Heat shock protein 60 (HSP60) is a mitochondrial protein constitutively expressed in the majority of cells, and its expression is up-regulated by a variety of stressors. In heart failure, HSP60 is released from cardiomyocytes. The authors speculate that increased serum HSP60 (sHSP60) may be related to the severity of heart failure. This investigation sought to assess the association between sHSP60 and the composite end point of death/readmission in patients with acute heart failure (AHF). A total of 132 consecutive patients were admitted for AHF. The independent association between sHSP60 and the end point was assessed with Cox regression. During a median follow-up of 7 months (interquartile range, 3–14), 35 (26.5%) deaths, 40 (30.3%) readmissions, and 65 (49.2%) deaths/readmission were identified. Patients who exhibited the outcome showed higher median sHSP60 values (6.15 ng/mL [8.49] vs 4.71 ng/mL [7.55] \( P = .010 \)). A monotonic increase in the incidence of the composite end point was observed when moving from lower to higher tertile (4.74, 4.76, and 6.98 per 10 patients-years of follow-up, \( P \) for trend <.001). After adjusting for established risk factors, only patients in the upper tertile showed an increased risk of death/readmission (hazard ratio, 2.63; 95% confidence interval, 1.29—5.37; \( P = .008 \)). In patients with AHF, high sHSP60 was related to a higher risk for subsequent death/readmission for AHF. ©2012 Wiley Periodicals, Inc.

Heat shock protein 60 (HSP60) is an intracellular protein constitutively expressed in the majority of cells, although primarily located inside the mitochondria, 15% to 20% of HSP60 has been found in the cytosol. Its expression is up-regulated by a variety of stressors such as infection, anoxia, oxidative stress, and inflammation. It has a protective role against stress-induced injury by maintaining cellular homeostasis and 3-dimensional structure of proteins. HSP60 has been reported to translocate to the myocardial cell surface before being released into the plasma in patients with heart failure (HF). In fact, increased levels of HSP60 in plasma membrane and serum HSP60 (sHSP60) in rat and human failing hearts have paradoxically been associated with an increase in myocardial apoptosis (measured by caspase activation and DNA fragmentation) and immune modulation due to its antigenic role.

Detectable sHSP60 levels have been reported in healthy control human patients and patients with cardiovascular diseases (diabetes and coronary heart disease). Various studies have shown that sHSP60 levels have been associated with unfavorable psychosocial measures (socioeconomic status, psychological distress, and social isolation), proinflammatory status (increase of tumor necrosis factor \( \alpha \) [TNF-\( \alpha \)]), atherosclerosis burden, endothelial dysfunction (flow-mediated vasodilation assessment), and higher risk of coronary heart disease, suggesting an important role in the activation of vascular cells and the immune system.

In patients with advanced chronic heart failure (CHF), sHSP60 was related to the severity of the disease and associated with a high risk of cardiac events. Nevertheless, no studies have examined the clinical implications of sHSP60 during an acute phase of the disease. Therefore, we sought in this study to evaluate whether sHSP60 was associated with the risk of the composite end point of death/readmission for acute heart failure (AHF) in a nonselected sample of patients admitted for AHF.

MATERIALS AND METHODS

Study Group
We prospectively studied 138 patients with AHF admitted to the cardiology department of the Hospital Clinico Universitario from November 1, 2008, to May 19, 2009. Following current guidelines, diagnosis of AHF was defined as the rapid onset of symptoms and signs secondary to abnormal cardiac function and the presence of objective evidence of structural or functional abnormality of heart at rest (cardiomegaly, third heart sound, cardiac murmur, abnormality on echocardiography, and/or elevated natriuretic peptides). This diagnosis was confirmed by a cardiologist in the emergency department. Exclusion criteria were a final diagnosis of acute coronary syndrome (n=2), known or suspected infection (n=1), and any systemic
inflammatory or malignant disease different from HF (n=1). Additionally, 2 patients died before blood sample drawing, leaving 132 patients to be studied (Figure 1). Data regarding demographic information, medical history, vital signs, 12-lead electrocardiography, laboratory data, and drug utilization were obtained in the emergency department and throughout hospitalization by means of predefined registry questionnaires. Left ventricular ejection fraction (LVEF) was assessed with 2-dimensional echocardiography in all patients during the index hospitalization. All patients received intravenous treatment with furosemide at least during the first 48 hours of admission. Treatment with angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, β-blockers, aldosterone antagonists, anticoagulants, and other therapeutic strategies was individualized following current guidelines.16,17

The composite end point of all-cause mortality and readmission for AHF was selected as the main clinical end point. This study was approved by the hospital ethics committee and written consent was obtained from the patients.

sHSP60 Measurement
Following an overnight fast, venous blood samples were drawn from the antecubital vein between 8:30 AM and 9:30 AM with the patient in a resting supine position for at least 15 minutes prior to sampling. sHSP60 levels were quantified by a technician completely blinded to patient diagnosis and evolution. An enzyme-linked immunosorbent assay using commercially available kits was used to that end.

Statistical Analysis
Continuous variables were expressed as median (interquartile range [IQR]). Discrete variables were presented as percentages. In the absence of established cut points, baseline characteristics were compared among tertiles of sHSP60. Rates for the composite end point of mortality/AHF rehospitalization were depicted (among tertiles of sHSP60) using the Kaplan-Meier method and their differences were tested by the log-rank test.

The independent association between sHSP60 categories and the main outcome was assessed with Cox regression analysis. Candidate covariates for the initial multivariable model included all variables listed in the Table. Then, a reduced and parsimonious model was derived by using backward step-down selection. The final Cox model included the following covariates: age, HF etiology, systolic blood pressure on admission, heart rate on admission, serum creatinine, serum hemoglobin, and serum sodium.

The proportional assumption for the hazard function over time was tested by means of the Schoenfeld residuals. The model’s discriminative accuracy was assessed by the Harrell’s C statistic, while its calibration tested by Gronnesby and Borgan test. A 2-sided P value of <.05 was considered to be statistically significant for all analyses. All analyses were performed using STATA 11.2 (Stata Corp 2009; STATA Statistical Software: Release 11; College Station, TX).

RESULTS
The mean age in our sample was 76 ± 14 years; 51.5% were men, 39.4% had history of ischemic heart disease, 50.8% exhibited LVEF ≤ 50%, and 71.5% were admitted for acute decompensated heart failure. The median IQR for sHSP60 was 5.74 ng/mL (0.45–8.53).

Baseline Characteristics and sHSP60
Overall, there was an inverse and monotonic association between tertiles of sHSP60 and parameters indicative of HF severity (Table). Indeed, a higher proportion of patients with peripheral edema and history of dyslipidemia were found from T1 to T3. Likewise, a higher median of heart rate, troponin I serum values, and lower relative lymphocyte count on admission and median plasmatic cholesterol levels were observed, paralleling the increase in sHSP60 tertiles (Table). Other important risk factors such as sex, comorbidity, blood pressure, and left ventricular function were not associated with sHSP60 tertiles.

sHSP60 and Prognosis
During a median follow-up of 7 months (IQR 3–14 months), 35 deaths (26.5%), 40 readmissions for AHF (30.3%), and 65 death/readmission for AHF (49.2%) were identified. Median (IQR) sHSP60 levels were higher in patients exhibiting the main outcome (6.15 ng/mL [8.49] vs 4.71 ng/mL [7.55], P=.010). A monotonic increase in the incidence of the composite end point was observed from T1 to T3 (4.75, 4.76, and 6.98 per 10 patients-years of follow-up, P for trend <.001) with tangible differences observed since
the first months after hospital admission (Figure 2a). A similar increase and direction of this trend was also observed for all-cause mortality when evaluated as an isolated end point (1.27, 1.76, and 2.41 per 10 patients-years of follow-up for T1, T2, and T3, respectively, \( P \) for trend <.001) as is depicted in Figure 2b.

In the multivariate analysis, after adjusting for well-known prognostic factors (age, HF etiology, systolic blood pressure on admission, heart rate on admission, serum creatinine, serum hemoglobin, and serum sodium), only patients in the upper tertile (>7–71 ng/mL) showed an adjusted increased risk of death/readmission for AHF (heart rate [HR], 2.63;
95%, confidence interval [CI], 1.29–5.37; \( P = .008 \) as compared with T1. No significant difference was found between T2 vs T1 (HR, 1.21 95%; CI, 0.55–2.64; \( P = .634 \)) (Figure 3). Harrell’s C statistics (0.746) and Gronnesby and Borgan test (0.834) showed adequate discriminative accuracy and calibration of the multivariable model.

In a sensitivity analysis, variables that were not included in the multivariable model but were known as potential confounders were forced in the analysis. Indeed, the inclusion of LVEF, serum C-reactive protein, serum troponin, serum N-terminal pro-brain natriuretic peptide, relative lymphocyte count, serum cholesterol, and presence of peripheral edemas (regardless of their \( P \) value), did not change the strength nor the direction of the reported association between sHSP60 levels and the composite end point (T3 vs T1: HR, 2.48; 95% CI, 1.21–5.10; \( P = .013 \)).

**DISCUSSION**

In the present study we have found a significant positive association between sHSP60 levels (determined during an episode of decompensation) and the risk of subsequent death/readmission for AHF. These results are consistent with a previous study carried out in stable patients with CHF, and we wanted to investigate whether this also applies to patients with acutely decompensated HF.

**Previous Studies**

HSP60 was previously considered an intracellular protein mainly located in the mitochondria but also in the cytosol. It has a protective role against stress-induced injury by maintaining cellular homeostasis with an antia apoptotic effect in these locations. However, recent reports have identified HSP60 in the blood of healthy persons and there was evidence of an association between this stress protein level, psychosocial stress measures, and impaired endothelial function. Furthermore, circulating HSP60 has also been associated with the pathogenesis of atherosclerosis, diabetes, and cardiovascular disease by modulating the immune response.

In the setting of HF, there is only one recent small case-control study of 112 patients with ischemic or idiopathic dilated cardiomyopathy, where the authors reported that sHSP60 not only was related to the

**FIGURE 2.** Serum heat shock protein 60 (sHSP60) levels and prognosis. (a) Cumulative risk of the composite end point (mortality/readmission for acute heart failure) across sHSP60 tertiles. (b) Cumulative risk of all-cause mortality across sHSP60 tertiles.

**FIGURE 3.** Adjusted risk for mortality/readmission for acute heart failure and serum heat shock protein 60 (sHSP60) tertiles. Multivariate model adjusted by age, heart failure etiology, systolic blood pressure on admission, heart rate on admission, serum creatinine, serum hemoglobin, and serum sodium. HR indicates hazard ratio; sHSP60, serum heat shock protein 60.
severity of the disease but was also related to a higher risk of adverse cardiac events (cardiac death or worsening CHF requiring readmission). 15

HSP60 and Prognosis in HF: Another Epiphenomenon? In AHF, it is now widely accepted that an elevation in HSP60 (mainly when located intracellular), it is known that it might induce apoptosis in myocardial cells of failing human and rat hearts, when it translocates to the plasma membrane. 6,7 Moreover, some studies have demonstrated that sHSP60 could activate the innate immune system because it constitutes a ligand for the toll-like receptor 4 and, through this mechanism, stimulate TNF-α production. 20,21 These authors proposed that the proapoptotic ability of sHSP60 might be mediated through the immune system activation. 20,21 In support of their conclusions, we found that sHSP60 correlated to parameters indicative of myocardial damage (such as high troponin I levels) and higher inflammatory activity (such as low relative lymphocyte count and presence of signs of congestion) as shown in the Table.

We thus speculate that sHSP60, in addition to reflecting underlying cell stress levels and myocardial damage, also plays an antigenic role by promoting the activation of the immune system. Whether this biomarker is just another epiphenomenon or has a direct pathogenic role in the progression of HF remains unclear. Further studies are warranted in order to clarify the exact role of this biomarker for risk stratification and even as a therapeutic target.

Limitations There are a number of limitations that we have to address: (1) this is a single-center small observational study, where by design, different types of bias and other confounding factors may be introduced; (2) with the present data, we cannot address the complex pathogenic mechanisms that may be operating between HSP60 and prognosis in HF; and (3) because no repeated measures were available for sHSP60, it is not possible to study the effect that changes which occurred at follow-up might have on the incidence of the clinical end points.

CONCLUSIONS In patients admitted for AHF, sHSP60 levels are associated with increased risk of the composite end point of death/readmission. Further studies are needed to confirm our findings and provide more insights into the pathophysiology and potential clinical utility of this biomarker in HF.

Conflict of Interest: None.

Funding: This work was supported by the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III RED HERACLES (Madrid, Spain) (grant number RD 06/009/1001).

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