

Decreased Oxidative Stress in Patients With Idiopathic Dilated Cardiomyopathy One Year After Immunoglobulin Adsorption

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OBJECTIVES	In a substudy to a recently reported investigation that demonstrated the benefit of immunoglobulin adsorption (immunoabsorption) for patients with idiopathic dilated cardiomyopathy (IDC), we tested whether this benefit is associated with a reduction of oxidative stress.
BACKGROUND	The progression of cardiomyopathy is believed to be related to the increase of oxidative stress. Therefore, reduction of oxidative stress could be one of the effects of immunoabsorption for improvement of cardiac performance and clinical status.
METHODS	Plasma markers for oxidative stress—thiobarbituric acid-reactive substances (TBARS), lipid peroxides (LPO), anti-oxidized low-density lipoprotein-autoantibodies (anti-oxLDL-AB), thiol groups and vitamin E—were compared in 31 patients, of whom 16 underwent immunoabsorption and 15 received conventional treatment (controls). All patients received a daily supplement of vitamins, minerals and trace elements.
RESULTS	After one year, TBARS ($p = 0.026$), LPO ($p = 0.026$) and anti-oxLDL-AB ($p = 0.044$) were decreased in the immunoabsorption group but not in the controls. Thiols were unchanged in the immunoabsorption group but were decreased in the controls ($p = 0.001$). Vitamin E accumulated in both groups (immunoabsorption: $p = 0.001$; controls: $p = 0.031$) with a trend for stronger accumulation after immunoabsorption ($p = 0.09$). Prior to the study, the anti-oxLDL-AB to left ventricular ejection fraction (LVEF) ($p = 0.05$) were inversely correlated. After one year, correlations with borderline significance were calculated for TBARS to New York Heart Association functional class ($p = 0.081$) and inversely for LPO to LVEF ($p = 0.083$).
CONCLUSIONS	Effective therapy in patients with IDC, such as immunoabsorption which improved cardiac performance and clinical status, is associated with a reduction of oxidative stress. (J Am Coll Cardiol 2001;38:178–83) © 2001 by the American College of Cardiology

The benefit of immunoglobulin adsorption (immunoabsorption) for the elimination of cardiac-specific autoantibodies in patients with dilated cardiomyopathy was recently reported. Müller et al. (1) found that cardiac performance and clinical status improved one year after a cycle of

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immunoabsorption, which was performed on five consecutive days. Improved hemodynamics after immunoabsorption, which was combined with subsequent immunoglobulin G (IgG) substitution, was demonstrated by Felix et al. (2), who performed immunoabsorption in four courses, at one-month intervals, until month 3.

However, heart failure is associated with increased oxi-

dative stress (3–9), the extent of which correlates positively with the class of heart failure (6,7,9) and negatively with cardiac performance (4). Therefore, oxidative stress is believed to be involved in the development and progression of heart failure (10,11). Consequently, beneficial treatment, such as immunoabsorption, which improves cardiac performance and clinical status (1,2), could result in the reduction of oxidative stress that might contribute to the therapy benefit.

To test this hypothesis, which would substantiate a close relationship between oxidative stress and heart failure, we compared in a substudy to Müller et al. (1) the prestudy and one-year levels of plasma markers for oxidative stress in patients with idiopathic dilated cardiomyopathy (IDC) treated with immunoabsorption according to Müller et al. (1) versus conventionally treated patients.

METHODS

Patient characteristics. In the present study, we analyzed plasma samples that were collected from patients with IDC who were characterized by Müller et al. (1) with regard to

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Abbreviations and Acronyms

ACE	=	angiotensin-converting enzyme
ANOVA	=	analysis of variance
anti-beta1A-AB	=	anti-beta 1-adrenoceptor antibodies
anti-oxLDL-AB	=	anti-oxidized low-density lipoprotein-autoantibodies
IDC	=	idiopathic dilated cardiomyopathy
IgG	=	immunoglobulin G
LPO	=	lipid peroxides
LVEF	=	left ventricular ejection fraction
LVIDd	=	left ventricular internal diameter in diastole
LVIDs	=	left ventricular internal diameter in systole
NYHA	=	New York Heart Association
TBARS	=	thiobarbituric acid-reactive substances

the effects of immunoadsorption versus conventional treatment on cardiac performance and clinical status.

From the 32 patients with IDC who finished the Müller et al. study (1), we included 31 patients (mean age: 47.8 ± 10.3 years; range: 21 to 61 years, men: 29; women: 2) in our study. One patient was excluded owing to a hemolytic prestudy sample. All patients were accepted as candidates for heart transplantation and had New York Heart Association (NYHA) functional class II or worse, left ventricular ejection fraction (LVEF) <0.29, left ventricular internal diameter in diastole (LVIDd) >64 mm and evidence for cardiac-specific autoantibodies indicated by the presence of anti-beta1-adrenoceptor autoantibodies (anti-beta1A-AB). There was no evidence of coronary artery disease or any other cardiac disease. Exclusion criteria were atrial fibrillation, infectious diseases, alcohol-induced cardiomyopathy, previous allergic reaction to sheep protein and signs of malignancy. Sixteen patients (mean age 45.6 ± 10.0 years; range: 30 to 59 years; men: 14; women: 2) were treated with immunoadsorption and 15 (mean age: 50.1 ± 10.6 years; range: 21 to 61 years; men: 15) were conventionally treated. One year after immunoadsorption, the anti-beta1A-AB level (prestudy level: 5.9 ± 1.2 laboratory units) was reduced in the treatment group by approximately 90%. This was accompanied by a highly significant improvement ($p < 0.0001$) of cardiac performance (LVEF: 22.1 ± 3.3 vs. 37.8 ± 8.2%; LVIDd: 74.4 ± 7.3 vs. 63.8 ± 6.2 mm; left ventricular internal diameter in systole [LVIDs]: 66.0 ± 6.3 vs. 55.6 ± 6.2 mm) and clinical status (NYHA functional class I/II/III/IV: 0/2/13/1 vs. 7/9/0/0). By comparison, no significant changes of the controls in anti-beta1A-AB (prestudy level: 4.8 ± 1.0 laboratory units) or in cardiac performance (LVEF: 24.1 ± 3.0 vs. 25.2 ± 5.9%; LVIDd: 75.5 ± 5.3 vs. 73.7 ± 7.4 mm; LVIDs: 67.0 ± 5.4 vs. 65.1 ± 8.4 mm) and clinical status (NYHA functional class I/II/III/IV: 0/4/11/0 vs. 0/7/6/2) were seen after conventional treatment.

Written, informed consent was obtained from all pa-

tients. The study protocol was approved by the Human Ethics Committee of the Humboldt University of Berlin, Germany.

Medical treatment. At baseline, medical treatment for heart failure was standardized in that all patients were administered maximal tolerated dosages of angiotensin-converting enzyme (ACE) inhibitors, digitalis, diuretics and oral anticoagulants. In addition, patients were treated with the beta-blocker bisoprolol for the first time. Dosage adjustments were made to achieve a systolic blood pressure of 100 to 110 mm Hg and a heart rate of 60 to 80 beats/min within three months. All patients received a daily supplement of a moderate dose of vitamins, minerals and trace elements (OrthoCorPlus; Orthomol, Langenfeld, Germany).

Extracorporeal immunoglobulin adsorption, measurement of anti-beta1A-AB and echocardiographic evaluation. For the immunoadsorption, which was performed according to the established method of low-density lipoprotein (LDL) elimination, adsorption columns containing polyclonal anti-human immunoglobulin antibodies produced in sheep (12) were used.

Immunoadsorption was performed on five consecutive days, and serum IgG levels were monitored. To eliminate anti-beta1A-AB with an acceptable risk of infection, immunoglobulin G (IgG) reduction to <120 mg/dl was planned.

For the measurement of anti-beta1A-AB, a bioassay recently described by Wallukat et al. (13) was used.

Transthoracic echocardiography was performed according to the guidelines of the American Society of Echocardiography (14). (For more details, see Müller et al. [1]).

Sampling and measurement. As markers for oxidative stress, we determined the levels of thiobarbituric acid-reactive substances (TBARS) (15), lipid peroxides (LPO) (PerOx; Immundiagnostik, Bensheim, Germany) (16), anti-oxidized low-density lipoprotein-autoantibodies (anti-oxLDL-AB) (oLab Elisa; Biomedica, Wien, Austria), thiol groups (17) and vitamin E (18) in venous plasma samples (anticoagulated with 14,300 U/l heparin) collected prior to the study and after one year.

To avoid unspecific marker changes by *in vitro* oxidation, especially relevant to TBARS, LPO and thiol groups, and to guarantee high precision in the analytical procedures, adequate conditions for sampling, storage and measurement were ascertained in prestudy experiments. Using the findings of these experiments, samples and aliquots were prepared under a nitrogen atmosphere, immediately frozen and stored in liquid nitrogen until measurement. Both the prestudy and one-year sample of each subject of both groups were handled identically and—for high precision—analyzed in parallel.

Statistical analysis. Data are expressed as mean ± SD. A log-transformation was applied to stabilize the variance. A two-way repeated-measures analysis of variance (ANOVA) was used to test for effect of time, group and their interaction followed by the Student *t* test for paired and unpaired

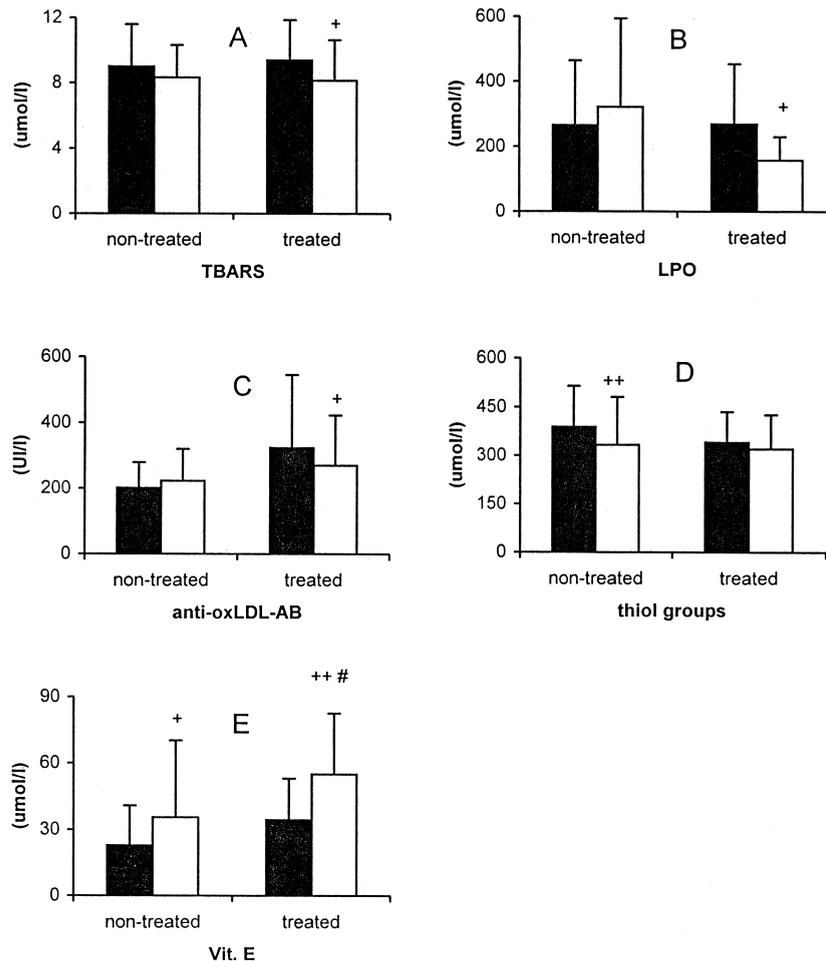


Figure 1. Prestudy (black columns) and one-year levels (white columns) of plasma markers for oxidative stress in patients with idiopathic dilated cardiomyopathy conventionally treated (nontreated, n = 15) and treated with immunoglobulin adsorption (treated, n = 16). Prestudy level versus one-year level: +p ≤ 0.05; ++p ≤ 0.001; nontreated versus treated: #p ≤ 0.1. (A) Thiobarbituric acid-reactive substances (TBARS); (B) lipid peroxides (LPO); (C) anti-oxidized low-density lipoprotein-autoantibodies (anti-oxLDL-AB); (D) thiol groups; (E) vitamin E (Vit. E).

data. Linear regression was performed using the SPSS software package (SPSS Inc., Chicago, Illinois).

RESULTS

Oxidative stress in patients with IDC one year after immunoglobulin adsorption. The prestudy and one-year levels of indicators for oxidative stress were widely scattered in both patient groups, as shown by their SD (Fig. 1). However, there were no significant group-specific differences prior to the study. Two-way ANOVA for analysis of the overall effect of group allocation and repeated measures on the markers for oxidative stress revealed significant time effects but no interaction effects for TBARS (p = 0.0001 [time effect p value] p = 0.99 [interaction effect p value]), thiol groups (p = 0.001; p = 0.1) and vitamin E (p = 0.001; p = 0.43). In contrast, no time effects but a trend to interaction was found for LPO (p = 0.14; p = 0.08) and anti-oxLDL-AB (p = 0.89; p = 0.09).

In addition to highly significantly reduced anti-beta1A-AB and improved cardiac performance and NYHA functional class in the patients who underwent immunoad-

sorption, as demonstrated by Müller *et al.* (1), a significant lowering was observed in these patients over the study period by $1.24 \pm 0.5 \mu\text{mol/l}$ for TBARS (9.38 ± 2.48 vs. $8.14 \pm 2.50 \mu\text{mol/l}$; p = 0.026), by $110.6 \pm 44.5 \mu\text{mol/l}$ for LPO (268.6 ± 186.1 vs. $157.9 \pm 71.7 \mu\text{mol/l}$; p = 0.026), and by $53.2 \pm 24.0 \text{ U/l}$ for anti-oxLDL-AB (323.1 ± 221.4 vs. $269.9 \pm 153.1 \text{ U/l}$, p = 0.044). In contrast, TBARS decreased only by $0.87 \pm 0.6 \mu\text{mol/l}$ (9.19 ± 3.36 vs. $8.33 \pm 2.49 \mu\text{mol/l}$, p = 0.165) in the controls. Both LPO (265.4 ± 198.9 vs. $321.4 \pm 272.5 \mu\text{mol/l}$, p = 0.364) and anti-oxLDL-AB (200.4 ± 78.7 vs. $222.6 \pm 97.3 \text{ U/l}$; p = 0.334) increased by $56.0 \pm 59.6 \mu\text{mol/l}$ and $22.2 \pm 22.6 \text{ U/l}$, respectively, in the controls. However, these changes were insignificant.

For the thiol groups, there was only an insignificant change of $20.8 \pm 12.4 \mu\text{mol/l}$ (341.1 ± 94.4 vs. $320.3 \pm 105.1 \mu\text{mol/l}$; p = 0.140) in the immunoadsorption group, but thiols decreased significantly in the controls by $53.2 \pm 12.9 \mu\text{mol/l}$ (386.1 ± 129.0 vs. $332.9 \pm 147.4 \mu\text{mol/l}$, p = 0.001). Vitamin E increased significantly over the study period in the treated group by $20.56 \pm 2.9 \mu\text{mol/l}$ ($34.44 \pm$

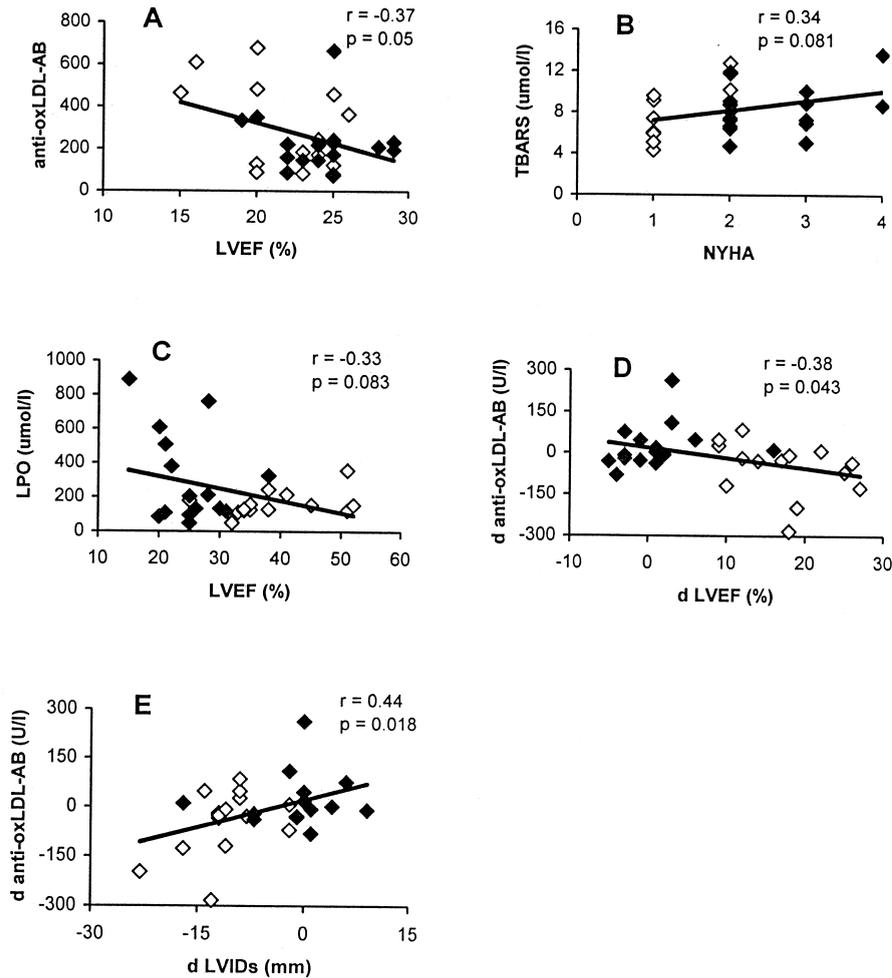


Figure 2. Relationships between markers of oxidative stress and clinical status and cardiac performance in the complete population of patients conventionally treated (**solid diamonds**) and those treated with immunoglobulin adsorption (**open diamonds**). **(A)** Anti-oxidized low density lipoprotein autoantibodies (anti-oxLDL-AB) versus left ventricular ejection fraction (LVEF) prior to the study. **(B)** Thiobarbituric acid-reactive substances (TBARS) versus New York Heart Association functional class one year after immunoadsorption. **(C)** Lipid peroxides (LPO) versus LVEF one year after immunoadsorption. Prestudy to one-year change of anti-oxLDL-AB (d anti-oxLDL-AB) versus change of **(D)** LVEF (d LVEF), and **(E)** LVIDs (d LVIDs).

18.64 vs. $55.0 \pm 27.56 \mu\text{mol/l}$, $p = 0.001$) as well as in the control group by $12.75 \pm 5.2 \mu\text{mol/l}$ (22.85 ± 18.23 vs. $35.60 \pm 34.42 \mu\text{mol/l}$, $p = 0.031$) with a trend for stronger accumulation in the immunoadsorption group ($p = 0.09$). **Oxidative stress versus NYHA functional class and cardiac performance.** Prior to the study, a significant inverse correlation of anti-oxLDL-AB to LVEF was calculated for the complete population of patients (Fig. 2A). After one year (Fig. 2B, C) the best correlations were found for TBARS to NYHA functional class and inversely for LPO to LVEF. Additionally, the anti-oxLDL-AB changes over the study period correlated inversely to LVEF and directly to LVIDs changes.

DISCUSSION

To our knowledge for the first time in humans, we demonstrated that improvement of cardiac performance and clinical status in heart failure, such as shown after immu-

noadsorption (1), is in parallel associated with a reduction of oxidative stress.

This was demonstrated by measurement of markers adaptable to clinical chemistry for analysis of oxidative stress in human plasma (19) such as TBARS, LPO, and anti-oxLDL-AB as indicators for lipid peroxidation; thiol groups, for which a decrease signals thiol oxidation; and vitamin E, an important nonenzymatic plasma antioxidant for which a decrease indicates consumption due to oxidative stress.

Oxidative stress in patients with IDC one year after immunoglobulin adsorption. Decreased oxidative stress one year after immunoadsorption was indicated by a lowering of TBARS, LPO and anti-oxLDL-AB, which was not seen in the conventionally treated patients. With respect to anti-oxLDL-AB, immunoadsorption is not antigen selective and could—in addition to anti-beta1A-AB—eliminate other IgG class antibodies such as anti-oxLDL-

AB. Therefore, a delayed reincrease of anti-oxLDL-AB could also explain the low anti-oxLDL level in the one-year samples. Müller et al. (1) have speculated that antioxidant supplementation could modulate the autoantibody reincrease. Although all patients received equal amounts of OrthoCorPlus, which supplied vitamin E, there was a trend to stronger accumulation of vitamin E in the plasma of the patients who underwent immunoadsorption. We attribute this effect to a lower consumption of vitamin E, due to decreased oxidative stress in these patients. The significant lowering of thiol groups over the study period, but only in the controls, is a further indication of different levels of oxidative stress in both groups.

Oxidative stress versus NYHA functional class and cardiac performance. Close correlations for our patients between the intensity of oxidative stress and clinical status and cardiac performance would substantiate the relationship between oxidative stress and heart failure. Recently, such correlations were demonstrated in studies that enrolled patients with widespread data for cardiac performance and clinical status (4,6,7,9), which favor regression analysis. Regression analysis was limited by the small range of these parameters in our patients, especially prior to the study, and also by the relatively small number of patients in our study. However, the correlations for oxidative stress, clinical status and cardiac performance, demonstrated in Figure 2A-C, supplement the findings of the other studies (4,6,7,9). At present, it cannot be decided whether the correlations, which were calculated for the prestudy to the one-year changes of anti-oxLDL-AB and cardiac performance (Fig. 2D-E), support a close relationship between the intensity of oxidative stress and the severity of heart failure. For that, investigations must clarify the exact reasons—such as discussed above—for low anti-oxLDL-AB levels one year after immunoadsorption.

Potential reasons for reduced oxidative stress after immunoglobulin adsorption. The reasons for reduced oxidative stress in patients with IDC one year after immunoadsorption are unknown. Predisposing events of heart failure, such as mechanical stress, ischemia, intoxication, viral myocarditis, reduced antioxidative defense due to malnutrition and neurohumoral hyperstimulation, are thought to drive myocardial oxidative stress (10,11), which can damage—via thiol oxidation and lipid peroxidation—myocardial structures and functions (20,21) and in this way join the initial pathophysiologic events and the development of heart failure. Because of stimulated myocyte growth, apoptosis and fibroblast proliferation during increased oxidative stress (22), progression of heart failure could be favored.

Reduced mechanical stress, probably reduced inflammation after immunoadsorption together with the vitamin E supplement, may minimize oxidative stress. Furthermore, hyperstimulation of the myocardial beta-adrenergic pathway, which is linked to heart failure (23), could increase the oxygen radical generation by blockade of the electron transport in the mitochondrial complex I (24). Therefore,

immunoadsorption of anti-beta1A-AB, believed to be involved in the hyperstimulation of the myocardial beta-adrenergic pathway, could reduce oxidative stress.

Study limitations. Decreases of plasma indicators for oxidative stress do not inevitably reflect reduced oxidative stress in the heart, which is favorable for an improvement of function. Apart from the myocardium, perhaps poorly perfused peripheral muscles and activated neutrophils may be responsible for the oxidative stress found in plasma. Future studies analyzing myocardial biopsies in parallel to plasma seem to be warranted.

Conclusions. Improvement of clinical status and cardiac performance in patients with IDC due to immunoadsorption is associated with a reduction of oxidative stress. This could contribute to the benefit of immunoadsorption in these patients.

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