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Biomarker panel in sleep apnea patients after an acute coronary event

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ABSTRACT

Background: Acute coronary syndrome (ACS) is a major cause of death and closely related with obstructive sleep apnea (OSA). Our hypothesis is that several cardiovascular-related biomarkers could have a differential prognostic value for ACS severity in patients with OSA, and could also help (individually or combined) in the detection of OSA in patients after a coronary event.

Methods: Up to 361 consecutive individuals admitted due to ACS were included in the study. All of them were evaluated for ACS severity (Killip score, number of diseased vessels, ejection fraction) and further classified as OSA or non-OSA. Medical records were registered and eleven blood biomarkers were measured, including heart-type fatty acid-binding globulin, N-terminal pro-brain natriuretic peptide, matrix metalloproteinase-9 (MMP9), placental growth factor (PlGF) and high-sensitivity C-reactive protein.

Odds ratios of every biomarker for ACS severity-related parameters were calculated and adjusted for age, gender, body-mass index (BMI), hypertension, diabetes, smoking and drinking. The use of clinical measures and biomarkers for the diagnosis of OSA in ACS patients was evaluated both alone and combined using ROC curves.

Results: Several biomarkers showed a significant association with ACS severity, which remained after adjusting for OSA and other potentially confounding variables.

The mathematical combination of age, BMI, PlGF and MMP9 showed an area under the ROC curve (AUC) for OSA identification of 0.741, which was greater than any individual parameter or combination assessed: AUC(BMI):0.687, AUC(age):0.576, AUC(PlGF):0.584, AUC(MMP9):0.555.

Conclusions: The usefulness of biomarkers in the assessment of ACS severity was independent of OSA and the other variables evaluated. In patients admitted after a coronary event, the combination of clinical measures and biomarkers showed a significant discriminating power for the detection of OSA.

Clinical trial registration: NCT01335087 (clinicaltrials.gov).

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1. Introduction

Coronary artery disease has become the leading cause of death worldwide. Its main manifestation is the acute coronary syndrome (ACS), whose symptoms are due to an insufficient oxygen supply to the cardiac muscle caused by an obstruction in the coronary vessels [1,2]. A myriad of cardiovascular risk factors have been described and helped in the understanding of the pathophysiology of ACS, as well as in its prediction and characterization.

Obstructive sleep apnea (OSA), a very prevalent respiratory disease, stands out as one of the main modifiable cardiovascular risk factors [3,4]. It results from a narrowing in the upper airway during sleep, which causes a reduction in the oxyhemoglobin saturation (hypoxemia), an increase in partial carbon dioxide pressure in blood (hypercapnia) and small awakenings aiming to restore normal airflow (arousals) [5,6]. These intermittent events of airway collapse lead to the development of comorbidities associated with OSA, especially cardiovascular, cerebrovascular and neurocognitive diseases, and a higher global mortality rate [4,7,8], which might be explained by the complex pathophysiology of OSA, including oxidative stress and metabolic dysfunction, systemic inflammation and immune activation, sympathetic activation and endothelial dysfunction, among others [6].

A very powerful tool for the objective assessment of these links are biomarkers in blood, the measurement of which may also help in the prediction and screening, diagnosis and staging to the monitoring of a therapeutic response of almost any pathology [9–11]. The use of known biomarkers reflecting the abovementioned individual pathophysiological aspects of OSA could be a great aid to explain its close relationship with coronary disease and interpret the underlying molecular mechanisms.

Myeloperoxidase (MPO) is a known and well-established biomarker of oxidative stress [12]. The concentration of soluble CD40 (sCD40) may approach endothelial dysfunction and immune activation [13], while systemic inflammation may be studied through the quantification of the high-sensitivity C-reactive protein (hsCRP) and the matrix metalloproteinase-9 (MMP9), which is also implicated in angiogenesis and tissue remodeling and plays a key role in atherosclerotic plaque development and atherothrombosis [14]. Other biomarkers for an integral assessment of cardiovascular-related diseases include N-terminal probrain natriuretic peptide (Nt-proBNP) as hemodynamic marker, and the placental growth factor (PlGF) and the soluble fms-like tyrosine-kinase domain-1 (sFlt1) as angiogenesis-related biomarkers [15].

Therefore, the two objectives of this study were *(1)* to evaluate the impact of OSA on the association between cardiovascular risk biomarkers and ACS severity and *(2)* to assess the relationship of these biomarkers with OSA in patients after an acute coronary event.

2. Materials and methods

2.1. Study participants and procedures

This is an ancillary study of the ISAACC Study (NCT01335087), which is an open-label, parallel, prospective, randomized and controlled clinical trial and aims to evaluate whether CPAP treatment reduces the incidence of new cardiovascular events in OSA patients after a first ACS episode [16]. It started in April 2011, and patients were recruited across the country (15 hospitals), and provided written, informed consent. The study was approved by the Ethics Board of each participating center. ACS was defined as the acute presentation of coronary disease, with or without ST-elevation, unstable angina or MI type I[17]. The diagnosis of OSA was performed using the apnea-hypopnea index (AHI), based on the guidelines of the Spanish National consensus on the apnea-hypopnea syndrome [18]. An apnea was defined as the absence of airflow lasting ≥10 s, whereas a hypopnea was defined as a reduction in the airflow (between 30% and 90%) lasting ≥10 s accompanied by an oxyhemoglobin desaturation (≥4%). AHI was calculated as the number of apneas and hypopneas per hour of sleep, and a threshold of 15 h^{-1} was used for the diagnosis of OSA, thus classifying participants as either ACS or ACS + OSA.

Moreover, demographic and anthropometric data were recorded from all participants, and a cardiorespiratory polygraphy was performed (Embletta, ResMed, Australia) the night following the coronary event. Oronasal flow, thoracoabdominal movements, an electrocardiogram and a pulse oximetry were continuously recorded.

ACS severity was routinely performed in terms of Killip score (upon hospital admission), ejection fraction and number of diseased vessels. The cut-off values used were previously described in detail [17]. Other ICU-related complications, such as infections or venous thrombosis, were excluded.

2.2. Blood sampling and biochemical analyses

Fasting venous blood samples were drawn from all subjects the morning following the sleep study. After centrifugation, plasma and serum samples were stored at −80 °C until analysis. The serum concentration of heart-type fatty acid-binding protein (HFABP) and non-esterified fatty acids (NEFA) were quantified using the H-FABP Immunoturbidimetric and NEFA Colorimetric kits, respectively (Randox Laboratories, UK). Insulin, the soluble fms-like tyrosine kinase-1(sFlt1), the placenta growth factor (PlGF) and the N-terminal probrain natriuretic peptide (Nt-proBNP) were measured on the Cobas e411 platform (Roche, Switzerland). The high-sensitivity C-reactive protein (hsCRP) was measured in serum at the Immulite 2000 platform (Siemens Diagnostics, Germany), whereas plasma myeloperoxidase (MPO), soluble CD40 and matrix metalloproteinase-9 (MMP9) were analyzed using the respective Human Quantikine ELISA kits (R&D Systems, USA). Analytical coefficients of variation were below 10% for all biomarkers.

2.3. Data analysis

All anthropometric, biochemical and sleep-related data were included in the ISAACC database. The mean values (and standard deviations) or the frequencies (and percentages) were compared for every variable between the ACS and ACS + OSA groups using the two-tailed Student's *t*-test (if normally distributed) or the Mann Whitney's *U* test (if not).

A binary logistic regression was performed for every individual biomarker for the evaluation of the three ACS severity-related variables. Using the optimal cut-off values, odds ratios (OR) and their confidence intervals were calculated. An OR-adjustment was performed for the presence of OSA as well as for other potentially confounding variables (age, gender, BMI, hypertension, diabetes, smoking status and drinking).

Receiver operating characteristics (ROC) curve analyses were performed for every single biomarker in the detection of OSA among our ACS patients, along with age and BMI. Areas under the ROC curves (AUCs) were integrated for the assessment of the diagnostic accuracy. Biomarker concentrations and clinical measurement combinations were assayed in the same fashion towards a maximization of the discriminating power. All statistical analyses were performed using the SPSS v19 software (IBM Corporation, USA), and the type α error was set at 0.05.

Univariate and multivariate logistic regression analyses were used to investigate independent associated factors to the presence of IAH < 15. All the previously listed factors were included initially in the model before stepwise and backward elimination. Crude and adjusted odds ratio (OR) were calculated for the detection of OSA.

Table 1

Anthropometric, sleep-related data, prevalence of ACS complications.

	No OSA	OSA	*p*-Value
	N = 152	*N* = 209	
Anthropometric data			
Age, years	57.1 ± 11.9	60.2 ± 10.6	0.014 *
Male, N	132 (86.8%)	168 (80.4%)	0.119
BMI, kg/m^2	26.75 ± 3.77	29.33 ± 4.42	< 0.001 *
Hypertension, N	57 (37.5%)	127 (60.8%)	< 0.001 *
Diabetes, N	32 (21.1%)	51 (24.4%)	0.527
Dyslipidemia, N	82 (53.9%)	125 (59.8%)	0.282
Smoking status, N			0.041 *
Yes	72 (47.4%)	93 (44.5%)	
No	36 (23.7%)	73 (34.9%)	
Former	44 (28.9%)	43 (20.6%)	
Current or former drinker, N	50 (32.9%)	46 (22.0%)	0.022 *
Sleep-related variables			
AHI, h^{-1}	6.2 ± 4.1	34.8 ± 17.5	< 0.001 *
Mean O$_2$ saturation, %	93.1 ± 2.1	93.2 ± 2.0	< 0.001 *
O$_2$ desaturation index, h^{-1}	9.9 ± 36.2	32.7 ± 41.7	< 0.001 *
Time with SatO$_2$ < 90%, min	16.6 ± 44.3	41.0 ± 67.2	< 0.001 *
Epworth Sleepiness Score	5.3 ± 2.4	5.4 ± 2.5	0.646
ACS severity-related variables			
Killip score > 1, N	8 (5.9%)	16 (8.9%)	0.323
Affected vessels ≥3, N	22 (15.7%)	48 (25.5%)	0.041 *
Ejection fraction < 51.5%, N	30 (27.8%)	58 (42.0%)	0.023 *
Biomarker concentrations			
HFABP, ng/mL	6.73 ± 3.40	6.56 ± 3.42	0.635
NEFA, mmol/L	0.64 ± 0.44	0.67 ± 0.51	0.558
Albumin, g/L	37.54 ± 3.59	37.00 ± 3.53	0.138
sCD40, pg/mL	431.1 ± 147.6	467.6 ± 255.2	0.470
Insulin, μIU/mL	11.9 ± 9.2	15.0 ± 15.5	0.018 *
Nt-proBNP, pg/mL	956.0 ± 1677.6	1120.1 ± 2396.6	0.339
PlGF, pg/mL	18.83 ± 5.72	20.57 ± 5.97	0.006 *
sFlt1, pg/mL	95.14 ± 77.43	105.09 ± 163.13	0.044 *
MMP9, ng/mL	856.2 ± 1414.6	952.62 ± 1225.1	0.075
MPO, ng/mL	318.6 ± 230.7	359.5 ± 251.2	0.128
hsCRP, mg/L	36.08 ± 61.19	41.34 ± 60.87	0.077

Non-standard abbreviations: BMI (body-mass index), AHI (apnea-hypopnea index), HFABP (heart fatty acid-binding protein), NEFA (non-esterified fatty acids), Nt-proBNP (N-terminal region of the brain natriuretic peptide), PlGF (platelet growth factor), sFlt1 (soluble fraction of fms-like tyrosine kinase 1), MMP9 (matrix metalloproteinase 9), MPO (myeloperoxidase), hsCRP (high-sensitivity C-reactive protein).

* Significant at $p < .05$ level.

3. Results

3.1. Study population

A total of 361 individuals with an ACS diagnosis evaluated in coronary care units or cardiology hospitalization were classified into two groups according to the presence of OSA, resulting in 152 participants without OSA and 209 participants with OSA.

The anthropometric, demographic and sleep-related characteristics of the participants are outlined on Table 1, together with the mean and standard deviations of the 11 biochemical markers. In the OSA group, individuals were older, with a higher BMI and more frequently hypertensive. More smokers (current and former) were detected in the non-OSA group. No differences in the prevalence of diabetes or dyslipidemia were seen, nor in the number of medications taken (data not shown). Among the biomarkers assayed, only PlGF and sFlt1 showed significant differences between groups ($p = .006$ and $p = .044$, respectively), even after adjusting for age and BMI. A positive significant correlation was seen between these angiogenesis-related biomarkers ($r = 0.396$, $p < .001$). Both hsCRP and MMP9 showed a tendency, but did not reach statistical significance between participants with and

Table 2

Odds ratios for the prediction of ACS severity-related variables by means of biomarkers (binary logistic regression).

	Killip score > 1[a]			Number of diseased vessels ≥3[b]			Ejection fraction < 51.5%[c]		
	Non-adjusted OR (95% CI)	OSA-adjusted OR (95% CI)	Adjusted OR* (95% CI)	Non-adjusted OR (95% CI)	OSA-adjusted OR (95% CI)	Adjusted OR * (95% CI)	Non-adjusted OR (95% CI)	OSA-adjusted OR (95% CI)	Adjusted OR * (95% CI)
hFABP	2.907 (1.170–7.222)	2.916 (1.172–7.253)	2.818 (1.097–7.238)	2.309 (1.350–3.947)	2.436 (1.414–4.196)	2.072 (1.153–3.721)	NS	NS	NS
NEFA	NS	NS	NS	NS	NS	NS	NS	NS	NS
Albumin	4.472 (1.904–10.502)	4.311 (1.820–10.214)	3.646 (1.468–9.057)	NS	NS	NS	2.674 (1.471–4.861)	2.761 (1.507–5.058)	3.530 (1.831–6.803)
sCD40	2.850 (1.228–6.614)	2.807 (1.208–6.525)	2.176 (0.883–5.364)	1.932 (1.132–3.297)	1.872 (1.093–3.207)	1.459 (0.798–2.669)	NS	NS	NS
Insulin	NS	NS	NS	2.662 (1.536–4.615)	2.513 (1.442–4.380)	2.938 (1.580–5.461)	NS	NS	NS
Nt-proBNP	5.432 (1.812–16.284)	5.362 (1.787–16.093)	4.756 (1.544–14.650)	NS	NS	NS	4.994 (2.765–9.019)	5.070 (2.787–9.223)	5.355 (2.847–10.073)
PlGF	5.662 (1.888–16.975)	5.518 (1.836–16.582)	5.056 (1.64515.539)	2.547 (1.477–4.392)	2.477 (1.431–4.287)	2.131 (1.191–3.813)	2.271 (1.332–3.871)	2.177 (1.270–3.731)	2.369 (1.334–4.208)
sFlt1	NS	NS	NS	NS	NS	NS	2.099 (1.211–3.638)	2.035 (1.167–3.548)	2.125 (1.186–3.805)
MMP9	NS	NS	NS	2.501 (1.435–4.361)	2.392 (1.367–4.186)	3.122 (1.690–5.767)	NS	NS	NS
MPO	NS	NS	NS	2.307 (1.337–3.978)	2.275 (1.314–3.940)	2.062 (1.132–3.756)	NS	NS	NS
hsCRP	7.827 (3.271–18.732)	7.635 (3.155–18.475)	9.753 (3.480–27.336)	2.008 (1.158–3.482)	1.939 (1.114–3.375)	1.780 (0.990–3.201)	2.810 (1.641–4.811)	2.749 (1.597–4.731)	2.997 (1.694–5.301)

Abbreviations: NS: not significant, OR: odds ratio, CI: confidence interval, HFABP (heart fatty acid-binding protein), NEFA (non-esterified fatty acids), Nt-proBNP (N-terminal region of the brain natriuretic peptide), PlGF (platelet growth factor), sFlt1 (soluble fraction of fms-like tyrosine kinase 1), MMP9 (matrix metalloproteinase 9), MPO (myeloperoxidase), hsCRP (high-sensitivity C-reactive protein).

[a] Optimal cut-off values: HFABP > 5.9 ng/mL, albumin < 34.7 g/L, sCD40 > 469.7 pg/mL, Nt-proBNP > 485.6 pg/mL, PlGF > 18.6 pg/mL, hsCRP > 59.0 mg/L.

[b] Optimal cut-off values: HFABP > 6.7 ng/mL, sCD40 > 441.1 pg/mL, insulin > 13.2μIU/mL, PlGF > 19.2 pg/mL, MMP9 > 501.8 ng/mL, MPO > 418.2 ng/mL, hsCRP > 14.2 mg/L.

[c] Optimal cut-off values: albumin < 39.0 g/L, Nt-proBNP > 461.4 pg/mL, PlGF > 19.6 pg/mL, sFlt1 > 81.72 pg/mL, hsCRP > 20.3 mg/L.

* Adjusted for 8 variables: OSA, age, gender, BMI, hypertension, diabetes, smoking, drinking status.

without OSA.

3.2. Biomarkers associated with ACS severity

The number of patients with a reduced ejection fraction or a greater number of diseased vessels was different depending on the presence of OSA (Table 1), whereas no significant difference was seen in the prevalence of an increased Killip score.

Several biomarkers showed a significant association with the different ACS severity-related variables, as shown on Table 2.

A Killip score above 1 was best associated with PlGF, Nt-proBNP or hsCRP as biochemical markers. A number of diseased vessels ≥3 could also be approached when using MMP9, insulin, PlGF or MPO, whereas a low ejection fraction was associated with Nt-proBNP or hsCRP. These observations remained significant when adjusted for OSA alone, as well as when adjusted for 8 potentially confounding variables (age, gender, BMI, hypertension, diabetes, OSA, smoking status and drinking status) (Supplemental Table). The statistical significance after adjustment was reduced only for sCD40 on the Killip score and the number of diseased vessels.

3.3. Biomarkers for OSA detection

The parameter with the highest discrimination power for the detection of OSA in our population was BMI (AUC = 0.687), which showed an OR for the detection of OSA of 4.39 after adjusting for age, and the best blood biomarkers for the classification of OSA in our cohort were PlGF, insulin, sFlt1 and MMP9 (AUC > 0.555 for all of them), as outlined on Table 3.

Odds ratios of PlGF and MMP9 for the diagnosis of OSA were still significant after adjusting for age and BMI (PlGF adjusted OR: 1.64 (1.00–2.69); and MMP9 adjusted OR: 1.42 (1.13–2.08)).

Clinical data and biomarker concentrations were combined in different algorithms using mathematical and optimization models. The combination with the highest power of discrimination included age, BMI, PlGF and MMP9 after a log-transformation (0.870·log (age) + 1.479·log(BMI) + 0.475·log(PlGF) + 0.353·log(MMP9) - 1.942). This combination yielded an AUC = 0.741 (95% CI: 0.688–0.764; $p < .001$) for the diagnosis of OSA, hence maximizing the power of discrimination over the individual variables in patients after an acute coronary event (Fig. 1). The inclusion of any further variable in the algorithm did not improve the discriminating power. The biomarker-free combination of the three clinical variables (BMI, age and hypertension), although useful, showed a lower discrimination power than the combinations including also biomarkers (AUC = 0.710, 95% CI: 0.654–0.765).

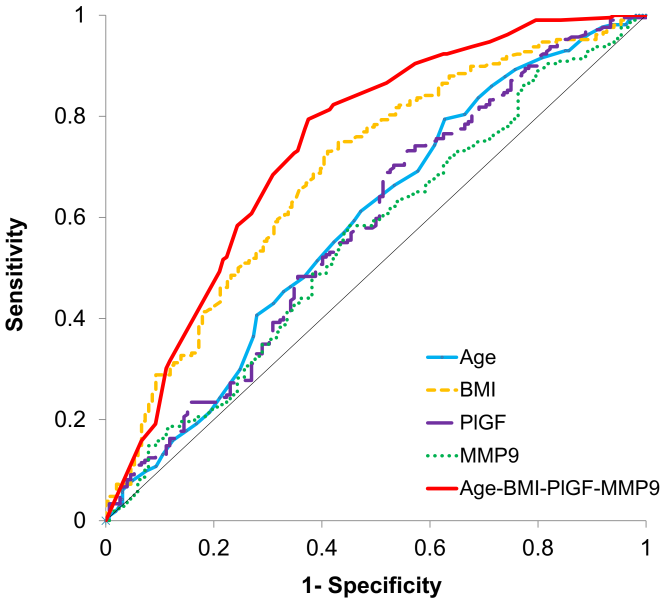

Fig. 1. Receiver operating characteristics (ROC) curve for the diagnosis of OSA after a coronary event.

Crude and adjusted odds ratio for the individuals variables are displayed on Table 4. The calculation potential contribution of each

Table 3
Area under the ROC curve for the diagnosis of OSA in patients after an ACS (biomarkers not reaching statistical significance are not shown).

	AUC	CI 95%	p-value	Optimal cut-off value (sensitivity and specificity, %)
Clinical measures				
Age	0.576	0.515–0.636	0.014	48.5 (Se 85%, Sp 29%)
HT	0.616	0.558–0.675	< 0.001	–
BMI	0.687	0.631–0.742	< 0.001	26.8 (Se 75%, Sp 57%)
Individual biomarkers				
Insulin	0.573	0.513–0.633	0.018	8.8 (Se 66.5%, Sp 47.4%)
PlGF	0.584	0.524–0.645	0.006	17.2 (Se 73.2%, Sp 44.7%)
sFlt1	0.562	0.501–0.623	0.044	68.2 (Se 76.1%, Sp 37.5%)
MMP9	0.555	0.495–0.615	0.075	459.9 (Se 58.4%, Sp 55.3%)
hsCRP	0.554	0.494–0.614	0.077	32.7 (Se 34.9%, Sp 75.7%)
Clinical and biomarker combinations				
BMI + Age	0.692	0.636–0.748	< 0.001	NA
Age + HT	0.636	0.578–0.694	< 0.001	NA
BMI + HT	0.695	0.639–0.752	< 0.001	NA
BMI + Age + PlGF	0.702	0.646–0.757	< 0.001	NA
BMI + Age + MMP9	0.709	0.654–0.764	< 0.001	NA
BMI + Age + HT	0.710	0.654–0.765	< 0.001	NA
BMI + Age + HT + hsCRP	0.712	0.657–0.767	< 0.001	NA
BMI + Age + HT + sFlt1	0.714	0.659–0.769	< 0.001	NA
BMI + Age + HT + Insulin	0.717	0.663–0.772	< 0.001	NA
BMI + Age + HT + PlGF	0.719	0.665–0.774	< 0.001	NA
BMI + Age + HT + MMP9	0.733	0.678–0.788	< 0.001	NA
BMI + Age + HT + PLGF + MMP9	0.740	0.687–0.794	< 0.001	NA
BMI + Age + PlGF + MMP9	0.741	0.688–0.764	< 0.001	NA
BMI + Age + HT + Insulin + PlGF + MMP9	0.742	0.689–0.795	< 0.001	NA

Abbreviations: AUC: area under the ROC curve, CI: confidence interval, BMI: body mass index, HT: hypertension, PlGF: placental growth factor, sFlt1: soluble fraction of fms-like tyrosine kinase 1, MMP9: matrix metalloproteinase 9, hsCRP: high-sensitivity C-reactive protein, NA: not applicable.

Table 4

Multivariate logistic regression of risk factors associated to IAH < 15. Multivariate analysis was performed using stepwise backward method. Crude and adjusted odds ratio (OR) are indicated on the table. Comparison of the expected and observed frequencies by the Hosmer-Lemeshow goodness-of-fit test (*p* = .866) and by ROC curve (AUC = 0.740; p < .001) indicated a good fit for the model.

	Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-Value
Age	1.025	(1.006–1.045)	0.010	1.024	(1.004–1.044)	0.016
Hypertension	2.581	(1.678–3.968)	< 0.001	1.933	(1.169–3.197)	0.010
BMI > 26.8	3.940	(2.518–6.164)	< 0.001	3.776	(2.341–6.089)	< 0.001
Insulin > 8.8	1.787	(1.164–2.744)	0.008	–	–	> 0.05
PlGF > 17.2	2.212	(1.421–3.443)	0.000	1.448	(1.141–1.837)	0.002
MMP9 > 4599	1.316	(1.066–1.625)	0.011	1.713	(1.048–2.802)	0.032

individual variable for the detection of OSA in our population shows that BMI, hypertension and MMP9 have the greatest OR.

4. Discussion

The results of this study have shown that although some biomarkers were associated with ACS severity-related parameters, this association was independent of the presence of OSA or any other confounding variable assessed. Besides, individual clinical measures and biomarkers could distinguish between individuals with and without OSA in our population, and our results reveal that the combination of 4 parameters in a diagnostic algorithm could improve and maximize their discriminating power: age, BMI, PlGF and MMP9.

4.1. Biomarkers associated with ACS severity

Several biomarkers were found to be associated ACS severity in our population, especially those related with angiogenesis and hemodynamics (placental growth factor, Nt-proBNP…), which reached OR values up to 9 for hsCRP in the prediction of a high Killip score, up to 5 for Nt-proBNP in the prediction of a reduced ejection fraction and up to 3 for MMP9 in the prediction of an increased number of diseased vessels (Table 2). However, their association with ACS severity was independent of the presence of OSA or any relevant parameter assessed. Therefore, neither OSA nor the other variables seem to play any important role in the association between these biomarkers and ACS severity-related parameters.

Our findings are in accordance with previous studies assessing associations between non-myocardial necrosis biomarkers and ACS severity [19,20]. As previously discussed by Ramasamy, FABP has been evaluated for the early prediction of adverse clinical outcomes in patients with suspected ACS, being a good predictor for long-term effects including cardiac death. Nt-proBNP and BNP measured upon admission were found to correlate with the magnitude of cardiac damage after AMI [21], and to predict left ventricular function [22]. Higher levels were also associated with a more severe coronary vascular disease [23]. Myeloperoxidase and hsCRP also showed prognostic value for short-term adverse cardiac events [24,25], whereas controversial findings have been described for sCD40 [26]. Other biomarkers, such as β_2-microglobulin, have also been related with the number of stenotic vessels and with coronary artery disease severity [27]. Nevertheless, to our knowledge, this is the first study assessing the impact of OSA of the clinical association between biochemical markers and angiographic and echographic features following a coronary event.

4.2. Biomarkers for OSA detection

The highest accuracy for the diagnosis of OSA was achieved when age, BMI, MMP9 and PlGF were combined in a simple mathematical model. By doing this, each variable reflects a different pathophysiological element from the development of OSA. The addition of further variables did not significantly increase the ability to distinguish individuals with OSA from individuals without OSA among our population with coronary disease.

Age is a crucial risk factor, due to anatomical alterations and changes in the sleep patterns and quality [28]. BMI is the major contributor to our algorithm. Obesity is currently accepted as one of the major risk factors for OSA and, in turn, OSA induces intermittent hypoxemia and may exacerbate hypoxia at a tissue level, thus causing metabolic dysfunction and oxidative stress [5,29,30]. As clear and reciprocal interactions have been extensively described, it has been suggested that obesity and OSA might be understood not as separate pathologies, but as a complex individual entity [31].

As reported elsewhere, PlGF is associated with the presence of OSA in patients after a coronary event and plays a role in pathological angiogenesis [32–34]. MMP9 levels in plasma have been associated with cardiovascular disease and mortality, probably by contributing to the weakening and rupture of the atherosclerotic plaques [35–37]. Therefore, high MMP9 levels in patients without cardiovascular disease might represent low-grade systemic inflammation or initiation of atherosclerotic plaque development.

Previous studies have also assessed the combination of biomarkers for the evaluation of OSA in individuals with and without heart disease. Fleming et al. [38] demonstrated that three different biomarkers combined (glycated hemoglobin, hsCRP and erythropoietin) could effectively predict OSA severity. Another study with several biomarkers concluded that the combination with highest accuracy for the distinction between moderate/severe OSA from mild/no OSA were insulin (measured before sleep) and IL-6 (measured after sleep) [39]. Although our population was different, we believe that the inclusion of clinical variables in the algorithm could further improve diagnostic accuracy. To the best of our knowledge, this is the first study suggesting a biomarker combination for the screening of OSA in individuals with ACS.

The main limitations of our study include the implementation of these blood biomarkers in clinical practice, due to their limited access and the duration of their analytical quantification. The short-term nature of this study might not bring to light the rationale for the selection and true impact of the biomarkers, which will be further evaluated after a long-term follow-up of the patients with a bigger population. In addition, lack of harmonization in the measurements of troponin (the current most specific cardiac biomarker) made it not feasible to include it in the study. Moreover, although the suggested 4-parameter combination could be a good aid for OSA, it cannot substitute sleep polysomnography, as it is the gold standard not only for the diagnosis of OSA but also for the staging and prognosis of such sleep-breathing disorder.

The fact that patients had non-sleepy OSA also represents a limitation on the potential clinical implications, since the benefit of identifying and treating OSA for the prevention of cardiovascular outcomes in these patients is uncertain.

5. Conclusions

In patients admitted after a coronary event, despite a combination of clinical measures and biomarkers could be useful for the detection of OSA, such biomarkers did not show any differential prognostic value in patients with sleep-disordered breathing.

Our mathematical model for the diagnosis of OSA integrates different aspects of the complex pathophysiology and epidemiology of the sleep apnea syndrome. Nevertheless, the presence of OSA in patients with ACS does not affect the association between biomarkers and severity-related variables.

The validation of our results would include the performance of a prospective study with cardiorespiratory polygraphy in the absence of conditions that place the patient at increased risk of non-obstructive sleep-disordered breathing like a significant cardiopulmonary disease, in order to confirm the presence of OSA.

Author contributions

Conceived and designed the experiments (AB, FB), collected samples/reagents (MP, AS, MS, JA, JD, OM, MJM, AU, JT), performed the experiments (JMB, LF, JP, MA), analyzed the data (JMB, PS, AB), wrote the manuscript (JMB, AB, AS, MS), guarantor of the study (FB).

Conflict of interest disclosures

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2019.03.015.

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