Increased Serum Levels of Oxidative Stress Are Associated with Hospital Readmissions due to Acute Heart Failure

Ori Rogowski\textsuperscript{a}  Sergei Shnizer\textsuperscript{b}  Rafael Wolff\textsuperscript{c}  Basil S. Lewis\textsuperscript{c,d}  Offer Amir\textsuperscript{c,d}

\textsuperscript{a}Department of Internal Medicine ‘E’, Tel Aviv Sourasky Medical Center affiliated with the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, and \textsuperscript{b}Carmel Diagnostics Ltd. (formerly Lumitest), \textsuperscript{c}Department of Cardiovascular Medicine, Heart Failure Center, Lady Davis Carmel and Lin Medical Centers, and \textsuperscript{d}The Ruth and Bruce Rappaport School of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

**Abstract**

**Objectives:** Inflammation and serum oxidative stress (OS) are important components in heart failure (HF) deterioration. In this study we tested the hypothesis that an increase in patients’ sera OS levels is associated with acute HF (AHF) readmissions. **Methods:** Thirty consecutive patients (mean age 71 ± 10 years) admitted with AHF were included in the study. Serum OS in these patients was measured in-hospital and repeatedly after discharge over a period of 8 weeks of follow-up in which we reordered patients’ HF readmissions. Of the 30 patients, 13 (43%) were readmitted (RAD group) and 17 (57%) did not require readmission (NRAD group). **Results:** OS levels before discharge from the first hospital admission in the 2 groups were similar (p = 0.84 and p = 0.56, respectively). However, using repeated measures ANOVA, we found that the interaction between the time points and the 2 groups of patients (RAD and NRAD) was statistically significant (p = 0.037). It is important to note that OS serum levels were more predictive of HF readmissions than were repeated simultaneous serum measurements of NT-proBNP (p = 0.97). **Conclusions:** Increased OS levels in AHF patients, after they have been discharged from the hospital, are associated with higher HF readmission rates. In AHF, OS is a dynamic parameter associated with HF deterioration.

**Key Words**

Oxidative stress  ·  Heart failure  ·  Readmissions

**Objectives**

Heart failure (HF) is a major cardiovascular health problem associated with high mortality and morbidity. Several studies suggest that inflammation plays an important role in HF progression. Serum oxidative stress (OS) is a crucial element of the inflammatory process, owing to the accumulation of reactive oxygen/nitrogen species that might provoke and exacerbate the myocardial damage in the already failing heart [1].

Patients with HF are frequently readmitted to the hospital because of exacerbation of their symptoms, with a prevalence of up to 40% following the previous hospital discharge [2–4]. In this study we analyzed sera OS levels to test the hypothesis that an increase in patients’ sera OS levels may be associated with acute HF (AHF) readmissions. This was done by measuring OS levels in the serum
of patients who had been admitted due to AHF, both in-hospital and repeatedly after discharge, over a period of 8 weeks of follow-up.

**Patients and Methods**

**Study Population**

A total of 31 patients who were admitted to the hospital due to AHF were recruited. One patient developed cholangitis upon admission, requiring surgical intervention, and was excluded from further analysis. Consequently, the final analysis included 30 consecutive patients (mean age 71 ± 10 years; 23 (77%) males and 7 (23%) females) who were admitted with a diagnosis of AHF with no evidence of acute coronary syndrome. Only patients with symptomatic HF (stage C) for at least 30 days prior to the first index hospital admission due to AHF were considered for inclusion.

The exclusion criteria were any of the following: (1) acute coronary syndrome, (2) having been an active smoker at least 3 years prior to inclusion, (3) taking either antioxidant medications/vitamins or any form of nitrates regimens, (4) a recent history (2 weeks prior to inclusion) of active infection, and (5) a history of inflammatory disease or treated malignancy in the last 2 years prior to recruitment.

We followed our cohort of patients for 2 months and recorded their readmissions due to AHF. Of the 30 patients, 13 (43%) were readmitted (RAD group) and 17 (57%) did not require readmission (NRAD group). We compared the OS and the N-terminal pro-brain natriuretic peptide (NT-proBNP) serum levels between these 2 groups as well as other relevant prognostic markers. The patients’ characteristics are described in table 1.

For the purpose of OS analysis, we used repeated fresh sera samples of the patients and measured OS levels and NT-proBNP from the same blood sample. The sample collection analysis for serum OS and NT-proBNP was based on the samples taken upon admission, prior to hospital discharge, and thereafter at 2, 4, 6, and 8 weeks after discharge. A total of 6 blood samples from each patient were planned. However, 2 samples (6 and 8 weeks postdischarge) from 1 patient in the RAD group and 2 samples (6 and 8 weeks postdischarge) from 1 patient in the NRAD group were unavailable for analysis. Accordingly, the final analysis was based on 176 samples, representing 98% of the planned target number of samples.

**OS Measurements**

The OS level was analyzed via thermochemiluminescence (TCL) oxidizability assay (Carmel Diagnostics Ltd., Haifa, Israel). TCL measures the OS level via photon emission counting from excited carbonyls in biological macromolecules [5, 6]. TCL measurements were validated previously by comparing them to other well-accepted methods which measure serum OS levels via oxidation of lipids or proteins [6–9]. We have previously demonstrated that high serum OS levels, measured via TCL assay, correlated with an advanced New York Heart Association class, worsening renal function, and higher serum hs-CRP and NT-proBNP levels in HF patients [9].

Briefly, the principle of TCL assay measurement is based on the measurement of unstable carbonyls in the sample and correlates with the degree of oxidation. The process of TCL assay measurement requires heating a sample to 80 ± 0.20°C, which leads to molecular oxidative modification. There is a resulting formation of electronically excited species of unstable carbonyls of lipid and protein origins. The latter decompose into stable carbonyls and light energy (low chemiluminescence) in the wavelength range of 350–600 nm. During the heating period, the most significant change in the photon reading occurs between 50 and 280 s from starting. There is no significant change in oxidation level before or after this time period. Accordingly, TCL reading is performed at these 2 time references with H1 defined as 50 s and H3 defined as 280 s – the TCL reading. The TCL serum OS level is expressed by the H-3/H-1 ratio (TCL ratio). It is important to note that, because of the method TCL employs to measure the OS level, there is an inverse relation between the TCL measurement values and the level of serum OS. Accordingly, low TCL values actually represent a high level of serum OS and high TCL values represent a low serum OS level.

<table>
<thead>
<tr>
<th>Table 1. Patients’ characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Mean age ± SD, years</td>
</tr>
<tr>
<td>Male/female ratio</td>
</tr>
<tr>
<td>History of diabetes mellitus, n (%)</td>
</tr>
<tr>
<td>Etiology of coronary artery disease, n (%)</td>
</tr>
<tr>
<td>New York Heart Association class III/IV(^a), n (%)</td>
</tr>
<tr>
<td>Mean left ventricular ejection fraction ± SD, %</td>
</tr>
<tr>
<td>Mean hemoglobin ± SD, g/dl</td>
</tr>
<tr>
<td>Mean creatinine(^a) ± SD, mg/dl</td>
</tr>
<tr>
<td>log NT-proBNP(^a)</td>
</tr>
</tbody>
</table>

\(^a\) Level on admission at the first index hospital admission.
In this study serum NT-proBNP was measured with an Elecsys proBNP electro-chemiluminescence immunoassay run on an Elecsys 1010 (Roche Diagnostics, Indianapolis, Ind., USA).

The study conformed to the principles outlined in the Helsinki Declaration and was approved by the Institutional Review Board (Helsinki committee) of Lady Davis Carmel Medical Center. All patients gave their written informed consent before inclusion into the study.

**Results**

As shown in table 1, both groups of patients were similar in terms of their clinical profile, except for female gender which was more prevalent in the RAD group.

Table 2 shows the TCL measurements of the OS levels of patients. The OS levels obtained in the emergency room and before discharge from the first hospital admission in the 2 groups were not different (p = 0.84 and p = 0.56, respectively). However, there was a trend towards a decrease in serum OS levels in the RAD group during the time from discharge.

Using repeated measures ANOVA with the OS level as the dependent variable, we found that the interaction between the time points and the 2 groups of patients (RAD and NRAD) was statistically significant (p = 0.037) (fig. 1).

During the follow-up period, the OS level in the RAD group was significantly higher compared to their OS level at first index admission (average difference 14.8 units; 95% CI 5.71–22.6, p = 0.038). Moreover, the OS serum level measured 2 weeks prior to their readmission was significantly elevated compared to the lowest OS level these patients had during follow-up as shown by a decreased TCL ratio of 21.1 units (95% CI 11.8–30.5, p = 0.004).

It is important to note that we found a relatively strong correlation between OS levels and serum NT-proBNP (r = 0.551; p < 0.001). Interestingly, although serum NT-proBNP was higher in the readmitted (RAD) patients (fig. 2), it was not as accurate as OS in differentiating between the RAD and NRAD groups. The receiver operat-
ing characteristic (ROC) curve reflecting the changes in OS levels over the 8 weeks after discharge from the hospital due to the index admission is presented in figure 3.

**Discussion**

Our main finding in this study is that rising OS levels in AHF patients, after they have been discharged from the hospital, are associated with increased HF readmission rates. Moreover, among our patients who were readmitted, the OS serum level in samples was significantly elevated approximately 2 weeks prior to readmission when compared to the lowest OS level during follow-up. This is compatible with the concept that OS is a dynamic parameter in AHF and is associated with HF readmission.

It is worth noting that the OS serum level was more predictive of HF readmission than was a simultaneous NT-proBNP measurement. There are studies that support the notion that natriuretic peptides can reliably predict future HF deterioration [10–12]. Nevertheless, there are other reports which emphasize major limitations of natriuretic peptides in predicting HF deterioration. Interestingly, we noted that during the first index hospital stay there was no significant change in OS levels in our patients, and OS levels started rising only several weeks after hospital discharge.

The OS level measurements in our study were done using the TCL technique. This method has the advantage of assessing the total oxidation capacity; however, it does not sort out the specific biochemical source of the oxidation, namely protein and/or lipid oxidation. Therefore, it is possible that measurement of specific oxidized lipid metabolites could result in the detection of significant OS level changes during hospital admission.

OS is considered a biomarker in HF in general. It is well established that increased levels of OS result from an imbalance between reactive oxygen species such as superoxide anion, hydrogen peroxide, hydroxyl radical, and endogenous antioxidant defense mechanisms [13]. Moreover, a mechanism of systemic ‘endothelitis’ was suggested to have an important role in the pathogenesis of AHF, with formation of peroxynitrate from superoxide scavenging nitric oxide [14].

Several studies found a correlation between different parameters of oxidation and HF severity including metabolites of increased xanthine oxidase activity and biopyrrins. Moreover, as demonstrated previously by several reports, including ours, OS level parameters were shown to have a significant correlation with hemodynamic measurements, natriuretic serum peptides, and other prognostic parameters of HF [9, 15–18].

However, although OS has been studied in chronic HF, fewer studies have assessed the role of OS in AHF. Recently, myeloperoxidase, a biomarker of inflammation and OS produced by neutrophils, was shown to be an independent predictor of 1-year mortality in AHF patients with an additive value to BNP [19]. Another small study done in patients with AHF of nonischemic etiology showed elevated serum thiobarbituric acid-reactive substances (TBARS) and a decreased total antioxidant status. The authors suggested that in AHF the increased oxygen-free radicals could be responsible for arrhythmias and complications of AHF [20].

Several mechanisms of OS implications for the remodeling process in HF were proposed. OS participates in the myocardial injury of necrosis and apoptosis. Oxidation is associated with endothelial dysfunction, abnormal redox signaling, and intracellular energy malproduction, and it is directly related to increased matrix metalloproteinase activity [21–24].

To the best of our knowledge, our data is distinctive as we measured OS levels in AHF and in a discrete period following onset. The association between increased OS levels and readmission may be interpreted 2 ways: either
OS facilitates AHF deterioration via direct damage to myocardial cells or OS is a reflection of the ongoing clinical deterioration. The specific nature of this observed association is beyond the scope of this study.

Several study limitations should be noted. Our study cohort was relatively small and included patients with diverse clinical settings such as systolic and diastolic HF. The OS role in these different clinical entities may not be the same. Larger-scale studies are needed to clarify the importance of OS in the acute setting of HF.

In conclusion, continuous increased OS levels in the serum of AHF patients, after they have been discharged from the hospital, are associated with higher HF readmission rates. It seems that OS is a dynamic parameter in AHF and is associated with HF clinical deterioration.

**Acknowledgment**

We thank Mrs. Hagar Paz (RN) for her assistance in this study.

---

**References**


2. Reznick AZ, Lee DS, Tu JV, Newman AM, McAlister FA: Comparison of one-year outcome (death and rehospitalization) in hospitalized heart failure patients with left ventricular ejection fraction >50% versus those with ejection fraction <50%. Am J Cardiol 2008;102:79–83.


