Fractional heat shock protein 27 urine excretion as a short-term predictor in acute exacerbation of chronic obstructive pulmonary disease

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Background: Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality and is characterized by episodes of acute exacerbations. Finding a systemic biomarker that reliably predicts outcome after an acute exacerbation remains a major challenge. Heat shock protein 27 (HSP27) has been previously studied in COPD, however, urine excretion trajectory and prognostic value after an exacerbation is unknown.

Methods: In this retrospective post hoc analysis of a prospective study that included 253 COPD patients who were hospitalized for acute exacerbation, 207 patients were analyzed. Urine and serum were sampled at admission, discharge, and 180 days after discharge; urine excretion trajectory was analyzed and correlated with clinicopathological and survival data.

Results: HSP27 urine excretion increased after an exacerbation episode [1.8% admission, 1.8% discharge, 2.3% 180 days after discharge (P=0.091)]. In severely ill patients (GOLD IV) this course was even more distinct [1.6% admission, 2.1% discharge, 2.8% 180 days after discharge (P=0.007)]. Furthermore, fractional HSP27 urine excretion at discharge was increased in GOLD IV patients (P=0.031). In Kaplan-Meier and

univariable Cox proportional hazard models patients with HSP27 urine excretion below 0.845% showed significantly worse survival at 30, 90 and 180 days after discharge. In a multivariable Cox proportional hazard model including established COPD outcome parameters fractional HSP27 urine excretion remained a significant predictor of survival at 30 and 90 days after discharge. Comparing this model to our already published model that includes HSP27 serum concentration we could show that fractional HSP27 urine excretion performs better in short-term survival.

Conclusions: Our findings provide novel information about fractional HSP27 urine excretion trajectory in acute exacerbation of COPD. Fractional HSP27 urine excretion may be significantly reduced during an episode of acute exacerbation in COPD patients and may be used as a predictor of short-term all-cause mortality.

Keywords: Biomarkers; heat shock protein 27 heat-shock proteins (HSP27 heat-shock proteins); pulmonary disease; chronic obstructive; urine

Introduction

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death worldwide (1). It is characterized by acute exacerbations (AECOPD) that significantly affect morbidity and mortality both short- and long-term after an exacerbation episode (2). AECOPD, defined as acute worsening of respiratory symptoms associated with physiological deterioration beyond day-to-day variations, is an event in the natural disease progression (3). Hitherto COPD has been understood as a pulmonary disease that is characterized by structural abnormalities of airways and pulmonary parenchyma. However, recent studies indicate systemic inflammatory reactions to be associated with these pathologies. T cell mediated autoimmunity has been suggested in COPD patients. An increased number of $CD28^{null}CD8^+$ and $CD28^{null}CD4^+$ has been reported in COPD and this T cell senescence is associated with the loss of HSP90 (4-6). As a consequence, this immune activation can also be detected systemically by elevation of inflammatory mediators (7).

Diagnosis and monitoring of COPD patients is mainly performed using spirometry, however, in acute exacerbation this method may not be recommended as it is too difficult to perform (8). Apart from a variety of clinical parameters (9-11) several blood biomarkers e.g., procalcitonin (12), CRP (13), uric acid (14), cardiac troponin T (cTnT) and N-terminal pro-brain natriuretic protein (NT-proBNP) (15) have been studied in AECOPD. Finding a systemic marker that offers a prognostic value is of enormous importance as identifying COPD patients at high risk of poor outcome could help intensifying interventions to improve quality of life and reduce morbidity in those patients. However, identifying such a biomarker is difficult, as no gold standard to definitively clarify its value has been established, yet.

Heat shock proteins (HSPs) are a group of highly preserved stress proteins that are usually found intracellularly. As part of the intracellular stress response HSPs are released from cells to interact with adjacent cells or may enter the blood stream. They are directly involved in chaperoning and regulating cell death pathways (16). Heat shock protein 27 (HSP27) is a member of the small HSP family with a molecular weight of approximately 27 kDa. It is involved in a multitude of cellular functions such as stress tolerance, protein degradation and cell death (17). It further provides protective mechanisms against oxidative-inflammatory conditions (18). Extracellular HSP27 can act anti-inflammatory by increasing the level of the anti-inflammatory cytokine interleukin-10 and attenuating migration and adhesion of macrophages (19,20). It is thought to exert an important role in many different conditions such as cancer and cardiovascular disease (21,22). Its role as a diagnostic and prognostic marker in COPD has already been studied extensively with partly contradictory results (23-28).

As a consequence of observations on the expression of HSP27 in COPD a possible role of this protein as a useful marker also in episodes of acute exacerbation is suggested as HSP27 is released from PBMCs (29). Previous work

on HSP27 in serum and plasma included only a small set of patients. Recently published data showed increased serum concentration during hospitalization for an acute exacerbation, further elevated serum HSP27 was associated with worse survival (28). Nonetheless no data are available on fractional HSP27 urine excretion in AECOPD. We hypothesize that fractional HSP27 urine excretion might provide prognostic value in those patients. To test this hypothesis, we performed serum and urine measurements and correlated them with clinicopathological and survival data.

We present the following article in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/atm-20-3683).

Methods

Patient population

The study has been approved by the National Ethics Committee of the Republic of Slovenia (EC number: 65/02/09), was conducted according to the declaration of Helsinki (as revised in 2013) and is registered at ClinicalTrials.gov (NCT01225627). Informed consent was provided by all participants. Study protocol and main findings were published previously (30,31). In brief, 253 patients that were admitted for AECOPD between 2009 and 2011 at the University Clinic of Pulmonary and Allergic Diseases Golnik, Slovenia were prospectively enrolled. After informed consent was given, patients were screened for inclusion and exclusion criteria as described previously (30) and enrolled according to their eligibility. Blood and urine samples were collected at admission, discharge, and 180 days after discharge. A post hoc analysis of 207 patients was performed, as samples were not available for 28 patients and 18 patients were excluded from the final analyses due to missing values. HSP27 serum levels have recently been analyzed in this patient cohort (28), in this re-analysis four patients were excluded due to missing urine samples. 3-year-mortality data (all cause) were available for all patients. Blood was taken at admission for full blood count, CRP, cTnT, NT-proBNP and other measurements as per good clinical practice.

Quantification of urine HSP27

A commercially available ELISA Kit (DYC1580, R&D Systems, Minneapolis, MN, USA) was used to assess serum

and urine total HSP27 concentrations according to the manufacturer's protocol. This kit was previously compared to other commercial kits and showed superior performance of diagnostic accuracy in diagnosis of pathologies of the lung (32). Inter- and intraassay variability for urine samples of this ELISA kit has been previously assessed (33).

Fractional HSP27 excretion was calculated as previously described (33) with the formula: [(urine HSP27 × serum creatinine)/(serum HSP27 × urine creatinine) ×100 to eliminate varying water excretion and resorption as confounding factors as well as to compensate the influence of comprised renal capillary endothelial permeability during exacerbation (34).

HSP27 cut off determination

Cut off values of HSP27 urine excretion at admission for Kaplan-Meier curve analysis and Cox proportional hazard models for all cause mortality calculations were identified using Cutoff Finder version 2.1, a freely available R functions based web application (35).

Statistics

Data obtained were evaluated statistically using IBM SPSS Statistics version 23 (SPSS Inc., Chicago, USA) and GraphPad Prism 6 software (GraphPad Software Inc., LA Jolla, CA, USA). Mann-Whitney U test and Kruskal-Wallis test with each pairwise P value correction were used to compare non-parametric, unpaired variables and Friedman test with each pairwise P value correction was used to compare non-parametric paired variables and expressed as median and interquartile range (IQR). Kaplan-Meier curves and log-rank test were used to evaluate event-free (time to death) interval. Cox proportional hazard models were built to evaluate prognostic significance of established factors and fractional HSP27 urine excretion at 30 days, 90 days, 180 days, 1 year and 3 years and were expressed as hazard ratio (HR) with corresponding 95% confidence intervals (CI). As this is an explorative reanalysis of an already published dataset, we aimed to compare this model to the published one (HSP27 serum concentration as a prognostic predictor) (28). Harrell's c-Index was assessed for both models at all above given time points. Binary logistic regression was performed to obtain predicted probability values of the models and ROC curves with those values were plotted. Area under the curve (AUC) was used to compare both models at all time

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points. All tests were performed in a two-sided manner. P values equal or below 0.05 were considered as statistically significant.

Results

Study population

All 207 patients were included into this explorative re-analysis. HSP27 urine excretion values were available at admission of 207 patients, at discharge of 189 patients and at 180 days after discharge of 107 patients. A summary of clinicopathological parameters of all included patients from admission is depicted in *Table 1*. Patients are predominately male (72%) and suffered from advanced COPD (GOLD II–IV). Median age was 72 years (IQR: 64–77). All patients received COPD specific optimal medical therapy. Follow up time was 1095 days, the number of deaths at 30 days after discharge was 7 (3.4%), 13 (6.3%) at 90 days, 25 (12.1%) at 180 days, 70 (33.8%) at 2 years and 98 (47.3%) at 3 years.

HSP27 urine excretion in an episode of acute exacerbation in COPD

Both HSP27 urine and serum concentration were measured at admission, discharge, and 180 days after discharge. Both showed decreasing values during the observed time frame. HSP27 urine concentration (median) was 3,300.6 pg/mL (IQR: 2,160.1–4,939.2 pg/mL) at admission, 2,857.3 pg/mL (IQR: 1,491.9–4,783.1 pg/mL) at discharge and 2,533.9 pg/mL (IQR: 1,557.4–4,836.8 pg/mL) 180 days after discharge (P=0.091, *Figure 1A*). HSP27 serum concentration (median) was 2,204.0 pg/mL (IQR: 1,545.7–3,013.1 pg/mL) at admission, 1,993.6 pg/mL (IQR: 1,504.4–2,686.5 pg/mL) at discharge and 1,635.6 pg/mL (IQR: 1,333.5–2,405.2 pg/mL) 180 days after discharge (P=0.101, *Figure 1A*). Serum and urine concentration of HSP27 did not significantly correlate at admission (r=–0.05, P=0.471).

Levels of proteins in urine are highly dependent on individually varying water excretion and resorption as well as renal capillary endothelial permeability during an episode of exacerbation. To eliminate those confounding factors fractional urine excretion of HSP27 was calculated. Fractional HSP27 urine excretion exhibited a trend towards significantly decreased values during and immediately after an episode of acute exacerbation. Fractional HSP27 urine excretion (median) was 1.8% (IQR: 1.0–4.1%) at admission, 1.8% (IQR: 1.1–3.9%) at discharge and 2.3% (OQR:

1.4–4.7%) 180 days after discharge (P=0.091, *Figure 1A*). Further admission serum HSP27 and fractional HSP27 urine excretion correlated significantly (r=–0.241, P<0.001).

If only severely ill patients (GOLD IV) are included, a more distinct course could be demonstrated. Fractional HSP27 urine excretion (median) in GOLD IV patients was 1.6% (IQR: 1.0–3.2%) at admission, 2.1% (IQR: 1.1–5.2%) at discharge and 2.8 % (IQR: 1.6–6.6%) 180 days after discharge (P=0.007, *Figure 1B*). A statistical difference could be detected between admission and 180 days after discharge (P=0.007). HSP27 serum and urine concentration did not differ in GOLD IV patients *(Figure 1B)*.

As serum HSP27 allows discrimination between mild and severe COPD (24), but no data on fractional HSP27 urine excretion in different GOLD stages is available we compared HSP27 urine excretion between GOLD stages. Fractional HSP27 urine excretion was elevated in GOLD IV patients after an episode of acute exacerbation (GOLD II *vs.* GOLD IV, P=0.031) and 180 days after admission (GOLD III *vs.* GOLD IV, P=0.035), however not at admission (P=0.819). However, HSP27 urine excretion did not correlate significantly with age at any time point.

By comparing fractional HSP27 urine excretion, serum, and urine HSP27 and eGFR in decedents and survivors at 180 days after admission we observed significantly increased serum HSP27 levels in decedents *(Figure 1C)*.

Fractional HSP27 urine excretion at admission as a predictor of survival

To assess admission fractional HSP27 urine excretion's impact as a biomarker that predicts all-cause mortality we performed Kaplan Meier curve analyses and built a Cox proportional hazard model. Survival data were available for all patients for up to 3 years after admission. Using the R functions-based web application Cutoff Finder 2.1 we could identify a cut off level for admission fractional HSP27 urine excretion that predicts survival within the first 180 days after discharge (0.845%). We further compared HSP27 to the well-known biomarkers NT-proBNP (*Figure 2A*) and cTnT (*Figure 2B*). Median survival time for patients with values below the cut off was 1309 days (95% CI: 806–1,812 days) and for patients with values above the cut off was 1185 days (95% CI: 943–1,427 days). Patients with fractional HSP27 urine excretion at admission below or equal 0.845% had significantly worse survival 30 days (P=0.007, Log rank =7.292), 90 days (P=0.006, Log rank =7.608) and 180 days (P=0.043, Log rank =4.100) after discharge (*Figure 2C*).

**Table 1** Patients' baseline demographic and laboratory values at admission

Baseline	All patients, n=207	HSP27 high (>0.854%), n=169	HSP27 low (≤0.854%), n=38	P value	Survivors at 180 days, n=186	Decedents at 180 days, n=26	P value
Gender (male/female)	148 (72%)/59 (28%)	113 (70%)/56 (33%)	35 (92%)/3 (8%)	0.002	130 (71%)/52 (29%)	18 (72%)/7 (28%)	0.953
Age (years; median ± IQR)	72.4 (63.5–77.3)	71.9 (63.4–77.1)	74.4 (66.0–78.4)	0.284	71.2 (62.8–76.9)	76.1 (71.2–81.2)	0.009
GOLD				0.491			0.064
GOLD II	25 (12%)	22 (13%)	3 (8%)		24 (13%)	1 (4%)	
GOLD III	93 (45%)	73 (43%)	20 (53%)		85 (47%)	8 (32%)	
GOLD IV	89 (43%)	74 (44%)	15 (39%)		73 (40%)	16 (64%)	
BMI (kg/m^2; median ± IQR)	25.7 (23.3–30.4)	25.0 (23.3–30.3)	28.0 (23.2–32.5)	0.401	25.8 (23.4–30.4)	24.2 (21.2–30.0)	0.252
Tiffeneau index (%)	41 (32.0–52.0)	41 (33.0–53.0)	38 (30.0–50.0)	0.364	41 (32.0–51.0)	42 (30–54)	0.748
LTOT (%)	46 (22%)	39 (23%)	7 (18%)	0.533	38 (21%)	8 (32%)	0.210
Comorbidities							
Heart failure	54 (26%)	43 (25%)	11 (29%)	0.657	43 (24%)	11 (44%)	0.030
Arterial fibrillation	19 (16%)	14 (16%)	5 (16%)	0.985	17 (17%)	2 (13%)	0.662
Ischemic heart disease	17 (15%)	13 (15%)	4 (13%)	0.764	14 (14%)	3 (19%)	0.606
Arterial hypertension	51 (44%)	35 (41%)	5 (16%)	0.293	44 (44%)	7 (44%)	0.989
Diabetes mellitus	27 (23%)	20 (23%)	7 (23%)	0.939	23 (23%)	4 (25%)	0.844
CRP (mg/L, median ± IQR)	23.1 (3.8–77.2)	22.3 (4.2–87.6)	32.7 (3.4–67.3)	0.988	21.5 (3.7–75.6)	37.9 (14.0–77.2)	0.312
cTnT (ng/L, median ± IQR)	0.0 (0.0–0.017)	0.0 (0.0–0.015)	0.0 (0.0–0.029)	0.854	0.0 (0.0–0.015)	0.0 (0.0–0.03)	0.140
NT-proBNP (pg/mL, median ± IQR)	471.2 (138.7–1,692.0)	489.5 (132.8–1,643.0)	451.9 (171.0–2,378.0)	0.472	363.4 (120.0–1,493.0)	1323.0 (471.2–3789.0)	0.002
Serum creatinine-admission (mg/dL, median ± IQR)	0.9 (0.7–1.1)	0.9 (0.7–1.1)	0.9 (0.8–1.0)	0.710	0.9 (0.7–1.1)	0.8 (0.6–1.1)	0.525
Urine creatinine-admission (mg/dL, median ± IQR)	72.9 (32.5–128.4)	63.3 (29.3–114.4)	130.4 (100.2–198.4)	<0.001	71.3 (32.5–125.8)	90.5 (33.4–132.4)	0.748
Urea–admission (mmol/L, median ± IQR)	6.9 (5.3–9.7)	6.7 (5.3–9.7)	7.35 (5.4–9.6)	0.613	6.7 (5.3–9.7)	7.5 (5.7–9.2)	0.429
eGFR (CDK-EPI) (mL/min/1.73 m^2, median ± IQR)	81.7 (65.2–93.5)	81.7 (64.9–93.6)	83.1 (72.5–91.3)	0.767	82.3 (66.8–93.3)	76.0 (63.6–93.5)	0.667
HSP27 urine excretion: admission (%, median ± IQR)	1.8 (1.0–4.1)	2.2 (1.3–5.1)	0.6 (0.4–0.7)	<0.001	1.8 (1.0–4.4)	1.6 (0.8–2.4)	0.145
HSP27 urine excretion: discharge (%, median ± IQR)	1.8 (1.1–3.9)	2.0 (1.2–4.7)	1.2 (0.8–1.9)	0.001	1.8 (1.1–3.9)	1.7 (1.1–3.2)	0.606
HSP27 urine excretion: 180 days after discharge (%, median ± IQR)	2.3 (1.4–4.7)	2.7 (1.6–5.3)	0.9 (0.7–1.8)	0.000	2.3 (1.4–4.7)	–	–
HSP27 serum: admission (pg/mL, median ± IQR)	2,204.0 (1,545.7–3,013.1)	2,030.7 (1,487.9–2,719.2)	2,817.6 (2,226.6–3,543.0)	0.001	2,099.9 (1,521.3–2,802.4)	3100.1 (2286.7–3462.6)	0.003

Table 1 (continued)

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Table 1 *(continued)*

Baseline	All patients, n=207	HSP27 high (>0.854%), n=169	HSP27 low (≤0.854%), n=38	P value	Survivors at 180 days, n=186	Decedents at 180 days, n=26	P value
HSP27 serum: discharge (pg/mL, median ± IQR)	1,993.6 (1,504.4–2,686.5)	1,948.0 (1,520.4–2,675.5)	2,224.9 (1,500.4–2,872.0)	0.670	1,931.1 (1,500.4–2,675.5)	2458.7 (1680.7–2827.5)	0.195
HSP27 serum: 180 days after discharge (pg/mL, median ± IQR)	1,635.6 (1,333.5–2,405.2)	1,653.6 (1,333.5–2,126.8)	1,991.4 (1,416.6–2,881.0)	0.215	1,635.6 (1,333.5–2,405.2)	–	–
HSP27 urine–admission (pg/mL, median ± IQR)	3,330.6 (2,160.1–4,939.2)	3,592.4 (2,372.4–5,243.6)	2,211.7 (1,077.5–3,484.0)	<0.001	3,363.2 (2,160.1–4,977.8)	3129.9 (2178.1–4417.2)	0.784
HSP27 urine: discharge (pg/mL, median ± IQR)	2,857.3 (1,491.9–4,783.1)	2,959.5 (1,533.4–5,511.7)	1,914.7 (926.6–3,466.1)	0.014	2,884.1 (1,413.2–4,783.1)	2074.8 (1528.3–4979.1)	0.720
HSP27 urine: 180 days after discharge (pg/mL, median ± IQR)	2,533.9 (1,557.4–4,836.8)	2,629.7 (1,670.7–5,340.0)	1,764.1 (1,173.9–3,818.2)	0.038	2,533.9 (1,557.4–4,836.8)	–	–

HSP27, heat shock protein 27; IQR, interquartile range; GOLD, global initiative for chronic obstructive lung disease; BMI, body mass index; LTOT, long-term oxygen therapy; CRP, C-reactive protein; cTnT, cardiac Troponin T; NT-proBNP, n-terminal pro-brain natriuretic peptide.
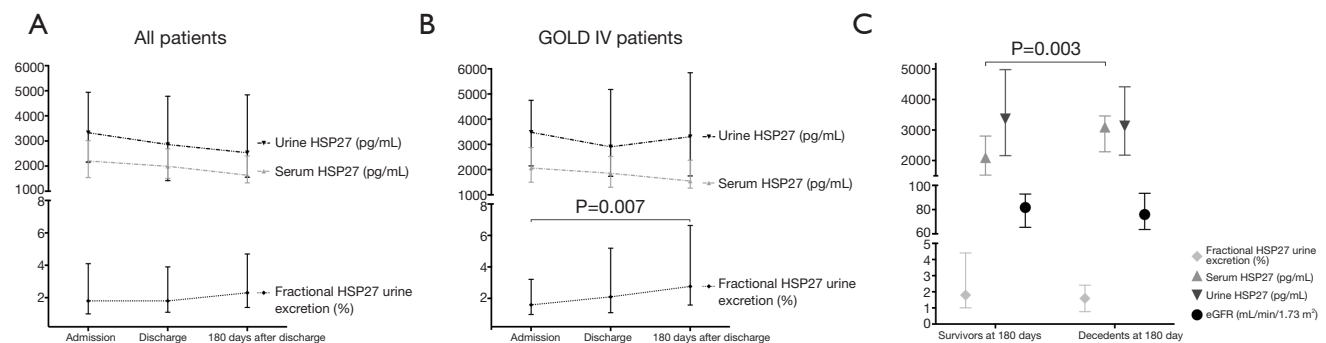

Figure 1 Time course of HSP27 urine and serum concentration as well as fractional HSP27 urine excretion in all patients (A) and GOLD IV patients (B). Differences of HSP27 urine and serum concentration, fractional HSP27 urine excretion and eGFR between survivors and decedents at 180 days after admission (C). HSP27, heat shock protein 27; eGFR, estimated glomerular filtration rate.

There was no significant difference of survival for longer follow up periods (1 year: P=0.219, Log rank =1.511; 3 years: P=0.952, Log rank =0.004). Combination of fractional HSP27 urine excretion and NT-proBNP in a Kaplan-Meier survival analysis showed a worse survival in the NT-proBNP-high/HSP27-low group after 180 days (log-rank test =12.709, P=0.005) (*Figure 2D*).

Adjusted Cox proportional hazard models were built using established COPD outcome parameters (age, gender, GOLD class, CRP, cTnT and NT-proBNP, *Tables 2,3*). In a univariable analysis admission fractional HSP27 urine excretion did significantly predict 30 day (HR: 6.1,

95% CI: 1.4–27.2, *Figure 3A*), 90 day (HR: 4.1, 95% CI: 1.4–12.2, *Figure 3B*) and 180-day mortality (HR: 2.3, 95% CI: 1.0–5.4, *Figure 3C*). For the timepoints 1 year and 3 years HSP27 did not predict mortality (*Figure 3D,E*). In a multivariable analysis fractional HSP27 urine fraction remained significant at 30 days (HR: 7.1, 95% CI: 1.6–32.3, *Figure 3A*) and 90 days (HR: 4,3, 95% CI: 1.4–12.8, *Figure 3B*) after admission.

We have previously analyzed HSP27 serum concentration as a prognosis predictor in this patient subset and have identified its power in mid-term survival (90 days to 1 year follow-up time) (28). In contrast HSP27 fractional
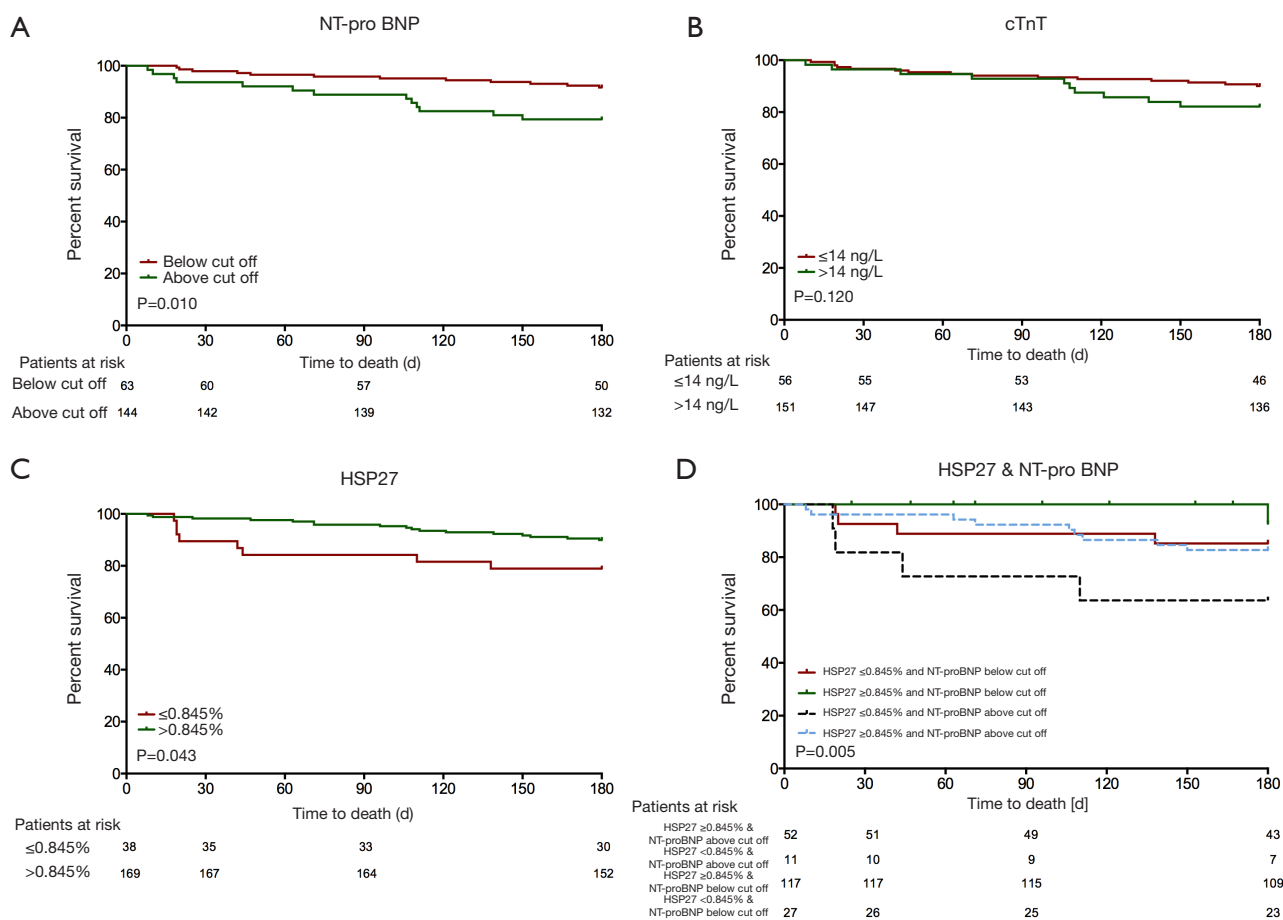
Figure 2 Kaplan-Meier survival stratified by (A) NT-proBNP, (B) cTnT, (C) HSP27 and (D) combined concentrations of HSP27 and NT-proBNP at admission. NT-proBNP, n-terminal pro-brain natriuretic peptide; HSP27, heat shock protein 27; cTnT, cardiac troponin T.

urine excretion seems to reveal its strength in short-term survival (30 days). We compared both Cox proportional hazard models and observed higher c-indices of the models including HSP27 fractional urine excretion compared to the serum model in short-term survival (urine *vs.* serum at 30 days: c-index: 0.850 *vs.* 0.500). This is supported by the AUC of both models (urine *vs.* serum at 30 days: AUC: 0.701 *vs.* 0.500). In contrast both c-index and AUC were superior in the serum model on year 1 (*Table 4*).

Association with other markers

Increased NT-proBNP concentrations at admission were significantly associated with fatal outcome over the follow-up period (*Figure 2A*), in contrast elevated cTnT levels were not associated with worse outcome (*Figure 2B*). As CRP, NT-proBNP and cTnT have already been identified as

prognostic markers in AECOPD we correlated them with admission fractional HSP27 urine excretion (15,36-40). We did not observe a correlation of admission fractional HSP27 urine excretion with any marker (CRP: r=−0.060, P=0.394; NT-proBNP: r=−0.052, P=0.454, cTnT: r=0.106, P=0.130).

Discussion

In the present study we demonstrated decreased factional HSP27 urine excretion at admission due to an episode of acute exacerbation in COPD patients and an increase after 180 days after discharge. This course was even more distinct in advanced COPD patients (GOLD IV). Furthermore, we could identify an admission fractional HSP27 urine excretion cut off value serving as predictors for short-term mortality in Kaplan-Meier curve analyses and a univariable and multivariable Cox proportional hazard model including

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Table 2 Univariable Cox proportional hazards analysis for prognostic factor of outcome data are presented as hazard ratios and 95% CI

| Factor	Mortality at 30 days		Mortality at 90 days		Mortality at 180 days		Mortality at 1 year		Mortality at 3 years	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Age (≥72 years)	2.4 (0.5–2.3)	0.300	1.5 (0.5–4.7)	0.458	2.5 (1.0–6.0)	0.040	3.1 (1.5–6.6)	0.003	1.9 (1.2–2.8)	0.003
Gender (male *vs.* female)	0.4 (0.1–3.4)	0.413	0.7 (0.2–2.7)	0.648	1.0 (0.4–2.3)	0.929	0.9 (0.4–1.9)	0.817	0.5 (0.3–0.9)	0.016
GOLD										
GOLD II *vs.* III	0.3 (0.0–4.2)	0.341	1.0 (0.1–9.4)	0.966	2.1 (0.3–17.2)	0.472	1.1 (0.3–3.9)	0.896	1.8 (0.8–3.9)	0.164
GOLD II *vs.* IV	1.4 (0.2–11.8)	0.767	2.3 (0.3–18.1)	0.442	4.7 (0.6–35.8)	0.131	2.2 (0.7–7.5)	0.188	2.5 (1.2–5.6)	0.021
CRP (≥0.5 mg/L)	$20.5\ (0.0{-}1.8{\times}10^{13})$	0.830	$20.5\ (0.0{-}1.0{\times}10^{10})$	0.767	$20.6\ (0.0{-}3.0{\times}10^{7})$	0.677	$20.6\ (0.0{-}2.0{\times}10^{6})$	0.606	0.8 (0.2–3.2)	0.731
Cardiac TnT (≥14 ng/L)	1.1 (0.2–5.7)	0.912	1.2 (0.4–3.9)	0.750	1.9 (0.8–4.2)	0.127	2.8 (1.5–5.3)	0.002	2.0 (1.3–2.9)	0.001
NT-proBNP (age-adjusted)	3.2 (0.7–14.1)	0.132	2.8 (0.9–8.3)	0.066	2.7 (1.2–5.9)	0.014	4.3 (2.2–8.3)	<0.001	2.4 (1.6–3.6)	<0.001
Fraction HSP27 urine excretion (<0.845%)	6.1 (1.4–27.2)	0.0018	4.1 (1.4–12.2)	0.011	2.3 (1.0–5.4)	0.049	1.6 (0.8–3.4)	0.223	1.0 (0.6–1.7)	0.952

GOLD, global initiative for chronic obstructive lung disease; CRP, C-reactive protein; cTnT, cardiac Troponin T; NT-proBNP, n-terminal pro-brain natriuretic peptide; HSP27, heat shock protein 27.

Table 3 Multivariable Cox proportional hazard analysis for 180 days mortality (backward LR selection)

Factor	Mortality at 30 days		Mortality at 90 days		Mortality at 180 days		Mortality at 1 year		Mortality at 3 years	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Age (≥72 years)	–	n.s.	–	n.s.	2.0 (0.8–5.0)	0.127	2.7 (1.7–6.6)	0.013	1.5 (1.0–2.3)	0.073
Gender (male *vs.* female)	–	n.s.	–	n.s.	–	n.s.			0.6 (0.4–1.1)	0.084
GOLD										
GOLD II *vs.* III	0.2 (0.0–2.7)	0.207	–	n.s.	1.6 (0.2–13.4)	0.631	0.8 (0.2–3.0)	0.772	1.5 (0.5–3.2)	0.418
GOLD II *vs.* IV	0.9 (0.1–8.2)	0.908	–	n.s.	3.8 (0.5–28.9)	0.196	1.8 (0.5–5.9)	0.360	2.3 (1.0–5.1)	0.041
CRP (≥ 0.5 mg/L)	–	n.s.	–	n.s.	–	n.s.				
TnT (≥14 ng/L)	–	n.s.	–	n.s.	–	n.s.				
NT-proBNP (age-adjusted)	3.1 (0.7–14.4)	0.153	2.9 (1.0–8.7)	0.054	2.2 (0.9–4.8)	0.067	3.4 (1.7–6.6)	<0.001	2.0 (1.4–3.1)	0.001
Fraction HSP27 urine excretion cut off (<0.845%)	7.1 (1.6–32.3)	0.011	4.3 (1.4–12.8)	0.009	2.1 (0.9–5.1)	0.080				

Data are presented as hazard ratios and 95% CI. GOLD, global initiative for chronic obstructive lung disease; CRP, C-reactive protein; cTnT, cardiac Troponin T; NT-proBNP, n-terminal pro-brain natriuretic peptide; HSP27, heat shock protein 27.
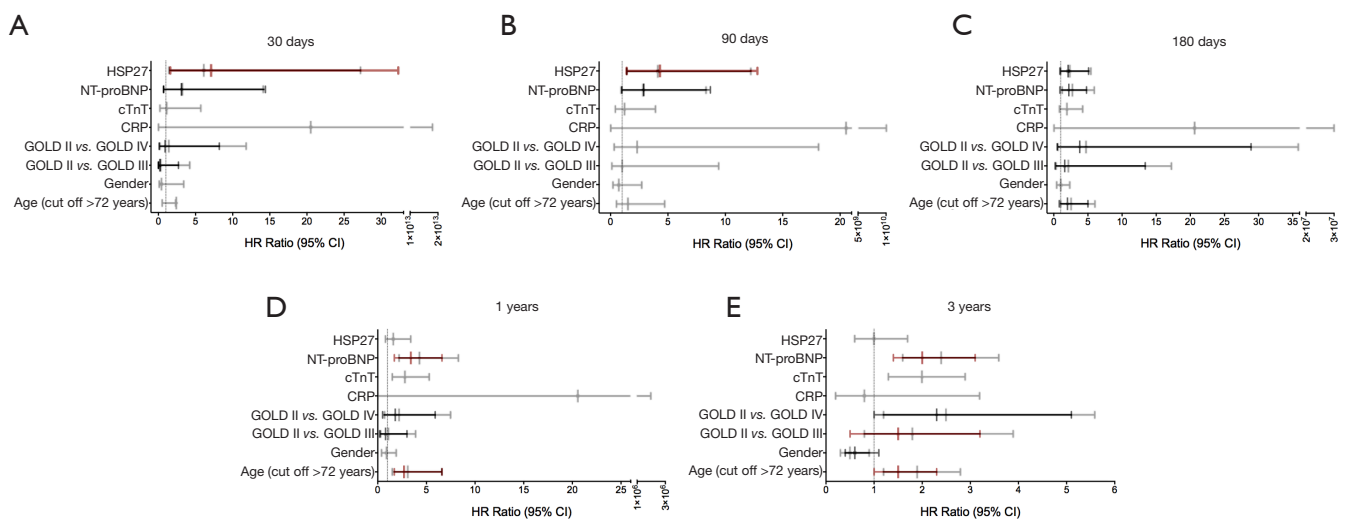
Figure 3 HR and 95% CI in univariable (grey bar) and multivariable (black and red bar, for the latter P<0.05) Cox regression analysis after 30 days (A), 90 days (B), 180 days (C), 1 year (D) and 3 years (E). Variables remaining in the model after multivariable analyses are presented in red/black. HSP27, heat shock protein 27; NT-proBNP, n-terminal pro-brain natriuretic peptide; cTnT, cardiac troponin T; CRP, C-reactive protein; GOLD, Global Initiative for Chronic Obstructive Lung Disease; HR, hazard ratios.

Table 4 Comparison of c-indices and AUC of the cox proportional hazard model including HSP27 urine excretion and the previously published cox proportional hazard model including HSP27 serum excretion (28)

Time period	HSP27 fractional urine excretion		HSP27 serum excretion	
	C-index	AUC	C-index	AUC
30 days	0.850	0.701	0.500	0.500
90 days	0.717	0.720	0.657	0.664
180 days	0.735	0.726	0.699	0.724
1 year	0.740	0.773	0.758	0.790
3 years	0.669	0.701	0.665	0.701

AUC, area under the curve; HSP27, heat shock protein 27.

established outcome parameters. Recently published data on HSP27 serum concentration in this patient cohort suggest a role as a predictor of all cause survival for mid- and long-term follow up (up to 1 year). We hypothesized that fractional HSP27 urine excretion might be a superior prognosis marker especially for the period of 30 days after discharge. Therefore, we compared both models and observed higher Harrell's c-index and AUC in the model including fractional HSP27 urine excretion for 30-day all-cause mortality, suggesting a superior performance of the fractional HSP27 urine excretion-based model. However, in 1-year survival analysis the model including HSP27 serum concentration was superior. Survival data up to 3 years

were available for our patient cohort. In none of the models HSP27 was a predictor of 3-year mortality, in contrast, we observed NT-proBNP, a well-known cardiac marker, as the main predictor in a multivariable Cox proportional hazard model.

Several previous studies attempted to identify biomarkers that predict clinical outcome after hospitalization for AECOPD. Acute exacerbations are both common and serious conditions in COPD patients and frequency and severity of exacerbation episodes are a major factor limiting survival and quality of life (3). Prevention of exacerbations is generally considered a key strategy in managing COPD (1). Thus, identifying patients who are at high risk for further

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exacerbations or death is of particular importance (3).

The heat shock response is characterized by increased expression of HSPs due to stress to ensure cell integrity and minimize the extent of apoptosis (17). HSPs can be spilled into the blood system by cell damage to mediate a response by the innate immune system but also by immune cells themselves (29,41). A variety of syndromes e.g., stable COPD (24), sepsis (42) and coronary artery disease (43) are associated with altered HSP27 concentration. Elevated extracellular HSP27 levels are found under inflammatory conditions. Especially oxidative stress induces HSP27 expression. Copper (Cu^{2+}), a toxic metal ion that is contained in cigarette smoke, results in increased expression of HSP27. Hence, HSP27 binds Cu^{2+} and inhibits Cu^{2+} mediated generation of reactive oxygen species and oxidative stress (17,44,45). Oxidative stress derived from cigarette smoke or air pollutants plays a pivotal role in the pathogenesis of COPD and especially during an exacerbation episode the burden of oxidative stress increases (46). Increased HSP27 expression and extracellular secretion in COPD may reflect mechanisms to protect bronchial epithelial cells from apoptosis under oxidative stress (47). Even though previous studies clearly implicate a role for HSP27 in the pathogenesis of COPD it is still unclear. Increased HSP27 levels in serum of stable COPD patients were demonstrated (24,48) whereas Cui *et al.* showed decreased plasma HSP27 concentrations but increased lymphocyte HSP27 levels in coal miners suffering from COPD compared to healthy coal miners (25). In another study increased expression of HSP27 in bronchial epithelium of COPD patients was seen (26). In contrast, Cappello *et al.* could not detect any differences in HSP27 expression in bronchial epithelium of COPD patients and control subjects (27). Elevated intracellular HSP27 expression may result in spillage into the vascular bed. Thus, it is not surprising that we could detect a trend towards increased serum and urine HSP27 concentrations during exacerbation and decreasing concentrations in the stable phase. As HSP27 belongs to the family of small HSPs and is of a size of about 27 kDa, renal elimination of HSP27 may be suggested (17). Fractional HSP27 urine excretion reflects the percentage of filtered HSP27 that is excreted. Lebherz-Eichinger *et al.* demonstrated increased fractional HSP27 urine excretion in severe kidney injury (33). Patients included in our cohort did not suffer from kidney injury and did not observe a difference in eGFR in both survivors and decedents (*Figure 1C*) as well as in fractional HSP27 urine excretion low or high group. Increased fractional

HSP27 urine excretion in patients suffering from kidney injury may be a result of direct release of the failing kidney. Thus, we hypothesize that increasing HSP27 urine excretion and simultaneously decreasing serum and urine HSP27 concentrations after an exacerbation represent less production of HSP27 due to decreasing stress and successful renal elimination of HSP27. This dynamic may reflect a persistent stable condition of those patients.

Additionally to increased HSP27 serum concentration in COPD patients compared to healthy smokers and non-smokers Hacker *et al.* demonstrated also continuous increase with disease severity (24). In accordance with these findings, we could detect a significant increase of HSP27 urine excretion in GOLD IV patients compared to GOLD II patients. Increased HSP27 urine excretion in GOLD IV patients may reflect augmented excretion by the kidney to eliminate elevated HSP27 production due to increasing stress levels in severely ill patients.

We provide significant evidence that fractional HSP27 urine excretion could serve as a biomarker to identify COPD patients that are at high risk for death within 180 days after discharge after an episode of acute exacerbation with superior performance especially in the first 30 days after discharge in comparison to our already published serum HSP27 based model. Nevertheless, some limitations of our study should be mentioned. First of all, we conducted a single-center study and one must carefully translate our results into other patient groups. Second, we included only GOLD II–IV patients. No intervention apart from standard care was performed so we can only hypothesize that the availability of fractional HSP27 urine excretion to physicians offers a useful tool for the management of AECOPD.

Conclusions

In conclusion, we found that fractional HSP27 urine excretion is decreased during an episode of acute exacerbation and can serve as a mortality predictor within a short follow up time. We propose that obtaining HSP27 urine and serum concentration at admission and calculating fraction HSP27 urine excretion might offer valuable information for the management of COPD patients admitted to a hospital due to an acute exacerbation within the first year. Whereas fractional HSP27 urine excretion is superior within the first 30 days after discharge, HSP27 serum concentration might provide valuable information for patient management up to 1 year after discharge.

Nevertheless, further studies including a larger sample size are needed to confirm our results.

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at http://dx.doi.org/10.21037/atm-20-3683

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-20-3683). MF reports personal fees from Astra Zeneca, personal fees from Boehringer Ingelheim, outside the submitted work. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study has been approved by the National Ethics Committee of the Republic of Slovenia (EC number: 65/02/09) and conducted according to the Declaration of Helsinki (as revised in 2013). Informed consent was provided by all participants.

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