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Biomarkers of carcinogenesis and tumour growth in patients with cutaneous melanoma and obstructive sleep apnea

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Take-Home Message: In patients with cutaneous melanoma, OSA is associated with an increased expression of a biomarker of tumorigenesis.

Abstract

The goal of this study was to assess the relationship between the severity of OSA and the levels of carcinogenesis- and tumour growth-related biomarkers, in patients with cutaneous melanoma (CM).

This multicentre observational study included patients who were newly diagnosed with melanoma. The patients were classified as non-OSA (apnoea–hypopnoea index (AHI); 0-5 events/h), mild-OSA (AHI; 5-15 events/h), and moderate to severe OSA (AHI>15 events/h). Enzyme-linked immunosorbent assays (ELISAs) were performed to analyze the serum levels of hypoxia- and tumour adhesion-related biomarkers (VEGF, IL8, ICAM-1, VCAM-1), and markers of tumour aggressiveness (S100B, MIA). A logistic model adjusted for age, sex and body mass index (BMI) was fitted to each biomarker, and the AHI served as the dependent variable.

A total of 360 patients were included (52.2% male, median [IQR]; 55.5 [43.8-68.0] years, AHI of 8.55 [2.8-19.5] events/h). The levels of VEGF, IL-8, ICAM-1, S100B and MIA were not related to the severity of OSA. The levels of VCAM-1 were higher in patients with OSA than those without OSA (mild OSA: OR=2.07,p=0.021; moderate-severe OSA: OR=2.35,p=0.013).

In patients with cutaneous melanoma, OSA was associated with elevated circulating levels of VCAM-1 that could indicate the contribution of OSA in tumorigenesis via integrin-based adhesion.

Introduction

Obstructive sleep apnea (OSA) is a prevalent disease that affects at least 10% of the adult population [1–3]. OSA is characterized by a repetitive obstruction of the upper airway that leads to intermittent hypoxia and sleep disruption. OSA is associated with an increased risk of cardiovascular and neurocognitive consequences [4].

Cutaneous melanoma (CM) is a tumour with a melanocytic origin. The incidence of melanoma has increased worldwide, and 10-30 new melanoma cases per 100,000 inhabitants are estimated to occur in Europe and the USA. Melanomas account for 90% of deaths associated with cutaneous tumours [5]. No effective treatments are available once this malignant tumour has spread [6–8]. The oncogenic transformation of melanocytes to melanoma is the result of complex changes in multiple physiopathological pathways that control cell cycle progression and apoptosis [9]. The identification of treatable diseases that accelerate the progression of melanoma and the pathways involved in this progression can be crucial for reducing the mortality from melanoma.

Recently, OSA has been shown to be associated with a higher cancer prevalence, incidence and mortality [10–12]. Specifically, two recent studies have demonstrated the existence of a relationship between OSA and CM aggressiveness [6, 13]. Systemic inflammation due to hypoxia-reoxygenation cycles in OSA may activate diverse physiopathological pathways that enhanced tumour progression [14, 15]. Both diseases are associated with elevated circulating levels of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) [16–18] and vascular cell adhesion molecule-1 (VCAM-1) [17, 19, 20], inflammation cytokines, such as vascular endothelial growth factor (VEGF) [21, 22] and interleukin-8 (IL-8)

[7, 16, 23], and biomarkers of the prognosis and severity of melanoma, such as S100 calcium binding protein B (S100B) [7, 24] and melanoma inhibitory activity (MIA) [7].

Patients with OSA could be at an increased risk of suffering from neoplastic processes, but the specific roles played by OSA have not been clearly established to date [25]. The objective of the present study was to evaluate the relationship between the biomarkers of carcinogenesis and tumour growth and OSA. Therefore, we measured six biomarkers that represent different physiopathological pathways that may link OSA and melanoma.

Methods

Study design

This ancillary, observational, prospective, cross-sectional and multicentre clinical study was conducted in 29 teaching hospitals in Spain and included patients with CM. Briefly, the relationship between the presence and severity of sleep disordered breathing and melanoma aggressiveness was analysed. This study was approved by the ethics committees of each of the participating hospitals. All participants provided signed informed consent to participate in the study.

Patient selection

The patients were consecutively recruited at the dermatology or oncology units of each participating centre. The patients were initially eligible for participation in the study if they had a diagnosis of invasive CM, were older than 18 years and signed the informed consent to participate in the study. The initial exclusion criteria included primary unknown melanoma, extracutaneous melanoma, *in situ*

melanoma, pregnancy, daytime respiratory or heart failure, and current or previous use of continuous positive airway pressure therapy (CPAP). Because the metastatic status of the patients may strongly influence the levels of the circulating biomarkers of cancer, those patients with metastatic status were excluded (Figure 1).

Sleep studies

The patients included in the study underwent polygraphy for a maximum of 6 months after the CM diagnosis at the sleep unit of each centre. The respiratory polygraphy included a continuous recording of the oronasal flow and pressure, heart rate, thoracic and abdominal respiratory movements, and oxygen saturation (SaO_2). Trained personnel manually scored the polygraphy data. Apnea was defined as an interruption of the oronasal airflow for >10 seconds. Hypopnea was defined as a 30-90% reduction in the oronasal airflow for >10 seconds, which was associated with an oxygen desaturation ≥3%. The apnea-hypopnea index (AHI) was defined as the number of apnea events plus hypopnea events per hour during the recording, while the $Tsat_{90}$ was defined as the percentage of the recording time with an SaO_2<90%. The baseline saturation, mean saturation, minimum saturation, and oxygen desaturation index (ODI) at 3% and 4% were also measured.

The patients were divided into the following three groups: the non-OSA group (AHI: 0-5 events·h^{-1}), the mild sleep apnea group (AHI: 5-15 events·h^{-1}) and the moderate-severe sleep apnea group (AHI: >15 events·h^{-1}).

Evaluation of the growth rate of CM

CM growth rate was calculated as the Breslow index (in millimetres) divided by the difference between the date of the excision of the tumour and the date on which the patient noticed changes suggestive of malignant transformation in a stable, pre-existing lesion or the time he/she noticed the appearance of a new and changing lesion [6]. CM growth rate was categorized as follows: slow-intermediate growth rate (<0.49 mm·month^{-1}) and fast growth rate (≥0.5 mm·month^{-1}) [26].

Measurements of circulating molecules

Fasting venous blood samples were drawn between 08.00 and 09.00 a.m. The blood samples were centrifuged to separate the serum, and all specimens were immediately aliquoted, frozen, and stored at -80°C.

Two different methods, i.e., multiplex analyses and traditional enzyme-linked immunosorbent assays (ELISAs), were used to quantify the circulating molecules. Briefly, the Q-Plex TM ELISA technology allows for the simultaneous measurement and detection of multiple proteins at the microscale level. This technology involves an array of quantitative sandwich ELISAs that use standard curves for data interpolation. Using the sandwich ELISAs, capture antibodies are spotted into a 96-well microtiter plate and bind to target proteins in the sample, and secondary or detection antibodies bind to the different epitopes on the target proteins. Streptavidin-HRP is pre-conjugated to secondary antibodies and used to generate a signal. The measured signal is proportional to the amount of the target protein in the sample.

The two multiplexed proteins (including VEGF and IL-8) are analysed using the Q-View Imager. Individual ELISAs were performed to detect VCAM-1, ICAM-1, MIA

and S100B following the manufacturer's recommendations. For each assay, standard curves were generated to determine the concentrations of the proteins. The sensitivity and ELISA trademark for each protein are presented in e-Table 1.

Measurements of molecules in postexcisional melanoma tumour tissues

The expression levels of VEGF and the levels of hypoxia inducible factor 1 alpha (HIF-1α) were measured and previously reported in this cohort of patients [27] to determine whether assessments of the abundance of these biomarkers in post-excisional melanoma tumour tissues may lead to a more accurate determination of tumour aggressiveness. For the present study, we evaluated the association of the expression levels of these biomarkers with OSA severity. The levels of VEGF and HIF-1α were assessed by immunohistochemistry on 4-μm-thick sections of formalin-fixed, paraffin-embedded melanoma samples. Immunostaining was performed on a Leica Bond-III stainer (Leica Biosystems, Newcastle, UK) using the Leica Bond Polymer Refine Kit (Leica Biosystems). Melanoma samples were stained with a 1:2 dilution of anti-VEGF prediluted antibody (ab27620; Abcam, Cambridge, UK) and with a 1:100 dilution of anti-HIF-1α antibody (ab51608; Abcam). The staining results were analysed independently by two expert pathologists who were blinded to the staging and clinical features of the patients. VEGF-specific staining was scored considering both the percentage of the positive (<25, 25–50, 51–75, and >75%) and the expression intensity (negative-low or high). HIF-1α-specific nuclear staining was scored considering the presence or absence of positive cells.

Statistical analysis

The quantitative variables are expressed as the medians and interquartile ranges (IQR) since these variables were non-normally distributed. Absolute and relative frequencies were used to describe the qualitative variables. We performed a bivariate analysis to analyze the studied variables according to the AHI group. The Kruskal-Wallis test was performed to compare the quantitative variables, and the Pearson's chi-squared test or Fisher exact test was performed to compare the qualitative variables. A logistic regression model (adjusted for age, sex and body mass index (BMI)) was fitted for each of the ELISAs as the explanatory variable(s), and the AHI, which was categorized by severity (non-OSA, mild OSA, and moderate/severe OSA), was the dependent variable. The calibration was assessed using the Hosmer-Lemeshow goodness-of-fit test. All tests were two-tailed, and a statistical significance level of 0.05 was used. The statistical analyses were performed using R version 3.3.1.

Results

Patient characteristics

Of the 476 patients who were initially recruited, 360 patients with a diagnosis of CM who underwent a sleep study and provided at least one serum sample were included in the final analysis (Figure 1). The clinical characteristics and demographic variables of the patients are shown in Table 1. The study population included middle-aged men and women who were overweight and had mild sleep apnea (an AHI of 8.55 events·h^{-1} [range: 2.80-19.5 events·h^{-1}]).

Analysis of serum markers

The results of the measurements of the circulating levels of VEGF, IL-8, ICAM-1, VCAM-1, S100B and MIA are presented in Figure 2. The serum levels of IL-8, ICAM-1, S100B and MIA were similar among the non-OSA, mild OSA and moderate/severe OSA patients ($p = 0.436$, $p = 0.259$, $p = 0.560$, and $p = 0.328$, respectively). These biomarkers, which are related to the inflammation, progression, and prognosis in CM, were not significantly related to the severity of OSA. In contrast, the serum levels of VEGF exhibited an increased tendency ($p = 0.165$) in both the mild OSA (odds ratio (OR) = 1.26 [95% C.I.: 0.70-2.28]) and moderate-severe sleep apnea (OR = 1.61 [95% C.I.: 0.85-3.06]) groups (see Figure 2 and Table 2). Similarly, we observed that the serum levels of VCAM-1 were significantly higher in the sleep apnea patients than those in the non-OSA patients, for mild OSA (OR = 2.07 [95% C.I.: 1.12-3.89]; $p = 0.021$) and for moderate-severe OSA (OR = 2.35 [95% C.I.: 1.20-4.66]; $p = 0.013$) after adjusting for BMI, age, and gender (Table 2 and Figure 2). Similar results were obtained by comparing the groups according to the ODI terciles (data not shown).

Analysis of tumour tissues samples

Assessment of the levels of VEGF and HIF-1α expression in the tissue samples from the resected tumour are presented in Table 3. Although a tendency was observed in the HIF-1α proportion of positive stained tumours in patients with moderate/severe OSA, the adjusted analysis reported similar proportions among non-OSA patients, mild OSA (OR = 1.52 [95% C.I.: 0.64-3.75]; $p = 0.35$) and moderate/severe OSA (OR = 1.82 [95% C.I.: 0.73-4.77]; $p = 0.21$) (Table 3). For VEGF, the proportion of stained cells in tumour tissues samples was higher than

75% for the three groups. OR was obtained for VEGF expression in tissue samples of the resected tumour and no significant differences emerged between groups (Table 3).

Relationship between CM growth rate and VCAM-1

VCAM-1 levels were categorized as follows according to the mean levels: high levels of VCAM-1 (above the mean) and low levels of VCAM-1 (below the mean). It was observed a tendency of high serum levels of VCAM-1 positively associated with a fast CM growth rate (Figure 3) (1.59 [95% C.I.: 0.91-2.76], but this association did not reach statistical significance ($p = 0.112$).

Discussion

In the present study we described that in a cohort of patients with cutaneous melanoma, the OSA severity was related to the serum levels of the VCAM-1 tumour adhesion-related biomarker, but not to the serum levels of markers related with tumour aggressiveness, such as VEGF, IL-8, ICAM-1, S100B and MIA, or levels of VEGF and HIF-1α expression in tissue samples of the tumour.

The increase in the circulating levels of ICAM-1 and the correlations between ICAM-1 and AHI have been previously reported [16, 19, 28]. The circulating levels of VEGF are frequently increased in OSA patients and play a role in the regulation of tissue oxygen delivery [22, 28]. The levels of IL-8 have been reported to be higher in OSA patients than those in healthy controls [16, 29], while the S100B levels are increased in OSA patients but do not correlate with the severity of the disease [24, 30]. We only observed increased levels of VCAM-1 and moderate increased levels of VEGF, which may be due to the differences between the

populations since our cohort included patients with CM and OSA; the CM effect, that is independently related with the increase of VEGF [31, 32], together with IL-8 [28, 32], ICAM-1 [18, 33], S100B [7, 34, 35] and MIA [7, 35, 36], could mask the OSA effect on these proteins, which may explain why we did not observe the previously reported differences in these biomarkers in the patients with OSA. Although the possibility exists that the presence of CM may exert a potential ceiling effect on the contributions of OSA to the biomarkers of interest, the study was not designed to address this issue, which therefore must remain speculative at present. In the present study, we were not able to evaluate the specific separate effects of OSA and CM. This further buttresses the need to disassociate the relative contributions of OSA and VM to biomarkers such as those explored in the study. However, the study was not designed to address this specific possibility. Indeed, we would have needed to include two additional matched groups, no CM and no OSA, OSA alone in the absence of CM, and then further evaluate the effect of treating OSA on the biomarkers of interest to segregate the independent and overlapping contributions of CM and OSA to these biomarkers.

Several pathophysiological mechanisms, including key intermediate molecules, link OSA to the aggressiveness of cancer. Patients with OSA have higher serum levels of VCAM-1 than healthy controls [17, 19, 28], which is primarily due to hypoxic stress. OSA could enhance the aggressiveness of CM and increase the levels of VCAM-1.

The secreted form of VCAM-1 is due to proteolytic cleavage upon its release from the cell surface via the activity of neutrophil-derived serine proteases. VCAM-1 is mainly involved in leukocyte transendothelial migration and leukocyte retention

into tissues [37]. VCAM-1 plays a central role in the recruitment of inflammatory cells, and its expression is rapidly induced by proinflammatory cytokines, such as VEGF [38]. VCAM-1 plays important roles due to its multiple functionalities in directing the spread of tumours, the formation of metastatic niches and supporting the angiogenic process [20]. Thus, OSA due to hypoxic stress could elevate the serum levels of VEGF and stimulate the expression of VCAM-1, which promotes tumorigenesis. This hypothesis is supported by the relationship between the VCAM-1 circulating levels and the growth rate of melanoma. Even though the result was not statistically significant, a tendency to a positive association could exist, and should be further explored.

For the first time, a biomarker for tumorigenesis has been reported to be elevated in patients with OSA who already suffer from CM. These findings could highlight the importance of VCAM-1 in tumorigenesis in patients with OSA, as a possible pathway that relates OSA with CM. In the present study, we observed a tendency toward increased HIF-1α in post-excisional melanoma tumour tissues as the severity of OSA increased, although the adjusted analysis yielded similar expression levels between mild OSA and moderate/severe OSA. No significant association was observed between VEGF levels in tumour samples and OSA severity. The fact that the biomarkers that were significantly associated with OSA in patients with CM consisted of levels measured in circulating blood samples may be relevant as far as their clinical utility, considering their accessibility. The circulating levels of VEGF and VCAM-1 could be helpful in the identification of patients with melanoma and OSA that are at a higher risk of carcinogenesis and tumour growth. Additionally, measuring the VEGF and VCAM-1 circulating levels is important not only in patients with CM but also in patients with only OSA because

these biomarkers play an important role in the initial stages of tumorigenesis, and OSA patients with elevated serum levels of VCAM-1 and VEGF could be more likely to suffer from tumorigenesis. These findings could support the hypothesis that treating patients with CM and OSA, with increased levels of circulating VEGF and VCAM-1 could reduce tumorigenesis and improve tumour outcomes.

Intermittent hypoxia and arousal, the hallmark features of OSA, are recognized as the major pathophysiological consequences of OSA that promote the activation of intermediate mechanisms that associate OSA with its deleterious consequences and may contribute to an increased cancer risk [25]. Consistent with the evidence from laboratory and animal experiments [39], previous observational trials have reported cancer incidence to be augmented with increasing OSA levels [40, 41]. These observational studies suggested an association between nocturnal hypoxemia and cancer mortality in patients with OSA. Notably, in patients who remained untreated, the association between the hypoxemic index and cancer incidence was slightly stronger. The application of treatment that allows the abolition of the apnea may contribute to the reduction in these angiogenic biomarkers associated with cancer. Although there are data clearly suggesting an association between sleep apnea and cancer risk, there is an absolute lack of evidence of the possible positive effect of CPAP treatment on decreasing tumour aggressiveness in patients with OSA. Although the effects of CPAP treatment in patients with OSA and cancer have not been explored, basic research studies have identified the role of CPAP treatment in mechanisms related to tumorigenesis. Indeed, Gharib *et al.* [42] studied the whole-genome expression of peripheral blood leucocytes in 18 patients with OSA before and after one month of CPAP treatment. Gene-set enrichment analysis comparing leucocytes in each patient

from pre- and post-CPAP treatment indicated that some gene sets involved in neoplastic processes were down-regulated by OSA treatment. Moreover, Hernández-Jiménez *et al*. [43] demonstrated that OSA induces changes in the functionality of circulating monocytes and NK cells, which could favour tumour immune escape. This finding was not observed in CPAP-treated patients. Finally, Almendros *et al*. [44] evaluated in a murine model whether the chronic intermittent hypoxia that characterizes OSA leads to release of tumor-promoting exosomes in the circulation. These investigators observed that the exosomes released during IH mimicking OSA increase the malignancy properties of TC1 lung tumour cells in vitro. In this study, the authors also evaluated exosomes from 10 adult human patients with OSA for 6 weeks before and after CPAP treatment and reported that exosomes from patients with OSA increase proliferation and migration when compared with the same patients after 6 weeks of adherent CPAP treatment.

CPAP treatment is the gold standard for treating OSA and should be considered the first line therapeutic option for OSA. Mandibular advancement devices have also been considered for OSA treatment, particularly in mild-to-moderate OSA [45]. Effective OSA treatment in patients with CM and OSA may contribute to the reduction of the levels of markers related to cancer. Because of the lack of evidence of the possible positive effects of CPAP treatment in cancer progression this possibility should be considered speculative at present. Further studies are necessary to evaluate the effect of CPAP treatment in tumorigenic biomarkers and the prognosis of patients with OSA and cancer.

The main strengths of the study are related to its prospective nature and

the relatively large cohort size that enables appropriate gauging of the potential effects of OSA severity on biomarkers related with carcinogenesis and tumour growth. Nevertheless, the present study present several limitations that deserve comments. First, the sleep habits and shift-work was not reported, variables that are related to cancer [12]. Second, the results of this study are specific related to cutaneous melanoma. Because of that the relationship observed for VCAM-1 and VEGF could not be present in other types of cancer. Third, the present study evaluates the relationship between the severity of OSA and the levels of cancer-related biomarkers, in patients with cutaneous melanoma; however, no information is available regarding the effect of CPAP treatment on these biomarkers.

In conclusion, our study suggests that in patients with cutaneous melanoma, OSA is independently associated with high levels of circulating VCAM-1 tumour adhesion-related biomarker, but not to circulating levels of markers related with tumour aggressiveness. The activation of pathways related with tumour adhesion could be related with the observed relationship between OSA severity and melanoma. Further studies should be explored to identify the intermediate pathways linking OSA severity and cancer.

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Tables and Figures

Table 1. Anthropometric and clinical characteristics of the study groups.

	All	Non-OSA	Mild OSA	Moderate/severe OSA	p-value
	N = 360	N = 132	N = 109	N = 119	
Physiological characteristics					
Gender *n (%)*					
Male	188 (52.2%)	51 (38.6%)	54 (49.5%)	83 (69.7%)	**<0.001**
Age (years) *median [IQR]*	55.0 [43.8-68.0]	44.0 [37.0-52.2]	59.0 [46.0-68.0]	67.0 [56.0-72.5]	**<0.001**
BMI (kg/m^2) *median [IQR]*	27.0 [24.2-29.7]	24.5 [22.9-27.2]	28.3 [25.1-30.8]	28.3 [26.2-31.5]	**<0.001**
Tobacco habit *n (%)*					0.303
Non-smoker	184 (51.3%)	62 (47.0%)	60 (55.0%)	62 (52.5%)	
Active smoker	69 (19.2%)	32 (24.2%)	20 (18.3%)	17 (14.4%)	
Former smoker	106 (29.5%)	38 (28.8%)	29 (26.6%)	39 (33.1%)	
Melanoma site *n (%)*					0.457
Head/neck	52 (14.4%)	16 (12.1%)	16 (14.7%)	20 (16.8%)	
Arms	50 (13.9%)	17 (12.9%)	18 (16.5%)	15 (12.6%)	
Legs	93 (25.8%)	34 (25.8%)	33 (30.3%)	26 (21.8%)	
Trunk	148 (41.1%)	60 (45.5%)	35 (32.1%)	53 (44.5%)	
Acral	16 (4.44%)	5 (3.79%)	7 (6.42%)	4 (3.36%)	
Sleep apnea variables					
AHI (events/h) *median [IQR]*	8.55 [2.80-19.5]	1.95 [0.70-3.02]	9.00 [7.00-12.0]	25.7 [19.6-40.5]	**<0.001**
ODI (events/h) *median [IQR]*	4.90 [1.10-12.8]	0.80 [0.25-1.60]	5.05 [3.38-8.38]	15.4 [10.5-25.8]	**<0.001**

IQR: interquartile range; BMI: body mass index; AHI: apnea-hypopnea index (number of events·h^{-1}); ODI: oxygen desaturation index. The statistically significant p values (p values <0.05) are denoted in bold.

Table 2. Adjusted logistic regression models of the concentrations of the circulating biomarkers in the study groups

	OR [95% C.I.]*	p-value
VEGF		
Mild OSA	1.26 [0.70-2.]	0.431
Moderate/severe OSA	1.61 [0.85-3.06]	0.143
IL-8		
Mild OSA	0.75 [0.42-1.35]	0.345
Moderate/severe OSA	1.04 [0.55-1.97]	0.902
ICAM		
Mild OSA	1.11 [0.62-1.96]	0.724
Moderate/severe OSA	0.69 [0.37-1.30]	0.253
VCAM		
Mild OSA	2.07 [1.12-3.89]	**0.021**
Moderate/severe OSA	2.35 [1.20-4.66]	**0.013**
S100B		
Mild OSA	1.27 [0.70-2.34]	0.434
Moderate/severe OSA	0.94 [0.49-1.76]	0.883
MIA		
Mild OSA	1.20 [0.63-2.31]	0.582
Moderate/severe OSA	0.70 [0.34-1.40]	0.312

VEGF; Vascular endothelial growth factor. IL-8: Interleukin 8, ICAM: Intracellular adhesion molecule, VCAM: Vascular cell adhesion molecule 1, S100B: S100 calcium-binding protein B, MIA: Melanoma inhibitory activity.
*The odds ratios (OR) were calculated using a logistic regression; the response variables were the individual biomarkers, and the explanatory variables were the categories of OSA. The model was adjusted for gender, age and BMI. The statistically significant p values (p values <0.05) are denoted in bold.

Table 3. Adjusted logistic regression models of the expression of the biomarkers in the resected tumour samples in the groups analysed.

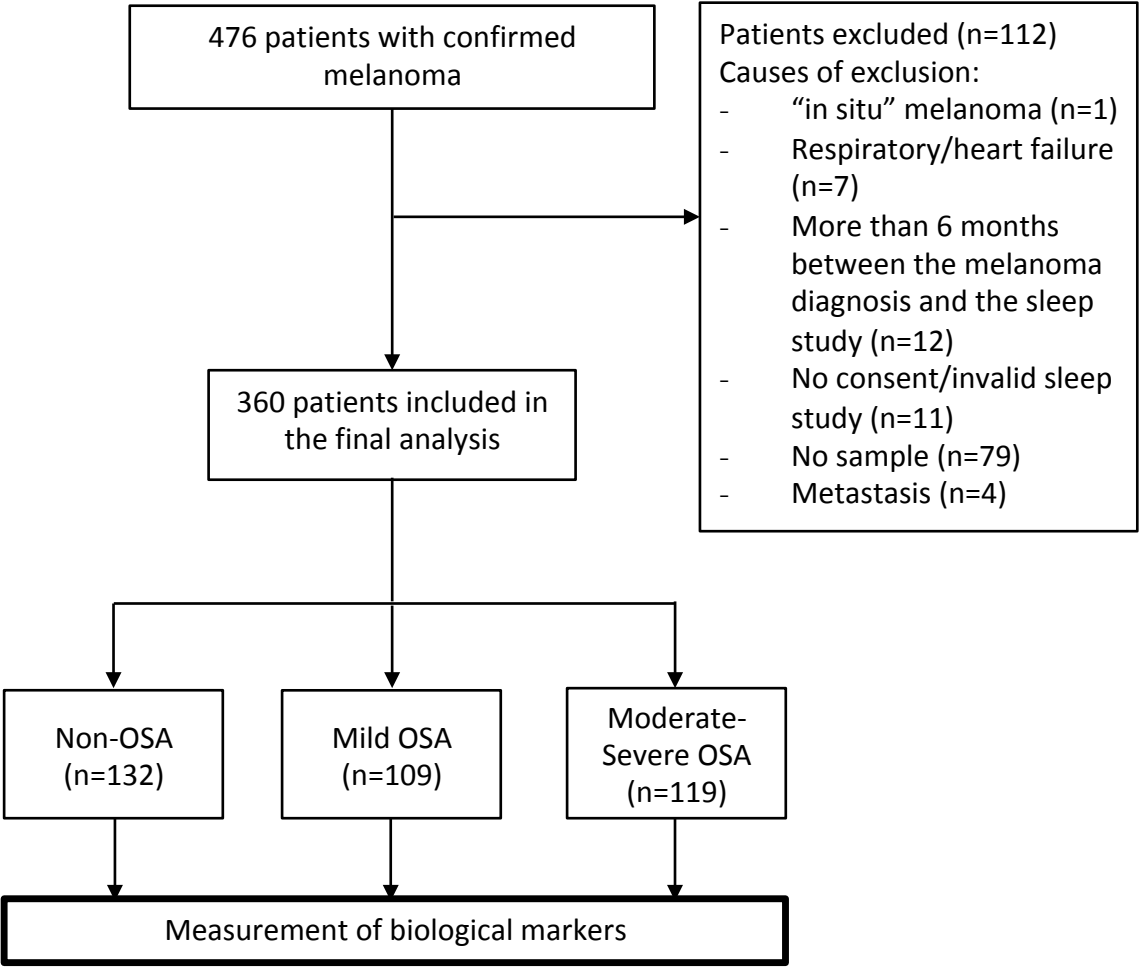
	OR [95% C.I.]*	p-value
VEGF		
Mild OSA	1.69 [0.85-3.39]	0.14
Moderate/severe OSA	1.35 [0.64-2.85]	0.43
HIF-1α		
Mild OSA	1.52 [0.64-3.75]	0.35
Moderate/severe OSA	1.82 [0.73-4.77]	0.21

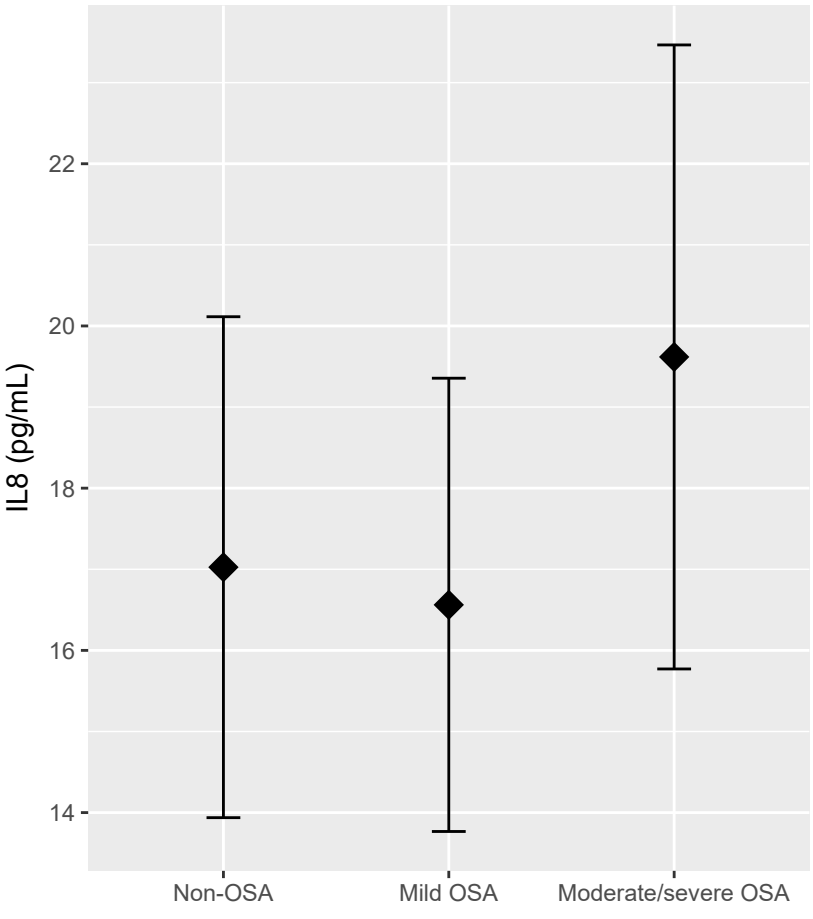
VEGF; Vascular endothelial growth factor. HIF-1α; Hypoxia inducible factor 1 alpha.
*The odds ratio (OR) was calculated using a logistic regression; the response variables were the individual biomarkers, and the explanatory variables were the categories of OSA. The model was adjusted for gender, age and BMI.

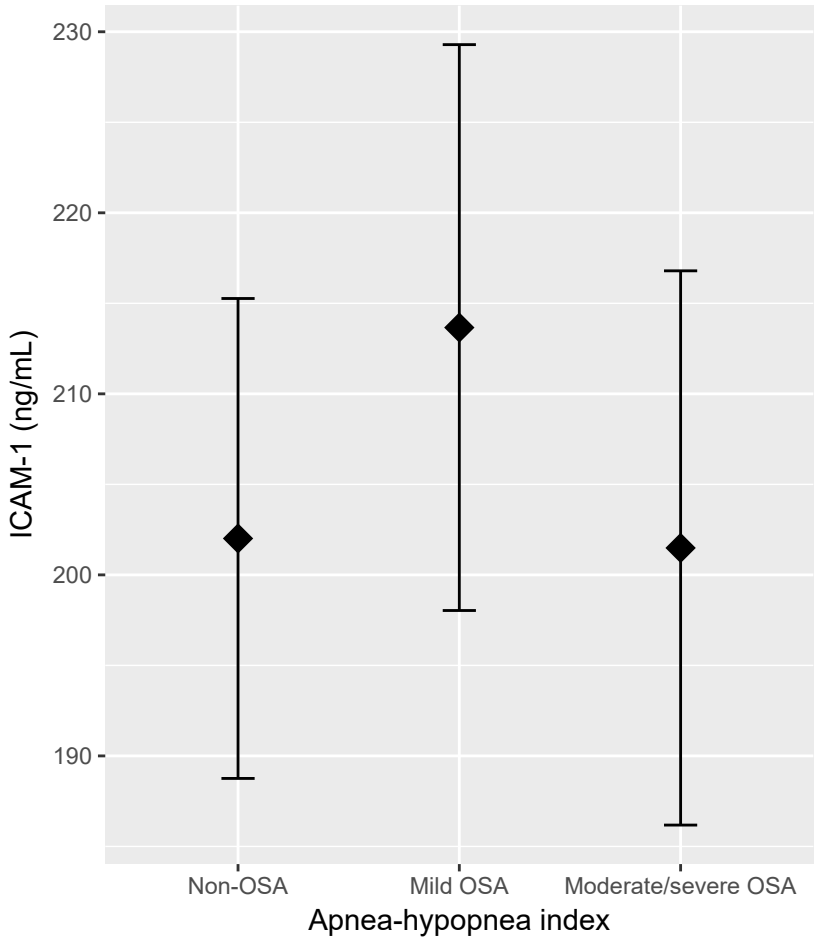
Figure 1. Flowchart of the study

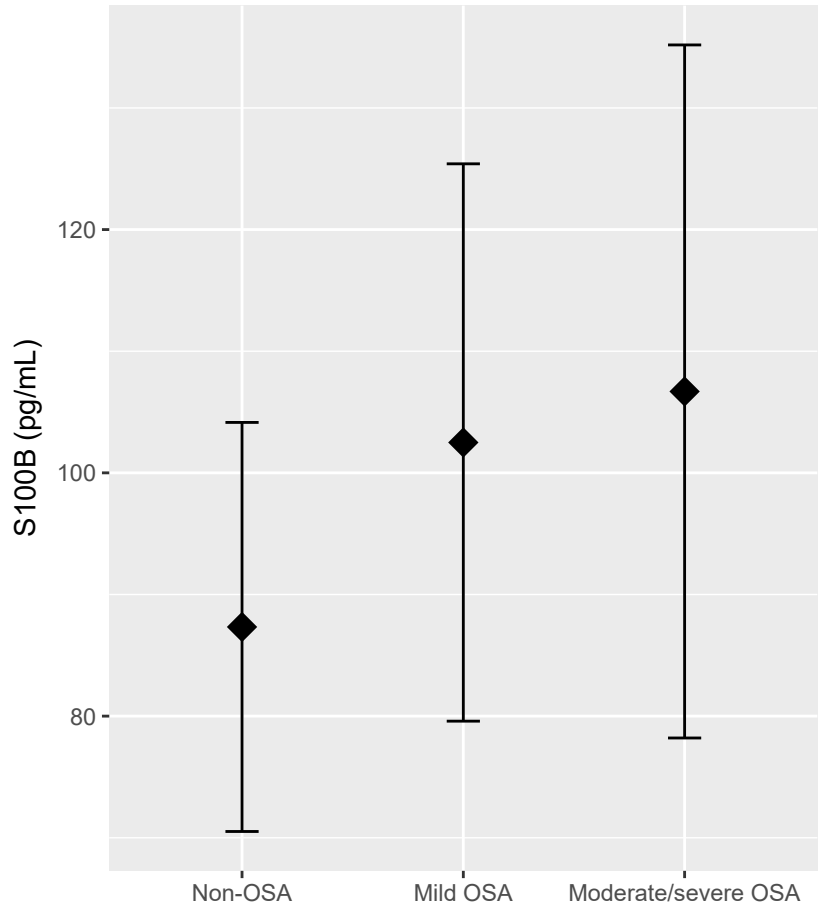
Figure 2. Circulating levels of the biomarkers

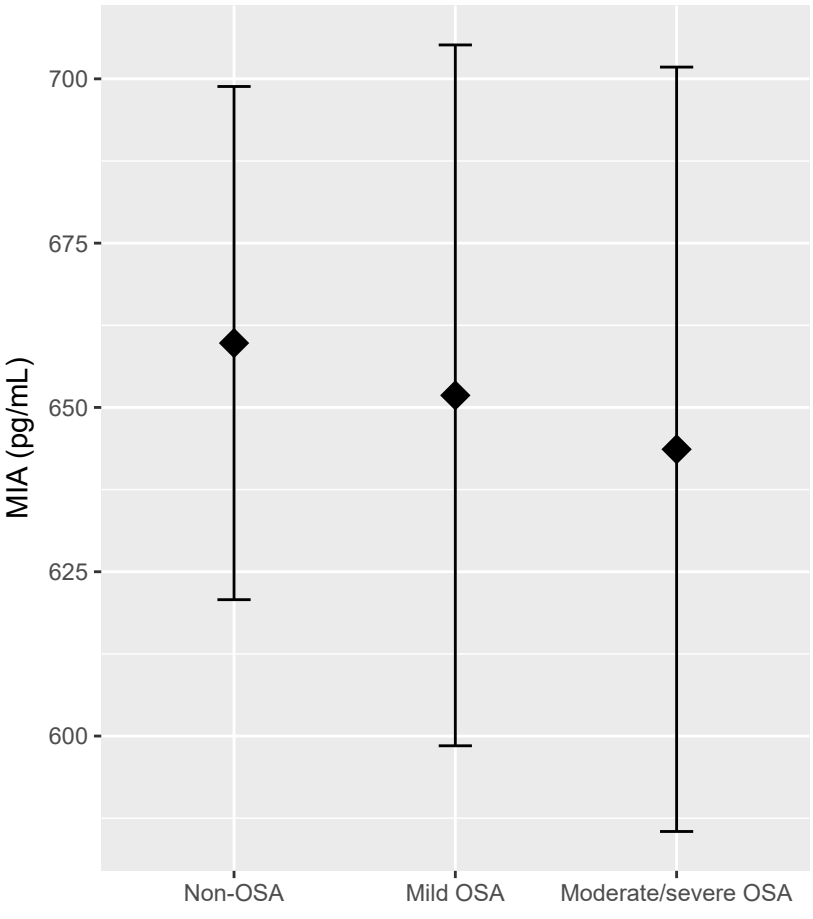
Figure 3. Relationship between the circulating levels of VCAM-1 and the CM growth rate

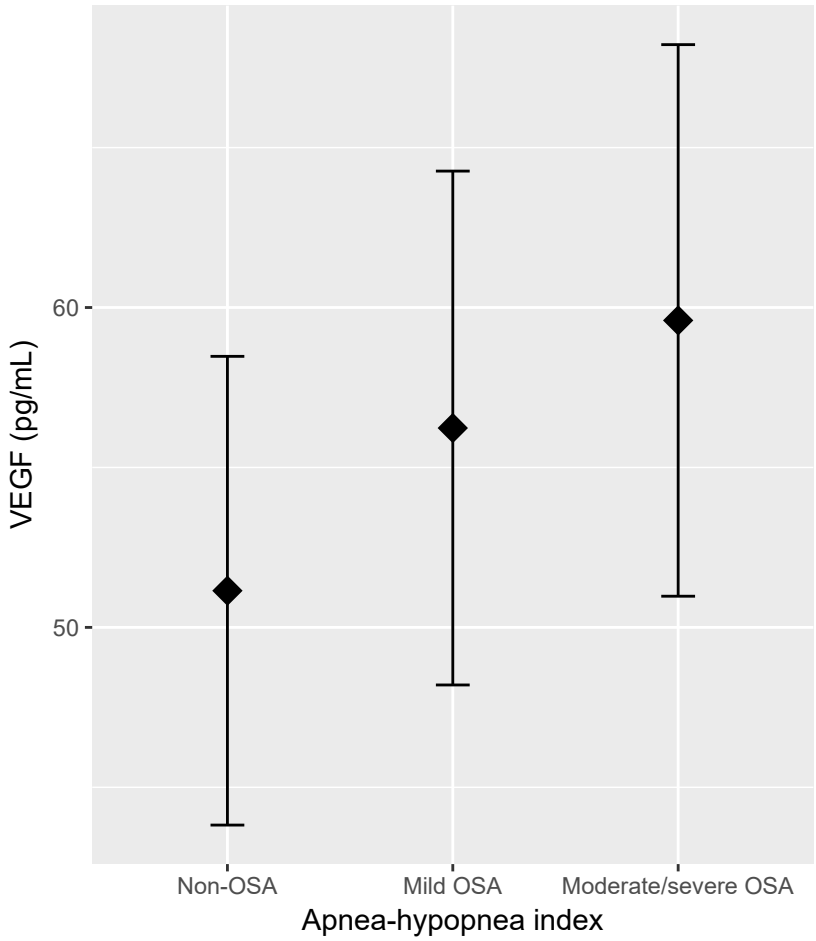

476 patients with confirmed melanoma
Patients excluded (n=112)
Causes of exclusion:
- "in situ" melanoma (n=1)
- Respiratory/heart failure (n=7)
- More than 6 months between the melanoma diagnosis and the sleep study (n=12)
- No consent/invalid sleep study (n=11)
- No sample (n=79)
- Metastasis (n=4)
360 patients included in the final analysis
Non-OSA (n=132)
Mild OSA (n=109)
Moderate-Severe OSA (n=119)
Measurement of biological markers

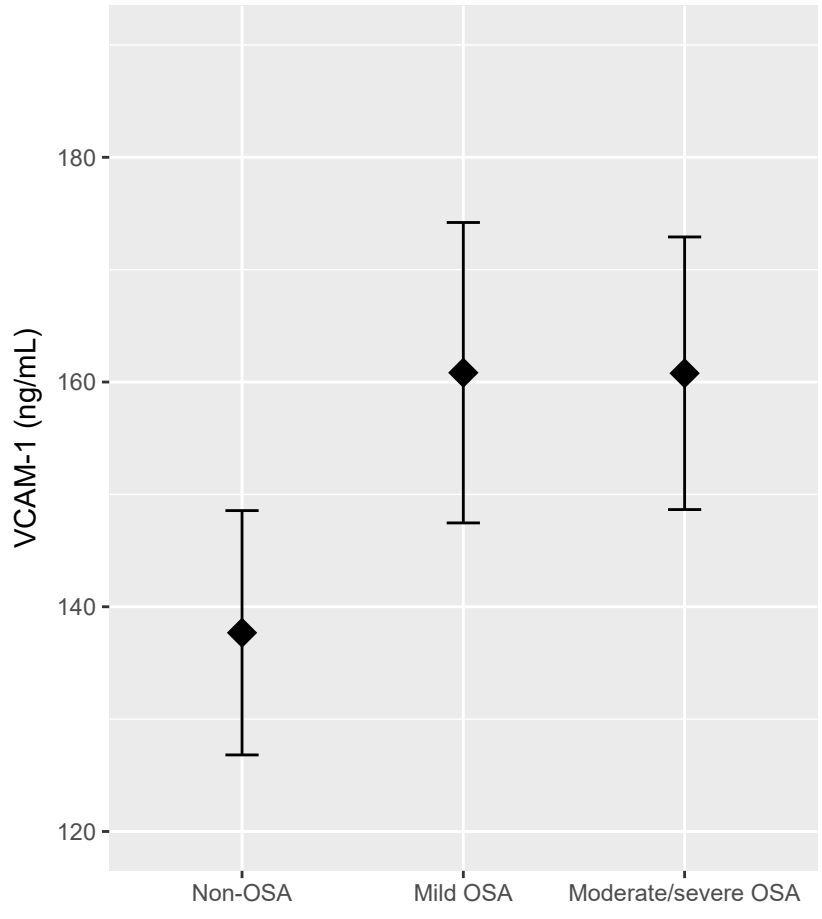

IL8 (pg/mL)
22
20
18
16
14
Non-OSA
Mild OSA
Moderate/severe OSA

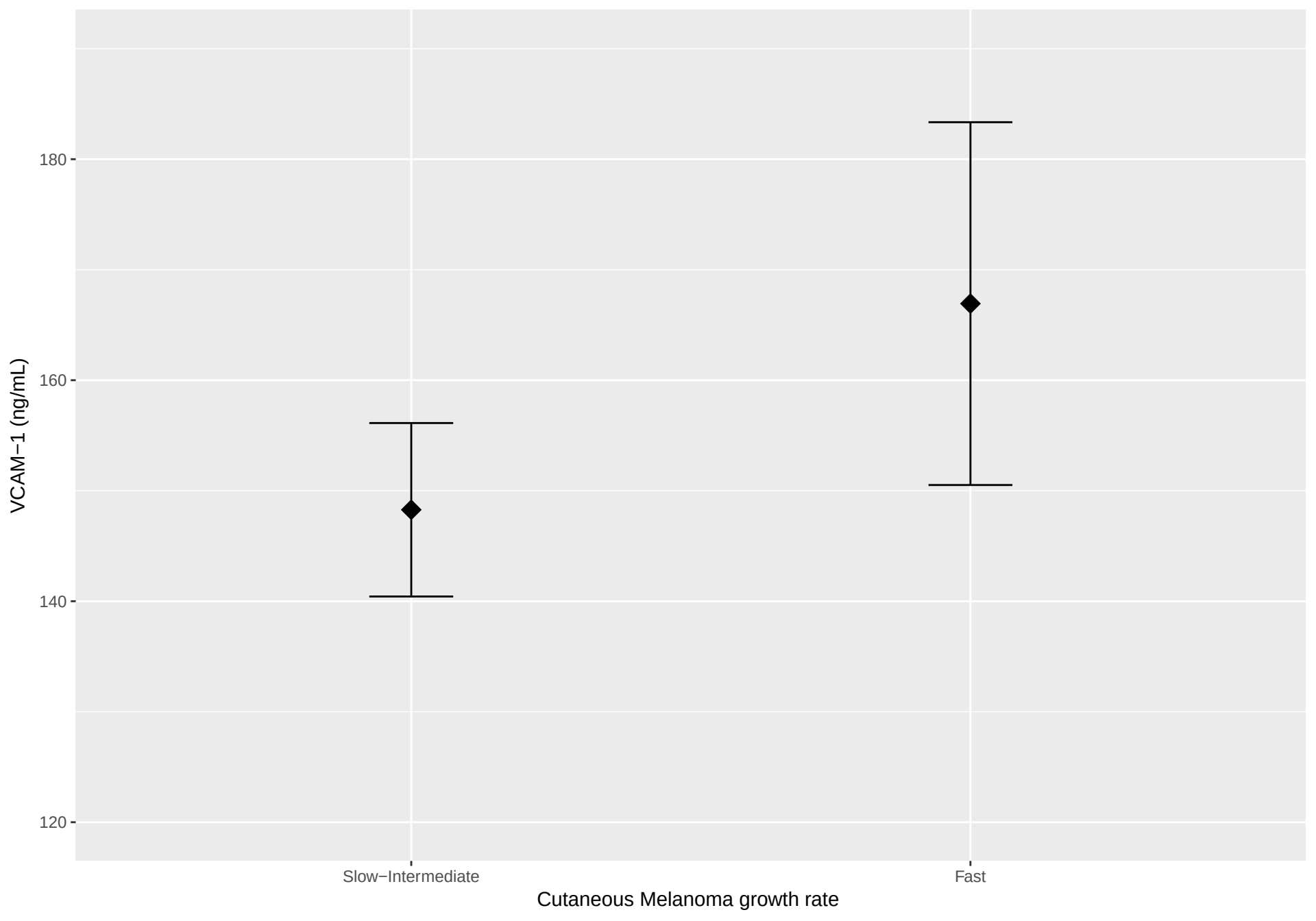

ICAM-1 (ng/mL)
230
220
210
200
190
Non-OSA
Mild OSA
Moderate/severe OSA
Apnea-hypopnea index


S100B (pg/mL)
120
100
80
Non-OSA
Mild OSA
Moderate/severe OSA


MIA (pg/mL)
700
675
650
625
600
Non-OSA
Mild OSA
Moderate/severe OSA


VEGF (pg/mL)
60
50
Non-OSA
Mild OSA
Moderate/severe OSA
Apnea-hypopnea index


VCAM-1 (ng/mL)
180
160
140
120
Non-OSA
Mild OSA
Moderate/severe OSA


VCAM−1 (ng/mL)
180
160
140
120
Slow−Intermediate
Fast
Cutaneous Melanoma growth rate

e-Table 1. ELISA trademark and sensitivity

Biomarker	Sensitivity	Trademark
VEGF	4.76 pg/ml	Quansys Biosciences, USA
IL-8	1.31 pg/ml	Quansys Biosciences, USA
VCAM-1	300 pg/ml	RayBiotech,USA
ICAM-1	150 pg/ml	RayBiotech,USA
MIA	10 pg/ml	Boster, USA
S100B	18.75 pg/ml	Elabscience, USA