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Authors: Tomasz Urbanowicz, Ewelina Wojtasińska, Anna Olasińska-Wiśniewska, Krzysztof J Filipiak, Małgorzata Ładzińska, Jędrzej Sikora, Ewa Straburzyńska-Migaj, Andrzej Tykarski, Marek Jemielity, Joanna Rupa-Matysek

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Neutrophil to extracellular traps (NET) as an early marker of right ventricular dilatation in patients with left ventricular assist device (LVAD)

Short title: NETs in LVAD patients

Tomasz Urbanowicz[1]*, Ewelina Wojtasińska[2]*, Anna Olasińska-Wiśniewska[1], Krzysztof J Filipiak[3, 4], Małgorzata Ładzińska[1], Jędrzej Sikora[5], Ewa Straburzyńska-Migaj[6], Andrzej Tykarski[3], Marek Jemielity[1], Joanna Rupa-Matysek[2]
*Both authors equally contributed to the study.

[1]Department of Cardiac Surgery and Transplantology, Poznan University of Medical Sciences, Poznań, Poland
[2]Department of Hematology and Bone Marrow Transplantation, Poznan University of Medical Sciences, Poznań, Poland
[3]Department of Hypertensiology, Angiology and Internal Medicine, Poznan University of Medical Sciences, Poznań, Poland
[4]Institute of Clinical Science, Maria Sklodowska-Curie Medical Academy, Warszawa, Poland
[5]Poznan University of Medical Sciences, Poznań, Poland
[6]1st Department of Cardiology, Poznan University of Medical Sciences, Poznań, Poland

Correspondence to:
Tomasz Urbanowicz, MD, PhD,
Department of Cardiac Surgery and Transplantology,
Poznan University of Medical Sciences,
Długa 1/2, 61–848 Poznań, Poland,
phone: +48 61 854 92 10,
e-mail: turbanowicz@ump.edu.pl

INTRODUCTION

The prevalence of heart failure (HF) is increasing due to the ageing of the population and improved survival of patients with optimal treatment of cardiovascular diseases [1]. Congestive HF is a progressive multi-faceted disorder characterized by symptomatic stability punctuated by episodes of deterioration. Despite novel models of pharmacological optimalization [2], the

gradual circulatory insufficiency may indicate left ventricular assist devise (LVAD) support as the optimal option. The right ventricular failure (RVF) following LVAD implantation is still a challenging problem associated with adverse postoperative outcomes.

Activated neutrophils have a potential to programmed cell death and may decondense their entire nuclear chromatin/DNA forming neutrophil extracellular traps (NETs) [3]. Histones, the protein components of chromatin subjects are involved in NETs formation. NETs accumulation has been reported in chronic and acute manifestations of several cardiovascular diseases.

The aim of the study was to assess the possible role of NETs generation evaluated by citrullinated histone 3 (citH3) serum concentration as an early biomarker of RV dysfunction in patients after LVAD implantation.

METHODS

Twenty-five consecutive male patients (median age of 59 [51–63] years) who underwent HeartMate 3 (Abbot Corp., US) implantation in Cardiac Surgery and Transplantology Department in Poznan between 2021 and 2023 due to end-stage left sided congestive HF, were included to the single center analysis. Preoperative right ventricular (RV) dysfunction, oncological disease and signs of infection were exclusion criteria.

Pre-operative assessment included clinical evaluation, echocardiographic examination, 6-minute walk test (6MWT), coronary angiography, right heart catheterization, and computed tomography. Patients were qualified for LVAD implantation by the heart team of cardiologists, cardiac surgeons and transplantologists.

All patients were treated according to current recommendations for HF management. Moreover, warfarin with adequate levels of international normalized ratio between 2.0–3.0 with aspirin at the dose of 75 mg per day were used after LVAD implantation. During follow-up visits, we meticulously assessed patients at every 3 months, with clinical, laboratory, echocardiographic evaluation, and 6MWT. Moreover, LVAD function parameters were controlled. Moreover, non-planned hospitalizations were recorded. Blood samples were collected before procedure and during follow-up visits for citH3 serum concentration, describing NET generation.

Transthoracic echocardiography was performed during the qualification to LVAD implantation, after the procedure, and at the follow-up. The exam was performed by experienced echocardiographers according to the same protocol. Special attention was pointed to the symptoms of RV abnormalities. RV diameter was assessed in the parasternal long axis

view and in the apical 4-chamber view. The preoperative RV diameters were compared to the results obtained in the follow-up.

Patients were subsequently divided according to the echocardiographic comparison of RV diameters obtained preoperatively and during follow-up, into group 1 (no changes in RV diameter) and group 2 (RV dilatation) (Supplementary material, *Table S1*).

NETs methodology

To quantify CH3 in the plasma we employed the Human (CH3) Elisa Kit from Shanghai Sunred Biological Technology Company Ltd. Study wells were precoated with human monoclonal anti-citH3 antibody. Calibrators and patient samples were simultaneously incubated with secondary anti-citH3 antibody labeled with biotin and combined with streptavidin-HRP to form immune complex. After incubation and washing unbound enzyme was removed. The enzyme bound to the solid phase was incubated with the substrate, tetramethylbenzidine. An acid stopping solution was then added to stop the reaction and convert the color from blue to yellow. The intensity of the yellow color was measured using a spectrophotometer at a wavelength of 450 nm. A dose response curve of absorbance versus concentration was generated using results obtained from calibrators. Concentration of human citH3 were determined from this curve.

Bioethics committee

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of Poznan University of Medical Sciences (695/20).

Statistical analysis

Continuous variables were reported as medians and interquartile ranges (Q1–Q3) since data did not follow normal distribution. Categorical data were presented as numbers and percentages. Numerical variables were compared by Mann–Whitney test, or repeated measures ANOVA, where applicable. Categorical data were analyzed by Fisher's exact test. Spearman correlation analysis was used. Statistical analysis was performed using JASP statistical software (JASP Team; 2023. Version-0.18.1). $P < 0.05$ were considered statistically significant.

RESULTS

All patients were diagnosed with end-stage HF due to coronary artery disease (n = 13) or dilated cardiomyopathy (n = 12).

On regular checkups, there were no significant clinical symptoms of RVF, with satisfactory 6MWT of 405 (370–443) meters. The pump function was uneventful generating output of 4.3 (4.0–4.4) liters/minute. None of the patients was transplanted nor suffered from thromboembolic events. During a median (Q1–Q3) follow-up of 180 (89–1004) days, echocardiography revealed clinically silent RV dysfunction based on its diameter distension in 7 (28%) patients. The citH3 median (Q1–Q3) concentrations before LVAD implantation were within median (Q1–Q3) citH3 values of 2255 (1533–2633) pg/ml. Postoperatively, in the follow-up visits, significant citH3 decrease with median values (Q1–Q3) of 563 (474–615) pg/ml was observed and compared to preoperative values (P <0.001).

The echocardiographic pre-implantation and post-implantation results were compared (Supplementary material, *Table S2*). The significant difference was observed between RV diameter (P = 0.01) between both groups in the follow-up. The change in the RV diameters obtained before implantation and in the follow-up was statistically significant between both groups (Supplementary material, *Table S2*).

The assessment of pump function was performed during protocolar checkups and accompanied by laboratory tests (Supplementary material, *Table S3*). The only significant difference was related to citH3 serum concentration (P = 0.003). The relations between citH3 pre- and postoperative concentrations between both groups were presented in Figure 1A–B. Increased citH3 serum concentration positively correlated with RV dilatation (r = 0.471; P = 0.01) (Supplementary material, *Table S4*).

DISCUSSION

This is the first study, to our best knowledge, presenting the relationship between increased NETs formation and RV diameter in LVAD patients.

Our analysis was based on a group of patients with mechanical circulatory support (MCS) supported by magnetically levitated centrifugal-flow pumps. MCS disturbs the hematologic and coagulation system, that leads to platelet and contact pathway of coagulation activation.

We point out the significance of citH3 as a marker for proceeding events and may indicate one of the most ominous long-term complications as RVF. Though there are several approaches for prediction of RV dysfunction in LVAD patients, no single marker was established as safe in clinical practice.

In our analysis, the significant decrease between preoperative and follow-up citH3 plasma concentrations after LVAD implantation was observed. After LVAD, NETs formation

measured by citH3 plasma concentration decreased to the levels comparable to levels observed in healthy controls presented in other studies [4]. Interestingly, according to our study, in the postoperative follow-up, the citH3 derangements can be regarded as a possible early marker of increased risk of RVF. The early recognition of progression to RVF is difficult and is based on clinical scrutiny evaluation and echocardiographic assessment. Even mild echocardiographic changes may precede RV function deterioration. The increase in RV diameter in outwardly clinically stable LVAD patients and with satisfactory pump punction, should raise clinical attention due to possible increased inflammatory state that may progress into hypercoagulable conditions.

The RV dysfunction in LVAD patients is claimed to be related to coagulation disturbances [5]. The pro-coagulation state in patients with MCS can be also secondary to inflammatory activation as we reported in our previous study [6]. However, the presented analysis excluded the significant differences in inflammatory markers related to infection. Yang et al. [7] in their analysis pointed out the distinction in the inflammatory milieu in patients with RVF. Since a large body of evidence suggests association between HF and cytokines and chemokines induction, the NETs role in HF progression is inevitable.

In our analysis, we did not find significant relation between pre-implantation pulmonary circulation characteristics and neither NETs concentration nor RV diameter dilatation. We believe that subtle RV dysfunction presented in our analysis is of a different entity than clinical scenarios focused on severe stages. The dynamic metrics of pulmonary artery pressure were claimed predictive for RV dysfunction by Gulati et al. [8].

The LVAD implantation is a relatively unique procedure performed in patients presenting with the most advanced stage of HF, and therefore the analysis of limited number of patients should be treated with caution and rules out the possibility to performed multivariable analysis. We did not assess other specific biomarkers of NETs, such as complexes of cell free DNA or myeloperoxidase (MPO-DNA) or neutrophil elastase, which may be treated as a limitation of our study.

CONCLUSION

Concentration of citrullinated histone 3 describing NETs correlates with changes in RV diameter and may enable differentiating of patients at higher risk of RVF.

Supplementary material

Supplementary material is available at https://journals.viamedica.pl/polish_heart_journal.

Article information

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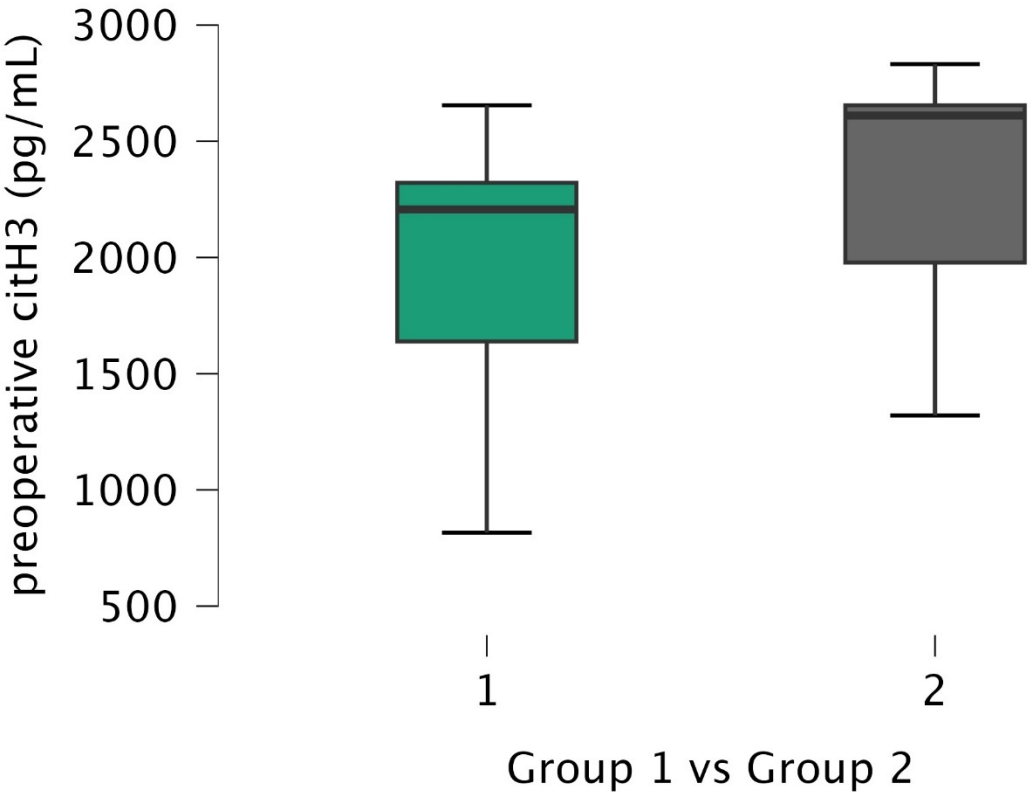

Figure 1. The citH3 plasma concentration differences (*P* <0.001) between group 1 (no changes in RV diameter) and group 2 (RV dilatation) on 180 days follow-up

Abbreviations: citH3, citrullinated histone 3; RV, right ventricular