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

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DOI: 10.33963/v.phj.100884

Received:
April 22, 2024

Accepted:
May 26, 2024

Early publication date:
June 5, 2024

INTRODUCTION

The prevalence of heart failure (HF) is increasing due to the aging 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 optimized models of pharmacological care [2], gradual circulatory insufficiency may be an indication for left ventricular assist device (LVAD) support as the optimal option. Right ventricular failure (RVF) following LVAD implantation is still a challenging problem associated with adverse postoperative outcomes.

Activated neutrophils have the potential to trigger 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 NET formation. NET accumulation has been reported in chronic and acute manifestations of several cardiovascular diseases.

This study aimed to assess the possible role of NET 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 59 [51–63] years) who underwent HeartMate 3 (Abbot Corp., US) implantation in the Cardiac Surgery and Transplantology Department in Poznań between 2021 and 2023 due to end-stage left-sided congestive HF, were included in this single-center analysis. Preoperative right ventricular (RV) dysfunction, oncological disease, and signs of infection were exclusion criteria.

Preoperative 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 transplant surgeons.

All patients were treated according to current recommendations for HF management. Moreover, warfarin with adequate levels of the international normalized ratio between 2.0–3.0 with aspirin at the dose of 75 mg per day was used after LVAD implantation. During follow-up visits, every 3 months, we meticulously assessed patients using clinical and laboratory, echocardiographic evaluation, as well as the 6MWT. Moreover, LVAD function parameters were controlled. Moreover,

non-planned hospital admissions were recorded. Blood samples were collected before the procedure and during follow-up visits for citH3 serum concentration describing NET generation.

Transthoracic echocardiography was performed during the qualification for LVAD implantation, after the procedure, and during the follow-up. The exam was performed by experienced echocardiographers according to the same protocol. Special attention was paid to symptoms of RV abnormalities. RV diameter was assessed in the parasternal long-axis and apical 4-chamber views. The preoperative RV diameters were compared to the results obtained in the follow-up.

Patients were subsequently assigned 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*).

NET methodology

To quantify citrulinated histone H3 (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 a secondary anti-citH3 antibody labeled with biotin and combined with streptavidin-HRP to form an 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. The concentration of human citH3 was 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 (IQR) because data did not follow a normal distribution. Categorical data were presented as numbers and percentages. Numerical variables were compared by Mann–Whitney test, Wilcoxon, and Friedman tests for 2 and 3 repeated measurements, respectively. 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*-values of <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 a satisfactory 6MWT of 405 (370––443) meters. Pump function was appropriate and generated an output of 4.3 (4.0–4.4) liters/minute. None of the patients had a transplant or suffered from thromboembolic events. During a median (IQR) 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 (IQR) concentrations before LVAD implantation were within median (IQR) citH3 values of 2255 (1533–2633) pg/ml. Postoperatively, in the follow-up visits, a significant citH3 decrease with median values (IQR) 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*). A significant difference was observed between RV diameter (P = 0.01) between both groups in the follow-up. The difference in the RV diameters obtained before implantation and in the follow-up was statistically significant between both groups (Supplementary material, *Table S2*).

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 relationships 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*).

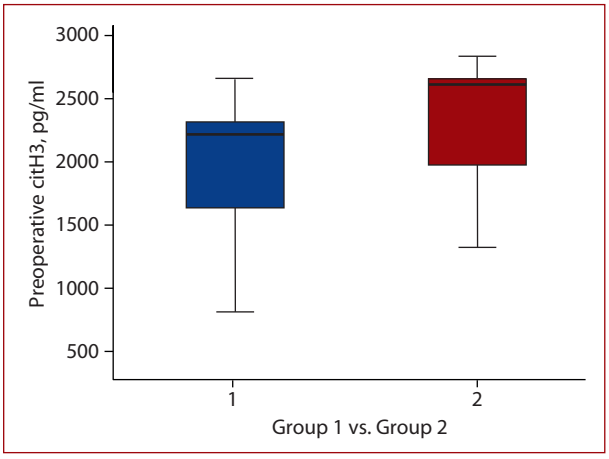

Figure 1. The citH3 plasma concentration differences (P <0.001) between group 1 (no changes in RV diameter) and group 2 (RV dilatation) on 180 days of follow-up

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

DISCUSSION

To our best knowledge, this is the first study, presenting the relationship between increased NET formation and RV diameter in LVAD patients.

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

We have highlighted the significance of citH3 as a marker for unfolding events, and it may indicate one of the most ominous long-term complications such as RVF. Though there are several approaches to prediction of RV dysfunction in LVAD patients, no single marker has been established as safe in clinical practice.

In our analysis, a significant decrease between preoperative and follow-up citH3 plasma concentrations after LVAD implantation was observed. After LVAD, NET formation measured by citH3 plasma concentration decreased 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 and echocardiographic assessment. Even mild echocardiographic changes may indicate RV function deterioration. The increase in RV diameter in seemingly clinically stable LVAD patients 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 MCS patients 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 an association between HF and cytokines and chemokines induction, the NET's role in HF progression is inevitable.

In our analysis, we did not find a significant relationship between pre-implantation pulmonary circulation characteristics and either NET concentration or RV diameter dilatation. We believe that subtle RV dysfunction presented in our analysis is of a different nature than clinical scenarios focused on severe stages. Dynamic metrics of pulmonary artery pressure were considered predictive of RV dysfunction by Gulati et al. [8].

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

CONCLUSION

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

Supplementary material

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

Article information

Conflict of interest: None declared.

Funding: None.

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