ORIGINAL ARTICLE

Novel potential biomarkers with predictive power on response to therapy in cardiac resynchronization therapy – cardiac oxidative stress assesment

Sergiu Sipos[1], Radu Ciudin[1], Corina Grigore[2], Carmen Ginghina[1]

Abstract: CRT represents the transition from the heart rhythm therapy, started more than 60 years ago with the first pacemakers, to the optimization therapy of myocardial contractility in heart failure. It is estimated that about a quarter of the population of patients with heart failure have electrical and mechanical criteria for cardiac asynchrony. They are the target of resynchronization therapy. The current indications for resynchronization therapy use basic selection criteria, without having high predictive power in terms of response to treatment. About one-third of patients undergoing resynchronization are found to be non-responsive to therapy. In this study we tested a new direction in our effort to increase the number of post-resynchronization beneficiaries, using markers of oxidative stress in patients with heart failure, assessed before and after intervention, with promising results.
Keywords: heart failure, cardiac resynchronization therapy (CRT), response to therapy, oxidative stress.

Rezumat: Terapia de resincronizare cardiacă (CRT) reprezintă trecerea de la terapia ritmului cardiac, începută acum mai bine de 60 de ani cu primele stimulatoare cardiace, la terapia de optimizare a contractilității miocardice în insuficiența cardiacă. Se estimează că aproximativ un sfert din populația pacienților cu insuficiență cardiacă prezintă criterii electrice și mecanice de asincronism cardiac. Aceștia reprezintă ținta terapiei de resincronizare. Indicațiile actuale ale terapiei de resincronizare folosesc criterii bazale de selectie, fără putere predictivă mare în ceea ce privește răspunsul la tratament. Aproximativ o treime dintre pacienții supuși procedurii de resincronizare se dovedesc a fi non-responderi la terapie. În acest studiu am testat o nouă direcție în încercarea de a crește numărul beneficiarilor post-resincronizare, apelând la markeri ai stresului oxidativ, înainte și după implant, la pacienți cu insuficiență cardiacă, cu obținerea unor rezultate promițătoare.
Cuvinte cheie: insuficiență cardiacă, terapie de resincronizare cardiacă, răspuns la terapie, stres oxidativ.

INTRODUCTION

Over the past few decades, clinical and experimental studies have provided substantial evidence that oxidative stress, defined as an overproduction of reactive oxygen species (ROS) relative to the body's antioxidant capacity, has implications for HF[1,2,3,4,8,9,10,12,13,14]. Excess ROS causes cellular dysfunction, protein and lipid peroxidation[8], and DNA damage and can lead to irreversible damage and cell death (processes described in a wide range of cardiovascular diseases). The importance of oxidative stress is increasingly emphasized in terms of a physiopathological mechanism of cardiac remodeling, responsible for the emergence and progression of IC[15]. Specifically, ROS can directly affect contractile function, modifying proteins that play a role in excitation-contraction coupling[16]. Furthermore, ROS activates a wide variety of kinases with a role in signaling myocyte hypertrophy, transcription factors and mediated apoptosis[17]. It also stimulates the proliferation of cardiac fibroblast and activation of matrix-metalloproteinases (MMP), which leads to the remodeling of the extracellular matrix[18]. These cellular events are involved in the emergence, progression, remodeling and, finally, in the working myocardium dysfunction. The balance between ROS production and their removal from the body by using specific antioxidant mechanisms defines the „redox" system. Increased oxidative stress is defined as an excess production of ROS relative to levels of antioxidants. ROS are hi-

[1] „Prof. Dr. C.C. Iliescu" Emergency Institute for Cardiovascular Diseases, „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2] „Coltea" Clinical Hospital, „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

Contact address:
Sergiu Sipos, MD, „Prof. Dr. C.C. Iliescu" Emergency Institute for Cardiovascular Diseases, „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.
E-mail: sersip@yahoo.com

ghly reactive chemical oxygen species. Their representatives are free radicals, such as superoxide (O_2 -•), hydroxyl radical (- OH) and nonhydroxyl, capable of generating free dangerous radicals, such as hydrogen peroxide (H_2O_2)[10,11,12,18] All of these known theoretical aspects can be used for better defining heart failure patients and could be of good use when selecting the best suited candidates for CRT.

OBJECTIVES

The aim of the study was to evaluate the total antioxidant capacity and the degree of damage of the DNA structure using specific biochemical markers 8-hydroxy 2'-deoxyguanosine (8-OHdG) and total antioxidant capacity (TAC)) in patients with low EF <35% and indication for CRT, in 3 distinct steps: sample 1 collected from peripheral blood at the time of resynchronization; sample 2 collected from the blood taken from the coronary sinus at the time of cardiac resynchronization; sample 3 collected from peripheral blood 6-9 months after intervention. The basis for our research was the behavior of the REDOX system in patients with heart failure[15,16,19]. There is a growing interest in literature for assessing the role played by oxidative stress agents in promoting organ damage, but also in determining the oxidative stress as a marker of the target organ injury severity - in our case the heart[17,20]. Publications we have studied conclude that there is a relationship of direct proportionality between the degree of heart failure and the level of these plasma quantifiable markers[11-16]. On the other hand, the vast majority of data show the low discrimination between oxidative stress markers of cardiac or systemic origin[7,8]. In this regard, we considered appropriate to evaluate the markers of oxidative stress in both peripheral venous blood and the central venous blood (during the implant, from the coronary venous sinus). From our observations, a single study published in Circulation in 2015 (please note that at that time our study was already underway) analyzed the levels of oxidative agents (oxidized mitochondrial RNA of myocyte origin) sampled directly from the coronary sinus vs. the same marker extracted from peripheral blood, with promising results[2]. Other research looked at markers of oxidative stress extracted only from peripheral venous blood[5,6,7]. Our working hypothesis was based on these observations - that the level of oxidative stress is higher in patients with advanced heart failure. As such, we chose the quantification of 8-hydroxy 2'-deoxyguanosine (DNAd 8-OHdG) as an marker of oxidized myocytic DNA and we used the evaluation of total antioxidant capacity (TAC) as a measure to verify the former, well knowing that oxidative systems and antioxidants are complementary in the human body. Thus, any therapeutic intervention with impact on these patients (CRT in our case) would, on the one hand, influence the values of these biomarkers and on the other hand, it could even identify the profile of the future CRT responders.

METHODS

This prospective, dynamic, non-randomized clinical study enrolled 29 heart failure patients. These patients were admitted between 2013-2015 in the Emergency Institute for Cardiovascular Diseases Prof. Dr. "C.C. Iliescu" - Bucharest, with indication of cardiac resynchronization. There were 22 (75.86%) men and 7 (24.14%) women, with an average age of 64. Heart failure causes, for these patients, were divided into ischemic dilatative cardiomyopathy (DCM) and non-ischemic DCM. This study involved human subjects and was approved by the Ethics Committee of the Emergency Institute for Cardiovascular Disease Prof. Dr. "C.C. Iliescu" - Bucharest. All clinical investigations were performed in accordance with the principles set out in the Declaration of Helsinki and patients were included in the study after signing the informed consent. The enrolled patients were implanted in the department of Cardiac Electrophysiology and Pacemakers within the Emergency Institute for Cardiovascular Diseases "Prof. Dr. CC Iliescu" in Bucharest, according to the present European CRT guidelines. The implant procedure was performed following the operating protocol of our Electrophysiology Department, with the goal of obtaining the optimal position of the LV lead in respect to the individual anatomical variability. No significant procedural complications were reported during implantation. During the CRT procedure, blood samples were taken simultaneously from peripheral vein and from venous coronary sinus (CS). The third blood sample was obtained after 6 to 9 months during follow-up. We took into account the absence of obvious clinical signs and symptoms of infection as well as the absence of the infectious biological picture at the time of the oxidative stress biological samples collection (for all samples). We grouped the clinical and laboratory parameters of the diagnosis, as well as those related to the procedure into 3 categories:

1) Pre-procedural parameters: NYHA functional class, 6 minutes walking test, comorbidities, opti-

mal drug therapy, chest X-Ray, EKG, biochemistry, echocardiography analysis;

Echocardiography analysis:

- severity parameters: FE, degree of mitral regurgitation, VTDVS, AS dimensions;
- mechanical asynchronism data (presence of AV, VV, intra-ventricular asynchronism parameters);
- others: echogenicity of LV walls, regional kinetic disorders / affected territory;

2) Intra-procedural parameters

Device type:

- CRT-P (- defibrillator)
- CRT-D (+ defibrillator)

LV lead positioning: lateral, postero-lateral, inferior or anterior wall

3) Post-procedural parameters

Device programming:

Programming mode: VVI / VVIR; DDD / DDDR; DDI / DDIR

A-V interval: 80-90-100-110-120-140 ms

V-V interval: 0-10-20-30-40-50-60-70-80 ms

Scheduled frequency: 50-55-60-65-70-75-80 / min

Re-evaluation of biochemical, ECG and echocardiographic parameters both immediately after implantation and in dynamics during the study.

Blood samples were collected in sterile vacutainers without anticoagulant. Maximum 3 hours after sampling, the blood was centrifuged at 3000 rpm for 10 minutes to obtain the serum. The serum was immediately separated, brought into Eppendorf tubes and stored at -80° C until determinations were made. After thawing the serum and bringing it to room temperature, we performed the actual analisis by determining TAC and 8-OHdG biomarkers by ELISA (Enzyme-linked immunosorbent assay). The kits used in this experiment are from Abcam, USA.

As mentioned, the aim of the study was to evaluate the total antioxidant capacity and the degree of damage to the DNA structure (using 8-hydroxy 2'-deoxyguanosine (8-OHdG) as a specific biochemical marker) in patients with HF with low FE (<35%) and indication for cardiac resynchronization, in 3 distinct stages: Sample 1 from peripheral blood at resynchronization; Sample 2 of blood collected from the coronary sinus at the time of cardiac resynchronization; Sample 3 of peripheral blood at 6-9 months post-intervention.

Oxidative stress determinations were performed by the competitive ELISA method: OxiSelect™ Oxidative DNA Damage ELISA Kit - determination of 8-hydroxydeoxyguanosine (8-OHdG) as a marker of oxidative DNA damage. OxiSelect™ Total Antioxidant Capacity (TAC) - determination of total antioxidant capacity, based on the reduction of copper (II) to copper (I) by plasma antioxidants. Chemwell 2010 EIA and Chemistry Analyzer Automated Analyzer (Awarness Technology INC, USA) was used.

Statistical analysis

The statistical programs SPSS (Statistical Package for the Social Sciences), STATISTICA and, respectively, Microsoft Excel were used for the statistical analysis of the data. The following parameters were determined: arithmetic mean of the values in the data string; standard deviation (SD); variance (how the values in the data group are divided around the average value); probability (p); Pearson correlation coefficient.

In the case of the data groups with normal dispersion, the Student's and ANOVA's tests were used. The statistical interpretation of the analyzed results was the following:

if $p < 0.05$, the difference is significant;

if $p < 0.01$, the difference is highly significant;

if $p < 0.001$, the difference is very high significant;

if $p > 0.05$, the difference is insignificant.

RESULTS

Characterization of the studied population

This prospective, dynamic, non-randomized clinical study was attended by 31 patients admitted between 2013-2015 at the Emergency Institute for Cardiovascular Diseases Prof. Dr. "C.C. Iliescu" - Bucharest, with indication of cardiac resynchronization, with an average age of 64. It should be noted that two patients were excluded from the study for reasons related to the methodology of biology products processing - hemolyzed blood that did not allow efficient centrifugation.

Regarding sex distribution, most of the enrolled patients are male (Figure 1), which is explained partially by the higher incidence of ischemic DCM among males and also by the higher incidence of DCM in general, in males (67-72% male prevalence in different studies[21,22,23,24,26,29]).

Most patients prove to be from urban areas (58%), have non-ischemic DCM (84%) and have significant co-morbidities, in this order: hypertension (HBP), dyslipidemia, diabetes (DM), chronic kidney disease. Four patients had chronic lung disease.

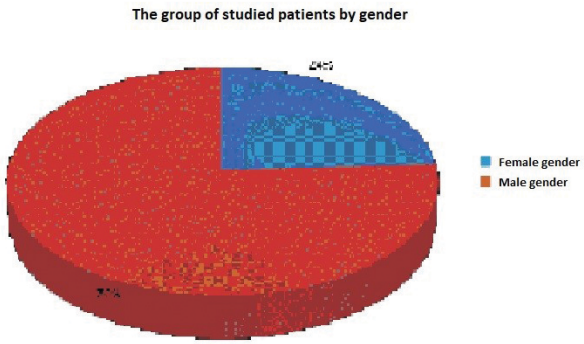

Figure 1.

In terms of clinical condition, the majority (78%) had ICC class III NYHA and 6 (20%) of the 29 patients were in atrial fibrillation (AF).

With the exception of one patient, all others had optimal drug therapy.

In terms of EKG, 4 of the 29 patients had nonspecific intra-ventricular conduction disorder (NIVCD) and one had iatrogenic LBBB (VVI pacing a few years back).

The mean duration of QRS was 162 ms, a single patient had a rather short QRS duration of 120 ms, all the others seemed to have durations over 130 ms.

From the echocardiography point of view, we note the presence of an average end-diastolic volume of 137 ml, an average left atrium diameter of 47.8 mm, the presence of grade III or IV mitral valve regurgitation in 70% of subjects and increased pulmonary artery pressure (PAPs over 40 mmHg) in 95% of patients. 22% had type I diastolic dysfunction, 67% restrictive type and 11% pseudo-normal type. Regarding the analysis of mechanical desynchrony, 55% of the patients had apical rocking present at the time of inclusion in the study; 88% had diffuse hypokinesia of LV walls, the others had segmental kinetics disorders.

The ejection fraction (EF), as the main echocardiographic parameter for inclusion in the study was initially evaluated as 25 ± 8.31 (SD), and in follow-up 27 ± 9.22 (SD) (Figure 2).

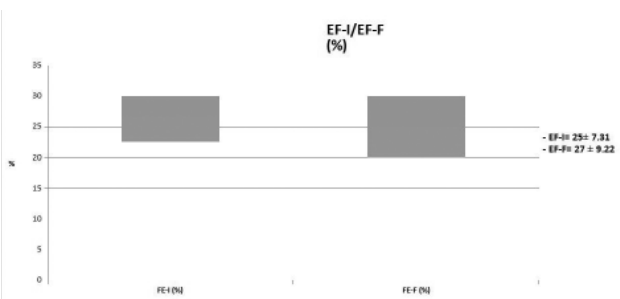

Figure 2. The mean initial EF and the later EF do no differ very much due to the decrease of value in the non-responder group, during follow-up.

Regarding the type of implanted device, 33% (10 subjects) received a CRT-D device, the rest of them CRT-P.

The location of the LV lead was in 47% of cases at the mid posterior-lateral wall, 21% had a basal posterior-lateral position, 5% apical posterior-lateral wall, 12% lateral basal wall, 7% mid lateral wall, 3% anterior-lateral wall and 5% inferior wall.

Device programming was performed in DDD (R) mode in 80% of patients and VVI (R) in 20% (those with AF). The average programmed heart rate (Lower rate interval) was 55 bpm. The mean programmed AV interval was 105 ms and the mean inter-ventricular interval was 35 ms.

Early post-implant cardiac ultrasound optimization control was performed in all patients, aiming especially the optimization of LV filling and abolishing of the apical rocking.

We can summarize our patients profile as follows: Male patient, from urban environment, around 65 years old, with non-ischaemic DCM, with a mean ejection fraction of 25%, in sinus rhythm, with complete LBBB of 160 ms duration, cls III NYHA and under optimal medical treatment. "Our patient" received a CRT-P type device with the left ventricle lead being placed in a posterior or lateral position with optimal pacing parameters. The device was programmed in DDD mode, with a short AV delay of 100 ms and a delay of 30 ms between left and right lead.

Regarding laboratory testing, it is important to emphasize that there was no anemia either before or after the implant. Neither the serum glucose nor the lipid profile parameters showed any significant statistically differences between the 2 distinct moments in time in which the evaluation was performed. The same observation was noticed in the case of hepatic, renal and other hematological parameters.

Uric acid is a clinical parameter of oxidative stress[25,26,30]. The plasma concentration of this compound, determined at the time of cardiac resynchronization collected from peripheral blood, was slightly increased compared to the concentration determined at 6 months post-intervention, but without statistical significance (Figure 3).

As can be seen from the table below (Figure 4), the patients included in the study have slightly to moderate impaired renal function, the level of creatinine clearance being generally above 50 ml/min. We do not notice significant differences between the sexes.

Biological profile		
Laboratory tested parameter (mean ± SD)	**Peripheral blood (at the time of CRT implant)**	**Peripheral blood (at 6-9 months after the implant)**
Hemoglobin (mg / dL)	13,75 ± 1,49	13,55 ± 1,77
Hematocrit (%)	40,55 ± 4,07	39,75 ± 4,85
Blood glucose (mg / dL)	102±23,35	102,5±33,39
HDL (mg / dL)	42 ± 14,73	42 ± 17,56
LDL (mg / dL)	88,5 ± 44,11	87,25± 39,51
BT (mg / dL)	128 ± 59,84	106,5 ± 53,46
Total cholesterol (mg / dL)	151 ± 47,56	155 ± 50,13
ALT (U / L)	21,25±11,53	18,2±13,5
AST (U / L)	20,05±4,58	20,5±8,72
Uric acid (mg / dL)	7,32±2,46	7,05±1,87
Urea (mg / dL)	51±29,03	51±32,95
Creatinine clearance (mL / min / 1.73m^2)	53±23,38	53±23,32

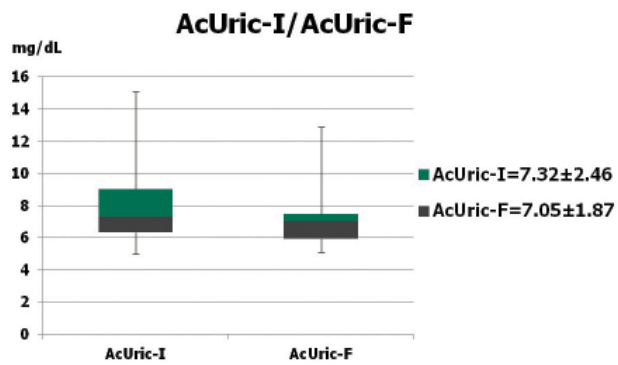

Figure 3. Dynamics of serum uric acid levels in the studied patient group.

Study of the status of the responder / non-responder to cardiac resynchronization therapy

The chosen criterion for considering a patient as a responder to CRT was a post-interventional increase of the left ventricle ejection fraction (LV) EF, with at least 5%, determined by cardiac ultrasound. However, the definition of responders vs. non-responders after cardiac resynchronization therapy is a non-standardized assessment in literature[16,18,27,28] and should take into account several clinical and laboratory elements, such as those listed below:

- „surrogate" clinical objectives: NYHA functional class, 6MWT, quality of life;
- „hard" clinical objectives: morbidity / mortality indices;
- reverse-remodeling echocardiography parameters: EF, EndSystolic/Diastolic LV volume, Mitral valve regurgitation, left atrium volume, mechanical asynchrony parameters (assessed by ultrasound, cardiac MRi, or other methods[29,30,31,32]).

Nevertheless, most of the authors of the major impact relevant studies use the dynamics of the echocardiographically evaluated LV ejection fraction. This is seen as a raw parameter easier to assess in daily practice[16,18,22,43].

Applying this echocardiographic parameter, to which we added the need of at least one NYHA class unit decrease in terms of clinical benefit, we assessed a number of 15 patients as clear responses to therapy (52% of all patients included in the study).What is particularly noteworthy is the fact that 4 of the total of 6 patients with AF, undergoing the intervention, proved to be respondents, which, within the limits of this small group of patients, brings a favorable argument for the inclusion of these patients in the group of those proposed for CRT. It is worth mentioning the unclear and sometimes contradictory data seen in literature[1,33,34,35] regarding this subject.

Regarding the morbidity / mortality indices, during the follow-up period the average number of hospitalizations was 3, which corresponds to a number of 1 hospitalization / year; as expected, a higher number of hospitalizations was noted in the case of patients with complicated evolution (observing a maximum of 7 hospitalizations / 3 years of follow-up).

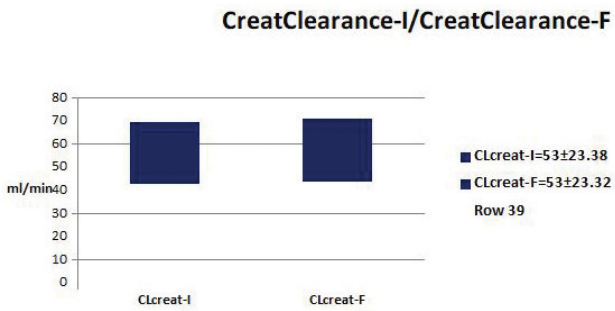

Figure 4. Renal function balance at the moment of the implant and at follow-up.

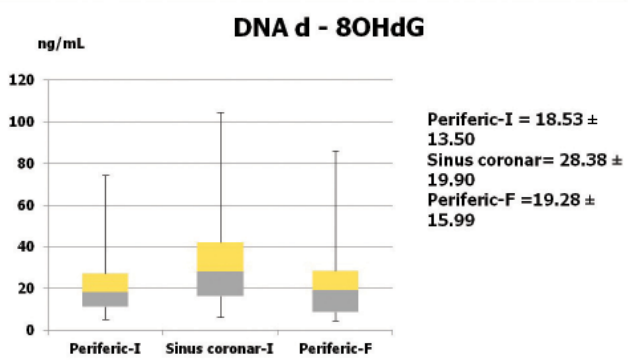

Figure 5. The results of the plasmatic levels of oxidized DNA, according to the 3 distinct moments of sampeling (periferic blood, coronary sinus, and periferic blood after 6-9 months).

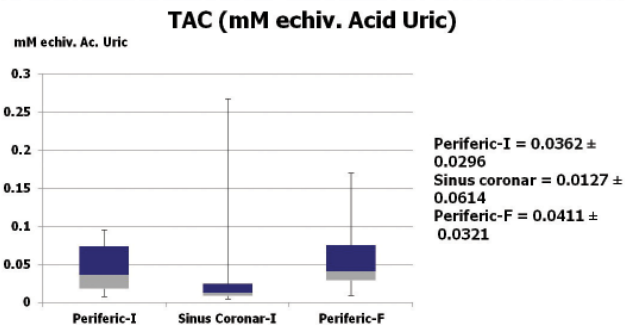

Figure 6. TAC assessment at the 3 moments of sampling.

In our group of patients, 3 died by the end of the follow-up period. One of them was part of the group of respondents.

Evaluation of oxidative stress biomarkers in the studied population

8-hydroxydeoxyguanosine (8-OHdG) is a marker of oxidative DNA damage. In the study group, we notice that the lowest 8-OHdG value was measured in the peripherally collected blood at the moment of resynchronization. The value increases slightly in peripherally collected blood at 6 months post-intervention. Oxidized DNA is significantly increased ($p < 0.05$) in the blood collected from the coronary sinus at the time of surgery, meaning increased oxidative stress at the time of surgery (Figure 5).

The total antioxidant capacity (TAC) collected from the coronary sinus has the lowest value compared to the blood collected from the periphery before and at 6 months post-intervention, correlating with the increased value of oxidized DNA (Figure 6).

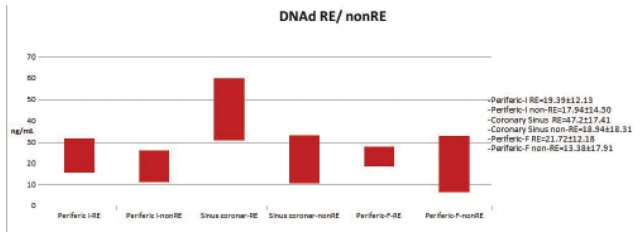

Figure 7. Plasma levels of oxidized DNA in patients with HF and CRT-responders and non-responders.

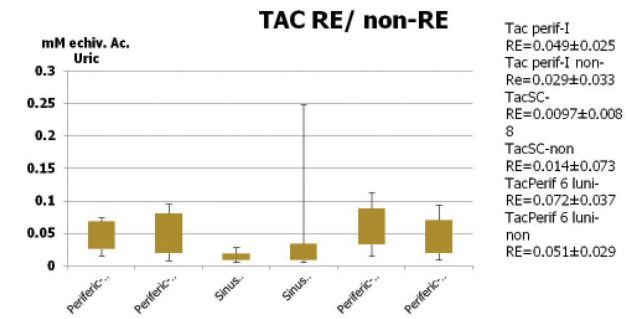

Figure 8. Total antioxidant capacity in responders and non-responders to CRT.

Oxidized DNA measured in blood collected from the coronary sinus has a statistically significant increased value in the future responder patients ($p < 0.01$). Oxidized DNA collected from the coronary sinus from non-responder patients does not differ statistically significantly from oxidized DNA collected from peripheral blood (Figure 7).

The total antioxidant capacity in the blood collected from the coronary sinus in future responder patients has a significantly lower value compared to non-responders and with the initial and final evaluations of peripheral blood. This low value is inversely proportional to the value of oxidized DNA in future responder patients (Figure 8).

In this study we described a novel bio-marker – in terms of extracellular damaged DNA – with possible impact in the CRT candidate selection. In the studied cohort, in the case of responders group (defined by more than 5% improvement of the EF and one NYHA class downgrading), we notice high levels of damaged DNA of cardiac origin (sampled from the coronary sinus blood). We would like to emphasize that this clearly separates this group from all the other groups of patients for whom 8-OHdG was measured and analyzed. DNAd 8-OHdG was independently related with response to device therapy, having superior dis-

criminative ability in comparison to other usual clinical variables (such as cardiac ultrasound characteristics or EKG measurements). The use of TAC was intended as a checking method for the DNAd 8-OHdG assessment, as it is well known that in REDOX system there is a balance between cellular and extracellular oxidative and antioxidative agents[15,18,20,35,36]. When misbalance occurs, this is due to insufficient levels of the antioxidative system agents or to the presence of high, overwhelming, pro-oxidative reactions[11,14,23,25,37,38]. Either-way it is expected to see a laboratory picture of high oxidative agents and low antioxidative capacities when disbalance occurs. Our results are clearly pointing to this. There is a clear correlation between high levels of oxidized products in the CS plasma and low values of TAC in the responder group, ensuring a good validation of the lab measurements we performed.

The use of certain biomarkers to predict response to therapy represents a cornerstone in modern electrophysiology struggle[39,40]. It is estimated that up to one third of CRT receivers fail to respond to therapy. Conventional prediction criteria (echo, ECG, other imaging methods) failed to improve the response rate to CRT[1,6,8]. In our extended framework, the newly considered biomarker - DNAd 8-OHdG determined in the blood of the coronary sinus - if validated in a large cohort study, could be a response to the unreliable selection of the CRT candidates we currently use[41,42]. Peripheral assessment of damaged DNA proved to be of no use, mainly because of its mixture with oxidated DNA of other origin. For practical reasons, this should not be a major inconvenient, if we take into account that all patients evaluated for the causes of DCM and all the candidates for CRT do benefit from coronary angiography prior to implant. At this moment, we could add to the procedure, the catheterization of CS. A blood sample could be obtained in this way and assessed for damaged DNA. We could, thus, avoid deploying an expensive device to a patient who will not benefit from it, or even worse, which could bring additional procedural risks and long term complications. The results of our study should be interpreted within the boundaries of its design. Human sample size was not sufficient to yield 80% power, except for large effects, or to provide adequate precision for odds ratio estimates. As a consequence of limited data, we used left ventricular ejection fraction as the major criteria to define response instead of more elaborate parameters as left atrium enlargement, end-systolic and end-diastolic volumes, mitral valve regurgitation and other reverse-remodeling parameters. Because of the limited number of patients, more refined clinical aspects regarding hospitalization or deaths in high damaged DNA patients, are difficult to be formulated, but a direct relationship between this patients, their positive response to CRT and a reduced number of hospitalization is clearly distinguishable in our cohort. Of course, many scientific questions, including the best method of plasma DNAd 8-OHdG assessment, techniques of normalization, and reference profiles in healthy individuals, need clarification before bringing this novel biomarker into the clinical practice. Our data supports the hypothesis established by recent studies that clinically useful extracellular damaged DNA or RNA may be functionally implicated in heart failure pathogenesis[1,2]. Oxidized DNA or mRNA appears to be dynamically regulated by the mechanical stretch of the cardiomyocite on the one hand and the number of viable cardiac cells on the other[4,5]. In other words the amount of oxidized products determined in the cardiac venous blood is dependent of the contractile reserve and the amount of cardiac fibrosis. The fewer the viable cardiomycites, the less measurable is the damaged DNA in cardiac extracted plasma[4,5]. These patients are less likely to reverse-remodel and consequently are candidates for non-response to CRT[43]. Of course, these are mainly theoretical hypothesis that need further research. The scientific literature regarding these subjects is quite poor. Using the widely used scientific search engines, we were able to identify a relatively small number of articles regarding oxidative stress in heart failure with less than 5 publications regarding this topic and CRT. Among those, only a few speak about the assessment of oxidative compounds in arterial coronary blood in acute ischemic heart disease1 and only one takes into account specific measurements from the venous CS in humans[2].

CONCLUSIONS

Heart failure (HF) patient selection for Cardiac Resynchronization Therapy (CRT) continues to be a real issue nowadays and still needs improvement. Up to 35% of CRT patients are currently non-responders to therapy. New bio-markers could be helpful in identifying CRT responders, before procedure. The assessment of damaged DNA from peripheral blood prior and after intervention showed no specific pattern and no obvious evolution, both in responders and non-responders. By contrast, the level of dama-

ged DNA taken from the coronary sinus identified a clear and distinct population – almost all responders to therapy seemed to have greater levels of Damaged DNA in contrast with the non-responder population. As expected, TAC was lower in the responder group vs. non-responders. Our theory is that greater levels of damaged DNA should be present in heart failures with high percent of remaining viable myocardium. On the contrary, hearts with extensive fibrosis/scar with no or very little viable myocardium are unable to produce high quantities of damaged DNA due to the lack of active cardiac fibers. These patients are less likely to reverse-remodel and consequently are candidates for non-response to CRT. There was no correlation between oxidative stress observed in peripheral and central cardiac blood. DNAd 8-OHdG and TAC measured from coronary sinus plasma could be a new biomarker to identify responders to CRT, but it needs further research. According to our study, peripheral oxidative stress markers seem to be of no use.

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