High D-dimer levels after stopping anticoagulants in pulmonary embolism with sleep apnoea

Angela García Suquia[1], Alberto Alonso-Fernández[2,3], Mónica de la Peña[2,3], David Romero[4], Javier Piérola[5], Miguel Carrera[2,3], Antonia Barceló[1,3], Joan B. Soriano[6], Meritxell Arque[5], Carmen Fernández-Capitán[7], Alicia Lorenzo[7] and Francisco García-Río[3,4]

Affiliations: [1]Dept of Clinical Analysis, University Hospital Son Espases, Palma de Mallorca, Spain. [2]Dept of Pneumology, University Hospital Son Espases, Palma de Mallorca, Spain. [3]CIBER Enfermedades Respiratorias, Palma de Mallorca, Illes Balears, Spain. [4]Dept of Pneumology, University Hospital La Paz, IdiPAZ, Madrid, Spain. [5]IdISPa-FISIB, Hospital Universitari Son Espases, Palma de Mallorca, Spain. [6]Instituto de Investigación Hospital Universitario de la Princesa (IISP), Universidad Autónoma de Madrid, Cátedra UAM-Linde, Madrid, Spain. [7]Dept of Internal Medicine, University Hospital La Paz, Madrid, Spain.

Correspondence: Alberto Alonso-Fernández, Servicio de Neumología. Hospital Universitario Son Espases, Carretera de Valldemossa 79, 07010 Palma de Mallorca, Spain. E-mail: alberto.alonso@ssib.es

ABSTRACT Obstructive sleep apnoea is a risk factor for pulmonary embolism. Elevated D-dimer levels and other biomarkers are associated with recurrent pulmonary embolism. The objectives were to compare the frequency of elevated D-dimer levels (>500 ng·mL^{-1}) and further coagulation biomarkers after oral anticoagulation withdrawal in pulmonary embolism patients, with and without obstructive sleep apnoea, including two control groups without pulmonary embolism.

We performed home respiratory polygraphy. We also measured basic biochemical profile and haemogram, and coagulation biomarkers (D-dimer, prothrombin fragment 1+2, thrombin-antithrombin complex, plasminogen activator inhibitor 1, and soluble P-selectin).

64 (74.4%) of the pulmonary embolism cases and 41 (46.11%) of the controls without pulmonary embolism had obstructive sleep apnoea. Plasmatic D-dimer was higher in PE patients with OSA than in those without obstructive sleep apnoea. D-dimer levels were significantly correlated with apnoea–hypopnoea index, and nocturnal hypoxia. There were more patients with high D-dimer after stopping anticoagulants in those with pulmonary embolism and obstructive sleep apnoea compared with PE without obstructive sleep apnoea (35.4% *versus* 19.0%, p=0.003). Apnoea–hypopnoea index was independently associated with high D-dimer.

Pulmonary embolism patients with obstructive sleep apnoea had higher rates of elevated D-dimer levels after anticoagulation discontinuation for pulmonary embolism than in patients without obstructive sleep apnoea and, therefore, higher procoagulant state that might increase the risk of pulmonary embolism recurrence.

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Introduction

There is increasing evidence from cross-sectional and longitudinal studies that obstructive sleep apnoea (OSA) is a risk factor for pulmonary embolism (PE) independent of potential confounders [1–5].

The annual incidence of PE is one to two cases per 1000 person-years, and it is strongly age dependent. PE and OSA share risk factors (advanced age, physical inactivity and obesity) [6, 7], its association represent a major public health burden, given its high prevalence and increased mortality rates [7, 8], which is likely to rise as the population ages and obesity rates increase in developing and emerging countries. Patients with a first episode of PE have a cumulative recurrence rate circa to 25% at 5 years [9]. Recurrent PE is associated with mortality rates of 4–9%, and it is also a risk factor for chronic pulmonary hypertension. Anticoagulants are highly effective in decreasing the PE recurrence rate; however, it increases the risk of bleeding complications and, therefore, the optimal length of anticoagulation following PE remains unclear.

D-dimer is a fibrin degradation product that is used to evaluate patients with suspected PE. It has also reported a higher risk of PE recurrence in patients with unprovoked PE who had a high post-anticoagulation D-dimer (>500 ng·mL^{-1}) than in patients with normal D-dimer levels [10]. A landmark randomised clinical trial found that patients with an abnormal D-dimer test who restarted anticoagulation had a significantly lower combined incidence of recurrent venous thromboembolism and bleeding than those who did not resume the treatment [11]. Thus, D-dimer has been proposed as a tool in guiding the length of therapy for PE.

The prognostic role of other biomarkers of haemostasis/fibrinolysis is less known. Elevated levels of prothrombin fragment 1+2 (F1+2) and soluble P-selectin (sP-selectin) have been explored as a risk factor for first and recurrent thromboembolic events, but studies have reported conflicting results [12–15].

Based on the aforementioned features and considering that OSA leads to a hypercoagulable state, we hypothesised that those patients discontinuing anticoagulation after a PE with OSA could have a greater risk of embolic recurrence, evidenced by the presence of a high level of D-dimer, than non-apnoeic subjects. The main objective of the present study was to compare the frequency of elevated plasma levels (>500 ng·mL^{-1}) of D-dimer after oral anticoagulation withdrawal for a first PE episode in patients with and without OSA, including two control groups without PE, one with OSA and one with no sleep–breathing problems.

Methods

Subjects, design and ethics

We performed a prospective matched case-control study (University Hospital Son Espases, Palma de Mallorca and University Hospital La Paz, Madrid, Spain). Eligible cases were all patients with a previous (6–12 months) PE episode diagnosed by computed tomography (CT) pulmonary angiography that had completed at least 3 months with a vitamin K antagonist. They were included if they stopped anticoagulation for at least 1 month. Patients were excluded if they were unwilling or unable to participate in the study, estimated survival <12 months, and/or severe daytime hypoxaemia (arterial oxygen tension <60 mmHg).

Controls were population-based volunteers. They were enrolled from the population area of the Primary Care Centres. They were never previously evaluated with a sleep study. We randomly selected a subject without previous diagnosis or clinical suspicion of thromboembolic disease, with similar in sex, age (±2 years), weight (±2 Kg) and height (±5 cm) with regard to the two preceding PE patients included in the study and who had no exclusion criteria *e.g.* a very serious illness (terminal or serious physical/mental disability) that might prevent monitoring and participation in the study, inability to sign the informed consent and/or treatment with vitamin K antagonists, and those with communication or comprehension difficulties.

The study was approved by the Institutional Ethics Committee at the hospital and all subjects (cases and controls) gave their written informed consent.

Measurements

A validated portable recording sleep monitoring system (Stardust II; Respironics, Inc., Murrysville, PA, USA) was used to perform a sleep study in all patients and control subjects. Every patient or control was classified as having OSA when the obstructive component was dominant and apnoea–hypopnoea index (AHI) was $\geqslant 5 \cdot h^{-1}$ (see online supplementary material).

In all subjects anthropometric, clinical and sleep data were collected. We recorded, for all subjects, all the potential confounders which included: sex, age, body mass index (BMI), neck diameter, physical activity level, classic risk factors for pulmonary embolism (prolonged immobilisation major surgery, trauma, cancer, major medical diseases, hormone replacement, oral contraceptive therapy and cerebrovascular disease), blood pressure values, and forced expiratory volume in 1 s (FEV_1) measured as % predicted. Physical activity was measured with a self-administered questionnaire (International Physical Activity Questionnaire

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long form). Office blood pressure was measured by a random zero sphygmomanometer. More details and descriptions of each of the respiratory events are available in the online supplementary material.

In PE patients 1 month after oral anticoagulation withdrawal, a venous blood sample was collected. Quantitative determination of D-dimer levels was performed by inmunoturbidimetric assay (INNOVANCE D-Dimer of SIEMENS Healthcare, Marburg, Germany). D-dimer was considered high when >500 $ng \cdot mL^{-1}$. Plasma levels of soluble P-selectin (sP-selectin), thrombin–antithrombin complex (TAT), plasminogen activator inhibitor 1 (PAI-1), and F1+2, were determined by an enzyme-linked immunoassay (MAGPIX Multiplex Immunoassay, Merck Millipore, Dormstadt, Germany) according to manufacturer's instructions with Xponent software 4.1. Commercial kit R&D Systems Quantikine (Minneapolis, MN, USA) was used to measure sP-selectin (Human soluble P-Selectin/CD62P Immunoassay) and PAI-1 (Human Serpin E1/PAI1) and for the quantitative determination of human prothrombin fragment F1+2 and TAT the commercial kit Enzygnost (SIEMENS Healthcare Diagnostics, Marburg, MN, Germany) was used. Measurements were always done in duplicate and the measured values were used for analysis. Intra-assay/inter-assay coefficient variation (CV) for the techniques used were: sP-selectin (5.2–8.8% CV); TAT (5–7.5% CV); PAI-1 (6.7–7.3% CV); F1+2 (4.5–7.8% CV); D-dimer (4.2–5% CV).

Statistical analysis

The normality of the distribution of variables was tested using the Kolmogorov–Smirnov test. Continuous variables were expressed as mean±SD or median (interquartile range), depending on their distribution. Categorical variables were reported as absolute numbers and percentages. Differences between study groups for qualitative and quantitative variables were analysed by the Chi-squared test and analysis of variance with Bonferroni *post hoc* test, respectively. The relationship between plasma levels of biomarkers and sleep parameters was determined using multiple linear regression analysis after normalisation of all variables by log transformation and adjustment for classic risk factors for PE. To examine associations between study groups and the study variables, odds ratios (OR) and 95% confidence intervals (CI) in univariate and multivariate analyses were calculated by logistic regression. Statistical significance was assumed for $p<0.05$. All analyses were performed using the Statistical Package for the Social Sciences, version 13.0 software (SPSS Inc., Chicago, IL, USA).

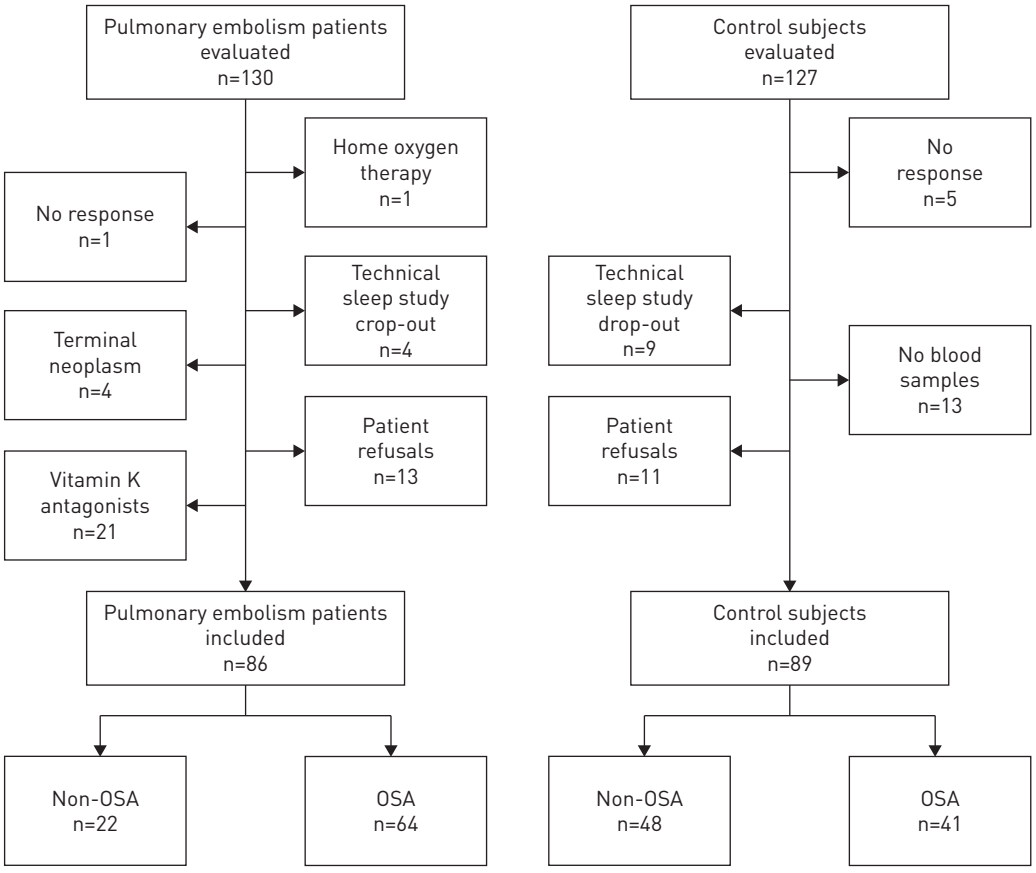

FIGURE 1 Flow diagram of all the study subjects.

Results

From the 107 patients with PE and the 102 control subjects [1] we excluded 21 PE patients, who were on vitamin K antagonist, and 13 control subjects, who were without blood samples. All patients were under oral anticoagulation with acenocoumarol. Therefore, 86 patients with PE and 89 control subjects were finally included in the study (figure 1). There were no significant differences between patients with PE and control subjects in the anthropometric characteristics (age, sex, BMI, smoking, and alcohol intake).

General characteristics

Overall, 64 (74.4%) PE patients and 41 (46.1%) control subjects had OSA (apnoea–hypopnoea index >5·h^{-1}) (figure 1). Subjects were classified in four study subgroups: 1) PE-OSA (PE patients with OSA), 2) PE non-OSA (PE patients without OSA), 3) C-OSA (control subjects with OSA) and 4) C non-OSA (control subjects without OSA). The four subgroups were homogenous in sex, BMI and daily physical activity level. OSA patients (PE-OSA and C-OSA) were older than non-OSA subjects (tables 1 and S1).

Sleep study variables

By definition AHI was significantly higher in OSA patients (PE-OSA and C-OSA) compared with non-OSA. In addition, OSA patients (PE-OSA and C-OSA) had worse nocturnal oxygen saturation indices than non-OSA subjects. PE-OSA also had higher desaturation index and lower mean night-time arterial oxygen saturation (S_{aO_2}) than C-OSA (22.9±17.2 event·h^{-1} *versus* 15.7±14.0 event·h^{-1}, and 92.6±2.4% *versus* 94.0±1.9%, respectively; p<0.05) (table 1). Lastly, no differences were found in daytime somnolence.

TABLE 1 Comparison of the demographic characteristics, sleep parameters, daily physical activity and plasma biomarkers between the study groups according to the presence of obstructive sleep apnoea (OSA)

	PE patient group		Control group		p-value
	OSA	Non-OSA	OSA	Non-OSA	
Subjects n	64	22	41	48	
Sex male	43 (67)	9 (41)	31 (66)	28 (51)	0.066
Age years	60±14$^{\#\#\#,\P\P}$	50±16	61±12$^{\#\#\#,\P\P}$	49±14	0.000
Body mass index kg·m^{-2}	27.8±5.6$^{\#\#\#}$	26.8±4.8	27.5±3.9$^{\#}$	25.7±3.9	0.168
Epworth Sleepiness Scale	7.1±3.9	5.8±3.5	6.0±3.9	6.5±2.5	0.359
Apnoea–hypopnoea index events·h^{-1}	22.0 (11.8–40.3)$^{\#\#\#,\P\P\P}$	3.4 (2.0–4.2)	12.9 (7.8–30.7)$^{\#\#\#,\P\P\P}$	2.4 (1.2–3.6)	0.000
CT$_{90\%}$ %	15.0 (1.5–43.0)$^{\#\#,\P\P}$	0 (0–0.9)	6.5 (0.5–33.4)	0.5 (0.0–2.4)	0.001
Mean S_{aO_2} %	92.6±2.4$^{\#\#\#,\P\P\P,+}$	94.9±1.2$^{\#}$	94.0±1.9$^{\#\#\#}$	95.5±1.4	0.000
Desaturation index events·h^{-1}	15.0 (10.5–35.0)$^{\#\#\#,\P\P\P,+}$	2.7 (2.2–4.0)	10.7 (7.9–19.3)$^{\#\#,\P\P}$	3.1 (1.9–5.4)	0.000
Minimum S_{aO_2} %	78.0 (70.0–84.0)$^{\#\#\#,\P\P\P}$	88.0 (84.5–89.5)	82.5 (75.0–86.8)$^{\#,\P\P}$	86 (80.3–89.0)	0.000
Physical activity level					0.440
Low	36.6	14.3	23.1	31.8	
Moderate	41.5	71.4	51.3	40.9	
High	22.0	14.3	25.6	27.3	
Glucose mg·dL^{-1}	103.0 (94.0–115.0)$^{\#}$	98.5 (91.0–104.3)	108.0 (95.5–120.0)$^{\#}$	97.0 (90.5–105.0)	0.056
Triglycerides mg·dL^{-1}	140±62$^{\#\#\#}$	153±87$^{\#\#\#}$	118±57¶	99±40	0.000
Cholesterol mg·dL^{-1}	204±41	217±35	208±31	203±36	0.4410
HDL cholesterol mg·dL^{-1}	49±12$^{\#\#\#,+}$	49±12$^{\#\#}$	54±12$^{\#}$	60±16	0.000
LDL cholesterol mg·dL^{-1}	127±33	141±30$^{\#}$	129±29	121±35	0.139
Uric acid mg·dL^{-1}	6.2 (4.8–7.1)$^{\#\#}$	5.3 (4.0–6.9)	5.4 (4.6–6.7)	4.6 (3.9–5.5)	0.035
Haemoglobin g·dL^{-1}	14.8 (13.2–15.9)	13.7 (13.2–15.3)	14.1 (13.6–15.7)	14.3 (13.5–14.9)	0.759
Haematocrit %	44.2 (39.1–46.9)	41.6 (38.9–46.4)	41.4 (39.3–45.1)	41.2 (39.3–43.4)	0.593
Leukocytes 10^3·uL^{-1}	6.944±1.926	6.898±2.064	6.800±1.634	6.862±2.266	0.983
Platelets 10^3·uL^{-1}	244±61	255±72	251±60	258±91	0.708
D-dimer ng·mL^{-1}	355 (230–615)$^{\#\#,\P}$	345 (238–520)	335 (215–498)	300 (200–380)	0.039
Soluble P-selectin ng·mL^{-1}	2.1±0.5	2.0±0.4	2.2±0.9	1.9±1.2	0.390
PAI-1 ng·mL^{-1}	4.2 (2.5–6.5)$^{\#,+}$	3.6 (2.0–6.1)	2.2 (1.2–5.0)	1.5 (1.1–5.1)	0.053
F1+2 pmol·L^{-1}	303 (197–435)$^{\#\#,+}$	143 (101–217)	193 (150–217)	162 (141–196)	0.002
TAT µg·L^{-1}	4.5 (3.2–12.1)$^{\#}$	3.0 (1.9–11.6)$^{\#}$	2.2 (1.0–10.2)¶	7.9 (6.2–10.1)	0.097

Data are presented as n (%), mean±SD, median (interquartile range) or %, unless otherwise stated. PE: pulmonary embolism; CT$_{90\%}$: percentage of total study time spent with arterial oxygen saturation (S_{aO_2}) <90%; HDL: high-density lipoprotein; LDL: low-density lipoprotein; PAI-1: plasminogen activator inhibitor 1; F1+2: prothrombin fragment 1+2; TAT: thrombin–antithrombin complex. $^{\#}$: p<0.05; $^{\#\#}$: p<0.01; $^{\#\#\#}$: p<0.001 for *versus* control group non-OSA. ¶¶¶: p<0.001; ¶¶: p<0.01; ¶: p<0.05 for *versus* PE group non-OSA. $^{+}$: p<0.05 for *versus* control group with OSA.

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Haematological and biochemical analysis

The number of circulating erythrocytes, leucocytes and platelets were similar between groups. Dyslipidaemia was significantly more frequent in the PE group than in controls, with both higher triglycerides and lower high-density lipoprotein (HDL)-C levels. C-OSA had lower HDL-C values compared with C non-OSA. Furthermore, uric acid levels were also higher in PE-OSA than C non-OSA subjects ($p<0.05$) (table 1).

Procoagulant, platelet activity and fibrinolysis biomarkers

The primary outcome of our study was to compare the percentage of patients with elevated D-dimer between the study groups. The prevalence of elevated levels of D-dimer (>500 ng·mL^{-1}) after stopping anticoagulant treatment for PE was higher in patients with OSA than in patients without OSA (35.4% *versus* 19%, p=0.003). In the same way, C-OSA had a higher percentage of subjects with high D-dimer levels than C non-OSA (figure 2). PE patients with OSA had significantly greater D-dimer plasma concentrations than non-OSA (with or without previous PE, 355 ng·mL^{-1} (230–615 ng·mL^{-1}) *versus* 345 ng·mL^{-1} (238–520 ng·mL^{-1}) and 300 ng·mL^{-1} (200–380 ng·mL^{-1}); p<0.01 and p<0.05, respectively). F1+2 and PAI-1 levels were also higher in patients with PE and OSA compared with those who did not have a PE (with or without OSA) (table 1). Furthermore, TAT was also higher in PE than controls without PE (table 1).

In patients with PE, AHI was related to high D-dimer levels (table 2). This association remained significant after adjusting for sex, age, BMI, neck diameter, physical activity level, classic risk factors for pulmonary embolism, blood pressure values and FEV_1 % pred, adjusted OR 1.114 (95% CI 1019–1218, p=0.018).

The relationships between plasma levels of haemostasis/fibrinolysis biomarkers and sleep parameters, adjusted for the classic risk factors for PE, are shown in table 3. In all study subjects, D-dimer levels were directly related to AHI (r=0.279, p=0.007), percentage of total time study spent with S_{aO_2} <90% and desaturation index and inversely related to the mean and minimum night-time S_{aO_2} (figure 3). A significant relationship was also found between AHI and the sP-selectin level. In addition, other significant relationships were found between some indices of nocturnal oxygen saturation and PAI-1 and F1+2 levels (table 3). In a multiple linear regression analysis, adjusted for sex, age, BMI and classic risk factors for pulmonary embolism, we found that D-dimer levels were mainly determined by AHI (β=0.22, p=0.02, ΔR^2=0.12) and minimum night-time S_{aO_2} (β=−0.25, p=0.011, and ΔR^2=0.04), whereas the mean sleep S_{aO_2}, Epworth Sleepiness Scale and the percentage of total study time spent with S_{aO_2} <90% were the main determinants of F1+2 levels (table 4).

Finally, we evaluated the relationship between procoagulant, platelet activity and fibrinolysis biomarkers and sleep characteristics in the subgroup of subjects with OSA and excessive sleepiness (AHI >5 h^{-1} and ESS >11). After adjusting for classic risk factors for PE, D-dimer remained significantly related with AHI (r=0.631, p=0.037) (figure S1), desaturation index (r=0.631, p=0.021) and mean and minimum night-time S_{aO_2} (r=−0.515, p=0.049; r=−0.534, p=0.049, respectively). In these patients, AHI was also related to sP-selectin levels (r=0.658, p=0.020) (figure S1), whereas ESS was significantly correlated with PAI-1 (r=0.651, p=0.041), F1+2 (r=0.746, p=0.008) and TAT (r=0.617, p=0.033).

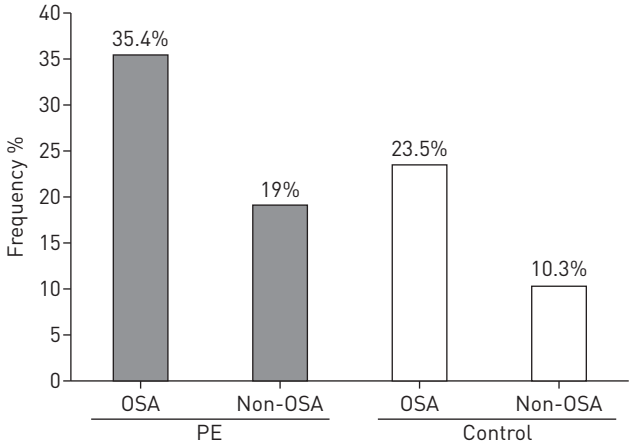

FIGURE 2 Frequencies of high plasmatic levels of D-dimer (>500 ng·mL^{-1}) in patients with pulmonary embolism after anticoagulation discontinuation and in control subjects. p=0.003.

TABLE 2 Risk factors related with the persistence of a high D-dimer plasmatic level after anticoagulant treatment in patients with pulmonary embolism

	Crude OR (95% CI)	p-value
Sex male	0.799 (0.318–2.008)	0.633
Age years	1.030 (0.995–1.067)	0.095
Body mass index	1.024 (0.934–1.123)	0.611
Neck diameter	1.036 (0.920–1.167)	0.558
Physical activity		0.248
Moderate	0.778 (0.176–3.431)	0.740
High	0.263 (0.047–1.476)	0.129
Forced expiratory volume at 1 s % pred	1.027 (0.995–1.060)	0.103
Blood pressure		
Systolic	1.006 (0.979–1.034)	0.653
Diastolic	1.009 (0.964–1.057)	0.692
Apnoea–hypopnoea index	1.030 (1.004–1.057)	0.024

OR: odds ratio; 95% CI: 95% confidence interval.

Discussion

In this study, we have shown that PE patients with OSA had higher rates of elevated D-dimer levels (>500 ng·mL^{-1}) after anticoagulation discontinuation for PE than in patients without OSA. Furthermore, D-dimer levels were higher in OSA patients than in patients without OSA. D-dimer concentrations were directly related to the severity of OSA, and inversely correlated with nocturnal hypoxia.

There is increasing evidence from cross-sectional and longitudinal studies, that OSA is an independent risk factor for PE [1–5]. We have recently shown in a group of 107 PE patients significantly higher prevalence in all OSA severity groups when compared with controls.

We found significant association between AHI and PE after adjustment for the main confounding factors. The adjusted OR using AHI as a continuous variable was 1.038, which indicated that for every 10-unit rise in AHI, PE risk increased by 45% [1].

The mechanisms explaining this novel association between PE and OSA are unclear. PE is the result of Virchow's classic risk triad, namely vascular endothelial impairment, stasis of blood flow, and/or increased coagulability [16]. OSA could potentially play a role in all these three mechanistic pathways. Apnoea increase oxidative stress [17], and activate inflammatory cascade that impairs endothelial function [18].

TABLE 3 Relationship between haemostasis/thrombolysis biomarkers and sleep characteristics in all study subjects[#]

	r[¶] (95% CI)	p-value
D-dimer		
Apnoea–hypopnoea index	0.279 (0.119–0.425)	0.007
Mean S_{aO_2}	−0.244 (−0.393– −0.082)	0.005
Desaturation index	0.274 (0.114–0.420)	0.001
$CT_{90\%}$	0.223 (0.060–0.375)	0.026
Minimum S_{aO_2}	−0.318 (−0.459– −0.161)	0.001
Soluble P-selectin		
Apnoea–hypopnoea index	0.202 (0.024–0.367)	0.032
PAI-1		
Desaturation index	0.201 (0.010–0.378)	0.047
F1+2 pmol·L^{-1}		
Mean S_{aO_2}	−0.266 (−0.426– −0.090)	0.004
Minimum S_{aO_2}	−0.262 (−0.422– −0.086)	0.008
Desaturation index	0.198 (0.019–0.365)	0.032

S_{aO_2}: arterial oxygen saturation; $CT_{90\%}$: percentage of total study time spent with S_{aO_2} <90%; PAI-1: plasminogen activator inhibitor 1; F1+2: prothrombin fragment 1+2. [#]: all variables are log normalised; [¶]: regression coefficient adjusted for classical risk factors for pulmonary embolism.

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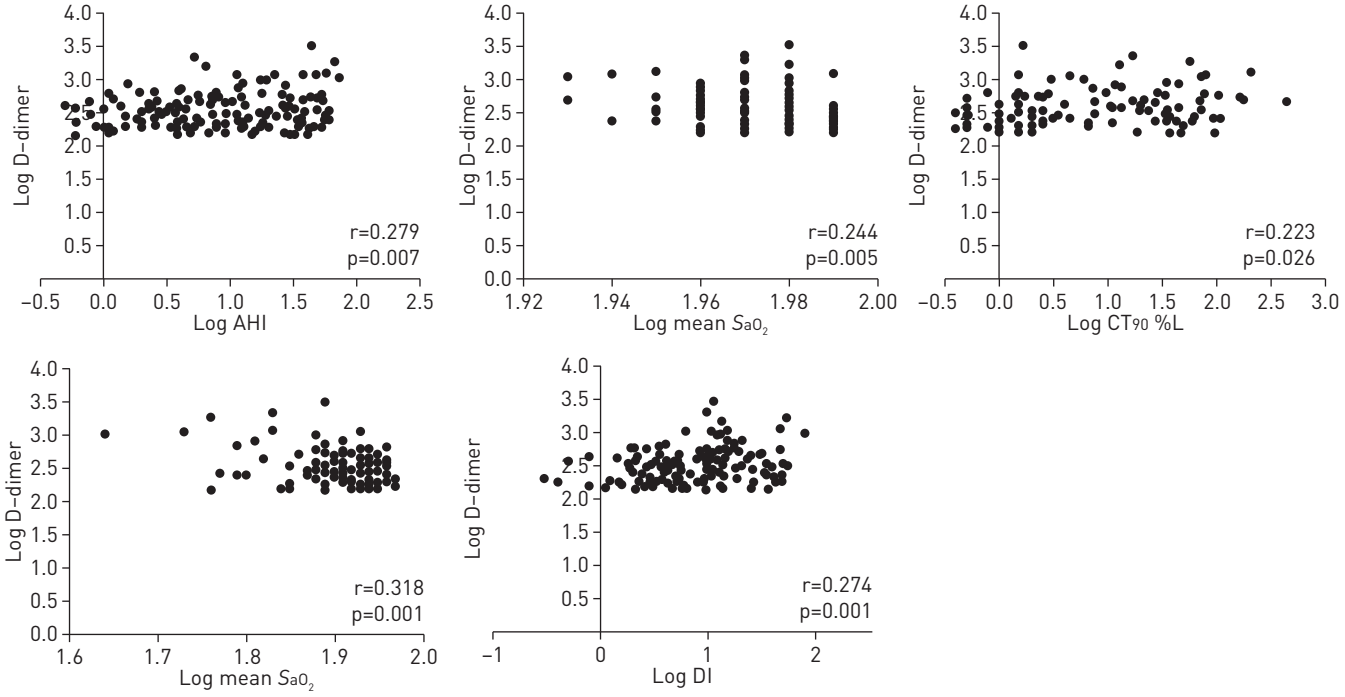

FIGURE 3 Relationship between the sleep parameters and levels of D-dimer in the whole study subjects. Regression coefficients are adjusted for classic risk factors for pulmonary embolism and all variables are log normalised. AHI: Apnoea–hypopnoea index; S_{aO_2}: arterial oxygen saturation; $CT_{90\%}$: the percentage of total study time spent with S_{aO_2} <90%; DI: desaturation index.

OSA-related haemodynamic alterations may slow intravenous flow [19], and lastly, cross-sectional studies support increased coagulability, platelet activity and aggregability [20, 21] and decreased fibrinolytic capacity in OSA. It has also been shown that that coagulant activity, platelet function and fibrinolytic system are improved after continuous positive airway pressure (CPAP) [20–25]. However, the associations with OSA and changes with CPAP have not been uniformly established for all biomarkers [24–26].

D-dimer is a major plasmin-derived degradation product of cross-linked fibrin. Its generation depends on the presence of fibrin and plasmin. The level of D-dimer is considered to reflect the global activity of clot formation and lysis [10].

SHITRIT *et al.* [27] selected 103 OSA patients and found high D-dimer levels in 14. The subgroup with high D-dimer levels had lower minimal oxygen saturation and a longer mean period with S_{aO_2} <90% in the sleep study compared with normal D-dimer OSA group [27]. In keeping with these results, it has been shown that D-dimer increased significantly when patients were exposed to hypoxic conditions [28]. Other investigators have found augmented fibrinogen levels in both adults and children with OSA [29–31],

TABLE 4 Independent determinants of D-dimer and prothrombin fragment 1+2 (F1+2) levels[#]

	Unstandardised regression coefficients B±SE (95% CI)	Standardised regression coefficients B	p-value	r^2	r^2 change
D-dimer mg·mL^{-1}					
Constant	1.325±0.396 (0.540–2.110)		0.001		
Minimum S_{aO_2} %	−0.0121±0.005 (−0.021– −0.003)	−0.246	0.011	0.120	0.120
AHI events·h^{-1}	0.005463±0.002 (0.00–0.010)	0.224	0.020	0.160	0.040
F1+2 pmol·L^{-1}					
Constant	7414.0±2214 (3015–11813)		0.001		
Mean S_{aO_2} %	−77.2±23.2 (−123.3– −31.1)	−0.427	0.001	0.092	0.092
ESS	29.9±11.9 (6.4–53.5)	0.251	0.013	0.144	0.052
$CT_{90\%}$ %	−2.3±1.0 (−4.3– −0.3)	−0.292	0.024	0.193	0.049

S_{aO_2}: arterial oxygen saturation; AHI: apnoea–hypopnoea index; ESS: Epworth Sleepiness Scale; $CT_{90\%}$: the percentage of total study time spent with S_{aO_2} <90%. [#]: values are adjusted for sex, age, body mass index and classic risk factors for pulmonary embolism.

which decreased after CPAP treatment [22]. However, these findings are not uniform with several studies showing no, or even inverse, associations between OSA and D-dimer [30, 32, 33], while a number of reports did not find changes after CPAP [24, 32–34]. We also found significantly higher D-dimer plasma levels in PE-OSA compared with non-OSA PE patients. Furthermore D-dimer was directly related to OSA severity and inversely correlated to nocturnal hypoxaemia. Nocturnal hypoxia plays an important role in the procoagulant state associated with OSA [23, 25, 27, 28, 31]. Similarly we have shown significant correlations between D-dimer, sP-selectin, PAI-1, F1+2 and TAT and some indices of nocturnal hypoxaemia. Venous thrombosis usually initiates at the venous valves in the legs, where stasis and hypoxia may occur, which can induce endothelial damage starting a potentially hypercoagulable microenvironment [35, 36]. OSA is associated to sedentary lifestyle and obesity that may induce a procoagulant state and venous stasis, which have been associated with an increased risk of PE [7]. Moreover, it is tempting to speculate that nocturnal hypoxaemia in OSA patients may lead to up-regulation of procoagulant activity in valvular sinus in deep veins.

The conflicting results in D-dimer in OSA may be explained by differences in populations and sampling (concomitant comorbidities, sex distribution, age, BMI and varying degrees of sleep apnoea severity). Besides, non-dipping blood pressure profile at night and emotional and mood factors are associated with elevated D-dimer [37, 38], and the impact of these aspects has not been systematically evaluated in the majority of the studies.

The main finding of our study (higher proportion of subjects with elevated post-anticoagulation D-dimer in PE-OSA compared with PE non-OSA) may have important clinical implications. In patients with a first PE event, an elevated D-dimer level after anticoagulation is stopped is a risk factor for recurrent PE [10]. A randomised clinical trial has shown that patients with an abnormal D-dimer test and who restarted anticoagulation had a significantly lower combined incidence of recurrent venous thromboembolism and bleeding, than those who did not resume the treatment [11]. Therefore, it has been suggested to use this biomarker as a tool to choose the extent of anticoagulation. We hypothesise that those patients with PE and concomitant OSA have an increased risk of future PE episodes, and it could be used to identify patients with a high risk of recurrence and who might require longer anticoagulant treatment.

Other global coagulation markers activation and fibrinolysis, such as TAT, sP-selectin, PAI-1 and F1+2, have been explored as predictors of recurrent venous thromboembolism, but are not yet ready for clinical use [12–15].

Several studies have shown increased sP-selectin, PAI-1 and TAT levels in OSA [23, 25, 26, 28, 30, 32]. However, there are conflicting results [24, 26]. But, overall, most studies have confirmed that OSA is associated with a procoagulant state.

We could not demonstrate any difference in sP-selectin, F1+2, PAI-1, and TAT between OSA and non-OSA subjects. Consequently, we found no imbalance in platelet activation, fibrinolysis system and thrombin generation. However, there were significant correlations between sP-selectin, PAI-1, F1+2 and TAT and some indices of nocturnal hypoxaemia. These could be explained because they are really unrelated, or because the present study might have had limited power to detect differences. The precise processes that initiate venous thrombosis remain uncertain. Several studies have shown increased markers of coagulation activation and platelet activity such as D-dimer, TAT and F1+2, and sP-selectin in conditions with enhanced thrombin formation [39]. A single abnormality factor is seldom enough to cause venous thrombosis, as it may require a synergy of several factors. Toukh *et al.* [40] used thromboelastography to assess coagulability, which provides information about the full spectrum of the haemostatic process, (from the initial formation of fibrin until lysis of the clot) and found that OSA patients had a procoagulant state that was reduced after 2 weeks of CPAP. As a result, there may be a thrombosis threshold where the trend to cause thrombin is not effectively regulated by antithrombotic mechanisms.

There are a number of limitations that should be considered in relation to our study, beyond sample size, which limits some ancillary subanalyses. First, there is a marked diurnal variability in procoagulant factors in OSA [32, 33, 41]. We did single time-point assessments, but whole samples in all subjects were collected after fasting overnight between 08:00 and 10:00 h, and single sample assessments of elevated D-dimer have been prospectively related with an increased risk of recurrent PE in several studies [10]. Second, although we designed our study to evaluate coagulation markers in addition to our primary outcome of association between OSA and PE, the selection of these biomarkers was determined *post hoc*, thus the study may have been underpowered to find out changes in some hypercoagulable state mediators. Third, although cross-sectionally high D-dimer concentration in OSA patients compared with non-OSA subjects is consistent with a procoagulant state, any cause–effect relationship would require confirmation in prospective longitudinal cohort studies. Fourth, although OSA diagnosis was based on type 3 portable sleep monitoring system without electroencephalographic signals, it has been previously validated. Both

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patients and controls were studied with the same device and protocol, which could underestimate the severity of OSA and the relationships that we have found between D-dimer level and OSA severity. Finally, we acknowledge our patients have comorbidities that could affect the values of some of the biomarkers tested. However, the groups were quite similar and we selected consecutive PE patients that correspond to the real-life entire clinical disease spectrum of PE.

To conclude, we have found that PE patients with associated OSA had a higher proportion of subjects with high post-anticoagulation D-dimer, than in those without OSA. Moreover, D-dimer levels were higher in PE patients with associated OSA than without, indicating that there is a persistent hypercoagulable state in OSA. Data from new studies are needed to definitively clarify if OSA is a risk factor for PE recurrence, particularly in those ones with high D-dimer values. Given the high prevalence of OSA in PE patients [1–5], the increased blood coagulability induced by OSA, and the raised percentage of patients with elevated D-dimer levels after the completion of anticoagulation, the potential of CPAP and/or resumption anticoagulant drugs to reduce incidence and mortality in patients with OSA clearly warrants further study, in particular for patients with previous PE.

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