify patients at risk of advanced fibrosis. Non-invasive screening tools for liver fibrosis, including blood tests and transient elastography devices, have recently been developed and validated in at-risk populations [7, 8]. Transient elastography is a recognized rapid and user-friendly technique that can be easily performed at the bedside or in the outpatient clinic with immediate results and good reproducibility [9]. Liver

stiffness measurement (LSM) by transient elastography (FibroScan, EchoSens, Paris, France) has been demonstrated to be one of the most accurate tests for the non-invasive diagnosis of liver fibrosis in NAFLD [10]. LSM has also been found to be a reliable prognostic marker for the stratification of NAFLD patients into several subgroups with significantly different prognosis [10]. The aim of this cross-sectional cohort study was to determine whether severe OSA is associated with significant liver disease evaluated by LSM.

Patients and Methods

Patients

This study was nested within the NUMEVOX Cohort (Clinicaltrials.gov No: NCT00997165) conducted at Angers University Hospital, France. The primary objective of the NUMEVOX Cohort was to evaluate the impact of fat distribution on vascular and metabolic progression in patients with at least one criterion for MS referred to Angers University Hospital [11, 12]. Exclusion criteria were age <18 or >65 years, cause of chronic liver disease other than NAFLD, complicated cirrhosis and insulin-treated diabetes. All patients included in the NUMEVOX Cohort were assessed by the BERLIN questionnaire to evaluate the clinical likelihood of OSA [13]. Patients subsequently investigated by overnight polysomnography based on a positive BERLIN questionnaire were included in the present study. The study was approved by the institutional ethics committee and oral and written consent was obtained from each patient by means of a process validated by the Angers University Hospital Research Ethics Committee (CPP OUEST II, ANGERS).

Clinical assessment and blood tests

Baseline evaluation included anthropometric data, medical history, and medication use. Patients receiving antidiabetic treatment were considered to have diabetes. Hypertension was defined as a physician's diagnosis combined with treatment with appropriate medication. Waist circumference (cm) was measured using a non-elastic measuring tape. Excessive daytime sleepiness was evaluated by the Epworth Sleepiness Scale [14]. All patients underwent fasting blood tests including blood glucose, insulin, liver enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST], gamma glutamyl transpeptidase [GGT], and alkaline phosphatase), and total bilirubin. The Homeostasis Model Assessment for Insulin Resistance index (HOMA-IR) was calculated from fasting glucose and insulin concentrations, as follows: $\text{insulin(mIU/L)} * \text{glucose(mmol/L)} / 22.5$ [15]. The presence of MS was defined according to the National Cholesterol Education Program Adult Treatment Panel III clinical criteria [11].

Liver stiffness measurement

LSM was performed with the FibroScan device under fasting conditions according to the manufacturer's recommendations [9] by a specialized nurse blinded to the patient's data. The examination was conducted using the standard M probe. Ten valid acquisitions were recorded and the result (kiloPascal: kPa) was expressed as the median of these valid acquisitions. Based on the meta-analysis published by Tsochatzis *et al* [16], an LSM cut-off value ≥ 7.3 kPa was used to identify patients with significant liver disease. Advanced liver fibrosis was defined by the classical ≥ 9.6 kPa cut-off value used in NAFLD [17, 18]. According to previously validated reliability criteria for LSM with FibroScan, patients with $\text{LSM} \geq 7.3$ kPa and interquartile

range/median (IQR/M) ratio >0.30 were considered to be poorly reliable and were excluded from the analysis [19, 20].

Polysomnography

As previously described [21], overnight in-laboratory polysomnography (PSG) (CID 102™, Cidelec, Angers, France) was performed (date of LSM \pm 1 month) with continuous recording of the following channels: electroencephalogram, electrooculogram, chin electromyogram, arterial oxygen saturation, nasal-oral airflow, electrocardiogram, chest and abdominal wall motion, and body position. Respiratory events were scored manually according to standard criteria [22]. Apnoea was defined as complete cessation of airflow and hypopnoea was defined as an at least 30% decrease in the nasal pressure signal combined with either \geq 3% arterial oxygen desaturation or an arousal, both lasting at least 10 s. The apnoea-hypopnoea index (AHI) and 3% oxygen desaturation index (ODI) were defined by the number of events per hour of sleep. The following commonly used cut-offs for AHI were used to define categories of OSA severity: 5 to <15 for mild OSA, 15 to <30 for moderate OSA, and ≥ 30 for severe OSA. The percentage of sleep time with oxygen saturation below 90% (T90) was also calculated.

Statistical analysis

All statistical analyses were performed with SAS software (SAS/STAT Package 2002–2003; SAS Institute Inc., Cary, NC). Results were expressed as percentages, mean (standard deviation, SD) and odds ratio (OR), 95% confidence interval (CI). A two-tailed p value <0.05 was considered significant. The primary dependent variables of interest were the presence of significant liver disease and advanced liver fibrosis as defined by LSM values ≥ 7.3 kPa and ≥ 9.6 kPa, respectively. The primary independent variable was the severity of OSA as assessed by the

AHI and two different indices of nocturnal hypoxemia (ODI, and T90). Patient characteristics across categories of OSA severity were compared using a Chi-square test for categorical variables and 2-sample t-test for continuous variables. Logistic regression procedures were performed to calculate adjusted OR (95%CI) for having $LSM \geq 7.3$ or ≥ 9.6 kPa according to the severity of OSA and nocturnal hypoxemia. To adjust for potential confounders, the following covariates that are likely to interfere with NAFLD were entered in the multivariate analysis: age, gender, waist circumference, diabetes, hypertension, MS, and HOMA-IR.

Results

Of the 414 patients included in the NUMEVOX Cohort between March 2008 and August 2017, 147 patients had a positive BERLIN questionnaire and were further investigated by overnight polysomnography. Twenty-three of these patients were excluded from the analysis due to unreliable transient elastography with LSM values ≥ 7.3 kPa and IQR/M ratio >0.30 . The final study sample size therefore comprised 124 patients (Figure 1). OSA was confirmed by polysomnography in all patients, including 34 (27.4%) with mild OSA, 38 (30.6%) with moderate OSA and 52 (42.0%) with severe OSA. As shown in Table 1, the study population consisted of typical OSA patients, predominantly male, obese or overweight, frequently presenting metabolic comorbidities. As expected, increasing OSA severity was associated with higher BMI ($p=0.06$), waist circumference ($p=0.02$), ODI ($p<0.0001$), T90 ($p=0.002$), and higher proportions of male patients ($p=0.02$) with MS ($p=0.02$). Increasing OSA severity was also associated with higher LSM values ($p=0.004$) with a marked increase between mild-to-moderate OSA and severe OSA.

Among the 124 patients with mild-to-severe OSA, 91 (73.4%) had LSM values <7.3 kPa, 18 (14.5%) had LSM values between 7.3 and <9.6 kPa indicating significant liver disease, and 15 (12.1%) had LSM values \geq 9.6 kPa indicating advanced liver fibrosis. As shown in Table 2, patients with elevated LSM values had significantly higher levels of liver enzymes (ALT, $p=0.01$; AST, $p=0.0003$; GGT, $p=0.01$) and total bilirubin ($p=0.002$). HOMA-IR increased with elevated LSM values, but this increase was not significant ($p=0.39$).

As shown in Figure 2, a dose-response relationship was observed between OSA severity and LSM values with a marked increase in the proportion of patients with significant liver disease (LSM between 7.3 and <9.6 kPa) and advanced liver fibrosis (LSM \geq 9.6 kPa) between mild-to-moderate and severe OSA. For subsequent analysis, the AHI variable was dichotomized into 2 categories (i.e. AHI \geq 30, severe OSA *versus* <30, mild-to-moderate OSA). The study sample was also dichotomized into 2 categories for physiologic indices of nocturnal hypoxemia according to the median value of the variable.

Unadjusted and adjusted OR for LSM \geq 7.3 kPa according to OSA severity are presented in Table 3. On univariate analysis, severe OSA (AHI \geq 30) was associated with a significantly increased risk of LSM \geq 7.3 kPa (OR [95%CI], 5.66 [2.32-13.79]). The association between OSA and significant liver disease remained highly significant after adjustment for age, gender, waist circumference, hypertension, and diabetes (model 1; OR 6.59 [2.35-18.49]); adjustment for age, gender and MS (model 2; OR 5.63 [2.17-14.63]); or adjustment for age, gender, MS and HOMA-IR (model 3; OR 7.17 [2.51-20.50]).

The associations between 2 different indices of nocturnal hypoxemia and LSM \geq 7.3 kPa were also investigated. As shown in Table 3, increasing hypoxemia during sleep, as reflected by ODI

and T90, was significantly associated with an increased risk of significant liver disease. On multivariate analysis, the association remained significant for ODI and T90 according to models 1, 2 and 3.

A subsequent analysis calculated the unadjusted and adjusted odds ratios for $LSM \geq 9.6$ kPa according to the severity of OSA and hypoxemia during sleep. On multivariate analysis, severe OSA and elevated ODI (≥ 19 events/h of sleep) were associated with an increased risk of advanced liver fibrosis after adjustment for age, gender, MS and HOMA-IR (OR, 4.73 [1.25-17.88] and 4.79 [1.18-19.54], respectively) (see e-Table 1 in supplementary data).

Discussion

To our knowledge, this is the largest study evaluating the relationship between OSA severity and LSM. This study demonstrated that patients with severe OSA and metabolic comorbidities are at higher risk of significant liver disease ($LSM \geq 7.3$ kPa) and advanced liver fibrosis ($LSM \geq 9.6$ kPa) after adjustment for the main factors contributing to the development and exacerbation of NAFLD, including age, central obesity, MS and insulin resistance. AHI and ODI were the factors with the strongest independent association with LSM, whereas T90 was not associated with advanced liver fibrosis. While ODI reflects the frequency of oxygen desaturations, T90 reflects the severity of hypoxemia and is less specific of OSA-related hypoxemia [23]. These data are in accordance with previously published clinical reports and basic research suggesting the major role of intermittent hypoxia in the hepatic consequences of OSA [3, 4].

This study underlines the high proportion of patients at risk of significant liver lesions, especially liver fibrosis, among patients with metabolic comorbidities investigated for suspected OSA, particularly among those with severe OSA. This finding is consistent with histological data from

previous studies performed in obese patients undergoing bariatric surgery showing that almost 50% of patients with severe OSA present significant fibrosis (\geq F2 stage) [4, 24]. These findings suggest that, apart from extremely obese patients, a significant proportion of severe OSA patients should be referred to hepatologists for specific management.

Identification and quantification of fibrosis is a key issue in patients with NAFLD because fibrosis stage, but no other histologic features of steatohepatitis, is independently associated with long-term outcomes [5]. However, the independent association between OSA severity and liver fibrosis remains the subject of debate. Biopsy-based studies evaluating liver fibrosis have reported conflicting results, as some demonstrated an independent association between liver fibrosis and markers of nocturnal hypoxemia [25], while others failed to demonstrate an independent association after adjustment for major NAFLD risk factors [26, 27]. These studies were conducted in the particular population of morbidly obese patients undergoing intraoperative needle liver biopsy during bariatric surgery, thereby limiting generalization of the findings and presumably contributing to the relative statistical weakness of the association observed, as up to 91% of the patients included were female, less prone to develop android obesity and its metabolic and respiratory consequences [4, 28]. Two other studies investigated the association between OSA and the various components of NAFLD in larger cohorts of patients investigated by polysomnography and blood tests for evaluation of NAFLD lesions. These studies demonstrated an independent association between OSA severity and steatosis, but the association with liver fibrosis markers was no longer significant after adjustment for major risk factors including age and waist circumference [29, 30]. The algorithms used to predict liver fibrosis included age, BMI, sex and serum transaminase levels. As these various parameters have been

shown to correlate with or be impacted by OSA, further adjustments in multivariate analysis would be of limited value.

This limitation can be overcome by evaluating liver lesions with LSM, a physical measurement based on elastography. To the best of our knowledge, only one study has evaluated LSM in patients with OSA and found an independent correlation between the severity of intermittent hypoxia and increased fibrosis as determined by LSM [31]. This study presented a major limitation due to the mix of two different cohorts of NAFLD and OSA patients and finally a very small sample of patients in which both polysomnography and LSM data were available (polysomnography was performed in only 31 of the 123 patients included in the analysis).

We acknowledge that liver biopsy was not available in our study. Despite it remains the reference to evaluate liver lesions in NAFLD, this procedure is seldom used in clinical practice because of its invasiveness [6]. In addition, liver biopsy is not a perfect gold standard because of sampling bias and suboptimal inter-observer reproducibility [32, 33]. Blood tests and elastography devices have been developed to facilitate the diagnosis of liver fibrosis and these non-invasive tests are now widely used in clinical practice. A recent meta-analysis has shown that LSM with FibroScan ≥ 9.6 kPa has excellent sensitivity and negative predictive value for the diagnosis of advanced liver fibrosis in NAFLD, but only a moderate 68% positive predictive value [18]. We therefore cannot affirm that all patients with LSM ≥ 9.6 kPa in our study had advanced liver fibrosis. However, blood fibrosis tests and FibroScan are highly correlated with liver fibrosis, and have been recently shown to be themselves prognostic markers able to identify NAFLD patients with impaired outcome [34, 35]. This represents a strong argument for the use of these tests in clinical practice to assess NAFLD severity and identify the subgroup of patients justifying referral to specialized hepatologists for specific evaluation and management [36]. For

the same reason, non-invasive tests of liver fibrosis are relevant tools in clinical studies such as the present work to evaluate the relationships between NAFLD severity and the different complications of obesity and MS.

Another potential limitation of the present study concerns the selection of the sample. We included patients presenting with a clinical suspicion of OSA and at least one criterion for MS. Our findings are in accordance with previous experimental and clinical studies demonstrating that OSA and intermittent hypoxia exacerbate the metabolic dysfunction of obesity [37]. However, the present findings should not be generalized to all OSA patients and further studies are required to investigate the impact of OSA severity on LSM in patients without metabolic comorbidities.

Finally, because of the cross-sectional design of this study, it cannot be determined whether OSA preceded onset of increased liver stiffness. Investigations into the causal link between OSA and liver damage will require prospective studies. In a recent randomized trial, 6 to 12 weeks of nasal continuous positive airway pressure, the reference treatment for moderate-to-severe OSA, did not improve blood markers of liver injury [38]. Further studies are required to determine whether long-term OSA therapy, by abolishing nocturnal oxygen desaturation, can help to prevent progression of liver stiffness.

In conclusion, severe OSA in patients with metabolic comorbidities is independently associated with increased liver stiffness, which may predispose to a higher risk of significant liver disease and poorer prognosis.

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Table 1: Patients characteristics according to the severity of obstructive sleep apnoea

	All	Mild OSA	Moderate OSA	Severe OSA	p value
n	124	34	38	52	
Female, %	34.4	52.9	24.3	29.4	0.0247
Age, year	52.4 (9.5)	49.7 (10.8)	52.5 (9.6)	53.7 (7.9)	0.1513
BMI; kg/m ²	29.9 (4.8)	28.6 (4.9)	29.6 (4.2)	31.0 (5.0)	0.0620
WC, cm	102.3 (12.7)	97.4 (14.7)	102.6 (10.3)	105.2 (12.1)	0.0195
Diabetes, %	16.4	17.6	10.8	19.6	0.5313
Hypertension, %	45.1	38.2	43.2	51.0	0.4939
Statin use, %	26.2	20.6	16.2	37.3	0.0584
MS, %	52.5	32.4	59.5	60.8	0.0218
ESS score	11.2 (5.5)	10.4 (5.2)	12.0 (5.6)	11.1 (5.6)	0.5984
AHI, n	29.1 (23.2)	6.6 (3.7)	20.7 (3.9)	50.2 (20.7)	<0.0001
ODI, n	23.2 (22.4)	7.4 (16.3)	16.3 (11.8)	38.7 (22.1)	<0.0001
T90, %	9.1 (16.9)	2.1 (7.4)	6.0 (16.8)	16.1 (19.1)	0.0002
LSM, kPa	6.6 (4.3)	5.2 (1.6)	5.8 (2.3)	8.1 (6.0)	0.0041

Data are expressed as percentages or mean (standard deviation).

Abbreviations: OSA, obstructive sleep apnoea, BMI, body mass index, WC, waist circumference; MS, Metabolic Syndrome; ESS, Epworth sleepiness scale; AHI, apnea-hypopnea index; ODI, oxygen desaturation index; T90, sleep time with oxygen saturation <90%; LSM, liver stiffness measurement.

Table 2: Biological data according to the liver stiffness measurement (LSM)

	LSM<7.3 (kPa)	7.3≤LSM<9.6 (kPa)	LSM≥9.6 (kPa)	p value
n	91	18	15	
Total bilirubin $\mu\text{mol/L}$	9.1 (4.3)	10.0 (4.8)	14.3 (9.5)	0.0023
ALT, IU/L	39.7 (24.5)	61.5 (33.2)	58.6 (33.6)	0.0014
AST, IU/L	28.0 (12.2)	42.6 (27.5)	40.1 (18.8)	0.0003
Alkaline phosphatase, IU/L	69.3 (23.7)	70.8 (18.6)	72.2 (25.4)	0.8924
GGT, IU/L	56.9 (81.8)	112.1 (109.6)	134.2 (216.9)	0.0145
HOMA IR	4.0 (6.9)	4.8 (2.4)	6.3 (2.8)	0.3899

Data are expressed as mean \pm standard deviation. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; HOMA-IR: homeostasis model assessment of insulin resistance

Table 3: Unadjusted and adjusted odds ratios for having a liver stiffness measurement (LSM) ≥ 7.3 kPa according to the severity of obstructive sleep apnoea and hypoxemia during sleep.

Variables	Unadjusted OR (95%CI)	Adjusted OR (95%CI)		
		Model 1	Model 2	Model 3
AHI, n				
<30	1	1	1	1
≥ 30	5.66 (2.32-13.79)	6.59 (2.35-18.49)	5.63 (2.17-14.63)	7.17 (2.51-20.50)
ODI, n				
<19	1	1	1	1
≥ 19	4.86 (2.01-11.78)	6.62 (2.17-20.22)	4.79 (1.80-12.74)	6.23 (2.11-18.42)
T90, %				
<2	1	1	1	1
≥ 2	4.46 (1.75-11.38)	4.51 (1.53-13.25)	3.75 (1.39-10.12)	4.29 (1.50-12.23)

Abbreviations: AHI, apnea-hypopnea index; ODI, oxygen desaturation index; T90, sleep time with oxygen saturation $< 90\%$

Model 1: adjusted for age, gender, waist circumference, diabetes and hypertension

Model 2: adjusted for age, gender and metabolic syndrome

Model 3: adjusted for age, gender, metabolic syndrome and homeostasis model assessment of insulin resistance

Figure 1: Flow diagram of subjects during the study.

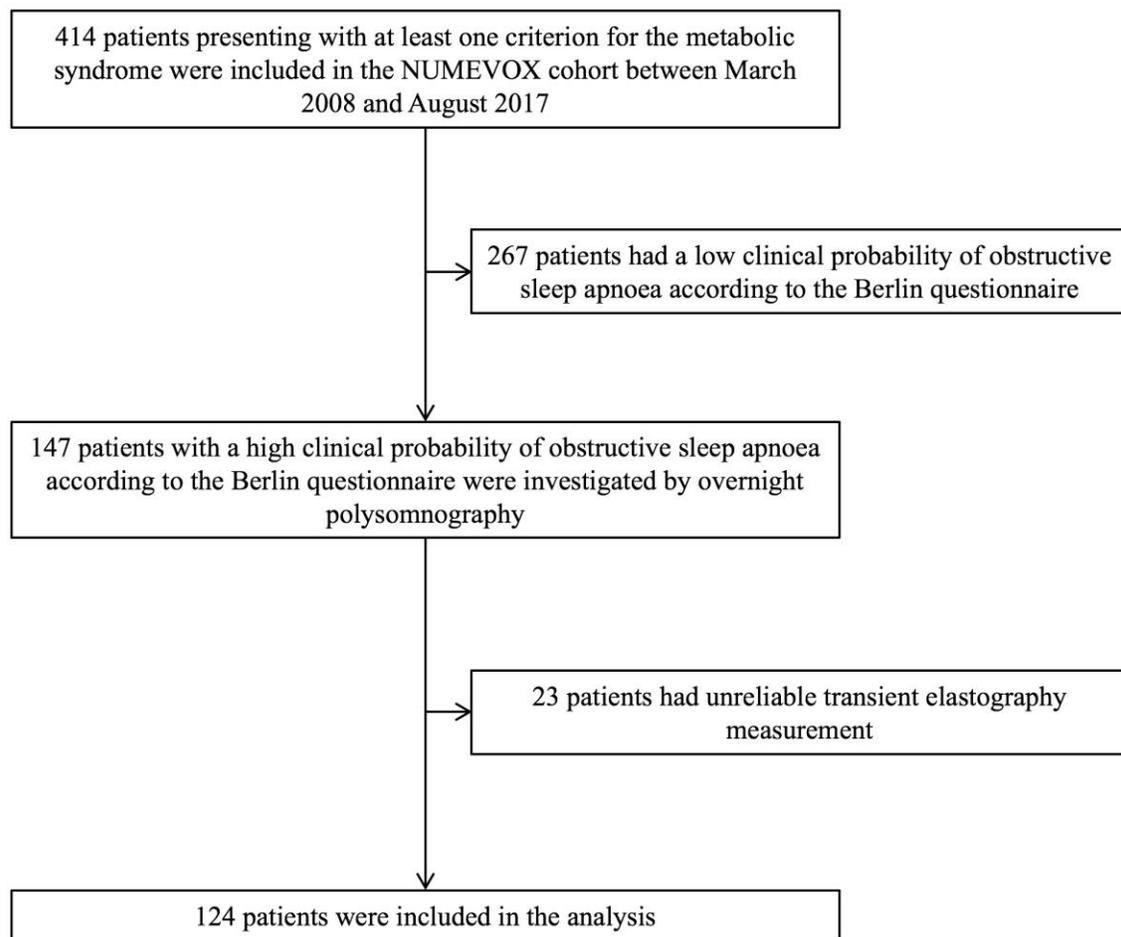
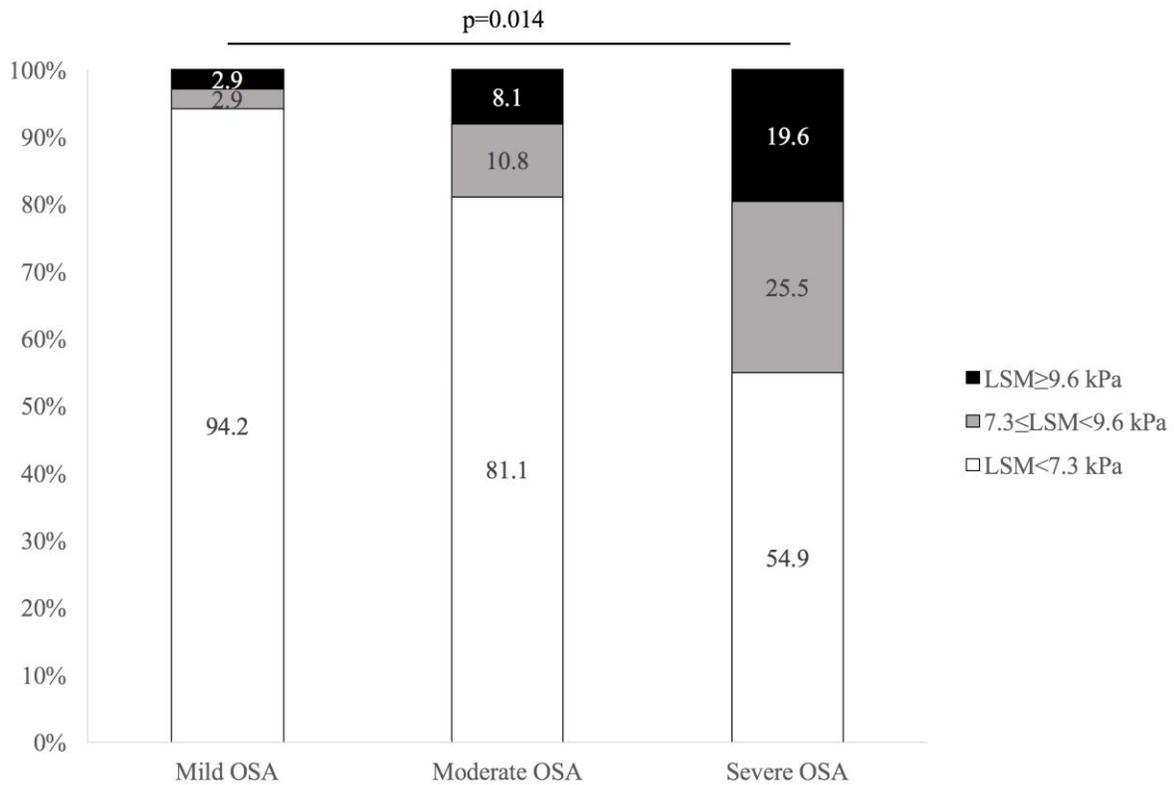


Figure 2: Relationship between obstructive sleep apnoea severity and liver stiffness measurement (LSM).

The following commonly used cut-offs for apnoea-hypopnoea index (AHI) were used to define categories of obstructive sleep apnoea (OSA) severity: 5 to <15 for mild OSA, 15 to <30 for moderate OSA, and ≥ 30 for severe OSA.



Supplementary data

e-Table 1: Unadjusted and adjusted odds ratios for having a liver stiffness measurement (LSM) ≥ 9.6 kPa according to the severity of obstructive sleep apnoea and hypoxemia during sleep.

Variables	Unadjusted OR (95%CI)	Adjusted OR (95%CI)		
		Model 1	Model 2	Model 3
AHI, n				
<30	1	1	1	1
≥ 30	4.08 (1.20-13.88)	4.73 (1.17-19.06)	4.04 (1.11-14.68)	4.73 (1.25-17.88)
ODI, n				
<19	1	1	1	1
≥ 19	3.63 (1.07-12.33)	5.17 (1.08-24.78)	3.61 (0.95-13.74)	4.79 (1.18-19.54)
T90, %				
<2	1	1	1	1
≥ 2	1.67 (0.53 – 5.31)	1.54 (0.39-6.02)	1.32 (0.38-4.61)	1.49 (0.42-5.25)

Abbreviations: AHI, apnea-hypopnea index; ODI, oxygen desaturation index; T90, sleep time with oxygen saturation $< 90\%$

Model 1: adjusted for age, gender, waist circumference, diabetes and hypertension

Model 2: adjusted for age, gender and metabolic syndrome

Model 3: adjusted for age, gender, metabolic syndrome and homeostasis model assessment of insulin resistance