Antibodies to Oxidized LDL as Predictors of Morbidity and Mortality in Patients With Chronic Heart Failure

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ABSTRACT

Background: Oxidative stress appears to play a significant role in the pathogenesis of heart failure (HF). Antibodies to oxidized low-density lipoprotein (Ox LDL Abs) reflect an immune response to LDL over a prolonged period and may thus represent oxidative stress over an extended time. Ox LDL Abs have been shown to correlate with clinical control in HF patients. We evaluated the predictive power of Ox LDL Abs on the outcome in patients with HF.

Methods and Results: Baseline levels of Ox LDL Abs were determined by enzyme-linked immunosorbent assay in 284 consecutive outpatients with severe chronic HF who were being treated in the cardiology services of our medical center. Their mean New York Heart Association (NYHA) Class was 2.8. The mean follow-up for the group was 3.7 years, during which 107 (37%) died. The mean time from symptom onset to first hospital admission from HF was 25.8 months. Ox LDL Abs were found to predict morbidity and mortality as evaluated by a Cox multivariate regression analysis with a hazard ration of 1.013 (P < .013), whereas N-terminal pro-B-type natriuretic peptide (NT pro-BNP) levels achieved a HR of 1.028 (P < .099).

Conclusions: Ox LDL Abs level maybe a useful parameter for monitoring and planning better management of patients with HF. It was superior to pro-BNP as a predictor of clinical course as expressed by time to hospitalization. (J Cardiac Fail 2009;15:770–774)

Key Words: Heart failure, lipoproteins, antibodies, natriuretic peptides.

“Oxidative stress” is a general term that denotes imbalance between factors that promote production of reactive oxygen species and the ability to oppose and scavenge and subsequently neutralize the byproducts of these reactive free radicals.1 It may also have a role in the genesis and progression of chronic myocardial dysfunction.2,3 There is considerable evidence that oxidative stress is increased in both ischemic and nonischemic congestive heart failure (CHF).2,3 Experimental studies in animal models of cardiac dysfunction, such as those produced by myocardial infarction after left anterior descending artery ligation, Adriamycin administration and pressure overload all demonstrated increased production of free radicals.4–6 Antioxidant therapy was shown to attenuate myocardial damage induced by Adriamycin in rats,7 but assessment of oxidative stress is more complex in humans because there is no reproducible, standardized methodology. Studies on patients with heart failure (HF) have used elevation of breath pentane levels, plasma thiobarbituric acid reactive substances, and malondialdehyde-like activity,8 all of which are lipid peroxidation markers reflecting oxidative stress. Isoprostanes, relatively new markers of oxidative stress, have been shown to be increased in the pericardial fluid of patients with HF;9 and neutrophil superoxide-generating capacity3 was also found to be elevated in patients with left ventricular dysfunction.

Oxidized low-density lipoprotein (Ox LDL) is present in the atheromatous plaque and correlates with the extent of atherosclerosis.10–12 Tsutsui et al13 using plasma level of Ox LDL by sandwich enzyme-linked immunosorbent assay with a specific
monoclonal antibody against Ox LDL showed plasma levels of Ox LDL as having prognostic value in predicting mortality in HF patients. Steinerova et al. suggested that assessment of antibodies to Ox LDL (Ox LDL Abs) may more reliably reflect the level of oxidative stress (reflect more generalized immunoresponse against Ox LDL). These antibodies have already been shown to correlate with the extent of atherosclerosis and predict future myocardial infarction.15–17

The aim of this prospective study was to assess the potential applicability of Ox LDL Abs in predicting morbidity and mortality in a cohort of outpatients with chronic severe HF.

**Methods**

**Study Population**

All the participants in the present prospective study were recruited from the specialized Heart Failure Outpatient Unit at Tel-Aviv Sourasky Medical Center. Follow-up started after obtaining blood samples, between January 2000 and July 2001. All consenting consecutive HF patients were included in the present study. Systolic HF was defined as a left ventricular ejection fraction (LVEF) ≤40% by echocardiography or isotopic Tc 99 ventriculography scan. Diabetes mellitus and hypertension were defined according to medical history and to World Health Organization diagnostic criteria.18 At the first visit, the patients underwent a physical examination and the following information was obtained: medical history, current and previous medications, resting blood pressure, heart rate, weight, and NYHA classification (based on the retrieved information, echocardiography, or isotopic ventriculography). The study patients were examined at least every 3 months and, not uncommonly, more often from the gravity of their medical condition. Patients who had malignant disease with diffuse metastases, severe cerebral vascular disease, patients with inflammatory disorders, connective tissue diseases, fever, infections including low-grade and acute infections, or were chronically bedridden were excluded. The end points of the study were time to first hospitalization, all-cause mortality, and a combination of the two. The study was approved by the local ethics committee of Tel Aviv Sourasky Medical Center and the Israeli Ministry of Health.

**Determination of Ox LDL Abs**

Native and Ox LDL were prepared as previously described. Ninety 6–well polystyrene plates (Nunc Maxisorp, Roskilde, Denmark) were coated with either copper Ox LDL, native LDL (at a concentration of 10 μg/mL in phosphate-buffered saline [PBS]), or PBS overnight at 4°C. After washing 4 times with PBS containing 0.05% Tween and 0.001% aprotinin (Sigma, St. Louis, MO), the plates were blocked with 2% bovine serum albumin (BSA) for 2h at room temperature. Serum samples were diluted to 1:50 in PBS 0.05% Tween 0.2% BSA. After additional overnight incubation, the sera were washed and alkaline phosphatase-conjugated goat anti-human IgG (Jackson ImmunoResearch laboratory Inc, West Grove, PA) was added diluted 1:10,000 in PBS 0.05% Tween-0.2% BSA for 1 hour at room temperature. After thorough washing, 1 mg/mL p-nitrophenol-phosphate (Sigma) in 50 mM carbonate buffer containing 1 mM MgCl₂ pH 9.8 was added as a substrate. The reaction was stopped after 30 minutes by adding 1 M of NaOH. The color was read at a 405 nm wavelength in a Titertek ELISA reader (S.L.T. Laboratory Instruments, Vienna, Austria), and the results were expressed as optical density at 405 nm. The ELISA for IgG Ox LDL Abs was performed from a single batch of Ox LDL, which was produced from the plasma of 20 healthy donors. Plate-to-plate variability, which was always <10%, was corrected by putting several control samples on all plates to serve as references. In addition, repeated assays of all sera showed an interassay variability of <10%.

**Determination of N-terminal pro-B-type Natriuretic Peptide Levels**

Serum pro-BNP levels were measured by automated immunosay (Elecsys pro-BNP, Roche Diagnostics, Mannheim, Germany) from the same sample drawn for Ox LDL Abs. The testing principle includes using two polyclonal antibodies directed against N-terminal pro-BNP; epitope 1: amino acid 1-21 and epitope 2: amino acid 39-50. The results are calibrated against a synthetic N-terminal pro—BNP (amino acid 1-76). The range of results is between 5 and 35,000 pg/mL.

**Statistical Analysis**

Ox LDL Abs were included in the statistical analysis as a continuous parameter and also as a dichotomous (based on median) parameter (ie, <193 vs. ≥193). Pearson correlation coefficients were calculated to study the relationship between continuous parameters (age, N-terminal pro-B-type natriuretic peptide [NT pro-BNP], hemoglobin [Hb], LVEF) and Ox LDL Abs. Ox LDL Abs and NT pro-BNP levels were analyzed as continuous variables. The values of Ox LDL Abs were calculated by subtracting the value obtained from binding to native LDL from the binding to Ox LDL. Optical density levels were multiplied by a factor of 1000.

Multivariate Cox regression models were applied to the data to simultaneously study the independent relationship between each risk factor and each outcome. Each model predicts the risk of occurrence of the outcome as a function of the explanatory variables. Several variable selection models were used to construct the Cox regression models. Significance was set at 0.05 and the SPSS (Chicago, IL) for Windows software, Version 13.0, was used for the analysis.

**Results**

A total of 324 consecutive outpatients with CHF—related symptoms were originally eligible for participation in this prospective study according to study criteria. Of them, 40 were excluded for noncompliance or lack of sufficient follow-up information. The remaining 284 patients were entered into the study. HF duration ranged between 8 months to 11.5 years and mean follow-up time was 3.7 years.

 Relevant data on the patients’ general characteristics are presented in Table 1. It shows the distribution of the NYHA class among the 284 patients. The mean number of clinical visits was 15.3. The mean time to first hospitalization was 25.8 ± 17.0 months. A total of 72.2% of patients suffered from systolic HF. The mortality rate was 37% (108 patients).
The main purpose of this prospective study was to evaluate Ox LDL Abs as a predictor of morbidity and mortality in HF patients. There has been a growing interest in brain natriuretic peptides as surrogate follow-up markers for patients with CHF. The pathogenesis of HF syndromes is multifactorial and includes enhanced oxidative stress. We recently observed that Ox LDL Abs correlated with past hospitalizations and mortality. In contrast, anti-Ox LDL Abs had a significant impact on mortality (P = .013). A multivariate linear regression analysis demonstrated that plasma NT pro-BNP was independently associated with Ox LDL Abs (P = .0352). No correlation emerged between Ox LDL Abs and NT pro-BNP, age, creatinine, NYHA Class, and LVEF.

Discussion

The main purpose of this prospective study was to evaluate Ox LDL Abs as a predictor of morbidity and mortality in HF patients. There has been a growing interest in brain natriuretic peptides as surrogate follow-up markers for patients with CHF. The pathogenesis of HF syndromes is multifactorial and includes enhanced oxidative stress. We recently observed that Ox LDL Abs correlated with past hospitalizations and mortality. In contrast, anti-Ox LDL Abs had a significant impact on mortality (P = .013). A multivariate linear regression analysis demonstrated that plasma NT pro-BNP was independently associated with Ox LDL Abs (P = .0352). No correlation emerged between Ox LDL Abs and NT pro-BNP, age, creatinine, NYHA Class, and LVEF.
tested the hypothesis that immunoglobulin G antibodies to oxidatively modified LDL (Ox LDL Abs) would predict outcome in a relatively large cohort of patients with CHF. The end points of the study were time to first hospitalization which reflects the morbidity, all-cause mortality, and a combination of the two.

A high plasma level of circulating Ox LDL was previously shown to be an important predictor of mortality, time to hospitalization (morbidity), and both of these two end points in combination, independent of clinical and laboratory determinants. Plasma levels of Ox LDL Abs were recently shown to be increased with severity of CHF in patients with systolic, diastolic, ischemic, and valvular HF and found no significant differences. This can be explained by assuming that oxidative stress is not specific to a distinct etiology of HF but rather to the functional myocardial compromise imposed by HF syndrome.1−3,10−12,15−17 In the present study, we show for the first time that Ox LDL Abs plasma levels were a significant and independent predictor of time to hospitalization and mortality. Moreover, NT pro-BNP plasma levels, LVEF, and NYHA class were also of prognostic value in the CHF patients as assessed by multivariate analysis.

Our results show that Ox LDL Abs levels were superior to NT pro-BNP levels as predictors for time to hospitalization. They were also better predictors of the combined end points, although NT pro-BNP was a better predictor of total mortality. The apparent differential predictive power of Ox LDL antibodies and NT pro-BNP may be attributable to the different mechanisms leading to their elevated levels. Thus, NT pro-BNP reflects the activation of the neurohormonal axis, whereas Ox LDL Abs mirrors oxidative stress. These 2 mechanisms governing HF progression are possibly activated in different patient subpopulations and predict different end points.

HDL appears to harbor anti oxidative properties that may directly influence the status of LDL oxidation and oxidative stress, in general. The average HDL level in the patients appears relatively high, despite only 58% use of statins. However, the patients in this study didn’t take any of the known medications that elevate HDL levels except for a small percentage of those who took bezafibrates. Many of them did however change or improve their life style by physical activities and diet.

We did not find a robust correlation between Ox LDL Abs and other factors related to progression of HF. Interestingly, creatinine levels did not appear as a significant prognostic factor. This apparently odd finding can be related to the fact that being in a specialized HF clinic with very strict care with regard to renal function by nephrologists, could have reduced oxidized stress and contributed to the lowering of the levels of Ox LDL Abs. Another possible explanation is that that most patients were in NYHA Classes III and IV and the number of patients in the other groups was too small to achieve statistical power.

An interesting point that was also tested here is whether activation of the neurohormonal axis is necessarily associated with increased oxidative stress. However, with regard to this question, there was no association between NT pro-BNP and Ox LDL Abs levels, suggesting that determination of the latter may have an incremental value over that provided by the former, although this was not evident in this specific study.

An additional noteworthy issue relates to the levels of Ox LDL in different HF populations. Indeed, we compared levels of Ox LDL Abs in subgroups of patients with systolic, diastolic, ischemic, and valvular HF and found no significant differences. This can be explained by assuming that oxidative stress is not specific to a distinct etiology of HF but rather to the functional myocardial compromise imposed by HF syndrome.

The potential advantage of Ox LDL Abs testing over other prognostic biomarkers lies in its inherent stability throughout time. Immunoglobulin G antibodies reflect the state of

### Table 3. Adjusted HR

<table>
<thead>
<tr>
<th>Variable</th>
<th>A. HR of Hospitalization</th>
<th>B. HR of Mortality</th>
<th>C. HR of Hospitalization and Mortality Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95% CI for Exp (B)</td>
<td>95% CI for Exp (B)</td>
<td>95% CI for Exp (B)</td>
</tr>
<tr>
<td>Clinical data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.016</td>
<td>1.033</td>
<td>1.006</td>
</tr>
<tr>
<td>Gender</td>
<td>0.003</td>
<td>2.234</td>
<td>1.317</td>
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<tr>
<td>Weight</td>
<td>0.255</td>
<td>1.099</td>
<td>0.993</td>
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<tr>
<td>Hyperlipidemia</td>
<td>0.911</td>
<td>0.973</td>
<td>0.608</td>
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<tr>
<td>Smoking</td>
<td>0.515</td>
<td>1.185</td>
<td>0.710</td>
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<td>HTN</td>
<td>0.839</td>
<td>1.048</td>
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</tr>
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<td>DM</td>
<td>0.062</td>
<td>1.531</td>
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<td>NYHA Class</td>
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<td>2.943</td>
<td>1.894</td>
</tr>
<tr>
<td>Ischemic CMP</td>
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<td>0.581</td>
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<tr>
<td>LVEF</td>
<td>0.598</td>
<td>0.986</td>
<td>0.968</td>
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<tr>
<td>Biochemical data</td>
<td></td>
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<tr>
<td>Creatinine</td>
<td>0.973</td>
<td>0.996</td>
<td>0.778</td>
</tr>
<tr>
<td>Ox LDL Ab</td>
<td>0.020</td>
<td>1.016</td>
<td>1.002</td>
</tr>
<tr>
<td>NT-pro BNP</td>
<td>0.841</td>
<td>1.005</td>
<td>0.960</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; HTN, hypertension; DM, diabetes mellitus; NYHA, New York Heart Association; CMP, cardiomyopathy; LVEF, left ventricular ejection fraction; Ox, oxidized; LDL, low-density lipoprotein; Ab, antibody; NT-pro BNP, N-terminal pro-B-type natriuretic peptide.
oxidative stress prevailing over prolonged time periods. Because plasma levels of immunoglobulin G antibodies are not significantly altered by any short self—limited events, they are more likely to both reflect and predict outcome compared with biomarkers, such as NT-proBPN or BNP, that are altered by the extent of stretching of the ventricular myocardium.

There are several limitations to the current study. The number of patients in different NYHA groups was unequal and in subgroups such as NYHA Class I, there was only a small number of patients. This is the reason for the difficulties encountered in comparing variables in different NYHA groups such as Ox LDL Abs, creatinine, systolic, diastolic, ischemic, or valvular HF. The HF population comprised patients with different etiologies including ischemic versus valvular systolic versus diastolic. The third limitation is that Ox LDL Abs ELISA method is not a standardized assay and cross-sectional comparative studies cannot be optimally conducted.

In conclusion, determination of Ox LDL Abs was found to be a useful parameter for monitoring clinical status and for distinguishing between well and poorly controlled HF patients. This large prospective study also demonstrates, for what we believe to be the first time, the superiority of Ox LDL Abs testing over NT pro-BNP in predicting future hospitalization in CHF patients. If complemented by additional prospective data and standardized, Ox LDL Abs levels may prove as bodies against oxidized LDL a predictor in patients with chronic congestive heart failure. J Am Coll Cardiol 2002;39:957–62.


