Acute pulmonary exacerbation and lung function decline in patients with cystic fibrosis: high-mobility group box 1 (HMGB1) between inflammation and infection

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Abstract

Airway inflammation plays a central role in cystic fibrosis (CF) lung disease, and biomarkers of inflammation, such as high-mobility group box 1 (HMGB1) could be used to monitor disease activity. The main aim of this study was to confirm the role of HMGB1 in CF patients, correlating its serum and sputum levels with pulmonary function and inflammation. Serum and sputum HMGB1 were evaluated in a cohort of 31 CF patients and 30 non-smoking healthy subjects (HS group). Acute pulmonary exacerbation events and lung function decline have been also evaluated during a 3-year follow-up period. Serum HMGB1 levels were significantly higher than those measured in HS, such as sputum HMGB1. Kaplan-Meier survival curves revealed that patients with high HMGB1 values experienced a significantly faster evolution to decline of lung function. A multiple Cox regression analysis assessed that an increase of serum HMGB1 was associated with 5% increased risk of pulmonary disease progression, whereas elevated sputum HMGB1 was related to a 10% increased risk of lung function decline. In CF patients, HMGB1 closely reflects the entity of pulmonary impairment and represents a strong and independent risk marker for progression of lung function decline.

Keywords: Acute pulmonary exacerbation, cystic fibrosis, HMGB1, lung function decline, pulmonary *Pseudomonas* infection
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Introduction

Survival in cystic fibrosis (CF) has increased significantly in the past 30 years due to therapeutic advances, with a consequent delay in the progressive decline in lung function [1].

However, *Pseudomonas aeruginosa* infection is still one of the most prevalent bacterial pathogens affecting the majority of CF patients and negatively influencing their outcomes, despite intensive antibiotic regimens and innovative therapies [2]. Several preventive strategies have been adopted in recent years to detect the infection early, such as serology tests adjunct to respiratory culture methods, but this infection is still closely tied to the progression of CF lung disease, frequency of hospitalization, and decreased survival [3,4].

The mechanisms of chronic airway inflammation are not fully elucidated, although many components of the innate immune response in CF lung disease have been typified [5] (Fig. 1).

Monitoring the expression of biomarkers that are predictive of pulmonary exacerbation and/or infection would potentially help to prolong the survival of CF subjects, by helping to dictate short- and long-term therapy, minimizing cumulative lung

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damage as a consequence of these cycles of infection and inflammation.

Forced expiratory volume in the first second (FEV_1) is universally adopted as a surrogate outcome, but it seems to be poorly sensitive in evaluating lung disease progression in CF [6]. Bronchoalveolar lavage (BAL) remains the primary method of interrogating the inflammatory status of the airway, but the procedure is invasive and the inflammatory profiles in the lung segments sampled may not accurately represent the lung as a whole. On the other hand, induced sputum analysis offers safety advantages over BAL fluid samples, providing effective measurements, although questions still exist regarding the reproducibility of this approach [7].

In recent decades, the widespread use of high-performance analytical technology and the development of miniaturized control and processing systems have opened enormous prospects for breath diagnosis as a non-invasive diagnostic tool for a variety of diseases, including CF [8,9]. In particular, proteomics provides the ability to characterize proteins in complex solutions, such as sputum, offering a greater understanding of the physiopathology of the lung environment in CF [10].

High-mobility group box 1 (HMGB1) could play a prominent role in the enhanced inflammatory responses in CF lungs, acting on tissue repair, neutrophilic recruitment, direct inhibition of macrophage ability to phagocytose bacteria, and activating multiple inflammatory signals through the interaction with its

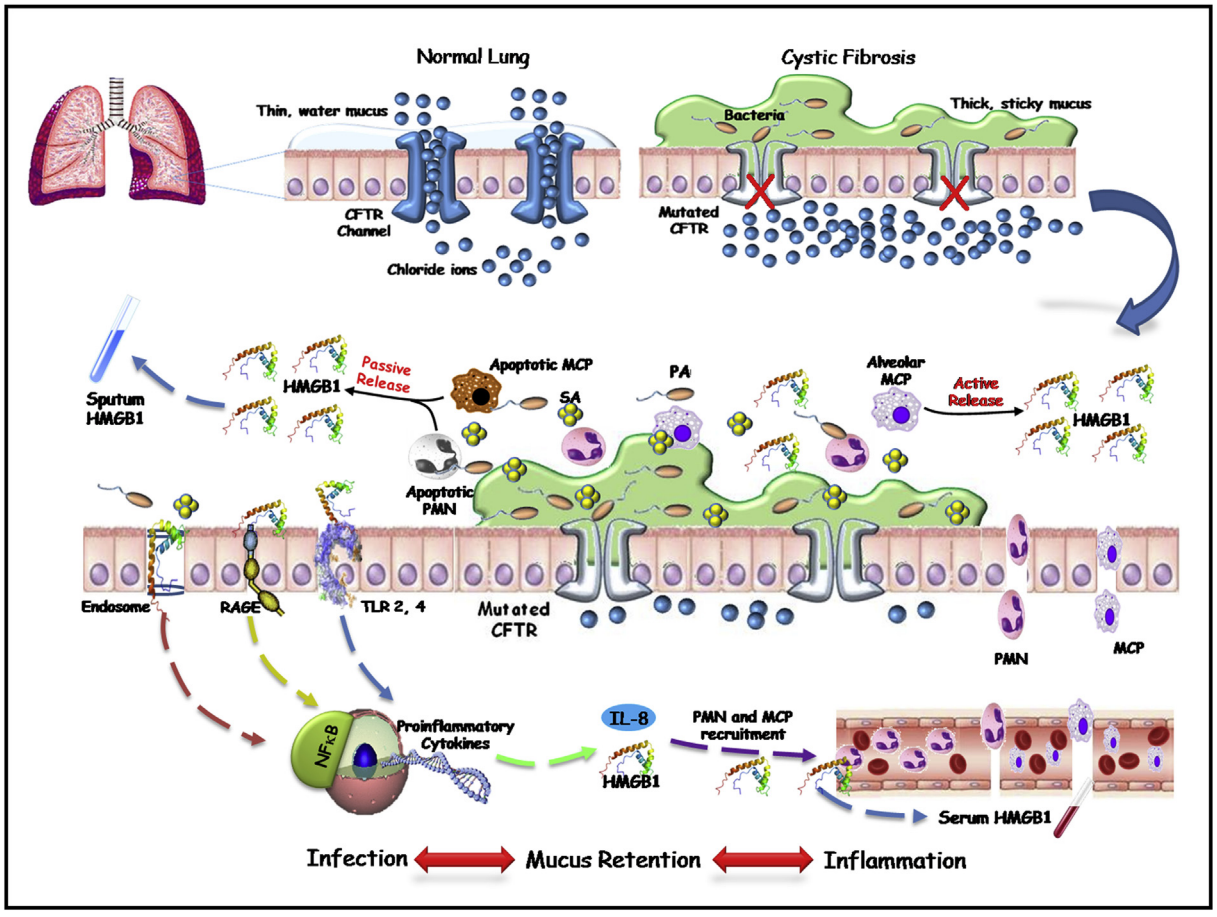

FIG. 1. Pathogenesis of inflammation, infection, and HMGB1 role in the CF airway. Inflammation and bacterial colonization initiate the pathological process, with the activation of neutrophils (PMNs) and macrophages and subsequent activation of NF-kappa B-mediated inflammatory response. This, in turn, leads to the release of prominent levels of interleukin-8, the major neutrophil chemoattractant in the lung, and HMGB1 in the CF airways. Both tissue and alveolar macrophages are activated with airway bacterial colonization, leading to the active release of a host of pro-inflammatory mediators, including HMGB1. PMNs actively release a variety of inflammatory products and, passively as a result of PMN apoptosis or necrosis, HMGB1. HMGB1 upregulates pro-inflammatory cytokine expression via its cellular receptors, the receptor for advanced glycation end products (RAGE) and toll-like receptor (TLR)-2 and -4. CFTR, cystic fibrosis trans-membrane conductance regulator; HMGB1, high-mobility group box 1; IL, interleukin; MCP, macrophage; PA, *Pseudomonas aeruginosa*; PMN, polymorphonuclear leukocytes; SA, *Staphylococcus aureus*.

specific receptors, such as toll-like receptor-4 and the receptor for advanced glycation end-product [11,12]. Previous reports of HMGB1 activity have focused on its importance as a mediator of acute inflammation of the lung, demonstrating its elevated levels in CF airways, closely relating with neutrophil influx and lung matrix degradation [13–15]. Moreover, it was also revealed in sputum samples, although it was not clear whether elevated levels of HMGB1 were mainly released from apoptotic leukocytes [16].

The main aim of this study was to confirm the role of HMGB1 in CF patients, correlating its serum and sputum levels with acute pulmonary exacerbation (APE) and lung disease severity. We have also analyzed the diagnostic and prognostic role of this biomarker, evaluating its ability to predict the lung function decline during a 3-year follow-up period.

Material and methods

Patient and study design

A cohort of 31 consecutive CF patients was prospectively enrolled at the Pediatric Bronchopneumology and Cystic Fibrosis Unit, Department of Pediatrics, University Hospital of Catania, Italy. All subjects with CF were diagnosed using accepted diagnostic criteria, including a minimum of two clinical features consistent with the diagnosis and either two sweat Cl^- values >60 mEq or two disease-causing CF trans-membrane conductance regulator (CFTR) mutations [17].

To increase the homogeneity of the CF population, patients with clinical signs of exacerbation (decline of lung function >10%, start of an antibiotic therapy, increase in coughing, clinical signs of infection, or signs of infection in terms of C-reactive protein elevation or differential counts) were excluded from analysis.

Thirty non-smoking healthy controls (HS group) were recruited without history of arterial hypertension, diabetes and neoplastic, cardiovascular, inflammatory, renal, lung, or endocrine diseases. None of these subjects was under medical treatment.

Every participant gave a fully informed approval to take part in the study. The local Ethics Committee approved the study protocol.

Collection of blood, procedures and definitions

Blood samples were drawn from all patients in fasting state in the morning during their regular visits.

All spirometric tests used in the present study were performed in a Pulmonary Physiology Unit every 4 months. Forced vital capacity (FVC) and the FEV_1 were recorded. During the spirometric test, three forced expiratory manoeuvres were performed, and the best result was registered. All values were expressed in percentage of the predicted values for age, height, and gender [18].

Screening for suspected exocrine pancreas insufficiency was assessed by faecal fat dosage, faecal chymotrypsin, and faecal elastase [19].

Patients with CF were classified according to spirometry results. In particular, an FEV_1 $\geq$80% of predicted was considered normal, whereas a mild respiratory dysfunction was defined by a FEV_1 level of 60–80%. A moderate respiratory disorder was indicated by a FEV_1 value of 40–60% of predicted; a severe respiratory disorder was identified by a FEV_1 <40% of predicted.

After spirometry, sputum induction was performed according to a standard operating procedure [20]. Sputum was collected into two containers. One specimen was submitted for comprehensive microbiology per CF consensus guidelines [21]. The second specimen was processed for the measurement of HMGB1, which was measured by ELISA, using commercially available antibodies (IBL Shino Test Corporation, Hamburg, Germany).

The number of prior-year APE and the incidence of hospitalization were also evaluated. In particular, CF patients hospitalized for APE had at least four of the following criteria: 10% or greater decrease in baseline FEV_1, increased cough or sputum production, change in sputum character, dyspnoea, tachypnea, fever, weight loss, 5% or greater decrease in O_2 saturation, new or worsening crackles on lung auscultation, or findings on chest X-ray study consistent with pneumonia [22].

Prospective follow-up

After baseline assessments, patients were followed prospectively until the end of the observation period of 3 years or until the primary study end point was reached. This latter was defined by a worsening of pulmonary function established by reduction of FEV_1 more than 10%.

Statistical analyses

Statistical analyses were performed with NCSS for Windows (version 4.0; NCSS LLC, Kaysville, UT, USA), the MedCalc software (version 11.0; MedCalc Software Acacialaan, Ostend, Belgium), and the GraphPad Prism package (version 5.0; GraphPad Software, Inc., San Diego, CA, USA). Differences between groups were established by unpaired *t* test or by analysis of variance followed by Bonferroni test for normally distributed values and by Kruskal-Wallis analysis followed by Dunn's test for nonparametric values. Dichotomized values were compared using the χ^2 test. Logistic regression analysis was performed to detect potential relationships between dichotomous variables and HMGB1. Pearson or Spearman correlation coefficients were used as appropriate to test correlations between HMGB1 and other variables. Receiver operating characteristics (ROC) analysis was employed to

calculate the area under the curve (AUC) for serum and sputum HMGB1 to find the best cut-off values capable of identifying the lung function decline and APE in CF patients. Kaplan-Meier curves were generated to assess incidence of the progression of lung disease in subjects with serum and sputum HMGB1 values above and below the optimal ROC-derived cut-off levels. Differences were evaluated using the log-rank test. Adjusted risk estimates for CF worsening were calculated using univariate followed by multivariate Cox proportional hazard regression analysis. All results were considered significant if p was <0.05.

Results

Patients' baseline characteristics

Baseline clinical characteristics of our study subjects are summarized in Table 1. The HS group was well matched for sex and age with the study group (HS group: 15 female and 15 male; age 23.2 ± 11.3 years; study group: 14 females and 17 males; age 21.2 ± 13.9 years).

Twenty-three patients (74%) were characterized by a Δ508 CFTR gene mutation, whereas eight patients (26%) had a non-F508del CFTR genotype. In particular, one patient had the genotype L1077P/D1152H, one patient had the mutation1717-1G>A/Q220X, two patients had the mutation W1282X/P5L, and four patients were characterized by the mutation N1303K/N1303K.

P. aeruginosa pulmonary isolation was assessed in 20 patients (64.5%; a primo-colonization was revealed in seven patients), whereas a positive culture for *Staphylococcus aureus* was detected in six (19%) patients. In four patients, we revealed a co-colonization.

The mean FEV_1 and the mean FVC were 61.1 ± 32.5% and 76.2 ± 27.5% of predicted, respectively. Of the 31 patients, eight (26%) presented FEV_1 ≥80%, nine (29%) had FEV_1 ≥60–80%, in seven (22.5%) cases FEV_1 was between 40% and 60%, and seven (22.5%) patients had FEV_1 <40%.

Serum HMGB1 levels were significantly higher than those measured in healthy controls (median (range): 9.2 (5.4–75.4) vs. 1.8 (1.4–2.5) ng/mL, p <0.0001), as well as sputum HMGB1 levels (5.1 (2.5–25.6) vs. 1.1 (0.8–1.8) ng/mL, p <0.0001). We analyse HMGB1 according to CF genotype, but we did not find statistical significant differences of its levels among the different groups.

We have also demonstrated that patients with worse pulmonary functions, revealed by spirometric data, such as low FEV_1 percentage, had high HMGB1 levels (77.2 (51.2–112.3) ng/mL; (28.9 (15.6–43.2) ng/mL), in both serum and sputum, respectively.

Moreover, according to APE events occurring during the study period, patients were subdivided into two groups: APE group (n = 12) and No-APE group (n = 19). In the first group, both serum and sputum HMGB1 values were significantly higher than those observed in patients without acute pulmonary exacerbation (serum HMGB1: 73.5 (5.4–103.3) vs. 7.6 (3–22.4) ng/mL, p 0.005; sputum HMGB1: 15.8 (11–35.4) vs. 3.2 (2.2–10.2) ng/mL, p 0.01).

In addition, 21 (68%) patients with exocrine pancreas insufficiency were characterized by higher levels of serum HMGB1 than those observed in patients with normal pancreas function (38.6 (3.9–81.3) vs. 8.8 (7.6–9.4) ng/mL, p 0.01), whereas no differences in HMGB1 levels assessed in the sputum have been detected. Fig. 2 summarizes these data.

Univariate correlations and multiple regression analysis

Serum HMGB1 was strictly correlated with markers of pulmonary dysfunction and inflammation. In particular, a direct correlation was demonstrated between serum HMGB1 and C-

TABLE 1. Main baseline characteristics of the study cohort and statistical differences between patients with or without lung disease progression assessed during the follow-up period

	Baseline (n = 31)	Progressor (n = 14)	Non-progressor (n = 17)	p
Gender (M/F)	17/14	9/5	8/9	0.35
Age, years	21.2 ± 13.9	21.5 ± 9.5	21 ± 17	0.93
BMI	20.2 ± 4.5	20.5 ± 5.3	20.1 ± 6.4	0.66
ΔF 508 / ΔF 508 /	23 (74%)	14 (100%)	9 (53%)	0.04
ΔF 508 / other	8 (26%)	0	8 (47%)	0.03
FVC % predicted	76.2 ± 27.5%	67.7 ± 28.3	85.2 ± 24.6	0.10
FEV_1 % predicted	61.1 ± 32.5%	47.7 ± 25.4	80.5 ± 26	0.002
Pseudomonas aeruginosa	20 (64.5%)	14 (100%)	6 (35%)	0.03
Staphylococcus aureus	6 (19%)	4 (28%)	2(11%)	0.28
APE events/year	0.8 ± 1.3	1.5 ± 1.7	0.2 ± 0.4	0.008
Exocrine pancreatic failure	21 (68%)	14 (100%)	7 (41%)	0.01
Chronic azithromycin	11 (35%)	5 (35%)	6 (35%)	0.45
Inhaled steroids	27 (87%)	13 (92%)	14 (82%)	0.31
Colistin inhalation	14 (45%)	8 (57%)	6 (35%)	0.22
C reactive protein, mg/L, median (IQR)	0.5 (0.2–0.8)	0.7 (0.4–2.6)	0.2 (0.1–0.6)	0.008
Serum HMGB1, ng/mL	9.2 (5.4–75.4)	76.5 (15.3–103.7)	6.9 (2.3–8.9)	0.0002
Sputum HMGB1, ng/mL	5.1 (2.5–25.6)	28.9 (12.9–34.5)	2.5 (2.1–4.1)	<0.0001

BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in the first second; APE, acute pulmonary exacerbation; HMGB1, high-mobility group box 1; IQR, interquartile range (25–75%).

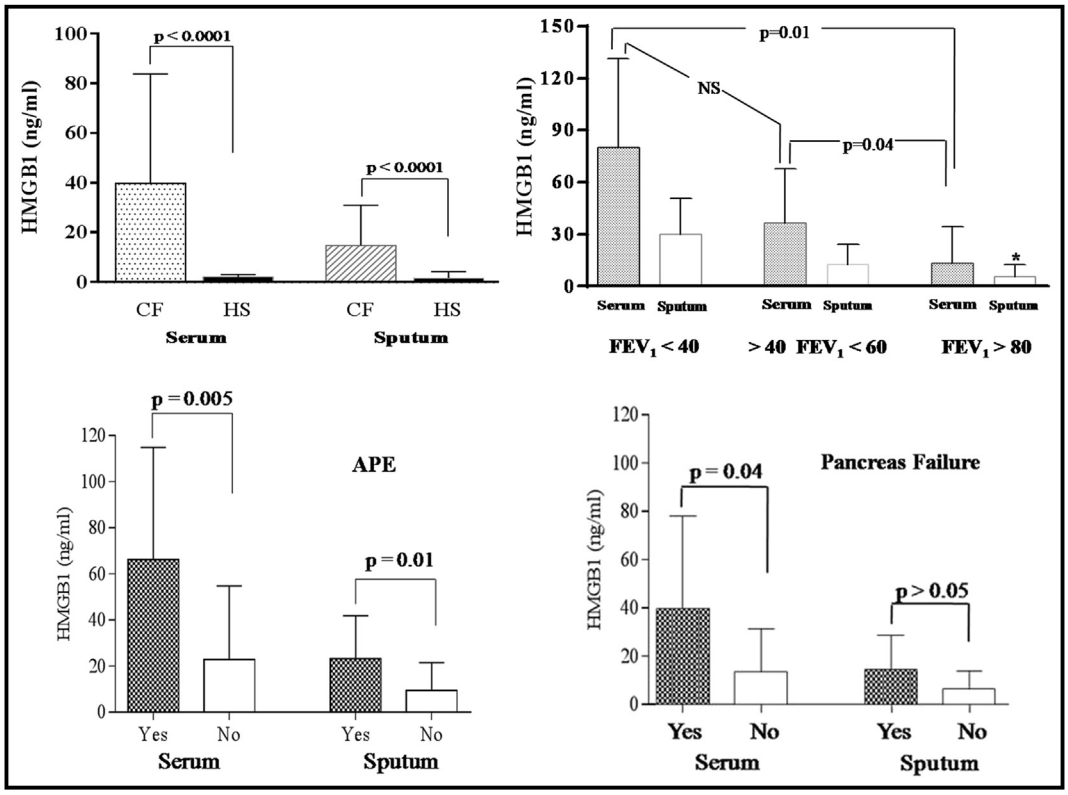

FIG. 2. HMGB1 levels in CF patients and control subjects, according to lung function, pancreas failure and pulmonary exacerbations. *FEV1 <40% vs. FEV1 >80%; p 0.03. APE, acute pulmonary exacerbation; CF, cystic fibrosis; FEV_1, forced expiratory volume in the first second; HMGB1, high-mobility group box 1; HS, healthy subjects; NS, p >0.05.

reactive protein (CRP) (*r* 0.65; p 0.0001), APE events (*r* 0.54; p 0.001), and number of hospitalizations (*r* 0.35; p 0.001), whereas an inverse correlation was found with baseline FEV_1 (*r* −0.48; p 0.01) and FVC (*r* −0.52; p 0.004). Serum HMGB1 was closely related to HMGB1 levels assessed in the sputum sample (*r* 0.88; p <0.0001). The same relationships found with serum HMGB1 were confirmed with sputum HMGB1 (Fig. 3).

Using serum and sputum HMGB1 as dependent variables in a multiple regression model, including all previously reported univariate correlates (CRP, APE events, number of hospitalizations, baseline FEV_1, and FVC), only the associations with CRP (β = 0.61, p 0.004), FVC (β −0.76, p 0.008), and FEV_1 (β −0.70, p 0.001) remained significant.

Moreover, to assess the relationship between *P. aeruginosa* infection and serum and sputum HMGB1 as independent variables, we performed a logistic regression analysis. We revealed that only sputum HMGB1 remained included in the model (standardized coefficient β 0.264; p 0.01).

HMGB1 as a diagnostic marker of APE and lung disease progression

ROC analyses were performed in order to define the diagnostic profile of serum and sputum HMGB1 in identifying APE events and decline of lung function among CF patients. Analysing these biomarkers for APE events, we have assessed that the AUC for serum HMGB1 was 0.792. When the cut-off values were set at 9.5 ng/mL, sensitivity and specificity of the marker used for the diagnosis were 75% and 73.7%, respectively. A similar profile was obtained evaluating sputum HMGB1. The AUC was 0.770 associated with 91.7% and 73.7% of sensitivity and specificity, respectively, when the cut-off value was set at 4.8 ng/mL. We did not find statistical differences between HMGB1 levels, both in serum and in sputum, and CRP in identifying APE events (p 0.06).

We have also assessed the role of HMGB1 in detecting the worsening of lung function. In particular, the AUC for serum HMGB1 was 0.973, with a sensitivity and specificity of 92.9% and 94.1%, respectively, when the cut-off value was set at 9.5 ng/mL. The AUC of sputum HMGB1 was 0.958. When the cut-off value was set at 5.3, the sensitivity and specificity were 92.9% and 94.1%, respectively. We did not find statistical differences between these two biomarkers, whereas their profiles were better than those obtained with CRP (AUC 0.773; sensitivity 92.9% specificity 58.8%; best cut-off value 0.3 mg/L; p 0.006). Figure 4 schematizes these data.

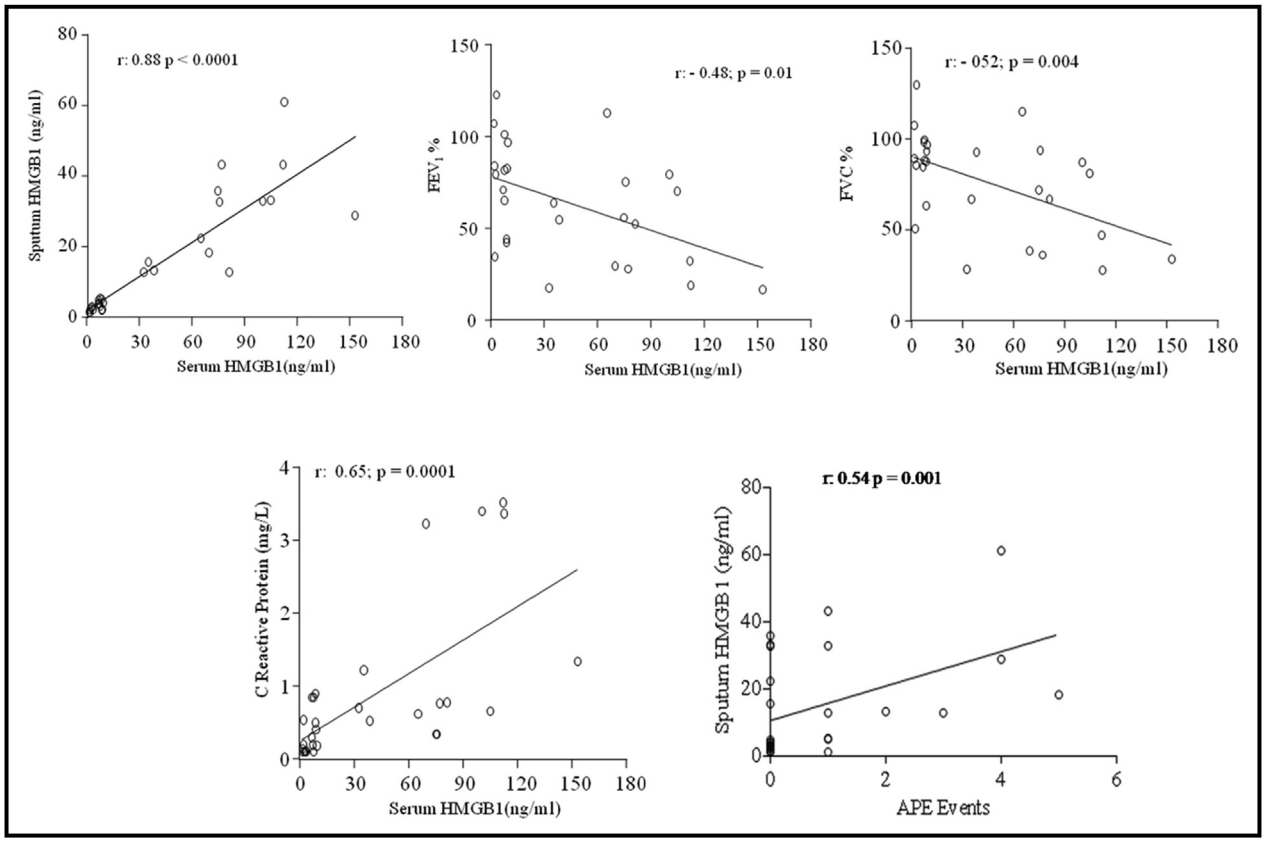

FIG. 3. Univariate baseline statistical correlations of sputum and serum high-mobility group box 1 (HMGB1).

HMGB1 and lung disease progression

During the observational period, 14 patients (45%) reached the end point, represented by pulmonary disease progression, established by the decreased FEV_1 >10%. The remaining 17 patients (55%), who did not experience a progression in CF, completed the whole observational period. Table I displays main data and statistical differences between patients with or without lung disease progression during the follow-up period.

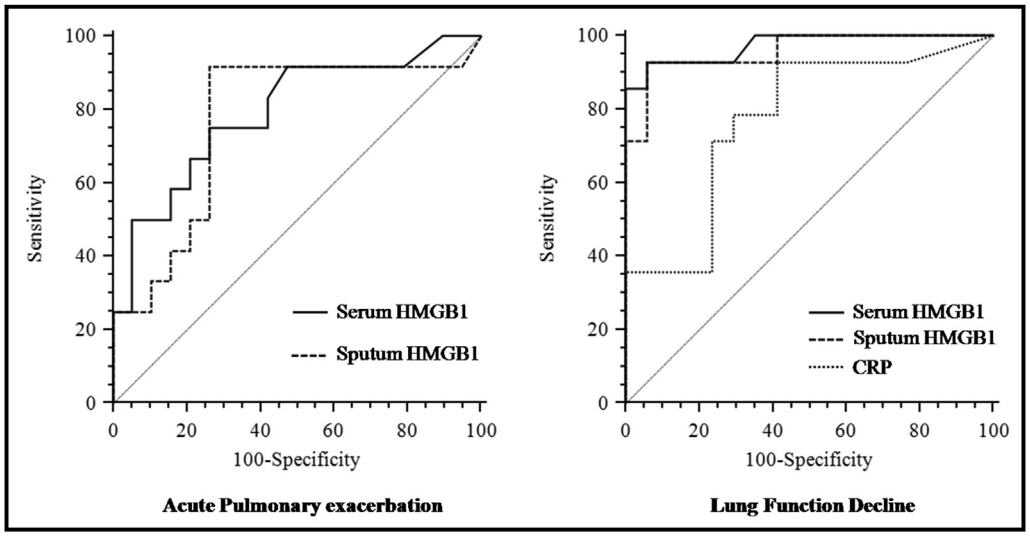

FIG. 4. Receiver operating characteristics curves of serum high-mobility group box 1 (HMGB1), sputum HMGB1, and C-reactive protein, considering acute pulmonary exacerbation and lung function decline as status variables.

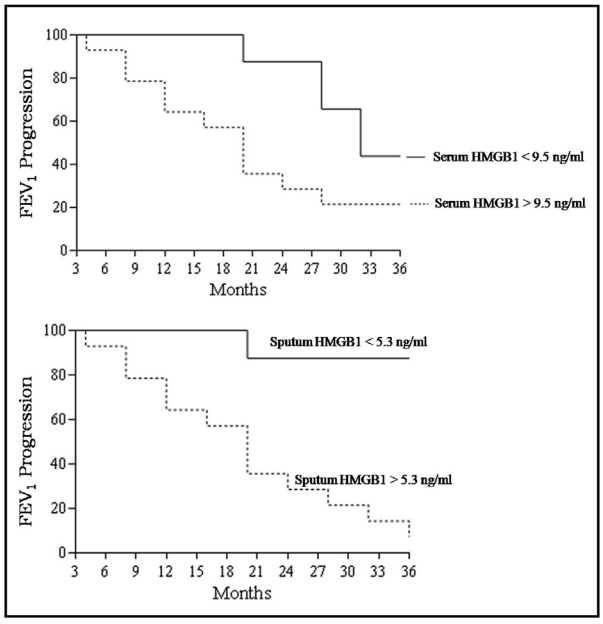

FIG. 5. Kaplan-Meier survival curves of end point (lung function decline during the follow-up period) in patients with serum and sputum high-mobility group box 1 (HMGB1) levels above and below the optimal receiver operating characteristics cut-off level.

In particular, progressor subjects presented significantly increased serum and sputum HMGB1 values at baseline compared with non-progressors (serum HMGB1: 76.5 (15.3–103.7) vs. 6.9 (2.3–8.9), p 0.0002; sputum HMGB1: 28.9 (12.9–34.5) vs. 2.5 (2.1–4.1), p <0.0001).

Kaplan-Meier survival curves in patients with serum and sputum HMGB1 levels above and below the optimal cut-off are presented in Fig. 5.

Patients with serum HMGB1 values above 9.5 ng/mL experienced a significantly faster evolution to decline of lung function (log-rank (χ^2) 7.2; p 0.007; hazard ratio 0.28; 95% CI 0.1–0.8) with a mean follow-up time to progression of 20 months, compared with 32 months for serum HMGB1 below the cut-off. A parallel trend was observed analysing sputum HMGB1 levels (log-rank (χ^2) 11; p 0.0009; hazard ratio 0.15; 95% CI 0.05–0.4).

Univariate/multiple Cox regression analysis and incidence of decline of lung function

To identify putative risk factors associated with incidence of decline of lung function, we performed a Cox regression analysis, inserting in the model all variables that were different at baseline in patients who reached the end point during the whole follow-up period (ΔF 508 genotype, FEV_1, *P. aeruginosa* infection, APE events per year, exocrine pancreatic failure, CRP, serum and sputum HMGB1).

Univariate analysis showed that ΔF508 genotype, FEV_1, *P. aeruginosa* infection, exocrine pancreatic failure, CRP, and serum and sputum HMGB1 values were significantly associated with end point. A multiple Cox regression was constructed, simultaneously inserting into the model all of the variables found to be significantly associated with end point at univariate analysis. Results from this analysis indicated that serum and sputum HMGB1 as well as genotype, *P. aeruginosa* infection, and CRP predicted higher risk of decline of lung function. In detail, an increase of serum HMGB1 was associated with a 5% increased risk of pulmonary disease progression (hazard ratio 1.05; 95% CI 1.02–1.09; p 0.002), whereas elevated sputum HMGB1 was related to a 10% increased risk (hazard ratio 1.10; 95% CI 1.03–1.18; p 0.0004). Table 2 summarizes data from univariate and multivariate Cox analysis.

Discussion

HMGB1 mediates inflammatory lung disease in CF and it is closely related with airway infection and chronic neutrophilic recruitment.

We demonstrated that CF patients are characterized by higher levels of this alarmin, both in sputum and in serum, than healthy subjects, supporting the hypothesis that HMGB1 plays a critical role in CF pathogenesis. Moreover, whereas serum

TABLE 2. Univariate and multivariate Cox proportional hazards regression model for incidence of lung disease progression

	Univariate analysis			Multivariate analysis		
	HR	95% CI	p	HR	95 % CI	p
ΔF508 genotype	1.03	1.01–1.06	0.02	1.02	1.01–1.04	0.03
FEV_1	0.98	0.96–0.99	0.03	0.97	0.96–1.02	0.10
APE events, *n*	1.08	1.03–1.16	0.01	1.04	1.01–1.07	0.02
PA infection	1.12	1.05–1.27	0.002	1.08	1.04–1.18	0.001
EPI	1.15	0.73–1.18	0.23			
CRP	1.02	1.01–1.04	0.04	1.01	0.98–1.03	0.08
Serum HMGB1	1.04	1.01–1.08	0.001	1.05	1.02–1.09	0.002
Sputum HMGB1	1.07	1.08–1.22	0.003	1.10	1.03–1.18	0.0004

APE, acute pulmonary exacerbation; CRP, C-reactive protein; EPI, exocrine pancreas insufficiency; FEV_1, forced expiratory volume in the first second; HMGB1, high-mobility group box 1; HR, hazard ratio; PA, Pseudomonas aeruginosa.

HMGB1 probably seems to reflect a systemic involvement, sputum HMGB1 is more specific for pulmonary damage, as demonstrated by its exclusive correlation with airway *P. aeruginosa* infection, FEV_1, and APE events.

It is well known that HMGB1 in CF airways plays a critical role for neutrophil recruitment and persistent infection in the lung, due to its proinflammatory properties and through a direct inhibition of macrophage ability to phagocytose bacteria, thus representing an ideal therapeutic target [23,24].

It was demonstrated that pulmonary neutrophil recruitment, tissue injury, and inflammation, induced by a direct HMGB1 instillation into the lung, have been inhibited by co-administration of anti-HMGB1 antibodies [25]. These effects have been confirmed by Entezari *et al.*, who revealed, in animals, that neutralizing anti-HMGB1 antibodies could rescue the macrophage dysfunction with a reduction of *P. aeruginosa*-induced neutrophilic inflammation, bacterial counts in the lung and alveolar injury, highlighting the role of HMGB1 on bacterial clearance beyond its inflammatory properties [26]. Moreover, HMGB1 could regulate inflammation and infection through the autophagy process. Recently, in CF patients, a functional impairment of autophagy, which plays a central regulator role of inflammatory responses modulating inflammasome activation and nuclear factor-κB activity, was demonstrated, with susceptibility to severe infections [27].

Our results revealed that HMGB1 represents a useful biomarker, able to detect exacerbations of pulmonary disease and lung function decline with high sensitivity and specificity. Patients with APE were, in fact, characterized by high HMGB1 levels, as well as CF patients with low FEV_1 values. Moreover, sputum HMGB1 gave a better diagnostic profile than that obtained by CRP, characterized by a low specificity.

There is a paucity of data in the literature examining the relationships between longitudinal changes in airway inflammation and lung function in CF. In a retrospective review of sputum biomarkers, measured in multiple CF clinical trials, longitudinal analyses revealed significant associations between increases in neutrophil elastase and decreases in FEV_1 [7]. BAL rather than sputum was also used to investigate biomarkers of airway inflammation, such as neutrophil counts, free neutrophil elastase, and interleukin-8, but none of these cytokines predicted a decline in lung function [28].

Being able to use biomarkers to monitor disease progression and predict those subjects at risk of a rapid decline in lung function would have a significant impact on clinical practice in CF.

Our findings suggest that sputum and serum HMGB1 are suitable predictive biomarkers of lung function decline over a 3-year follow-up period. Moreover, sputum and serum HMGB1 represent novel risk markers of respiratory disease progression in CF patients. If predictive value of baseline *P. aeruginosa* infection, altered FEV_1, and high CRP levels confirms the general suggestion that an already impaired lung function and chronic inflammation are important factors for the subsequent progression of pulmonary disease, remarkably, sputum and serum HMGB1 showed a most impressive predictive power in such a contest even after adjustment for confounding factors. HMGB1 would not be a simple surrogate index of inflammation, but a marker on its own, predicting lung disease progression, beyond the information provided by other inflammatory markers, such as CRP.

The present study has some limitations that should be mentioned. It was a single-centre and hypothesis-generating study, involving a relatively small cohort of patients. Confirmation in wider cohorts is indispensable to attribute general validity to our reports. However, the primary end point was reached by 45% of the participants, and the statistical model was powerful enough to establish independent relationships between HMGB1 and progression of lung disease.

In conclusion, HMGB1 represents an important inflammatory modulator in airway secretions of subjects with CF. The determination of sputum and serum HMGB1 has predictive value for APE and lung function decline among CF patients, allowing monitoring of the disease activity and the response to therapy. HMGB1 itself could be a feasible treatment target in CF. Inhibitors of HMGB1 are currently being developed for application in sepsis and other inflammatory diseases.

In CF, an intervention that blocks the dysregulated activation of the innate immune system could potentially ameliorate excessive chronic neutrophilic inflammation that contributes to disease progression.

Further studies are needed to clarify the predictive and therapeutic role of this alarmin in CF patients.

Transparency declaration

The authors have no conflicts of interest to disclose that could be perceived as prejudicing the impartiality of the research reported.

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