Original Article:
Novel Possible Biomarkers for the Cardiovascular Disease Prognosis

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ABSTRACT

Background: Cardiovascular disease (CVD), especially heart failure (HF) as its common final pathway, is the leading cause of morbidity and mortality worldwide. Furthermore, oxidative and inflammatory processes represent fundamental underlying mechanisms for the development and progression of HF. Of interest, in recent years the development of markers with diagnostic and prognostic value for this pathology and other related CVD has been revalued.

Objectives: This study was done to quantify and evaluate inflammatory markers, such as ultrasensitive C-reactive protein (uCRP), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and heat shock protein 70 (Hsp70) in the serum of patients with HF and to compare them with healthy individuals, also correlate the values obtained from oxidative stress markers and nitric oxide (NO) bioavailability previously investigated in these patients with the coexistence or not of secondary pulmonary hypertension (SPH) associated with HF.

Methods: The determination of all parameters was achieved with standardized, reproducible, accurate, and affordable biochemical methods.

Results: The values obtained for uCRP, IL-6, and TNF-α were following the pattern of oxidative markers previously found in these patients. These findings indicate the coexistence of oxidative stress and inflammation during HF. Of particular interest, such markers are more exacerbated when were associated with SPH, increasing its value as possible biomarkers in this pathology. However, the found levels of Hsp70 were controversial.

Conclusion: The pattern of oxidative-inflammatory markers suggests their value as possible biomarkers in this cardiovascular disease. Nevertheless, additional studies are needed to assess in greater detail the importance of the relationship between serum Hsp70 expression and SPH-associated or non-SPH morbidity in HF.

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Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide in current societies, being heart failure (HF) one of the most prevalent CVD [1, 2]. Of interest, the prevalence of HF depends on the applied definition but ranges at around 1-2% of the adult population in developed countries, increasing to ≥10% among people aged 70 years [3]. Like many other pathologies, the early diagnosis of HF allows an adequate treatment and a better prognosis [4]. HF is a clinical syndrome characterized by typical symptoms (difficulty breathing, swelling of ankles, and fatigue) that can be accompanied by signs as elevated, crackling and peripheral edema venous pressure, caused by a structural or functional cardiac anomaly, which results in reduced cardiac output and/or elevated intra-cardiac pressures at rest or during exercise [5].

The most common causes of central origin are affectations of the myocardium, coronary disease, valvulopathies, pericardiopathies, arrhythmias, or diastolic dysfunction. The diagnosis is made with the clinic, the complementary exams (echocardiogram, thorax Rx, among others), and evaluation of the biomarkers. To highlight, biomarkers have complemented existing detection methods as useful tools in HF diagnosis, evolution, and prognosis. In this sense, the role of biomarkers in HF is increasingly recognized. These markers are used as a non-invasive form of evaluating the status of a patient with HF and the possibility of monitoring the changes induced by the patient's treatment. Biomarkers can be used to evaluate a variety of physiopathological processes of relevance for a patient's disease with HF, such as fibrosis, inflammation, myocardial injury, and remodeling. Specifically, monitoring biomarkers in HF can be used to make an initial diagnosis, help in the stratification of the prognosis, and identify the patient's response to the therapeutic intervention [6].

Of particular interest, our group has investigated in previous work some biomarkers involved in oxidative processes of the HF, such as the superoxide anion, the enzyme reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), superoxide dismutase (SOD), and nitric oxide (NO) [7, 8]. Specifically, we demonstrated a significant decrease in serum SOD and NO levels in patients with HF, while the levels of reactive oxygen species (ROS) and NADPH increased. These results agree with the physiopathological changes of this CVD. Furthermore, we also showed that in patients with HF and associated secondary pulmonary hypertension (SPH), serum levels of nitrites and nitrates and SOD presented a more marked decrease even concerning HF without SPH, while ROS and NADPH were even more augmented. Therefore, our results suggested that NO, ROS, NADPH, and SOD could be considered possible markers in HF and characterize patients with associated SPH [7].

It is well established that, together with oxidative stress, exacerbated inflammation is another main phenomenon observed during HF. The physiopathological mechanisms involved in HF can be summarized in fibrosis, cell apoptosis, and ventricular dysfunction, which all are related to an exacerbated inflammatory reaction. In this sense, the activation of the monocytes, followed by the tissue infiltration and the differentiation of the macrophages, appear. This effect leads to an increase in the levels of pro-inflammatory cytokines, such as the tumor necrosis factor-alpha (TNF-α), interleukins (IL-1, IL-6, and IL-8), and monocyte chemotactic protein, both in peripheral and myocardial vessels. In addition, macrophages produce transforming growth factor-beta (TGF-β), associated with myocardial fibrosis and pathological remodeling, both frequently observed in HF [9].

The deep and parallel study of these oxidative and inflammatory processes as underlying mechanisms for the development and evolution of HF could constitute a valuable contribution to discovering new biomarkers that significantly improve medical interventions in this field [10]. Some of the pro-inflammatory molecules that have been evaluated so far as potential markers during HF are TNF-α [11], IL-6 [12], and ultra-sensible C reactive protein (uCRP) [13], and to a lesser extent, the heat shock protein 70 (Hsp70) [14]. It should be noted that the latter can be considered a mixed marker, that is, both oxidative and inflammatory [15]. These markers are constitutively expressed in the heart under normal conditions; however, given the damage to the myocardium, there is a significant increase in their serum levels [16].

TNF-α is a critical mediator in systemic inflammation. Its activity is varied and includes the production of interleukins. This cytokine directly affects the contractile function of the heart, decreasing it through various mechanisms, such as the alteration of calcium homeostasis and the excitation-contraction coupling. The physiopathology of the TNF-α in the HF has been described in the negative regulation of the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) myocardial protein, the induction of ventricular hypertrophy, affecting cardiac remodeling by regulating the angiotensin II type I receptor in the cardiac fibroblasts, and matrix metal-

loproteinases both in cardiac myocytes and in fibroblasts [17]. On the other hand, the TNF-α increases some metalloproteinases' expression and activity, allowing more significant proteolytic activity with collagen degradation and restructuring of the extracellular matrix, contributing to the cardiac remodeled that precedes the HF. TNF-α is also a pro-inflammatory cytokine that stimulates the growth of vascular smooth muscle cells [18].

IL-6 also produces negative inotropic effects. Elevated levels of this interleukin increase the risk of acute myocardial infarction and mortality in patients with coronary disease. In addition, IL-6 stimulates the synthesis of the CRP and promotes the proliferation of vascular smooth muscle cells, a distinctive seal of HF [19]. Of interest, CRP is synthesized at a liver level in response to the increase in mainly IL-6, as mentioned above. The ultra-sensible quantification of CRP, when it is below the detection limits of common trials, has a critical role in the early determination of vascular inflammation and cardiovascular risk prediction. In this regard, there is *in vivo* and *in vitro* evidence that CRP can induce cardiac fibrosis and inflammation in cardiac fibroblasts and promote remodeling mediated by angiotensin II through overexpression of the type I angiotensin (AT1) receptor and activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). The CRP is an acute phase reactant whose concentration increases from 4 to 6 hours after acute tissue injury or inflammation and decreases rapidly with the resolution of tissue damage [20]. The highest levels of CRP may increase blood pressure by reducing the production of not endothelial cells, resulting in vasoconstriction and increased endothelin 1 (ET-1) production, both critical events of endothelial dysfunction [21]. The usefulness of determining CRP is seen in predicting groups of populations associated with a worse prognosis in HF. However, their participation in several inflammatory routes does not make it specific and is recommended as a single marker for the follow-up of HF [22].

The aforementioned inflammatory mediators produce acutely beneficial effects. However, when they remain in high concentrations over time due to the lack of resolution of the pathophysiological process that caused their increase, they can cause deleterious effects [23].

It is currently known that heat shock proteins (HSPs) are also elevated in the serum of patients with HF; however, their physiological role and value in predicting the development and/or progression of the disease are not yet fully understood. Traditionally, Hsp70 is considered an intracellular molecule that acts as a chaperone and cytoprotection. These chaperones have been defined as a class of proteins that mediate the correct folding of other proteins. In the vascular and cardiac compartments, HSPs are present and can be induced by different stress factors. The type of proteins expressed in the vascular system is somewhat different from that expressed in the heart. Thus, in stress-free adult mice, there are some HSPs, such as Hsp27, Hsc70, Hsp70, and Hsp84 that are expressed in various tissues, including the heart [24]. However, these HSP are an early marker of CVD [25]. They are produced in large quantities by cells in response to mechanical or ischemic stress and cytokine stimulation. They act as a highly conserved ancestral mechanism of protection against various adverse conditions. The expression of Hsp70 has beneficial effects against oxidative stress injury, inflammation, and apoptosis. Low levels of Hsp70 at the serum level would be related to a healthy cardiovascular state. Higher circulating levels of Hsp70 in patients with established hypertension may pose a future risk of CVD progression [14, 26]. However, it is not yet known whether Hsp70 production is initiated by hemodynamic force per se or by cytokines *in vivo* [27].

Despite the knowledge of all these biomolecules as possible markers of HF, to date, there are no specific and determining data on their use as biomarkers of the development and progression of SPH associated with HF [28, 29], which represents an additional risk factor and significant when making the diagnosis and adequate therapeutic management of these patients. Therefore, the central hypothesis of the present work arises from the presumption that the serum values of TNF-α, IL-6, CRP, and Hsp70 are abnormally elevated in patients with HF. Furthermore, another assumption is that HF patients with associated SPH with pulmonary pressure (PP) >40 mmHg have higher levels of TNF-α, IL-6, uCRP, and Hsp70 levels than those with PP <40 mmHg. In this context, the present work focused mainly on the inflammatory process associated with HF, and specifically, on the possibility of the use of pro-inflammatory proteins as serum biomarkers of HF, with particular attention to the development or not of associated SPH, which allow early diagnosis, better therapeutic management of patients, and a better prognosis of CVD.

Materials and Methods

Protocol

Thirty patients with HF and reduced ejection fraction (EF) (<40%) were protocolized. Then, they were separated according to their values of the pulmonary systolic

pressure (PSP) measured by Doppler echocardiography: group A (PSP ≥40 mmHg) with 13 patients (43%) and group B (PSP <40 mmHg) with 17 patients (57%). In addition, eight healthy individuals were included as controls. According to the initial protocol, as inflammatory markers were quantified, only a sample or subgroup of the initial population was used, consisting of 20 patients (10 with SPH and 10 without SPH) and five healthy controls. The study was observational, and cross-sectional, with patients protocolized according to inclusion and exclusion criteria, a number that was not yet sufficient because it was a preliminary study. The patients studied were subjected to biochemical determinations by extracting blood samples. In the design of this work, the basic principles of bioethics were taken into account. Prior to the protocolization, the informed consent was signed for each patient. The protocol was approved by the Research Ethics Committee of the Central Hospital of Mendoza (Ethical Code: 1232/19).

Serum inflammatory markers determination

Ultrasensitive CRP was determined in serum after 8 hours of fasting, without lipemia or hemolysis, using the immunoturbidimetric method with latex (Wiener CRP Lab Turbitest AA. Reagents) and ARCHITEC c8000 analyzer.

TNF-α and IL-6 were quantified by ELISA technique (IL-6 or TNF-α immunoassay kit, BioSource International, USA) according to the manufacturer's protocols. Plates were read at 450 nm on an automated reader. The minimum detectable concentration was 2 pg/mL, with a sensitivity greater than 96%.

Serum Hsp70 concentrations were determined using a high-sensitivity ELISA kit (Enzo Life Sciences, USA). The tests were performed according to the manufacturer's instructions and with the purification step described by Pockley et al.with minor modifications [30]. The detection limit of the ELISA assay was 0.09 mg/mL.

Statistics

The data collection instrument was the Patient Inclusion Form. The results were expressed as percentages for categorical variables and a mean with their standard deviation for continuous variables. The paired t-test was used to compare all the patients with HF against five healthy volunteers (a comparison that passed the p normality test) and ANOVA II was used to compare inflammatory markers between groups A and B. The GraphPad InStat program 3® was used to carry out the corresponding statistical and graphical operations. To compare uCRP and Hps70 of patients in groups A and B, a correlation test was used. In all cases, a statistically significant difference was considered as $P<0.05$.

Results

The data used for the statistical analysis were the PSP (mmHg) and the serum levels of TNF-α and IL-6, expressed as pg/mL, as well as uCRP and Hsp70, expressed as mg/mL. The reference values, taken from five healthy volunteers were as follows: TNF-α (8±7), IL-6 (2.5±2), uCRP (0.6±0.3) , and Hsp70 (10±3). To highlight, all healthy volunteers (control) had inflammatory markers within normal limits, despite differences in age and gender.

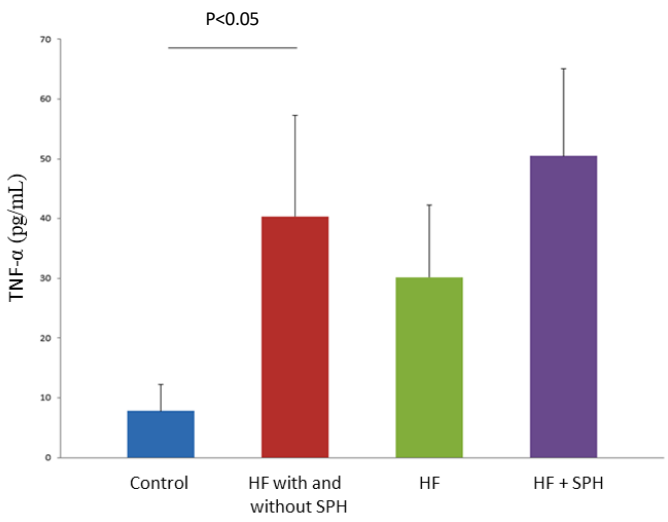

Figure 1. TNF-α serum levels were evaluated in healthy individuals (control), HF patients with and without SPH, HF patients without SPH, and HF patients with SPH

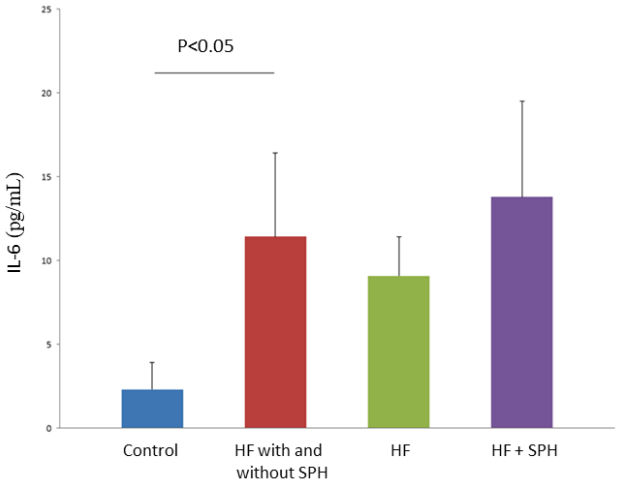

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Figure 2. Serum IL-6 levels were evaluated in healthy individuals (control), HF patients with and without SPH, HF patients without SPH, and HF patients with SPH

In group A, the mean PSP was 45±3 mmHg, while in group B, it was 30±4 mmHg. The values of TNF-α, IL-6, uCRP, and Hsp70 found for all patients (group A + group B) were as follows: 40.35±16.95, 11.45±4.96, 4.08±1.036, and 3.57±1.64, respectively. When these values were compared with healthy volunteers, the differences were significant for TNF-α (P<0.5) (Figure 1), IL-6 (P<0.5) (Figure 2), uCRP (P<0.01) (Figure 3), and Hsp70 (P<0.01) (Figure 4), respectively. On the other hand, the means with their corresponding mean standard error for each group in group A were 50.5±14.56; 13.8±5.72; 5.1±0.7, and 2.04±1, respectively, and 30.2±12.05, 9.1±2.34, 3.05±0.51, and 5.1±0.83 in group B, respectively. When these values were paired between both groups (A versus B), the results of group A showed statistically significant differences for uCRP, which increased (P<0.05), and for Hsp70, which decreased (P<0.05) (Figures 3 and 4). No significant differences were observed for TNF-α and IL-6; however, a clear tendency to increase these markers was demonstrated in group A compared to group B (Figures 1 and 2).

Likewise, the correlation test carried out to compare uCRP and Hsp70 (the only biomarkers that showed significant differences between groups A and B) showed an inverse correlation between both biomarkers (Figure 5), which reinforces the hypothesis that serum quantification of Hsp70 and uCRP could be used to differentiate

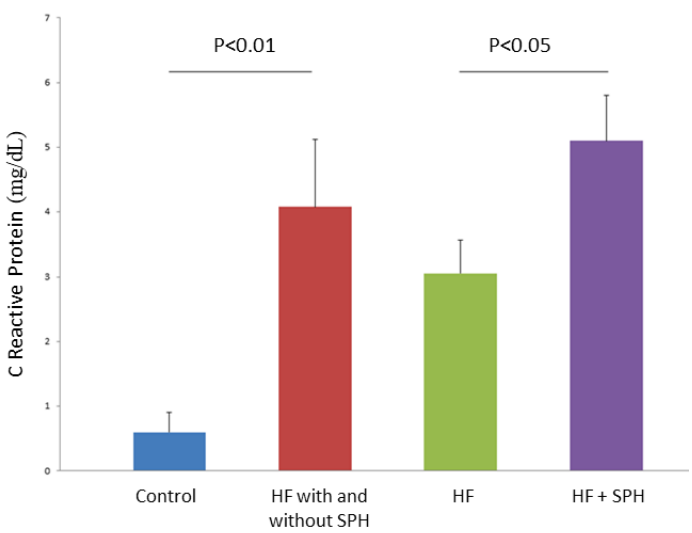

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Figure 3. Serum uCRP levels were evaluated in healthy individuals (control), HF patients with and without SPH, HF patients without SPH, and HF patients with SPH.

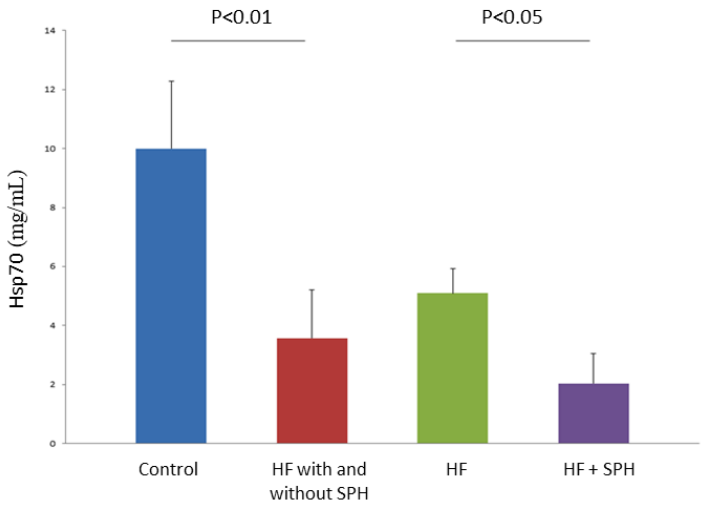

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Figure 4. Hsp70 serum levels were evaluated in healthy individuals (control), HF patients with and without SPH, HF patients without SPH, and HF patients with SPH

the coexistence of HF with SPH, from HF without associated SPH.

Discussion

The results obtained in the current study showed the oxidative and inflammatory link from the biochemical determinations carried out both in HF patients with SPH and in those with HF without SPH, in whom high levels of pro-oxidative markers (ROS and NADPH) had previously been found, as well as a decrease in NO levels with a low antioxidant marker (SOD) [7]. Specifically, the serum levels of IL-6, TNF-α, and uCRP significantly increased in patients with HF associated or not with SPH

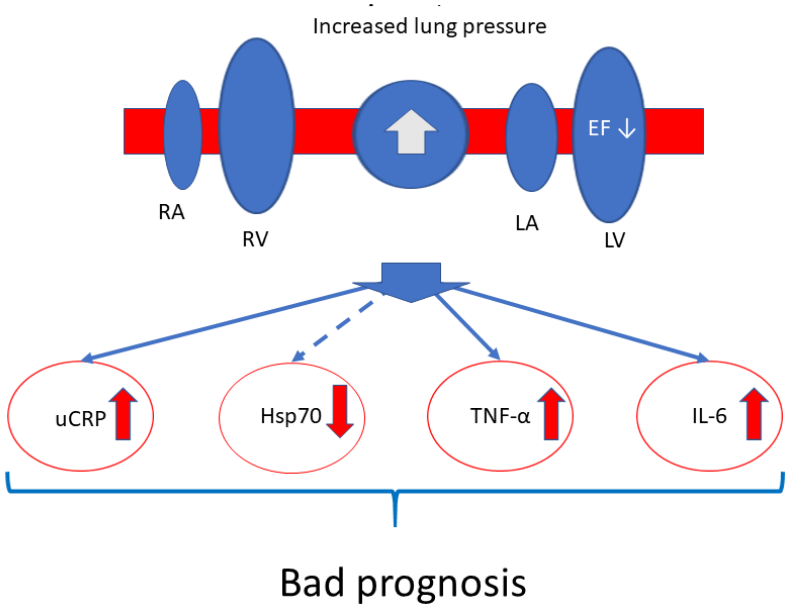

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Figure 6. Graphic summary of inflammatory markers in the context of HF with changes in pulmonary pressure and the worst prognosis of the disease

EF: ejection fraction; RA: right atrium; RV: right ventricle; LA: left atrium; LV: left ventricle.

The upper section of the figure shows that the pulmonary circuit is located in the center (RA, RV, LA, and VI). The fall in the ejection fraction (EF) in the left ventricle determines an increase in the pressure in the pulmonary circuit. In addition, the serial arrangement of the cavities and the changes in the markers with increases in uCRP, TNF-α, and IL-6 associated with a decrease in Hsp70 could determine the patient's prognosis.

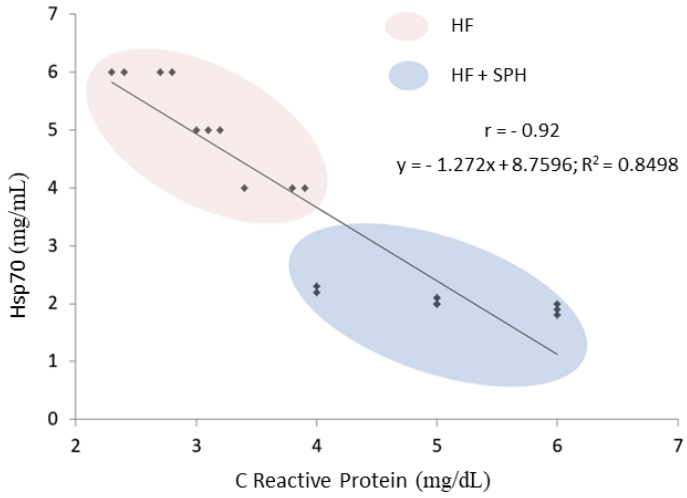

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Figure 5. Inverse statistical correlation between uCRP and Hsp70 in patients with HF without SPH, and patients with HF+SPH

compared to the control group. In this sense, several authors have quantified TNF-α, IL-6, and uCRP values as inflammatory markers present in the serum of patients with HF. In agreement with our results, these mediators were also significantly elevated in patients, compared to healthy individuals [31-38].

However, the number of articles found in the literature reporting the use of Hsp70 as a biomarker for HF is considerably lesser. It should be noted that in these studies, Hsp70 was significantly increased in individuals with this pathology [39-43]. It is a crucial finding considering that some authors have suggested that low bioavailability of NO can induce oxidative stress response proteins, such as Hsp70. This relation was shown in a previous study on the patients analyzed in the present work [7]. As a consequence, this response could promote protective effects against injury not only due to oxidative stress but also due to inflammation and apoptosis [44]. Unpublished and at the same time controversial, our results showed lower levels of Hsp70 in the serum of patients with HF compared to healthy individuals.

The interpretation of these results is even more complex since the opposite actions of Hsp70 at the extracellular versus intracellular level are known on the activation of the inflammatory pathway by NF-κB, where intracellular Hsp70 produces anti-inflammatory effects and extracellular Hsp70 mediates pro-inflammatory effects [45, 46]. However, and as a possible argument to the aforementioned alleged controversy about oxidative stress and inflammation, it is valid to consider the difference between acute and chronic events on the deterioration in the biological response capacity to these injuries (acute HF versus chronic HF). In this regard, previous reports studied these differences and demonstrated -in the chronic phase- that increased lipid peroxidation through higher levels of thiobarbituric acid reactive substances (TBARS) and increased oxidative stress produced a reduction in activity total antioxidant and increased NADPH oxidase activity, which was associated with a significant decrease in the expression of the inducible isoform of Hsp70, perhaps as a consequence of a negative feedback loop [47]. The claudication of antioxidant defense mechanisms and anti-inflammatory mechanisms in the chronicity of the disease could justify the low values found for Hsp70 in the serum of patients with HF compared to healthy volunteers.

Additionally, following our current results, our group reported in previous studies carried out on a model of kidney disease that the decrease in the bioavailability of NO with lower expression of inducible nitric oxide synthase (iNOS) was associated with Hsp70 deficiency and significant induction of apoptosis. Likewise, *in vivo* administration of L-arginine (a NO precursor) induced Hsp70 expression with a reduction in apoptosis and NADPH activity. On the other hand, the increase in Hsp70 due to the chronic elevation of NO sustained over time increases the phosphorylation of protein kinase B (AKT), 5' adenosine monophosphate-activated protein kinase (AMPK), and endothelial nitric oxide synthase (eNOS), improving the bioavailability of endogenous NO, resulting in optimizing the vasomotor and vasoprotective response in patients with CVD [48].

Other studies also agree that increased NO bioavailability induces the expression of HSP [49, 50]. There-

fore, the importance of the relationship between Hsp70 expression and morbidity in HF requires further studies that satisfactorily explain the existing controversy.

It has been established that inflammation is a characteristic of pulmonary arterial hypertension, with higher circulating levels of pro-inflammatory cytokines being observed in patients with this disease. However, to date, there is no concrete information on the importance of elevated cytokines or their potential as serum biomarkers in the development and progression of pulmonary arterial hypertension [28, 29]. For this reason, our group decided to quantify uCRP, IL-6, TNF-α, and Hsp70, not only in HF but also in SPH associated with HF. The analysis of our results showed that patients suffering from HF with SPH presented significantly higher values of uCRP, while IL-6 and TNF-α showed a trend, but without significant difference compared to HF without SPH. Consequently, the determination of oxidative and inflammatory markers in serum allowed us to differentiate healthy controls from patients with HF, and in turn, from patients with SPH associated with HF. Likewise, our unpublished and, at the same time, contradictory findings also showed lower serum levels of Hsp70 in patients with SPH associated with HF, compared to those quantified in the serum of patients with HF but without associated SPH. The marked inverse correlation observed between uCRP and Hps70 reinforces the results obtained. These two markers showed the most significant differences between patients with SPH and SPH associated with HF. However, our results are also controversial regarding other studies affirm that a higher expression of Hps70 is positively correlated with inflammatory markers in patients with CVD [27].

The present work results confirm a close link between oxidative stress and inflammation and both constitute a common pathway in the pathophysiology of HF. Likewise, these results also strengthen the hypothesis that the serum inflammatory mediators quantified in our current work, as well as the oxidative mediators evaluated in a previous work of our group, could be used as valuable biomarkers in the diagnosis and treatment of HF, allowing to detect, in addition, the coexistence or not of associated SPH.

To highlight although the inflammatory markers evaluated here are not specific for HF associated or not associated with SPH, but are also altered in other oxidative/inflammatory diseases, they have a very important predictive value in these CVDs, with detection costs considerably lower than those conventionally used in the clinic and, therefore, more affordable, especially in the field of public health [51].

Conclusion

The results obtained from serum inflammatory markers showed that the patients with HF presented an exacerbated inflammatory state. This process contributes to the pathophysiological changes developed in the chronic evolution of the disease. Of interest, these findings are consistent with those found in previous work by our research group. Specifically, Oxidative stress and decreased NO bioavailability cause the coexistence of inflammation and oxidative stress during HF. In addition, oxidative stress like inflammation is more exacerbated even when associated with SPH. Furthermore, this coexistence enhances the damage that underlies the progression of HF to advanced stages with a worse prognosis.

Regarding Hsp70, we conclude that although the serum levels of this marker are initially elevated in the early or acute stages of HF, these values may decrease significantly in late or chronic stages during this CVD. Interestingly, sometimes not well clarified, these results can be contradictory. Therefore, as a future perspective, we propose the need to carry out additional studies to evaluate in greater detail the importance of the relationship between the serum expression of Hsp70 and morbidity in HF.

Currently, among the emerging biomarkers in the diagnosis of HF are uCRP, TNF-α, Fas (APO-1), interleukins 1, 6, and 18, cytokines, procalcitonin, adipokines, and adiponectins [22]. The incorporation of Hsp70 would be advantageous by showing the associated presence of SPH, an association that impairs the evolution of patients in the short term [52]. The clinical and echocardiographic evaluation allows evidence of the adverse evolution of these patients later in the hands of experts. In addition, it is expressed earlier and with more specificity with these markers. The search for more specific inflammatory biomarkers of HF than those evaluated here should continue; however, the present study constitutes an initial contribution to these investigations. Figure 6 summarizes the main findings in the context of our study.

This study has methodological and design limitations. One is regarding how we measure pulmonary pressure (PP). The Central Hospital of Mendoza has a Hemodynamic Service, but for budgetary reasons, the performance of right catheterization has been restricted. On the other hand, the protocol's design did not allow for significant delays in management, and we had

to summon the patients as few times as possible. It was also necessary that they were not invaded. Therefore, PP's primary hemodynamic parameter measurement had to be done with a Doppler echocardiogram. In this sense, HP is defined as PPM>25 mmHg, which should be obtained by direct recording through right catheterization; however, PP was determined by indirectly measuring PSP by Doppler echocardiography, defining for our work HP as PSP≥40 mmHg. Undoubtedly, the direct recording of PP is more appropriate than that obtained indirectly. Still, the Doppler echocardiogram is a sensitive method to measure PSP and is advantageous because it is non-invasive. Stevenson compared the non-invasive measurements with simultaneous recordings made by right catheterization. PSP estimates were made in 89% of the patients with high security (r=0.97) [53]. Another limitation, for operational reasons at the Hospital Central de Mendoza, was that the same operator did not perform echocardiograms and did not always use the same equipment.

Data from some patients (6-minute walk, echocardiogram, and general laboratory) could not be obtained due to various inconveniences; however, this fact did not affect the search for the proposed central objective or the results obtained, and in this sense, we can affirm that the set of data informs us about chronic patients with severe structural deterioration and good functional class due to response to treatment. Finally, as mentioned in the protocol section on the small sample size, we recognize that the patient number was insufficient, and for this reason, it is a preliminary study.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the Mendoza Central Hospital (Code: 1232/19).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflicts of interest.

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References

[1] Savarese G, Lund LH. Global public health burden of heart failure. Card Fail Rev. 2017; 3(1):7-11. [DOI:10.15420/cfr.2016:25:2]

[2] Arrigo M, Jessup M, Mullens W, Reza N, Shah AM, Sliwa K, et al. Acute heart failure. Nat Rev Dis Primers. 2020; 6(1):16. [DOI:10.1038/s41572-020-0151-7] [PMID] [PMCID]

[3] Mosterd A, Hoes AW. Clinical epidemiology of heart failure. Heart. 2007; 93(9):1137-46. [DOI:10.1136/hrt.2003.025270] [PMID] [PMCID]

[4] Jones NR, Hobbs FR, Taylor CJ. Prognosis following a diagnosis of heart failure and the role of primary care: A review of the literature. BJGP Open. 2017; 1(3):bjgpopen17X101013. [DOI:10.3399/bjgpopen17X101013] [PMID] [PMCID]

[5] Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J. 2016; 37(27):2129-200. [DOI:10.1093/eurheartj/ehw128] [PMID]

[6] Spoletini I, Coats AJS, Senni M, Rosano GMC. Monitoring of biomarkers in heart failure. Eur Heart J Suppl. 2019; 21(Suppl M):M5-8. [DOI:10.1093/eurheartj/suz215] [PMID] [PMCID]

[7] Bonafede RJ, Calvo JP, Fausti JMV, Puebla S, Gambarte AJ, Manucha W. Nitric oxide: A new possible biomarker in heart failure? Relationship with pulmonary hypertension secondary to left heart failure. Clin Investig Arterioscler. 2017; 29(3):120-6. [DOI:10.1016/j.artere.2017.05.001] [PMID]

[8] Bonafede R, Manucha W. Nitric oxide and related factors linked to oxidation and inflammation as possible biomarkers of heart failure. Clin Investig Arterioscler. 2018; 30(2):84-94. [DOI:10.1016/j.artere.2017.12.003] [PMID]

[9] Polónia J, Gonçalves FR. The historical evolution of knowledge of the involvement of neurohormonal systems in the pathophysiology and treatment of heart failure. Rev Port Cardiol (Engl Ed). 2019; 38(12):883-95. [DOI:10.1016/j.repc.2019.05.008] [PMID]

[10] Milinković I, Polovina M, Simeunović DS, Ašanin M, Seferović PM. Oxidative stress and inflammation in heart failure: The best is yet to come. Eur J Prev Cardiol. 2020; 27(5):490-3. [DOI:10.1177/2047487319900294] [PMID]

[11] Schumacher SM, Naga Prasad SV. Tumor necrosis factor-α in heart failure: An updated review. Curr Cardiol Rep. 2018; 20(11):117. [DOI:10.1007/s11886-018-1067-7] [PMID] [PMCID]

[12] Markousis-Mavrogenis G, Tromp J, Ouwerkerk W, Devalaraja M, Anker SD, Cleland JG, et al. The clinical significance of interleukin-6 in heart failure: Results from the BIOSTAT-CHF study. Eur J Heart Fail. 2019; 21(8):965-73. [DOI:10.1002/ejhf.1482] [PMID]

[13] Pellicori P, Zhang J, Cuthbert J, Urbinati A, Shah P, Kazmi S, et al. High-sensitivity C-reactive protein in chronic heart failure: Patient characteristics, phenotypes, and mode of death. Cardiovasc Res. 2020; 116(1):91-100. [DOI:10.1093/cvr/cvz198] [PMID]

[14] Li Z, Song Y, Xing R, Yu H, Zhang Y, Li Z, et al. Heat shock protein 70 acts as a potential biomarker for early diagnosis of heart failure. PLoS One. 2013; 8(7):e67964. [DOI:10.1371/journal.pone.0067964] [PMID] [PMCID]

[15] Ren J, Liu C, Zhao D, Fu J. The role of heat shock protein 70 in oxidant stress and inflammatory injury in quail spleen induced by cold stress. Environ Sci Pollut Res Int. 2018; 25(21):21011-23. [DOI:10.1007/s11356-018-2142-8] [PMID]

[16] Kany S, Vollrath JT, Relja B. Cytokines in inflammatory disease. Int J Mol Sci. 2019; 20(23):6008. [DOI:10.3390/ijms20236008] [PMID] [PMCID]

[17] Chaikijurajai T, Tang WHW. Reappraisal of inflammatory biomarkers in heart failure. Curr Heart Fail Rep. 2020; 17(1):9-19. [DOI:10.1007/s11897-019-00450-1] [PMID] [PMCID]

[18] Rolski F, Błyszczuk P. Complexity of TNF-α signaling in heart disease. J Clin Med. 2020; 9(10):3267. [DOI:10.3390/jcm9103267] [PMID] [PMCID]

[19] Tanase DM, Gosav EM, Radu S, Ouatu A, Rezus C, Ciocoiu M, et al. Arterial hypertension and interleukins: Potential therapeutic target or future diagnostic marker? Int J Hypertens. 2019; 2019:3159283. [DOI:10.1155/2019/3159283] [PMID] [PMCID]

[20] Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. Front Immunol. 2018; 9:754. [DOI:10.3389/fimmu.2018.00754] [PMID] [PMCID]

[21] Zhang R, Zhang YY, Huang XR, Wu Y, Chung AC, Wu EX, et al. C-reactive protein promotes cardiac fibrosis and inflammation in angiotensin II-induced hypertensive cardiac disease. Hypertension. 2010; 55(4):953-60. [DOI:10.1161/HYPERTENSIONAHA.109.140608] [PMID]

[22] Sarhene M, Wang Y, Wei J, Huang Y, Li M, Li L, et al. Biomarkers in heart failure: The past, current and future. Heart Fail Rev. 2019; 24(6):867-903. [DOI:10.1007/s10741-019-09807-z] [PMID]

[23] Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2017; 9(6):7204-7218. [DOI:10.18632/oncotarget.23208] [PMID] [PMCID]

[24] Snoeckx LH, Cornelussen RN, Van Nieuwenhoven FA, Reneman RS, Van Der Vusse GJ. Heat shock proteins and cardiovascular pathophysiology. Physiol Rev. 2001; 81(4):1461-97. [DOI:10.1152/physrev.2001.81.4.1461] [PMID]

[25] Radons J. The human HSP70 family of chaperones: Where do we stand? Cell Stress Chaperones. 2016; 21(3):379-404. [DOI:10.1007/s12192-016-0676-6] [PMID] [PMCID]

[26] Ranek MJ, Stachowski MJ, Kirk JA, Willis MS. The role of heat shock proteins and co-chaperones in heart failure. Philos Trans R Soc Lond B Biol Sci. 2018; 373(1738):20160530. [DOI:10.1098/rstb.2016.0530] [PMID] [PMCID]

[27] Srivastava K, Narang R, Bhatia J, Saluja D. Expression of heat shock protein 70 gene and its correlation with inflammatory markers in essential hypertension. PLoS One. 2016; 11(3):e0151060. [DOI:10.1371/journal.pone.0151060] [PMID] [PMCID]

[28] Groth A, Vrugt B, Brock M, Speich R, Ulrich S, Huber LC. Inflammatory cytokines in pulmonary hypertension. Respir Res. 2014; 15(1):47. [DOI:10.1186/1465-9921-15-47] [PMID] [PMCID]

[29] Joshi AA, Davey R, Rao Y, Shen K, Benza RL, Raina A. Association between cytokines and functional, hemodynamic parameters, and clinical outcomes in pulmonary arterial hypertension. Pulm Circ. 2018; 8(3):2045894018794051. [DOI:10.1177/2045894018794051] [PMID] [PMCID]

[30] Pockley AG, Shepherd J, Corton JM. Detection of heat shock protein 70 (Hsp70) and anti-Hsp70 antibodies in the serum of normal individuals. Immunol Invest. 1998; 27(6):367-77. [DOI:10.3109/08820139809022710] [PMID]

[31] Abernethy A, Raza S, Sun JL, Anstrom KJ, Tracy R, Steiner J, et al. Pro-inflammatory biomarkers in stable versus acutely decompensated heart failure with preserved ejection fraction. J Am Heart Assoc. 2018; 7(8):e007385. [DOI:10.1161/JAHA.117.007385] [PMID] [PMCID]

[32] Kunin M, Carmon V, Arad M, Levin-Iaina N, Freimark D, Holtzman EJ, et al. Inflammatory biomarkers in refractory congestive heart failure patients treated with peritoneal dialysis. Biomed Res Int. 2015; 2015:590851. [DOI:10.1155/2015/590851] [PMID] [PMCID]

[33] DuBrock HM, AbouEzzeddine OF, Redfield MM. High-sensitivity C-reactive protein in heart failure with preserved ejection fraction. PLoS One. 2018; 13(8):e0201836. [DOI:10.1371/journal.pone.0201836] [PMID] [PMCID]

[34] Stumpf C, Sheriff A, Zimmermann S, Schaefauer L, Schlundt C, Raaz D, et al. C-reactive protein levels predict systolic heart failure and outcome in patients with first ST-elevation myocardial infarction treated with coronary angioplasty. Arch Med Sci. 2017; 13(5):1086-1093. [DOI:10.5114/aoms.2017.69327] [PMID] [PMCID]

[35] Noori NM, Shahramian I, Teimouri A, Keyvani B, Mahjoubifard M. Serum levels of tumor necrosis factor-α and interleukins in children with congenital heart disease. J Tehran Heart Cent. 2017; 12(1):15-22. [PMCID]

[36] Noori NM, Moghaddam MN, Teimouri A, Shahramian I, Keyvani B. Evaluation of serum level of tumor necrosis factor-alpha and interleukin-6 in patients with congenital heart disease. Niger Med J. 2016; 57(4):233-7. [DOI:10.4103/0300-1652.188353] [PMID] [PMCID]

[37] Ptaszynska-Kopczynska K, Szpakowicz A, Marcinkiewicz-Siemion M, Lisowska A, Waszkiewicz E, Witkowski M, et al. Interleukin-6 signaling in patients with chronic heart failure treated with cardiac resynchronization therapy. Arch Med Sci. 2017; 13(5):1069-77. [DOI:10.5114/aoms.2016.58635] [PMID] [PMCID].

[38] Su D, Li Z, Li X, Chen Y, Zhang Y, Ding D, et al. Association between serum interleukin-6 concentration and mortality in patients with coronary artery disease. Mediators Inflamm. 2013; 2013:726178. [DOI:10.1155/2013/726178] [PMID] [PMCID]

[39] Genth-Zotz S, Bolger AP, Kalra PR, von Haehling S, Doehner W, Coats AJ, et al. Heat shock protein 70 in patients with chronic heart failure: Relation to disease severity and survival. Int J Cardiol. 2004; 96(3):397-401. [DOI:10.1016/j.ijcard.2003.08.008] [PMID]

[40] Gombos T, Förhécz Z, Pozsonyi Z, Jánoskuti L, Prohászka Z. Interaction of serum 70-kDa heat shock protein levels and HspA1B (+1267) gene polymorphism with disease severity in patients with chronic heart failure. Cell Stress Chaperones. 2008; 13(2):199-206. [DOI:10.1007/s12192-007-0001-5] [PMID] [PMCID]

[41] Wei YJ, Huang YX, Shen Y, Cui CJ, Zhang XL, Zhang H, et al. Proteomic analysis reveals significant elevation of heat shock protein 70 in patients with chronic heart failure due to arrhythmogenic right ventricular cardiomyopathy. Mol Cell Biochem. 2009; 332(1-2):103-11. [DOI:10.1007/s11010-009-0179-1] [PMID]

[42] Jenei ZM, Gombos T, Förhécz Z, Pozsonyi Z, Karádi I, Jánoskuti L, et al. Elevated extracellular HSP70 (HSPA1A) level as an independent prognostic marker of mortality in patients with heart failure. Cell Stress Chaperones. 2013; 18(6):809-13. [DOI:10.1007/s12192-013-0425-z] [PMID] [PMCID]

[43] Zerikiotis S, Angelidis C, Dhima I, Naka KK, Kasioumi P, Kalfakakou V, et al. The increased expression of the inducible Hsp70 (HSP70A1A) in serum of patients with heart failure and its protective effect against the cardiotoxic agent doxorubicin. Mol Cell Biochem. 2019; 455(1-2):41-59. [DOI:10.1007/s11010-018-3469-7] [PMID]

[44] Camargo AB, Manucha W. [Potential protective role of nitric oxide and Hsp70 linked to functional foods in the atherosclerosis (Spanish)]. Clin Investig Arterioscler. 2017; 29(1):36-45. [DOI:10.1016/j.artere.2016.05.002]

[45] Mazzei L, Docherty NG, Manucha W. Mediators and mechanisms of heat shock protein 70 based cytoprotection in obstructive nephropathy. Cell Stress Chaperones. 2015; 20(6):893-906. [DOI:10.1007/s12192-015-0622-z] [PMID] [PMCID]

[46] Molina MN, Ferder L, Manucha W. Emerging role of Nitric Oxide and heat shock proteins in Insulin resistance. Curr Hypertens Rep. 2016; 18(1):1. [DOI:10.1007/s11906-015-0615-4] [PMID]

[47] Rinaldi Tosi ME, Bocanegra V, Manucha W, Gil Lorenzo A, Vallés PG. The Nrf2-Keap1 cellular defense pathway and heat shock protein 70 (Hsp70) response. Role in protection against oxidative stress in early neonatal unilateral ureteral obstruction (UUO). Cell Stress Chaperones. 2011; 16(1):57-68. [DOI:10.1007/s12192-010-0221-y] [PMID] [PMCID]

[48] Manucha W, Vallés PG. Cytoprotective role of Nitric Oxide associated with Hsp70 expression in neonatal obstructive nephropathy. Nitric Oxide. 2008; 18(3):204-15. [DOI:10.1016/j.niox.2008.01.005] [PMID]

[49] Mazzei L, García IM, Cacciamani V, Benardón ME, Manucha W. WT-1 mRNA expression is modulated by nitric oxide availability and Hsp70 interaction after neonatal unilateral ureteral obstruction. Biocell. 2010; 34(3):121-32. [PMID]

[50] Mazzei L, Manucha W. Wt-1 expression linked to nitric Oxide availability during neonatal obstructive nephropathy. Adv Urol. 2013; 2013:401750. [DOI:10.1155/2013/401750] [PMID] [PMCID]

[51] Chow SL, Maisel AS, Anand I, Bozkurt B, de Boer RA, Felker GM, et al. Role of biomarkers for the prevention, assessment, and management of heart failure: A scientific statement from the American Heart Association. Circulation. 2017; 135(22):e1054-91. [PMID]

[52] Santiago-Vacas E, Lupón J, Gavidia-Bovadilla G, Gual-Capllonch F, de Antonio M, Domingo M, et al. Pulmonary hypertension and right ventricular dysfunction in heart failure: Prognosis and 15-year prospective longitudinal trajectories in survivors. Eur J Heart Fail. 2020; 22(7):1214-25. [DOI:10.1002/ejhf.1862] [PMID]

[53] Stevenson JG. Comparison of several noninvasive methods for estimation of Pulmonary artery pressure. J Am Soc Echocardiogr. 1989; 2(3):157-71. [DOI:10.1016/S0894-7317(89)80053-7]

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