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Alterations in circulating mitochondrial signals at hospital admission for COPD exacerbation

Carlos A Amado[1,2,3], Paula Martín-Audera[4], Juan Agüero[1], Diego Ferrer-Pargada[1], Begoña Josa Laorden[1], Daymara Boucle[2,5], Ana Berja[4], Bernardo A Lavín[4], Armando R Guerra[4], Cristina Ghadban[3], Pedro Muñoz[3,6] and Mayte García-Unzueta[2,4]

Abstract

Background: Chronic obstructive pulmonary disease (COPD) exacerbation (ECOPD) alters the natural course of the disease. To date, only C-reactive protein has been used as a biomarker in ECOPD, but it has important limitations. The mitochondria release peptides (Humanin (HN), FGF-21, GDF-15, MOTS-c and Romo1) under certain metabolic conditions. Here, we aimed to evaluate the pathophysiologic, diagnostic and prognostic value of measuring serum mitochondrial peptides at hospital admission in patients with ECOPD.

Methods: A total of 51 consecutive patients admitted to our hospital for ECOPD were included and followed for 1 year; in addition, 160 participants with stable COPD from our out-patient clinic were recruited as controls.

Results: Serum FGF-21 ($p < .001$), MOTS-c ($p < .001$) and Romo1 ($p = .002$) levels were lower, and GDF-15 ($p < .001$) levels were higher, in patients with ECOPD than stable COPD, but no differences were found in HN. In receiver operating characteristic analysis, MOTS-c (AUC 0.744, 95% CI 0.679–0.802, $p < .001$) and GDF-15 (AUC 0.735, 95% CI 0.670–0.793, $p < .001$) had the best diagnostic power for ECOPD, with a diagnostic accuracy similar to that of C-RP (AUC 0.796 95% IC 0.735–0.848, $p < .001$). FGF-21 (AUC 0.700, 95% CI 0.633–0.761, $p < .001$) and Romo1 (AUC 0.645 95% CI 0.573–0.712, $p = .001$) had lower diagnostic accuracy. HN levels did not differentiate patients with ECOPD versus stable COPD ($p = .557$). In Cox regression analysis, HN (HR 2.661, CI95% 1.009–7.016, $p = .048$) and MOTS-c (HR 3.441, CI95% 1.252–9.297, $p = .016$) levels exceeding mean levels were independent risk factors for re-admission.

Conclusions: Most mitochondrial peptides are altered in ECOPD, as compared with stable COPD. MOTS-c and GDF15 levels have a diagnostic accuracy similar to C-RP for ECOPD. HN and MOTS-c independently predict future re-hospitalization.

Keywords

COPD, MOTS-c, humanin, FGF-21, GDF-15, Romo1 mitochondria, exacerbation

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[1]Department of Pulmonology, Hospital Universitario Marqués de Valdecilla, Santander, Spain
[2]Department of Medicine and Psychiatry, University of Cantabria, Santander, Spain
[3]IDIVAL (Instituto de Investigación Biomédica de Cantabria), Santander, Spain
[4]Department of Clinical Biochemistry, Hospital Universitario Marqués de Valdecilla, Santander, Spain
[5]Department of Internal Medicine, Hospital Universitario Marqués de Valdecilla, Santander, Spain
[6]Management of Primary Care of Cantabria, Servicio Cántabro de Salud, Santander, Spain

Corresponding author:
Carlos A Amado, Department of Pulmonology, Hospital Universitario Marqués de Valdecilla, Av Valdecilla SN, 39005 Santander, Spain.
Email: amadodiago.carlos@gmail.com

Introduction

Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide.[1] COPD exacerbation (ECOPD) is defined as an event characterized by dyspnea and/or cough and sputum that worsens over ≤14 days.[2,3] ECOPD is a major outcome of this disease and is associated with diminished quality of life,[4] FEV1 decline,[5] future exacerbation[6,7] and mortality.[8] The diagnosis of ECOPD is based on clinical criteria that can be subjective and variable. Several causes can induce ECOPD.[9] C-reactive protein (C-RP) is an unspecific biomarker of inflammation that can be altered in very heterogeneous conditions it is recommended by GOLD[3] to evaluate the severity of ECOPD. Therefore, new parameters must be investigated in clinical practice to establish a diagnosis of ECOPD, evaluate the cause of the exacerbation or predict future exacerbation.

Mitochondria are cellular organelles responsible for regulating energetic and oxidative metabolism, cellular respiration, inflammation, and cell death; these are all key elements in the pathogenesis of both COPD and ECOPD. Different mitochondrial peptides have been recently described. MOTS-c, regulates the antioxidant response of many target genes.[10] Humanin (HN), sends a systemic signal of mitochondrial stress, inducing a global cytoprotective effect.[11–17] FGF21 is considered a metabolic hormone and a marker of nutritional stress.[18] GDF15 is an inflammation and metabolism-associated pleiotropic hormone that is altered in several diseases including COPD, in which it has been associated with mucus hypersecretion, airway epithelial cell senescence, and impaired antiviral defenses.[19,20] Reactive oxygen species modulator 1 (Romo1) is a redox-sensitive protein that regulates the integrity of mitochondrial cristae and is regulated by oxidative stress.[21] Previous studies have shown that mitochondrial peptides are altered in stable COPD (SCOPD), and are associated with several COPD characteristics.[22,23] To date, no studies have evaluated the circulating levels of these proteins in the setting of ECOPD.

We hypothesized that circulating mitochondrial peptides would be altered during ECOPD and might be associated with certain characteristics of exacerbation. Therefore, we evaluated whether their levels at admission due to ECOPD could be used as diagnostic or prognostic factors for future ECOPD.

Methods

This was an observational prospective study performed at a hospital in Spain from November 2018 to December 2020. All patients provided informed written consent to participate in this study. Samples and data from patients included in this study were preserved by the Biobank Valdecilla (PT17/0015/0019) (COPD collection approved by the ethics committee of our institution (2018.189)) in the Spanish Biobank Network and were processed according to standard operating procedures. The Ethics Committee of our institution (2018.276) approved the study. This study included stable and exacerbated COPD patients as stated in the study protocol registered at ClinicalTrials.gov (https://clinicaltrials.gov/ct2/show/NCT04449419).

Participants

We recruited patients with SCOPD during routine visits to our dedicated COPD outpatient clinic. The ECOPD study population consisted of age, sex and FEV1 matched patients who were hospitalized at our institution between November 2018 and December 2020 for ECOPD. The distribution of SCOPD: ECOPD was 3:1.

Inclusion criteria were: (1) patients with SCOPD according to the GOLD Guidelines,[24] older than 40 years, and without an exacerbation 8 weeks before inclusion in the study, and (2) patients with COPD hospitalized because of ECOPD. ECOPD was defined by an increase in more than one of the following respiratory symptoms: dyspnea, sputum purulence, increased amount of sputum, cough, or wheezing; symptoms remaining present for at least two consecutive days; and symptoms requiring treatment with antibiotic and/or systemic steroid.[3] In all ECOPD patients' blood samples were obtained within the first 24 h after hospitalization.

Exclusion criteria: (1) patients receiving pulmonary rehabilitation during the study or 6 months before, (2) patients with COVID-19, (3) previous diagnosis of coronary artery disease, cancer or pulmonary arterial hypertension (4) glomerular filtration rate<30 mL/min/1.73 m^2, (5) treatment with systemic corticosteroids before hospital admission.

Measurements

Spirometry was performed according to the Spanish Society of Pulmonology and Thoracic Surgery protocol.[25] Patients were categorized as having high risk of exacerbation if they had had two or more moderate ECOPD or one severe ECOPD during the previous year.[3] The dyspnea-eosinopenia-consolidation-acidaemia-atrial-fibrillation (DECAF) score[26] and Anthonisen criteria[27] were calculated in all patients with ECOPD. The occurrence of increased dyspnea, sputum volume, and sputum purulence was defined as a type 1 exacerbation. Type 2 exacerbation was defined by the presence of two of these three symptoms. Type 3 exacerbation was defined by the presence of one of the three symptoms in addition to at least one of the following findings: upper respiratory infection within the past 5 days; fever without another cause; increased wheezing; increased cough; or a respiratory rate or heart rate 20%

above baseline. Routine hematological and biochemical analytes (serum creatinine, uric acid, creatine kinase and C-RP) were measured with Siemens traceable enzymatic method assays (Atellica Analyzer, Siemens, Germany).

Serum HN, GDF15, FGF21, MOTS-c and Romo1 levels were measured with specific sandwich immunoassays according to the manufacturer's instructions (Human Putative Humanin Peptide MT-RNR2 ELISA, CSB-EL015084HU, Cusabio Biotech, TX, USA; Human GDF-15 ELISA, Thermo Fisher Scientific, CA, USA; Human FGF-21 ELISA, RayBiotech, GA, USA; MOTS-c ELISA, Cloud-Clone Corp., TX, USA; and Romo1 ELISA, Elabscience® TX, USA).

We obtained early morning blood samples from all participants after they signed a consent form to participate in the study. Physicians of the Hospital Emergency Service unaffiliated with this study and blinded to the results of mitochondrial peptides made the diagnoses of exacerbation and the decisions to hospitalize the patients. After the patients were released from the hospital, they were followed for 1 year, and any additional hospitalizations were recorded.

Statistical analysis

Data are presented as mean ± SD for normally distributed data or median (interquartile range) for nonparametric data. We calculated sample sizes in G*Power 3.1 program (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). The calculations were made for differences in two independent groups with respect to the GDF15 variable, given the greater availability of previous studies. An α level of 0.05 and a β level of 0.2 were assumed, for a bilateral test, to detect differences between the groups of at least 800 ng/mL, that is, an effect size of 0.525. Furthermore, the ratio between the SCOPD and ECOP groups was 3:1. This represents a sample number of at least 153 subjects in the SCOPD group and 51 in the ECOPD group. Differences between groups were analyzed with unpaired t tests for parametric data or Mann-Whitney tests for nonparametric data. Correlations between data sets were examined using Pearson (r) correlation coefficient or Spearman rank (rs) correlation coefficient. Normal distribution was evaluated with the Kolmogorov-Smirnov test. A receiver operating characteristic (ROC) curve and the area under the ROC curve (AUC) were used to assess the diagnostic value of mitochondrial peptides for ECOPD. ROC curve analysis was performed in MEDCALC version 11.6.1.0 (MedCalc Software, Mariakerke, Belgium). Evaluation of HN, GDF15, MOTS-c, FGF-21 and Romo1 as dichotomized variables, with a cut-off at the median, resulted in the best discriminative power, as reported in our previous studies[22,23] and other similar studies.[24,28] We evaluated cross-sectional associations with high versus low circulating mitochondrial peptides through logistic regression with re-hospitalization as the outcome variable. We used Kaplan-Meier estimates to calculate the proportion of participants experiencing a new admission due to ECOPD over time. We performed Cox proportional risk analysis in SPSS version 25.00. We considered differences to be significant if the p values were less than 0.05. All reported p values are two-sided.

Results

Characteristics of patients with SCOPD and ECOPD

We included 160 patients with SCOPD and 51 sex, age and FEV1 (%) matched patients with ECOPD in this study (Figure 1). Table 1 shows participants' demographic, clinical and biochemical data. No differences were observed in age, sex or FEV1% between groups. The prevalence of patients with high risk of exacerbation and previous admission was lower in the SCOPD group than the ECOPD group ($p < .001$). C-RP was higher in the ECOPD group (2.9 (0.5–11.6) mg/dL) than the SCOPD group (0.4 (0.4–0.8) mg/dL, $p < .001$).

GDF-15 levels were higher in the ECOPD group than the SCOPD group (2481.50 (1160.0–3666.5) pg/mL versus 1209 (893–1665) pg/mL, $p < .001$). MOTS-c, FGF21 and Romo1 levels were lower in the ECOPD group than the SCOPD group (498 (372–628) ng/mL versus 706 (505–970) ng/mL, $p < .001$; 153.6 (105.3–274.2) pg/mL versus 317.1 (172.9–509.1) pg/mL, $p < .001$; and 3.5 (1.9–6.8) ng/mL versus 6.1 (3.1–9.5) ng/mL, $p = .011$, respectively). No differences in HN levels were observed between patients with ECOPD versus SCOPD.

Potential utility of mitochondrial peptides and C-RP in ECOPD diagnosis

In ROC analysis (Table 2, Figure 2), C-RP had the highest AUC for differentiating patients with ECOPD from patients with SCOPD (AUC 0.796 95% CI 0.735–0.848, $p < .001$). MOTS-c had the best diagnostic power for ECOPD among the mitochondrial proteins measured (AUC 0.744, 95% CI 0.679–0.802, $p < .001$), and was followed by GDF-15 (AUC 0.735, 95% CI 0.670–0.793, $p < .001$). Both MOTS-c and GDF-15 had a diagnostic accuracy like that of C-RP. FGF-21 (AUC 0.700, 95% CI 0.633–0.761, $p < .001$) and Romo1 (AUC 0.645 95% CI 0.573–0.712, $p = .001$) had lower diagnostic accuracy. Otherwise, HN levels did not differentiate patients with ECOPD from patients with SCOPD.

To improve the diagnostic accuracy for these molecules, we evaluated the AUC of the C-RP/MOTS-c ratio (AUC 0.850, 95% CI 0.789–0.911, $p < .001$) and GDF-15/MOTS-c ratio (AUC 0.780, 95% CI 0.705–0.855, $p < .001$). Both ratios showed better diagnostic accuracy than the mitochondrial peptides alone.

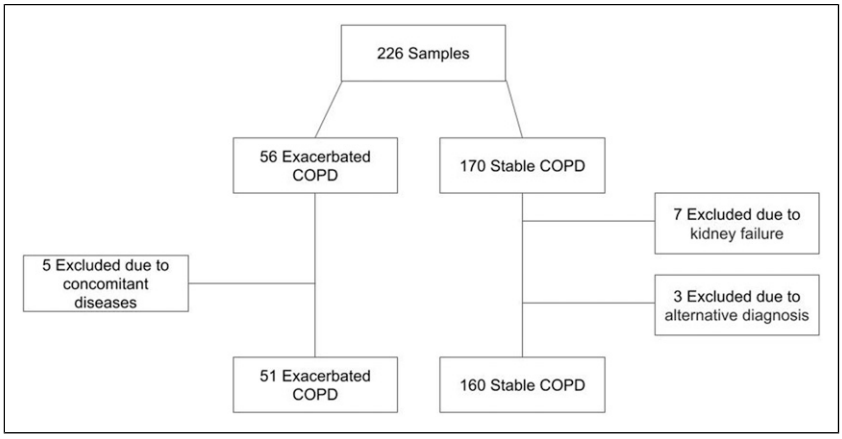

Figure 1. Flowchart for patient selection.

Table 1. Baseline characteristics of stable and exacerbated COPD patients.

Variable	Stable COPD *n* = 160	Exacerbated COPD *n* = 51	*p*
Age (years)	68.31+/−6.69	70.04+/−8.52	0.135
Sex Male *n* (%)	105 (65.6%)	36 (70.5%)	0.512
FVC (mL)	2684+/−825	2837+/−881	0.272
FVC (%)	84.1+/−20.7	88.5+/−21.9	0.217
FEV_1 (mL)	1265 (840–1845)	1220 (940–1630)	0.960
FEV_1 (%)	52 (35–73)	50 (39–71)	0.863
FEV_1/FVC	49.1 (38.2–61.1)	44.17 (34.4–57.9)	0.104
Weight (Kg)	74.2+/−16.0	73.3+/−16.1	0.757
BMI (Kg/m^2)	27.0 (24.1.8–31.2)	27 (22.8–31.3)	0.657
Charlson	1 (1–2)	2 (1–4)	0.523
Current smokers *n* (%)	47 (29.4)	20 (39.3)	0.189
GOLD 1/2/3/4 *n*(%)	29 (18.1)/61 (38.1)/46(28.8)/24(15.0)	6(11.8)/20(39.2)/21(41.2)/4(7.8)	0.228
GOLD A/B/C/D *n*(%)	**53 (33.1)/48(30.0)/13(8.1)/ 46(28.8)**	**5(9.8)/10(19.6)/10(19.6)/ 26(51)**	**<0.001**
High risk of exacerbation *n*(%)	**57 (35.6)**	**36(70)**	**<0.001**
≥1 admissions in the previous year *n* (%)	**30 (18.9)**	**22 (43.1)**	**<0.001**
Chronic treatment with Inhaled corticosteroids *n* (%)	89 (55.6)	27 (52.9)	0.941
Diabetes Mellitus *n* (%)	32 (20.0)	13 (25.4)	0.319
MOTS-c (ng/mL)	**706(505–970)**	**498 (372–628)**	**<0.001**
Humanin (pg/mL)	231 (43–496)	176 (102–357)	0.612
FGF21 (pg/mL)	**317.1 (172.9–509.1)**	**153.6 (105.3–274.2)**	**<0.001**
GDF-15 (pg/mL)	**1209 (893–1665)**	**2481.50 (1160.0–3666.5)**	**<0.001**
Romo1 (ng/mL)	**6.1 (3.1–9.5)**	**3.46 (1.9–6.8)**	**0.002**
Creatinine (mg/dL)	0.82 (0.7–0.94)	0.85 (0.66–0.98)	0.619
Uric acid (mg/dL)	6.23+/−1.83	6.33+/−1.84	0.751
CK (U/L)	64.5 (45.0–86.7)	69 (33–88)	0.318
C-RP(mg/dL)	**0.40(0 .4–0.8)**	**2.90(0.5–11.6)**	**<0.001**

FVC: Forced Vital Capacity; FEV1: Forced expiratory Volume in the first second; FVC (%) and FEV1 (%): percent of predicted; GOLD: Global initiative for Chronic Obstructive Lung Disease; BMI: Body Mass Index; FFMI: Fat Free Mass Index; HRE: high risk of exacerbation = two or more moderate COPD exacerbation events or one severe COPD exacerbation event during the previous year; CK: Creatine-kinase C-RP = C-reactive protein, **Bold font** indicates statistical significance.

Table 2. Area Under Curve stable versus exacebated COPD.

	AUC	CI 95%	*p*
MOTS-c (ng/mL)	0.744	0.679–0.802	<0.001
Humanin (pg/mL)	0.401	0.454–0.593	0.557
FGF-21 (pg/mL)	0.700	0.633–0.761	<0.001
GDF-15 (pg/mL)	0.735	0.670–0.793	<0.001
Romo1 (ng/mL)	0.645	0.573–0.712	0.001
C-RP (mg/dL)	0.796	0.735–0.848	<0.001

AUC: Area Under Curve; C-RP: C-reactive protein; CI 95%: 95% confidence interval.

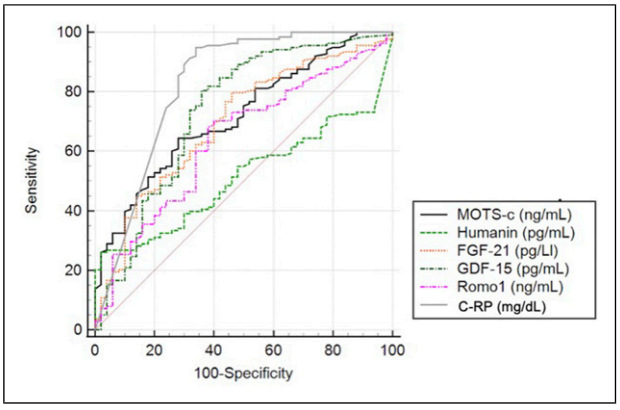

Figure 2. Diagnostic performance of mitochondrial peptides and C-reactive protein in receiver operating characteristic curve analysis. (a) Serum MOTS-c. (b) Serum Humanin. (c) serum FGF-21. (d) Serum GDF-15. (e) Serum Romo1. (f) Serum C-reactive protein.

Correlation of mitochondrial peptides and inflammatory biomarkers and blood gases during COPD exacerbation

GDF-15 negatively correlated with MOTS-c (r = −0.347, p = .013), but we did not find any correlations among the other mitochondrial peptides during ECOPD. Moreover, we observed no correlations of mitochondrial peptides with leukocyte, neutrophil or eosinophil counts. Romo-1 showed a negative correlation with pH (r = −0.415, p = .025) and PaO_2 (r = −0.525, p = .003), and a positive correlation with $PaCO_2$ (r = 0.506, p = .005). The other mitochondrial peptides did not correlate with gasometric parameters.

Mitokine levels stratified by exacerbation type, severity, and treatment

ECOPD was graded as type 1 in 25 participants (49.0%), type 2 in 12 participants (23.5%) and type 3 in 14 participants (27.5%). HN was higher in type 1 (288 (138–365) pg/mL, p = .047) and type 2 (279 (154–420) pg/mL, p = .047) than in type 3 (96 (58–174) pg/mL) exacerbation.

Eighteen participants (35.2%) had high DECAF scores (≥3). Participants with high DECAF scores had higher MOTS-c levels (562 (520–729) ng/mL) than patients with DECAF scores <3 (452 (357–538) ng/mL, p = .006). MOTS-c was also higher among patients with pneumonia (n = 12) versus the rest of the patients (n = 39) (547 (503–841) ng/mL versus 470 (358–568) ng/mL, p = .011). Patients with eosinophilic exacerbations (n = 11) (eosinophil count ≥300 cels/μL) had higher levels of GDF-15 3443 (2135–4250) pg/mL compared with patients with non-eosinophilic exacerbations (n = 40) 2186 (1116–3452) pg/mL, p = .026).

We did not find other differences in mitokine levels in ethiological characteristics or severity.

Twenty-five patients were treated with systemic corticosteroids and 36 patients received antibiotics before taking the samples. No differences were found in mitokine levels both between in patients receiving systemic corticosteroids before taking the sample and the rest of the patients (HN p = .591; GDF-15 p = .637; MOTS-c p = .585; FGF-21 p = .522 or Romo1 p = .580) or patients receiving antibiotics before taking the sample and the rest of the patients (HN p = .627; GDF-15 p = .301; MOTS-c p = .733; FGF-21 p = .710 or Romo1 p = .914).

Mitokines as predictors of second COPD hospitalization

Twenty-two participants in the ECOPD group (43.1%) required re-hospitalization because of new exacerbation within 1 year of follow-up. Mitochondrial peptides were dichotomized into high or low levels with the median as the cutoff. Sixteen patients with high MOTS-c levels and six patients with low MOTS-c levels were re-hospitalized because of exacerbation during the follow-up period. Fifteen patients (57.7%) with high HN levels and seven patients (28%) with low HN were re-hospitalized because of ECOPD during the follow-up period. A total of nine patients (34.6%) in the high GDF-15 group, 13 patients (52%) in the low GDF-15 group, 10 patients (38.5%) in the high FGF-21 group, 12 patients (48%) in the low FGF-21 group, 10 patients (38.5%) in the high Romo1 group and 12 patients (48%) in the low Romo1 group were re-hospitalized because of ECOPD during the follow-up period. Univariate Cox proportional risk analysis indicated that high MOTS-c levels (p = .020), high HN levels (p = .035) and the Charlson index (p = .05) were risk factors for re-hospitalization, whereas high GDF15 levels, high FGF-21 levels, high Romo1 levels, age, sex, smoking status, FEV1 (%) and the mMRC dyspnea score were not risk factors. Multivariate Cox proportional risk analysis indicated that age (HR 1.062, CI95% 1.001–1.126, p = .045) and high MOTS-c (HR 3.441, CI95% 1.252–9.297, p = .016) were independent risk

factors for hospital re-admission (Figure 3, MODEL A) (Table 3, MODEL A). Using the same model, we determined that high HN (HR 2.661, CI95% 1.009–7.016, p = .048) and baseline mMRC Dyspnea score (HR 0.546, CI95% 0.305–0.978, p = .042) were also independent risk factors for hospital re-admission (Figure 3, MODEL B) (Table 3, MODEL B). With the same model, FGF-21, GDF-15 and Romo1 were not found to be independent predictors of hospital re-admission.

Discussion

This study provides the first demonstration that circulating levels of mitochondrial peptides are heterogeneously different between patients with ECOPD and patients with SCOPD. GDF15 levels are higher while MOTS-c, FGF21 and Romo1 levels are lower and HN levels are not different in patients with ECOPD compared to patients with SCOPD. According to ROC curves, GDF15 and MOTS-c had similar diagnostic accuracy to that of C-RP, the established biomarker for diagnosing ECOPD. Use of the indexes GDF15/MOTS-c or C-RP/MOTS-c improved the accuracy beyond that of GDF15 or C-RP alone. In contrast, levels of HN or MOTS-c above the mean at admission were independent predictors of re-admission.

In previous studies,[22,23] we measured the serum levels of mitochondrial peptides in SCOPD. HN, GDF15 and Romo 1 levels in COPD patients were higher than those in smokers without COPD, MOTS-c levels were lower, and FGF21 levels did not differ. We hypothesized that these

Table 3. Multivariate Cox proportional risk analysis showing high MOTS-c levels (MODEL A) and high humanin levels (Model B) as predictors of readmission.

				95% CI HR	
MODEL A	B	*p*	HR	Lower	Upper
Age (years)	0.060	0.045	1.062	1.001	1.126
Sex	0.122	0.836	1.129	0.358	3.565
Current smoker	0.087	0.877	1.091	0.363	3.282
FEV1 (%)	0.003	0.808	1.003	0.980	1.026
Charlson index	0.106	0.532	1.112	0.797	1.552
mMRC Dyspnea score	−0.498	0.104	0.608	0.334	1.107
High MOTS-c levels	**1.227**	**0.016**	**3.411**	**1.252**	**9.297**
				95% CI HR	
MODEL B	B	*p*	HR	Lower	Upper
Age (years)	0.043	0.174	1.044	0.981	1.110
Sex	−0.330	0.584	0.719	0.220	2.347
Current smoker	0.391	0.479	1.478	0.501	4.365
FEV1 (%)	0.004	0.723	1.004	0.982	1.027
Charlson index	0.106	0.561	1.111	0.778	1.587
mMRC Dyspnea score	**−0.605**	**0.042**	**0.546**	**0.305**	**0.978**
High Humanin levels	**0.979**	**0.048**	**2.661**	**1.009**	**7.016**

High MOTS-c levels= >498 ng/mL (higher than the median in exacerbated group); FEV1: Forced expiratory Volume in the first second; mMRC Dyspnea score: Baseline modified Medical research council dyspnea score; **Bold font** indicates statistical significance. High Humanin levels= >177 pg/mL (higher than the median in exacerbated group); FEV1: Forced expiratory Volume in the first second; mMRC Dyspnea score: Baseline modified Medical research council dyspnea score; **Bold font** indicates statistical significance.

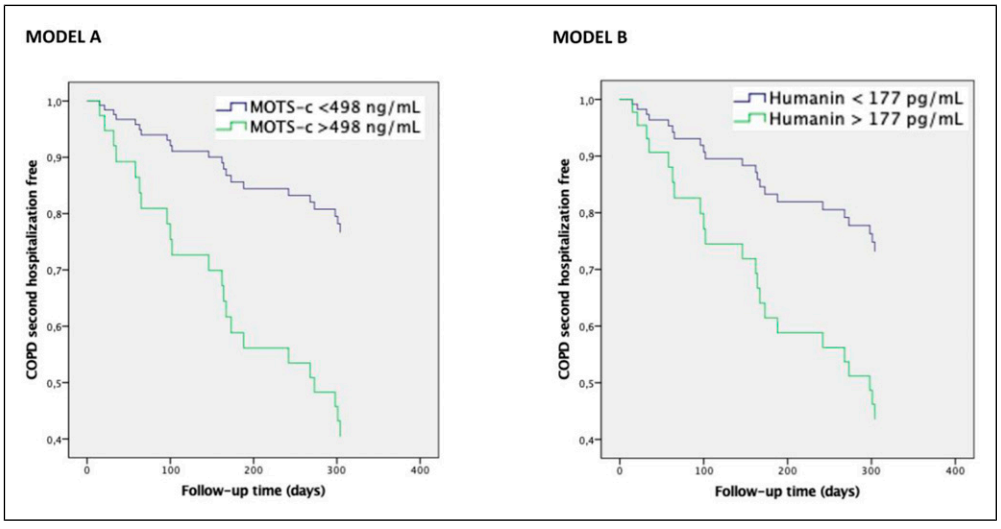

Figure 3. Kaplan-Meier curve of time to readmission through 1 year of follow-up according to MOTS-c (MODEL A) and humanin levels (MODEL B).

changes in mitochondrial peptides would be intensified in patients with ECOPD. Our findings supported our hypothesis, in that GDF15 was elevated and MOTS-c was diminished in ECOPD; however, the other parameters did not support this hypothesis (FGF21 and Romo1 were diminished, and HN remained unchanged).

GDF15 is the only mitokine that has been measured in ECOPD before.[29–31] In agreement with those studies, our data confirmed that patients with ECOPD had higher GDF15 levels than patients with SCOPD.[29,30] The study by Mutlu et al.[30] included a short number of patients and showed that GDF15 had an AUC of 0.78 for the identification of ECOPD. In contrast, a study by Kim et al.,[31] which did not compare ECOPD with SCOPD, has shown that elevated GDF15 predicts adverse short-term outcomes. According to our data, GDF15 was higher in ECOPD than SCOPD and had a similar diagnostic accuracy to that of C-RP.

Previously, we demonstrated that MOTS-c levels are diminished in SCOPD.[23] Here, we showed that these levels are even lower in ECOPD than SCOPD. Furthermore, GDF15 and MOTS-c levels were negatively correlated, indicating that GDF15 and MOTS-c show opposite responses. According to our findings, MOTS-c levels had slightly better diagnostic power than GDF15, but the best diagnostic power was obtained with the indexes GDF15/MOTS-c or C-RP/MOTS-c.

In patients with ECOPD, Romo1 was negatively correlated with pH and PaO_2, and positively correlated with $PaCO_2$. This result is concordant with findings from our previous study indicating an association between Romo1 and oxygen saturation. Our data confirm that higher Romo1 levels correlate with poorer gasometrical parameters. We found a possible etiological relationship between MOTS-c and exacerbations with pneumonia and GDF-15 and eosinophilic exacerbations. We did not find a relationship between treatments received and the mitokines in patients with ECOPD.

Only HN and MOTS-c levels at hospital admission had prognostic utility regarding re-admission due to ECOPD. In our previous study,[22] HN levels above the median in patients with SCOPD have been found to be independent predictors of ECOPD development. In this study, although HN levels did not increase during ECOPD, they were found to be independent predictors of re-admission. This finding suggests that increased HN levels due to mitochondrial dysfunction generally indicate poor prognosis in patients with COPD. Patients with ECOPD and higher levels of MOTS-c seem to have higher risk of re-admission. Unfortunately, due to the characteristics of our study we can only hypothesize about these results. One of the possibilities is that somehow, the decrease of MOTS-c during ECOPD is a protective signal for the mitochondria, and in some patients, this protective mechanism does not work in an appropriate way favoring future exacerbations. In any case, the relationship between MOTS-c and immunological response has not been studied before.

This study has several strengths. First, it simultaneously evaluated a wide range of mitochondrial molecules not previously measured in ECOPD. Second, the patients included in this study were carefully selected and well-characterized, and patients with diseases or therapies that could have influenced our results were excluded.

However, these data should be replicated in other settings, in a larger number of patients, with standardized therapy and multiple samples to evaluate the time course of the responses of these molecules. No patients included in the study died during hospitalization; therefore, we could not perform mortality analysis. Although stable patients were similar to patients with ECOPD in terms of spirometric values, sex, age, comorbidities and BMI, we did not consider other potential confounders such as frailty, previous exacerbations of fat-free mass index. Finally, our study reveals associations but not causality.

Our study reports the first evidence of alterations in circulating levels of MOTS-c, FGF21 and Romo 1 in ECOPD, and confirms that GDF15 levels are elevated in patients with ECOPD. MOTS-c, GDF15 and particularly the index GDF15/MOTS-c had high diagnostic accuracy in differentiating ECOPD from SCOPD. HN and MOTS-c levels are independent predictors of re-hospitalization.

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Author contributions

Guarantor of the paper: C.A.A. Conceptualization: C.A.A. and M.G.-U. Data curation: C.G., D.F., C.A.A., and P.M.-A. Formal analysis: D.F., C.A.A., and P.M. Project administration: C.A.A. and P.M. Methodology: C.A.A., M.G.-U., D.B., and A.B. Resources: C.A.A., M.G.-U., A.B., and P.M.-A. Visualization: C.A.A., B.A.L., and A.R.G. Supervision: M.G.-U., and P.M.-A. Software: D.B. Writing—original draft: C.A.A., P.M., and B.J.L. Writing—review and editing: C.A.A., P.M.-A., A.B., B.A.L., A.R.G., and D.B.

Declaration of conflicting interests

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Ethical statement

Ethical approval

This study complies with internationally accepted standards for research practice and reporting. The Ethics Committee of our Institution approved the study (2018.276). All patients gave informed written consent to take part in this study.

ORCID iDs

Carlos A Amado https://orcid.org/0000-0002-5598-1753
Cristina Ghadban https://orcid.org/0009-0003-9262-3247

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