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# Severity of oxidative stress and inflammatory activation in end-stage heart failure patients are unaltered after 1 month of left ventricular mechanical assistance

Raffaele Caruso [a], Alessandro Verde [b], Jonica Campolo [a], Filippo Milazzo [b], Claudio Russo [b], Chiara Boroni [a], Marina Parolini [a], Salvatore Trunfio [b], Roberto Paino [b], Luigi Martinelli [b], Maria Frigerio [b], Oberdan Parodi [a,*]

[a] CNR Clinical Physiology Institute, Cardiovascular Department, Niguarda Cà Granda Hospital, Piazzale Ospedale Maggiore, 3-20162 Milan, Italy
[b] Cardiovascular Department, Niguarda Cà Granda Hospital, Piazzale Ospedale Maggiore, 3-20162 Milan, Italy

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ABSTRACT

This study investigates the impact of early left ventricular (LV)-mechanical unloading on systemic oxidative stress and inflammation in terminal heart failure patients and their impact both on multi organ failure and on intensive care unit (ICU) stay. Circulating levels of urinary 15-isoprostane-$F_{2t}$ (8-epi-$PGF2_{\alpha}$) and pro-inflammatory markers [plasma interleukin (IL)-6, IL-8, and urinary neopterin, a monocyte activation index] were analyzed in 20 healthy subjects, 22 stable end-stage heart failure (ESHF) patients and in 23 LV assist device (LVAD) recipients at pre-implant and during first post-LVAD (PL) month. Multi-organ function was evaluated by total Sequential Organ Failure Assessment (tSOFA) score. In LVAD recipients the levels of oxidative-inflammatory markers and tSOFA score were higher compared to other groups. After device implantation 8-epi-$PGF2_{\alpha}$ levels were unchanged, while IL-6, and IL-8 levels increased during first week, and at 1 month returned to pre-implant values, while neopterin levels increased progressively during LVAD support. The tSOFA score worsened at 1 PL-week with respect to pre-implant value, but improved at 1 PL-month. The tSOFA score related with IL-6 and IL-8 levels, while length of ICU stay related with pre-implant IL-6 levels. These data suggest that hemodynamic instability in terminal HF is associated to worsening of systemic inflammatory and oxidative milieu that do not improve in the early phase of hemodynamic recovery and LV-unloading by LVAD, affecting multi-organ function and length of ICU stay. This data stimulate to evaluate the impact of inflammatory signals on long-term outcome of mechanical circulatory support.



## 1. Introduction

In the last decades left ventricular assist device (LVAD) implantation has proven effectiveness in the management of end-stage heart failure (ESHF) patients [1], not only as bridge to transplant (BTT), but also as potential destination therapy. An amelioration of hemodynamic and a decrease of LV filling pressure are more or less regularly observed since the first hours after LVAD implantation. Moreover, mechanical LV-unloading by LVAD, decreasing the LV cavitary pressures, results in the decreased of wall stress-related signals, which promote favorable changes of myocardial and peripheral neurohormonal milieu [2], potentially associated also to cardiac recovery [3].

Clinical studies have shown that many inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) increase in HF patients [4–6], suggesting their important roles in the underlying pathophysiology of HF [7, 8]. IL-6/C-reactive protein (CRP) pathway is found more elevated in acute decompensated HF patients with left ventricular systolic dysfunction than in patients with preserved LV ejection function [9]. Furthermore, HF is complicated also by oxidative stress, with elevated levels of lipid peroxides, such as malondialdehyde (MDA) [10], and alteration of redox state [11], that suggest a link with inflammatory pathways in the HF pathogenesis [12–14].

In ESHF patients the hemodynamic recovery, amelioration of neurohormonal milieu, and LV-unloading, by LVAD, might potentially interrupt the cycle of inflammation and oxidative stress, contributing to favorable conditions both to outcome of the mechanical circulatory support (MCS), and to clinical course during hospitalization. However, the effects of LVAD implant on

*Abbreviations:* LVAD, left ventricular assist device; ESHF, end-stage heart failure; BTT, bridge to transplant; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; MDA, malondialdehyde; MCS, mechanical circulatory support; ICU, intensive care unit; NYHA, New York Heart Association; GSH, glutathione; Cys, cysteine; GPx-3, GSH peroxidase type 3; RAP, right atrial pressure; tSOFA, total Sequential Organ Failure Assessment; HPLC, high-performance liquid chromatography; Neo/Cr, neopterin to creatinine ratio; CI, cardiac index; PCWP, pulmonary capillary wedge pressure; MOFS, multi-organ failure syndrome.

* Corresponding author. Tel.: +39 02 6473407; fax: +39 02 66116990.
*E-mail address:* ifcnig@tin.it (O. Parodi).

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circulating inflammatory cytokines, oxidative stress and redox state markers in ESHF patients have not yet well investigated. Moreover, the data about relationship between inflammatory-oxidative state and clinical course of LVAD patients are sparse.

Thus, the purposes of this study were to: (1) define the oxidative stress grade and severity of inflammatory milieu in ESHF patients candidates to LVAD implantation, comparing their pre-implant levels with those of stable HF patients and healthy subjects, (2) assess the impact of hemodynamic recovery by LVAD on redox state, oxidative stress, and inflammatory milieu during first month of LVAD support, and (3) assess whether the dynamic of multi-organ function and the duration of the stay in intensive care unit (ICU), reflecting the clinical course of patients, are affected from oxidative stress and inflammatory markers.

## 2. Methods

### 2.1. Patients

Twenty-three ESHF patients underwent LVAD implantation as BTT were enrolled in the study. In all patients axial continuous-flow devices were implanted [5 De Bakey-LVADs (MicroMed Technology, Inc., Houston, TX), 6 Incor-LVADs (Berlin Heart AG) and 12 HeartMateII-LVADs (Thoratec, Pleasanton, CA)].

Twenty-two chronic HF patients, who had been admitted for routine re-evaluation or for treatment of advanced HF and assigned to New York Heart Association (NYHA) classes III and IV, were enrolled as stable ESHF group.

Twenty healthy subjects with normal LV function (LV ejection fraction >55%) and no history of cardiovascular events were enrolled as control group.

### 2.2. Study design

Redox state [blood and plasma, total and reduced, GSH, Cys levels, and plasma GSH peroxidase type-3 (GPx-3) activities], oxidative stress [plasma free MDA (fMDA) and urinary 15-isoprostane-$F_{2t}$ (known as 8-epi-$PGF2_\alpha$) levels] and inflammatory variables [plasma IL-6, IL-8, TNF-α, serum C-reactive protein (CRP) concentrations, and urine levels of neopterin, a specific marker of monocyte activation] were assessed in LVAD-patients serially from pre-implant, and subsequently at 1, 7 and 30 days since LVAD placement. Hemodynamic was assessed preoperatively and daily up to first postoperative week by pulmonary artery Swan-Ganz catheter. Right heart function was monitored by assessment of right atrial pressure (RAP) and inotropic need [15, 16]. Inotropic equivalent >10 [17] and/or RAP >10 mmHg were criteria to assess right heart dysfunction [18].

The overall condition of the multi-organ function was daily monitored according to the Sequential Organ Failure Assessment (SOFA) system [19]. The SOFA system is a daily score from 0 to 4 assigned in proportion to the severity of functional deterioration for each of six individual organ systems (cardiovascular, respiratory, hepatic, renal, neurological, and hemocoagulative). The aggregate total SOFA (tSOFA) score was calculated by adding the scores for each of the organ systems during the observation period [19].

In stable ESHF group, echocardiographic, haemodynamic and clinical data were collected during hospitalization. Before haemodynamic assessment, fasting venous blood and urine samples were collected for biochemical determinations.

In all control subjects, after an overnight fast, venous blood and urine samples were withdrawn for biochemical determinations before echocardiographic assessment, and clinical data were collected at admission.

The study conformed the principles outlined in the Declaration of Helsinki and the study protocol was approved by local ethics committee. All subjects gave written informed consent to participate to the study.

### 2.3. Redox state assessment

Blood reduced GSH level was determined by prompt acidification of whole blood immediately after blood sample collection according to method previously described [11]. As reduced GSH levels in plasma are low (1–2%), the reduced GSH concentration in whole blood can reflect GSH content inside the cellular fraction of blood. Plasma reduced and total forms of Cys and blood total GSH were determined according to methods validated in our laboratory [11]. The total form of GSH measured in our study includes the oxidized GSH, all conjugated forms of GSH (protein-bound GSH and GSH-mixed disulfides), produced through oxidative processes or thiol-disulfide exchange reactions, and reduced free GSH. Thiol separation was performed by high-performance liquid chromatography (HPLC) method (ProStar-Varian, Surrey, UK). GPx-3 activity was determined in plasma samples stored frozen up to analysis that was performed within 1 month from storage. GPx-3 activity was measured as previously described using t-butyl hydroperoxide (SIGMA, Steinheim, Germany) as substrate [20].

### 2.4. Oxidative stress

fMDA levels, the reactive non conjugated MDA form, were determined by isocratic HPLC assay with fluorescence detection (ChromSystemsGmbH, München, Germany). Urinary 8-epi-$PGF2_\alpha$ levels were measured by enzyme-linked immunosorbent assay (Oxford Biomedical Research, Inc., Oxford, MI, USA).

### 2.5. Inflammation parameters

Plasma IL-6, IL-8, and TNF-α levels were measured according by enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN, USA). Urinary neopterin levels were measured by an isocratic HPLC method previously described [19] and normalized by urine creatinine concentrations (Neo/Cr), while sCRP concentrations were measured using a Roche/Hitachi 917 Analyzer by high-sensitive immuno-nephelometric method (Roche Diagnostic GmbH, Mannheim, Germany).

### 2.6. Statistical analysis

Data are expressed as median and interquartile range (I, III) or frequency (percentage). Differences among patient groups were assessed by nonparametric Kruskal–Wallis test for continuous variables and by Chi-square for categorical variables. Changes of the redox and inflammatory parameters during the time were analyzed by non-parametric Friedman test; post-hoc analysis with Wilcoxon Signed-Rank tests was conducted with a Bonferroni correction applied, resulting in a significance level set at $P < 0.017$. The association between variables was tested by Spearman's correlation test. Data were analyzed using SPSS for Windows (Version 17.0, Chicago, IL).

## 3. Results

### 3.1. Clinical findings in LVAD recipients, stable ESHF patients and controls

The clinical characteristics of LVAD recipients, at the pre-implant time, stable ESHF patients and controls are described

**Table 1**
Clinical and echocardiographic parameters among LVAD-recipients, stable ESHF patients and controls.

| | Controls ($n$ = 20) | Stable ESHF patients ($n$ = 22) | LVAD recipients at pre-implant time ($n$ = 23) | $P$ value for group |
|---|---|---|---|---|
| Age, years | 51 (34, 63) | 56 (49, 60) | 51 (45, 61) | 0.300 |
| Male gender, $n$ (%) | 14 (70) | 20 (91) | 22 (96) | 0.038 |
| Hypertension, $n$ (%) | 8 (40) | 6 (27) | 5 (22) | 0.409 |
| Hyperlipidemia, $n$ (%) | 5 (25) | 12 (55) | 5 (22) | 0.040 |
| Diabetes, $n$ (%) | 5 (25) | 4 (18) | 5 (22) | 0.865 |
| History of smoking, $n$ (%) | 2 (10) | 3 (14) | 9 (39) | 0.037 |
| Etiology, $n$ (%) | | | | 1.000 |
| IDC | – | 15 (68) | 16 (70) | |
| ICM | – | 7 (32) | 7 (30) | |
| NYHA class | | | | <0.001 |
| III | – | 15 (68) | 2 (9) | |
| IV | – | 7 (32) | 21 (91) | |
| LVEF, % | 65 (63, 69)*,§ | 27 (19, 33) | 23 (18, 25) | <0.001 |
| LVEDV, mL | 88 (76, 108)*,§ | 186 (151, 289) | 263 (181, 345) | <0.001 |
| LVEDD, mm | 46 (42, 48)*,§ | 67 (61, 75) | 70 (60, 78) | <0.001 |
| Treatments, $n$ (%) | | | | |
| ACEi + ATII | 5 (25%) | 16 (73%) | 16 (70%) | 0.002 |
| Beta-blocker | 2 (10%) | 19 (86%) | 13 (57%) | <0.001 |
| Statins | 2 (10%) | 10 (46%) | 4 (17%) | 0.017 |
| Diuretics | 1 (5%) | 21 (96%) | 23 (100%) | <0.001 |
| Inotropic therapy | – | – | 17 (74%) | |
| Creatinine, g/dL | 0.90 (0.84, 0.98) | 1.08 (0.90, 1.22) | 1.02 (0.84, 1.36) | 0.060 |
| BUN, mg/dL | 40 (34, 47)§ | 48 (41, 65) | 46 (35, 63) | 0.045 |
| t-Bil, mg/dL | 0.51 (0.33, 0.69)*,§ | 1.27 (0.66, 1.99) | 1.43 (0.63, 2.10) | <0.001 |
| tSOFA-score, $n$ | 0 (0, 0)*,§ | 2 (1, 2)* | 5 (3, 7) | <0.001 |

Data are expressed as median and interquartile range (I, III) or number (percentage). ACEi, ACE inhibitor; ATII, angiotensin II receptor antagonists; t-Bil, total bilirubin; BUN, blood urea nitrogen; IDC, idiopathic dilated cardiomyopathy; ICM, ischemic cardiomyopathy; LVEDV, left ventricular end-diastolic volume; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; tSOFA, total Sequential Organ Failure Assessment.
* $p < 0.05$ vs. LVAD recipients by post-hoc test with Bonferroni correction.
§ $p < 0.05$ vs. stable ESHF patients by post-hoc test with Bonferroni correction.

in Table 1. Median age of LVAD-recipients was comparable to those of stable ESHF patients and controls. The cardiovascular risk factors were comparable among the groups with the exception of dyslipidemia, more present in stable ESHF patients, and smoking history among LVAD recipients. LV-impairment, as evaluated by echocardiographic measurements, was comparable between LVAD recipients and stable ESHF patients, however, NYHA class was more advanced in the former (Table 1).

Besides optimal medical and electrical therapy for chronic heart failure, terminal HF patients candidate to LVAD implantation were treated with inotropic support, while controls with one or more cardiovascular risk factors received specific treatment (Table 1).

Blood urea nitrogen, plasma creatinine and total bilirubine levels were comparable between stable ESHF patients and LVAD-recipients at pre-implant time; tSOFA score of LVAD-recipients, at pre-implant time, was significantly higher with respect to those of other groups (Table 1).

Cardiac index (CI) and RAP values were comparable between stable ESHF patients and LVAD recipients [1.88 (1.76, 2.07) vs. 1.69 (1.37, 2.00) L/min/m$^2$ of CI, respectively, $p = 0.102$; 7 (2, 9) vs. 6 (4, 10) mmHg of RAP, respectively, $p = 0.901$] while pulmonary capillary wedge pressure (PCWP) levels were lower in stable ESHF patients with respect to LVAD recipients [16 (10, 24) vs. 26 (15, 31) mmHg, respectively, $p = 0.046$].

### 3.2. Redox, oxidative and inflammatory states in LVAD recipients at baseline, stable ESHF patients and controls

Plasma total Cys levels were higher, while blood and plasma total GSH levels were lower in LVAD recipients, at pre-implant time, with respect to controls (Table 2). In LVAD recipients the blood reduced GSH levels and blood reduced to total GSH (r/tGSH) ratio, an index of GSH bioavailability, were lower with respect to other groups. The GPx-3 activities were higher in LVAD recipients with respect to other groups (Table 2).

The LVAD recipients showed higher plasma fMDA levels with respect to other groups and higher urinary 8-epi-PGF2$_\alpha$ concentrations only with respect to controls (Table 2).

Plasma IL-6, IL-8 and serum CRP levels were higher in LVAD recipients with respect to other groups, while urinary Neo/Cr levels were higher in LVAD recipients only with respect to controls. Plasma TNF-$\alpha$ levels in LVAD recipients were not significantly different with those of stable ESHF patients (Table 2).

### 3.3. Effect of LVAD implant on hemodynamic and clinical profile

Median duration of ICU stay of LVAD recipients was 14 (11, 20) days, ranging from 5 to 40 days. LVAD implantation improved CI and reduced PCWP with respect to pre-implant hemodynamic condition (Table 3). RAP values during first postoperative week did not change compared to pre-implant condition, signs of right heart failure were present in 11 patients only during first postoperative week.

The main morbid events occurred during early phase of mechanical assistance are described in Table 4. During first postoperative week, 14 patients showed elevated levels of serum creatinine [1.35 (1.36, 1.45) mg/dL of serum creatinine in patients with signs of renal dysfunction at 1 week], still observable in five patients after 1 month [1.40 (1.34, 1.65) mg/dL], while 19 patients present signs of hepatic dysfunction [6.73 (2.48, 9.63) mg/dL of serum total bilirubine in patients with signs of hepatic dysfunction at 1 week], still persistent in seven patients after 1 month [1.88 (1.61, 2.38) mg/dL]. Likewise, white blood cells increased during first postoperative week (20/23 patients), but significantly decreased after 1 month at values comparable to those of pre-implant time (Table 3). During ICU stay, only two patients

**Table 2**
Redox, oxidative stress and inflammatory parameters among LVAD recipients, stable ESHF patients and controls.

| | Controls ($n$ = 20) | Stable ESHF patients ($n$ = 22) | LVAD recipients at pre implant time ($n$ = 23) | $P$ value for group |
|---|---|---|---|---|
| Redox state | | | | |
| r-$Cys_{pl}$, μmol/L | 8.43 (5.93, 10.58) | 9.05 (7.13, 12.22) | 9.61 (7.00, 11.50) | 0.361 |
| t-$Cys_{pl}$, μmol/L | 239 (205, 258)*,§ | 313 (282, 383) | 316 (258, 358) | <0.001 |
| r-$GSH_{pl}$, μmol/L | 2.04 (0.46, 2.65) | 1.06 (0.68, 1.46) | 1.73 (1.07, 2.28) | 0.071 |
| t-$GSH_{pl}$, μmol/L | 6.02 (4.95, 6.75)*,§ | 3.18 (2.28, 4.45) | 3.68 (2.78, 5.66) | <0.001 |
| r-$GSH_{bl}$, μmol/L | 758 (585, 948)*,§ | 557 (479, 725)* | 384 (315, 500) | <0.001 |
| t-$GSH_{bl}$, μmol/L | 968 (814, 1168)* | 891 (781, 997) | 790 (666, 915) | 0.006 |
| r/t$GSH_{pl}$ | 0.337 (0.083, 0.468)* | 0.321 (0.231, 0.427) | 0.464 (0.343, 0.631) | 0.016 |
| r/t$GSH_{bl}$ | 0.736 (0.646, 0.904)* | 0.668 (0.518, 0.793)* | 0.477 (0.418, 0.641) | <0.001 |
| GPx-3, IU/L | 621 (540, 703)* | 561 (506, 662)* | 780 (587, 981) | 0.003 |
| Oxidative stress | | | | |
| fMDA, μmol/L | 0.15 (0.11, 0.19)* | 0.17 (0.13, 0.20)* | 0.24 (0.13, 1.06) | 0.086 |
| 8-epi$PGF_{2t}$/Cr, ng/mg | 1.70 (0.22, 2.49)* | 1.87 (1.66, 3.00) | 3.49 (0.92, 9.82) | 0.045 |
| Inflammatory parameters | | | | |
| IL-6, pg/mL | 0.53 (0.0, 0.69)*,§ | 1.97 (0.91, 6.69)* | 12.8 (3.60, 37.2) | <0.001 |
| IL-8, pg/mL | 2.95 (2.50, 4.72)* | 4.40 (3.25, 7.43)* | 7.80 (5.50, 11.3) | <0.001 |
| TNF-α, pg/mL | 1.25 (0.60, 9.88)§ | 10.6 (4.68, 18.5) | 7.63 (4.55, 12.6) | 0.012 |
| CRP, mg/dL | 0.20 (0.10, 0.40)* | 0.40 (0.18, 0.63)* | 2.20 (0.40, 3.82) | <0.001 |
| Neo/Cr, μmol/mmoL | 0.12 (0.10, 0.21)*,§ | 0.25 (0.15, 0.44) | 0.37 (0.28, 0.71) | <0.001 |

Data are expressed as median and interquartile range (I, III). Bl, Blood; CRP, C-reactive protein; Cys, cysteine; GSH, glutathione; GPx-3, glutathione peroxidase type-3; IL, interleukin; fMDA, free malondialdehyde; Neo/Cr, neopterin to creatinine levels ratio; pl, plasma; r, reduced; t, total.
* $p < 0.05$ vs. LVAD recipients by post-hoc test with Bonferroni correction.
§ $p < 0.05$ vs. stable ESHF patients by post-hoc test with Bonferroni correction.

**Table 3**
Haemodynamic and clinical changes after LVAD implantation.

| | Pre-implant | 24 PL hours | 1 PL week | 1 PL month | $P$ value for time |
|---|---|---|---|---|---|
| CI, L/min/$m^2$ | 1.69 (1.37, 2.00) | 2.75 (2.25, 3.48)* | 2.95 (2.53, 3.85)* | nd | <0.001 |
| PCWP, mmHg | 26 (15, 31) | 10 (9, 17)* | 10 (8, 14)* | nd | 0.037 |
| RAP, mmHg | 6 (4, 10) | 8 (7, 10) | 7 (5, 9) | nd | 0.090 |
| $SvO_2$, % | 54 (46, 61) | 73 (69, 80) | 70 (66, 80) | nd | 0.023 |
| Diuretics, $n$ (%) | 23 (100) | 9 (39)* | 10 (44)* | 4 (17)* | <0.001 |
| Inotropic eq, $n$ | 7 (0, 10) | 8 (5, 18)* | 3 (1, 10) | 0 (0, 0)* | <0.001 |
| tSOFA-score, $n$ | 5 (3, 7) | 7 (6, 9)* | 7 (4, 9)* | 0 (0, 1)* | <0.001 |
| Creatinine, g/dL | 1.02 (0.84, 1.36) | 1.35 (0.87, 2.01)* | 1.00 (0.80, 1.35) | 0.94 (0.62, 1.14)* | <0.001 |
| WBC, $10^9$/L | 8.9 (8.2, 11.6) | 15.6 (12.7, 19.9)* | 14.5 (12.3, 16.3)* | 7.5 (6.3, 9.4) | <0.001 |

Data are expressed as median and interquartile range (I, III) or number (percentage). CI, cardiac index; PCWP, pulmonary capillary wedge pressure; PL, post-LVAD; RAP, right atrial pressure; $SvO_2$, mixed venous oxygen saturation; tSOFA, total Sequential Organ Failure Assessment; WBC, white blood cells.
* $p < 0.05$ vs. pre-implant by post-hoc test with Bonferroni correction.

**Table 4**
Morbid events during early phase of LVAD support.

| | LVAD patients |
|---|---|
| Bleeding | |
| Requiring surgery | 1 (4) |
| Requiring >2 PRBC units | 20 (87) |
| Hemorrhagie | 7 (30) |
| Embolism | 1 (4) |
| Arrhytmias | |
| Atrial | 4 (17) |
| Ventricular | 3 (13) |
| Ventricular tachycardia | 3 (13) |
| Infection | |
| Sepsis | 0 (0) |
| Local nondevice-related infection | 2 (9) |
| SIRS | 2 (9) |
| Respiratory failure | 8 (35) |
| Renal failure | 14 (61) |
| Hepatic dysfunction | 19 (83) |
| Right heart failure | 11 (48) |
| TIA | 1 (4) |
| Psychological | 3 (13) |
| Other neurological | 0 (0) |

Data are expressed as number (percentage). PRBC, packed red blood cells; SIRS, systemic inflammatory response syndrome; TIA, transient ischemic attack.

experienced infections because of decubitus wound. No patient with infections was present at 1 month.

Pre-implant tSOFA-score, index of multi-organ function grade, worsted during first postoperative week, but significantly decreased after 1 month of MCS (Table 3), with lower tSOFA-score with respect to those of stable ESHF-patients [0 (0, 1) vs. 2 (1, 2), $p = 0.025$].

### 3.4. Changes of redox state and oxidative stress after LVAD implantation

Plasma total Cys, GSH and blood total GSH levels decreased during the first month of MCS (Table 5), the former decreased to levels comparable to controls ($p = 0.513$). Blood and plasma r/tGSH ratios increased during first month of LVAD support, while GPx-3 activities decreased following LVAD implantation (Table 5).

Levels of plasma fMDA and urinary 8-epi-$PGF2_\alpha$ remained unchanged during the first month of MCS (Table 5).

### 3.5. Post-LVAD inflammatory changes

All inflammatory variables increased during the first week of MCS, with the exception for TNF-α levels (Table 5), but at 1 month,

**Table 5**
Redox, oxidative stress and inflammatory parameters after LVAD implantation.

| | Pre-implant | 24 PL hours | 1 PL week | 1 PL month | *P* value for time |
|---|---|---|---|---|---|
| Redox state | | | | | |
| r-$Cys_{pl}$, μmol/L | 9.61 (7.00, 11.50) | 11.80 (8.81, 16.46)* | 9.02 (7.60, 10.40) | 9.20 (7.90, 11.20) | 0.003 |
| t-$Cys_{pl}$, μmol/L | 316 (258, 358) | 216 (191, 304)* | 274 (230, 314)* | 240 (205, 314)* | <0.001 |
| r-$GSH_{pl}$, μmol/L | 1.73 (1.07, 2.28) | 1.76 (1.33, 2.16) | 1.66 (1.15, 2.91) | 1.62 (1.30, 2.51) | 0.168 |
| t-$GSH_{pl}$, μmol/L | 3.68 (2.78, 5.66) | 3.64 (2.81, 5.13) | 2.31 (1.79, 3.99) | 2.81 (1.64, 3.63)* | 0.034 |
| r-$GSH_{bl}$, μmol/L | 384 (315, 500) | 351 (295, 453) | 374 (310, 433) | 421 (307, 592) | 0.241 |
| t-$GSH_{bl}$, μmol/L | 790 (666, 915) | 754 (673, 856) | 630 (542, 736)* | 627 (548, 734)* | <0.001 |
| r/t$GSH_{pl}$ | 0.464 (0.343, 0.631) | 0.444 (0.337, 0.673) | 0.642 (0.378, 0.894)* | 0.716 (0.494, 0.970)* | 0.003 |
| r/t$GSH_{bl}$ | 0.477 (0.418, 0.641) | 0.499 (0.404, 0.566) | 0.587 (0.490, 0.667) | 0.685 (0.557, 0.799)* | <0.001 |
| GPx-3, IU/L | 780 (587, 981) | 647 (555, 832)* | 711 (511, 908) | 627 (450, 772)* | 0.003 |
| Oxidative stress | | | | | |
| fMDA, μmol/L | 0.24 (0.13, 1.06) | 0.35 (0.16, 0.99) | 0.50 (0.19, 0.87) | 0.43 (0.18, 1.30) | 0.398 |
| 8-epi$PGF_{2t}$/Cr, ng/mg | 3.49 (0.92, 9.82) | 2.89 (0.74, 6.01) | 4.43 (1.24, 16.80) | 2.85 (1.44, 8.48) | 0.235 |
| Inflammatory parameters | | | | | |
| IL-6, pg/mL | 12.8 (3.6, 37.2) | 376.5 (177.4, 590.4)* | 61.0 (30.3, 123.3) | 20.2 (8.2, 30.6) | <0.001 |
| IL-8, pg/mL | 7.8 (5.5, 11.3) | 20.1 (11.7, 35.9)* | 19.3 (16.0, 36.5)* | 10.4 (8.4, 14.0) | <0.001 |
| TNF-α, pg/mL | 7.63 (4.55, 12.9) | 9.75 (3.00, 19.8) | 6.50 (3.08, 12.8) | 9.95 (6.75, 13.3) | 0.682 |
| CRP, mg/dL | 2.2 (0.4, 3.8) | 16.7 (11.6, 22.4)* | 10.2 (4.7, 13.9)* | 2.4 (1.3, 3.6) | <0.001 |
| Neo/Cr, μmol/mmol | 0.37 (0.28, 0.71) | 0.49 (0.35, 0.74) | 0.74 (0.53, 1.02)* | 0.97 (0.49, 1.71)* | <0.001 |

Data are expressed as median and interquartile range (I, III). For abbreviations see Tables 2 and 3.
* $p < 0.05$ vs. pre-implant by post-hoc test with Bonferroni correction.

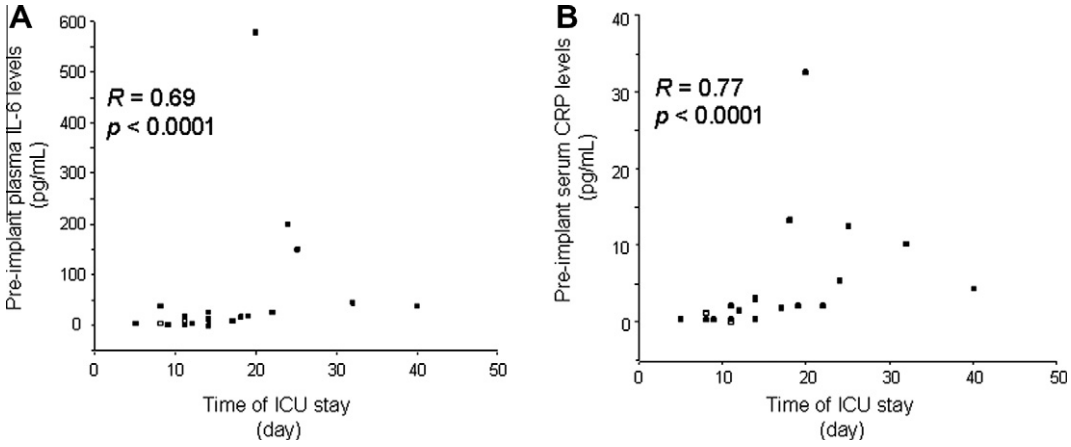


**Fig. 1.** Correlations of duration of ICU stay with pre-implant plasma IL-6 (A), and serum CRP (B) levels in LVAD patients.

their levels returned comparable to pre-implant values. Differently, urinary Neo/Cr levels progressively increased during LVAD support with higher levels at 1 month with respect to pre-implant values (Table 5).

### *3.6. Correlations with tSOFA-score and with duration of ICU stay in LVAD-patients*

Among biomarkers, tSOFA-score was positively correlated with plasma IL-6 ($R = 0.64$, $p < 0.0001$), IL-8 ($R = 0.54$, $p < 0.0001$), and serum CRP ($R = 0.63$, $p < 0.0001$) levels.

On the other hand, duration of ICU stay of LVAD patients was positively correlated with tSOFA score, assessed both at pre-implant time ($R = 0.57$, $p = 0.005$) and at first postoperative week ($R = 0.82$, $p < 0.0001$). Among biomarkers, duration of ICU stay was positively correlated with pre-implant levels of plasma IL-6 and serum CRP (Fig. 1). Likewise plasma IL-8 levels, measured after 24 h following LVAD implantation, positively correlated with duration of ICU stay ($R = 0.66$, $p = 0.001$).

### *3.7. Outcomes*

At 1 year following LVAD implantation, eight of the 23 enrolled LVAD patients were bridged to HT, four died while on the device [three died because of cerebral hemorrhage and one because of multi-organ failure syndrome (MOFS)] and one was weaned off device due to cardiac recovery (after 192 days from LVAD implantation). The other patients were awaiting for HT.

## 4. Discussion

The present study shows that advanced HF patients are characterized by a worsening of redox state and systemic inflammation, with concomitant increase of oxidative stress and monocyte activation. These alterations are more aggravate in ESHF patients with hemodynamic instability needing an LVAD implantation as BTT, when compared to stable ESHF patients. However, the severity of inflammatory milieu and oxidative stress present in LVAD recipients persist after 1 month of LVAD support, with further activation of monocytes, even though adequate hemodynamic recovery and amelioration of multi-organ function by LVAD implant. Early post-operative changes of multi-organ function resulted related with IL-6 and IL-8 level flotation, while duration of ICU stay related with pre-implant IL-6 and CRP levels.

Increasing evidences indicate oxidative stress and altered inflammatory processes as potential mechanisms involved in the pathogenesis and clinical course of chronic HF [7, 8, 21]. Elevated TNF-α and IL-6 levels were reported in patients who required

LVAD assistance compared with patients with less severe HF [22]. In agreement with these findings, in our ESHF patients needing LVAD implantation the levels of pro-inflammation cytokines were markedly higher when compared to patients with stable ESHF. Only TNF-α levels were comparable between patients with different severity of disease. Our LVAD recipients showed even elevated levels of IL-8, a neutrophil- and macrophage-attracting chemokine on endothelial cells, and neopterin, a pteridine-derivative produced by activated monocytes. These data support that monocyte activation is implicated in the hemodynamic instability of end-stage phase of HF. Moreover, also the grade of systemic oxidative stress was markedly elevated in LVAD recipients compared to stable ESHF patients. In particular, systemic oxidative stress seems to involve membrane damage since isoprostane 8-epi$PGF_2$ is a prostaglandin-like compound, synthesized through the free radical catalyzed peroxidation of arachidonic acid, while MDA is a marker of membrane damage due to peroxidation of polyunsaturated lipids. In addition, our LVAD recipients showed even a worsening of systemic redox state with lower GSH bioavailability with respect to stable ESHF patients. Altogether these findings indicate that end-stage HF disarrangement (acute hemodynamic instability and multi-organ function collapse), that addressed to LVAD placement, is properly characterized by intensification of oxidative stress and pro-inflammatory milieu.

The LVAD implantation has become an effective therapeutic option for hemodynamic recovery of advanced HF patients, but post-implant morbid events impact on successful outcome and on clinical course of patients [1]. Several studies have focused in findings the right indications and timing for LVAD implant to minimize postoperative adverse events, mainly evaluating clinical and hemodynamic variables [23–26]. The MCS seems to be associated to a better prognosis, even as functional recovery, when LV-unloading, attenuating LV wall stress, and the restoration of a normal neurohormonal milieu are obtained [3]. The inflammatory network activated following LVAD implantation have been reported to play an important role in the development of MOFS [19, 27], supporting that the attenuation or normalization of inflammatory state by hemodynamic recovery is a condition for favorable outcome. In this context the search for useful systemic biomarkers to predict the main complications that occur during MCS is generating interest.

In our patients supported by LVAD, the high pre-implant levels of inflammatory mediators further increased during the first week of LVAD support, with marked IL-6 and CRP levels, probably due to the impact of surgery and cardiopulmonary by-pass. Moreover, the activation of IL-6–CRP pathway probably stimulates the increased expression of chemokine IL-8 and the further activation of monocytes, as evidenced by profile of neopterin during the first month of LVAD. Indeed neopterin is a known pteridine mainly synthesized by macrophages and monocytes at levels reflecting their degree of activation [28]. In this phase of LVAD support these elevated levels of pro-inflammatory mediators might promotes vascular inflammation, a mechanism also demonstrated in animal models [29], favoring the development of morbid events. The relationship found between tSOFA score and IL-6 levels suggests a correlation between IL-6-dependent signaling pathways, their degree of activation and change of overall organ function during MCS. Indeed, in our LVAD recipients, the early postoperative phase was characterized by deterioration of multi-organ function, as evidenced by high tSOFA score, and complications observed during ICU stay. Moreover, the length of stay in ICU, that reflects the clinical course, was found related with pre-implant levels of IL-6, CRP, tSOFA score and, at 24 post-LVAD hours, also with the levels of IL-8. Although requiring validation in a larger series, these data support a role of inflammatory milieu and grade of multi-organ dysfunction, at pre-implant, and of consequent inflammatory post-LVAD response on the onset of morbid events and their severity, factors that together might affect the duration of hospitalization. Likewise, postoperative vasodilatation and hemodynamic instability were observed also in patients undergoing elective coronary artery by-pass grafting with increased circulating levels of IL-6 and IL-8 [30].

In a previous study [19] we reported that LVAD patients who died because of adverse MOFS during early MCS showed levels of pro-inflammatory cytokines and tSOFA score much higher than complication-free LVAD-patients. Altogether these data suggest that the severity of end-organ dysfunction reflects the grade of inflammatory response. Patients with less aggressive inflammatory response showed minor and transitory complications, as observed in our patient series.

After 1 month of LVAD support, the inflammatory status and oxidative stress in our patients maintain levels comparable to those pre-intervention, representing an unfavorable condition potentially affecting the long-term outcome. Moreover, the progressive increment of monocyte activation, as found in our LVAD-patients, might contribute for late thromboembolic events, a condition that might be enhanced also by pro-inflammatory stimuli [31]. Note that at the same time multi-organ function was already improved to physiological condition with respect to pre-implant time. Our findings support the hypothesis that LV-unloading and hemodynamic recovery after 1 month of MCS do not attenuate the levels of pro-inflammatory cytokines and oxidative stress implicated in the terminal phase of HF. These data are in agreement with previous report that found TNF-α and IL-6 levels after 3 months of MCS comparable with those at pre-implant time [32]. Only circulating redox state was ameliorated by LVAD. Indeed, increase in GSH bioavailability, normalization of Cys levels and decrease of GPx-3 activity, observed during 1 month of MCS, are evidences of the establishment of the partial restoration of a balanced redox state.

The sources of cytokine production in HF are likely multiple and include the immune system, peripheral tissues, and the failing heart itself. The amelioration of multi-organ function observed at 1 month by LVAD seems to exclude an involvement of peripheral tissues as sources of cytokine and production of reactive oxygen species. The decrease of myocardial TNF-α in LVAD patients that experienced cardiac functional recovery suggests the heart as a source of inflammatory activation and its reduction as signal associated with the inverse remodeling [33]. However, true myocardial recovery is very uncommon and, when it occurs, it takes weeks to months to reach a level that allows LVAD removal. Only in one of our LVAD patients, hemodynamic improvement allowed for weaning from the device, but after long-term MCS. Therefore, the persistence of elevated levels of pro-inflammatory cytokine and of oxidative stress at 1 month is probably imputable to failing heart and immune system, as potential sources of production of inflammatory cytokines and reactive oxygen species, not still altered by early phase of LVAD support. However, future studies are recommended to evaluate the effect of LVAD implant on inflammation and oxidative stress in a longer time of ventricular unloading.

It is noteworthy that levels of TNF-α, differently from those of the other inflammatory mediators, were unchanged during the overall period of the first month of LVAD. This finding suggests that circulate TNF-α levels, in LVAD patients, are not affected by peripheral tissues or immune system potentially implicated into transitory and reversible multi-organ dysfunction, as observed in the early phase of LVAD support.

## 5. Conclusions

In terminal ESHF patients, hemodynamic recovery, LV unloading and amelioration of multi-organ function obtained following

1 month of MCS did not improve the grade of oxidative stress and severe inflammatory milieu pre-existent at pre-implant. Pre-implant levels of IL-6 may contribute to worsening of multi-organ function during early phase of MCS, and to a worse duration of ICU stay, as well as the postoperative change of IL-8. Thus, further studies with longer monitoring period are needed to evaluate whether and to what degree LVAD reduces both oxidative stress and expression of pro-inflammatory cytokines, potential signs of adverse remodeling.

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