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Lifestyle Walking Intervention in Patients With Heart Failure With Reduced Ejection Fraction: The WATCHFUL Trial

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Abstract

Background: Physical activity is pivotal in managing heart failure with reduced ejection fraction (HFrEF), and walking integrated into daily life is an especially suitable form of such physical activity. This study aimed to determine if a 6-month lifestyle walking intervention combining self-monitoring and regular phone counseling improves functional capacity assessed by the six-minute walk test (6MWT) in stable patients with HFrEF compared to usual care.

Methods: The WATCHFUL trial was a 6-month, multicenter, parallel-group, randomized, controlled trial recruiting HFrEF patients from six Czech cardiovascular centers. Eligible participants were ≥18 years old with left ventricular ejection fraction <40% and NYHA class II/III symptoms, on guidelines-recommended medication, excluding those exceeding 450m in the baseline 6MWT. Patients in the intervention group were equipped with a Garmin vívofit activity tracker and received monthly phone counseling from research nurses who encouraged them to employ behavior change techniques such as self-monitoring, goal-setting, and action planning to increase their daily step count. The control group patients continued usual care. The primary outcome was the difference between groups in the distance (in meters) walked during the 6MWT at 6 months. Secondary outcomes included daily step count and minutes of moderate-to-vigorous physical activity (MVPA) as measured by the hip-worn Actigraph wGT3X-BT accelerometer, NT-proBNP and hsCRP biomarkers, ejection fraction, anthropometric measures, depression score, self-efficacy, quality of life, and survival risk score. The primary analysis was conducted by intention-to-treat.

Results: From 218 screened patients, 202 were randomized (65 years; 22.8% female; 90.6% NYHA II; left ventricular ejection fraction 32.5%; 6MWT 385m; 5071 steps/day; 10.9 minutes of MVPA per day). At six months, no between-group differences were detected for the 6MWT (7.4 m, 95% CI -8.0 to 22.7, p=0.345, N=186). The intervention group increased their average daily step count by 1420 (95% CI: 749; 2091) and daily minutes of MVPA by 8.2 (95% CI: 3.0; 13.3) over the control group. No between-group differences were detected for any other secondary outcomes.

Conclusions: While the lifestyle intervention in patients with HFrEF improved daily steps by about 25%, it failed to demonstrate a corresponding improvement in functional capacity. Further research is needed to understand the disconnect between increased physical activity and functional outcomes.

Clinical Trial Registration: ClinicalTrials.gov (NCT03041610, https://clinicaltrials.gov/ct2/show/NCT03041610).

Keywords: physical activity; behavior change; activity tracker; Garmin; self-monitoring; phone counseling; functional capacity; six-minute walk test; step count; moderate-to-vigorous physical activity; cardiac rehabilitation

Non-standard Abbreviations and Acronyms

BDI-II: Beck depression inventory-II
GSE: General self-efficacy scale
HFrEF: heart failure with reduced ejection fraction
hsCRP: high-sensitivity C-reactive protein
MAGGIC: Meta-Analysis Global Group in Chronic Heart Failure
MVPA: moderate-to-vigorous physical activity
NT-proBNP: N-terminal pro–B-type natriuretic peptide
PA: physical activity
SAP: statistical analysis plan
SF-36: 36-item short-form health survey
WATCHFUL: Walking in Chronic Heart Failure Trial
6MWT: six-minute walk test

Clinical Perspective

What is new?

- A simple lifestyle walking intervention, combining self-monitoring with an activity tracker and phone counseling, can increase daily step count of heart failure patients by approximately 25%.
- The intensity of lifestyle walking interventions may not be sufficient to elicit improvements in the functional capacity of patients with heart failure with reduced ejection fraction.

What are the clinical implications?

- Traditional supervised, structured exercise-based cardiac rehabilitation programs continue to be the standard strategy for improving functional capacity, quality of life, and prognosis in patients with heart failure with reduced ejection fraction.
- These programs can be supplemented with simple lifestyle physical activity interventions, utilizing tools such as activity trackers, to support long-term behavioral change and enhance health outcomes.

Introduction

Heart failure with reduced ejection fraction (HFrEF) represents a substantial burden on global health, contributing significantly to hospital admissions, healthcare costs, and mortality.[1] Physical activity (PA) and exercise are fundamental in managing HFrEF, with potential benefits in improving functional capacity and quality of life and enhancing patient prognosis.[2–5] However, the optimal strategy to increase PA in this population remains elusive.[6]

While traditional supervised, structured exercise-based cardiac rehabilitation programs have demonstrated benefits,[7,8] their availability and accessibility are often limited, reaching only a fraction of eligible patients.[9,10] Additionally, the demanding intensity of the exercises, logistical issues such as transportation difficulties and inconvenient scheduling, and individual time constraints contribute to lower adherence rates.[11,12] Lastly, these programs often fail to induce sustained behavioral change, resulting in their impact being frequently short-term.[13]

In contrast, lifestyle PA interventions have emerged as an alternative approach.[14] These interventions aim to seamlessly integrate increased PA levels into daily life, often promoting walking as a natural, accessible form of exercise.[15] By focusing on incorporating walking into everyday routines, lifestyle walking interventions offer a more flexible and sustainable solution, potentially addressing the limitations associated with more structured programs.[16] Typically, these interventions employ various behavior change techniques such as goal setting, action planning, and self-monitoring.[13] They commonly utilize activity trackers to facilitate self-monitoring and often combine this technology with human support, i.e., phone counseling, to enhance adherence and effectiveness.[17,18] Prior research involving patients with conditions like chronic respiratory diseases, cardiovascular diseases, or type 2 diabetes

has shown that similar interventions can result in substantial step count increases ranging from 1,000 to 2,000 steps daily.[17,19–23] Moreover, these step count increments have been linked to significant health benefits, including reduced systolic blood pressure, decreased waist circumference, and lowered low-density lipoprotein cholesterol levels, as well as enhanced functional capacity.[16,23–25]

Yet, whether lifestyle walking interventions can attain a substantial step count increase among patients with HFrEF and whether such increases transform into enhanced functional capacity remains unexplored.[26] The WAlking in Chronic Heart FailUre (WATCHFUL) trial aimed to address this gap by investigating whether a 6-month lifestyle walking intervention, compared to usual care, can elicit improvements in 6-minute walk test (6MWT) distance, a measure of functional capacity, in stable HFrEF patients.

Methods

Trial Design

The WATCHFUL trial was a 6-month, multicenter, parallel-group, randomized, controlled trial performed from August 2018 to June 2023 at six clinical centers in the Czech Republic, of which four were tertiary cardiovascular centers, one regional cardiovascular center, and one large outpatient ambulatory center, all providing specialized outpatient heart failure services (e-Appendix 1). The trial protocol received approval from the multicenter Ethics Committee of the General University Hospital in Prague (20/16 Grant VES 2017 AZV VFN) and has been registered at ClinicalTrials.gov, NCT03041610. The trial protocol and statistical analysis plan (SAP) have been published in detail elsewhere.[27,28] Details regarding the deviations from the published protocol can be found in e-Appendix 2. The data that support the findings of this study are available from the corresponding author upon reasonable request (e-Appendix 3).

Participants

The trial recruited patients aged ≥18 years with stable HFrEF (left ventricular ejection fraction <40%) and New York Heart Association (NYHA) class II or III symptoms, on evidence-based medication with maximally tolerated dosages. We excluded patients who had signs or symptoms of decompensated heart failure, uncontrolled arrhythmia or effort-induced angina, severe or symptomatic aortic stenosis, persistent hypotension, and recent events (<3 months) such as myocardial infarction, percutaneous coronary intervention, implantation of an implantable cardioverter defibrillator or bi-ventricular pacemaker or shocks delivered by the automated implantable cardioverter defibrillator. Potential participants were screened using the 6MWT and those exceeding 450 m or unable to complete the test were excluded. For the full set of inclusion and exclusion criteria, see e-Appendix 4.

Sample Size

To detect a clinically meaningful change of 45 m on the 6MWT[29] with 80% power using a two-sided 0.05 significance level (alpha) and assuming a standard deviation of within-group change of 100 m, 79 participants were required in each group. Anticipating an attrition rate of 20%, our recruitment goal was set at 100 patients per group, leading to a total of 200 patients. The clinically meaningful change of 45 m was derived from the work of Shoemaker et al., who triangulated the minimum clinically meaningful difference using various methods of analyzing existing data.[29] The standard deviation for within-group change, set at 100 m, was based on findings from previous studies.[29–31]

Randomization and Blinding

Patients were randomized in a 1:1 ratio to either the intervention or control group. Centralized randomization was executed using a computer-automated system to ensure proper allocation concealment. The trial employed a permuted block randomization scheme, stratified by center, NYHA class, sex, and age (18–65, ≥66) to guarantee balanced group

representation.

Given the trial's design, blinding of patients and researchers was not feasible, as both were aware of the allocation due to their active involvement in the intervention. Nonetheless, all assessments were conducted by assessors who remained blinded to treatment allocation.

Intervention and Control Groups

During the clinical visits at baseline, 3 months, and 6 months, all patients were educated about the health benefits of regular PA and encouraged to integrate walking into their daily routine.

Patients in the intervention group participated in a 6-month behavioral lifestyle intervention aimed at seamlessly integrating additional PA, primarily walking, into their daily routines. This intervention utilized behavior change techniques, including self-monitoring, goal-setting, and action planning, facilitated by one of two research nurses through regular phone consultations.

At the baseline visit, patients were equipped with a wrist-worn Garmin vívofit activity tracker to self-monitor their daily step count. The Garmin vívofit was chosen for its simplicity, functioning primarily as a pedometer, making it suitable for our study population of older heart failure patients, many of whom had limited experience with advanced smart devices. To maintain this simplicity, we configured the Garmin device to avoid any prompts or feedback. However, we did not prohibit participants from altering the Garmin settings or using the Garmin app, allowing them the discretion to personalize their device experience. This approach mirrors how older individuals might use such devices in real-world settings outside of a controlled study environment. Furthermore, the Garmin vívofit's extended battery life of at least eight months alleviated the burden of frequent charging for patients. Lastly, the Garmin vívofit's step-counting accuracy has been previously validated in both lab-based and free-living environments. Among the devices tested, it emerged as the top-

performing tracker for heart failure patients.[32] Nevertheless, the inherent simplicity of the device meant it lacked features like a heart rate monitor or wear sensor, which posed challenges in verifying consistent wear.

The patients were advised to wear the Garmin device from waking to sleeping. Importantly, participants were instructed not to intentionally increase their activity levels during the initial week to ensure an accurate capture of their habitual daily step count. Furthermore, to bolster patients' adherence to self-monitoring, they were instructed to record their daily steps as indicated by the Garmin device in a paper diary and to review this diary at least once a week.

Approximately two weeks after the baseline visit, patients in the intervention group were contacted by phone by one of the two nurse counselors. The nurse reviewed the patients' average daily step count as recorded by the tracker during the initial week. The patients were then guided to aim for an incremental increase of at least 3,000 steps above their baseline gradually over six weeks. Targeting an additional 3,000 steps daily is a common goal in behavioral interventions.[33] This increment equates to approximately 30 minutes of walking, assuming a pace of 100 steps per minute—a heuristic estimate for a moderate-intensity threshold.[33] As a result, this represents more than 150 minutes of moderate-intensity PA each week, in line with the WHO's guidelines for adults with chronic conditions.[34] Regular engagement at this activity level has consistently been associated with notable health advantages.[34] If patients found the 3,000-step increase challenging, they were encouraged to propose a more achievable goal. Studies have shown that 'goal ownership,' or a deep personal commitment to a set target, often has a stronger influence on behavior change than the exact numerical value of the goal.[35] Therefore, the counselor ensured that patients felt a sense of ownership over their goals rather than feeling they were externally imposed. Additionally, patients were prompted to identify opportunities to incorporate these additional

steps into their daily routines and to formulate their own action plans. Should they encounter challenges in creating these plans, the counselor offered suggestions, such as incorporating walking into daily commutes, post-meal or evening strolls, walking with grandchildren, dog walking, or walking meetings.

In the follow-up phone counseling sessions at 1, 2, 4, and 5 months, the counselor assisted patients in revisiting their step goals and action plans. These sessions focused on addressing any barriers faced, emphasizing the importance of social support, and offering tailored feedback on progress. The counselor also discussed the patients' personal goals, monitored adherence, reviewed their step diaries, and provided guidance on overcoming challenges to PA. Based on the patient's progress, the counselor had the flexibility to adjust the step goals. For example, if a patient regularly surpassed their goal, a higher target might be set. On the other hand, if a patient found the goal unachievable, it could be adjusted downward to maintain motivation and avoid discouragement.

Patients in the control group received usual care, which included education about the health benefits of PA and encouragement to augment their walking routine during clinical visits. However, they did not receive the activity tracker, specific step goals, or regular phone consultations from the research nurse.

Outcomes

The primary outcome was the difference between groups in the distance (in meters) walked during the 6MWT at 6 months.

Secondary outcomes at 6 months encompassed: (a) PA measures: average daily step count and minutes of moderate-to-vigorous PA (MVPA); (b) biomarkers: N-terminal pro–B-type natriuretic peptide (NT-proBNP) and high-sensitivity C-reactive protein (hsCRP); (c) left ventricular ejection fraction; (d) patient-reported outcomes: Beck Depression Inventory-II (BDI-II), 36-item Short-Form Health Survey (SF-36), and General Self-Efficacy Scale

(GSE); (e) anthropometric measures: body mass index, waist and hip circumference; (f) Meta-Analysis Global Group in Chronic Heart Failure (MAGGIC) risk score. The 6MWT, NT-proBNP, and body weight were additionally evaluated at 3 months.

For both the intervention and control groups, PA was objectively measured using the Actigraph wGT3X-BT accelerometer. Participants were instructed to wear the device on their right hip during waking hours for a consecutive 7-day period at baseline and 6 months, ensuring consistent measurement across both groups.[36] This device recorded raw accelerometry data which were then aggregated into 60-second epochs. Non-wear time was identified using Choi's algorithm,[37] and a valid day was defined as having a minimum of 600 minutes of wear time. Only measurements with at least four valid days, including at least one weekend day, were considered for analysis.[38] The average daily step count was determined using the manufacturer's proprietary algorithm as implemented in the ActiLife software, and minutes of moderate-to-vigorous PA (MVPA) were calculated based on Freedson's cut-points.[39] It's important to note that while the intervention group also wore the Garmin activity tracker, the reported values of step count and minutes of MVPA for both groups were obtained solely through the Actigraph accelerometer.

Adherence to the intervention was gauged based on the percentage of subjects who either (a) declined to wear the wrist-worn activity tracker, (b) missed the clinic visit at 3 and 6 months, or (c) participated in fewer than 3 out of the 5 planned phone counseling sessions. Additionally, during each phone contact, we assessed adherence to self-monitoring with the activity tracker by asking patients if they maintained their paper diary and inquiring about their recent step count.

Adverse events were consistently monitored and documented throughout the trial duration. Data concerning hospitalizations, heart failure decompensation, cardiovascular events, falls and injuries, musculoskeletal issues, and fatalities were gathered at each time

point.

Detailed descriptions of all outcomes, including exploratory, can be found in the published protocol and SAP.[27,28]

Statistical Methods

The primary analyses were conducted by the strict intention-to-treat principle utilizing a linear mixed-effect model, accounting for clustering at the center level as a random effect and adjusting for baseline value of the respective variables, age, sex, and NYHA class as fixed effects. Additionally, supplementary analyses were conducted on the per-protocol population using the same approach; the definition of the per-protocol population is detailed in the published SAP.[28] Furthermore, for the primary outcome, a supplementary analysis was undertaken in which missing data were imputed using multiple imputations by chained equations (MICE) with the predictive mean matching method.

Pre-specified subgroup analysis was conducted to assess the differential effect of the intervention on patients participating before (prior to March 11, 2020) versus after the onset of the COVID-19 pandemic. Additional post-hoc subgroup analyses investigated patient subgroups according to baseline 6MWT, daily step count, age, sex, NYHA class, body mass index, NT-proBNP, and MAGGIC risk score. Whether intervention effects were significantly different between the complementary subgroups was assessed by conducting interaction tests, with interaction terms incorporated into the linear mixed-effect models, adjusted for covariates as in the primary analysis.

The intervention effect for the primary outcome is presented as the mean, accompanied by a two-sided 95% confidence interval (95% CI) and the associated p-value (determined at a two-sided 5% significance level). For secondary outcomes, only the mean and 95% CI are reported, as adjustments for multiple testing were not made. For the within-group changes, both means (and 95% CI) and medians (and IQR) are reported, given that the variables did

not exhibit a normal distribution. The normality of the variables was assessed using the Shapiro-Wilk test.

All analyses were conducted using the R statistical software (version 4.1.2) and the packages nlme (3.1-153) and mice (3.15.0).

Results

Study Participants

Out of the 218 patients screened, 11 were excluded and 5 refused to participate (Figure 1). A total of 202 patients were randomized, with a median age of 65 years; 22.8% were women, 90.6% classified as NYHA class II (Table 1). Complete primary outcome data were available for 186 patients, representing 92.1% (Table S1). A CONSORT flowchart detailing the trial's progression is provided in Figure 1. Major and minor protocol violations are detailed in Table S2.

Adherence to Intervention

Of the 101 patients allocated to the intervention group, 19 did not adhere to the pre-specified criteria. Specifically, 3 patients declined to wear the Garmin activity tracker immediately post-randomization, another 3 later during the intervention period, 7 missed their clinic visits at either three or six months, and 14 participated in fewer than three of the five planned counseling sessions. As a result, 82 patients (81%) fully adhered to the intervention.

Of the 84 patients in the intervention group who participated in at least three of the five planned counseling sessions and did not decline to wear the tracker, 70 (83%) consistently kept their diaries, and 77 (92%) consistently provided estimates of their recent step counts during all phone contacts.

Primary Outcome

In the intention-to-treat analysis of the complete cases, the intervention effect on the 6MWT

at 6 months was +7.4 m (95% CI -8.0 to 22.7; p = 0.345). The supplementary analysis following the imputation of missing cases and the per-protocol analysis yielded similar results (Table 2).

Secondary Outcomes

In the intention-to-treat analysis of secondary outcomes at 6 months (Table 3), notable findings include the positive intervention effect on average daily step count (+1420; 95% CI 749 to 2,091) and minutes of MVPA (+8.2; 95% CI 3.0 to 13.3). Results for the intention-to-treat analysis at 3 months are in Table S3. Per-protocol analyses at 6 months (Table S4) and 3 months (Table S5) produced similar results as those conducted by intention-to-treat.

Subgroup Analyses

In the exploratory subgroup analyses of the primary outcome, no significant differences between the subgroups were observed (Figure 2), although there was a numerical improvement in younger, non-obese patients with better functional status and milder disease severity.

Adverse Events

Adverse events for each trial group are detailed in Table 4, where the number of occurrences for each event is presented separately for each trial group. The most frequently recorded adverse events were cardiovascular events including hospitalization for cardiovascular reasons (13 patients). Five patients either visited the emergency room or were hospitalized due to heart failure decompensation and four patients required an increased diuretic dose. Importantly, no adverse events directly related to the intervention were reported.

Discussion

In this randomized controlled trial of a 6-month walking intervention in stable HFrEF patients, no significant improvement in the primary outcome of 6MWT distance was

observed. Among the secondary outcomes, both objective PA measures (step count and time in MVPA) showed significant improvements, as did self-reported general health score; but there were no significant differences in levels of NT-proBNP and hsCRP, ejection fraction, anthropometric measures, depression scores, self-efficacy, most domains of quality of life, and survival risk scores.

The observed improvement in PA by 1420 steps/day—representing an approximately 25% increase—is substantial and equivalent to cardiac rehabilitation studies in cardiovascular patients.[20] It also aligns with findings from other trials evaluating various PA interventions across a range of populations and settings, including patients with chronic conditions[23] as well as healthy[21] and older[22] adults in community settings. In cohort studies with long-term follow-up, the difference of just 1000 steps per day has been associated with a significant decrease in all-cause mortality by 15% in both general population[40] and heart failure patients.[41] Furthermore, the minimum clinically important difference for the physically inactive general population[42] as well as patients with chronic conditions such as chronic obstructive pulmonary disease[43] or peripheral artery disease[44] has been estimated to lie between 500 and 1100 steps per day. Thus, it is noteworthy that while successfully enhancing PA levels, our intervention did not yield a corresponding improvement in functional capacity assessed by the 6MWT.

A plausible explanation for this disconnect could be as simple as the fact that despite a substantial increase in the volume of PA as indicated by step count, the pattern, duration, and intensity of PA were insufficient to elicit changes in the 6MWT.[30,45] Indeed, most cardiac rehabilitation studies showing improvement in 6MWT for HFrEF patients utilized supervised, structured exercises with rigorously prescribed duration and intensity.[8,31] In contrast, our lifestyle walking intervention was more natural, solely focused on increasing the daily number of steps; it was intentionally neither supervised nor structured, and importantly,

did not require specific duration and intensity of PA. As a result, patients in our study could accumulate additional steps in very short bouts and at low intensities, differing from the approach of exercised-based rehabilitation programs, which typically prescribe walking or other exercise modalities in bouts of at least 20 minutes at an intensity above 60% of heart rate reserve.[8,26,46–48] Notably, our study observed an increase of 8 minutes of MVPA per day—equivalent to approximately 800 steps—indicating that the intervention group actually did achieve some of their additional PA at higher intensities. However, the use of minutes of MVPA as a cut-point-based measure has inherent limitations,[49] leaving it uncertain whether the patients reached the intensities typically prescribed in exercised-based cardiac rehabilitation programs.[26,30,48]

An alternative explanation might be that the baseline 6MWT distance of 385 m was near the patients' maximum potential for improvement, suggesting a possible ceiling effect.[50] However, this hypothesis is not supported by our exploratory analysis, which compared subgroups of patients below and above the median baseline 6MWT. Interestingly, patients above the median exhibited greater improvements in 6MWT. This underscores the importance of initial functional capacity in interpreting the potential benefits of interventions on 6MWT outcomes. Another conceivable explanation could be the relatively high initial PA levels of the participants. The benefits of an increased step count are most pronounced in individuals who are the least active, typically averaging around 3000 steps per day.[51] In contrast, our participants had a baseline of approximately 5000 steps, representative of the step count observed in heart failure patients in other studies.[52] Nonetheless, this explanation seems less plausible, as our subgroup analysis revealed that patients with step counts above the median tended to benefit more from the intervention compared to those below the median. Yet another explanation could be the COVID-19 pandemic, which significantly impacted patients' activity levels and could have confounded the results.[53] Exploratory

subgroup analysis indicated that patients who participated before the onset of the pandemic experienced greater (albeit insignificantly) gains in 6MWT than those who participated after the onset. Nonetheless, the improvement of 19 m in the former group was still considerably below the minimal clinically important difference. Therefore, while the COVID-19 pandemic may have had some influence, it is unlikely to fully explain the observed lack of significant improvement in 6MWT in our study.

Results in Context of Other Literature

A limited number of studies have investigated the impact of interventions aiming to enhance the PA of heart failure patients in everyday life outside of structured exercise-based programs, with the majority showing limited success.[6,13] For instance, the REACH-HF trial, which incorporated a progressive walking program, did not elevate overall PA levels nor showed any between-group difference in the incremental shuttle walk test, although it did improve disease-specific health-related quality of life, its primary outcome.[54] The HF-Wii trial adopted a different approach, utilizing home-based exergaming to encourage increased PA in heart failure patients. The intensity of the exergames varied from 2.0 to 4.2 METs, but most participants opted for the lower-intensity games. After adjusting for baseline 6MWT, the trial found no significant differences in the 6MWT, leading the authors to conclude that the intervention might not have been sufficiently intense,[55] rather similar to our results.

In contrast to the aforementioned lifestyle PA interventions, the results from structured exercise-based programs have demonstrated significant improvements in both the 6MWT[30,56] and quality of life.[57,58] Nevertheless, even in these trials, the improvements observed in the 6MWT are often modest and tend to be short-term. For example, the individual participant meta-analysis ExTraMATCH II reported improvements in the 6MWT at 12-month follow-up of 21 meters.[59] Moreover, HF-ACTION, the largest trial of exercise-based rehabilitation to date, identified a significant intervention effect of 15 meters at 3 months, but this effect

disappeared at 12 months.[8] Furthermore, a recent large 9-week telerehabilitation trial, TELEREH-HF, found a significant difference in the 6MWT of only 9 meters.[31] Our trial was not powered to detect such small differences in the 6MWT, and it is debatable whether these differences are clinically relevant.[29]

Strengths and Limitations

Our study has several important strengths. Firstly, our recruitment strategy drew from a range of centers across the Czech Republic, enhancing generalizability. Secondly, our participants' average baseline step count was representative of heart failure patients as observed in other studies,[43] further bolstering external validity. Thirdly, we employed objective assessment methods for PA, utilizing accelerometers, which minimized subjective bias and provided accurate and reliable data on participants' activity levels. Lastly, our study exhibited minimal losses to follow-up, with only an 8% dropout rate, thereby reducing the risk of attrition bias and ensuring a comprehensive analysis of the collected data. These strengths collectively contribute to the robustness and real-world applicability of our study findings.

Several limitations warrant consideration and may influence the interpretation of the findings. Firstly, our study was designed to detect a clinically meaningful change of 45 m in the 6MWT. However, smaller changes that might still be of clinical significance could have been missed. Furthermore, the observed between-group difference of 1,420 steps might not have been substantial enough to elicit a 45-meter improvement in the 6MWT. Consequently, a study powered to detect smaller 6MWT differences, while also achieving more pronounced step count increases, might discern a significant effect on the 6MWT. Besides, the study might not have been powered to detect differences in secondary outcomes. Secondly, the follow-up period of 6 months might not be sufficient to observe the long-term effects of the intervention on functional capacity and other outcomes. The ongoing 12-month follow-up will provide more insights into the long-term sustainability of the observed changes in PA.

Thirdly, due to practical reasons, we could not record the number and characteristics of all patients who were considered for inclusion but did not enter the formal screening using the baseline 6MWT. Fourthly, our study population included a notable proportion of patients with NYHA functional class II and higher baseline 6MWT, which might not be entirely reflective of the broader HFrEF population in more recent clinical trials. This could potentially limit the generalizability of our findings to populations with more pronounced functional limitations. Fifthly, due to the constraints of the study design and to ensure a manageable assessment burden on participants, the study did not include heart failure-specific patient-reported outcomes such as the Kansas City Cardiomyopathy Questionnaire (KCCQ) or the Minnesota Living with Heart Failure Questionnaire (MLWHF), which might have offered a more nuanced assessment of the patients' quality of life in the context of heart failure. Finally, the absence of patient blinding constitutes a significant limitation. Being aware of their group assignment could potentially influence participants' behavior, especially during assessments, thereby introducing bias into the reported outcomes.

Conclusions

Integrating a simple lifestyle walking intervention into the daily life of stable HFrEF patients did not improve their functional capacity, despite having increased their objectively measured PA levels. Achieving improvements in functional capacity likely necessitates the implementation of traditional supervised, structured exercise-based rehabilitation programs with specified durations and intensities. As these programs often fail to induce sustained behavioral change in the long term, future studies should explore the potential of a comprehensive approach that combines exercise-based rehabilitation with simple lifestyle PA interventions, utilizing tools such as activity trackers and mobile apps, to support long-term behavioral change and enhance health outcomes for HFrEF patients.

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Disclosures

The corresponding author (JB) reports receiving lecture honoraria and participation on advisory boards for Novartis, BoehringerIngelheim and AstraZeneca. AL reports receiving lecture honoraria and participation on advisory boards for Novartis, BoehringerIngelheim and AstraZeneca. JV reports receiving lecture honoraria and participation on advisory boards for Novartis, BoehringerIngelheim and AstraZeneca. IS reports receiving lecture honoraria from Novartis, BoehringerIngelheim and AstraZeneca and participation on advisory board for BoehringerIngelheim. RP reports receiving lecture honoraria from Novartis, BoehringerIngelheim and AstraZeneca. All other investigators report no conflict of interests.

Supplemental Materials

e-Appendices 1 to 4

Tables S1 to S5

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Table 1. Baseline Characteristics.

Characteristic	All Patients (n = 202)	Intervention Group (n = 101)	Control Group (n = 101)
Age at time of randomization, years, median (IQR)	65.0 (56.0–72.8)	65.0 (56.0–72.0)	65.0 (56.0–73.0)
Sex, n (%)			
Male	156 (77.2%)	78 (77.2%)	78 (77.2%)
Female	46 (22.8%)	23 (22.8%)	23 (22.8%)
Marital status, n (%)			
Married	131 (65.5%)	69 (69.7%)	62 (61.4%)
Divorced	24 (12.0%)	10 (10.1%)	14 (13.9%)
Single	20 (10.0%)	10 (10.1%)	10 (9.9%)
Widowed	17 (8.5%)	8 (8.1%)	9 (8.9%)
Living with a partner	8 (4.0%)	2 (2.0%)	6 (5.9%)
Education level, n (%)			
Elementary	16 (8.0%)	7 (7.1%)	9 (8.9%)
Secondary	153 (76.9%)	73 (74.5%)	80 (79.2%)
University	30 (15.1%)	18 (18.4%)	12 (11.9%)
Employment status, n (%)			
Employed	52 (25.9%)	31 (31.0%)	21 (20.8%)
Unemployed	11 (5.5%)	4 (4.0%)	7 (6.9%)
On old age pension	109 (54.2%)	55 (55.0%)	54 (53.5%)
On disability pension	29 (14.4%)	10 (10.0%)	19 (18.8%)
Smoking status, n (%)			
Never smoked	63 (31.2%)	31 (30.7%)	32 (31.7%)
Ex-smoker	105 (52.0%)	55 (54.5%)	50 (49.5%)
Current smoker	34 (16.8%)	15 (14.9%)	19 (18.8%)
Alcohol intake, n (%)			
Does not consume	65 (32.2%)	33 (32.7%)	32 (31.7%)
Occasionally	112 (55.4%)	55 (54.5%)	57 (56.4%)
Regularly consumes	25 (12.4%)	13 (12.9%)	12 (11.9%)
Average daily step count, median (IQR)	5,071 (3,148–7,357)	4,851 (3,049–7,357)	5,343 (3,168–7,265)
Average daily minutes of MVPA, median (IQR)	10.9 (3.2–27.3)	11.1 (3.6–27.3)	10.2 (2.6–27.7)
Distance walked during the 6MWT, m, median (IQR)	385.0 (329.0–425.0)	390.0 (325.0–430.0)	371.0 (329.8–420.0)
Systolic blood pressure, mmHg, median (IQR)	120.0 (109.0–130.0)	120.0 (109.0–130.0)	120.0 (110.0–131.0)
Diastolic blood pressure, mmHg, median (IQR)	77.0 (70.0–80.0)	78.0 (69.0–80.0)	77.0 (70.0–80.0)
Body mass index, kg/m^2, median (IQR)	29.0 (25.8–33.4)	29.7 (26.0–33.6)	28.5 (25.1–33.0)
Waist circumference, cm, median (IQR)	107.0 (97.0–118.0)	109.0 (99.0–118.0)	104.0 (96.8–117.2)

NYHA class, n (%)			
II	183 (90.6%)	92 (91.1%)	91 (90.1%)
III	19 (9.4%)	9 (8.9%)	10 (9.9%)
Left ventricular ejection fraction, %, median (IQR)	32.5 (25.0–36.8)	34.0 (26.0–37.0)	32.0 (25.0–36.0)
NT-proBNP, ng/L, median (IQR)	597.0 (287.0–1,483.0)	597.0 (276.0–1,483.0)	613.5 (293.5–1,480.0)
hsCRP, mg/L, median (IQR)	2.1 (1.0–5.3)	1.9 (1.0–4.8)	2.3 (1.1–5.4)
Creatinine, µmol/L, median (IQR)	93.0 (79.2–113.8)	93.0 (79.5–115.5)	93.0 (79.5–110.5)
eGFR <60 mL/min/1.73 m², n (%)	78 (38.6%)	42 (41.6%)	36 (35.6%)
Ischemic heart disease, n (%)			
Yes	121 (59.9%)	67 (66.3%)	54 (53.5%)
No	81 (40.1%)	34 (33.7%)	47 (46.5%)
MAGGIC score, mean (SD)	19.0 (5.7)	18.6 (5.7)	19.5 (5.7)
Comorbidities, n (%)			
Arterial hypertension	128 (63.4%)	65 (64.4%)	63 (62.4%)
Prior myocardial infarction	96 (47.5%)	51 (50.5%)	45 (44.6%)
History of atrial fibrillation/flutter	63 (31.2%)	33 (32.7%)	30 (29.7%)
Atrial fibrillation/flutter at screening	23 (11.4%)	13 (12.9%)	10 (9.9%)
Peripheral artery disease	21 (10.4%)	9 (8.9%)	12 (11.9%)
History of cardiac arrest	19 (9.4%)	9 (8.9%)	10 (9.9%)
Stroke	15 (7.4%)	8 (7.9%)	7 (6.9%)
Valve procedure	13 (6.4%)	4 (4%)	9 (8.9%)
Type 2 diabetes	78 (38.6%)	37 (36.6%)	41 (40.6%)
COPD	21 (10.4%)	13 (12.9%)	8 (7.9%)
Depression	16 (7.9%)	10 (9.9%)	6 (5.9%)
Bronchial asthma	12 (5.9%)	4 (4%)	8 (7.9%)
History of malignancy	11 (5.4%)	6 (5.9%)	5 (5%)
Device therapy, n (%)			
ICD or CRT-D	114 (56.4%)	57 (56.4%)	57 (56.4%)
CRT-P or CRT-D	52 (25.7%)	31 (30.7%)	21 (20.8%)
Medication, n (%)			
ß blocker	195 (96.5%)	99 (98%)	96 (95%)
ARNI	114 (56.4%)	58 (57.4%)	56 (55.4%)
ACEi	63 (31.2%)	32 (31.7%)	31 (30.7%)
ARB	16 (7.9%)	8 (7.9%)	8 (7.9%)
MRA	159 (78.7%)	78 (77.2%)	81 (80.2%)
SGLT2i	37 (18.3%)	17 (16.8%)	20 (19.8%)
Loop diuretics	160 (79.2%)	77 (76.2%)	83 (82.2%)
Digoxin	27 (13.4%)	13 (12.9%)	14 (13.9%)
Ivabradine	18 (8.9%)	7 (6.9%)	11 (10.9%)

IQR indicates interquartile range; MVPA, moderate-to-vigorous physical activity; 6MWT, six-minute walk test; eGFR, estimated glomerular filtration rate; COPD, chronic obstructive pulmonary disease; ICD, implantable cardioverter-defibrillator; CRT-D, cardiac resynchronization therapy-defibrillator; CRT-P, cardiac resynchronization therapy-pacemaker; ARNI, angiotensin receptor neprilysin inhibitor; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; MRA, mineralocorticoid receptor antagonist; and SGLT2i, sodium-glucose cotransporter 2 inhibitor.

Table 2. Analyses of the Primary Outcome (6-Minute Walk Test) at 6 Months.

	Intervention group				Control group					
Analysis (number of patients included in the analysis)	**Value at baseline, median (IQR)**	**Value at six months, median (IQR)**	**Change, median (IQR)**	**Change, mean (95% CI)**	**Value at baseline, median (IQR)**	**Value at six months, median (IQR)**	**Change, median (IQR)**	**Change, mean (95% CI)**	**Adjusted between-group difference (95% CI)**	**P-value**
Intention-to-treat analysis of the complete cases (n = 186)	388.0 (324.2; 427.0)	417.5 (375.5; 475.5)	40.0 (0.0; 70.0)	35.5 (22.7; 48.3)	373.5 (331.0; 420.0)	400.0 (357.0; 450.0)	34.5 (0.0; 53.8)	28.8 (18.6; 39.0)	7.4 (-8.0; 22.7)	0.345
Intention-to-treat analysis following the imputation of missing cases (n = 202)	390.0 (325.0; 430.0)	420.0 (358.0; 474.0)	38.0 (5.0; 70.0)	35.2 (23.5; 47.0)	372.0 (330.0; 420.0)	400.0 (350.0; 450.0)	31.0 (-4.4; 53.0)	26.9 (17.2; 36.5)	8.7 (-5.6; 22.9)	0.231
Per-protocol analysis (n = 137)	394.0 (330.0; 430.0)	420.0 (374.0; 477.0)	40.0 (2.5; 70.0)	37.1 (21.3; 52.9)	382.5 (331.0; 420.0)	401.5 (365.0; 453.8)	36.0 (-0.0; 58.5)	30.1 (18.9; 41.2)	5.7 (-12.6; 24.0)	0.539

Change in the 6-minute walk test was not normally distributed.

Table 3. Intention-to-treat Analysis of the Secondary Outcomes at 6 Months.

Outcome (number of patients with complete data)	Change in intervention group, median (IQR)	Change in intervention group, mean (95% CI)	Change in control group, median (IQR)	Change in control group, mean (95% CI)	Adjusted between-group difference (95% CI)
Average daily step count (n = 131)	631.4 (-617.1; 1,639.2)	790 (332; 1,247)	-488.0 (-1,899.3; 728.7)	-667 (-1,183; -152)	1420 (749; 2,091)
Average daily minutes of MVPA (n = 131)	0.4 (-3.3; 9.5)	4.9 (1.0; 8.7)	-0.5 (-7.1; 1.5)	-3.1 (-6.8; 0.6)	8.2 (3.0; 13.3)
NT-proBNP, ng/L (n = 190)	-23.0 (-260.0; 162.0)	114 (-370; 598)	-35.0 (-321.0; 155.0)	-220 (-516; 75)	349 (-193; 892)
hsCRP, mg/L (n = 126)	0.0 (-0.6; 0.8)	1.7 (-0.9; 4.2)	-0.1 (-1.5; 0.5)	-0.3 (-2.2; 1.7)	2.1 (-1.0; 5.1)
LVEF, % (n=189)	2.0 (0.0; 6.0)	3.9 (2.5; 5.4)	3.0 (-0.3; 9.0)	3.7 (2.4; 5.0)	0.3 (-1.5; 2.2)
BDI-II (n=177)	-1.0 (-3.0; 1.0)	-0.7 (-1.6; 0.1)	0.0 (-2.0; 1.0)	0.1 (-0.8; 1.0)	-0.8 (-1.9; 0.4)
SF-36: Physical functioning (n=177)	0.0 (-5.0; 10.0)	1.3 (-1.3; 4.0)	0.0 (-5.0; 10.0)	0.2 (-2.9; 3.3)	1.3 (-2.5; 5.2)
SF-36: Role-Physical (n=177)	0.0 (0.0; 25.0)	3.5 (-4.0; 10.9)	0.0 (-25.0; 0.0)	-4.7 (-12.4; 3.0)	8.3 (-1.3; 17.8)
SF-36: Bodily pain (n=177)	0.0 (-20.0; 12.5)	-4.1 (-10.0; 1.8)	0.0 (-10.0; 10.0)	0.3 (-3.4; 4.0)	-4.9 (-11.0; 1.2)
SF-36: General health (n=177)	5.0 (-3.8; 15.0)	6.3 (3.2; 9.4)	0.0 (-5.0; 10.0)	1.3 (-2.0; 4.5)	4.5 (0.7; 8.4)
SF-36: Vitality (n=177)	0.0 (-5.0; 10.0)	2.6 (-0.2; 5.3)	0.0 (-10.0; 10.0)	-0.5 (-3.3; 2.2)	2.4 (-1.3; 6.0)
SF-36: Social functioning (n=177)	0.0 (-12.5; 12.5)	-1.2 (-5.6; 3.3)	0.0 (-12.5; 12.5)	-1.8 (-5.7; 2.1)	0.5 (-4.9; 5.9)
SF-36: Role-Emotional (n=177)	0.0 (0.0; 33.3)	8.9 (0.7; 17.1)	0.0 (0.0; 0.0)	0.7 (-7.3; 8.8)	4.3 (-5.0; 13.7)
SF-36: Mental health (n=177)	0.0 (-8.0; 4.0)	-1.2 (-4.2; 1.8)	0.0 (-8.0; 4.0)	-1.6 (-3.9; 0.6)	0.8 (-2.7; 4.3)
GSE (n=177)	0.0 (-2.5; 3.5)	0.2 (-1.0; 1.3)	0.0 (-2.0; 2.0)	-0.2 (-1.7; 1.3)	0.5 (-1.1; 2.1)
Weight, kg (n=193)	0.0 (-2.0; 2.0)	0.1 (-0.7; 1.0)	1.0 (-0.8; 3.0)	1.1 (0.3; 1.9)	-1.0 (-2.1; 0.1)
Waist circumference, cm (n=190)	0.0 (-2.0; 2.0)	-0.3 (-1.2; 0.5)	0.0 (-1.0; 3.0)	1.8 (-0.5; 4.2)	-1.5 (-3.7; 0.8)
Hip circumference, cm (n=190)	0.0 (-2.0; 2.0)	0.0 (-1.3; 1.2)	0.0 (-1.0; 3.0)	1.9 (-0.4; 4.1)	-0.9 (-3.1; 1.3)
MAGGIC risk score (n = 189)	-1.0 (-2.0; 1.0)	-0.5 (-1.1; 0.0)	-1.0 (-3.0; 0.0)	-1.1 (-1.7; -0.5)	0.4 (-0.4; 1.2)

The change in none of the variables followed a normal distribution.
MVPA indicates moderate-to-vigorous physical activity; LVEF, left ventricular ejection fraction; BDI-II, Beck depression inventory-II; SF-36, 36-item short-form health survey; and GSE, General self-efficacy scale.

Table 4. Adverse Events.

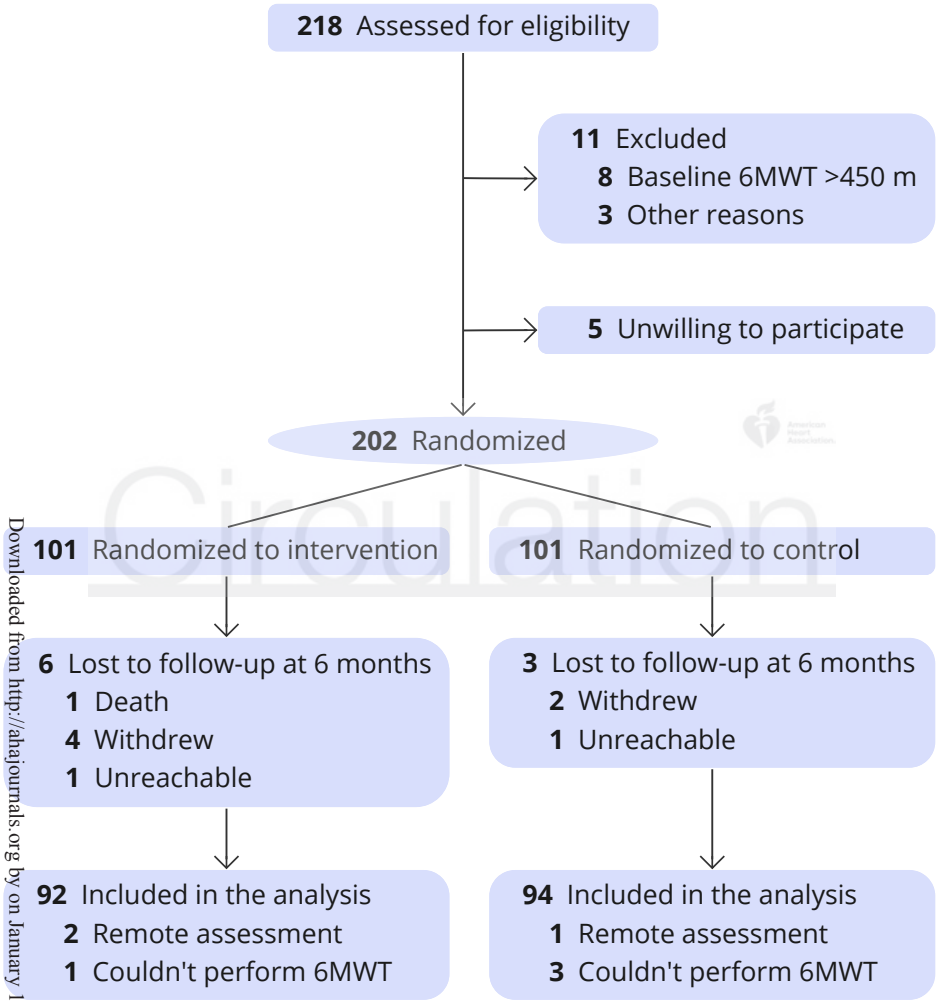
Adverse event category	All patients (n = 202)	Intervention group (n = 101)	Control group (n = 101)
Hospitalization for heart failure	4	1	3
Visit to the emergency room for heart failure	1	0	1
Increase in diuretic dose	4	1	3
Other CV events including hospitalizations for CV reasons	13	5	8
Non-CV events including hospitalizations for non-CV reasons	9	8	1
ICD discharge	6	4	2
Fall, injury	2	0	2
Infection	9	5	4
Others	3	2	1
Death	1	1*	0
Total	**52**†	**27**	**25**

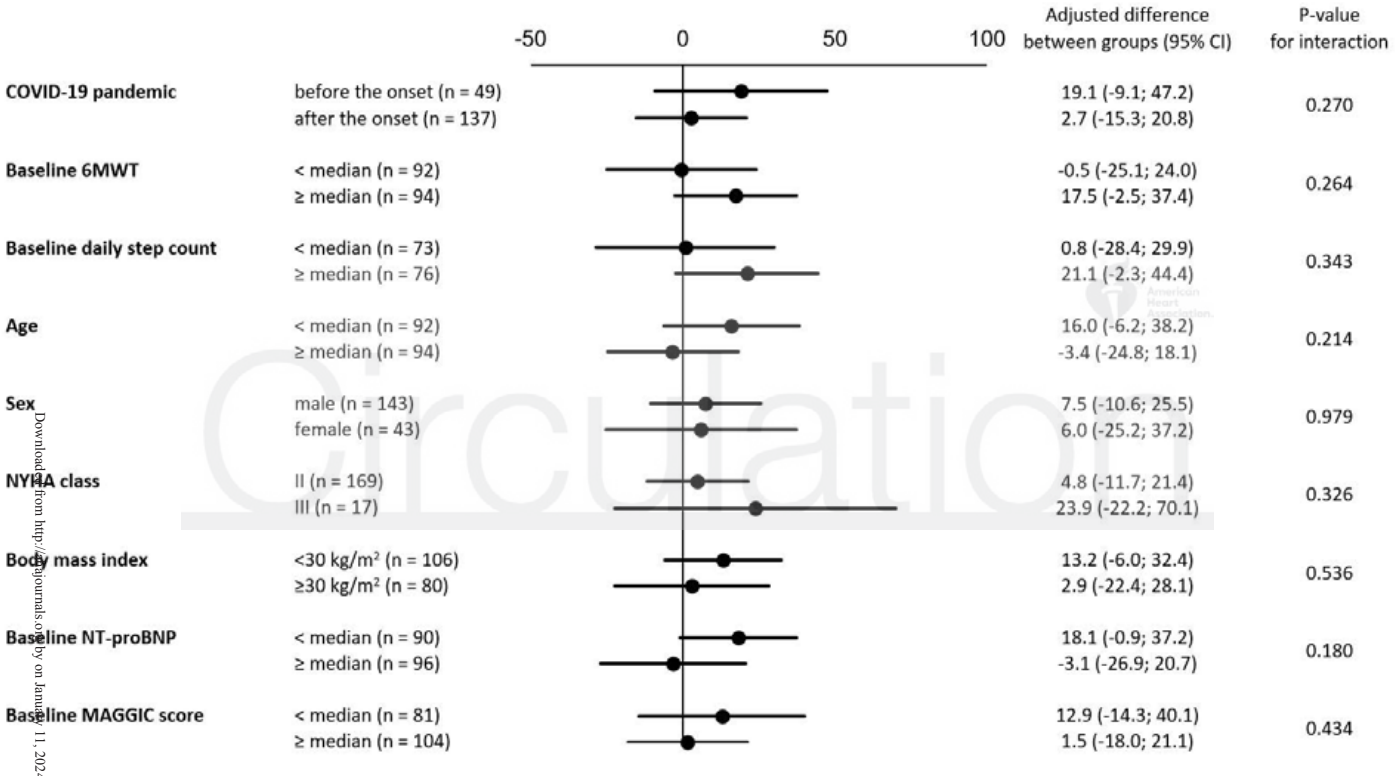
CV indicates cardiovascular; and ICD, implantable cardioverter-defibrillator.
*The only death was for non-CV reason.
†In total, 52 adverse events were recorded in 42 patients.

Figure Legends

Figure 1. Study enrollment, randomization, and follow-up

Figure 2. Subgroup analyses of the primary outcome (6-minute walk test) at 6 months





D. Rob et al.

Effect of Intra-arrest Transport, Extracorporeal Cardiopulmonary Resuscitation, and Invasive Treatment: a post-hoc Bayesian re-analysis of a randomized clinical trial

Chest Journal
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Effect of Intraarrest Transport, Extracorporeal Cardiopulmonary Resuscitation, and Invasive Treatment

A Post Hoc Bayesian Reanalysis of a Randomized Clinical Trial

To the Editor:

Evidence for the effect of extracorporeal cardiopulmonary resuscitation (ECPR) from randomized controlled trials (RCTs) on survival with a favorable neurologic outcome is inconclusive.[1-3] The Prague Out-of-Hospital Cardial Arrest study was an RCT evaluating the use of an invasive strategy, including early intraarrest transport, ECPR, and immediate invasive management to standard resuscitation in refractory out-of-hospital cardiac arrest.[1] The study enrolled 256 patients with a median age of 58 years, 83% were men, and the median time of resuscitation was 52.5 min. The primary outcome of 180 days' survival with a favorable neurologic outcome (cerebral performance category 1 or 2) was reached in 31.5% of patients in the invasive strategy group and 22.0% of patients in the standard resuscitation strategy group (OR, 1.63; 95% CI, 0.93-2.85; absolute difference, 9.5%; 95% CI, −1.3% to 20.1%; $P = .09$).[1] This difference was not statistically significant using the frequentist approach, and the primary outcome result was interpreted as neutral.[1] An unreasonable, simplistic, yet common, practice is to label a trial as either positive or negative based on a difference in the primary outcome evaluated by a P value threshold of .05.[4] Bayesian analysis may provide a comprehensive view of the data, especially when the benefits of an intervention are uncertain.[5] Therefore, we performed a previously unplanned Bayesian reanalysis of the prespecified primary outcome while adhering to the intention to treat principle.

Overall, normal priors were specified for the log(OR), and seven scenarios were considered which determined the prior mean and SD. As a reference, a weakly informative prior was considered assuming no intervention effect (zero mean of log[OR]) and a large prior SD of 10. This weak prior was used to produce results that relied strongly on data from the RCT alone. Three (mildly, moderately, and strongly) enthusiastic priors were established with prior ORs of 1.70, 2.15, and 2.65 corresponding to 10%, 15%, and 20% improvement in the primary outcome in the invasive arm. The prior SD of log(OR) was equal to 0.2, 0.5, and 1.0. That is, with increasing prior value of the OR, a lower degree of prior belief was considered. These three priors were based on three scenarios published in the statistical analysis plan prior to the study enrollment in 2012.[6] These scenarios were established based on the consensus of three experts, each of whom conducted independent literature reviews and made estimates. Finally, we considered three (mildly, moderately, and strongly) skeptical priors which assumed no effect of the invasive strategy (zero prior mean of log [OR]). The degree of skepticism was expressed by varying the prior SD of the log(OR) of 1.0, 0.5, and 0.2. We chose a range of priors to represent a wide spectrum of beliefs regarding the treatment effect. We did not assume a negative effect of the invasive strategy because of the findings of published observational studies and clinical trials in this field, which have shown either positive or neutral results of the ECPR strategy compared with standard treatment.[1-3] The analysis was performed using the R version 4.0.4 software (R Core Team), using the packages runjags and JAGS.[7,8]

The main results for the primary outcome of 180 days' survival with minimal or no neurologic impairment are shown in Table 1. Distribution of the logarithm of the OR for different scenarios is shown in Figure 1. The weakly informative scenario corresponds to the results of a frequentist analysis with an OR of 1.65, an effect difference of 9.6%, and a posterior probability of the effect difference > 0 of 96.1% in favor of the invasive arm. In the three enthusiastic scenarios reflecting qualified estimates before the study initiation, the ORs were 1.68, 1.76, and 1.70, with effect differences of 9.9%, 10.8%, and 10.2%, and posterior probabilities of 99.9%, 98.9%, and 97.4%, in favor of the invasive arm. In the three skeptical scenarios, the ORs were 1.58, 1.45, and 1.18, with effect differences of 8.9%, 7.2%, and 3.2%, and posterior probabilities of 95.3%, 93.6%, and 84.5%, in favor of the invasive arm.

TABLE 1] Bayesian Analysis of the Primary Outcome of Survival With Minimal or No Neurologic Impairment at 180 d

Scenario	Prior OR	Prior SD of log(OR)	OR (95% CI)	Effect Difference, % (95% CI)	Posterior Probability of the Effect Difference > 0, %
Weakly informative	1.00	10.0	1.65 (0.83-2.71)	9.6 (−1.2 to 20.2)	96.1
Mildly enthusiastic	1.70	0.2	1.68 (1.18-2.25)	9.9 (3.8 to 16.2)	99.9
Moderately enthusiastic	2.15	0.5	1.76 (1.01-2.73)	10.8 (1.7 to 20.2)	98.9
Strongly enthusiastic	2.65	1.0	1.70 (0.89-2.76)	10.2 (−0.3 to 20.4)	97.4
Mildly skeptical	1.00	1.0	1.58 (0.84-2.58)	8.9 (−1.7 to 19.0)	95.3
Moderately skeptical	1.00	0.5	1.45 (0.83-2.24)	7.2 (−1.7 to 16.9)	93.6
Strongly skeptical	1.00	0.2	1.18 (0.83-1.58)	3.2 (−3.1 to 9.2)	84.5

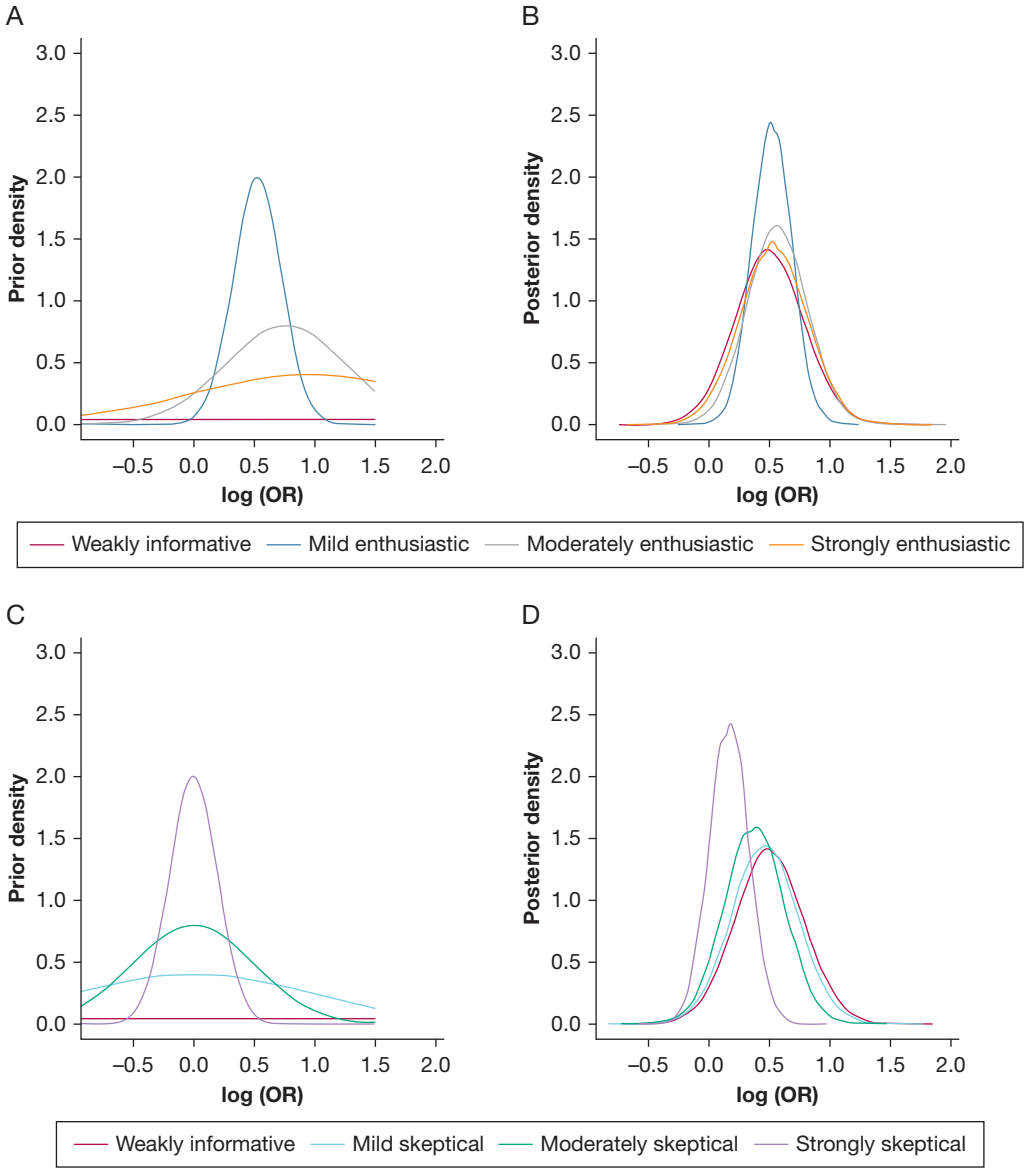

Figure 1 – *A and C, Prior and posterior (B and D) distribution for the logarithm of the OR for different scenarios.*

Limitations of this analysis include those of the primary trial.[1] Specifically, the study had a single-center design and was conducted in an experienced center, which limits the generalizability of our results. Additional limitations are specific to the Bayesian reanalysis. First, this is an unplanned post hoc analysis of the trial data. Furthermore, the accuracy of the results obtained from Bayesian analysis is always dependent on the prior distributions used. Recognizing this limitation, a wide range of potential prior beliefs was included in this analysis.

In conclusion, this Bayesian reanalysis of the study primary outcome showed a benefit of the invasive approach compared with standard resuscitation under a broad set of scenarios. This finding may help in the interpretation of the study results and underscores the importance of considering Bayesian analysis to complement frequentist approaches when evaluating the efficacy of interventions in clinical trials. Further research is warranted to gather additional evidence on the applicability, patient selection, and effectiveness of ECPR strategies across various centers and systems in improving outcomes for refractory out-of-hospital cardiac arrest.

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T. Muzafarová et al.

The Prognosis of Cardiogenic Shock Following Acute Myocardial Infarction—an Analysis of 2693 Cases From a Prospective Multicenter Registry

Deutsches Ärzteblatt Int.
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CORRESPONDENCE

Research Letter

The Prognosis of Cardiogenic Shock Following Acute Myocardial Infarction—an Analysis of 2693 Cases From a Prospective Multicenter Registry

Cardiogenic shock (CS) is the most common cause of death in patients with acute myocardial infarction (AMI) and complicates 5–12% of cases [1]. In-hospital mortality from AMI complicated with CS (CS-AMI) remains consistently high at about 50%[2]. Our analysis aims to examine the incidence, outcomes, and predictive factors in a large cohort of patients with CS-AMI.

Methods

The analysis is based on data from the national all-comers registry, the cardiovascular interventions module of which is a prospective multicenter registry that has collected data on all percutaneous coronary interventions (PCI) performed in all PCI centers in the Czech Republic since 2005.

Standard descriptive statistics were applied in the analysis: absolute and relative frequencies for categorical variables, means with standard deviations for continuous variables. Univariate and multivariate logistic regressions adjusted for the centers were used for the descriptive analysis of predictors of mortality. Kaplan–Meier methodology and the hazard ratio (HR), based on the Cox proportional hazards model, were applied for the description of time to event during the evaluated time window. Analyses were conducted using SPSS 28.0.1.1. For the evaluation of the association with comorbidities, the Deyo-Charlson Comorbidity Index based on the International Classification of Diseases codes was used.

Results

The initial dataset included 50 745 AMI patients from 2016–2020 (58.2% of them with ST-elevation myocardial infarction [STEMI] and 41.8% with non-ST-elevation myocardial infarction [NSTEMI]), of whom 2822 patients had CS-AMI. Patients with available information on 30-day mortality (N = 2693) were used in the detailed analysis. On average, 56.7% of CS-AMI patients required cardiopulmonary resuscitation (CPR) (both out- and in-hospital), 67.1% mechanical ventilation and 53.5% both. The HR for the 30-day mortality of patients with CS against patients without CS based on survival analysis is 15.25 with 95% confidence interval (CI) [14.24; 16.33].

The basic characteristics of patients are presented in *Table 1*. The univariate logistic regression identified female sex (odds ratio [OR] 1.23, age (for each one-year increment: OR 1.04, chronic kidney disease/failure (OR 1.67), diabetes mellitus (OR 1.68), subacute STEMI (OR 1.48), resuscitation (OR 1.23), mechanical ventilation (OR 1.35), three-vessel disease (OR 1.79), left main disease (OR 1.42) , and more than 8 hours delay from symptom onset to revascularization (OR 1.48) as the factors with the highest predictive power for 30-day mortality. Although the mortality rate was numerically higher during autumn and winter (54.2% vs. 45.8% and 51.45% vs. 48.55%, respectively, $p = 0.020$) and during the weekend vs. the working week (51.45% vs. 48.55%, $p < 0.001$) a predictive role of these factors was not confirmed neither in univariate nor in multivariate analysis.

TABLE 1

Basic characteristics of patients with cardiogenic shock in the period 2016–2020 with 30-day mortality

		Amount (%)	30-day mortality (%)
Total N (%)		2693 (100.0%)	1357 (50.4%)
Sex	Men	72.6%	49.0%
Age	< 40	1.1%	40.0%
	40–49	6.5%	33.7%
	50–59	16.1%	32.8%
	60–69	31.7%	46.1%
	70–79	28.3%	58.5%
	≥ 80	16.3%	69.5%
Diabetes mellitus		23.1%	59.8%
Previous PCI		17.7%	54.1%
Previous CABG		5.6%	46.7%
Chronic kidney disease/failure		8.1%	61.9%
Post CPR		57.4%	52.6%
Artificial lung ventilation		68.0%	52.8%
Time from symptom onset to PCI (in hours)			
	< 2	14.4%	46.3%
(acute STEMI only)	2–3	18.8%	42.8%
	3–4	10.3%	49.6%
	4–8	11.7%	53.2%
	> 8	7.5%	55.9%
	Not known	37.4%	54.0%
No. of diseased vessels	1	25.7%	2.3%
	2	28.5%	47.5%
	3	40.5%	56.8%
	Not known	5.2%	56.4%
Left main stenosis >50%	No	81.3%	48.7%
	Yes	17.3%	57.4%
	Not known	1.4%	60.5%
DCCI	0–1	10.7%	10.6%
	2–3	31.3%	29.0%
	4–6	37.9%	39.8%
	> 6	20.2%	20.7%

CABG, Coronary artery bypass graft; CPR, cardiopulmonary resuscitation; DCCI, Deyo Charlson Comorbidity Index; STEMI, ST-elevation myocardial infarction; PCI, percutaneous coronary intervention

Multiple logistic regression showed that age (> 80 years), diabetes mellitus, cardiopulmonary resuscitation, mechanical ventilation, three-vessel disease and left main disease were the independent factors with the highest predictive power for 30-day mortality (*Table 2*).

TABLE 2

Characteristics influencing 30-day mortality of patients with cardiogenic shock*

	OR [95% CI]
Women vs. men	1.00 [0.83; 1.21]
Age (years) (p<0.001)	
60–69 vs. < 60 years	1.67 [1.33; 2.11]
70–79 vs. < 60 years	2.65 [2.07; 3.40]
≥ 80 vs. < 60 years	4.69 [3.50; 6.28]
Previous PCI	1.28 [1.03; 1.60]
Previous CABG	0.65 [0.45; 0.94]
Diabetes mellitus	1.45 [1.17; 1.79]
Chronic kidney disease/failure	1.66 [1.21; 2.30]
MI type (p=0.062)	
Subacute STEMI vs. acute STEMI	1.23 [0.97; 1.57]
NSTEMI vs. acute STEMI	0.87 [0.69; 1.09]
Post CPR	1.27 [1.02; 1.59]
Artificial lung ventilation	1.54 [1.21; 1.95]
Number of diseased vessels (p=0.006)	
2 vs. 1	1.09 [0.87; 1.36]
3 vs. 1	1.36 [1.09; 1.70]
Left main stenosis ▯ 50%	1.36 [1.09; 1.71]
TIMI flow before PCI: 3 vs. 0–2	1.08 [0.86; 1.36]
TIMI flow after PCI: 3 vs. 0–2	2.68 [2.18; 3.29]
DCCI (p=0.137)	
2–3 vs. 0–1	0.79 [0.59; 1.06]
4–6 vs. 0–1	0.82 [0.61; 1.10]
> 6 vs. 0–1	0.68 [0.49; 0.95]

*multivariate model adjusted for centers
CABG, Coronary artery bypass graft; CPR, cardiopulmonary resuscitation; DCCI, Deyo–Charlson Comorbidity Index; HR, hazard ratio; MI, myocardial infarction; NSTEMI, Non-ST-elevation myocardial infarction; PCI, percutaneous coronary intervention; STEMI, ST-elevation myocardial infarction; TIMI, thrombolysis in myocardial infarction

Discussion

Our data are valuable because they include a large group of consecutive patients with CS-AMI. Analysis showed a 30-day mortality rate of 50.4%, similar to previous findings [3], [4]. These high numbers show that CS is an important target for further improvements in the management of patients with AMI.

The key finding of our study is that the outcome of patients with CS-AMI is highly affected by the patient's degree of instability, as documented by mechanical ventilation and resuscitation, and the timing of successful revascularization. The independent impact of comorbidity and nontraditional factors on the prognosis of these patients has not been confirmed. The analysis of predictors of 30-day mortality may be helpful in creating profiles of CS-AMI patients and in triage, which is important in the decision of management strategies.

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PK received lecture honoraria from Chiesi.

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Impact of the COVID-19 pandemic on the occurrence and outcome of cardiogenic shock complicating acute myocardial infarction

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Letter to the Editor

Impact of the COVID-19 pandemic on the occurrence and outcome of cardiogenic shock complicating acute myocardial infarction

Dear Editor,

SARS-CoV-2 predisposes patients to thrombotic disease due to excessive inflammation, platelet activation, endothelial dysfunction, and stasis [1]. The infection was recognized as an independent risk factor for acute myocardial infarction (AMI), which is considered part of the clinical picture of the COVID-19 disease [2]. Cardiovascular disease increases both the susceptibility to SARS-COV-2 and the risk of death in patients with COVID-19 [3]. Among those who died and were COVID PCR-positive, almost one-third (30%) suffered from ischemic heart disease [4].

Viral infections in general and SARS-Cov-2, even more so, increase the risk of atherosclerotic plaque destabilization and atherothrombosis [2,3]. The most significant reduction in the risk of cardiovascular events associated with influenza vaccinations was observed in patients post-MI, with an average reduction in the risk of subsequent MI by two-thirds and a decrease in the risk of cardiovascular death of more than a half [5].

Behavior changes, conditioned by fear of contact with health care facilities, led to patients neglecting care and treatment of diseases other than COVID-19 [6]. At the same time, the burden posed by the pandemic, in terms of increased numbers of patients, had the power to paralyze medical services, thus fundamentally affecting the overall functionality and availability of healthcare. There was an across-the-board decline in the utilization of in-person patient care [7]. In the Czech Republic, a significant decrease in hospital admissions for AMI was documented with an incidence ratio of 0.949 (0.911;0.989) for acute STEMI and 0.949 (0.911;0.989) for NSTEMI [8].

The changes caused by the COVID-19 pandemic led to the presumption of a higher incidence of cardiogenic shock complicating acute myocardial infarction. The presumption was primarily based on the higher risk of (repeated) atherothrombotic events in consequence to the infection- and pandemic-related stress, longer time to search- and impaired availability of health care, including reperfusion therapy, which plays a strategic role in patient prognosis. This study aimed to assess the impact of the COVID-19 pandemic on the incidence and prognosis of acute myocardial infarction complicated by cardiogenic shock (CS-AMI).

The analysis is based on data from the National Registry of Cardiovascular Surgery and Interventions (NRCSI) in the Czech Republic combined with data from other registries of the National Health Information System (NHIS), namely the registry of deaths for mortality analysis and the Information System of Infectious Diseases (ISID) for COVID-19 data. NHIS data were collected by the Institute of Health Information and Statistics in the Czech Republic and provide data on the health status of the population, and on activities of healthcare providers. Data in the ISID are collected in compliance with Act No. 258/2000 Coll. on Protection of Public Health, and data in the NHIS are collected under Act No. 372/2011 Coll., on Health Services and Conditions of Their Provision. Due to this legal mandate, the retrospective analyses did not require either approval by an ethics committee or informed consent from participants.

Standard descriptive statistics were applied in the analysis; absolute and relative frequencies for categorical variables and means supplemented with standard deviations or medians supplemented with IQR for continuous variables. Statistical significance of differences among groups of patients was computed using the Fisher exact test for categorical variables, and odds ratios supplemented with 95% confidence intervals were adopted to describe changes in risk events occurring between the studied periods. Linear regression was used for the prediction of the expected number of CS-STEMI. The level of statistical significance was set at 0.05 in all analyses. Statistical computations were done using SPSS 27.0.0.0. (IBM Corporation 2022).

From 1/2016 to 12/2020, 50,745 AMI patients were included in the NRCSI all-comers registry, and 2822 (5.6%) were complicated by CS.

The mean incidence of CS-AMI was significantly higher during the COVID period (2020) than the incidence from 2016 to 2019 (6% vs. 5.5%, $p = 0.032$, odds ratio (OR) for CS-AMI in 2020 was 1.118 (95% C. I. 1.019; 1.227)). The difference was caused by significant increase in acute STEMI complicated by CS (8.7% vs. 7.6%, $p = 0.011$, OR for CS-STEMI in 2020 was 1.166 (1.039; 1.309)); it was 7.1% in 2016, 7.8% (2017), 7.6% (2018), 7.8% (2019), and 8.7% (2020). NSTEMI complicated by CS was 2.3% (2016), 2.7% (2017), 2.7% (2018), 2.8% (2019), and 2.8% (2020). The observed rise in the incidence of CS-STEMI during each month of the pandemic (compared to the average incidence in non-pandemic years) correlated with national lockdowns and the substantial increase in the number of COVID infected/hospitalized patients (Fig. 1).

A significant prolongation in time delay to reperfusion (from the onset of symptoms to balloon/reperfusion) was only observed in October 2020, compared to non-COVID Octobers in 2016–2019. October 2020 saw (1) a peak in the national epidemic with the highest growth of infected and hospitalized patients and (2) a partial lockdown (Fig. 1). During the partial lockdown (October–December), measures affecting schools, shopping malls, and restaurants were in place (starting on October 5, 2020). However, no complete lockdown was implemented, and most measures ended before Christmas. In other months of the COVID year, no significant changes in the onset of symptoms to reperfusion time were observed in CS-STEMI patients.

Except for a less frequent history of previous PCI (13.9% vs. 8.2%, $p<0.001$), we found no significant differences in CS-STEMI patient characteristics in 2016–2019 vs. 2020; men 72.7% vs. 75.4%, mean age (SD) 66.3 (12.3) years vs. 66.3 (12.2) years, diabetes 20.9% vs. 19.1%, chronic kidney disease 5.4% vs. 5.7%, previous CABG 4.5 vs. 4.2%, left main disease 14.3% vs. 16%, one vessel disease 24.9% vs. 32.1%, pre-

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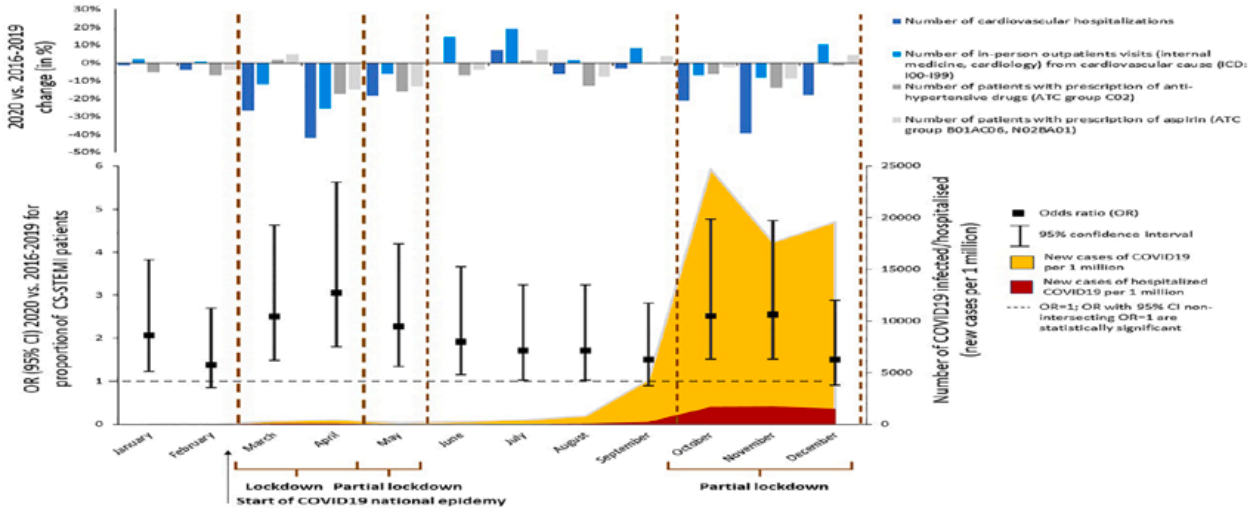

Fig. 1. The COVID-19 and the occurrence of cardiogenic shock (CS) complicating acute myocardial infarction (AMI).
- The upper part of the figure: The number of hospitalizations for cardiovascular diseases; in-person outpatient visits to cardiologists or internal medicine specialists, patients with prescriptions for anti- hypertensive drugs and aspirin (with a cardiovascular indication), in relation to lockdowns and the number of COVID-19 infected/hospitalized patients
- The lower part of the figure: Impact of national lockdowns and numbers of COVID-19 infected/hospitalized patients as it relates to the risk of CS-STEMI.

PCI TIMI flow 0 64.4% vs. 66.2%, and post- PCI TIMI flow 3 76.7% vs. 76.9%. PCR positivity for COVID-19 infection within 30 days before hospitalization for CS-AMI was confirmed in 3.6% (21) of patients.

The COVID pandemic did not influence the proportions of pre-hospital resuscitated CS-AMI patients (57.5% vs. 58.7%, p = 0.6) nor the number of patients on mechanical ventilation (67.8% vs. 68.3%, p = 0.8).

CS-AMI 30-day mortality was 53.7% in 2016, 51.6% (2017), 49.7% (2018), 49.3% (2019), and 47.9% (2020). For CS-STEMI it was 50.8%, 47.1%, 46.4%, 44.1%, and 45.3%, respectively (P for 2019 vs. 2020 = 0.8). Odds ratios for 30-day mortality in 2020 vs. 2016–2019 was 0.929 (0.738; 1.170), p = 0.534 for CS-AMI and 0.915 (0.541; 1.548), p = 0.740 for CS-STEMI.

During the national COVID epidemic, there was a meaningful decline in the number of hospitalizations for cardiovascular diseases (an absolute reduction of 4.606 per million population above 18 years), in-person outpatient visits to cardiologists or internal medicine specialists (fell by 5.207 per million population above 18 years), and prescription medications to prevent the risk of major cardiovascular events (anti-hypertensives fell by 2.549 and aspirin (for cardiovascular reasons) fell by 4.261 per million population above 18 years) (Fig. 1). This was especially true during lockdown periods, where the absolute reductions in the mentioned cardiovascular care parameters were −27.8%, −8.7%, −8.4%, and −5%, respectively.

Cardiogenic shock is the leading cause of death in AMI. Therefore, analyzing its incidence is essential to identifying preventable factors that diminish survival probabilities in AMI, which is the leading cause of cardiovascular death worldwide.

Our national all-commers registry data collected over the years showed no impact of the pandemic, across all societal and regulatory levels, on the mortality of patients admitted to hospital with STEMI complicated by CS. We found no differences in patient cardiovascular risk profiles, time to reperfusion, angiographic findings, and procedural results. We also failed to observe a significant increase in prehospital resuscitations.

We found a significant decline in the number of patients receiving in-hospital and out-of- hospital cardiovascular care and the number of patients taking prescription medication to prevent major cardiovascular events, i.e., hypertensives and aspirin (Fig. 1). We noted that adherence to outpatient care, lifestyle recommendations, and drug treatment was severely compromised during the pandemic [7]. Cardiovascular pharmacotherapy was linked to both greater susceptibility to coronavirus infection and higher mortalities after infection [9]. This appeared to be the case because patients with cardiovascular disease were more vulnerable to both these aspects [3,4]. Although randomized trials have not confirmed a relationship between drugs that interfere with neurohumoral regulation of the renin-angiotensin-aldosterone system [9] and antithrombotic agents [10]; however, alarming messages in media [11] led patients to distrust these medications and ultimately affected adherence.

The availability of comprehensive health care information makes it possible to present a detailed analysis of the incidence of CS-AIM during the COVID-19 pandemic [8]. We were able to document an increase in the proportion of patients admitted to hospitals with STEMI complicated by CS in 2020, the year of the COVID pandemic. The rise in CS correlated with lockdown periods and increases in the number of people infected. Considering the presented results, we concluded that the pandemic substantially impacted the availability of health care and patient treatment adherence. We found no evidence that the survival of those admitted to the hospital was affected by the specific and unusual circumstances created by the COVID-19 pandemic.

What are the implications of our findings? There is an essential need to prevent an unjustified violation of the patient's trust in treatment and thus their adherence to evidence-based recommendations, especially those impacting survival. In periods of social isolation, should mass and social media, with their power and close cooperation with cardiovascular care experts, take responsibility for building confidence in the health care system. Furthermore, it is also imperative for informing the public about the dangers and consequences of misinformation.

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Conflict of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that can influence the work reported in this paper.

Data availability statement

Anonymized data are available on request from the Institute of Health Information and Statistics of the Czech Republic (IHIS).

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Endothelin type A receptor blockade increases renoprotection in congestive heart failure combined with chronic kidney disease: Studies in 5/6 nephrectomized rats with aorto-caval fistula

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Endothelin type A receptor blockade increases renoprotection in congestive heart failure combined with chronic kidney disease: Studies in 5/6 nephrectomized rats with aorto-caval fistula

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ABSTRACT

Background: Association of congestive heart failure (CHF) and chronic kidney disease (CKD) worsens the patient's prognosis and results in poor survival rate. The aim of this study was to examine if addition of endothelin type A (ET_A) receptor antagonist to the angiotensin-converting enzyme inhibitor (ACEi) will bring additional beneficial effects in experimental rats.

Methods: CKD was induced by 5/6 renal mass reduction (5/6 NX) and CHF was elicited by volume overload achieved by creation of aorto-caval fistula (ACF). The follow-up was 24 weeks after the first intervention (5/6 NX). The treatment regimens were initiated 6 weeks after 5/6 NX and 2 weeks after ACF creation.

Results: The final survival in untreated group was 15%. The treatment with ET_A receptor antagonist alone or ACEi alone and the combined treatment improved the survival rate to 64%, 71% and 75%, respectively, however, the difference between the combination and either single treatment regimen was not significant. The combined treatment exerted best renoprotection, causing additional reduction in albuminuria and reducing renal glomerular and tubulointerstitial injury as compared with ACE inhibition alone.

Conclusions: Our results show that treatment with ET_A receptor antagonist attenuates the CKD- and CHF-related mortality, and addition of ET_A receptor antagonist to the standard blockade of RAS by ACEi exhibits additional renoprotective actions.

1. Introduction

Congestive heart failure (CHF) presents an extreme burden to the public healthcare worldwide. Almost 40% of CHF patients die within 1 year from the diagnosis and 70% within 5 years, even under adequate modern therapy [1,2]. The incidence and prevalence of chronic kidney disease (CKD) is also increasing [3] and CKD is one of the strongest risk factors for the development of CHF [4,5]. CHF coexists with CKD in approximately half of CHF patients [4–8]. Unfortunately, patients with estimated glomerular filtration rate $\leq$ 30 ml/min/1.73 m^2 have now largely been excluded from randomized control trials in HF, which limits the information on patients with combined CHF and CKD [4,5,7,8]. Therefore, although the patients with combined CHF and advanced CKD represent probably the highest cardiovascular risk population, their exclusion from CHF trials is a serious deontological error. Even the newest guidelines of the European Society of Cardiology for the treatment of CHF admit that there is little direct evidence to support any recommendation for the treatment of these patients [7,9]. Obviously,

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new therapeutic strategies for the treatment of CHF combined with CKD are urgently needed, and focused experimental studies should be performed to define new pharmacotherapeutic targets for prospective clinical research.

The rat with aorto-caval fistula (ACF) presents a well-defined model of CHF due to volume overload, which in many respects imitates the course of CHF in untreated humans [10–13]. The model is endorsed by the American Heart Association and the European Society of Cardiology for preclinical testing to identify new targets for CHF treatment [13]. In addition, the rat that undergoes the 5/6 renal mass reduction (5/6 NX) is acknowledged as a standard model of CKD and it thus provided a majority of the knowledge about the pathophysiology of CKD [14,15]. Importantly, we have introduced and validated a combination of 5/6 NX and ACF as a model to study the pathophysiology of CHF combined with CKD [16]; therefore the model was used in the present study.

In search for novel pharmacological strategies targeting the systems beyond the treatment currently considered and underlying fundamental drug therapy of CHF and/or CKD [3,7,9], attention has been directed to the endothelin (ET) system. Endothelin-1 (ET-1) is the most powerful mammalian vasoconstrictor [17] which plays an important role in the pathophysiology of hypertension, end-organ damage, CKD and CHF [18–21]. ET-1 actions are mediated via ET type A (ET_A) and ET type B (ET_B) receptors whose activation causes, respectively, vasoconstriction or vasodilatation with natriuresis [19,20]. ET system is markedly upregulated in animals with CHF and CKD and preclinical studies showed that activation of ET_A receptors contributes to the pathophysiology of CHF and CKD [19,21,22]. Therefore pharmacological blockade of ET might be a new approach to the treatment of CHF and CKD. Disappointingly, initial clinical studies evaluating the effects of ET blockade in patients with CHF and CKD failed to bring beneficial effects, and many of them had to be terminated prematurely, mainly due to fluid overload and worsening of CHF and even the development of acute heart failure [22–26]. Such adverse events might result from blockade of ET_B receptors in the kidney, a notion indirectly corroborated by recent results of SONAR study, which excluded patients at risk of fluid retention; pharmacological ET_A receptor blockade provided also renoprotection in type 2 diabetic patients with CKD [27]. Nevertheless, effects of selective ET_A receptor blockade on the course CHF when combined with CKD have not been thoroughly evaluated, even in preclinical studies. Therefore, utilizing our suitable experimental model and the available atrasentan, orally active and highly selective ET_A receptor antagonist [19], we first examined the effects of chronic atrasentan treatment on the morbidity and mortality in 5/6 NX + ACF normotensive Hannover Sprague-Dawley (HanSD) rats. Given that the pharmacological blockade of the renin-angiotensin system (RAS) is currently a cornerstone therapy for CHF as well as CKD, we compared the effects of pharmacological blockade of ET_A receptor antagonist with the standard RAS blockade (ACEi) as described earlier in studies in CHF and CKD [11,28–30]. Since previous experimental and clinical studies showed that renoprotective actions of ET_A receptor blockade in CKD are also present in individuals undergoing pharmacological blockade of the RAS, our third aim was to find out if the addition of the selective ET_A receptor blockade to standard treatment with ACEi would attenuate the progression of combined CHF and CKD in 5/6 NX + ACF HanSD rats. To further evaluate our hypothesis that beneficial actions of ET_A receptor blockade on the mortality in 5/6 NX + ACF HanSD rats are mediated predominantly by renoprotective actions, we assessed the degree of renal glomerular and kidney tubulointerstitial damage and organ weights in separate groups of animals after eight weeks of treatment, because at this stage the untreated 5/6 NX + ACF HanSD rats began markedly to die.

2. Methods

2.1. Ethical approval and animals

The studies were performed in accordance with the guidelines and practices established by the Animal Care and Use Committee of the Institute for Clinical and Experimental Medicine, Prague, which accord with the national law and the European Union policy and were approved by the Ministry of Health of the Czech Republic (project decision 21984/2021–5/OZV). The animals were bred at the Institute's Center of Experimental Medicine, accredited by the Czech Association for Accreditation of Laboratory Animal Care. The animals were kept on a 12-hour/12-hour light/dark cycle. Throughout experiments rats were fed a normal salt, normal protein diet (0.45% NaCl, 19–21% protein) produced by SEMED (Prague, Czech Republic) and had free access to tap water.

2.2. CHF and CKD models, exclusion criteria, therapeutic regimes and general analytic procedures

Male HanSD rats at initial age of 8 weeks, derived from several litters, were randomly assigned to experimental groups. In order to obtain reliable data regarding the effects of two treatment regimens on the survival rate, high initial *n* values were used, established using statistical power analysis [31].

The present studies were performed only in male rats, even though important sex-related differences in the progression of CHF as well as CKD are recognized and documented [2,3,32,33]. The American Physiological Society and the British Pharmacological Society recently recommended that "sex" should no longer be ignored as an experimental variable. Indeed, this is crucially important in preclinical research as a prerequisite for successful translation of the results into clinical practice, unless a strong rationale is provided for not incorporating both sexes [34–36]. In the present study, the justification for single-sex investigation is based on our original validation studies indicating no significant sex-related differences in the evaluated parameters [34–36]. Since, specifically, we found no such differences with respect to CKD- and CHF-related morbidity and mortality in 5/6 NX + ACF HanSD rats [16], we decided to perform the present study in one "sex" only, and the random choice was "male sex".

Animals were anesthetized (tiletamine + zolazepam, Virbac SA, Carros Cedex, France, 8 mg/kg; and xylasine, Spofa, Czech Republic, 4 mg/kg intramuscularly) and CHF was induced by volume overload from ACF created using needle technique as described previously [10–12,16,37–40]. Sham-operated rats underwent an identical procedure but without creating ACF.

To develop the CKD model, rats were anesthetized as usual and 5/6 NX was performed as described previously [28,29]. Briefly, the right kidney and both poles of the left kidney were excized in order to remove 5/6 of renal parenchyma. Sham-operated animals underwent the same procedure without removing renal parenchyma. Post-operative analgesia (meloxicam) wasadministered subcutaneously for two days.

To inhibit the angiotensin-converting enzyme (ACE), trandolapril (Gopten; Abbot, Prague, Czech Republic), was given at 2 mg/L in drinking water, the dose was previously shown to provide maximal blockade of the RAS [11,30]. ET_A receptor blockade was achieved with atrasentan (Abbot, Illinois, USA), 5 $mg.kg^{-1}.day^{-1}$ in drinking water; the dose was adjusted weekly to actual water intake and was previously found to effectively block ET_A receptors [28,29,41]. Importantly, there were no significant differences in water intake between experimental groups that were exposed to the treatment protocols with ET_A receptor antagonist and ACEi, alone or combined.

Albumin excretion was determined in 24-hour urine collections in individual metabolic cages; urinary albumin was measured by a quantitative sandwich enzyme immunoassay technique, using the commercially available ELISA kit (ERA3201–1, AssayPro, MO, USA). Before placing in metabolic cages, rats' body weight (BW) was monitored as well as the presence of CHF symptoms using a scoring system (used also for assessment of the onset of decompensation of heart failure in the model of spontaneously hypertensive heart failure rats) [42]. The method was found suitable also for the ACF model of CHF [16,38]. The

scoring referred to the five most apparent aspects of CHF phenotype: (1) presence of raised fur (piloerection), (2) diminished activity (lethargy), (3) peripheral cyanosis, (4) rapid or labored breathing (dyspnea), and (5) abdominal swelling (ascites). Every symptom was scored on the scale from 0 to 3 and a total CHF score was calculated for every animal as the sum of individual points; thus, the theoretical maximum that can be reached is 15. Once the score reached a threshold of ≥ 3, the advanced phase of CHF was diagnosed. It was demonstrated in the original characterization study that when an animal reached the score of 5, the onset of decompensation of CHF was seen and animals typically died within the next 7 days [43]. At the end of the study the survived animals were killed and individual organ weights were obtained, and the kidneys were used to assess glomerular damage and tubulointerstitial injury. The kidneys were fixed in 4% formaldehyde, dehydrated and embedded in paraffin. The sections stained with hematoxylin-eosin and PAS (periodic acid, for Schiff reaction) were examined and evaluated in a blind-test fashion. Fifty glomeruli in each kidney were examined on a semi-quantitative scale. The evaluation was as follows: *grade 0*, all glomeruli normal; *grade 1*, sclerotic area up to 25% (minimal sclerosis); *grade 2*, sclerotic area 25–50% (moderate sclerosis); *grade 3*, sclerotic area 50–75% (moderate-to-severe sclerosis); *grade 4*, sclerotic area 75–100% (severe sclerosis). The glomerulosclerosis index (GSI) was calculated using the following formula: $GSI = [(1 \times n_1) + (2 \times n_2) + (3 \times n_3) + (4 \times n_4)]/(n_0 + n_1 + n_2 + n_3 + n_4)$, where n_x is the number of glomeruli in each grade of glomerulosclerosis. Kidney cortical tubulointerstitial injury was evaluated as defined by Nakano et al. [44], to determine inflammatory cell infiltration, tubular dilatation, atrophy, or interstitial fibrosis. The injury was graded semi-quantitatively using the following scale of lesions: grade 0, no abnormal findings; 1, mild (<25% of the cortex); 2, moderate (25 – 50% of the cortex); 3, severe (>50% of the cortex). The lesions were assessed in at least 30 random and non-overlapping fields in the renal cortex. Thus, the maximum score for GSI is 4 and for the index of kidney tubulointerstitial injury is 3. This method is always employed in our studies evaluating the degree of kidney damage [12,28,29,45].

Systolic blood pressure (SBP) was measured with automated tail cuff system (Hatteras Instruments, Cary, N.C., USA). In accordance with recommendations for blood pressure (BP) measurements in experimental animals [46], this method is adequate for detecting intergroup differences in SBP over time, and therefore is optimal for long-term studies. Beginning from the initial age (i.e. 8 weeks), three times per week (Monday, Wednesday and Friday) the animals were immobilized for 20 min in the, heated cage designed for tail cuff SBP measurement, On the day of SBP measurement, after 5 min adaptation period, three values of SBP were taken, with 3–5 min' pause between individual measurements, and the average from these measurements was calculated. This method of training and SBP measurement was previously used and validated in our and professor Salazar's laboratory [41,45,47]; a close correlation was found between measurements by tail-plethysmography and direct BP measurements using indwelling catheter in conscious rats [36,40,42]. Admittedly, the method does not allow accurate measurements of diastolic BP and mean arterial pressure, in contrast to radiotelemetry which, however, could not be implemented in long-term studies in large groups of 5/6 NX + ACF animals. Nevertheless, as repeatedly shown, this method provides valuable information regarding the role of BP in mediating cardiovascular and renal effects of various treatment regimens in rats [48]. While being aware of this methodological limitation, we have demonstrated in preliminary experiments that the tail-cuff method and radiotelemetry provided similar results in 5/6 NX rats [41,45]. At the end of the study, mean arterial pressure (MAP) was monitored under anesthesia for 10 min using an arterial indwelling catheter. Finally, the animals were killed with an overdose of thiopental sodium and organs were collected.

2.3. Detailed experimental design

2.3.1. Series 1: Effects of long-term treatment (18 weeks) with ETA receptor antagonist and ACEi, alone or combined, on the survival rate and morbidity

The details of the design and timing of the experimental manoeuvres in this series are given in Fig. 1A. The following groups were evaluated:

(1): Sham-operated HanSD rats + placebo (initial n = 12).

(2): 5/6 NX + ACF HanSD rats + placebo (initial n = 26).

(3): 5/6 NX + ACF HanSD rats + ET_A receptor antagonist (initial n = 25).

(4): 5/6 NX ACF HanSD rats + ACEi (initial n = 24).

(5): 5/6 NX + ACF HanSD rats + ACEi + ET_A receptor antagonist (initial n = 24).

2.3.2. Series 2: Effects of 8-week treatment with ET_A receptor antagonist and ACEi, alone or combined, on kidney damage and organ weights

Animals were prepared as in series 1 and the design of this series is given in Fig. 1B. The following groups were examined:

(1): 5/6 NX + ACF HanSD rats + placebo (initial n = 16 and, final n = 10).

(2): 5/6 NX + ACF HanSD rats + ET_A receptor antagonist (initial n = 10, final n = 9).

(4): 5/6 NX ACF HanSD rats + ACEi (initial n = 10, final n = 9).

(5): 5/6 NX + ACF HanSD rats + ACEi + ET_A receptor antagonist (initial n = 11, final n = 10).

2.4. Statistical analysis

Graph-Pad Prism software (Graph Pad Software, San Diego, California, USA) was used. Comparison of survival curves was performed by log-rank (Mantel-Cox) test followed by Gehan-Breslow-Wilcoxon test. Statistical comparison of other results was made by Student's *t*-test, Wilcoxon's signed-rank test for unpaired data or one-way ANOVA when appropriate. Values are means ± S.E.M. P value < 0.05 was considered statistically significant.

3. Results

3.1. Series 1: Effects of long-term treatment (18 weeks) with ET_A receptor antagonist and ACEi, alone or combined, on the survival rate and morbidity

All sham-operated HanSD rats survived until the end of experiment (not shown in Fig. 2). Untreated 5/6 NX + ACF HanSD rats began to die at week + 2 (8 weeks after 5/6 NX operation, 4 weeks after ACF creation) and the final survival rate was 15% (4 of 26 animals) (Fig. 2). ET_A antagonist alone and ACEi alone treatments improved the survival up to the final rate of 64% (16/25) and 71% (17/24), respectively. The combined treatment improved the survival to 75% (18/24). The effectiveness of the three treatment regimens did not significantly differ.

In all groups BW progressively increased throughout study, but this weight gain was significantly arrested in untreated 5/6 NX + ACF HanSD rats between weeks + 5 to + 10; as we observed here and previously [16,38] the onset of mortality is associated with a profound BW drop (Fig. 3A).

The threshold for the advanced phase of CHF (heart failure score ≥ 3) was reached in untreated 5/6 NX + ACF HanSD rats at week + 5, and remained so until the end of the study (Fig. 3B). The score increased slightly and progressively with each of three treatment regimens but not sooner than at the end of study (week +18) did it come near the threshold value, still without reaching it (Fig. 3B). Thus, the animals that died in connection with ACF-induced CHF, in the weeks preceding the death showed the highest CHF score, hence the death caused its paradoxical improvement (as was also seen with BW changes). Therefore, reaching the HF score ≥ 3 in the whole group indicates that a significant

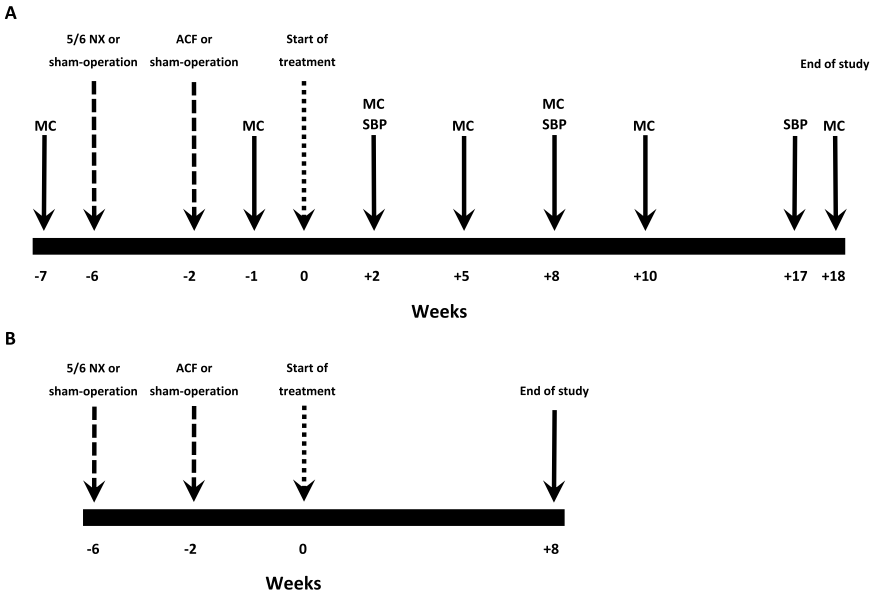

Fig. 1. The experimental design, especially the time sequence of experimental manoeuvres. 5/6 NX: 5/6 renal mass reduction, ACF: creation of aorto-caval fistula, MC: placement into an individual metabolic cage for 24-hour urine collection, SBP: systolic blood pressure measurement by tail-cuff plethysmography.

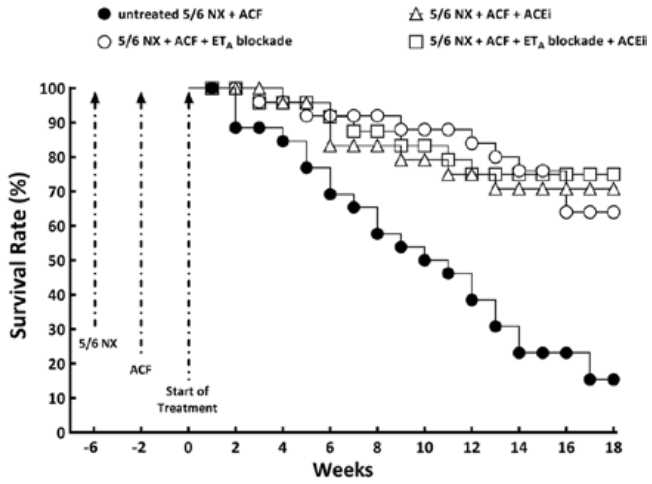

Fig. 2. Series 1: The effects of long-term treatment (18 weeks) on the survival rate in sham-operated normotensive Hannover-Sprague Dawley (HanSD) rats, in untreated HanSD rats undergoing combination of 5/6 renal mass ablation (5/6 NX) and creation of the aorto-caval fistula (ACF), in 5/6 NX + ACF HanSD rats treated with endothelin type A (ET_A) receptor antagonist, or with angiotensin-converting enzyme inhibitor (ACEi), alone or combined.

majority of animals were in the advanced phase of CHF.

At week + 2 in all 5/6 NX + ACF HanSD rats the systolic blood pressure was markedly lower than in sham-operated HanSD rats (Fig. 3C). Similar as with BW, in untreated 5/6 NX + ACF HanSD rats at weeks + 8 and + 17 SBP was significantly lower than in all other groups of 5/6 NX + ACF HanSD rats that were exposed to either treatment regimen; this SBP decrease was most pronounced in the animals that subsequently died within a few days.

Albuminuria, expressed in absolute values or normalized to urinary creatinine excretion, was negligible before 5/6 NX operation and ACF creation but progressively increased slightly with time, also in sham-operated animals. (Figs. 4A and 4B).

Untreated 5/6 NX + ACF HanSD showed a dramatic increase in proteinuria, up to the end of experiment, its values being 120-times higher than in sham-operated HanSD rats. ET_A antagonist treatment alone attenuated the progressive rise in albuminuria, however, at the end of the study no beneficial action was seen compared with untreated 5/6 NX + ACF HanSD rats. ACEi treatment attenuated the increase in albuminuria throughout the study, and in the end the animals showed ~45% reduction compared with untreated 5/6 NX + ACF HanSD rats. Remarkably, the combined treatment with ET_A antagonist and ACEi had additional beneficial effect as compared with the ACEi alone, and the reduction in albuminuria was about 70% as compared with untreated 5/6 NX + ACF HanSD rats (Figs. 4A and 4B).

Fig. 5 summarizes GSI and kidney tubulointerstitial injury at the end of study (in animals that survived until the end of experiment). Untreated 5/6 NX + ACF HanSD rats displayed marked renal glomerular and tubulointerstitial injury. ET_A antagonist treatment alone did not significantly reduce the renal glomerular damage (Fig. 5A), but significantly reduced the kidney tubulointerstitial injury (Fig. 5B). In contrast, ACE inhibition alone significantly reduced the renal glomerular damage as well as kidney tubulointerstitial injury. Remarkably, combined application of the two regimens brought additional reduction in GSI and kidney tubulointerstitial injury in 5/6 NX + ACF HanSD rats as compared with ACE inhibition alone.

Representative images of renal kidney sections of sham-operated HanSD rats and untreated as well as treated 5/6 NX + ACF HanSD rats are shown in Figs. 6 and 7.

Figs. 8 and 9 summarize MAP and organ weights at the end of study. As shown in Fig. 8A, in all groups of 5/6 NX + ACF HanSD rats MAP was significantly lower than in sham-operated HanSD rats, but in the untreated 5/6 NX + ACF HanSD rats it equalled only 71 ± 4 mmHg, markedly lower than in treated 5/6 NX + ACF HanSD rats irrespective of the treatment regimen. All groups of 5/6 NX + ACF HanSD rats exhibited marked bilateral cardiac hypertrophy as seen from whole heart weight, left ventricle (with septum) weight (Figs. 8B and 8C) and right ventricle weight (Fig. 9A), and the treatments did not attenuate it. Interestingly, the degree of right ventricle hypertrophy in 5/6 NX + ACF HanSD rats was higher than that of the left ventricle (higher right-to-left ratio) (Fig. 9B). All groups of 5/6 NX + ACF HanSD rats displayed increased lung weight as compared with sham-operated HanSD rats, and this was not altered by any of the treatment regimens (Fig. 9C).

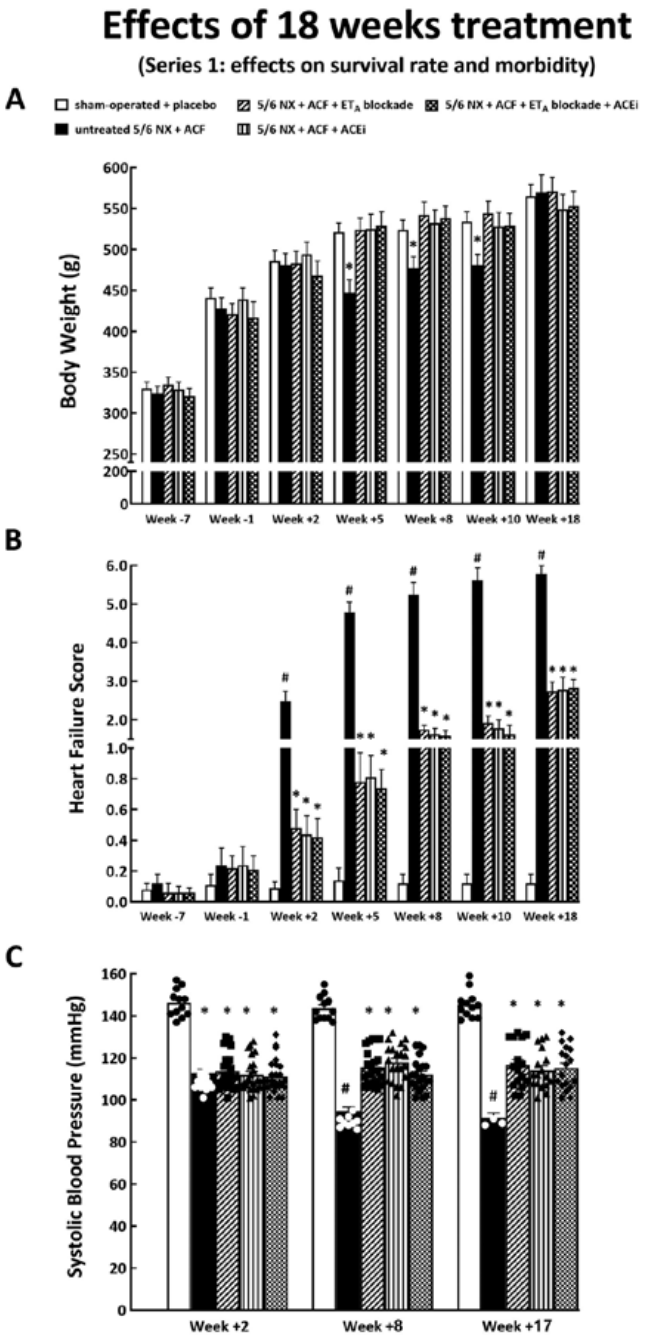

Fig. 3. Series 1: The effects of long-term treatment (18 weeks) on body weight changes (A), total heart failure score (B) and systolic blood pressure (C), in sham-operated normotensive Hannover-Sprague Dawley (HanSD) rats, in untreated HanSD rats undergoing combination of 5/6 renal mass ablation (5/6 NX) and creation of the aorto-caval fistula (ACF), in 5/6 NX + ACF HanSD rats treated with endothelin type A (ET_A) receptor antagonist, or with angiotensin-converting enzyme inhibitor (ACEi), alone or combined. Week − 7: basal values, before surgical interventions, week − 1: values 5 weeks after 5/6 NX operation and 1 week after ACF creation. * $P < 0.05$ sham-operated HanSD rats. # $P < 0.05$ versus all other groups.

3.2. Series 2: Effects of 8-week treatment with ET_A receptor antagonist and ACEi, alone or combined, on kidney damage and organ weights

Fig. 10 summarizes GSI and kidney tubulointerstitial injury 14 weeks after 5/6 NX and 10 weeks after ACF creation, the rats were treated for 8 weeks. Untreated 5/6 + ACF HanSD rats showed exceptionally high degree of renal glomerular and tubulointerstitial injury. ET_A antagonist

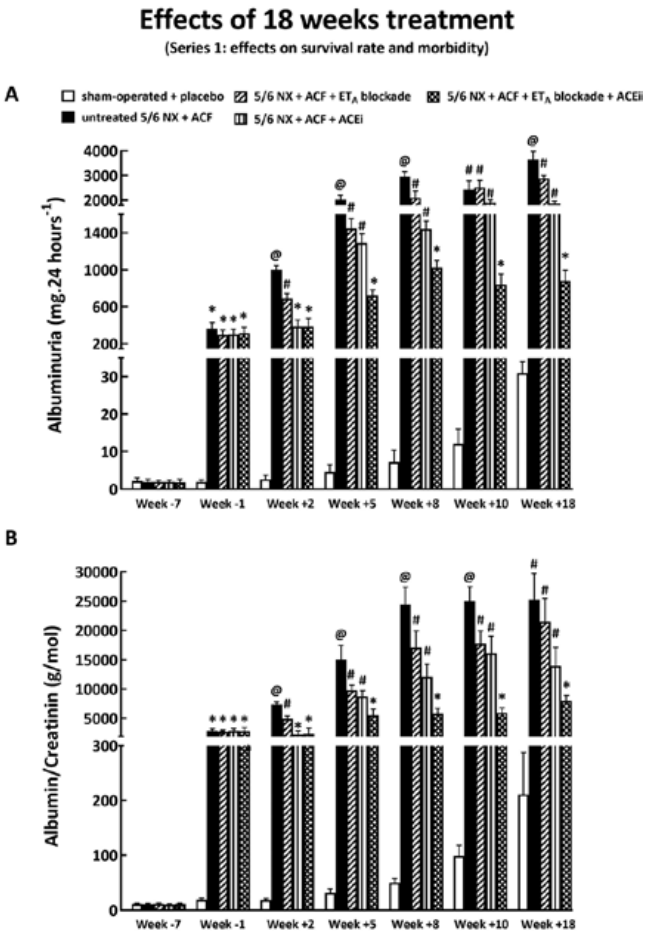

Fig. 4. Series 1: The effects of long-term treatment (18 weeks) on albuminuria (A) and albumin to creatinine ratio (B) in sham-operated normotensive Hannover-Sprague Dawley (HanSD) rats, in untreated HanSD rats undergoing combination of 5/6 renal mass ablation (5/6 NX) and creation of the aorto-caval fistula (ACF), in 5/6 NX + ACF HanSD rats treated with endothelin type A (ET_A) receptor antagonist, or with angiotensin-converting enzyme inhibitor (ACEi), alone or combined. Week − 7: basal values, before surgical interventions, week − 1: values 5 weeks after 5/6 NX operation and 1 week after ACF creation. * $P < 0.05$ sham-operated HanSD rats. # $P < 0.05$ versus 5/6 NX + ACF HanSD rat treated with ET_A receptor antagonist + ACEi. @ $P < 0.05$ versus all other groups.

treatment alone as well as ACE inhibition alone substantially reduced both GSI and kidney tubulointerstitial injury in 5/6 NX + ACF HanSD rats (Figs. 10A and 10B). Remarkably, combined application of the two drugs caused additional reduction in GSI and kidney tubulointerstitial injury in 5/6 NX + ACF HanSD rats (Figs. 10A and 10B), roughly to the levels that were observed in sham-operated HanSD rats at the end of study i.e. ten weeks later (Figs. 5A and 5B).

Fig. 11 summarizes organ weights measured at the same time point of the study as GSI and tubulointerstitial injury were analysed. As shown in Fig. 11A, the treatment with ET_A antagonist alone as well as ACE inhibition alone did not reduce the whole heart weight in 5/6 NX + ACF HanSD rats as compared with untreated counterparts, but the combined treatment reduced it significantly. As shown in Fig. 11B, in 5/6 NX + ACF HanSD rats ET_A antagonist treatment alone did not reduce left ventricle heart weight whereas ACE inhibition alone reduced it significantly. The combined treatment with ET_A receptor antagonist and ACE inhibitor tended to reduce it even more, but the difference from ACE inhibition alone did not reach statistical significance. Interestingly, only the combined treatment with ET_A receptor antagonist and ACE inhibitor significantly reduced right ventricle weight in 5/6 NX + ACF HanSD rats

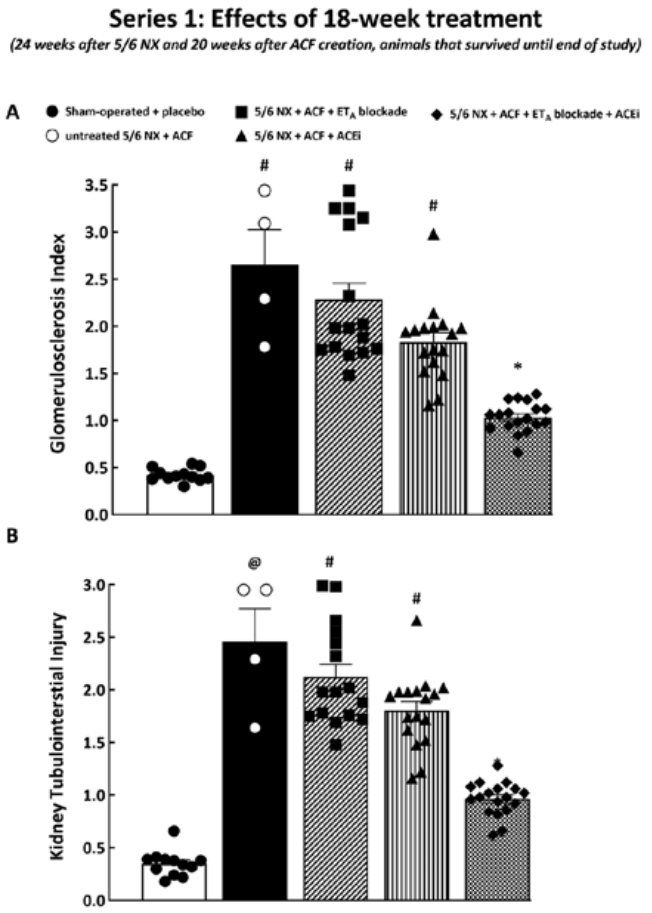

Fig. 5. Series 1: The effects of long-term treatment (18 weeks) on glomerulosclerosis index (A) and kidney tubulointerstitial injury (B) in sham-operated normotensive Hannover-Sprague Dawley (HanSD) rats, in untreated HanSD rats undergoing combination of 5/6 renal mass ablation (5/6 NX) and creation of the aorto-caval fistula (ACF), in 5/6 NX + ACF HanSD rats treated with endothelin type A (ET_A) receptor antagonist, or with angiotensin-converting enzyme inhibitor (ACEi), alone or combined. * $P < 0.05$ sham-operated HanSD rats. # $P < 0.05$ versus 5/6 NX + ACF HanSD rat treated with ET_A receptor antagonist + ACEi. @ $P < 0.05$ versus all other groups.

(Fig. 11C). There were no significant differences in the right-to-left ventricle ratio between experimental groups. In the group under ACE inhibition alone there was a tendency for an increase in this ratio as a consequence of a reduction in the left ventricle weight without alterations in the right ventricle weight. However, the difference did not reach significance level (data now shown). As shown in Fig. 11D, all treatment regimens significantly reduced the lung weight as compared with untreated 5/6 NX + ACF HanSD rats.

4. Discussion

This is the first experimental study that evaluates the role of the ET system in the progression of CHF combined with CKD. Specifically, it examines if selective ET_A receptor blockade attenuates the disease. The crucial question was if the addition of a selective ET_A receptor antagonist to the standard ACE inhibition would have additional beneficial effects in 5/6 NX + ACF HanSD rats, a model of combined CHF and CKD.

The first issue to consider was the effects of the "two-organ damage" on the mortality and morbidity and on albuminuria in this model. We found that in 5/6 NX + ACF HanSD rats the survival rate was dramatically worsened as compared with normotensive animals that were exposed only to ACF creation. The survival median in 5/6 NX + ACF animals was 12 weeks after ACF creation whereas in our original study

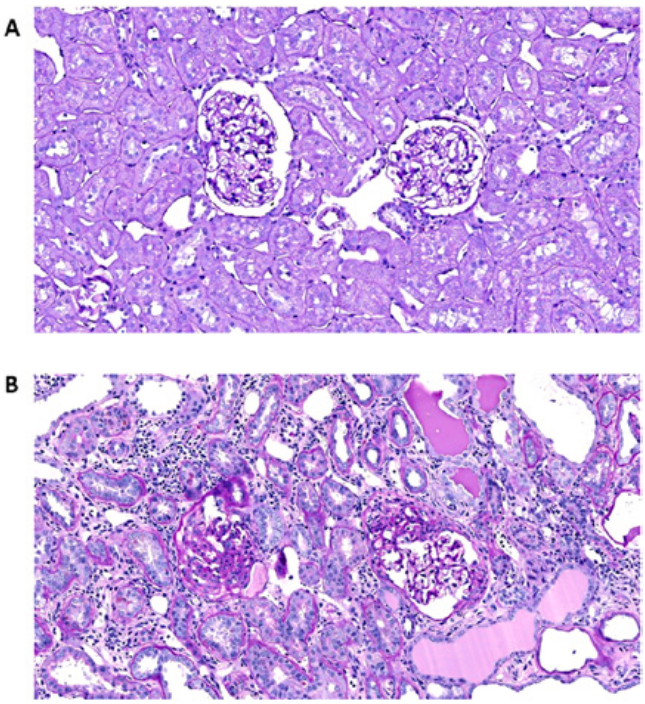

Fig. 6. Representative histological images of the renal cortex from the end of series 1, i.e. after long-term treatment (18 weeks) and 24 weeks after 5/6 renal mass ablation (5/6 NX) and 20 weeks after creation of the aorto-caval fistula (ACF) in (A) sham-operated normotensive Hannover-Sprague Dawley (HanSD) rats and in untreated HanSD rats undergoing combination of 5/6 NX and ACF creation (B).

with animals after ACF creation alone the median was 43 weeks [43]. Moreover, 5/6 NX + ACF HanSD rats exhibited albuminuria that was spectacularly higher than observed by us in Ren-2 transgenic hypertensive rats (TGR) after single organ damage (after 5/6 NX or ACF creation alone). This was so even though TGR display both features crucial for the progression of CKD and CHF: hypertension and inappropriately activated RAS [3,4,9,15]. Remarkably, in untreated 5/6 NX + ACF HanSD rats albuminuria was about 6-fold higher than in untreated 5/6 NX TGR [28,29,45,49], and even about 60-fold higher than in untreated ACF TGR [30]. Evidently, CKD is an exceptionally strong and independent risk factor for the progression of CHF. Furthermore, the present findings and long-term mortality and morbidity studies [2,4,5,7,8] indicate that 5/6 NX + ACF HanSD rats present an optimal model to study the pathophysiology and perform preclinical testing aimed to identify new targets for the treatment of combined CKD and CHF.

The albuminuria observed in the sham-operated and untreated rats also deserves some comment. The progressive increase in albuminuria in the normotensive rats without any intervention is a natural phenomenon of aging and age-related end-organ damage and in hypertensive rats, it is in addition a generally acknowledged marker of age- and hypertension-related end-organ damage. We have seen this age-dependent progression in sham-operated normotensive, i.e. fully healthy animals and also in two models of hypertensive rats, i.e. in healthy animals with "hypertension only" in our previous studies [12,29,30,49,50]. In this context, it is important to acknowledge that even if the albuminuria in sham-operated HanSD rats increased with age, it was still considerably lower than that observed in the sham-operated hypertensive rats, as we recently observed in the sham-operated Fawn-hooded hypertensive rats, a genetic model of spontaneous hypertension associated with CKD [12].

The second set of findings helps to evaluate the effects of atrasentan, a highly selective ET_A receptor antagonist, on the course of combined CKD and CHF. We found here a considerable improvement of survival rate in 5/6 NX + ACF HanSD rats, to the same level as observed in the

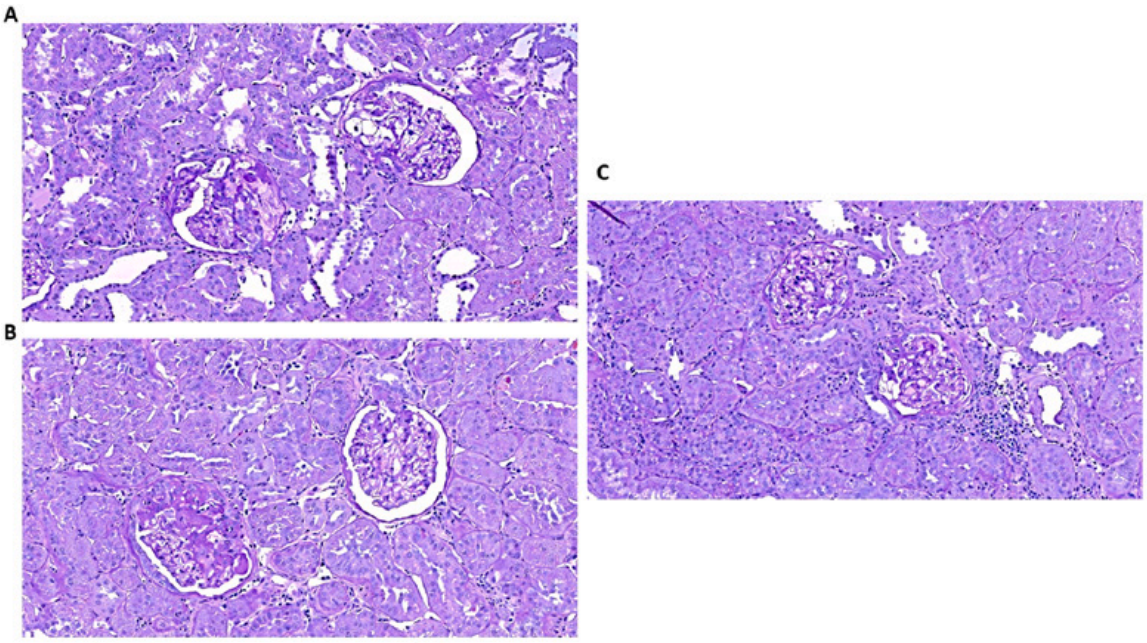

Fig. 7. Representative histological images of the renal cortex from the end of series 1, i.e. after long-term treatment (18 weeks) and 24 weeks after 5/6 renal mass ablation (5/6 NX) and 20 weeks after creation of the aorto-caval fistula (ACF) in 5/6 NX + ACF HanSD rats treated with endothelin type A (ET_A) receptor antagonist alone (A), or with angiotensin-converting enzyme inhibitor (ACEi) alone (B), or with combination of ET_A receptor antagonist and ACEi (C).

animals treated with ACEi alone. In addition, ET_A antagonist alone attenuated the progressive rise in albuminuria as compared with untreated 5/6 NX + ACF HanSD rats even though, admittedly, at some phase of the study ACEi alone was more effective. Moreover, at the end of the study the animals treated with ET_A alone did not show any significant attenuation of renal glomerular damage, bilateral cardiac hypertrophy and lung congestion, and showed only slight amelioration of the kidney tubulointerstitial injury. Nevertheless, approximately the same levels of albuminuria and end-organ damage were found in 5/6 NX + ACF HanSD rats treated with ACEi alone. This requires consideration for several reasons.

First, our data support the evidence that in the advanced stage of CKD as well as CHF organ-protection afforded by RAS blockade is far from complete [3,4,6,8,9,15,28,29,49], especially when both diseases are combined. Second, our present findings emphasize the difference between organ-protective effects of treatment regimens when initiated immediately after the onset of the damaging insult, and when some degree of CKD and CHF has already beenestablished. For instance, with RAS blockade initiated directly after 5/6 NX ("early treatment protocol"), the renoprotective actions are considerably stronger than with some postponement of such therapy ("late treatment protocol") [15,51,52]. Therefore, new pharmacological strategies should be evaluated (as in the present study) in late treatment protocols, the experimental setting more relevant to patients with established CKD or CHF and particularly in patients with combined diseases.

Our first conclusion is that the ET system via activation of ET_A importantly contributes to the progression of CHF coexisting with CKD. Very probably, selective inhibition of ET_A receptor mediated the beneficial actions by: (i) preventing direct vasoconstrictor actions of ET-1, (ii) preventing ET-1 induced glomerular large-pore hyperpermeability and glomerular barrier (podocyte) damage, (iii) unmasking ET_B receptor-mediated vasodilatory and natriuretic actions of ET-1, in the light of the current knowledge about the pathobiology of ET-1 in the kidney [18–20,53]. Remarkably, previous studies using less selective ET_A blockade failed to improve the course of CHF and CKD. Admittedly, our conclusion is only tentative due to the complex interplay of ET-1 with ET_A and ET_B receptors [54] but there is no doubt that ET_A receptor blockade attenuates the progression of CHF combined with CKD.

How to interpret the effects of combined treatment with ET_A receptor antagonist and ACEi on the progression of CHF occurring in tandem with CKD? Since we found that ET_A blockade alone and ACEi alone considerably attenuated mortality in 5/6 NX + ACF HanSD rats, and both treatment regimens affect different neurohormonal system, we expected additive beneficial effects with the combined treatment. However, this hope has not been fulfilled: the protection against CKD- and CHF-related mortality was not improved.

Nevertheless, the combined treatment displayed marked additional beneficial actions on albuminuria and important renoprotective effects, such as alleviation of the renal glomerular and cortical tubulointerstitial injury. Increased albuminuria is a strong and independent predictor for all-cause mortality in CKD as well as in CHF [3–5,7,8,53,55], hence with the prolonged treatment the additive beneficial actions on the course of CKD- and CHF-related mortality could occur. Such reasoning is supported by the favourable actions on renal glomerular and tubulointerstitial morphology: 5/6 + ACF HanSD rats treated within the late treatment protocol with either ET_A antagonist alone or ACEi alone showed marked renal glomerular damage, similar as observed in untreated animals. In contrast, the rats receiving combined therapy displayed only slight renal glomerular and tubulointerstitial injury, similar to that seen in sham-operated animals of the same age. To finally confirm such conclusions, 60-week follow-up studies would be necessary, and for reliable analysis of the survival curves, the initial n values should be at least 42 per group, to fulfil the power analysis requirements [31]. Such demanding thorough studies, admittedly long-standing, time consuming and costly will be needed in future.

In this context, our findings from the second series of experiments when the effects of 8-weeks´ treatment on renal glomerular and tubulointerstitial morphology and organ weights were evaluated are of particular importance. At this time point, i.e. 14 weeks after 5/6 NX creation and 10 weeks after ACF creation, untreated 5/6 NX + ACF HanSD rats showed an onset of high mortality whereas all the treated groups showed an almost complete survival (rates 92%, 84% and 88%, respectively). Our results show that at that time point, untreated 5/6 NX + ACF HanSD rats displayed remarkably high degree of renal glomerular and tubulointerstitial injury: both indices of kidney injury reached almost the arithmetically possible maximum (e.g. for GSI such

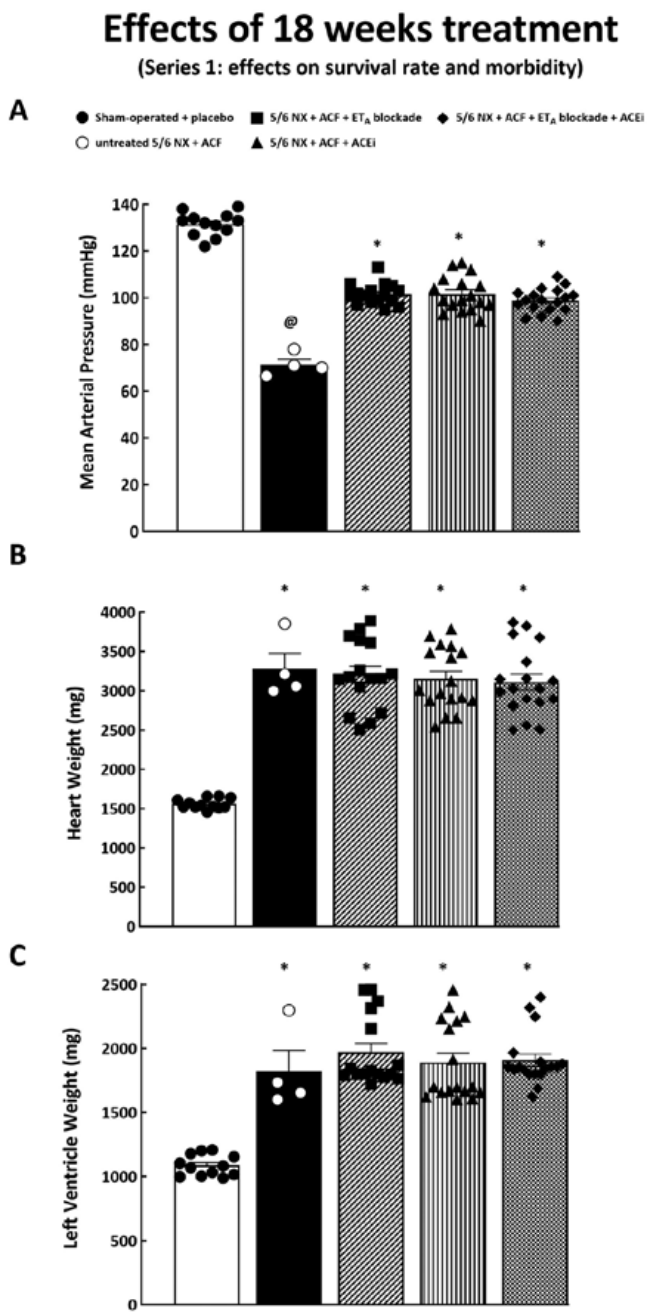

Fig. 8. Series 1: mean arterial pressure (A), whole heart weight (B) and left ventricle weight (C) 24 weeks after 5/6 renal mass reduction (5/6 NX) and 20 weeks after aorto-caval fistula (ACF) creation or sham operation, and after 18 weeks' treatment with endothelin type A (ET_A) receptor antagonist, or with angiotensin-converting enzyme inhibitor (ACEi), alone or combined. * $P < 0.05$ sham-operated HanSD rats. @ $P < 0.05$ versus all other groups.

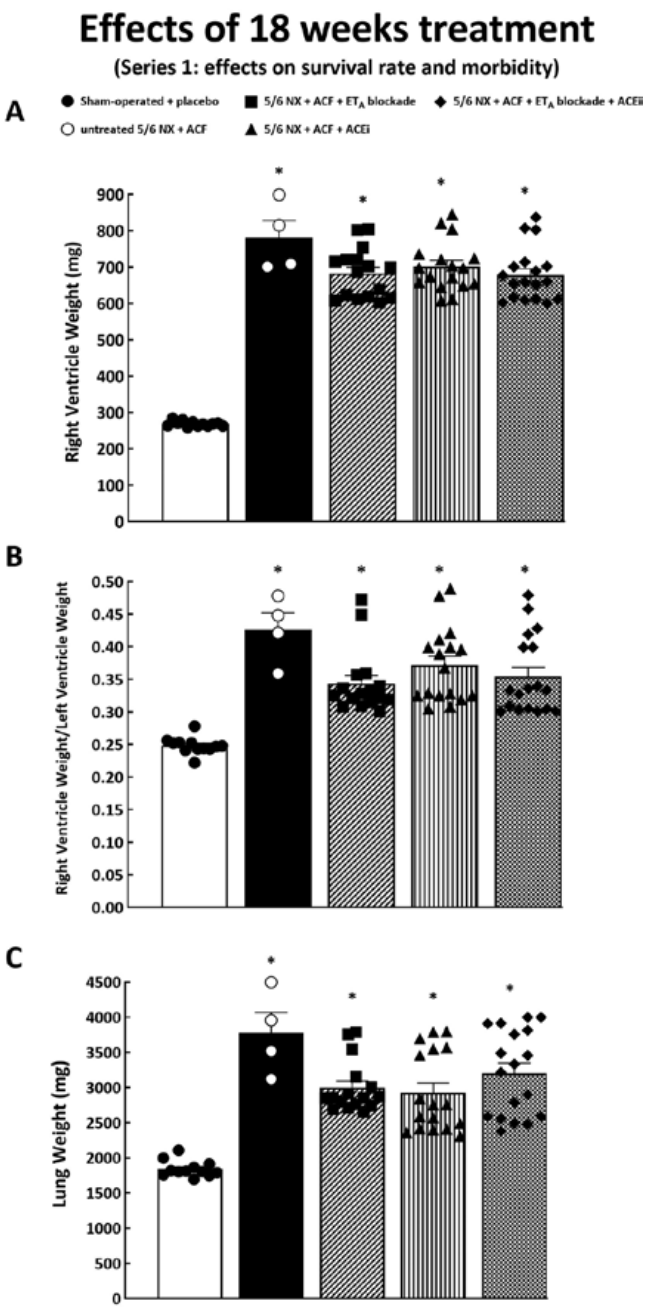

Fig. 9. Series 1: right ventricle weight (A), ratio of right ventricle weight to left ventricle weight (B) and lung weight (C) 24 weeks after 5/6 renal mass reduction (5/6 NX) and 20 weeks after aorto-caval fistula (ACF) creation or sham operation, and after 18 weeks' treatment with endothelin type A (ET_A) receptor antagonist, or with angiotensin-converting enzyme inhibitor (ACEi), alone or combined. * $P < 0.05$ sham-operated HanSD rats.

maximum equals 4 and in the animals that survived until the stated time point GSI was 3.16 ± 0.18). The treatment with ET_A receptor alone as well as ACEi alone substantially reduced renal glomerular and tubulointerstitial injury and, importantly, the animals receiving combined therapy showed only minimal degree of renal glomerular and tubulointerstitial injury. The indices at this time point were similar as observed in sham-operated HanSD rats at the end of series 1 experiment (the animals that served as healthy controls). Moreover, the combined treatment was the only regimen that reduced left ventricle as well as right ventricle hypertrophy as compared with untreated 5/6 NX + ACF HanSD rats. Since albuminuria and cardiac hypertrophy are regarded as independent risk factors for cardiovascular morbidity and mortality (including CKD- and CHF-related mortality) [2–4,55–58], greater beneficial effects on albuminuria, renal morphology and bilateral cardiac hypertrophy further support the notion that the addition of ET_A receptor antagonist to the treatment with ACEi exhibits supplementary protective effects.

Our present findings indicating additional protective effects of the combined treatment using the ET_A receptor antagonist and ACEi (as compared with standard pharmacological ACE inhibition alone) are in accordance with the preliminary results of two ongoing clinical trials evaluating the effects of the dual ET_A receptor plus angiotensin II

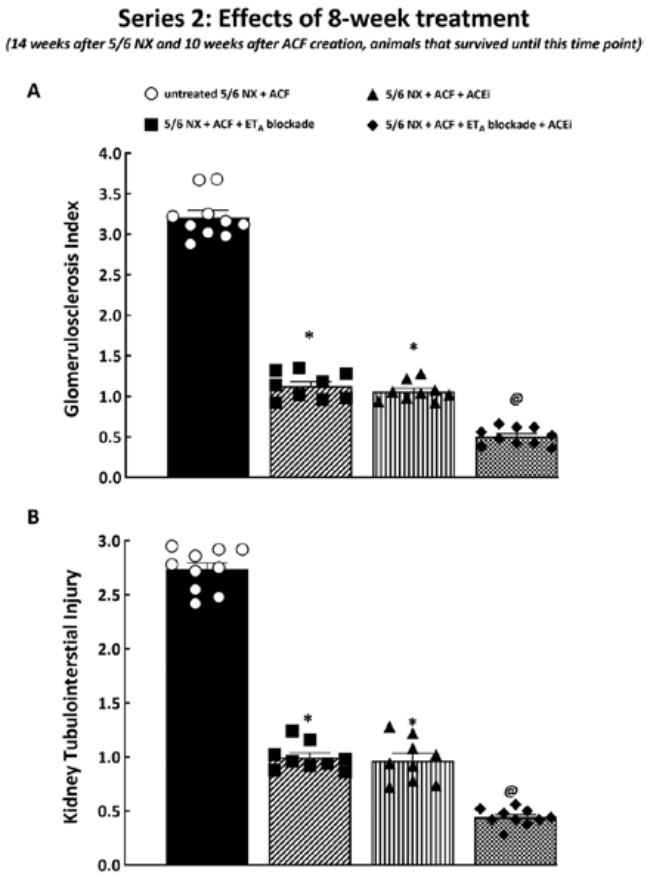

Fig. 10. Series 2: glomerulosclerosis index (A) and kidney tubulointerstitial injury (B) 14 weeks after 5/6 renal mass reduction (5/6 NX) and 10 weeks after aorto-caval fistula (ACF) creation or sham operation, and after 8 weeks' treatment with endothelin type A (ET_A) receptor antagonist, or with angiotensin-converting enzyme inhibitor (ACEi), alone or combined in untreated Hannover-Sprague Dawley (HanSD) undergoing combination of 5/6 NX and creation of the ACF, in 5/6 NX + ACF HanSD rats treated with endothelin type A (ET_A) receptor antagonist, or with angiotensin-converting enzyme inhibitor (ACEi), alone or combined. * $P < 0.05$ versus untreated 5/6 NX + ACF HanSD rats. @ $P < 0.05$ versus all other groups.

receptor type 1 (AT_1) antagonist (sparsentan) versus AT_1 receptor antagonist alone (irbesartan), which were obtained in individuals with either IgA nephropathy or with focal segmental glomerulosclerosis. Thus, both studies demonstrate better renoprotective actions of the dual blockade [59,60].

5. Limitations and strengths of the study

In addition to the uncertainties already discussed above, several important limitations should be here mentioned.

The first is that additional markers of kidney injury were not determined, such as urinary excretion of kidney injury molecule-1, which is recognized as a sensitive and specific biomarker for proximal tubular injury [61], and neutrophil gelatinase-associated lipocalin, another established marker for renal injury [62].

The second limitation relates to the absence of histological examination of the lungs and hearts, which would be especially relevant in the second series of experiments (evaluation of the effects of 8-week treatment), because here beneficial effects of the treatment on the cardiac hypertrophy were found, particularly in animals receiving combined therapy. Evidently, the evaluation of additional biomarkers of renal injury and the complex histological examination of the organs is needed to a further support of our conclusions.

The first strength of our work is that it is a complex in vivo study. Evidently, we are witnessing at present an explosion of tools, resources and publicly available data, which reflects an incredible progress of the molecular biological techniques. This appears to obscure the value of in vivo biological models in the biomedical research. However, as recently emphasized [63], basic animal models with strictly defined pathophysiological alterations are still irreplaceable in the search for new therapeutic targets in cardiovascular diseases. Let it be recalled that Claude Bernard, a founder of modern experimental medicine, stated more than 150 years ago that before generalization of scientific findings, results should by validated by employing various experimental models [64]. Fortunately, this view is increasingly accepted by the cardiovascular community in the development of new therapeutic measures for CHF [13,63].

Another strength of our present study is that it addresses the issue of the treatment with ET_A receptor antagonist when CHF is combined with CHF, the situation which was neglected until recently, particularly due to the concern that administration of ET_A receptor antagonist in patients with CKD, who are permanently in the status of subclinical volume overload, would cause excessive fluid retention, thereby increasing the risk of CHF decompensation. However, the recent post hoc analyses of the SONAR trial have also shown that the treatment with ET_A receptor antagonist could have some protective effects in patients with CHF and CKD [65]. Therefore, more information is needed regarding mechanisms underlying potential beneficial actions of ET_A receptor blockade in the situation when CHF is combined with CKD; and our present study offers such insight from in vivo experiments.

6. Translational perspective

In general, our present results demonstrated that the combined blockade of the ET system and the RAS should be considered in attempts to develop new pharmacological strategies for the treatment of combined CKD and CHF.

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CRediT authorship contribution statement

All authors conceived and designed the study. All authors have read and approved the final version of the manuscript.

P.K.: *Conceptualization, Methodology, Software, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Project administration, Funding acquisition;* **Z.V.**: *Investigation, Visualization;* **P.S.**: *Investigation;* **E.K-J.**: *Writing - Review & Editing;* **J.S.**: Writing - *Review & Editing;* **A.W.**: *Writing - Review & Editing;* **J.V.**: *Validation, Writing - Review & Editing;* **M.T.**: *Validation, Resources, Writing - Review & Editing, Funding acquisition;* **H.M.**: *Software, Visualization;* **I.V.**: *Writing - Review & Editing*: **L.C.**:*Conceptualization, Methodology, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Supervision, Funding acquisition.*

Declaration of Competing Interest

The authors of this manuscript have nothing to declare.

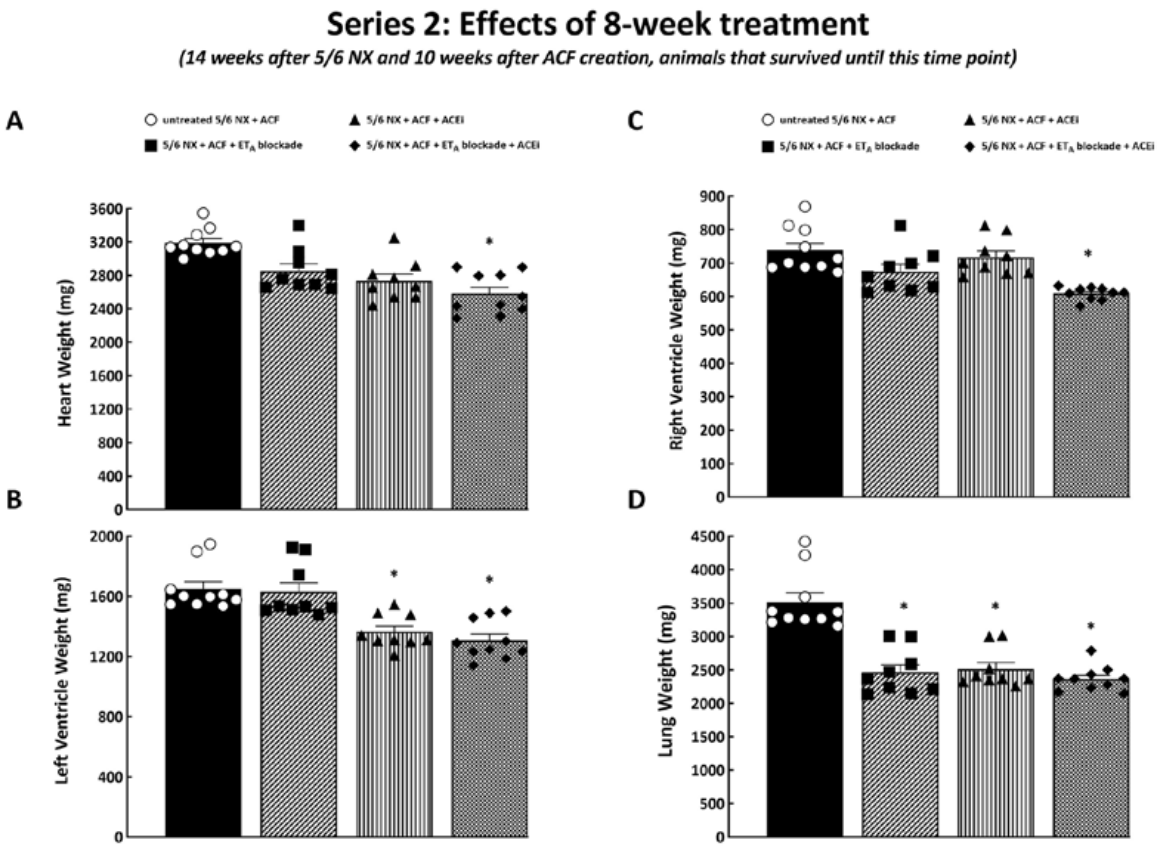

Fig. 11. Series 2: whole heart weight (A), left ventricle weight (B), right ventricle weight (C) and lung weight (D) 14 weeks after 5/6 renal mass reduction (5/6 NX) and 10 weeks after aorto-caval fistula (ACF) creation or sham operation, and after 8 weeks' treatment with endothelin type A (ET_A) receptor antagonist, or with angiotensin-converting enzyme inhibitor (ACEi), alone or combined in untreated Hannover-Sprague Dawley (HanSD) undergoing combination of 5/6 NX and creation of the ACF, in 5/6 NX + ACF HanSD rats treated with endothelin type A (ET_A) receptor antagonist, or with angiotensin-converting enzyme inhibitor (ACEi), alone or combined. * $P < 0.05$ versus untreated 5/6 NX + ACF HanSD rats.

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2022.114157.

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Acute Hemodynamic Effect of a Novel Dual-Vein, Multisite Biventricular Pacing Configuration

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Acute Hemodynamic Effect of a Novel Dual-Vein, Multisite Biventricular Pacing Configuration

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ABSTRACT

BACKGROUND Biventricular pacing (BVP) from multiple left ventricular (LV) sites could enhance the efficacy of cardiac resynchronization therapy (CRT) by engaging a greater myocardial mass.

OBJECTIVES The goal of this study was to evaluate the acute hemodynamic effect of various multisite pacing (MSP) configurations against conventional BVP.

METHODS Twenty patients with nonischemic dilated cardiomyopathy and left bundle branch block (mean age: 59 ± 14 years; LV ejection fraction: 27% ± 6%; native QRS: 171 ± 16 milliseconds) were investigated during a routine CRT implant procedure. In addition to conventional right atrial and right ventricular leads, 2 quadripolar leads were placed in the distant coronary venous branches. LV hemodynamics was evaluated by using a micromanometer-tipped catheter during atrioventricular BVP with 4 LV lead configurations: single-lead conventional BVP; single-lead multipoint pacing; triventricular pacing from distal dipoles of 2 LV leads; and maximum MSP (MSP-Max) from 4 dipoles of 2 LV leads.

RESULTS Compared with right atrial pacing, any BVP configuration produced a significant increase in the maximal LV diastolic pressure rise ($LVdP/dT_{Max}$) (a median relative increase of 28% [IQR: 8%-45%], 25% [IQR: 18%-46%], 36% [IQR: 18%-54%], and 38% [IQR: 28%-58%], respectively; all, $P < 0.001$). MSP-Max but no other multisite BVP generated a significant increase of the maximal $LVdP/dT_{Max}$ than conventional BVP ($P = 0.041$). Increased $LVdP/dT_{Max}$ during MSP-Max was associated with greater LV diameter and lower LV ejection fraction, independently of the QRS width.

CONCLUSIONS The study shows the hemodynamic advantage of a novel dual-vein MSP-Max configuration that could be useful for CRT in patients with advanced LV remodeling. (J Am Coll Cardiol EP 2023;9:2329-2338) © 2023 by the American College of Cardiology Foundation.

Cardiac resynchronization therapy (CRT) is an established treatment for heart failure with reduced left ventricular (LV) ejection fraction (LVEF) and left bundle branch block (LBBB) that can improve the patient's survival, cardiac function, and well-being.[1] However, some individuals respond less favorably to CRT because of intrinsic factors such as non-LBBB morphology, ischemic

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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ABBREVIATIONS AND ACRONYMS

BVP = biventricular pacing

CRT = cardiac resynchronization therapy

LBBB = left bundle branch block

LV = left ventricular

LVEF = left ventricular ejection fraction

MPP = multipoint pacing

MSP = multisite pacing

MSP-Max = maximum multisite pacing

RV = right ventricular

TVP = triventricular pacing

etiology, or extensive LV dilatation.[2,3] In these clinical nonresponders, pacing from multiple LV sites (ie, multisite pacing [MSP]) may be a viable alternative to conventional single-site biventricular pacing (BVP).[4]

The hemodynamic benefit of MSP has been explained by engaging a greater myocardial mass and shortening of LV activation time.[4,5] Animal studies on MSP showed correlation of hemodynamic improvement with increasing number of LV pacing sites[6] and larger interelectrode distances,[7] whereas several (although not all) studies in humans reported greater acute improvement of LV contractility and better long-term response rates compared with conventional BVP, especially in patients with advanced LV remodeling.[8] These studies conducted pacing through 2 dipoles of a single quadripolar LV lead (multipoint pacing [MPP]),[5,8-13] or distal dipoles of 2 conventional LV leads placed in separate coronary veins (dual-vein or triventricular pacing [TVP]),[14-18] or through a combination of MPP and TVP.[19]

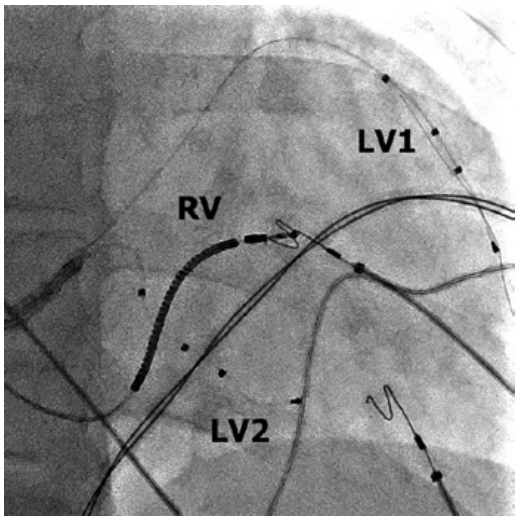
In the present study, we extended the combined TVP + MPP approach and developed a new maximum MSP configuration (MSP-Max) that uses pacing from 4 LV dipoles of 2 quadripolar LV leads. Based on the available evidence, we hypothesized that pacing by MSP-Max would generate a more significant acute hemodynamic response compared with that of conventional BVP and other MSP configurations. In addition, we verified whether the effect would be influenced by baseline LV remodeling.

METHODS

STUDY POPULATION. The study enrolled adult patients with heart failure with reduced LVEF due to idiopathic cardiomyopathy, who had sinus rhythm and LBBB and who underwent clinically indicated CRT. The hemodynamic study protocol was conducted during the implant procedures (January 2020-March 2022), during which the patients received a conventional permanent CRT device. Clinical checkup, echocardiography, and device interrogation were repeated after 6 months by examiners who were unaware of the hemodynamic data. Reduction of LV end-systolic diameter ≥15% at 6 months was considered a good response to CRT.[20]

The study was approved by the institutional ethics committee, and all patients signed informed consent.

FIGURE 1 Typical Positions of the Pacing Leads

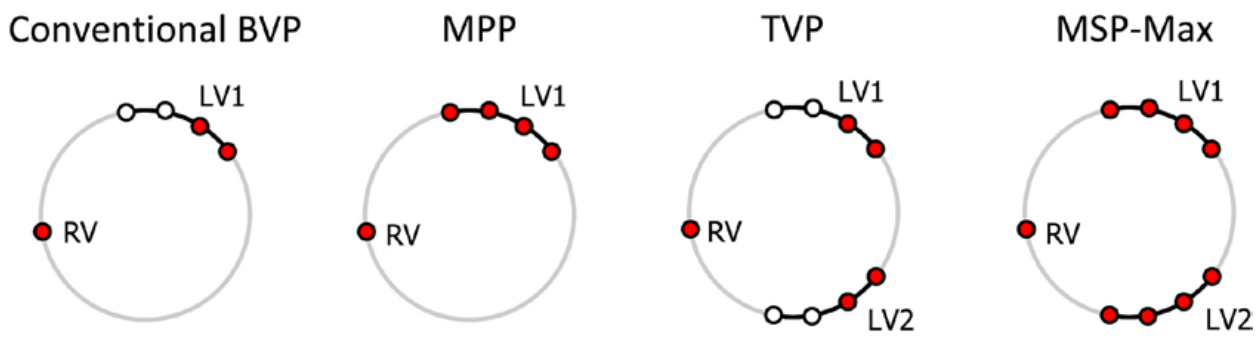


The figure shows a fluoroscopy image of a study patient with an optimal hardware setup with a wide separation of the left ventricular leads (LV1 and LV2). RV = right ventricle.

IMPLANT PROCEDURE AND INSTRUMENTATION. Conventional leads were positioned in the right atrial appendage and right ventricular (RV) mid septum. LV leads were implanted according to our previously described technique using electrophysiological guidance, balloon-occlusive angiography, and angiographic guidewires or subselectors when needed.[21] Two quadripolar leads (Quartet, Abbott) were introduced into distant coronary veins: one preferable position was in the anterior or anterolateral coronary vein and the other in the lateral or posterolateral vein, depending on the individual anatomy (Figure 1). A micromanometer-tipped pressure catheter (Micro-Cath, Millar) was inserted through a pig-tail catheter via transfemoral access into the left ventricle, and a sphygmomanometer cuff for continuous measurement of arterial blood pressure and stroke volume was strapped on the patient's third finger (Finometer Pro, FMS). After the hemodynamic study, the LV lead-generating lower $LVdP/dT_{Max}$ was removed, and the remaining LV lead was connected to a permanent CRT device.

PACING PROTOCOL. Patients were paced with the output of 5 V/0.75 millisecond at 90/min (or 10/min above the intrinsic heart rate) by 6 pacing configurations: right atrial pacing with native QRS, sequential RV pacing, conventional BVP, single-lead MPP,

FIGURE 2 Diagram Summary of the Used Biventricular Pacing Sequences



The diagram represents the biventricular pacing (BVP) sequences used. The red circles represent active electrodes of the left ventricular (LV) quadripolar leads. For simplification, the right atrial lead is not depicted, although all the configurations used atrioventricular sequential pacing with an atrioventricular delay of 150 milliseconds. Conventional BVP was performed from 1 right ventricular (RV) site and 1 LV site (distal dipole of the LV lead). Multipoint pacing (MPP) was performed from 1 RV site and 2 LV sites using a single quadripolar LV lead (proximal and distal dipoles of the LV lead). Triventricular pacing (TVP) was performed from 1 RV site and 2 LV sites using 2 LV leads (distal dipoles of 2 LV leads). Maximum multisite pacing (MSP-Max) was performed from 1 RV site and 4 LV sites using 2 quadripolar LV leads (proximal and distal dipoles of two LV leads).

dual-vein TVP, and MSP-Max; details are provided in Figure 2. Atrioventricular delay was set to 150 milliseconds, and RV-LV and intra-LV delays were set to 0 millisecond. BVP based on a single LV lead was conducted with the LV lead that generated greater $LVdP/dT_{Max}$. To ensure reliable capture from all electrodes, each electrode dipole was connected to a separate external pulse generator (Model 3085, Abbott) through a custom-made analog switcher. Each pacing sequence lasted approximately 1.5 minutes, and for each patient, the order of the sequences was changed randomly. To minimize measurement errors, the entire protocol was run twice.

DATA ACQUISITION AND PROCESSING. Analog signals from the LV pressure catheter and continuous sphygmomanometer were recorded at 1 kHz using a data acquisition system (PowerLab, ADInstruments) and analyzed in dedicated software (LabChart 8, ADInstruments). Measured hemodynamic variables included $LVdP/dT_{Max}$, LV end-diastolic pressure, and tau obtained from the pressure catheter; arterial systolic, mean, and pulse pressure acquired by using a sphygmomanometer; and stroke volume obtained by analysis of the arterial pressure waveform using a 3-parameter Windkessel model implemented in the software. For each pacing sequence, a 30-second steady-state interval was analyzed beat-by-beat and averaged over the selected interval, then averaged over 2 sequence runs. Electrocardiography was recorded at 1 kHz by using the CardioLab system (GE Healthcare). QRS and Q-LV intervals were obtained from the average of 3 measurements by an electronic caliper, as previously described.[21]

STATISTICAL ANALYSIS. Continuous variables are reported as mean ± SD or median (IQR). Comparisons of hemodynamic variables between various pacing configurations were performed by using a paired Student's t-test with the Holms correction for repeated measures. The primary endpoint was the change in $LVdP/dT_{Max}$ during BVP compared with right atrial pacing. Baseline factors associated with the change in $LVdP/dT_{Max}$ were evaluated by linear regression for continuous variables and logistic regression for categorical variables. Correlations between $LVdP/dT_{Max}$ and noninvasive hemodynamic variables were evaluated by using Pearson's test with pooled data from all patients at all pacing configurations. The pooled data were also used for linear regression to evaluate the relation among $LVdP/dT_{Max}$, (as a dependent variable) and QRS duration, QRS change, and BVP as independent variables.

The analyses were performed in R version 4.1 (R Foundation for Statistical Computing). The values of $P < 0.05$ were considered statistically significant.

RESULTS

STUDY POPULATION. From the initially enrolled 22 consecutive patients, 2 patients were excluded during the implantation procedure for unattainable myocardial capture from one of the proximal LV dipoles. The final study population comprised 20 patients with a completed study protocol (Table 1).

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TABLE 1 Patient Clinical Characteristics (N = 20)

Age, y	62 ± 13
Female	7 (35.0)
Body mass index, kg/m^2	28 ± 4
Arterial hypertension	11 (55.0)
Diabetes mellitus	5 (25.0)
Coronary artery disease	1 (5.0)
NYHA functional class I-IV	2 ± 1
HF diagnosis duration, y	1.5 (0.5-3.2)
Previous hospitalization for acute HF	6 (30.0)
ACE inhibitor/ARB/ARNI	19 (95.0)
Beta-blockers	17 (85.0)
Mineralocorticoid receptor antagonists	16 (80.0)
Loop diuretics	16 (80.0)
Antiarrhythmic drugs	4 (20.0)
B-type natriuretic peptide, μg/L	231 (131-375)
LV ejection fraction, %	25 ± 5
LV end-diastolic diameter, mm	65 ± 8
LV end-systolic diameter, mm	49 ± 8
Native QRS width, ms	177 ± 15
Native QLV delay, ms	133 ± 24
QLV/QRS ratio	0.74 ± 13

Values are mean ± SD, n (%), or median (IQR).

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; ARNI = angiotensin receptor neprilysin inhibitor; HF = heart failure; LV = left ventricular.

All patients had idiopathic dilated cardiomyopathy (LV diastolic diameter ranging from 58-90 mm) and a typical LBBB (a mean QRS of 177 ± 15 milliseconds). One patient had a history of coronary artery stenting of chronic stenosis of the circumflex artery (single-vessel disease) without a prior myocardial infarction. All patients were in sinus rhythm throughout the study.

IMPLANTATION PROCEDURE. Implantation of the 2 LV leads into separate coronary vein branches was successful in all cases. The achieved locations of the first LV leads (as appearing in right anterior oblique and left anterior oblique sciascopy views) were: lateral-medial (n = 5 [25%]), lateral-apical (n = 1 [5%]), anterior-basal (n = 5 [25%], anterior-medial (n = 7 [35%]), and anterior-apical (n = 2 [10%]). The locations of the second LV leads were: posterior-medial (n = 6 [30%]), posterior-apical (n = 5 [25%]), lateral-medial (n = 8 [40%]), and lateral-apical (n = 1 [5%]). The average duration of the entire implantation procedure, including the hemodynamic study, was 118 ± 21 minutes. The total fluoroscopic time reached 20 ± 8 minutes, and the radiation dose was 506 ± 207 μGy/m^2. No procedure-related complications occurred.

QRS AND HEMODYNAMICS DURING BVP. Compared with right atrial pacing, QRS complexes were significantly prolonged during RV pacing and significantly shortened during any BVP configuration (Table 2, Figure 3A). Paced QRS were shorter during TVP and MSP-Max compared with conventional BVP by a median of -10 milliseconds [IQR: -7 to -17 milliseconds] and -14 milliseconds [IQR: -10 to -18 milliseconds] (both, $P < 0.001$), whereas there was no significant difference in QRS duration between conventional BVP and MPP (Table 2, Figure 3A).

Hemodynamic changes at the different pacing configurations are summarized in Table 2 and Figure 3B. Compared with right atrial pacing, any BVP induced a significant increase in LVdP/dTMax; however, the increase was greatest during MSP-Max (median percent change, conventional BVP: 28% [IQR: 8%-45%]; MPP: 25% [IQR: 18%-46%]; TVP: 36% [IQR: 18%-54%]; and MSP-Max: 38% [IQR: 28% to 58%]; all, $P < 0.001$). LVdP/dTMax during MSP-Max was significantly greater compared with conventional BVP (by a median of 9% [IQR: 2% to 13%]; adjusted $P = 0.041$), whereas there was no significant difference in LVdP/dT_{Max} among conventional BVP and other MSP configurations (Table 2). No BVP configuration significantly affected LV diastolic function, as represented by the LV end-diastolic pressure and tau.

Of the noninvasive hemodynamic variables, only the mean arterial pressure increased significantly during TVP and MSP-Max (Table 2). No significant changes were observed in any other noninvasive hemodynamic variables for any pacing configuration. Moreover, there was only a modest correlation between LVdP/dT_{Max} and noninvasively measured mean arterial pressure, systolic pressure, or pulse pressure (n = 120, $r = 0.31$, $r = 0.30$, and $r = 0.20$; $P < 0.001$, $P < 0.001$, and $P = 0.043$, respectively), and no correlation was found between LVdP/dT_{Max} and noninvasive stroke volume ($r = 0.10$; $P = 0.30$).

FACTORS ASSOCIATED WITH A GREATER INCREASE IN LVdP/dT_{Max}. From the baseline variables listed in Table 1, only greater LV end-diastolic diameter and lower LVEF were significantly associated with the increase in LVdP/dT_{Max} and only during TVP and MSP-Max (β, 1.6 [95% CI: 0.2-2.9] and 1.5 [95% CI: 0.3-2.8] for LV end-diastolic diameter, and -2.6 [95% CI: -4.6 to -0.6] and -2.2 [95% CI: -4 to -0.2] for LVEF, respectively). Of note, LV end-diastolic diameter did not differ between male (66 ± 10 mm, n = 13) and female (64 ± 7 mm, n = 7) subjects.

TABLE 2 QRS Width and Hemodynamics and During Different Pacing Configurations

	RA Pacing (Native QRS)	RV Pacing	Conventional BVP	MPP	TVP	MSP-Max
QRS width, ms	177 ± 15	192 ± 20 [b,f]	165 ± 18[a]	157 ± 12[c]	151 ± 11[c,f]	149 ± 9 [c,f]
LVdP/dT$_{Max}$, mm Hg/s	1,884 ± 545	2,027 ± 687	2,440 ± 809[c]	2,541 ± 696[c]	2,533 ± 795[c]	2,685 ± 847[c,d]
SBP, mm Hg	127 ± 26	128 ± 24	130 ± 25	131 ± 24	132 ± 26	130 ± 29
PP, mm Hg	56 ± 19	56 ± 18	58 ± 19	58 ± 19	58 ± 21	59 ± 20
MAP, mm Hg	87 ± 19	89 ± 20	89 ± 19	89 ± 19	91 ± 19[a,e]	91 ± 18[a,d]
Stroke volume, mL	62 ± 14	61 ± 14	64 ± 14	61 ± 14[d]	62 ± 12	63 ± 13
LV EDP, mm Hg	16 ± 7	16 ± 7	15 ± 8	15 ± 8	15 ± 8	16 ± 8
LV tau index, ms	0.29 ± 0.23	0.24 ± 0.19	0.18 ± 0.09	0.32 ± 0.64	0.41 ± 1.18	2.52 ± 10.3

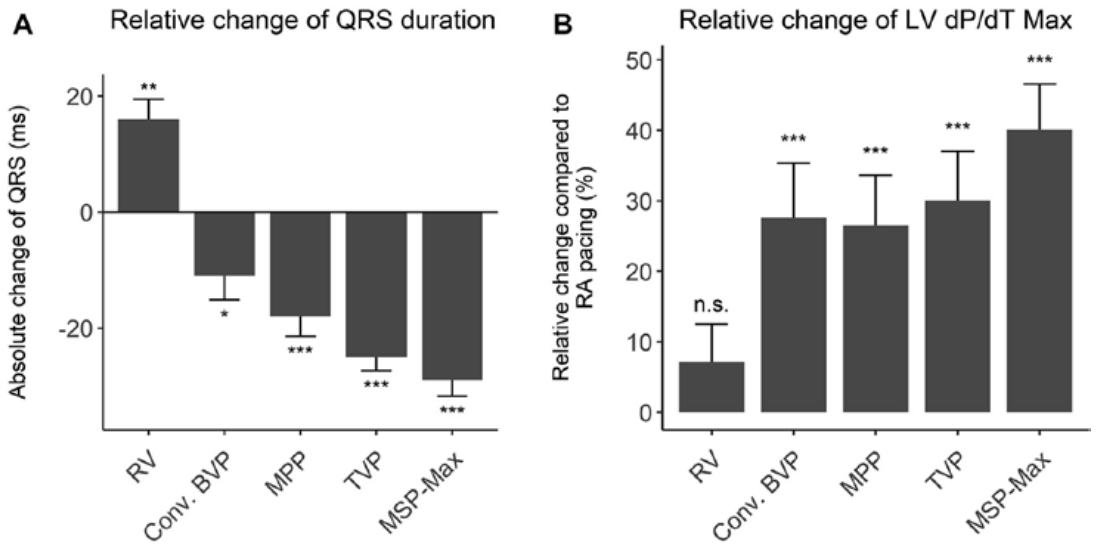
Values are mean ± SD. [a,b,c]Adjusted $P < 0.05/P < 0.01/P = 0.001$ against right atrial (RA) pacing. [d,e,f]Adjusted $P < 0.05/P = 0.01/P = 0.001$ against conventional biventricular pacing (BVP).

EDP = end-diastolic pressure; LV = left ventricular; LVDP/dT$_{Max}$ = maximal LV diastolic pressure; MAP = mean arterial pressure; MPP = multipoint pacing; MSP-Max = maximum multisite pacing; PP = pulse pressure; RV = right ventricular; SBP = systolic blood pressure; TVP = triventricular pacing.

In univariate regression analyses of pooled data from all sequences (n = 120), the only factors associated with the increase in LVdP/dT$_{Max}$ were the QRS duration, the absolute change of QRS duration, and pacing with any BVP configuration (β, -0.4 [95% CI: -0.7 to 0.2], -0.5 [95% CI: -0.8 to -0.3], and 576 [95% CI: 404-748]).

FOLLOW-UP AT 6 MONTHS. Clinical follow-up at 6 months, including echocardiography and device interrogation, was available in all patients. No delayed device-related infection or device failures were observed. Good response to conventional CRT was noted in 13 (65%) patients, whereas 7 (35%) patients responded poorly. A comparison between the

FIGURE 3 Effect of Multisite Pacing on QRS Duration and Hemodynamics



Absolute change of QRS width (A) and percent change in LVdP/dT$_{Max}$ (B) during different pacing configurations compared with right atrial pacing with native QRS. */**/***Adjusted $P < 0.05/0.01/0.001$. Details on the pacing configurations are provided in Figure 2. Conv. BVP = conventional biventricular pacing; RV = right ventricular pacing; other abbreviations as in Figure 2.

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responders and poor responders is presented in Supplemental Table 1. In a pooled analysis of all BVP configurations (80 data points), LVdP/dT_{Max} measured during BVP at baseline predicted good response to conventional CRT and relative change of LV end-systolic diameter (OR: 1.1 [95% CI: 1.02-1.21] and 2.0 [95% CI: 1.3-3.2] per 100 mm Hg/s, respectively). This relationship did not reach statistical significance when each BVP configuration was analyzed separately (20 data points).

DISCUSSION

This study evaluated acute hemodynamic effects of conventional BVP and 3 different MSP configurations in patients with dilated cardiomyopathy and LBBB. Although there was a significant increase in LV contractility during any of the BVP configurations, the greatest improvement was achieved by the newly proposed MSP-Max configuration that used 4 dipoles of 2 LV leads. In fact, our findings indicate hemodynamic superiority of MSP-Max over conventional BVP, which can be explained by more effective electric activation of a larger myocardial mass. Moreover, there was a significant association between LV diastolic diameter and improvement in LV contractility during TVP and MSP-Max, suggesting a possible role of the dual-vein approach in patients with advanced LV remodeling. In this limited sample, LVdP/dT_{Max} measured during BVP at baseline seemed to be associated with response to CRT at 6 months. However, the study found a limited effect of BVP on noninvasive hemodynamic parameters and a weak correlation of the noninvasive parameters with LVdP/dT_{Max}. These findings have relevant implications for assessment of hemodynamics in future studies on MSP (**Central Illustration**).

MPP VS CONVENTIONAL BVP. The concept of MSP has been explored for more than a decade in an effort to surpass the response rate of CRT. It is presumed that pacing from multiple LV sites can engage larger myocardial mass, thereby improving intra-LV synchrony with ensuing shorter LV activation time.[4] One approach to MSP is pacing from multiple dipoles of a single quadripolar LV lead, MPP. Four invasive hemodynamic studies reported greater acute improvement of LV contractility by MPP compared with conventional BVP.[9,12,13,19] Pappone et al[22] followed up their patients for additional 12 months and observed greater LV reverse remodeling in the MPP group compared with the conventional BVP group. Another study reported more pronounced LV reverse remodeling and an improved composite clinical score at 3 months with MPP, although this was mostly observed in patients with more advanced baseline LV remodeling.[8]

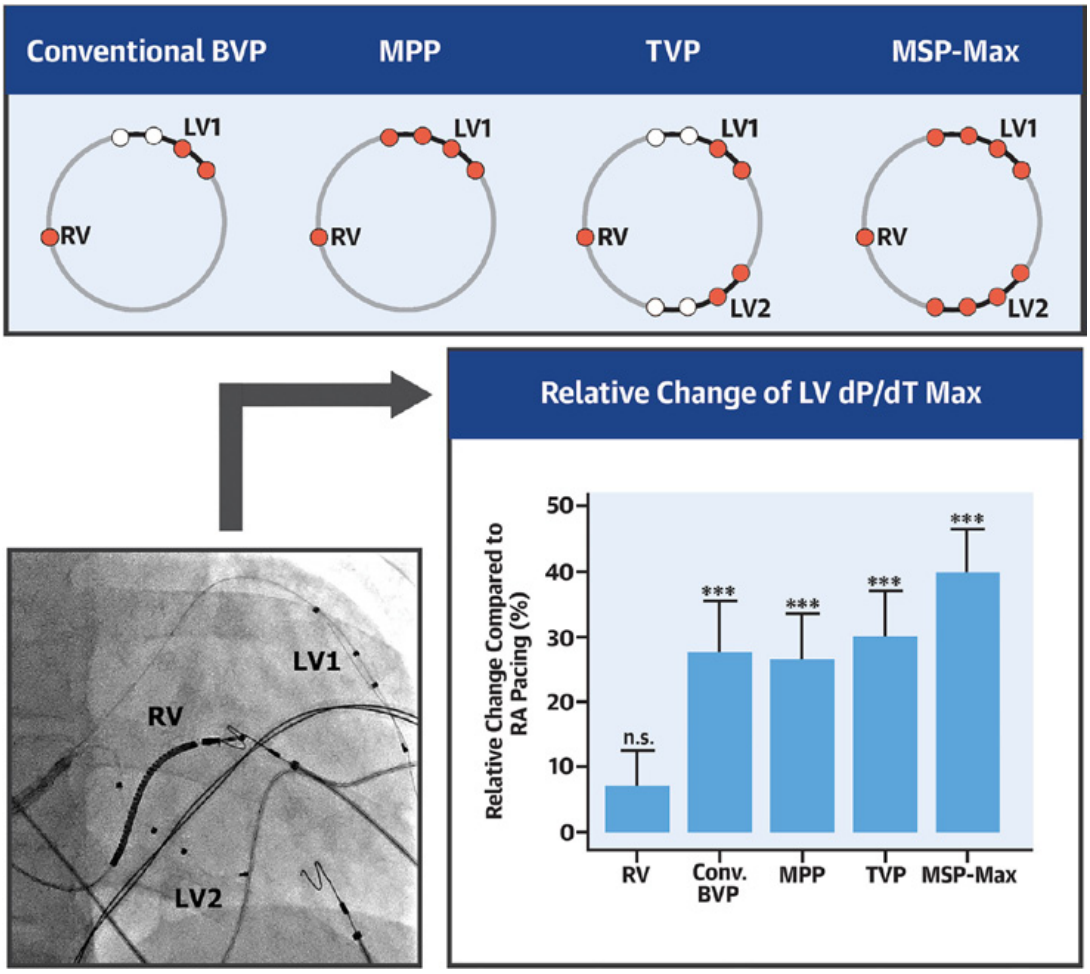
In contrast, 3 comparable hemodynamic studies together with our study found significant differences in LVdP/dT_{Max} between MPP and right atrial pacing but did not confirm hemodynamic superiority of MPP over conventional BVP.[5,10,11] We can only speculate that the lack of hemodynamic advantage of MPP found in the present study may be related to the distinct population of our patients with dilated cardiomyopathy who could have better responded to conventional BVP than patients with ischemic scar.[23] Given the conflicting evidence, further studies are needed to establish the role of MPP in clinical practice.

DUAL-VEIN APPROACH VS CONVENTIONAL BVP. Another strategy of MSP is implantation of a second LV lead to a separate coronary vein branch, the so-called dual-vein approach. The delayed effect of the dual-vein approach has been tested by 5 randomized trials with 3 to 12 months of echocardiographic follow-up. The pacing was carried through 1 RV site and 2 LV sites (ie, TVP). Three of the trials observed greater improvement of LVEF in the TVP group,[16-18] and 2 of them found additional improvement in functional capacity.[17,18] In contrast, the V3 and STRIVE-HF (Triventricular Pacing in Heart Failure) trials found no difference between the 2 groups in a composite clinical score, functional capacity, or LV remodeling.[14,15] However, the V3 trial could have been biased by selecting nonresponders to conventional BVP.

In the current study, the difference in LVdP/dT_{Max} between TVP and BVP did not reach statistical significance. Similarly as for MPP, this could have been related to an already good response to conventional BVP that was difficult to exceed. Nevertheless, our study contributes an important finding of greater hemodynamic response to dual-vein MSP in patients with larger LV diameter and lower LVEF. This association could be explained by the fact that a larger LV surface requires more pacing sites and wider electrode separation to effectively improve LV activation.[7,8] The finding also suggests the possible use of dual-vein MSP in patients with more advanced LV remodeling.

Zanon et al[19] combined MPP by a quadripolar LV lead with TVP by a second LV lead. The combination of TVP and MPP generated a greater acute increase in LV contractility than conventional BVP or TVP alone. This finding corroborates results of an experimental animal study, in which progressive pacing from up to

CENTRAL ILLUSTRATION Summary of Methods and Results



Sramko M, et al. J Am Coll Cardiol EP. 2023;9(11):2329–2338.

The upper panel shows evaluated pacing configurations: conventional biventricular pacing (BVP), multipoint pacing (MPP) using 2 dipoles of 1 quadripolar left ventricular (LV) lead, triventricular pacing (TVP) using distal dipoles of 2 LV leads, and a novel maximum multisite pacing (MSP-Max) using 4 dipoles of 2 quadripolar LV leads. The lower left panel shows hardware setup as appearing on sciascopy. The lower right panel shows the relative increase in $LVdP/dT_{Max}$ during the evaluated pacing configurations. *** $P < 0.001$ compared to RA pacing. $LVdP/DT_{max}$ = maximal LV diastolic pressure; n.s. = not significant; RV = right ventricle.

9 LV epicardial sites led to an incremental increase in $LVdP/dT_{Max}$.[6] Our study extended these observations. By adding an additional LV site, whereby obtaining 5 ventricular pacing sites, we showed significantly greater improvement in LV contractility that went beyond conventional BVP and all other MSP configurations.

CLINICAL FEASIBILITY OF DUAL-VEIN MSP. It is conceivable that implantation of an additional LV lead could increase the risk of periprocedural complications. However, except for 1 study in which complications were observed in up to 20% of patients,[14] most studies on dual-vein pacing reported a high success rate and a low rate of

complications.[16,17,19] Our study adds important safety data to the evidence base. We showed that the implantation of 2 LV leads could be achieved in 22 consecutive cases without any clinical complications. Technically, the implant procedure was successful in 20 of the patients (91%), as there were 2 cases of unattainable capture from one of the LV lead dipoles. Our data, together with the prevailing evidence, indicate the feasibility and acceptable safety of dual-vein CRT.

TECHNICAL CONSIDERATIONS. Although there are clinically available CRT devices that enable controlling different vectors of a single quadripolar LV lead, it should be emphasized that currently there is no clinically available 4-channel CRT device that could separately control right atrial, RV, and 2 LV leads. In the previous studies, this technical hurdle was partially overcome by connecting 2 LV leads through a Y-connector.[14-19] However, such an approach requires equal electrical impedance on all LV dipoles used. Any impedance mismatch would cause preferential flow of the electric current through the LV lead with the lower resistance, thereby hampering capture by the other LV lead or causing premature battery depletion due to the required higher voltage output. Implantation of 2 LV leads could also carry an increased risk of delayed lead dislocation. Conversely, this complication could be prevented by using new-generation quadripolar leads with active fixation.[24] Hopefully, studies such as the current one will stimulate device manufacturers to develop new CRT systems for dual-vein MSP.

STUDY LIMITATIONS. The small sample size limited analyses of baseline predictors of acute hemodynamic response to MSP or baseline predictors of long-term LV reverse remodeling. Nevertheless, the study was adequately powered for the evaluation of significant within-subject changes in $LVdP/dT_{Max}$, which was the main hemodynamic endpoint. Although the relation between acute increase in $LVdP/dT_{Max}$ during conventional CRT procedure and long-term LV reverse remodeling has been previously reported,[20] it would need to be explicitly confirmed for MSP-Max. In our limited sample size, we observed a significant association between BVP and future response to conventional BVP. However, the study was not designed for evaluation of the long-term effect of the individual MSP configurations. We did not measure the achieved interelectrode distances, which could potentially bring more insights into the effect of MSP. Such analysis would require dedicated sciascopy projections or computed tomography imaging, which were not designed in the study protocol.

To identify best responders to the MSP, we believe further studies should also implement electrocardiographic imaging and measurement of paced LV conduction times.[25] It should also be highlighted that our study investigated only patients with idiopathic dilated cardiomyopathy. Although this approach avoided the bias of inefficient pacing from postinfarct scar,[23] it limits the applicability of the results to this specific patient population. Lastly, to ensure similar conditions across the study group, we set a fixed atrioventricular delay of 150 milliseconds for the BVP sequences. It is conceivable that optimization of the atrioventricular delay for each patient could further improve the hemodynamic response.[26]

CONCLUSIONS

Dual-vein MSP with a novel MSP-Max configuration generated significantly greater acute increase in LV contractility than conventional BVP, especially in patients with greater LV diameter and lower LVEF. Conversely, the single-lead MPP approach did not seem to be hemodynamically superior. These preliminary findings provide foundations for designing new strategies for nonresponders to conventional CRT due to advanced LV remodeling.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Patients with more advanced LV remodeling seem to have hemodynamic benefit from dual-vein MSP, particularly from a newly proposed MSP-Max pacing configuration. Clinical nonresponders to conventional BVP due to advanced LV remodeling could be considered for implantation of a second LV lead and MSP-Max as an alternative strategy. However, such a strategy would need to be confirmed in a large, long-term prospective trial.

TRANSLATIONAL OUTLOOK: This study provides the clinical rationale for the manufacturers of CRT devices to develop new solutions that would enable reliable long-term dual-vein MSP in clinical practice. Further research would need to verify the benefits, longevity, and cost-efficacy of such devices.

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KEY WORDS cardiac resynchronization therapy, heart failure, hemodynamics, multipoint pacing, multisite pacing

APPENDIX For a supplemental table, please see the online version of this article.

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Serum lactate in refractory out-of-hospital cardiac arrest: Post-hoc analysis of the Prague OHCA study

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Clinical paper

Serum lactate in refractory out-of-hospital cardiac arrest: Post-hoc analysis of the Prague OHCA study

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Abstract

Background: The severity of tissue hypoxia is routinely assessed by serum lactate. We aimed to determine whether early lactate levels predict outcomes in refractory out-of-hospital cardiac arrest (OHCA) treated by conventional and extracorporeal cardiopulmonary resuscitation (ECPR).

Methods: This study is a post-hoc analysis of a randomized Prague OHCA study (NCT01511666) assessing serum lactate levels in refractory OHCA treated by ECPR (the ECPR group) or conventional resuscitation with prehospital achieved return of spontaneous circulation (the ROSC group). Lactate concentrations measured on admission and every 4 hours (h) during the first 24 h were used to determine their relationship with the neurological outcome (the best Cerebral Performance Category score within 180 days post-cardiac arrest).

Results: In the ECPR group (92 patients, median age 58.5 years, 83% male) 26% attained a favorable neurological outcome. In the ROSC group (82 patients, median age 55 years, 83% male) 59% achieved a favorable neurological outcome. In ECPR patients lactate concentrations could discriminate favorable outcome patients, but not consistently in the ROSC group. On admission, serum lactate >14.0 mmol/L for ECPR (specificity 87.5%, sensitivity 54.4%) and >10.8 mmol/L for the ROSC group (specificity 83%, sensitivity 41.2%) predicted an unfavorable outcome.

Conclusion: In refractory OHCA serum lactate concentrations measured anytime during the first 24 h after admission to the hospital were found to correlate with the outcome in patients treated by ECPR but not in patients with prehospital ROSC. A single lactate measurement is not enough for a reliable outcome prediction and cannot be used alone to guide treatment.

Keywords: Out-of-hospital cardiac arrest, Cardiopulmonary resuscitation, Extracorporeal membrane oxygenation, Lactate

Introduction

The survival rates for out-of-hospital cardiac arrest (OHCA) remain low, with only 8% of patients surviving.[1] The short low-flow duration and early return of spontaneous circulation (ROSC) are crucial for a favorable neurological outcome.[2] Prolonged resuscitation in refractory OHCA leads to more severe ischemia and reperfusion injury, resulting in higher mortality and a worse neurological outcome.[3] The reported survival for patients transported to the hospital without ROSC is <4%.[4,5] Extracorporeal cardiopulmonary resuscitation (ECPR) seems promising to increase the survival of properly selected refractory OHCA patients.[6–12]

Serum lactate level is an easily measured variable[13,14] commonly used for outcome prediction in critically ill patients.[15–18] Considering the devastating metabolic consequences of cardiac arrest, lactate

Abbreviations: ACLS, advanced cardiovascular life support, AUC, area under the curve, AUROC, area under the receiver operating characteristic curve, COPD, chronic obstructive pulmonary disease, CA, cardiac arrest, CPC, Cerebral Performance Category, CPR, cardiopulmonary resuscitation, ECLS, extracorporeal life support, ECMO, extracorporeal membrane oxygenation, ECPR, extracorporeal cardiopulmonary resuscitation, EMS, emergency medical services, h, hours, ICD, implantable cardioverter defibrillator, ICU, intensive care unit, IQR, interquartile range, Lac, serum lactate, OHCA, out-of-hospital cardiac arrest, min, minutes, ROC, receiver operating characteristic, ROSC, return of spontaneous circulation, TTM, target temperature management.

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measurements routinely evaluate the severity of hypoperfusion and tissue hypoxia.[19–21] Still, data on the association between early lactate levels after cardiac arrest and patient outcomes are inconsistent and inconclusive.[22–25] Moreover, the evaluation of lactate in OHCA treated by ECPR has rarely been reported.[26–28]

In this post-hoc analysis of the randomized Prague OHCA study[7] we evaluated the association between early lactate levels and neurological outcomes in refractory OHCA. We hypothesized that lactate serum concentrations might be a prognostically valuable marker in conventionally- and ECPR-managed populations.

Methods

This study is a post-hoc analysis of the prospective open-label randomized clinical trial, the Prague OHCA study (NCT01511666) which compared an invasive approach (early transport to hospital under mechanical cardiopulmonary resuscitation (CPR), ECPR and immediate invasive assessment and therapy) to standard advanced cardiac life support (ACLS) in patients with refractory OHCA. The study was conducted at a single center in Prague, Czech Republic between March 2013 and October 2020. All procedures were followed in accordance with the ethical standards of the institutional review board of the General University Hospital and First Faculty of Medicine, Charles University in Prague (IORG0002175 – General University Hospital in Prague, IRB00002705, 192/11S-IV) and the Helsinki Declaration. Each participant's legal representative and patients who regained normal neurological function were informed of the study enrolment and requested written informed consent.[7]

The main study protocol and results have been published elsewhere.[7,29] In brief, adults aged 18–65 with witnessed OHCA of presumed cardiac etiology were enrolled after a minimum of 5 minutes (min) of ACLS without ROSC. Patients were electronically randomized to invasive or standard treatment arms during the ongoing CPR. In the invasive arm patients were immediately transported directly to the cardiac center catheterization laboratory under continuous mechanical CPR with the intention of proceeding with ECPR if ROSC was not achieved en route or upon hospital admission. Patients allocated to the standard arm were managed by continued ACLS on-site. If ROSC were achieved (defined as a cardiac electrical activity with a palpable pulse), transport to the hospital was initiated for post-CPR care.[7]

The current post-hoc analysis aims to assess the feasibility of predicting the neurological outcome in refractory OHCA patients based on serial serum lactate levels measured during the first 24 hours (h) after hospital admission.

Neurological outcome of patients was assessed blindly by a neurologist at 30 and 180 days after the initial OHCA event. The best Cerebral Performance Category (CPC) score reached anytime within the study period was used to determine the outcome. The CPC score is a five-point scale from 1 (good cerebral performance) to 5 (brain death).[30] CPC scores of 1 and 2 were recognized as favorable and CPC scores 3, 4 and 5 as unfavorable outcomes.

Study population

This post-hoc analysis compares two distinct groups of patients from the original Prague OHCA study population regardless of the initial randomized group assignment. The ECPR group includes patients who presented with the absence of ROSC upon admission to the hospital and had undergone ECPR. The ROSC group comprises patients with standard ACLS therapy who achieved ROSC after randomization. In other words, the ECPR group includes all non-ROSC patients from the original invasive group and those who crossed over from the standard to the invasive group and ultimately received ECPR. The ROSC group contains all patients with ROSC in both the original standard and invasive arms. From the original study population, patients without laboratory data, including those who died on site and patients who presented on admission to the hospital without ROSC and were not treated by ECPR, were excluded from the present analysis.

Study procedures

The serum lactate sampling followed a prespecified protocol, and admission samples were obtained from each patient as soon as possible after admission. In addition, +4-h, +8-h, +12-h, +16-h, +20-h and +24-h samples were collected during the first 24 h at the intensive care unit. In the ECPR group the first blood sample was drawn before or during cannulation, i.e., before ECPR was established. The analyzed blood samples were preferably taken from the femoral artery; if this was not available, then central venous blood was analyzed instead. Lactate concentrations were determined using the blood gas analyzer ABL90 Flex (Radiometer Medical ApS, Brønshøj, Denmark), with values <2.0 mmol/L considered normal.[31–34]

Statistical analysis

The data were analyzed using MedCalc® Statistical Software version 20.211 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2023). Continuous variables were expressed as medians with interquartile range (IQR). The Shapiro-Wilk test and a manual check of skewness and kurtosis were used to verify the normality of the distribution of the continuous variables. A non-normal distribution was found for all continuous variables. Therefore, the Mann-Whitney U test was used to evaluate the differences in continuous variables between the favorable and unfavorable subgroups. Categorical variables were expressed as frequencies and percentages. The chi-square and Fisher's exact test (for data in 2×2 pivot tables) were computed to compare categorical variables between subgroups. For Fisher's exact test, a two-tailed p-value was calculated as the doubling of the one-tailed p-value of the Fisher exact test. A two-tailed p-value of <0.05 was considered statistically significant. The ECPR and ROSC groups were analyzed separately using identical statistical procedures. Participants from both study groups were divided according to the outcome reached. First, the lactate concentrations of patients with favorable and unfavorable outcomes from each group were directly compared at each time point (i.e., on admission and every 4 h). Second, the area under the curve (AUC) of the lactate concentration in the first 24 h was calculated for each patient. Next, the Mann-Whitney test was again used to compare the AUC values between the subgroups. Using ROC analysis, specific lactate values for each time point were determined to divide the study population based on the outcome. The value with the highest Youden index (J = sensitivity + specificity − 1) was employed as the cut-off value.

All measured lactate values were used for the direct comparison of lactate concentrations as well as for the ROC analysis. No specific technique was used for the imputation of missing data. However, based on the methods used, the AUC analysis was calculated only for patients with the initial and +24 h lactate values available. If a missing value was found in those patients, the AUC calculation algo-

rithm performed linear interpolation between the closest known values.

Results

Of the 256 participants in the original Prague OHCA study, 174 (68%) were included in this analysis. Some 82 patients did not meet the inclusion criteria for this post-hoc analysis and were excluded. Fig. 1 illustrates the study flow diagram.

ECPR group

Of 92 patients, 24 (26%) achieved a favorable neurological outcome within 180 days. As listed in Table 1, patients with a favorable outcome more often presented with a shockable rhythm, had a shorter time from collapse to emergency medical service arrival and were given lower doses of adrenaline.

Fig. 2 depicts the lactate levels observed in the ECRP group. Generally, lower lactate levels were seen in the favorable outcome patients. The median lactate values for a favorable vs. unfavorable outcome for patients on admission were 11.2 (IQR 9.2–13.8) mmol/L vs. 14.8 (IQR 12.1–17.5) mmol/L, p <0.001, respectively. In both subgroups the lactate values decreased rapidly during the first 4 h after admission, with a continued decrease up to 24 h. Still, the median lactate concentrations after 24 h of hospitalization did not normalize in either group. The difference in serum lactate concentrations between the favorable and unfavorable outcome patients was significant in each measurement during the first 24 h. The median total AUC of lactate levels was 107.8 (IQR 72.8–146.0) in the favorable outcome patients compared to 153.5 (IQR 102.2–207.8) in the unfavorable outcome patients (p = 0.006).

ROSC group

The ROSC group comprised 82 patients, with 48 (59%) reaching a favorable outcome. Baseline characteristics are given in Table 1. Diabetes was more frequent in the unfavorable group. Similar to the ECPR group, favorable outcome patients presented more often with a shockable rhythm and received fewer doses of adrenaline. The median time from collapse to the first emergency medical service crew arrival was equal between favorable and unfavorable patients. However, the favorable patients' total time from collapse to advanced cardiac life support (physician on site) was shorter.

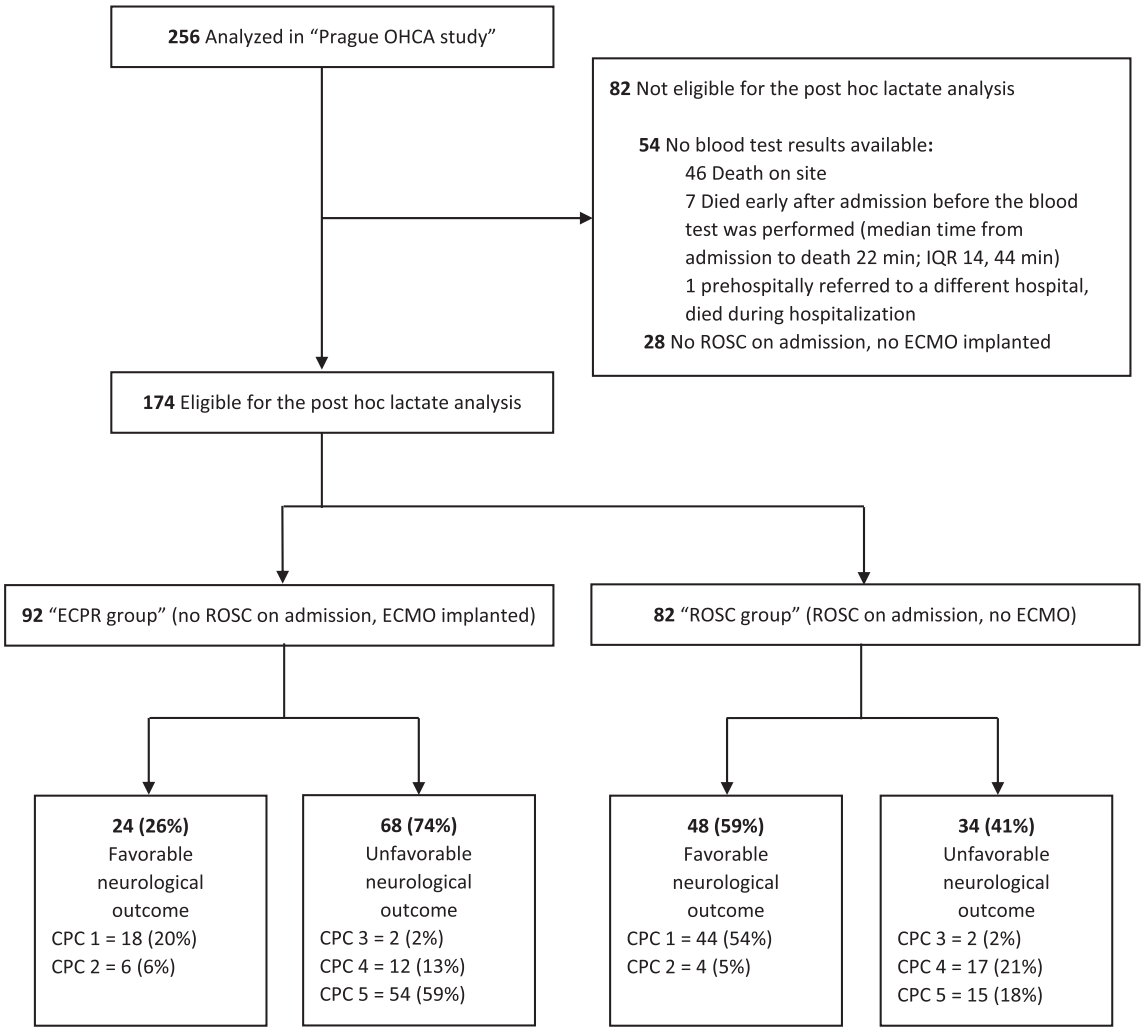


Fig. 1 – Consort flow diagram. Abbreviations: CPC – Cerebral Performance Category; ECMO – extracorporeal membrane oxygenation; ECPR – extracorporeal cardiopulmonary resuscitation; IQR – interquartile range; OHCA – out-of-hospital cardiac arrest; ROSC – return of spontaneous circulation.

Table 1 – Baseline population characteristics according to study group and outcome.

	ECPR group (*N* = 92)			ROSC group (*N* = 82)		
	Favorable (*N* = 24)	Unfavorable (*N* = 68)	*P* value	Favorable (*N* = 48)	Unfavorable (*N* = 34)	*P* value
Age, median (IQR), years	57.5 (40–64.5)	58.5 (47–65.5)	0.25	53.0 (45–61)	58.0 (51–66)	0.32
Sex						
Woman	3 (12.5)	13 (19.1)	0.7	7 (14.6)	7 (20.6)	0.68
Man	21 (87.5)	55 (80.9)		41 (85.4)	27 (79.4)	
Medical history						
Hypertension	9 (37.5)	29 (42.6)	0.44	19 (39.6)	18 (52.9)	0.12
Diabetes	3 (12.5)	10 (14.7)	0.9	4 (8.3)	10 (29.4)	**0.01**
Coronary artery disease	2 (8.3)	13 (19.1)	0.25	9 (18.8)	4 (11.8)	0.82
Chronic heart failure	2 (8.3)	7 (10.3)	1.0	2 (4.2)	1 (2.9)	1.0
COPD	0	2 (3)	1.0	5 (10.4)	2 (5.9)	0.94
Chronic kidney disease	0	2 (3)	1.0	1 (2.1)	2 (5.9)	0.63
ICD implanted	0	2 (3)	1.0	0	0	–
Location of cardiac arrest						
Public place	15 (62.5)	31 (45.6)	0.28	32 (66.7)	20 (58.8)	0.76
Home	4 (16.7)	22 (32.4)		14 (29.2)	12 (35.3)	
EMS	5 (20.8)	15 (22.1)		2 (4.2)	2 (5.9)	
Initial rhythm						
Ventricular fibrillation	23 (95.8)	34 (50)	**<0.001**	44 (91.7)	17 (50)	**<0.001**
Asystole	0	18 (26.5)		2 (4.2)	12 (35.3)	
Pulseless electrical activity	1 (4.2)	16 (23.5)		1 (2.1)	5 (14.7)	
Bystander CPR	23 (95.8)	68 (100)	0.52	48 (100)	32 (94.1)	0.34
Time from collapse to EMS arrival, median (IQR), minutes	6.0 (5.3–8)	9 (7–11)	**0.004**	9 (7–10)	9 (7.5–11.5)	0.34
Time from collapse to ACLS, median (IQR), minutes	8 (6–11)	10.5 (6.5–13)	0.09	10 (8–12)	12 (10–16)	**0.03**
Telephone assisted bystander CPR	18 (75)	47 (69.1)	0.79	44 (91.7)	31 (91.2)	1.0
Time to telephone assisted CPR, median (IQR), minutes	2.5 (2–4)	3 (2–4.8)	0.88	3 (2–4)	3 (2–4)	0.71
Number of adrenalin doses prehospitally, median (IQR), mg	2.5 (1.5–5)	4 (3–6)	**0.03**	2 (1–4)	4 (3–6)	**<0.001**
Number of defibrillations prehospitally, median (IQR)	6 (4–8)	5 (2–7)	0.14	4 (3–5)	3 (1.5–5)	0.17
Time from collapse to hospital arrival, median (IQR), minutes	45.5 (39.5–51)	48 (41.5–57)	0.16	60 (51–66)	63 (55–75)	0.06
Time of CPR (time to death/ROSC or ECLS), median (IQR), minutes	56.5 (52–63)	61 (51–71.5)	0.11	28 (22–36)	33 (25–44)	**0.04**
Target temperature management	24 (100)	66 (97.1)	1.0	48 (100)	30 (88.2)	0.05
Hypothermia initiated prehospitally	4 (16.7)	14 (20.6)	0.93	9 (18.8)	3 (8.8)	0.35

The continuous variables were expressed as medians with interquartile range (IQR). The categorical variables were presented as frequencies and percentages. Statistically significantly different values ($p < 0.05$) are in bold.

Abbreviations: ACLS – advanced cardiovascular life support; COPD – chronic obstructive pulmonary disease; CPR– cardiopulmonary resuscitation; ECLS – extracorporeal life support; EMS – emergency medical services; ICD – implantable cardioverter defibrillator; ROSC – return of spontaneous circulation.

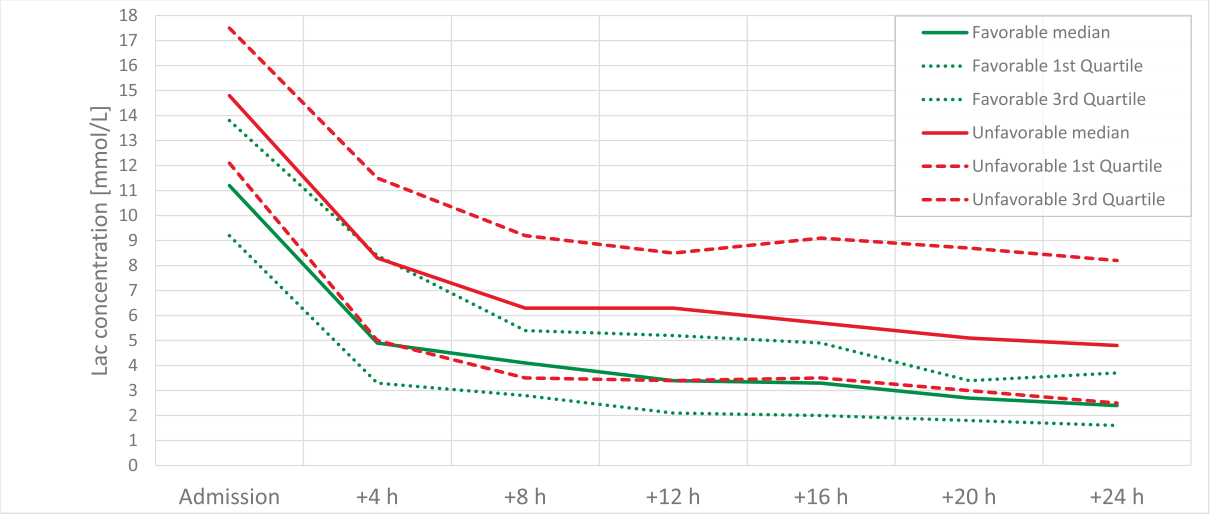
The lactate levels for the ROSC favorable outcome subgroup were generally lower, similar to the ECPR group (Fig. 3). The median serum lactate concentrations on admission were 7.8 (IQR 5.7–10.2) mmol/L in the favorable outcome subgroup compared to 9.7 (IQR 7.1–12.1) mmol/L in the unfavorable outcome subgroup, $p = 0.055$. The median lactate concentration normalized during the first 8 h of hospitalization in the favorable subgroup, which was not the case in the unfavorable outcome patients. We found no difference between the favorable and unfavorable subgroups in serum lactate levels on admission and after 20 and 24 h. For the remaining measurements, differences were present. The total lactate AUC was again lower in favorable patients (57 [IQR 46.7–74.7] vs. 68 [IQR 50.4–143]; $p = 0.04$).

Serum lactate concentration and outcome prediction

Fig. 4 delineates the suggested lactate cut-off values for the outcome prediction. In the ECPR group, a lactate value >14.0 mmol/L on admission predicts an unfavorable outcome with a specificity of 87.5% and a sensitivity of 54.4%. Similarly, a lactate value >10.8 mmol/L (specificity 83.0%, sensitivity 41.2%) predicts an unfavorable outcome for the ROSC group. After 24 h, the suggested cut-off values are 3.9 mmol/L (specificity 81.8%, sensitivity 61.9%) for the ECPR group and 1.6 mmol/L (specificity 61.9%, sensitivity 63%) for the ROSC group.

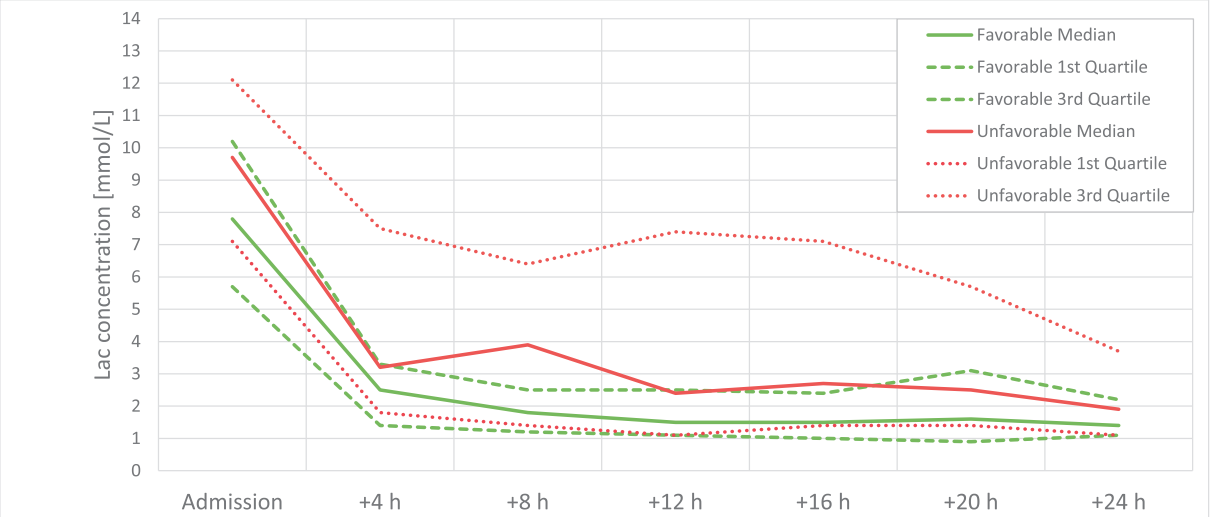
Discussion

This post-hoc analysis of a randomized controlled trial showed the long-term prognostic value of early serum lactate concentrations for neurological outcome prediction in refractory OHCA patients who were treated by ECPR or achieved ROSC with a conventional ACLS.



	Admission	+4 h	+8 h	+12 h	+16 h	+20 h	+24 h
Favorable	11.2 (9.2-13.8)	4.9 (3.3-8.4)	4.1 (2.8-5.4)	3.4 (2.1-5.2)	3.3 (2.0-4.9)	2.7 (1.8-3.4)	2.4 (1.6-3.7)
Unfavorable	14.8 (12.1-17.5)	8.3 (5.0-11.5)	6.3 (3.5-9.2)	6.3 (3.4-8.5)	5.7 (3.5-9.1)	5.1 (3.0-8.7)	4.8 (2.5-8.2)
p-value	<0.001	0.001	0.03	<0.001	0.005	0.002	0.001

Fig. 2 – ECPR group – Serum lactate concentration during the first 24 hours of intensive care according to the outcome. Data are expressed as median (IQR); serum lactate concentration is listed in mmol/L. Abbreviation: Lac – serum lactate.

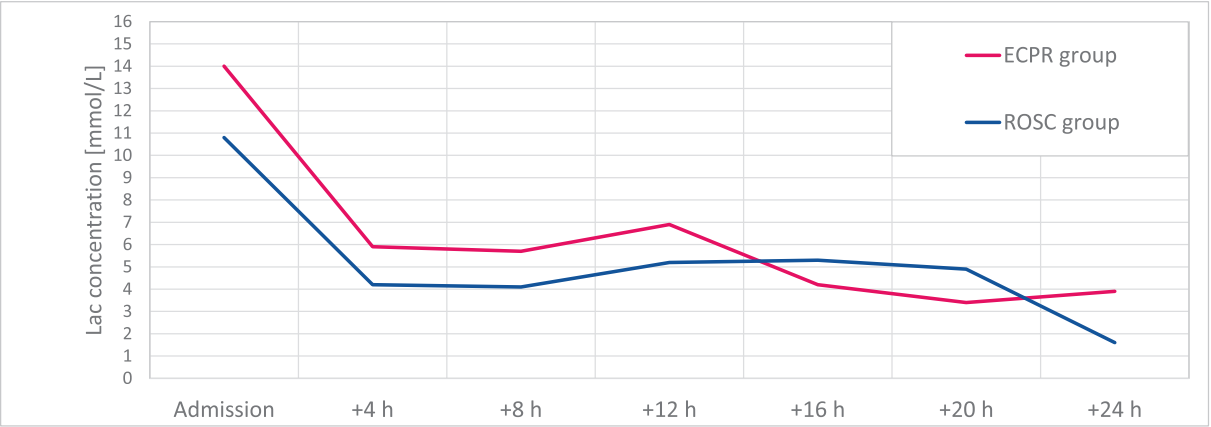


	Admission	+4 h	+8 h	+12 h	+16 h	+20 h	+24 h
Favorable	7.8 (5.7-10.2)	2.5 (1.4-3.3)	1.8 (1.2-2.5)	1.5 (1.1-2.5)	1.5 (1.0-2.4)	1.6 (0.9-3.1)	1.4 (1.1-2.2)
Unfavorable	9.7 (7.1-12.1)	3.2 (1.8-7.5)	3.9 (1.4-6.4)	2.4 (1.1-7.4)	2.7 (1.4-7.1)	2.5 (1.4-5.7)	1.9 (1.1-3.7)
p-value	0.055	0.017	0.02	0.04	0.03	0.07	0.14

Fig. 3 – ROSC group – Serum lactate concentration during the first 24 hours of intensive care according to the outcome. Data are expressed as median (IQR); serum lactate concentration is listed in mmol/L. Abbreviation: Lac – serum lactate.

ECPR patients showed consistently higher lactate values, reflecting a more severe state of organ hypoperfusion. The reasons for these higher lactate values are multiple, with the time sequence of ROSC and the first blood sampling being the most important. ECPR patients were admitted during ongoing CPR and the first blood sample was drawn before the extracorporeal circulation was launched. At the same time, ROSC patients had already restored spontaneous circulation. ROSC achieved prehospital and shorter overall CPR durations are the most likely determinants for the significantly higher number of patients with a favorable outcome in the ROSC group.

Despite the dissimilarities between the main study groups, which were to be expected, higher serum lactate levels in the ECPR and ROSC patients were associated with an unfavorable outcome. For the ECPR group, a significant difference was observed between



ECPR group							
Lac cut-off	>14.0	>5.9	>5.7	>6.9	>4.2	>3.4	>3.9
Sensitivity	54.4	70.5	62.3	49.1	68.9	66.7	61.9
Specificity	87.5	70.8	84.2	95.8	72.2	78.9	81.8
AUROC	0.74	0.73	0.67	0.75	0.73	0.76	0.74
ROSC group							
Lac cut-off	>10.8	>4.2	>4.1	>5.2	>5.3	>4.9	>1.6
Sensitivity	41.2	48.1	50	35.7	41.2	33.3	63
Specificity	83	89.1	97.6	95.2	96.7	94.4	61.9
AUROC	0.63	0.67	0.67	0.64	0.69	0.64	0.61

Fig. 4 – Cut-off values of serum lactate concentration. Higher values than the cut-off predict an unfavorable outcome. Serum lactate cut-off values are listed in mmol/L. Abbreviations: AUROC – area under the receiver operating characteristic curve; Lac – serum lactate.

the favorable and unfavorable outcome patients in all measurements in the first 24 h of hospitalization. The values continuously decreased after admission in both subgroups. Our results align with a German retrospective cohort study on cardiac arrest patients treated with ECPR, which showed increased serum lactate concentrations associated with higher 30-day mortality.[27] A retrospective study from Japan also proved the high prognostic value of early lactate metabolism after ECPR, despite the different methodologies (lactate clearance calculation).[26]

The significant difference in serum lactate values was proven at 4/7 time points and in the total AUC for the lactate concentrations in the first 24 h for the ROSC patients. The lactate values on admission did not differ significantly. We hypothesize that the initial lactate values are unreliable predictors as different transport times occur from the field to the hospital. The actual time between ROSC and the first lactate measurement differs from patient to patient. Further persistent high lactate levels without clearance may reflect not only the severity of an initial insult but also the severity of reperfusion syndrome or ongoing tissue hypoperfusion and hypoxia. In the ROSC unfavorable outcome subgroup a temporary lactate level increase at +8 h was even observed, possibly suggesting an early phase of postcardiac arrest syndrome.[35] The results indicate the need for serial lactate measurements and careful evaluation of the concentration trend over time, given that a single and randomly measured value can be misleading. This finding is supported by numerous trials showing conflicting data on the prognostic value of the single initial lactate value.[25,36–39]

To translate the results into a parameter that might be used in daily routine we analyzed the ROC curves and calculated the exact lactate values, which might help to separate patients based on the outcome. Of note, the AUROC curves of the measurements made at various times after admission were relatively consistent. Despite the good prognostic performance of lactate levels, no single parameter can currently serve as a decision surrogate to initiate or not the therapy (especially ECPR).[8,40] Even within our study, we identified three patients from the ECPR group and eight from the ROSC group who survived with a favorable neurological outcome despite higher initial lactate levels than the suggested cut-off values.

Several findings make our study valuable and clinically relevant. This study is based on data from a prospectively enrolled and randomized population. Patients were treated both conventionally and with ECPR. The study was focused on true refractory OHCA, documented by long CPR durations with a median time reaching 60 minutes (min) in the ECPR and 30 min in the ROSC group. This excessive length of CPR is also the most probable reason for reported higher on-admission lactate values in our population compared to other analyses with unselected OHCA patients. For example, in Donnino's study the reported median initial lactate level was 4.1 mmol/L for OHCA survivors compared to 7.3 mmol/L for non-survivors.[41] Dadeh and Nuanjaroan performed a retrospective study on lactate in OHCA patients arriving at the hospital with ongoing CPR, which is analogous to the ECRP group from our analysis. However, their suggested initial lactate cut-off of 9.1 mmol/L to predict mortality at 24 h is substantially lower than our proposed value of 14.0 mmol/L.[42] Lastly, we used the outcome assessment during the 180 days after the OHCA event to highlight the predictive value of initial lactate levels in a long-term context.

Limitations

The most important limitations are the single-center design, limited sample size and the post-hoc data analysis. Another limitation is that the medical staff in charge of each patient was not blinded; hence, the actual lactate level could affect the decision-making process. The blood samples were preferably drawn from the artery, but even

if some values were taken from the vein, data showed no significant difference in lactate levels between arterial and central venous samples.[43,44]

Conclusion

In refractory OHCA, serum lactate concentrations measured anytime during the first 24 h after admission to the hospital were found to correlate with the outcome in patients treated by ECPR but not consistently in patients with prehospital ROSC. An admission lactate value >14.0 mmol/L for ECPR and >10.8 mmol/L for ROSC patients predicted an unfavorable neurological outcome with a specificity of 87.5% and 83%, respectively. Considering specificity <100%, single lactate values should not be the only parameter in deciding to withdraw or continue the treatment for both ECPR and ROSC patients, but serial lactate measurements as part of a multimodal approach may help to guide the therapy.

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CRediT authorship contribution statement

Milan Dusik: Conceptualization, Methodology, Data Curation, Writing – Original Draft. **Daniel Rob:** Writing – original draft, Methodology, Investigation, Conceptualization. **Jana Smalcova:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Stepan Havranek:** Validation, Investigation, Data curation. **Jiri Karasek:** Writing – review & editing, Validation, Investigation. **Ondrej Smid:** Investigation, Data curation. **Helena Lahoda Brodska:** Investigation, Data curation, Conceptualization. **Petra Kavalkova:** Validation, Project administration, Investigation, Data curation. **Michal Huptych:** Formal analysis, Data curation. **Jan Bakker:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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ORIGINAL RESEARCH PAPER

Myocardial Damage, Inflammation, Coagulation, and Platelet Activity During Catheter Ablation Using Radiofrequency and Pulsed-Field Energy

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ABSTRACT

BACKGROUND Pulsed-field ablation (PFA) represents a new, nonthermal ablation energy for the ablation of atrial fibrillation (AF). Ablation energies producing thermal injury are associated with an inflammatory response, platelet activation, and coagulation activation.

OBJECTIVES This study aimed to compare the systemic response in patients undergoing pulmonary vein isolation (PVI) using pulsed-field and radiofrequency (RF) energy.

METHODS Patients with AF indicated for PVI were enrolled and randomly assigned to undergo PVI using RF (CARTO Smart Touch, Biosense Webster) or pulsed-field (Farapulse, Boston-Scientific) energy. Markers of myocardial damage (troponin I), inflammation (interleukin-6), coagulation (D-dimers, fibrin monomers, von Willebrand antigen and factor activity), and platelet activation (P-selectin, activated GpIIb/IIIa antigen) were measured before the procedure (T1), after trans-septal puncture (T2), after completing the ablation in the left atrium (T3), and 1 day after the procedure (T4).

RESULTS A total of 65 patients were enrolled in the pulsed-field ablation (n = 33) and RF ablation (n = 32) groups. Both groups were similar in baseline characteristics (age 60.5 ± 12.7 years vs 64.0 ± 10.7 years; paroxysmal AF: 60.6% vs 62.5% patients). Procedural and left atrial dwelling times were substantially shorter in the PFA group (55:09 ± 11:57 min vs 151:19 ± 41:25 min; $P < 0.001$; 36:00 ± 8:05 min vs 115:58 ± 36:49 min; $P < 0.001$). Peak troponin release was substantially higher in the PFA group (10,102 ng/L [IQR: 8,272-14,207 ng/L] vs 1,006 ng/L [IQR: 603-1,433ng/L]). Both procedures were associated with similar extents (>50%) of platelet and coagulation activation. The proinflammatory response 24 h after the procedure was slightly but nonsignificantly higher in the RF group.

CONCLUSIONS Despite 10 times more myocardial damage, pulsed-field ablation was associated with a similar degree of platelet/coagulation activation, and slightly lower inflammatory response. (The Effect of Pulsed-Field and Radiofrequency Ablation on Platelet, Coagulation and Inflammation; NCT05603637) (J Am Coll Cardiol EP 2023;■:■–■) © 2023 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

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ABBREVIATIONS AND ACRONYMS

AF = atrial fibrillation
CS = coronary sinus
CTI = cavotricuspid isthmus
DOAC = direct oral coagulants
ICE = intracardiac echocardiography
IL = interleukin
LA = left atrium
LIPV = left inferior pulmonary vein
PAC = procaspase-activating compound
PF = pulsed-field
PFA = pulsed-field ablation
PV = pulmonary vein
PVI = pulmonary vein isolation
RF = radiofrequency
RFA = radiofrequency ablation
RSPV = right superior pulmonary vein
vW = von Willebrand
vWF = von Willebrand factor

Pulmonary vein isolation (PVI) is the most effective treatment modality for atrial fibrillation (AF).[1] The rationale for PVI is to electrically isolate the pulmonary veins (PVs) using different energy sources. The ultimate mechanism for most energy sources, ie, radiofrequency (RF), cryo or laser energy, is similar, ie, it leads to thermal myocardial injury and coagulation necrosis. Therefore, all these energy sources are associated with a proinflammatory response, as well as the activation of platelets and the coagulation cascade.[2]

Pulsed-field (PF) energy is a new energy source for treating AF. It uses high-energy, ultra-short electrical pulses (of microsecond or nanosecond duration) to selectively and irreversibly increase the permeability (electroporation) of cardiomyocyte membranes, which leads to nonthermal cell death. In contrast to cryo- or radiofrequency ablation (RFA), lesions are made using nonthermal destruction of myocardial tissue. In vitro experiments show that the induction of myocardial cell death differs in pulsed-field ablation (PFA); in contrast to thermal energies, cell death is related to the induction of apoptosis in cardiomyocytes. Very importantly, coagulation necrosis induced with thermal energy is always associated with a proinflammatory response and activation of platelets and the coagulation cascade.[3]

The first clinically tested pentaspline catheters for PFA were approved by regulatory authorities for clinical praxis in 2021. Since then, the number of PFA procedures has grown exponentially. Despite the excellent safety profile reported in clinical studies,[4,5] many factors associated with PFA remain unknown. For instance, lower platelet and coagulation activation was expected due to the absence of coagulation necrosis. However, for example, the rate of silent strokes and small cerebral lesions caused by periprocedural microembolization was not lower in PFAs compared with RFAs. The exact myocardial and systemic response to PFA has yet to be described. The study aimed to compare markers of cell damage, platelet activation, and coagulation activation in patients undergoing PVI using PF and RF energy.

METHODS

TRIAL DESIGN. Ours was a prospective, randomized, single-center study to evaluate and compare the systemic effect of PFA vs RFA for AF. The study was approved by the Ethics Committee of the University Hospital Kralovske Vinohrady and was conducted in accordance with the Declaration of Helsinki. Each participant signed informed content before enrollment. The study was registered on clinicaltrials.gov (NCT05603637).

STUDY PARTICIPANTS. Patients with symptomatic paroxysmal or nonparoxysmal AF indicated for a first ablation were recruited and randomized to ablation using PFA or RFA. Several comorbidities, such as chronic heart failure or chronic obstructive pulmonary disease, are associated with higher proinflammatory activity or greater activation of platelets or coagulation systems. Therefore, such comorbidities were exclusion criteria for enrollment. Inclusion criteria were the presence of symptomatic paroxysmal or nonparoxysmal AF, age >18 years, and signed informed content. The exclusion criteria were heart failure with reduced ejection fraction (irrespective of NYHA functional class status), heart failure with preserved ejection fraction worse than NYHA functional class II, treated chronic obstructive pulmonary disease, history of left atrial ablation, presence of malignant, any hematologic, or systemic inflammatory disease, and treatment with antiplatelet agents (aspirin or adenosine-diphosphate antagonists). Because directl oral anticoagulants (DOAC) distinctively affect coagulation parameters compared with warfarin,[6] patients prescribed warfarin were also excluded. The randomization was done using a web-based electronic system and was stratified by AF type (paroxysmal vs nonparoxysmal AF), left atrial size, and age. Patients prescribed DOACs before the procedure were given their last dose of DOAC in the evening before (apixaban, dabigatran) or in the morning (rivaroxaban) on the day before the procedure.

ABLATION PROCEDURES. All procedures were performed under analgosedation with sufentanil, midazolam, propofol, and ketamine; sedation was mild in RFA patients and deep in PFA patients. Femoral

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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venous access was achieved using ultrasound guidance. In patients randomized to RFA, 2 sheaths (F6 and F11) were inserted in the left femoral vein, 1 for a 10-F phased-array intracardiac echocardiography (ICE) probe (AcuNav, Siemens) and the other for a decapolar catheter, which was inserted into the coronary sinus (CS) (Dynamic XT Catheter, Boston Scientific,). In patients randomized to PFA, the left femoral vein was used for the decapolar CS catheter only in patients for whom a cavotricuspid or mitral isthmus ablation was planned; otherwise, the left femoral vein was left untouched. In all patients, 2 sheaths were inserted in the right femoral vein: 1 11-F sheath for the ICE and an 8-F for the trans septal puncture in the PFA group, and 2 8-F sheaths, both for a trans septal puncture, in the RFA group.

Trans septal punctures were performed using a nonsteerable trans septal sheath (SL1, Abbott) under ICE guidance. In PFA patients, the SL1 sheath was replaced by a 13-F deflectable trans septal sheath (Faradrive, Boston Scientific) using the over-the-wire technique. Peri-procedural anticoagulation was managed using heparin at a dose of 5,000 IU before the trans septal puncture in both groups; another bolus of 5,000-10,000 IU was given immediately after the trans septal puncture. The activated clotting time was assessed every 10 min with a target value of 300 s; when this target was achieved, further activated clotting time checks were done every 20 min in the RFA group.

Patients in the PFA group underwent ablation using a pentaspline catheter (Farawave, Boston Scientific, Inc) with a PFA generator (Farastar, Boston Scientific, Inc). Ablations were performed using a biphasic bipolar waveform in the following order: 4 applications with the ablation catheter in the "basket" configuration and 4 applications with the ablation catheter in the "flower" configuration for each PV ostium. For the right superior PV, 2 additional applications in the flower configuration were used on the anterior aspect of the right superior PV. All PVs were checked for entrance (and exit, in sinus rhythm) block; if the block was not present, additional PF applications were made. In patients with nonparoxysmal fibrillation, ablation of the posterior wall and mitral isthmus was conducted at the discretion of the treating physician. Regarding the posterior wall, 2 applications were delivered at each overlapping posterior wall location to connect the right superior with the left superior PV and the right inferior with the left inferior pulmonary vein (LIPV). In nonparoxysmal patients, the mitral isthmus was ablated between the LIPV and the mitral annulus using 4-8 PF applications.

In patients randomized in the RFA group, all procedures were done using the CARTO 3 mapping system (Biosense-Webster). A circular mapping catheter (Lasso, Biosense-Webster) was inserted in all PVs for verification of entrance and exit blocks; in nonparoxysmal patients, left atrium (LA) mapping was done using an Octarey Mapping Catheter (Biosense-Webster). A 3.5-mm irrigated-tip CARTO catheter (ThermoCool SmartTouch, Biosense-Webster) was also used for mapping and ablation. Ablations were done using an ablation index with a target value of 400-450 on the anterior and superior aspects of the PVs and 350-400 and the posterior and inferior aspects, with RF ablation power of 30-35 W on the anterior/superior, and 25-30 W on the posterior/inferior parts of PVs. The surface areas of the isolated left- and right-sided veins were quantified. The CARTO system enables the calculation of the surface area from manually selected points. Because no voltage maps were done after ablations, the isolated areas were depicted through the middle of the ablation points. In nonparoxysmal patients, additional ablations were added at the discretion of the surgeon. These additional ablations involved the ablation of fractionated signals within the scar areas in the LA, the ablation of complex fractionated signals, and linear ablations.

BLOOD SAMPLING. All blood samples were taken while patients were in a fasting state. Four blood samples were drawn: 1) at the beginning of the procedure from the femoral vein before any intravenous anticoagulation was given; 2) from the LA immediately after the transseptal puncture (the first transseptal puncture in the RFA group); 3) from the LA at the end of the left-atrial ablations; and 4) in the morning on the day after the ablation (the antecubital vein). In all 4 samples, the first 5 mL of blood was discarded. The samples from antecubital veins were drawn without tourniquets. Samples for flow cytometry, troponin I, interleukin (IL)-6, and coagulation markers were analyzed immediately or within 3 h of collection.

BIOMARKER ANALYSIS. Markers of myocardial necrosis. Troponin I was quantified using a commercial Atellica IM High-Sensitivity Troponin I (TnIH) TR chemiluminescence test in a Siemens Atellica Solution Analyzer (Siemens Healthineers). The institutional physiological reference range for females was set as 0-34 ng/L and for males 0-53 ng/L, with a cut-off value of 2.5 ng/L.

Markers of platelet activation. Samples of citrated whole blood were used to determine the expression of platelet surface markers CD41a/CD61 (gpIIb/

IIIa complex), procaspase-activating compound (PAC)-1 (extracellular activation-induced conformational epitope on gpIIb/IIIa complex), CD62P (P-selectin), and CD42b (GPIbα) based on flow cytometry. Flow cytometry analysis was performed within 3 h of blood collection without adding ex vivo platelet agonists. Five microliters of citrated whole blood was diluted 1:9 in Tris-buffered saline (10 mmol/L TRIS, 0.15 mol/L sodium chloride) and then stained for 30 min with the following monoclonal antibodies: fluorescein isothiocyanate-conjugated anti-PAC1 (clone SP2), BV510-conjugated anti-CD41a (clone VI-PL2), BV510-conjugated anti-CD61 (clone HIP8), phycoerythrin-conjugated anti-CD62P (clone AK-4), and activated allophycocyanin-conjugated anti-CD42b (clone HIP1). All antibodies were purchased from Beckton Dickinson Biosciences. After incubation, samples were fixed using 400 μL of 1% paraformaldehyde solution. Platelets were acquired using a Navios EX (Beckman Coulter). Forward scatter and side scatter were set at a logarithmic gain, and platelets were identified based on the size and expression of CD41a and CD61. In each sample, platelets were further identified using the platelet-specific CD42b antibody. Expression of CD62P and PAC-1 was then evaluated on CD41a/CD61$^+$ CD42b$^+$ platelets.

Markers of coagulation. D-dimers were determined using a commercial INNOVANCE D-Dimer immunoturbidimetric assay (Siemens Healthineers) in a Sysmex CS-5100 (Sysmex Corporation) automated blood coagulation analyzer. The institutional physiological reference range was set to 0-500 ng/L, with an institutional cut-off set to 190 ng/L. Fibrin monomers were determined using a commercial STA-Liatest FM immunoturbidimetric test (Stago) in a Stago STA Compact Hemostasis System (Stago), with a reference range set to 0-5 mg/l and with an institutional cut-off set to 2.5 mg/L. Von Willebrand (vW) antigen and von Willebrand factor (vWF) activity were measured using commercial immunoturbidimetric assays (ie, von Willebrand Antigen-hemo-RGT and INNOVANCE VWF Ac assay) in a Sysmex CS-5100 (Sysmex Corporation) automated blood coagulation analyzer. Both assays were purchased from Siemens Healthineers. Results were reported as percentages, with the institutional reference range for vWF activity being 50%-150% and vW antigen being 50%-150%.

Markers of inflammation. IL-6 was analyzed using commercial Atellica IM IL-6 chemiluminescence tests in a Siemens Atellica Solution Analyzer (Siemens Healthineers). The institutional physiological reference range was 0-4.4 ng/L, with the cut-off value set to 2.7 ng/L.

STATISTICAL ANALYSIS AND POWER CALCULATION. Standard descriptive statistics were used for the analysis. Binary or categorical parameters of patients were characterized by absolute and relative frequencies, whereas continuous parameters were described as mean ± SD. Because most markers did not follow a normal probability distribution, the median (IQR) were used to describe those parameters. The Mann-Whitney test was used to assess the statistical significance of the differences between groups (PFA vs RFA) for continuous parameters and the Fisher exact test for categorical parameters. In related samples, Friedman 2-way analysis of variance by ranks with a Bonferroni correction for post hoc testing was used for evaluating the progression of individual markers. However, when only baseline (T1) and discharge (T4) marker levels were assessed (specifically for troponin I hs and IL-6), the related samples Wilcoxon signed-rank test was used. Univariate and multivariate stepwise linear regression was performed to predict the maximum biomarker value obtained during the procedure with selected clinical and procedural characteristics used as predictors. A log transformation of dependent variable was applied where appropriate.

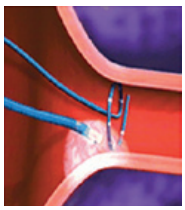
The level of statistical significance used in all analyses was $P = 0.05$. Analyses were performed in SPSS 28.0.1.1 (IBM Corporation).

Based on a previous observations of troponin levels between PFA and RFA,[7] we assumed at least a 50% relative increase in troponin levels after PFA. So far, no studies have compared platelet activation or inflammatory response during RFA and PFA procedures. Therefore, based only on the known physical principles and the expected biological response to PFA and RFA, which cause entirely different kinds of cell death and could lead to differing degrees of inflammatory response, we expect 50% less activation of proinflammatory markers and markers of platelet and coagulation activation in PFA patients. Because the concentrations of troponin, markers of platelet activation, and inflammation after RFA in published studies were reported as mean ± SD, power calculation was done using t test. Using a 2-tailed value of 0.05 and a power of 80% resulted in a sample size of 25 patients per group for troponin concentrations, and 30 patients per group for markers of platelet activation and inflammation. No power calculation was done for markers of coagulation.

RESULTS

PATIENTS AND PROCEDURES. Sixty-five patients were enrolled: 33 in the PFA group and 32 in the RFA group (Central Illustration). The baseline

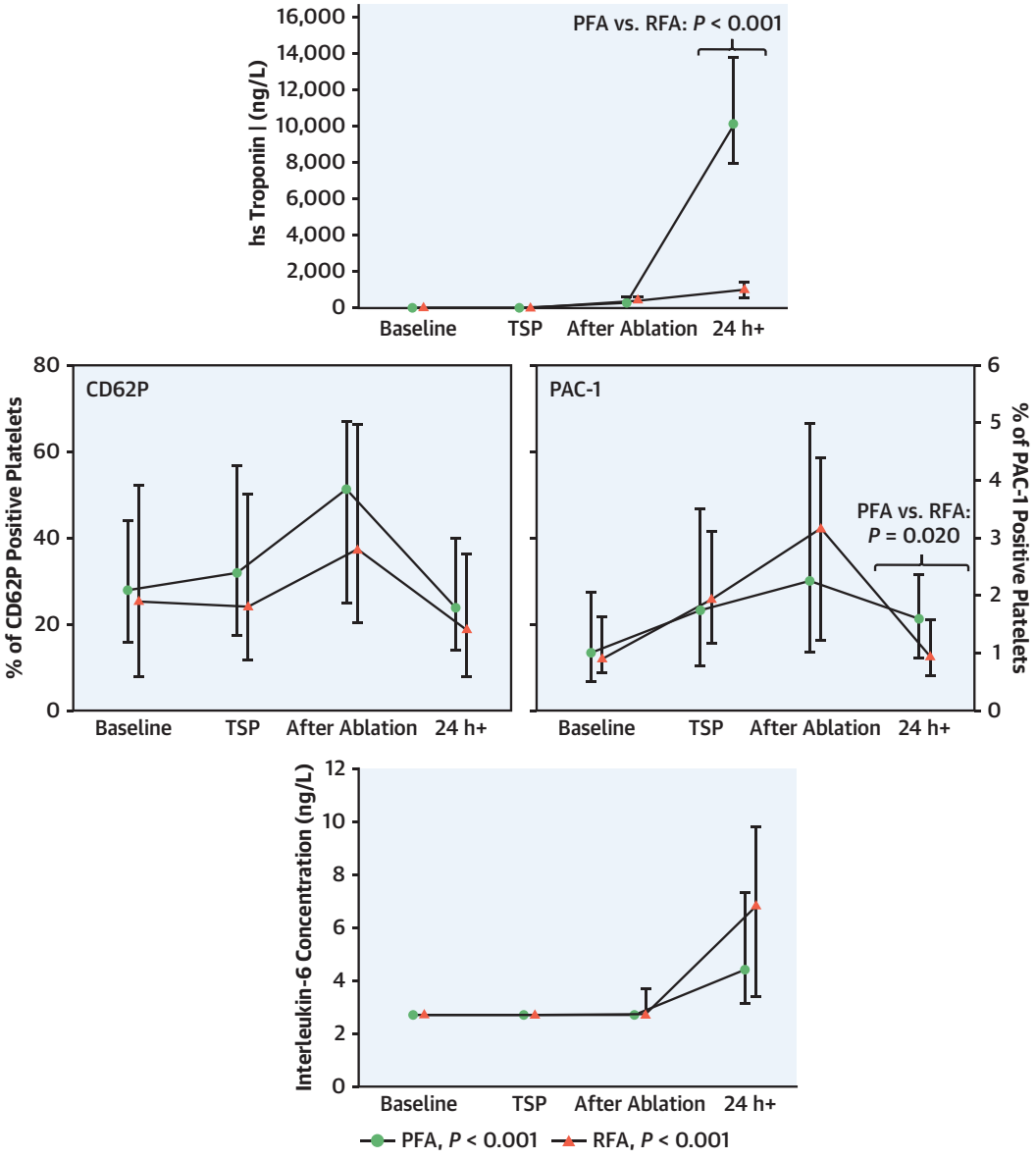
CENTRAL ILLUSTRATION Summary of Results



65 patients with atrial fibrillation
(age 62.2 ± 11.8 yrs, CHA_2DS_2VASc 2.2 ± 1.8)

RANDOMIZED

- Blood samples drawn before the procedure, after transeptal puncture, after the completion of ablation, 24h after the procedure
- Assessment of markers of myocardial damage, platelet activity (CD62P, PAC-1), coagulation (von Willebrand activity, D-dimers, fibrin monomers) and inflammation (IL-6)



Osmancik P, et al. J Am Coll Cardiol EP. 2023;■(■):■-■.

Figures display the concentration of troponin, platelet activity determined as the percentage of CD62P and PAC-1 positive platelets, and the concentration of IL-6 during PVI performed by PF and RF energy. PFA = green circles; RFA = red triangles. IL = interleukin; PAC- = procaspase-activating compound; PF = pulsed-field; PFA = pulsed-field ablation; PVI = pulmonary vein isolation; RF = radiofrequency; RFA = radiofrequency ablation.

TABLE 1 Baseline Clinical Characteristics of Patients

	PFA Group (n = 33)	RFA Group (n = 32)	*P* Value
Age (y)	60.5 ± 12.7	64.0 ± 10.7	0.23
Female	12 (36.4)	7 (21.9)	0.27
Hypertension	22 (66.7)	22 (68.8)	0.99
Diabetes mellitus	6 (18.2)	10 (31.3)	0.26
Body mass index, kg/m^2	28.9 ± 4.9	30.5 ± 5.2	0.20
Coronary artery disease	4 (12.1)	2 (6.3)	0.67
CHA_2DS_2VASc score	2.39 ± 1.68	2.28 ± 1.69	0.79
Stroke	3 (9.1)	1 (3.1)	0.61
AF type			
Paroxysmal	20 (60.6)	20 (62.5)	0.99
Persistent	10 (30.3)	9 (28.1)	
Long-lasting persistent	3 (9.1)	3 (9.4)	
Electrical cardioversion	18 (54.6)	17 (53.1)	0.99
Echocardiography			
LVEF (%)	58.7 ± 4.8	58.2 ± 4.9	0.75
LA size (mm)	41.7 ± 5.8	43.3 ± 5.1	0.25
Medication			
No. of antihypertensive drugs	1.97 ± 1.31	1.75 ± 1.32	0.41
Current AAD use	15 (45.5)	17 (53.1)	0.62
Current anticoagulation	32 (97.0)	30 (93.8)	0.61
Rivaroxaban	20 (60.6)	21 (65.6)	0.79
Apixaban	11 (33.3)	8 (25.0)	
Dabigatran	1 (3.0)	1 (3.1)	

Values are mean ± SD or n (%).

AAD = antiarrhythmic drug; AF = atrial fibrillation; LA = left atrium; LVEF = left ventricular ejection fraction; PFA = pulsed-field ablation; RFA = radiofrequency ablation.

TABLE 2 Procedural Characteristics

	PFA Group (n = 33)	RFA Group (n = 32)	*P* Value
Procedure duration (min)	55:09 ± 11:57	151:19 ± 41:25	<0.001
Procedure duration, PVI-only patients (min)	52:06 ± 11:19	136:23 ± 27:54	<0.001
Fluoroscopy duration (min)	9.4 ± 3.3	6.8 ± 2.4	<0.001
LA dwelling time (min)	36:00 ± 8:05	115:58 ± 36:49	<0.001
Total heparin dose (IU)	15,697 ± 2,023	16,565 ± 4,185	0.50
Maximum ACT (s)	357 ± 37	338 ± 24	0.02
Midazolam dose (mg)[a]	2.4 ± 1.1	1.8 ± 1.4[a]	0.01
Sufentanil dose (mg)	9.7 ± 2.8	16.3 ± 6.4	<0.001
Propofol dose (mg)[b]	277.4 ± 144.4	102.2 ± 81.1[b]	<0.001
Electrical cardioversion	8 (24)	9 (28)	0.78
SR at discharge	33 (100)	32 (100)	1.00
Ablation			
Total no. of LA PFA applications)/ total LA ablation time (min)	43.1 ± 11.8	31.7 ± 10.1	NA
No. of PFA applications on PVs (no)/PV ablation time (min)	34.6 ± 2.6	28.4 ± 8.4	NA
No. of patients with left atrial ablation outside PVs	12 (36.4)	11 (34.4)	1.00
No. of patients with CTI ablation	4 (12.1)	3 (9.4)	1.00
No. of patients with PVI only (without additional LA ablations or CTI ablation)	17 (51.5)	19 (59.4)	0.62

Values are mean ± SD or n (%). [a]Midazolam was used in 32 (97%) PFA and 18 (56.2%) RFA patients. [b]Propofol was used in 33 (100%) PFA and 18 (56.2%) RFA patients. [c]1 patient in the RFA group underwent both additional LA ablation and CTI ablation.

ACT = activated clotting time; CTI = cavotricuspid isthmus; NA = not available; PV = pulmonary vein; PVI = pulmonary vein isolation; SR = sinus rhythm; other abbreviations as in Table 1.

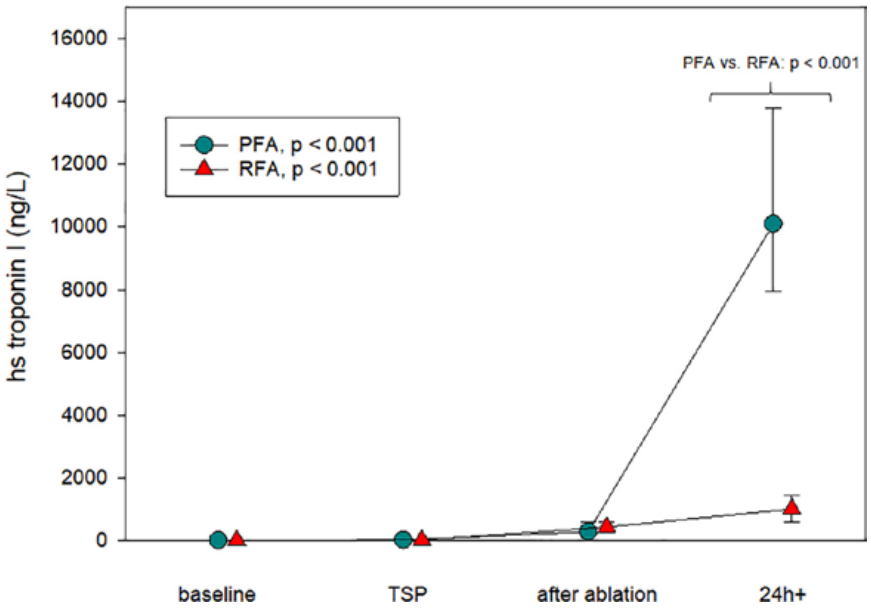
characteristics are shown in Table 1; both groups were similar in important baseline clinical characteristics. Twenty (60.6%) patients had paroxysmal AF and 13 (39.4%) had nonparoxysmal AF in the PFA group; in the RFA group, the proportion was 20 (62.5%) and 12 (37.5%) patients, respectively (Table 1). LA area was also similar between groups (23.0 ± 5.4 cm^2 PFA group vs 23.9 ± 5.5 cm^2 RFA group; $P = 0.54$).

Important procedural characteristics are shown in Table 2. As expected, the procedure was substantially shorter in the PFA group (55:09 ± 11.57 min vs 151:19 ± 41.25 min; $P < 0.001$), as was LA dwelling time (36:00 ± 8:05 min vs 115:58 ± 36:49 min; $P < 0.001$). Procedural durations were also shorter in PVI-only PFA patients compared with PVI-only RFA patients (52:06 ± 11.19 min vs 136:23 ± 27:54 min; $P < 0.001$). Electrical cardioversion was done in 8 (24.3%) patients in the PFA group, and in 9 (28.1%) patients in the RFA group. Blood samples (the "after ablation" samples) were drawn before electrical cardioversion in all 8 PFA patients. In the RFA group, blood samples were drawn before electrical cardioversion in 6 patients, and after cardioversion in 3 patients.

A local hematoma in a patient with RFA prolonged their hospitalization by 1 day but did not require blood products, surgical revision, or other procedures. Neither group had other complications (eg, cardiac tamponade, phrenic nerve palsy, stroke, or femoral access complications). Procedures were done under analgosedation, which was substantially deeper in the PFA group (Table 2). Although the dose of midazolam was higher in the RFA group, the doses of sufentanil and propofol were higher in the PFA group (Table 2). Propofol was used in 18 (56.2%) patients in the RFA group compared with 33 (100%) patients in the PFA group; the average dose of propofol in the patients with PFA was more than twice as high (Table 2). Additionally, 16 (48.5%) patients in the PFA group (but none in the RFA group) received ketamine (34.1 ± 9.2 mg/procedure). The surface areas of the isolated veins were quantified in the RFA group. The isolated areas were 5.67 ± 1.02 cm^2 for left-sided veins, 2.89 ± 0.51 cm^2 for right superior pulmonary vein (RSPV), and 2.32 ± 0.47 cm^2 for LIPV (left-sided PVs were isolated by a single circular ablation, right-sided veins were ablated separately). Because 3D mapping was not used in the PFA group, the ablated areas in the PFA group could not be quantified.

MARKERS OF MYOCARDIAL DAMAGE. Compared with baseline concentrations, there was a significant increase in high-sensitivity troponin I in both groups 24 hours after the procedure ($P < 0.001$). However,

FIGURE 1 The Time Course of High-Sensitivity Troponin I Concentrations During PFA and RFA



Figures display medians with IQRs (PFA = green circles; RFA = red triangles). The results of progression in each group are shown in rectangles. RFA = radiofrequency ablation; PFA = pulsed-field ablation.

the increase was significantly higher in the PFA (Figure 1) compared with the RFA group. The maximum troponin I concentrations were obtained 24 hours postprocedure in patients with PFA (median: 10,102; IQR: 8,272-14,207) and were almost 10 times higher than those in patients with RFA (median: 1,006; IQR: 603-1,433) with $P < 0.001$.

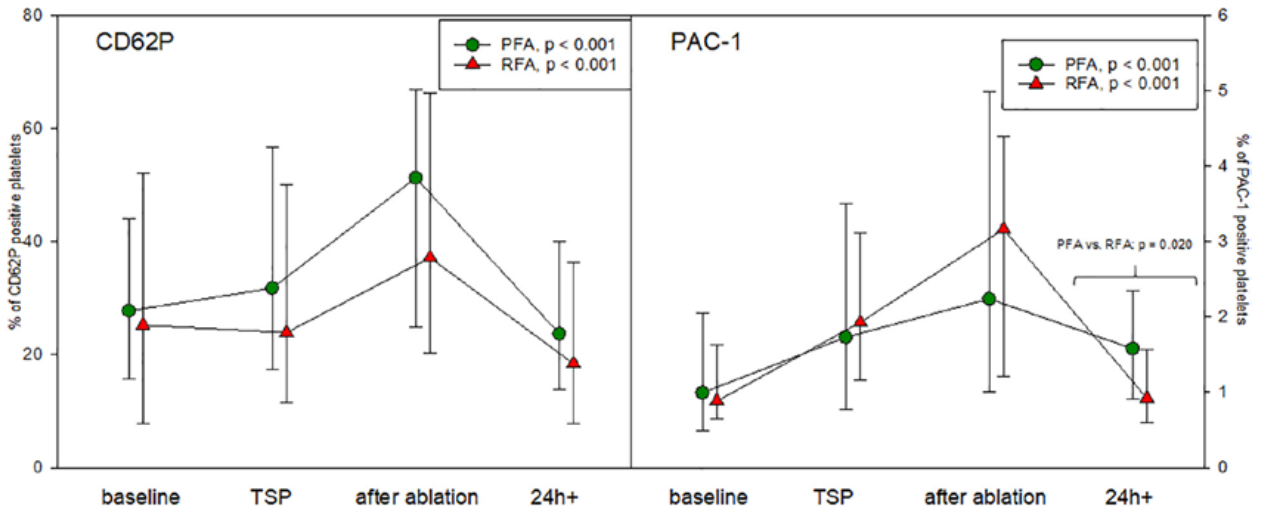
MARKERS OF PLATELET ACTIVATION. The time course of CD62P (P-selectin) during the procedure was very similar in both groups. There were similar significant increases by ~50% in CD62P after trans-septal punctures and at the end of the ablation; a return to preprocedural values occurred the following day in both groups ($P < 0.001$ for each group) (Figure 2). Similarly, a significant increase in PAC-1 was observed during the procedure in both groups ($P < 0.001$ for each group) (Figure 2). The time course of PAC-1 (ie, an increase during the procedure and a subsequent decrease to preprocedure values 1 day after the procedure) was very similar between groups. The only exception was the 24-hour value, which was higher in the PFA group than in the RFA group (Figure 2).

MARKERS OF COAGULATION ACTIVITY. There were no significant changes in the coagulation markers (ie, D-dimers, fibrin monomers, vW antigen, or vWF activity) between groups (Figure 3). There were no differences in D-dimer concentrations during the procedure in either group ($P = 0.46$ in the PFA or 0.43 in the RFA, respectively). The time course of fibrin monomers changed significantly during the procedure in both groups (Figure 3), with a slight decrease after trans-septal punctures, but without differences between groups. The time course of vW antigen and vWF activity changed significantly in both groups (Figure 3), with gradual increases in both vW antigen and vWF activity during the procedure, again without differences between groups.

MARKER OF INFLAMMATION. The concentrations of IL-6 increased significantly 24 hours postprocedure in both groups ($P < 0.001$) (Figure 4). The increase was slightly higher in the RFA group, although the difference did not reach statistical significance ($P = 0.07$).

SUBGROUP ANALYSIS AND LINEAR REGRESSION. In the PFA group, 17 patients underwent pulmonary vein isolation only (PVI-only), and 16 patients underwent left atrial ablations or cavotricuspid isthmus (CTI) ablations in addition to PVI (PVI-plus). The time-course of concentrations for all measured biomarkers was compared between PVI-only and PVI-plus subgroups of patients with PFA. The time course of D-dimers was not significant in either subgroup (but it was also not significant in the main analysis). The time course of all other analyzed

FIGURE 2 **The Time Course of Markers of Platelet Activation CD62P and PAC-1 During PVI Using PF and RF Energy**



Figures display the medians with IQRs and express the percentage of antigen-positive platelets (PFA = green circles; RFA = red triangles). The results of progression in each group are shown in rectangles. The difference between groups at time point 24 h+ is shown. PAC = procaspase-activating compound; PF = pulsed-field; PVI = pulmonary vein isolation; RF = radiofrequency; other abbreviations as in Figure 1.

biomarkers differed significantly during ablation in each subgroup, except for fibrin monomers ($P = 0.18$) and PAC-1 ($P = 0.058$) in the PVI-only PFA group. Importantly, no differences between PVI-only vs PVI-plus patients were found; additionally, the troponin I concentration did not reach statistical significance between PVI-only and PVI-plus PFA subgroups.

A similar analysis was performed for patients with RFA. In the RFA group, 19 patients only underwent PVI, and 13 patients had additional left atrial or CTI ablations. The time course of D-dimers was not significant in either subgroup (but was also not significant in the main analysis). The time course of all other analyzed biomarkers differed significantly in each subgroup, with exceptions of vW antigen ($P = 0.21$) and vW activity ($P = 0.70$) in PVI-only patients and fibrin monomers ($P = 0.15$) and CD6P ($P = 0.082$) in PVI-plus RFA patients. As with PFA patients, no differences between PVI-only vs PVI-plus RFA patients were found.

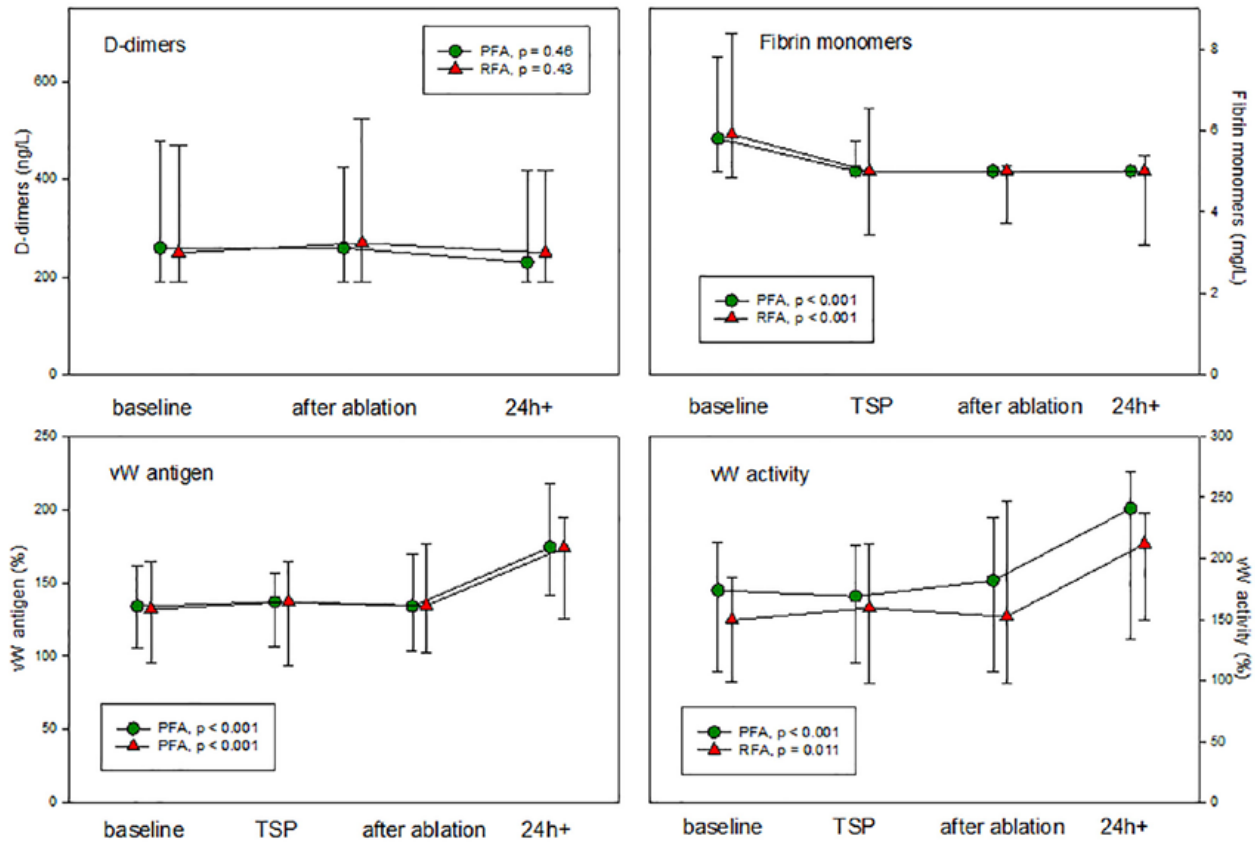
Finally, the concentrations of all measured biomarkers were compared between PFA (n = 17) and RFA (n = 19) patients who underwent the PVI-only procedure. Graphic presentations of biomarker concentrations are shown in Supplemental Figures 1 to 4. Similarly, as in the main analysis, the concentrations of hs-troponin I obtained 24 hours + after the procedure differed significantly between PFA and RFA PVI-only patients ($P < 0.001$). Furthermore, platelet expression of PAC-1 24 h+ after the procedure was slightly higher in the PFA patients compared with RFA patients ($P = 0.02$). No other biomarker differences were found between PFA and RFA PVI-only patients.

Univariate and multivariate linear regression was performed to predict the maximum biomarker value obtained during the procedure (the values immediately after ablations for CD62P and PAC-1 and for the remaining biomarkers at 24 hours+). A log transformation of dependent variable was applied where appropriate (eg, troponin concentration). Selected clinical (ie, age, sex, hypertension, coronary artery disease, and AF type) and procedural (ie, type of procedure, cardioversion) characteristics were used as predictors. Allocation to the PFA group was the only independent statistically significant predictor associated with high 24 hours + troponin levels in the multivariate linear regression model (exp(β) = 11.1; 95% CI: 7.9-15.6).

DISCUSSION

In this study, PVI performed using PF energy was associated with a substantially higher degree of myocardial damage compared with PVI performed using RF energy. Despite an almost 10 times higher degree of myocardial injury, it was not accompanied by a greater extent of platelet activation or coagulation. Moreover, the extent of inflammatory activation is slightly higher in RFA compared with PFA.

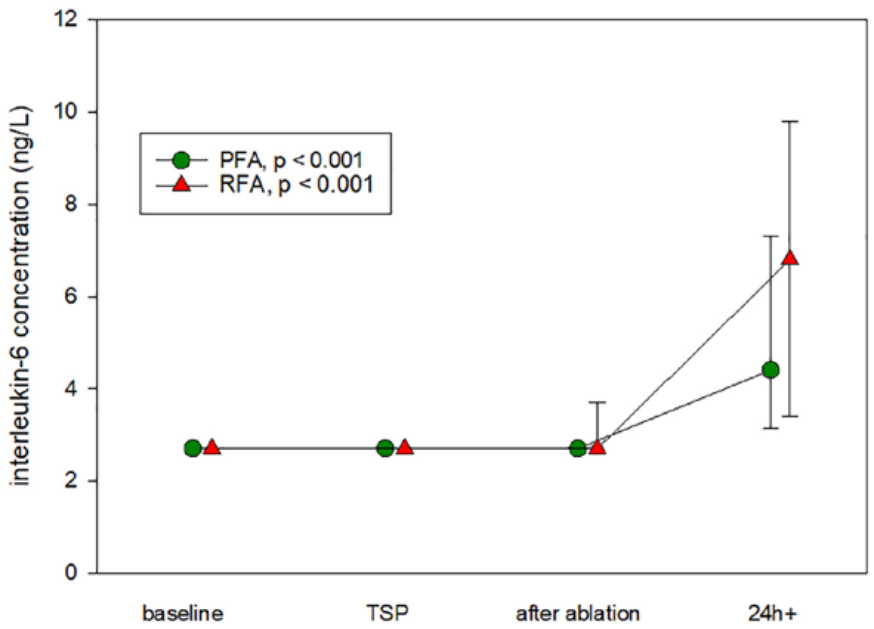
FIGURE 3 The Time Course of Markers of Coagulation During PFA and RFA



Figures display medians with IQRs (PFA = green circles; RFA = red triangles). The results of progression in each group are shown in rectangles. Abbreviations as in Figure 1.

MYOCARDIAL DAMAGE. As previously shown, the extent of myocardial injury in cryoablation and RFAs is similar. In this study, we found that the extent of myocardial injury was substantially greater in the PFA group. Kawamura et al[8] compared the isolated areas on voltage maps in patients after PVI performed using PFA and RFA and found that the isolated areas were similar between patients with PFA and patients with RFA. In our study, the isolated areas were measured only in RFA patients (3D mapping was not used in the PFA group), and the values of the isolated areas were similar to the values reported by Kawamura et al[8] (eg, 2.89 ± 0.51 cm^2 and 2.32 ± 0.47 cm^2 for RSPV and left superior pulmonary vein (LSPV) in our RFA patients, and 2.9 ± 1.1 cm^2 for RSPV and 2.5 ± 1.2 cm^2 for right inferior pulmonary vein (RIPV) in the report by Kawamura et al[8]). Because our PFA patients underwent ablation using a similar method as in Kawamura et al[8] and other reports on PFA ablation using the Farawave catheter (ie, 4 basket and 4 flower applications for each vein),[4,5] it is not probable that the areas in our patients with PFA would be significantly higher than in previous studies. In our opinion, the explanation for the difference in the greater extent of myocardial injury in the PFA group lies in the differences in the nature between PFAs and RFAs. In the RFA, the area around the PVs is isolated, but, in the PFA, the whole area is ablated. In RFA patients, only the circumference of the area around the PV is ablated, ie, a thin line (only a few millimeters wide) of cardiomyocytes around the vein is damaged. In contrast, PFA involves ablating the entire surface area surrounding the PVs. This means, that the number of damaged cardiomyocytes is significantly higher. Furthermore, as was shown in the preclinical and the first clinical studies, the degree of transmural injury is higher in ablations using PF energy, which, combined with a wide area of ablated cardiomyocytes, corresponds to the high degree of myocardial damage. In animal studies, markers of myocardial damage

FIGURE 4 The Time Course of Markers of IL-6 During PFA and RFA



Figures display medians with IQRs (PFA = green circles; RFA = red triangles). The results of progression in each group are shown in rectangles. IL = interleukin; other abbreviations as in Figure 1.

increased significantly after PFA, peaking 1-3 days after the procedure,[9] which corresponds to our results.

In a previous study, higher troponin release after RFA was associated with greater reversal of structural LA remodeling, and with a higher chance for sinus rhythm maintenance.[10] Whether this finding will also be confirmed in PFA ablation, which is associated with substantially higher troponin I concentrations, needs verification in further clinical studies. On the one hand, it could present a marker of high success rates in terms of durable PVI; on the other hand, it could represent more left atrial damage, which could produce a substrate for LA re-entry and could be associated with impaired left atrial function.

INFLAMMATORY RESPONSE AFTER PFA AND RFA. In an in vivo human study, Herrera Siklody et al[2] demonstrated that several proinflammatory markers increased significantly and similarly after ablation using cryo or RF energy. In a study by Yano et al,[11] cryoablation caused more myocardial injury than RFA; on the other hand, RFA was associated with a higher proinflammatory response. No study has yet compared the in vivo inflammatory response during RFA and PFA.

Previous in vitro studies using RF energy demonstrated that this kind of ablation energy triggers an inflammatory response. Histopathologic studies have established that RFA induces necrosis followed by infiltration of inflammatory cells leading to a fibrotic scar. In an animal study comparing PFA and RFA, PFA lesions were composed of organized, homogeneous fibrosis replacing the myocardium. In contrast, in RF lesions, fibrosis was disorganized and heterogeneous, and infiltration with mononuclear cells was present to a higher degree, which is consistent with a greater inflammatory response.[12] In another animal model of PFA, PFA caused selective atrial myocardial damage. The general architecture of the atrial wall was unaltered in histologic findings 7 days after the PFA procedure.[13] Also, 7 days after ablation, within regions where there was a loss of myocardial fibers, there were rare instances of slight thermal denaturation and mineralization with the presence of inflammatory cells as a consequence of occasional ongoing inflammation. Both these reports agree with our finding (ie, RFA was associated with a slightly higher degree of inflammation than PFA).

In nonparoxysmal AF patients, both troponins and proinflammatory markers were elevated, and a significant correlation between troponin and IL-6 was demonstrated.[14] A significant correlation between markers of myocardial necrosis and inflammation also exists in other cardiovascular disorders, such as acute coronary syndrome. In PFA, despite substantially

greater myocardial damage, there was no increase in the inflammatory response like that seen in myocardial injury caused by ischemia or thermal (RF) damage.

PLATELET ACTIVATION DURING PFA AND RFA. Previous studies have already shown that RFA induces platelet activation. In vitro studies have shown that RF lesions displayed higher thrombus formation than cryo-lesions,[15] and this finding was confirmed in clinical studies with human subjects. Hochholzer et al[16] reported significant platelet activation, indicated by platelet membrane CD62P expression, after ablation of the CTI using RF energy but not when using cryo-energy. Similarly, platelet activation after PVI using RF energy, but not after cryo-energy, was described by Bin Waleed et al.[17] Hererra Siklody et al[2] also described higher platelet activity during PVI in a randomized comparison between cryo-ablation and RFA; however, in this report, both kinds of ablation energies were associated with a similar extent of platelet activation during the ablation procedure.

In the past, myocardial necrosis has been reported to induce platelet activation in other clinical situations than catheter ablations (eg, due to ischemia). However, in our series of patients, despite a 10× increase in troponin after PFA compared with RFA, it was not associated with enhanced platelet activation.

Our data suggest that catheter ablations, in general, result in enhanced platelet activation. In studies comparing thermal energies (ie, cryo and RF energy), platelet activation was less dependent on the type of energy and more on the size of the lesion induced (indicated by peak troponin release). In contrast, in this study, the extent of platelet activation was similar using both RF and PF energy and was not related to the extent and size of the myocardial lesions, which confirms different biological responses to thermal and nonthermal ablation energy.

THE EFFECT OF RFA AND PFA ON COAGULATION MARKERS. The levels of D-dimers or vWF were higher in patients with AF than in controls without AF, and RFA was associated with a further increase in coagulation markers.[18] In our patients, neither PFA nor RFA significantly affected D-dimer concentrations.

Lee et al[19] described an elevation in coagulation markers after RFA. In 37 patients who underwent RFA, there was an increase in D-dimers after trans-septal punctures, with a further increase up to 24 h after the procedure. On the other hand, Lim et al[20] reported that, after RFA, D-dimers increased significantly but not earlier than 1 week after the ablation. Such a late increase would have been missed in our study because our last sample was taken 24 h after the procedure.

Kornej et al[21] described an elevation in the vWF after RFA, which peaked 24 h after the procedure. This agrees with our results (ie, both vW antigen concentrations and vWF activity were higher 24 h after the procedure compared with baseline values.

Importantly, in our patients, the time course of all coagulation parameters did not differ between patients with RF and patients with PF, and, from this point of view, PFA should not be associated with a higher risk of thrombus formation.

STUDY LIMITATIONS. The sheaths used for RFA and PFA differ in size and material, which could have influenced platelet or coagulation activation parameters. Intraprocedural blood samplings were performed from the LA, and the 24 h sampling was from a peripheral vein. The venipuncture site could have influenced platelet activation results; however, it would have influenced them similarly in both groups. The dose of drugs using for analgosedation differed between groups, and although it is known that anesthesia affects systemic coagulation, platelets, and inflammatory markers, it hardly explains the very similar results between groups. Patients with coronary artery disease, which can affect the levels of biomarkers, were not excluded. The patient sample was low and could be underpowered for secondary analyses, and no power calculation was done for markers of coagulation.

CONCLUSIONS

PFA is associated with significantly more myocardial necrosis. Despite greater myocardial damage, platelet activation during PFA was similar to RFA, whereas the inflammatory response was slightly greater after RFA.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: This study extends our knowledge of the effect of PFA on platelets, coagulation, and myocardial damage. It shows that myocardial damage after PFA is approximately 10 times higher compared with RFA. Despite significantly greater myocardial damage, platelet and coagulation activation is similar to RFA. Moreover, the inflammatory response is even slightly greater after RFA.

TRANSLATIONAL OUTLOOK: Similar extent of platelet and coagulation activation during PFA and RFA for AF implicates a pathophysiological background for similar antithrombotic regimens during RFAs and PFAs.

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KEY WORDS apoptosis, atrial fibrillation, coagulation, inflammation, platelet, pulsed-field ablation, radiofrequency ablation

APPENDIX For supplemental figures, please see the online version of this paper.

P. Wohlfahrt et al.

Attenuation of Hypocretin/Orexin Signaling Is Associated With Increased Mortality After Myocardial Infarction

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ORIGINAL RESEARCH

Attenuation of Hypocretin/Orexin Signaling Is Associated With Increased Mortality After Myocardial Infarction

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BACKGROUND: The hypocretin/orexin system has been shown to play a role in heart failure. Whether it also influences myocardial infarction (MI) outcomes is unknown. We evaluated the effect of the rs7767652 minor allele T associated with decreased transcription of the hypocretin/orexin receptor-2 and circulating orexin A concentrations on mortality risk after MI.

METHODS AND RESULTS: Data from a single-center, prospectively designed registry of consecutive patients hospitalized for MI at a large tertiary cardiology center were analyzed. Patients without previous history of MI or heart failure were included. A random population sample was used to compare allele frequencies in the general population. Out of 1009 patients (aged 64±12 years, 74.6% men) after MI, 6.1% were homozygotes (TT) and 39.4% heterozygotes (CT) for minor allele. Allele frequencies in the MI group did not differ from 1953 subjects from general population (χ^2 P=0.62). At index hospitalization, MI size was the same, but ventricular fibrillation and the need for cardiopulmonary resuscitation were more prevalent in the TT allele variant. Among patients with ejection fraction ≤40% at discharge, the TT variant was associated with a lower increase in left ventricular ejection fraction during follow-up (P=0.03). During the 27-month follow-up, there was a statistically significant association of the TT variant with increased mortality risk (hazard ratio [HR], 2.83; P=0.001). Higher circulating orexin A was associated with a lower mortality risk (HR, 0.41; P<0.05).

CONCLUSIONS: Attenuation of hypocretin/orexin signaling is associated with increased mortality risk after MI. This effect may be partially explained by the increased arrhythmic risk and the effect on the left ventricular systolic function recovery.

Key Words: hypocretin/orexin receptor-2 ■ hypocretin/orexin system ■ inflammation ■ mortality ■ myocardial infarction ■ outcomes ■ recovery

In the central nervous system, the hypocretin/orexin (H/O) system regulates sleep–wake cycles and metabolism. The loss of orexin-producing neurons in the hypothalamus causes narcolepsy with cataplexy (narcolepsy type I).[1] On the other hand, orexin receptor antagonists have been used for insomnia treatment.[2] Other studies suggested the role of the brain's H/O system in feeding behavior and propensity for weight gain, with orexin signaling acting through a net increase in energy expenditure.[3]

Outside of the central nervous system, the impact of the H/O system has been recently recognized in patients with heart failure (HF). In an unbiased systems-biology search, Perez et al identified the rs7767652 locus in the regulating domain of HCRTR-2 (hypocretin receptor-2) as the strongest predictor of left ventricular ejection fraction (EF) improvement in response to HF pharmacotherapy.[4] In the functional validation study, the rs7767652 minor allele T was associated with disruption of a transcription factor

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CLINICAL PERSPECTIVE

What Is New?

- In patients after myocardial infarction, attenuation of hypocretin/orexin signaling is associated with increased mortality risk.

What Are the Clinical Implications?

- Orexin receptor agonists may improve outcomes after myocardial infarction, but further research is needed.

Nonstandard Abbreviations and Acronyms

AMBITION	Institute for Clinical and Experimental Medicine Acute Myocardial Infarction Registry
HCRTR-2	hypocretin orexin receptor-2
H/O	hypocretin/orexin

4 binding site, leading to decreased transcription of HCRTR-2. Impact of the H/O system on HF was further confirmed in an animal model of HF, in which orexin administration improved left ventricular EF. In a human HF study, subjects with a higher circulating orexin A concentration (≥1.04 ng/mL) had more significant reduction in left ventricular end-diastolic and end-systolic volume and a trend toward greater improvement in left ventricular EF in response to HF therapy.[5]

Myocardial infarction (MI) is one of the most common causes of HF development and is associated with increased mortality risk.[6] Until now, no study has evaluated the effect of the H/O system on MI outcomes. The aim of the present prospective study was to describe the effect of the H/O system on total mortality among consecutive patients hospitalized for their first MI. To overcome typical biases associated with biochemical biomarker measurements that may impact results validity, we have used a genetic variant in the regulating domain of the HCRTR-2 gene, which is associated with attenuated HCRTR-2 signaling. Because of Mendelian inheritance laws, genetic variants are randomly distributed in the population. Unlike biochemical variables, genetic variants are not confounded by environmental and other factors. As a sensitivity analysis to further confirm the impact of the H/O system on survival, we have also measured circulating orexin A levels in a high-risk subgroup of patients with systolic dysfunction after MI.

METHODS

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Population

This study used data from the prospective AMBITION (Institute for Clinical and Experimental Medicine Acute Myocardial Infarction Registry) registry,[7] which has been collecting clinical data and biospecimens from all consecutive patients hospitalized for acute coronary syndrome at a tertiary heart center since June 2017. The methods of this study were previously described.[8] During the hospital stay, all patients underwent detailed interviews, and additional information was obtained through manual chart abstraction and laboratory studies.

For this analysis, data from individuals without a previous history of HF and coronary artery disease hospitalized for type 1 MI (caused by atherosclerotic plaque rupture and thrombosis)[9] between June 2017 and November 2021 were used. The institutional review board of the Institute for Clinical and Experimental Medicine approved the study, and all participants signed informed consent. The investigation conformed to the principles outlined in the Declaration of Helsinki.

To identify the impact of rs7767652 on MI risk, we compared allele frequencies in the general population and patients after MI. As a control group, we used data from the Czech post-MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) study, which examined a 1% random population sample in 9 districts of the Czech Republic. Methods of the Czech post-MONICA study were previously reported.[10]

Definition of Comorbidities

History of diabetes was defined by oral antidiabetic drugs or insulin use at the time of hospital admission or by glycated hemoglobin ≥48 mmol/mol at the time of hospitalization. Arterial hypertension was defined as self-reported use of antihypertensive drugs at admission. Self-reported history of smoking was used. A person was considered a current smoker if smoking at least 1 cigarette per day during the past 12 months. Positive family history of cardiovascular disease was defined by MI or stroke in first-degree relatives before age 55 years in men and before age 60 years in women, respectively.

rs7767652 Genotyping

DNA was isolated from peripheral blood. The rs7767652 locus in the regulating domain of HCRTR-2 was analyzed using the TaqMan SNP assay

No.C_29161754_20. Genotyping was performed according to the manufacturer's protocol on an ABI 7300 real-time polymerase chain reaction instrument.

Orexin A Concentration Measurement

In 245 patients with systolic dysfunction and EF <40% at hospital discharge, we measured the concentration of orexin A in blood samples drawn on the first day after hospital admission using the ELISA method (Phoenix Pharmaceuticals, Burlingame, CA).

Outcomes

The primary outcome of this study was all-cause mortality. Mortality data were provided by the Institute of Health Information and Statistics, which keeps a record of all deceased individuals by law.

Statistical Analysis

Data are presented as mean±SD, median (interquartile range [IQR]), or frequency (percent). ANOVA, Kruskal-Wallis, or χ^2 tests were used to compare differences across the 3 allele variants, as appropriate. A log-rank test was used to compare survival by allele variants. A Cox proportional hazard model was used to assess factors influencing survival after MI. The proportional hazard assumption was checked and fulfilled. Follow-up was defined as the time from hospital discharge to death ascertained to January 1, 2022, without censoring for any additional events.

Statistical analyses were performed using SPSS version 25.0 (IBM, Armonk, NY), Stata version 17 (StataCorp, College Station, TX), or R software version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was set at $P<0.05$. The same threshold was used for variables to enter the multivariable analyses.

RESULTS

Of the 1593 patients in the AMBITION registry, 1347 had type 1 MI. Of these, 268 had a previous history of coronary artery disease, and another 14 had chronic heart failure and were therefore excluded. Of the 1065 eligible patients, rs7767652 allele variants were available in 1009 patients. The study flowchart is shown in Figure 1. The main study findings are summarized in Figure 2.

rs7767652 Allele Variants in MI and the General Population

In 1009 patients from the MI population, 6.1% patients were homozygotes (TT) and 39.4% heterozygotes (CT) for the hypofunctional rs7767652 minor T allele. Similarly, in 1953 subjects from the general population of the Czech post-MONICA study, 6.6% were homozygotes and 37.8% were heterozygotes for the rs7767652 minor allele. Allele frequencies in the MI and general population were not statistically different (χ^2 P=0.62), suggesting that rs7767652 does no increase MI risk.

Traditional Risk Factors and MI Complications by rs7767652 Allele Variants

Demographic characteristics by allele variants are shown in Table 1. There were no statistically significant differences in traditional risk factors such as diabetes, body mass index, glycemia or low-density lipoprotein cholesterol by the rs7767652 allele variants. Similarly, there was no statistically significant difference in MI size as assessed by maximal troponin level or discharge EF. However, subjects with the TT variant more often experienced ventricular fibrillation (12.9% versus 4.8%, P=0.01) and more often required cardiopulmonary resuscitation (16.1% versus 7.0%, P=0.02), as compared with the CT and CC variants combined. Among the 243 patients with EF ≤40% at hospital discharge and available follow-up EF measured on a median of 128 days (IQR, 98–395 days) after the baseline EF measurement, minor allele homozygotes had a lower increase in EF during the follow-up (2.5±11.0% versus 8.4±9.4%, P=0.04, for TT versus CT and CC combined).

rs7767652 Allele Variants and Total Mortality

During the median follow-up of 27 months (IQR, 13–41 months), the total mortality rate was 8.4% (n=83). Homozygotes for the rs7767652 minor and hypofunctional allele had a higher mortality risk as compared with heterozygotes (P=0.001) and homozygotes for the major allele (P=0.001), with no statistically significant difference between heterozygotes and major allele homozygotes (P=0.836) (Figure 3). After multivariable adjustment, minor allele homozygotes remained at increased mortality risk (hazard ratio, 2.83 [95% CI, 1.55–5.19]) (Table 2).

Orexin A Concentration and Mortality

To further confirm the effect of the H/O system on mortality after MI, we measured orexin A levels in 245 patients with systolic dysfunction and EF ≤40% at hospital discharge. At baseline, patients with the rs7767652 TT allele variant had no statistically significant difference in orexin A concentrations from those with the CT (0.76±0.26 versus 0.80±0.28 ng/mL, P=0.82) and CC (0.76±0.26 versus 0.84±0.29 ng/mL, P=0.48) variants, respectively. In the analysis adjusted for age, mortality risk was lowest in subjects with orexin concentration ≥1.0 ng/mL (Figure 4). After multivariable adjustment,

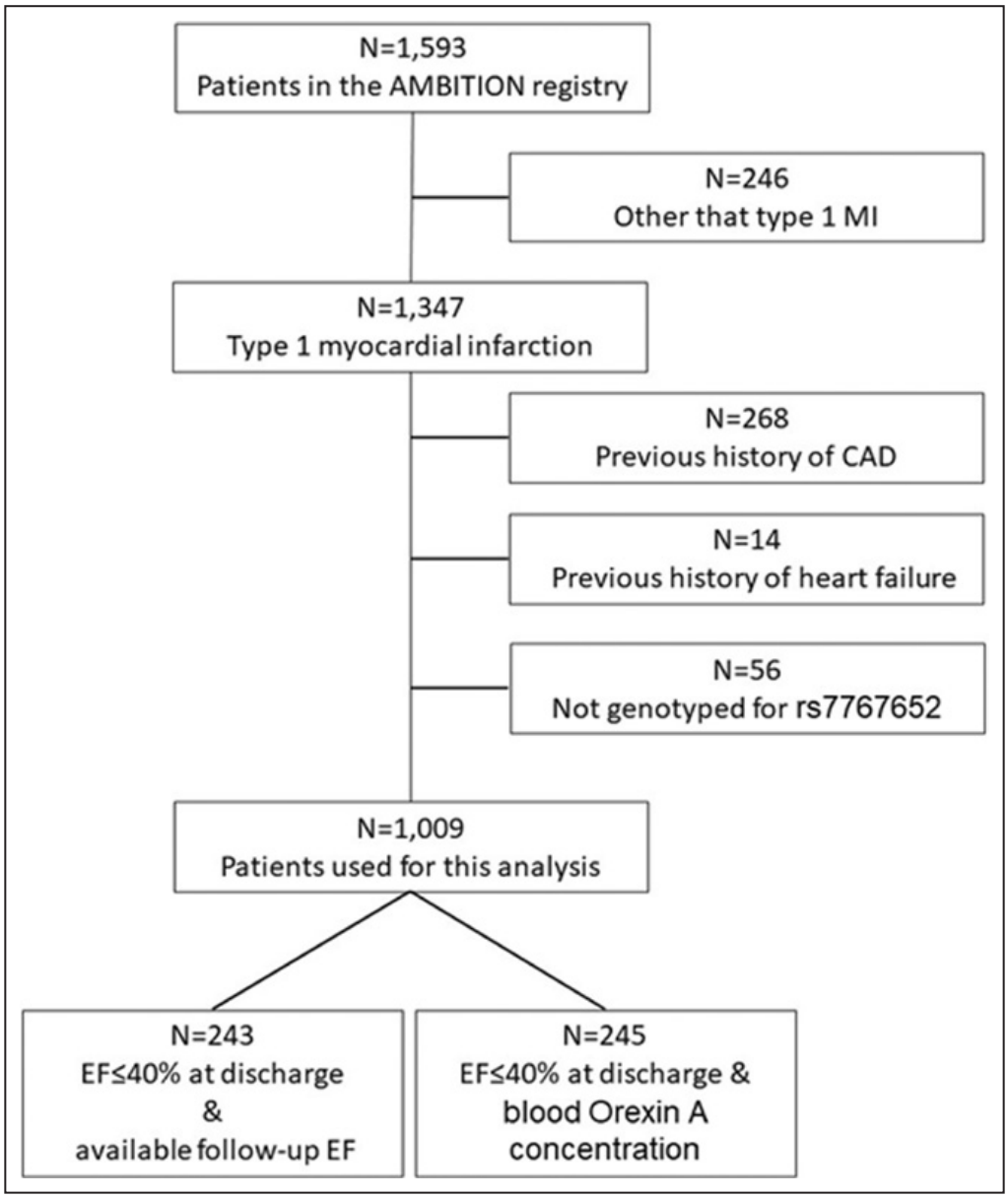

Figure 1. Study flowchart.
AMBITION indicates Institute for Clinical and Experimental Medicine Acute Myocardial Infarction Registry; CAD, coronary artery disease; EF, ejection fraction; and MI, myocardial infarction.

the mortality risk among patients with orexin A concentration ≥1.0 ng/mL was 59% lower than in patients with orexin <1.0 ng/mL (Table 3).

DISCUSSION

This is the first study to describe the influence of the H/O system on the prognosis of patients after the first MI. We have shown that the rs7767652 minor allele, which is associated with attenuated H/O signaling, does not increase MI risk and is not associated with traditional risk factors. Although MI size was similar, the long-term outcome differed by rs7767652 allele variants. Minor allele homozygous, which confers with lower HCRTR-2 transcription,[4] and subjects with lower circulating orexin A level were at increased total mortality risk. This effect of the H/O system on mortality may be partially explained by the increased arrhythmic risk and the impact on the left ventricular systolic function recovery.

The H/O system was first described almost 25 years ago by 2 independent groups searching for a possible treatment for obesity. De Lecea et al named discovered proteins hypocretins because of their location in the hypothalamus and amino acid similarities with a gut hormone secretin.[11] Sakurai et al named peptides

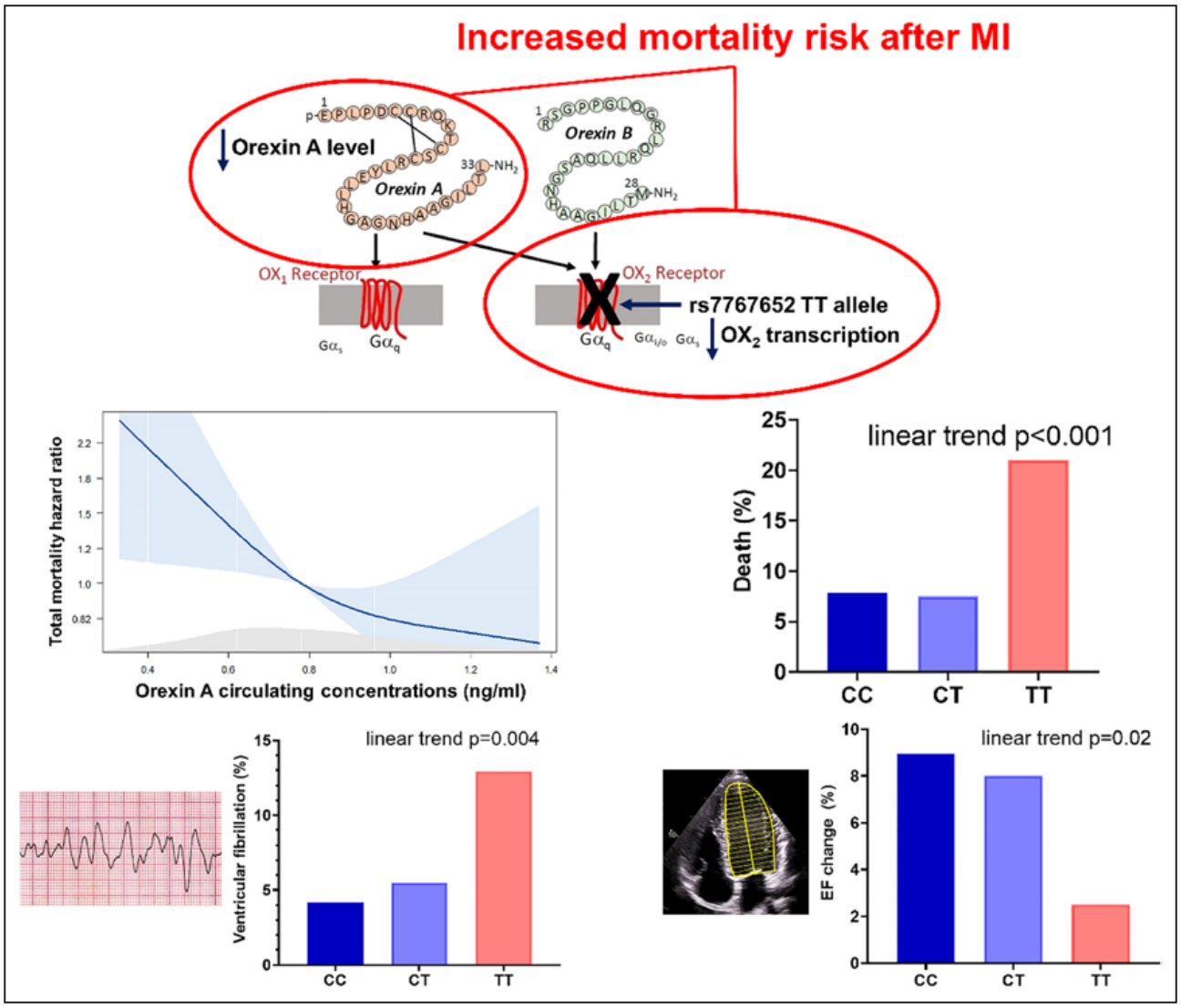

Figure 2. Main study findings.
EF indicates ejection fraction; and MI, myocardial infarction.

orexins after the Greek word for appetite, because their application induced feeding in rats.[12] It was discovered that the proteins identified by these groups were identical.

In the following years, the research on the central nervous system described involvement of the H/O system in maintaining wakefulness,[13] food intake, energy homeostasis, reward-seeking, stress, motivation, and drug addictions.[14] Outside the central nervous system, the H/O system may influence the risk of digestive tract cancer, inflammatory bowel syndrome, and glucose metabolism.[15] In humans, orexin deficiency is associated with glucose intolerance and insulin resistance.[16] In rodents, orexin overexpression protects from diet-induced obesity and improves glucose control.[17] This is related to orexin-induced increase in GLUT4 (glucose transporter type 4) expression in the liver and increased insulin secretion.[18,19]

The involvement of the H/O system in heart disease has been recognized only recently among patients with HF.[4,5,20] We added to this evidence by showing for the first time that the H/O system also influences prognosis after MI.

Several possible mechanisms may mediate the influence of the H/O system on mortality after MI. First, this effect may be caused by long-term exposure to classical risk factors. Association of the H/O system with metabolic syndrome and insulin sensitivity was described in previous studies,[15,16] both of which influence outcomes after MI.[21] However, we did not find any difference in diabetes, glucose, or body mass index by the rs7767652 allele variants in the present study. Although the H/O system influences drug addiction, including smoking,[14] there was no difference in the proportion of smokers by allele

Table 1. Demographic Characteristics by rs7767652 Allele Variants (N=1009)

Variable	CC, N=549	CT, N=398	TT, N=62	*P* for linear trend
Age, y	63.6±12.6	63.5±11.7	66.5±12.0	0.078
Male sex	414 (75.4)	294 (73.9)	46 (74.2)	0.843
Risk factors				
Arterial hypertension, n (%)	309 (56.4)	236 (59.4)	44 (66.1)	0.268
Diabetes, n (%)	131 (23.9)	106 (26.6)	18 (29.0)	0.491
Current smoking, n (%)	248 (45.2)	186 (46.7)	27 (43.5)	0.799
Statin use before admission, n (%)	91 (16.6)	72 (18.1)	14 (22.6)	0.242
Family history of CVD, n (%)	145 (26.4)	117 (29.4)	13 (21.0)	0.348
AF history, n (%)	24 (4.4)	20 (5.0)	4 (6.5)	0.470
Index event				
Cardiopulmonary resuscitation, n (%)	34 (6.2)	32 (8.0)	10 (16.1)	0.005
Ventricular fibrillation, n (%)	23 (4.2)	22 (5.5)	8 (12.9)	0.004
In-hospital AF, n (%)	65 (11.8)	52 (13.1)	7 (11.3)	0.890
STEMI, n (%)	361 (65.8)	262 (65.8)	38 (61.3)	0.481
Subacute MI, n (%)	78 (14.2)	54 (13.6)	10 (16.1)	0.674
Killip class >1, n (%)	108 (19.7)	78 (19.6)	14 (22.6)	0.584
Selective coronarography, n (%)	546 (99.5)	393 (98.7)	61 (98.4)	0.410
PCI, n (%)	460 (83.8)	346 (86.9)	53 (85.5)	0.744
CABG, n (%)	54 (9.8)	31 (7.8)	5 (8.1)	0.659
Pericarditis, n (%)	11 (2.0)	9 (2.3)	5 (8.1)	0.004
Intravenous diuretics, n (%)	130 (23.7)	91 (22.9)	15 (24.2)	0.922
Anterior MI, n (%)	241 (43.4)	173 (43.5)	26 (41.9)	0.769
Admission systolic BP, mm Hg	143.6±26.2	142.4±26.6	142.2±29.8	0.697
Admission diastolic BP, mm Hg	80.0±14.0	78.6±13.5	77.6±14.6	0.193
Admission heart rate, min^{-1}	77.9±19.2	76.9±17.0	77.4±16.2	0.851
Maximum troponin natural log, ng/L	7.00±1.53	7.01±1.54	6.76±1.38	0.242
Discharge EF, %	44.9±10.1	45.3±10.3	46.1±10.9	0.382
CKD EPI, mL/min per 1.73 m^2	77.6±22.2	77.9±22.7	75.8±19.9	0.528
BMI, kg/m^2	28.6±4.7	28.9±5.1	28.3±5.7	0.564
HbA1c, mmol/L per mol	44.5±11.6	45.8±14.5	44.9±11.8	0.852
Fasting glycemia, mmol/L	8.3±3.8	8.4±3.8	8.1±3.2	0.743
Total cholesterol, mmol/L	4.86±1.15	4.89±1.34	4.63±1.08	0.153
Triglycerides, mmol/L	1.7±1.0	1.8±1.4	1.9±1.3	0.031
HDL cholesterol, mmol/L	1.14±0.34	1.12±0.31	1.06±0.27	0.047
LDL cholesterol, mmol/L	3.25±1.11	3.21±1.11	2.99±0.97	0.075
Leukocytes, $10^9/L$	11.37±3.97	11.37±4.04	10.73±3.64	0.234
Hemoglobin, g/L	142.7±15.6	141.4±16.1	142.0±15.9	0.759
Discharge medication				
ACEi/ARB, n (%)	397 (73.7)	309 (78.2)	49 (81.7)	0.177
β-Blocker, n (%)	436 (80.9)	327 (82.8)	49 (81.7)	0.894
Furosemide, n (%)	106 (22.3)	67 (19.3)	13 (23.6)	0.792
Spironolactone, n (%)	100 (21.0)	67 (19.3)	13 (23.6)	0.634
Statin, n (%)	520 (96.5)	377 (95.4)	58 (96.7)	0.929
Echocardiography follow-up*				
EF change, %	9.0±8.7	8.0±9.2	2.5±11.0	0.019

(*Continued*)

Table 1. Continued

Variable	CC, N=549	CT, N=398	TT, N=62	*P* for linear trend
End-diastolic diameter change, mm	2.2±5.6	2.6±5.6	4.8±9.4	0.146
Outcome				
30-d mortality	14 (2.6)	9 (2.3)	3 (4.8)	0.276
Death, n (%)	43 (7.8)	30 (7.5)	13 (21.0)	<0.001

ACEi/ARB indicates angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; AF, atrial fibrillation; BMI, body mass index; BP, blood pressure; CABG, coronary bypass grafting; CKD EPI, Chronic Kidney Disease Epidemiology Collaboration; CVD, cardiovascular disease; EF, ejection fraction; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MI, myocardial infarction; PCI, percutaneous coronary intervention; and STEMI, ST-segment–elevation myocardial infarction.

*Analyzed in 243 patients with discharge EF ≤40% and available follow-up echocardiography.

variants. Second, infarct size, assessed by EF, troponin release, or medication use, was not affected by the rs7767652 polymorphism, indicating that infarct size per se does not explain the difference in survival. Third, the orexin system effected arrhythmia risk. We found a higher prevalence of ventricular fibrillation in rs7767652 minor allele homozygotes. Minor allele homozygotes had also higher triglyceride levels at the time of MI, which may reflect increased lipolysis in subcutaneous and epicardial adipose with release of nonesterified fatty acids and adipokines that promote arrhythmogenesis.[22] Fourth, on the effect on inflammation, we found that pericarditis was more prevalent in minor allele homozygotes despite a similar MI size, suggesting a higher inflammatory response to MI. This may be explained by an immunomodulatory effect of the orexin system.[23] Several recent studies have described the effect of the inflammatory response on outcomes after MI.[24] Fifth, we found that the H/O system affects EF recovery after MI, which is associated with improved survival.[25] Previously, variation in rs7767652 identified superresponders to pharmacotherapy, who had improved EF because of reverse remodeling.[4] In the present study among patients with EF ≤40% at hospital discharge, rs7767652 minor allele homozygotes had a lower increase in EF during follow-up. This is also supported by the fact that 30-day mortality did not differ by allele variants, whereas

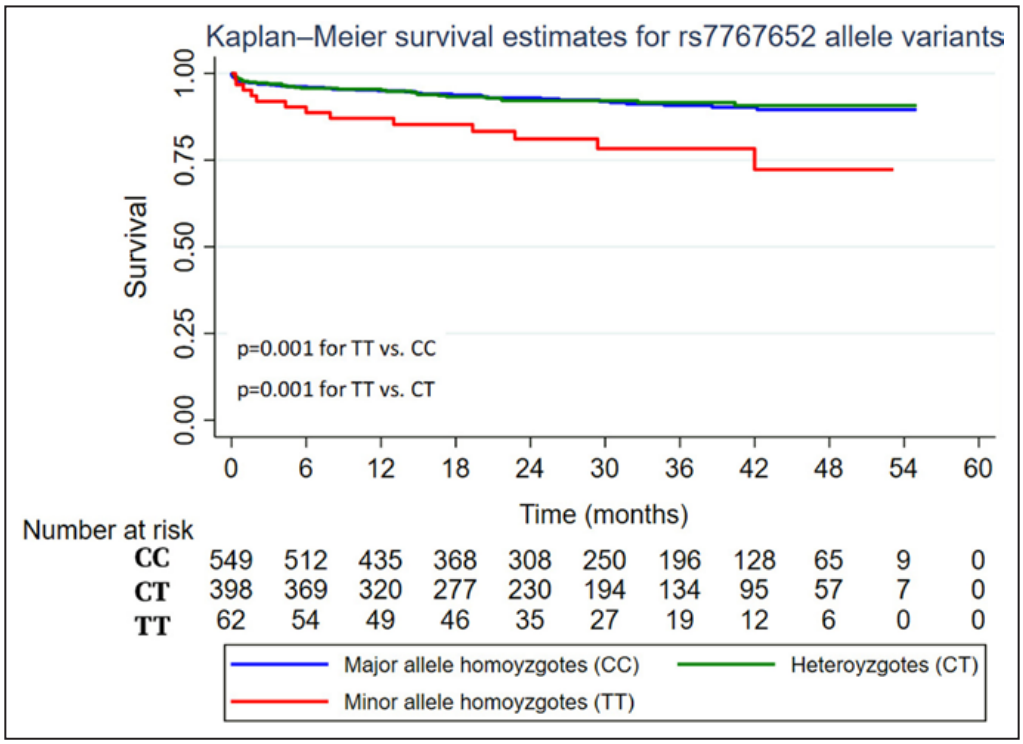

Figure 3. Kaplan-Meier survival estimates for rs7767652 allele variants.
Patients with the rs7767652 TT allele combination (red line) are at higher mortality risk as compared with those with the CC (blue line) and CT (green line) allele combination (both log-rank *P*<0.001).

Table 2. Factors Associated With Mortality Risk After Myocardial Infarction (N=1009)

Variable	HR (95% CI)	*P* value
Age	1.052 (1.027–1.78)	<0.001
CKD EPI	0.973 (0.962–0.984)	<0.001
Smoking	1.741 (1.080–2.807)	0.023
Left ventricular EF		0.016
EF <40% vs EF >50%	1.628 (0.977–2.714)	0.061
EF 40%–50% vs EF >50%	0.699 (0.378–1.294)	0.254
Glycemia	1.061 (1.016–1.108)	0.008
Killip class >I	2.551 (1.562–4.166)	<0.001
rs7767652 minor allele homozygote	2.833 (1.545–5.194)	0.001

CKD EPI indicates Chronic Kidney Disease Epidemiology Collaboration; EF, ejection fraction; and HR, hazard ratio.

Table 3. Factors Associated With Mortality Risk in Patients With Systolic Dysfunction at Hospital Discharge (n=245)

Variable	HR (95% CI)	*P* value
Age	1.029 (1.003–1.055)	0.030
CKD EPI	0.274 (0.140–0.535)	<0.001
Admission heart rate	1.012 (1.003–1.024)	0.013
Killip class >I	2.862 (1.710–4.792)	<0.001
Orexin ≥1.0 ng/mL	0.413 (0.186–0.914)	0.029

CKD EPI indicates Chronic Kidney Disease Epidemiology Collaboration; and HR, hazard ratio.

there was a difference in long-term mortality. The variety of mechanistic links connecting orexin signaling with increased mortality risk in our study may seem tricky at first. However, a multitude of downstream orexin signaling that involves SGK-1 (serum and glucocorticoid-regulated kinase-1)[26]; HIF-1 (hypoxia-inducible factor-1)[26]; phospholipase A2, C, and D; diacylglycerol lipase; Ca2+; and adenylyl cyclase cascades[23] may explain this variety of orexin signaling effects. In a recent study by Patel et al, orexin B but not orexin A had a direct cardioprotective effect in human heart samples that was mediated by the ERK1/2 (extracellular signal-regulated kinase 1 and 2) phosphorylation.[20] ERK1/2 phosphorylation is involved in the activation of contractile responses through direct phosphorylation of the Ca2+/calmodulin-dependent MLC (myosin light chain) kinase.[27]

Our findings have several clinical implications. Orexin receptor antagonists are widely used for insomnia treatment and in patients with MI. Whether their use may influence outcomes after MI needs to be examined. In addition, targeting the H/O system and increasing its activity by oral receptor antagonists, currently developed for treatment of narcolepsy, may be a novel therapeutic pathway to decrease the mortality risk and improve myocardial recovery after MI.

Study Limitations

Although this is an observational study, the use of a genetic instrumental variable with natural randomization of individuals under the Mendel law of segregation and independent assortment excludes the effect of confounding factors on our results. Although our

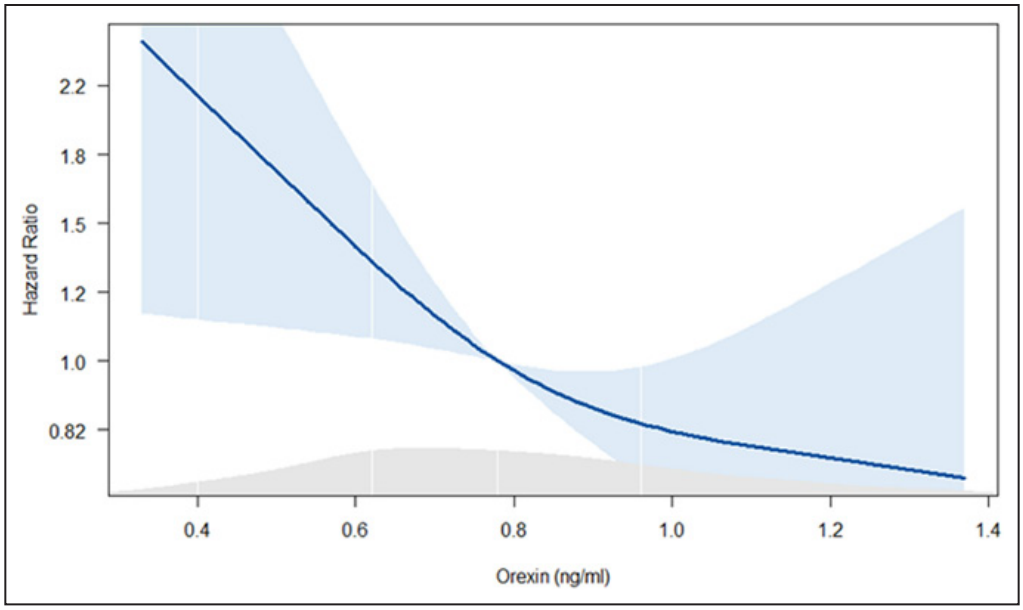

Figure 4. Circulating orexin A concentrations and mortality risk after myocardial infarction. The relationship between circulating orexin A concentration assessed at the time of myocardial infarction and total mortality hazard ratio. Data are adjusted for age. The gray area represents orexin A histogram, and the light blue area is the 95% CI.

study is relatively small by genetic standards and uses only a single nucleotide polymorphism, the effect of this polymorphism on survival is substantial, thus requiring a lower sample size. As a sensitivity analysis, we have confirmed the impact of the H/O system on survival using circulating orexin A concentrations. Our results are consistent with those observed in patients with HF.

CONCLUSIONS

The present study shows for the first time the effect of the attenuation of H/O signaling on increased mortality risk after myocardial infarction. Several mechanisms may mediate this association, of which the effect on left ventricular systolic function recovery and ventricular fibrillation risk seems promising. Future studies will have to address potential relevance of the H/O axis pharmacomodulation on post-MI remodeling and survival.

ARTICLE INFORMATION

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J.M., P.W., M.Š., M.Ž., and M.K. collected the clinical data. P.J. and D.D. analyzed blood specimens. P.W. and D.J. wrote the draft of the article. V.M., J.K., V.A., and J.P. critically revised the article. All authors read and approved the final version of the article.

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Disclosures

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Catheter ablation of atrial fibrillation and atrial tachycardia in patients with pulmonary hypertension: a randomized study

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CLINICAL RESEARCH

Catheter ablation of atrial fibrillation and atrial tachycardia in patients with pulmonary hypertension: a randomized study

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Aims Atrial fibrillation (AF), typical atrial flutter (AFL), and other atrial tachycardias (ATs) are common in patients with pulmonary hypertension. Frequently, several supraventricular arrhythmias are successively observed in individual patients. We investigated the hypothesis of whether more extensive radiofrequency catheter ablation of the bi-atrial arrhythmogenic substrate instead of clinical arrhythmia ablation alone results in superior clinical outcomes in patients with pulmonary arterial hypertension (PH) and supraventricular arrhythmias.

Methods and results Patients with combined post- and pre-capillary or isolated pre-capillary PH and supraventricular arrhythmia indicated to catheter ablation were enrolled in three centres and randomized 1:1 into two parallel treatment arms. Patients underwent either clinical arrhythmia ablation only (Limited ablation group) or clinical arrhythmia plus substrate-based ablation (Extended ablation group). The primary endpoint was arrhythmia recurrence >30 s without antiarrhythmic drugs after the 3-month blanking period. A total of 77 patients (mean age 67 ± 10 years; 41 males) were enrolled. The presumable clinical arrhythmia was AF in 38 and AT in 36 patients, including typical AFL in 23 patients. During the median follow-up period of 13 (interquartile range: 12; 19) months, the primary endpoint occurred in 15 patients (42%) vs. 17 patients (45%) in the Extended vs. Limited ablation group (hazard ratio: 0.97, 95% confidence interval: 0.49–2.0). There was no excess of procedural complications and clinical follow-up events including an all-cause death in the Extended ablation group.

Conclusion Extensive ablation, compared with a limited approach, was not beneficial in terms of arrhythmia recurrence in patients with AF/AT and PH.

Clinical Trials Registration ClinicalTrials.gov; NCT04053361.

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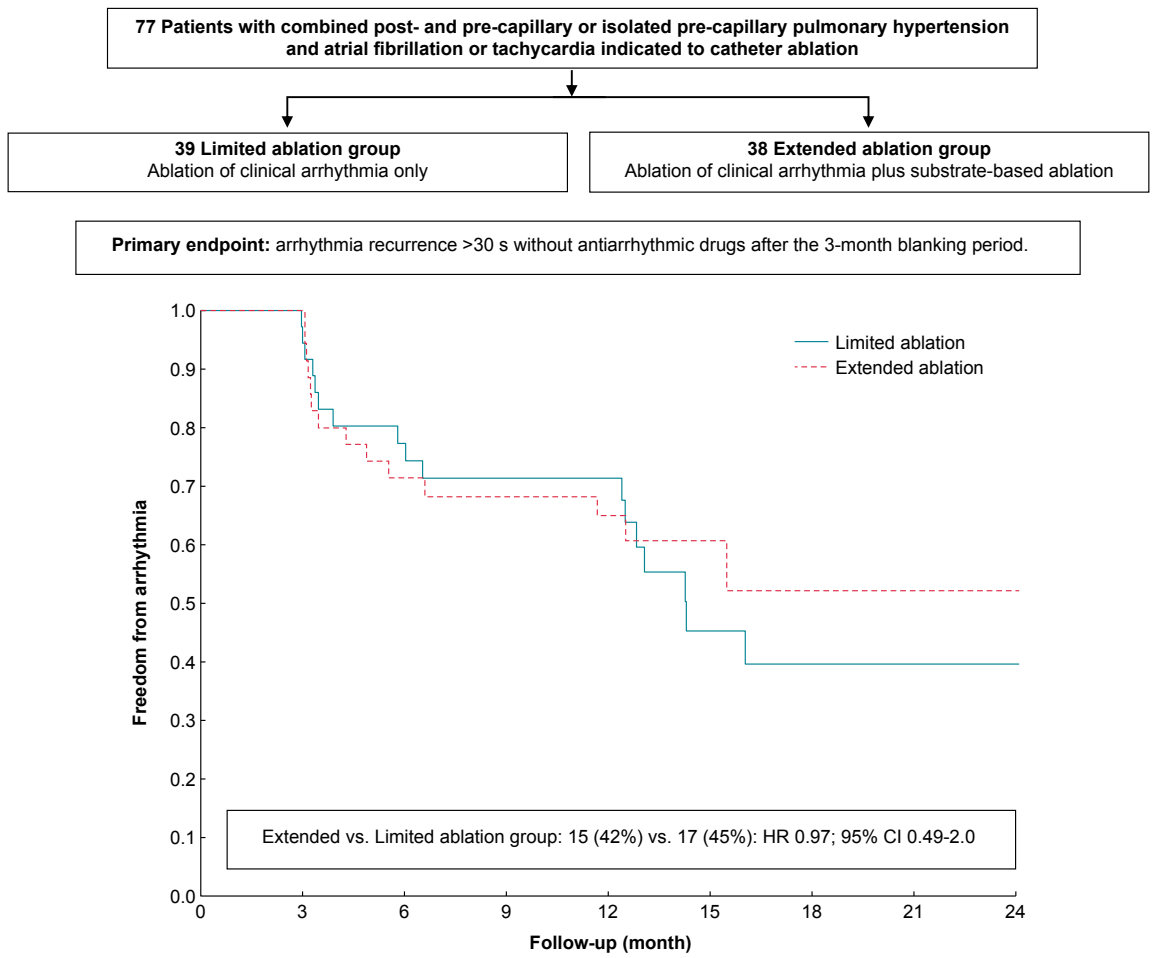
Graphical Abstract



Keywords Atrial fibrillation • Atrial tachycardia • Catheter ablation • Pulmonary hypertension

What's New?

- Extensive catheter ablation does not reduce arrhythmia recurrence in patients with pulmonary hypertension and atrial fibrillation/tachycardia.
- Despite enormous right atrial enlargement, regions with low-voltage and/or abnormal atrial electrograms are rare in patients with pulmonary hypertension.

Introduction

Various treatment strategies have been established in patients with pulmonary arterial hypertension (PH) that improve haemodynamics, exercise capacity, and quality of life.[1,2] Despite those advancements, PH is still a progressive disease with a generally inauspicious prognosis.

Supraventricular tachycardias (SVTs) have been frequently observed with a cumulative incidence of 10–29% in patients with both idiopathic[3] and secondary PH,[4–7] including chronic thromboembolic pulmonary hypertension, either inoperable[4,6] or treated with pulmonary endarterectomy.[8] The SVTs are associated with clinical deterioration and adversely impact the prognosis.[3,4,6,9] Conversely, maintenance of sinus rhythm (SR) appeared to improve the clinical outcome.[3,6,10] However, antiarrhythmic drugs may not be a feasible option because of their negative inotropic properties and interaction with specific therapy for PH.[11,12]

Radiofrequency catheter ablation (RFCA) of typical atrial flutter (AFL), atrioventricular nodal re-entrant tachycardia (AVNRT), and other focal or macroreentrant atrial tachycardias (AT) was reported to be effective (acute success rate of 86–100%) and safe in patients with PH according to retrospective studies with a limited number of patients.[5,10,13–16] However, the long-term results were much less favourable with freedom from arrhythmia in only 50–78% of patients.[13,14,16] Importantly, new-onset arrhythmias (different than their index SVT) were observed in 30–48% of recurrent cases.[13,14]

Although typical AFL can frequently be found as the first manifestation of SVT in patients with PH, atrial fibrillation (AF) is even more prevalent.[3–6] In this respect, data on the optimum rhythm control strategy of AF/AT, including RFCA, is lacking. Given the knowledge of the sequential manifestation of different SVTs in individual patients, it was plausible to hypothesize that first-line bi-atrial RFCA of all potentially arrhythmogenic substrates (i.e. not only ablation for index arrhythmia) could reduce the risk of arrhythmia recurrence and improve the clinical

outcomes compared to procedure targeting the clinical arrhythmia only. We investigated this hypothesis in a randomized fashion.

Methods

The study was a multicentre, parallel-group, open-label, randomized trial. It was performed according to good clinical practice and in compliance with the Helsinki declaration. The multicentric and local Ethics committees at all centres approved the study protocol. Individual written consent was obtained from each patient. The trial protocol is available in Supplementary material online, *Appendix S1*.

Patients

Participants were men or women, 18 years of age or older, who had pre-capillary or combined post- and pre-capillary PH of any aetiology, and documented symptomatic AF (paroxysmal, persistent, or long-standing persistent) or AT (typical AFL included) who were indicated for RFCA according to clinical practice guidelines.[17] Patients were excluded if they had any condition that might jeopardize patient safety or limit their participation in the study. The key exclusion criteria were complex congenital heart defects (corrected or uncorrected), isolated post-capillary PH, previous RFCA for AF, AT or AFL, NYHA Class IV, and life expectancy <1 year.

Study procedures and follow-up

Covariate adaptive 1:1 randomization was used to allocate enrolled patients into two parallel treatment arms to undergo clinical arrhythmia ablation only (Limited ablation group) or clinical arrhythmia plus substrate-based ablation (Extended ablation group). Covariates were applied as follows: age, gender, type of PH, and clinical arrhythmia.

Electrophysiological study

Patients were treated under conscious sedation or general anaesthesia at the discretion of the operator. The procedure was done on uninterrupted oral anticoagulation with the international normalized ratio between two and three in patients on vitamin K antagonists. In patients on direct oral anticoagulants, only the morning dose on the day of the procedure was omitted. All procedures were done under visual control of intracardiac echocardiography. Heparin was administered before transseptal puncture, and the doses were adjusted to achieve an activation clotting time of >300 s during the procedure.

In patients with SR at baseline, arrhythmia was induced by programmed, incremental, or burst atrial pacing. If present or induced arrhythmia differed from an arrhythmia that was documented non-invasively before the enrollment, the decision on what is 'clinical' arrhythmia was made by the operator. Point-by-point electroanatomical maps of both right (RA) and left (LA) atrium, each with a minimum of 100 mapping points, were acquired in consistent rhythm (SR/AF/AT) for meaningful assessment of low-voltage zones (CARTO 3, Biosense-Webster). Two-level quantification (bipolar voltages either <0.1 or <0.5 mV in SR; and either <0.04 or <0.2 mV in AF/AT) of low-voltage zones was applied separately for RA and LA.

Catheter ablation

Initial treatment was identical in both study arms. If clinical arrhythmia was fairly documented AF or typical AFL, pulmonary vein isolation (PVI) or cavotricuspid isthmus (CTI) ablation was performed. If AF persisted after PVI, electrical cardioversion was performed. When clinical arrhythmia was AT, it was induced (if not persistent), identified using activation and/or entrainment mapping, and ablated.

In patients in the Limited ablation group, no ablation was performed if AT was not inducible or if incidental (or induced) ATs were considered non-clinical. After clinical arrhythmia ablation, no induction protocols were attempted unless the non-inducibility was the principal endpoint of arrhythmia ablation, like in the case of AVNRT or microreentrant AT.

In patients in the Extended ablation group, substrate-based ablation continued after the initial ablation steps described above. This consisted of empirical lesion set within RA: superior vena cava (SVC) isolation, posteroseptal intercaval line, and CTI ablation (if not already done) and homogenization of low-voltage zones (if any) in LA/RA defined by bipolar voltage <0.5 mV in SR or <0.2 mV in AF/AT. These cut-off voltages were adapted (set lower) in severely diseased atria to identify reasonably smaller zones (<20% of the atrial surface) that were feasible to ablate. Arrhythmia induction protocol was performed consisting of 10-s burst atrial pacing with a cycle length of 300 ms decremented by 10 ms up to 1:1 atrial capture or cycle length of 200 ms. Induced ATs were mapped and ablated if feasible. In the case of inducible AF with a duration of >5 min, PVI was performed if not previously done as per protocol.

Follow-up and study objectives

During regular follow-up visits at 3-month intervals, symptoms and relevant clinical events were collected, and standard ECG was recorded. All class Ic or III antiarrhythmic drugs were discontinued at Month 3. Persistent arrhythmia (if observed at Month 3) was electrically cardioverted. Seven-day ECG monitoring was done 6 and 12 months after RFCA, and additional ECG monitoring was scheduled in patients with symptoms suggestive of non-documented arrhythmia. In case of arrhythmia recurrence, antiarrhythmic drugs were initiated and a repeated RFCA was considered.

The primary endpoint of the study was documented arrhythmia recurrence >30 s without antiarrhythmic drugs after the 3-month blanking period after the index ablation. Secondary endpoints were set up as follows: documented on-drugs arrhythmia recurrence, symptoms of arrhythmia, number of emergency visits, number of hospitalizations, mortality, procedure-related major complication rate, antiarrhythmic drugs, re-ablation, pacemaker implantation, and atrioventricular junction ablation. Major procedural complications were defined as events that occurred within 30 days of the ablation, were clearly or could probably be related to the procedure, and resulted in long-term disability, requiring intervention or prolonging hospitalization.

Statistical analysis

An independent statistician replicated and verified the analyses. All study objectives were analysed by standard statistical methods (*t*-test or Mann–Whitney U test for continuous variables or a Chi-square or two-tailed Fisher exact test for categorical variables). Time-to-event data were investigated by Kaplan–Meier analysis with log-rank statistics and by multivariate Cox regression models. A *P*-value <0.05 was considered significant. All analyses were performed using the STATISTICA vers.12 software (StatSoft, Inc., Tulsa, USA).

Results

From May 2018 to August 2021, a total of 77 patients (42 males) with a median age of 70 [interquartile range (IQR): 61; 75] years were enrolled at three sites in the Czech Republic. Thirty-nine patients were treated in the Limited ablation group, and 38 patients were treated in the Extended ablation group. At the time of randomization, the presumable clinical arrhythmia was AF in 38 and AT in 36 patients, including typical AFL in 23 patients (*Table 1*). One patient in the Limited ablation group (with left atrial appendage thrombosis) and two patients in the Extended ablation group (one with severe mitral regurgitation, and one who declined to participate) were later excluded (consort diagram, *Figure 1*). The baseline characteristics of the 74 patients who were scheduled for RFCA are shown in *Table 1*.

At the beginning of the index RFCA, arrhythmia different from that during the screening was seen in 5 of 38 and 8 of 36 patients from the Limited and Extended ablation groups, respectively. During the procedure, multiple distinct SVTs were observed in 5 and 4 patients from the Limited and Extended ablation groups, respectively.

In the Limited ablation arm, the RFCA procedure was completed per protocol in 36 (95%) out of the 38 patients. In one patient with enormous RA dilatation, transseptal puncture failed and CTI ablation only was performed. In another patient, extreme venous tortuosity prevented catheter insertion from the groin access. The RFCA was extended beyond assumed clinical arrhythmia in eight (21%) patients. This was done mainly because of conversion of the initial arrhythmia to a different one (three cases), spontaneous onset or induction of

Table 1 Baseline characteristics

	All patients $n = 74$	Limited ablation group $n = 38$	Extended ablation group $n = 36$	P
Age (years)	71 (61; 75)	70 (61; 75)	71 (60; 74)	NS
Males	41 (55%)	24 (63%)	17 (47%)	NS
Aetiology of PH				
– Idiopathic	41 (55%)	23 (61%)	18 (50%)	NS
– Chronic thromboembolic	22 (30%)	10 (26%)	12 (33%)	NS
– Lung disease/hypoxia	11 (15%)	5 (13%)	6 (17%)	NS
Index arrhythmia				
– Atrial fibrillation	38 (51%)	19 (50%)	19 (53%)	NS
– Paroxysmal	11 (15%)	6 (16%)	5 (14%)	NS
– Persistent	22 (30%)	10 (26%)	12 (33%)	NS
– Long-standing persistent	5 (7%)	3 (8%)	2 (6%)	NS
– Atrial tachycardia	36 (49%)	19 (50%)	17 (47%)	NS
– Typical atrial flutter	23 (31%)	12 (32%)	11 (31%)	NS
Symptoms of arrhythmia				
– Palpitation	28 (38%)	13 (34%)	15 (42%)	NS
– Dyspnea	47 (64%)	24 (63%)	23 (64%)	NS
– Peripheral oedema	26 (35%)	12 (32%)	14 (39%)	NS
Comorbidities				
– Arterial hypertension	59 (80%)	31 (82%)	28 (78%)	NS
– Diabetes mellitus	26 (35%)	12 (32%)	14 (39%)	NS
– Coronary artery disease	14 (19%)	5 (13%)	9 (25%)	NS
– Stroke/transient ischaemic attack	6 (8%)	4 (11%)	2 (6%)	NS
CHA_2DS_2-VASc score	3 (2; 4)	3 (2; 4)	3 (2; 4)	NS
HAS-BLED score	1 (0; 1)	1 (0; 1)	1 (0.5; 1)	NS
Treatment				
– Amiodarone	14 (19%)	8 (21%)	6 (17%)	NS
– Propafenone	3 (4%)	2 (5%)	1 (3%)	NS
– Sotalol	2 (3%)	1 (3%)	1 (3%)	NS
– Beta-blockers	27 (36%)	13 (34%)	14 (38%)	NS
– Warfarin	37 (50%)	18 (47%)	19 (53%)	NS
– Direct oral anticoagulants	31 (42%)	18 (47%)	13 (36%)	NS
– Specific therapy for PH	26 (35%)	14 (37%)	12 (33%)	NS
Functional status				
– NYHA I	0	0	0	NS
– NYHA II	17 (23%)	9 (24%)	8 (22%)	NS
– NYHA III	57 (77%)	29 (76%)	28 (78%)	NS
– NYHA IV	0	0	0	NS
– 6-minute walking test (m)	369 (280; 422)	363 (280; 413)	376 (300; 436)	NS
EQ-VAS	58 (40; 72)	56 (34; 74)	60 (42; 70)	NS
Laboratory				
– NT-proBNP (pg/mL)	1267 (732; 2317)	903 (724; 1979)	1587 (922; 3182)	NS
– Haemoglobin (g/L)	138 (128; 148)	145 (135; 148)	131 (117; 147)	NS
– Creatinine (μmol/L)	94 (80; 113)	95 (81; 112)	94 (73; 114)	NS
Echocardiography				
– LV end-diastolic diameter in PLAX (mm)	49 (44; 54)	49 (45; 54)	49 (44; 54)	NS
– LV ejection fraction (%)	60 (55; 63)	60 (55; 62)	60 (56; 64)	NS

Continued

Table 1 *Continued*

	All patients $n = 74$	Limited ablation group $n = 38$	Extended ablation group $n = 36$	P
– LA indexed volume (mL/m^2)	41 (31; 50)	39 (28; 51)	43 (32; 50)	NS
– RA diameter in A4C (mm)	53 (46; 59)	51 (46; 59)	54 (47; 59)	NS
– RV diameter in A4C (mm)	48 (41; 53)	49 (41; 52)	48 (42; 56)	NS
– Tricuspid annular plane systolic excursion (mm)	18 (14; 20)	17 (14; 20)	19 (14; 20)	NS
– Pulmonary artery systolic pressure (mmHg)	69 (50; 84)	72 (55; 87)	64 (48; 82)	NS
– LA appendage emptying velocity (m/s)	0.45 (0.34; 0.70)	0.49 (0.38; 0.70)	0.40 (0.30; 0.70)	NS
Haemodynamics				
– RA mean pressure (mmHg)	11 (6; 16)	13 (8; 18)	9 (5; 12)	0.02
– Pulmonary artery mean pressure (mmHg)	46 (38; 55)	47 (38; 54)	45 (36; 55)	NS
– Pulmonary capillary wedge pressure (mmHg)	11 (9; 15)	12 (10; 19)	11 (9; 13)	NS
– Cardiac index (L/min/m^2)	2.4 (2.0; 2.9)	2.35 (2.0; 2.8)	2.4 (2.0; 2.9)	NS

Data represent the number of cases (percentage) or median (interquartile range).
A4C, apical four-chamber view; EQ-VAS, European Quality of Life Group instrument self-report questionnaire visual analogue scale; NS, not significant; LA, left atrium; LV, left ventricle; PH, pulmonary hypertension; PLAX, parasternal long axis view; RA, right atrium; RV, right ventricle.

>1 arrhythmia during the procedure (two cases), and history of two clinically relevant arrhythmias in three cases, more details are in Supplementary material online, *Table S1*.

In the Extended ablation group, the RFCA procedure was completed per protocol in 33 (92%) out of 36 patients. Despite being assigned to extensive ablation, no RFCA was done in one patient without inducibility of any clinically relevant arrhythmia and lack of clear arrhythmogenic substrate. In two more patients, CTI block was not unequivocally demonstratable.

The RA lesions were significantly less frequently done in the Limited than in the Extended ablation group [22 (58%) vs. 33 (92%); $P < 0.001$]. The difference was mainly driven by the completion of per-protocol lesion set on top of CTI ablation (SVC isolation, posteroseptal intercaval line, and homogenization of low-voltage zones). On the other hand, the extent of LA lesions was comparable between the study groups. Electrical cardioversion for AF during the procedure was performed more often in the Limited than in the Extended ablation group [10 (26%) vs. 4 (11%) patients; $P = 0.04$]. Compared to patients in the Limited ablation group, procedural time and radiofrequency time were significantly prolonged in the Extended ablation group. The procedural details including performed lesions in both groups are provided in *Table 2* and Supplementary material online, *Table S1*.

The median duration of the follow-up period was 13 (IQR: 12; 18) months in the Limited ablation group and 14 (IQR: 12; 21) months in the Extended ablation group. The primary endpoint occurred comparably in 15 patients (42%) vs. 17 patients (45%) in the Extended vs. Limited ablation group [hazard ratio (HR): 0.97, 95% confidence interval (CI): 0.49–2.0], *Table 3*, *Figure 2*.

The secondary endpoints analysis is shown in *Table 3*. There were no other significant differences between the study groups except for the antiarrhythmic medication after the blanking period that was more frequently used in the Limited ablation group. There were 10 (28%) vs. 9 (24%) deaths in the Extended vs. Limited ablation group (HR: 0.92, 95% CI: 0.36–2.32). Corresponding Kaplan–Meier curves are presented in *Figure 3*.

Manifestation of new arrhythmia (different from all arrhythmias previously noticed) was seen in 9/31 patients with arrhythmia recurrence during the follow-up: 6 and 3 patients in Extended and Limited ablation groups, respectively. Typical AFL did not reoccur during the follow-up. Out of four patients with documented arrhythmia after CTI ablation, two had AF and two patients manifested atypical AFL (see Supplementary material online, *Table S2*).

Clear procedure-related complications were recognized in three patients: a prolonged severe vagal reaction during sheath removal at the end of the procedure with the necessity of short cardiopulmonary resuscitation, periprocedural progression of conservatively treated pericardial effusion, and surgically treated arteriovenous fistula. Three more adverse events could probably be related to RFCA. Of them, two patients manifested low cardiac output after RFCA, which led to prolonged hospitalization in one patient and slow progression to terminal heart failure and death in the second patient. One patient died suddenly (pulseless electrical activity) 1 day after the ablation of AVNRT without evidence of any periprocedural complication as assessed by autopsy. Hypoxia and end-stage heart failure were most likely responsible for this event. The other three patients manifested severe sinus bradycardia and sinus arrest episodes after the termination of persistent arrhythmia; however, ablation in these patients was not done in proximity to the sinus node. Major procedural complications and serious adverse events in the study are in more detail shown in Supplementary material online, *Table S3*.

Discussion

In this first multicentre randomized trial in a patient population with AF/AT and PH, extensive RFCA, compared with a limited approach, did not significantly reduce the recurrence of arrhythmia, symptoms, cardiovascular hospitalizations, and mortality.

Several retrospective studies with a limited number of patients have reported that RFCA of typical AFL or other less complex SVT was feasible, acutely effective, and safe in patients with PH.[5,6,10,13–16] Long-term clinical outcome after RFCA, however, was found to be less optimistic and more divergent. Bradfield *et al.*[13] reported that only 5 of 10 patients with acutely successful CTI ablation were completely arrhythmia-free at 3 months and three patients had recurrent arrhythmias different from their initial typical AFL. In 23 patients ablated for typical AFL and other organized SVTs, arrhythmia occurred in 12 patients during a 5-year follow-up, of whom the new onset of arrhythmia was seen in 10 cases.[14] On the contrary, a more favourable outcome was found in the recent retrospective study in 32 patients with successful ablation of typical AFL, who had a recurrence rate of ~20% during a follow-up of 3–108 months. However, the proportion of recurrence of index arrhythmia and onset of new arrhythmias was not provided.[16]

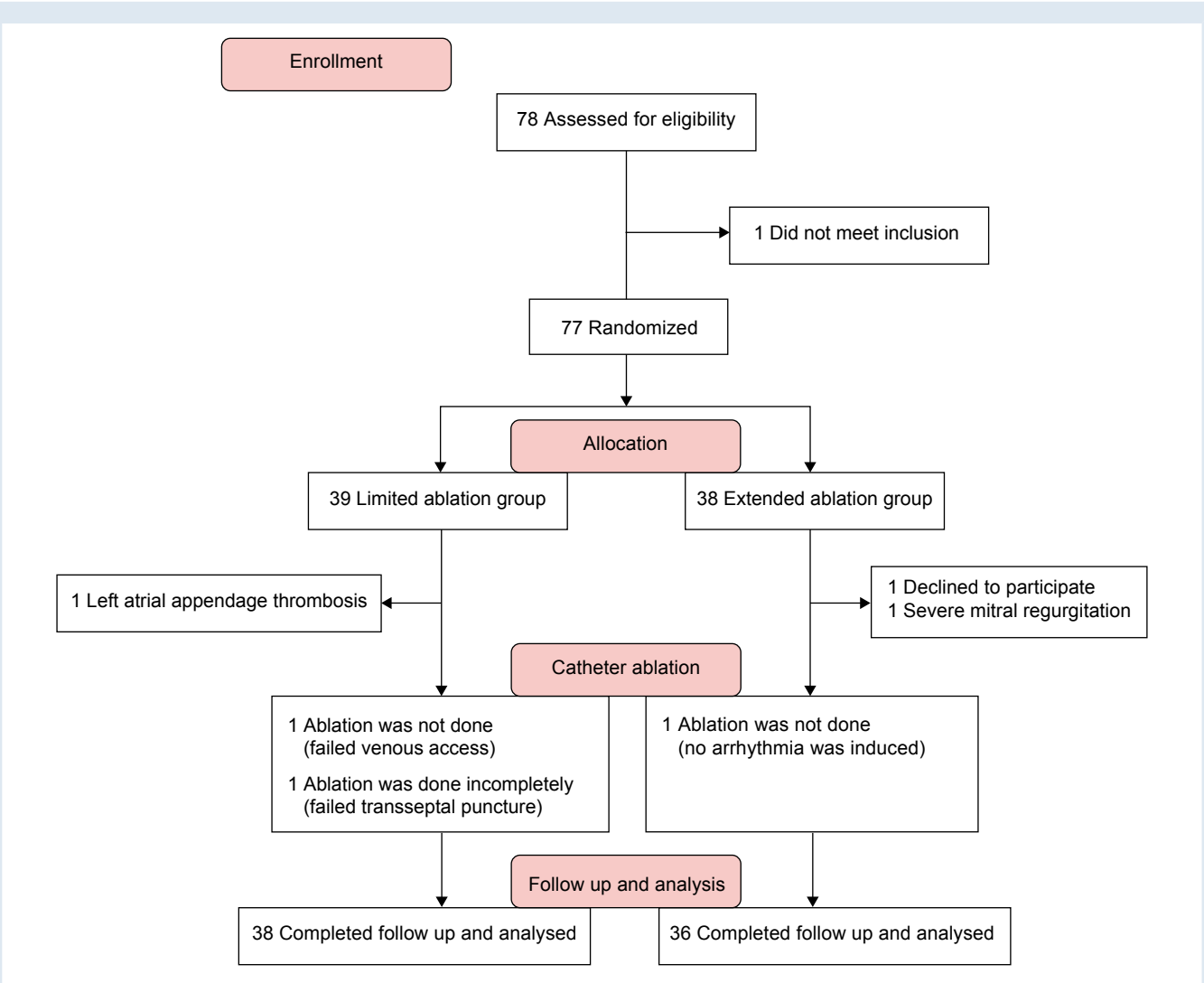


Figure 1 CONSORT flow diagram.

In our study, we prospectively evaluated the clinical outcome of two ablation strategies in PH patients with various types of supraventricular arrhythmia. The extensive ablation was intended to reduce a long-term arrhythmia recurrence rate by ablation of all inducible arrhythmias, including those that did not manifest clinically prior to the procedure, and by preventative modification of arrhythmogenic substrate for potentially new and currently non-inducible arrhythmias. Unlike previous studies, we also enrolled patients with AF.

The relatively high arrhythmia recurrence rate after the RFCA for AF/AT in patients with PH in both study arms was comparable to that in non-paroxysmal AF in non-PH patients with a low prevalence of structural heart disease.[18–20] A recent meta-analysis reported a pooled median success rate of 66.7% (95% CI 60.8–72.2%) after the single RFCA for non-paroxysmal AF.[18] Beyond PVI, a range of trigger and left atrial substrate modification ablation strategies have been proposed to improve success in non-paroxysmal AF. However, the randomized controlled trial STAR AF II indicated that adjunctive RF ablation strategies did not improve outcomes over PVI alone but were associated with higher fluoroscopy and procedure times.[19] Our study investigated different population of patients with highly suspected right over left atrial arrhythmogenic substrate because of right-sided pressure and volume overload, so tailored targeting of right atrial arrhythmogenic substrate seemed justified. Our study also included patients with paroxysmal AF (30% of all AF cases) and patients with AT including typical AFL.

No recurrence of typical AFL was observed in our cohort. This finding is far more favourable than previously reported long-term data.[13,14] We speculate that RFCA with 3D-electroanatomical mapping and direct visual control using intracardiac echocardiography could be responsible for such an outcome. Significant elimination of triggers (PVI in 62% of patients) may also play a role. The data overall indicate that CTI ablation in PH could be effective in patients with documented or highly suspected typical AFL. However, the reoccurrence of different arrhythmias in AFL patients in both study arms was noticed during follow-up, which is in concordance with previous results.[13,14]

Apart from the well-known reasons for the failure of additional substrate ablation in the general AF population, several other explanations for what is behind the lack of benefit from an extensive ablation in this trial can be offered. Although enlargement of RA, conduction slowing, reduced tissue voltage, and regions of electrical silence in RA were described in patients with PH,[21,22] we were not able, however, to detect a significant prevalence and extent of regions with low-voltage and/or abnormal atrial electrograms in our population despite expectedly dilated RA. We cannot exclude that our bipolar cut-off voltage for low-voltage zones was not sensitive and specific enough to identify RA arrhythmogenic substrate. We can

Table 2 Procedural characteristics

	All patients $n = 74$	Limited ablation group $n = 38$	Extended ablation group $n = 36$	*P*
Clinical arrhythmia present at baseline	28 (38%)	17 (45%)	11 (31%)	NS
SR present at baseline, clinical arrhythmia inducible	19 (26%)	11 (29%)	8 (22%)	NS
SR present at baseline, clinical arrhythmia non-inducible or not induced	14 (19%)	5 (13%)	9 (25%)	NS
Other than clinical arrhythmia present/induced at baseline	9 (12%)/4 (5%)	3 (8%)/2 (5%)	6 (17%)/2 (6%)	NS
>1 arrhythmia in the history	10 (14%)	6 (16%)	4 (11%)	NS
>1 arrhythmia during the procedure	9 (12%)	5 (13%)	4 (11%)	NS
RA mapping time (min)	18 (13; 24)	20 (13; 27)	16 (13; 22)	NS
LA mapping time (min)	18 (13; 23)	18 (13; 24)	17 (12; 22)	NS
Total procedure time (min)	173 (135; 210)	155 (130; 180)	205 (150; 225)	0.004
General anaesthesia	9 (12%)	4 (11%)	5 (14%)	NS
Fluoroscopy time (min)	2.4 (1.4; 5.1)	2.2 (1.4; 5)	3.3 (1.7; 7.3)	NS
Radiofrequency time (min)	39 (24; 56)	26 (14; 42)	49 (32; 65)	<0.0001
CARTO RA volume (mL)	196 (159; 250)	206 (155; 260)	191 (162; 249)	NS
CARTO LA volume (mL)	122 (99; 143)	122 (104; 142)	116 (62; 153)	NS
CARTO RA surface (cm^2)	198 (172; 228)	201 (173; 230)	182 (171; 213)	NS
CARTO LA surface (cm^2)	135 (120; 154)	137 (125; 156)	134 (119; 153)	NS
RA LVAs (% of the surface)	5 (1;12)	4 (1; 11)	5 (2; 13)	NS
LA LVAs (% of the surface)	2 (0; 10)	4 (1; 11)	2 (0; 22)	NS
Acute success of ablation	69 (93%)	36 (95%)	33 (92%)	NS
Ablation not done	3 (4%)	2 (5%)	1 (3%)	NS
Procedural ECV	16 (22%)	11 (29%)	5 (13%)	NS
Procedural ECV in AF patients	14/38 (37%)	10/19 (53%)	4/19 (21%)	0.04
LA ablation	48 (65%)	21 (55%)	27 (75%)	NS
– PVI alone	24 (32%)	10 (26%)	14 (39%)	NS
– PVI + additional lesions	22 (30%)	9 (24%)	13 (36%)	NS
– LA ablation without PVI	2 (3%)	2 (5%)	0 (0%)	NS
– LA foci	5 (7%)	3 (8%)	2 (3%)	NS
– CFAE	8 (11%)	3 (8%)	5 (14%)	NS
– LVAs	14 (19%)	4 (11%)	10 (28%)	NS
– CS	7 (9%)	4 (11%)	3 (8%)	NS
– Linear lesions	16 (%)	6 (16%)	10 (28%)	NS
RA ablation	55 (74%)	22 (58%)	33 (92%)	0.0009
– CTI alone	17 (23%)	14 (37%)	3 (8%)	0.004
– CTI + additional lesions	31 (42%)	2 (5%)	29 (81%)	<0.0001
– RA ablation without CTI	7 (9%)	6 (16%)	1 (3%)	NS
– SVC isolation	27 (36%)	1 (3%)	26 (72%)	<0.0001
– CFAE/LVA	14 (19%)	1 (3%)	13 (36%)	0.0002
– Intercaval line	26 (35%)	1 (3%)	25 (69%)	<0.0001
– RA/CS focal activity	4 (5%)	2 (5%)	2 (6%)	NS
– AVN slow pathway	3 (4%)	3 (8%)	0 (0%)	NS

Data represent the number of cases (percentage) or median (interquartile range).
AF, atrial fibrillation; AVN, atrioventricular node; CFAE, complex fragmented atrial electrograms; CS, coronary sinus; CTI, cavotricuspid isthmus; ECV, electrical cardioversion; LA, left atrium; LVA, low voltage area; NS, not significant; PVI, pulmonary vein isolation; RA, right atrium; SR, sinus rhythm; SVC—superior vena cava.

also speculate that elevated right-sided filling pressure with associated RA hypertrophy could mask the voltage-attenuation effects of spontaneous atrial scarring and dilatation. When abnormal myocardium could not be found, mainly empirical lesions (i.e. CTI block, SVC isolation, or intercaval line) constituted an extension of ablation, and such lesions alone might not be the most efficacious ablation targets in PH patients. We cannot also exclude the possibility that our strategy of extended ablation did not target sufficiently the uncommon type of ATs involving both atria and inter-atrial

Table 3 Study endpoints

	Limited ablation group $n = 38$	Extended ablation group $n = 36$	*P*
Primary endpoint			
– Documented arrhythmia recurrence >30 s without antiarrhythmic drugs after the 3-month blanking period	17 (45%)	15 (42%)	NS
Secondary endpoints			
– Documented on-drug arrhythmia recurrence	10 (26%)	7 (19%)	NS
– Symptoms of arrhythmia	13 (34%)	10 (28%)	NS
– Patients with emergency visits/number of emergency visits per patient	11 (29%)/2 (1; 3)	9 (25%)/2 (1; 2)	NS/NS
– Patients with hospitalization/number of hospitalizations per patient	14 (37%)/1 (1; 2)	13 (36%)/2 (1; 2)	NS/NS
– Patients with cardiovascular emergency visits or hospitalization/number of events per patient	13 (24%)/1 (1; 3)	11 (31%)/1 (1; 2)	NS/NS
– Mortality	9 (24%)	10 (28%)	NS
– Antiarrhythmic drugs (post-blanking period)	16 (42%)	7 (19%)	0.046
– Antiarrhythmic drugs (at the end of follow-up)	11 (29%)	7 (19%)	NS
– Reablation rate	5 (13%)	3 (8%)	NS
– Pacemaker implantation	3 (8%)	1 (3%)	NS
– AV junction ablation	0	1 (3%)	NS
Other objectives (12-month visit—baseline difference)			
– 6-minute walking test (m)	−10 (−27; 55)	9 (−28; 163)	NS
– EQ-VAS	−4 (−12; 14)	0 (−18; 22)	NS
– NT-proBNP (pg/mL)	239 (−312; 1120)	98 (−512; 695)	NS
Major procedural complications	5 (13%)	4 (11%)	NS

Data represent the number of cases (percentage) or median (interquartile range).
Details on major procedural complications are provided in Supplementary material online, *Table S3*.
EQ-VAS, European Quality of Life Group instrument self-report questionnaire visual analogue scale; NS, not significant.

connections.[23] Abnormal modulation of the intrinsic cardiac autonomic system has been identified as an arrhythmogenic mechanism in patients with PH.[24,25] The arrhythmia sources because of this mechanism are difficult to identify and modify by conventional ablation strategies. The high recurrence rate of arrhythmia in combination with PH, as a severely limiting underlying condition, was likely responsible for the absence of improvement in quality of life, functional capacity, and natriuretic peptides.

The results may be also biased by post-randomization deviations from protocol-specified care that could attenuate the difference in clinical outcome between study arms. For example, ablation on top of limited selective RFCA was done in eight (21%) patients in the Limited ablation group with more than one documented type of arrhythmias or when other arrhythmias were seen during the index procedure. This was done at the investigator's discretion if believed to be beneficial for the subject's welfare. Similarly, the investigators tended to perform more complex LA ablation in non-paroxysmal AF irrespective of the study treatment allocation, which finally resulted in a small difference in the LA lesion set between study arms. On the contrary, in the Extended ablation group, the lesion set was not completed in several patients. In one case with AT, ablation was not done when no arrhythmia was induced. Moreover, in two patients with AFL and two patients with AF, the full lesion set in RA was not completed mainly because of a prolonged and poorly tolerated procedure in combination with extreme enlargement of the right atrium preventing successful ablation.

It has been shown that the use of general anaesthesia could increase the single procedure success rate of RFCA of complex atrial arrhythmia, and shorten fluoroscopy and procedural time without increasing procedural complications.[26] However, the concern about severe complications related to general anaesthesia in PH patients exists. The PH is a serious condition, and the induction of general anaesthesia can incur additional sudden haemodynamic stress.[27] It is also known that patients with severe PH have increased rates of delayed extubating, heart failure, and mortality after non-cardiac surgery.[28] On the other hand, conscious sedation may result in inadvertent hypoventilation episodes with their consequences. Operators preferred to use conscious sedation, which is a common way of performing RFCA even for complex arrhythmias in our country.

Importantly, a considerable number of adverse events were recorded during the follow-up. There was no excess of clinical events including all-cause death in the Extended ablation group, and only a few events were directly procedure-related while all others could be considered the natural course of the underlying disease. Therefore, RFCA appeared safe even in the population of frailty PH patients when performed by experienced operators.

The left atrial stiff syndrome is a plausible long-term side effect of extensive complex RFCA in LA resulting in pulmonary venous hypertension[29,30] that can aggravate PH. The potentially higher risk of left atrial stiff syndrome with its consequences in PH patients is one of the arguments against routine extensive ablation in the left atrium in that population. This risk was not, however, investigated in our study.

Limitations

The study has several limitations. First, the patient population was heterogeneous in terms of the type and aetiology of PH. Second, high-

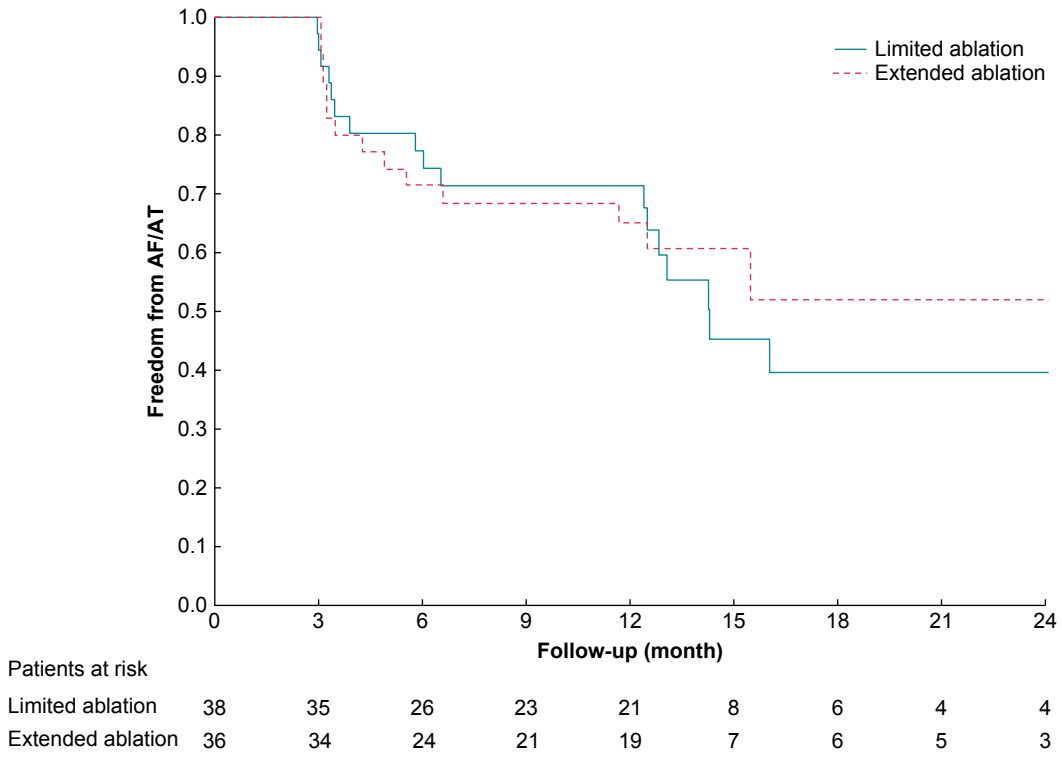


Figure 2 Event-free survival for arrhythmia recurrence (primary endpoint). Kaplan–Meier curves: solid blue for the Limited ablation group; dashed red for the Extended ablation group.

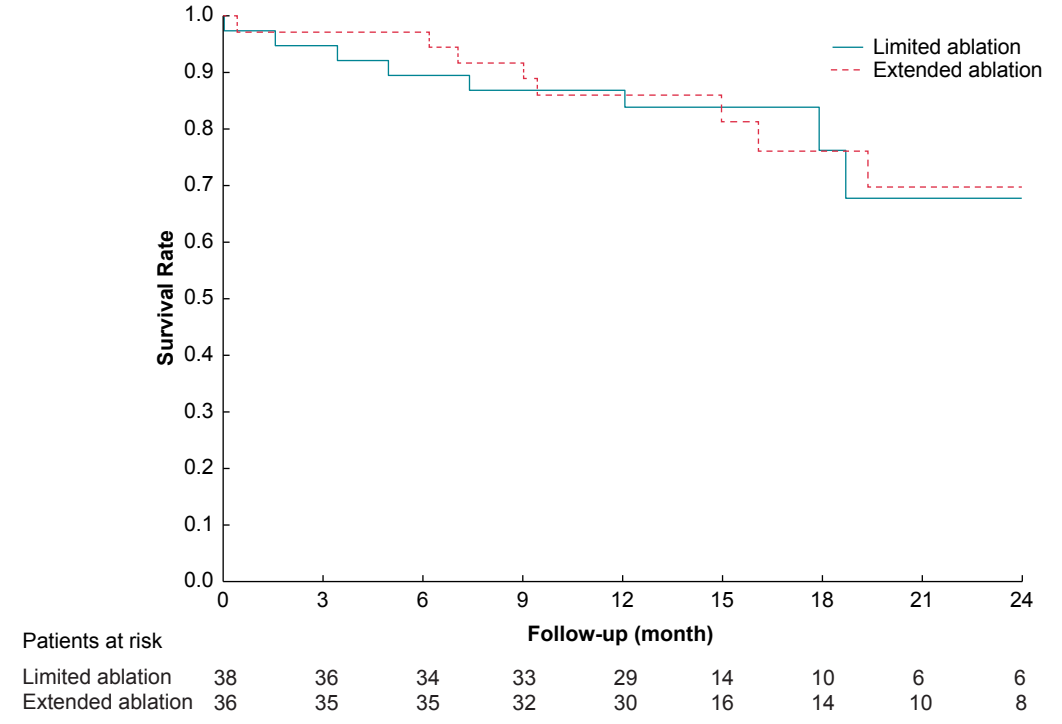


Figure 3 Event-free survival for all-cause mortality. Kaplan–Meier curves: solid blue for the Limited ablation group; dashed red for the Extended ablation group.

density mapping was not used to identify an arrhythmogenic substrate. Third, operators tended to deviate from the protocol (by performing more than simply PVI) in patients with persistent AF who were randomized to a limited ablation strategy. Fourth, pulsed electrical field ablation technology was not available during the study enrollment period. Fifth, the arrhythmia burden that would be a better procedural endpoint than the first arrhythmia recurrence was not assessed.

Conclusions

Extensive RFCA, compared with a limited approach, was not beneficial in terms of arrhythmia recurrence in patients with AF/AT and PH. The absence of clear advance in the context of the prolonged procedural time in the PH population warrants the conclusion that performing additional, and perhaps unnecessary, ablation lesions should be generally avoided.

Supplementary material

Supplementary material is available at *Europace* online.

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Conflict of interest: None declared.

Data availability

All relevant data are in the manuscript. Relevant dataset is available on request.

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Right ventricular global dysfunction score: a new concept of right ventricular function assessment in patients with heart failure with reduced ejection fraction (HFrEF)

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Right ventricular global dysfunction score: a new concept of right ventricular function assessment in patients with heart failure with reduced ejection fraction (HFrEF)

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Background: Right ventricular (RV) function is currently being evaluated solely according to the properties of RV myocardium. We have tested a concept that in patients with heart failure with reduced ejection fraction (HFrEF), RV assessment should integrate the information about both RV function as well as size.
Methods: A total of 836 stable patients with HFrEF (LVEF 23.6 ± 5.8%, 82.8% males, 68% NYHA III/IV) underwent echocardiographic evaluation and were prospectively followed for a median of 3.07 (IQRs 1.11; 4.89) years for the occurrence of death, urgent heart transplantation or implantation of mechanical circulatory support.
Results: RV size (measured as RV-basal diameter, RVD_1) was significantly associated with an adverse outcome independent of RV dysfunction grade ($p = 0.0002$). The prognostic power of RVD_1 was further improved by indexing to body surface area (RVD_1i, $p < 0.05$ compared to non-indexed value). A novel parameter named RV global dysfunction score (RVGDs) was calculated as a product of RVD_1i and the degree of RV dysfunction (1–4 for preserved RV function, mild, moderate and severe dysfunction, respectively). RVGDs showed a superior prognostic role compared to RV dysfunction grade alone (ΔAUC >0.03, $p < 0.0001$). In every subgroup of RVGDs (<20, 20–40, 40–60, >60), patients with milder degree of RV dysfunction but more dilated RV had similar outcome as those with more severe degree of RV dysfunction but smaller RV size (all $p > 0.50$), independent of tricuspid regurgitation severity and degree of pulmonary hypertension.
Conclusion: RV dilatation is a manifestation of RV dysfunction. The evaluation of RV performance should integrate the information about both RV size and function.

KEYWORDS

right ventricular function assessment, right ventricular size, right ventricular dysfunction, heart failure, outcome

Introduction

Echocardiographic evaluation of right ventricular (RV) function is complicated due to its complex geometry. Nevertheless, a correct RV function assessment is crucial as RV dysfunction is associated with an adverse outcome in multiple pathologic conditions including pulmonary artery hypertension and heart failure (1–4). RV function plays an

especially important role in patients undergoing LV-mechanical circulatory support implantation (5).

Traditional parameters for RV function assessment (TAPSE, Sm-TDI, fractional area change—FAC) are currently being replaced by more sophisticated measures (RV strain) (6, 7). However, all these parameters focus solely on the properties of RV myocardium, but RV size as well may have prognostic value in HFrEF patients (8). As both the size and the degree of RV dysfunction are related to the prognosis of HF patients, we propose that RV dilatation should be viewed as a manifestation of RV dysfunction.

The goal of the study was to test a novel concept that the information about RV size and function should be integrated into one parameter that would offer more accurate information about RV disease.

Methods

Study subjects

This is a retrospective analysis of prospectively enrolled patients; subjects with stable HFrEF (LVEF <40%) of at least 6 months duration (i.e., signs or symptoms of HF and LVEF <40% at least 6 months before enrollment and ongoing signs/symptoms of HF and ongoing LVEF < 40% at the time of enrollment) were enrolled in the study between 2008 and 2016 and prospectively followed. In all subjects, LVEF was assessed by echocardiography. Patients had to be on stable medical therapy for at least three months. Those with potentially reversible LV dysfunction (planned valve surgery, revascularization, or tachycardia-induced cardiomyopathy) were excluded. Patients were followed until July 2019.

Echocardiography and blood sample testing were performed upon enrollment. The protocol was approved by the Institutional Ethics Committee, and all subjects signed an informed consent. Patients were prospectively followed and the adverse outcome was defined as the combined endpoint of death, urgent heart transplantation, or ventricular assist device implantation. Due to the fact that time to non-urgent transplantation reflects donor availability rather than recipient's condition, patients who received a non-urgent heart transplant were censored as having no outcome event at the day of transplantation, as previously reported (9).

Echocardiography

Left ventricular size was measured in parasternal long axis (PLAX) as end-diastolic diameter, LV ejection fraction was assessed by the Simpson method (10). Right ventricular size was measured in apical 4-chamber view (A4C) as RV-basal diameter (RVD_1) (11). All sonographers were (as per institutional protocol) instructed to obtain the A4C projection with interventricular and interatrial septum perpendicular to the probe and to obtain the image of the "heart cross" with best available quality (**Figure 1**). RV dilatation was formally defined as RVD_1 > 42 mm, but in the analysis it was used as a continuous variable.

Right ventricular dysfunction was quantified semiquantitatively (preserved RV function, mild, moderate and severe RV dysfunction). The assessment of RV function was performed in an apical 4-chamber view by using tricuspid annular systolic excursion (M-mode TAPSE) (12) and tissue systolic velocity (Sm) (13) with the following cutoffs: normal RV function: TAPSE >20 mm, Sm >12 cm/s; mild RV dysfunction: TAPSE 16–20 mm, Sm 9–12 cm/s; moderate RV dysfunction: TAPSE 10–15 mm, Sm 6–9 cm/s; severe RV dysfunction: TAPSE <10 mm, Sm <6 cm/s. In case of disagreement between TAPSE and Sm, qualitative visual estimation of RV motion in apical 4-chamber was also taken into account. Similarly, in patients with the history of pericardial opening and decreased parameters reflecting longitudinal RV function, RV radial contraction was incorporated into the RV function assessment as well. Mitral and tricuspid regurgitation

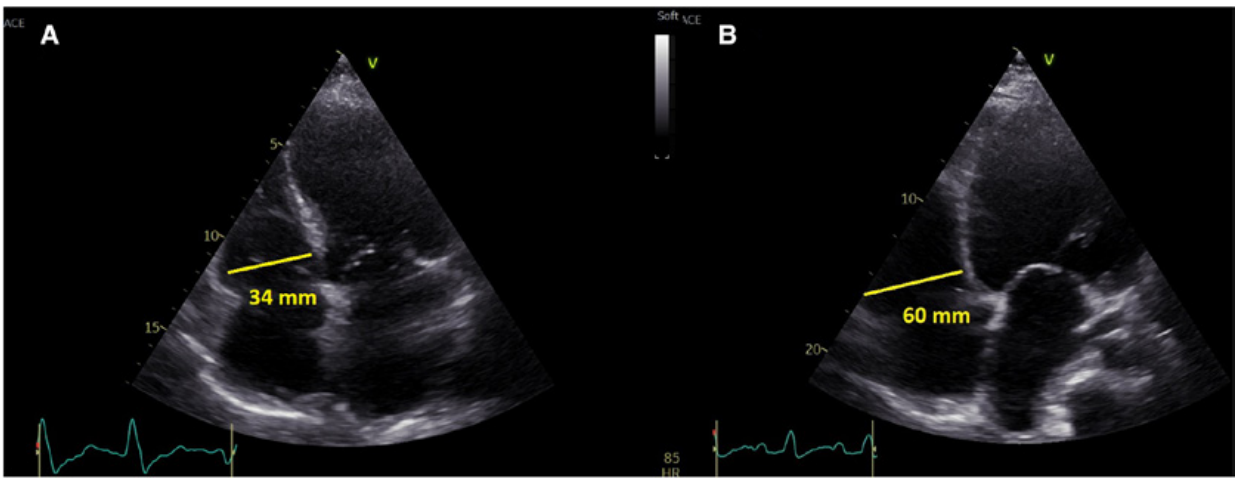

FIGURE 1
A representative presentation of non-dilated right ventricle (left) and dilated right ventricle (right).

severity was assessed semi-quantitatively in three degrees (mild-moderate-significant) (14). Vivid-7 and Vivid-9 (General Electric, Milwaukee, Wisconsin) were used for echocardiographic study. Indexation of RVD_1 was performed using body surface area (BSA) that was calculated as BSA = 0.007184. weight (kg)^(0.425). height(m)^(0.725).

Nuclear scintigraphy

RV end-systolic and end-diastolic volumes (and subsequently RV ejection fraction) was assessed using electrocardiogram-gated three-dimensional equilibrium Tc-labeled blood pool single photon emission computed tomography (SPECT). Patients received an injection of stannous pyrophosphate (Technescan PYP, Curium, the Netherlands) and 30 min later erythrocytes were *in vivo* labeled by intravenous injection of 740 MBq 99 mTc isotope. The heart chambers were imaged using a D-SPECT camera (Spectrum Dynamics, Israel) equipped with collimated, pixilated cadmium zinc telluride crystals detectors allowing rapid (7 min) data acquisition with superior spatial resolution. RV end-systolic and end-diastolic volumes were measured from three-dimensional reconstructed chambers using semiautomatic plug-in software (QBS Cedars-Sinai, Los Angeles, CA) by a single experienced physician.

Statistical analysis

Data are presented as mean ± standard deviation, median with interquartile ranges (IQRs), or frequency (percent). Unpaired *t*-test or Mann–Whitney test were used to compare continuous variables between groups as appropriate. Cox univariate and multivariable models were used to test the effect of analyzed variables on prognosis. Event-free survival of patients was analyzed by Kaplan–Meier analysis with log-rank test comparison between groups. Calculations were performed using JMP 11 (SAS Institute Inc., Cary, NC) and R (Vienna, Austria). Regression package for R (version 2020.12.08) was used to compare the area under the curve (AUC) of the Cox Proportional Hazard Regression models at four different time points.

Results

Patients

A group of 836 patients with HFrEF were enrolled in the study. Over the follow up of 3.07 (IQRs 1.11; 4.89) years 508 patients (60.8%) experienced an adverse outcome (death, urgent heart transplantation, MCS implantation). Furthermore, 35 patients (4.2%) underwent HTx as non-urgent recipients. Patients achieved a high degree of guideline-directed pharmacotherapy and device therapy—78.5% had ACEi/ARB, 87.4% beta-blockers, 76.7% mineralocorticoid receptor antagonist, 57.0% ICD, **Table 1**.

RV size and function

A total of 271 patients (32.4%) had preserved RV function, 190, 280 and 95 patients (22.7%, 33.5% and 11.4%) had mild, moderate and severe RV dysfunction, respectively.

RV dysfunction grade was associated with progressively deteriorating outcome ($p < 0.001$, **Supplementary Figure S1**). Compared to patients with preserved RV function, those with mild, moderate and severe RV dysfunction had 2-fold, 3-fold a 4.4-fold increased likelihood of an adverse outcome (HR 2.00 95% CIs 1.54–2.60 for mild RV dysfunction, HR 2.99 95% CI, 2.37–3.80 for moderate RV dysfunction and HR 4.42 95% CI, 3.29–5.91 for severe RV dysfunction, respectively, $p < 0.0001$). Similarly to RV dysfunction, RV size was also found to be significantly associated with adverse outcome (HR 1.02 95%CI, 1.01–1.04, $p = 0.0002$ after the adjustment for RV dysfunction grade). We have further analyzed whether an indexation of RV size brings any improvement in outcome prediction; absolute RV size was compared with RV size indexed to body surface area (RVD_1i) that showed significantly higher area under the curve (AUC), **Table 1**.

Moreover, RVD_1 was found to be independently associated with prognosis after the adjustment for TAPSE/PASP ratio (surrogate of RV-PA coupling), HR 1.02, 95% CI (1.006; 1.04), $p = 0.009$. RVD_1 showed a loose but significant correlation with TAPSE/PASP ratio ($r^2 = 0.14$, $p < 0.0001$); this correlation was tighter ($r^2 = 0.16$, $p < 0.0001$) in patients without the history of pericardial opening ($n = 632$) and looser ($r^2 = 0.06$, $p = 0.02$) in patients with the history of pericardial opening ($n = 202$).

Intraobserver and interobserver variability testing

Intraoberver and interobserver variability was tested on the sample of 25 patients. Because of the restrospective nature of the study, saved loops were used for the analysis. Inraobserver variability for RVD_1 was high ($r^2 = 0.97$, average difference 0.92 mm, SD 0.86, **Supplementary Figure S2A**), RV function was categorized the same in 24 cases (in one case it RV function was assessed to have a moderate dysfunction in one case and mild dysfunction in the second assessment). Interobserver variability (assessed by independent experienced sonographer) was acceptable as well, r^2 for RVD_1 was 0.93 (average difference 1.52 mm, SD 0.82 mm, **Supplementary Figure S2B**), RV function was assessed in the same category in 22 cases (in all three cases where the disagreement was observed in the second evaluation RV function was assessed in the adjacent category—mild dysfunction vs. normal function, moderate dysfunction vs. mild dysfunction, and moderate dysfunction vs. severe dysfunction).

Comparison of RV assessment by echocardiography and nuclear imaging

As echocardiography is a suboptimal method for evaluating RV size and function, we have performed a validation substudy; a

TABLE 1 Patients characteristics.

	Whole cohort ($n = 836$)	RVGDs <20 ($n = 204$)	RVGDs 20–40 ($n = 172$)	RVGDs 40–60 ($n = 183$)	RVGDs >60 ($n = 277$)	p for trend
Age (years)	57.34 ± 11.28	58.19 ± 11.30	58.15 ± 10.85	57.60 ± 11.17	55.92 ± 11.57	**0.02**
Males (%)	82.8	76.0	82.0	85.3	86.6	**0.002**
HF etiology (% ischemic)	49.9	48.3	45.6	47.5	56.1	0.06
BMI ($kg.m^{-2}$)	27.82 ± 5.09	29.45 ± 4.92	28.32 ± 5.20	27.60 ± 4.66	26.49 ± 5.11	**<0.0001**
NYHA (2–4, %)	32.1/60.5/7.5	48.5/49.0/2.5	39.0/56.4/4.7	25.1/65.6/9.3	20.6/67.5/11.1	**<0.0001**
Na ($mmol.L^{-1}$)	138.53 ± 3.58	138.81 ± 3.02	139.65 ± 3.29	138.55 ± 3.84	137.57 ± 3.73	**<0.0001**
BNP ($ng.L^{-1}$)	464 (207; 1076)	167 (89; 333.2)	329 (171; 686)	691 (343; 1213)	990 (567; 1720)	**<0.0001**
SBP (mmHg)	116.30 ± 19.10	124.39 ± 18.68	120.82 ± 19.93	112.4 ± 18.24	109.88 ± 16.38	**<0.0001**
Hemoglobin ($g.L^{-1}$)	140.85 ± 18.19	139.63 ± 15.85	140.22 ± 17.49	141.10 ± 18.01	141.99 ± 20.40	0.14
DM (%)	377 (45.1%)	70 (34.3%)	63 (36.6%)	90 (49.2%)	154 (55.6%)	**<0.0001**
eGFR ($ml.min^{-1}.1.73\ m^{-2}$)	68.92 ± 22.50	71.55 ± 23.38	70.13 ± 22.96	68.25 ± 21.58	66.60 ± 22.24	**0.01**
Previous cardiac surgery (n, %)	197, 23.6	21, 10.3	46, 26.7	58, 31.2	72, 26.0	**<0.0001**
Cardiac morphology and function						
LVEDD (mm)	69.42 ± 9.10	66.69 ± 8.69	69.24 ± 9.58	70.24 ± 8.66	71.03 ± 9.01	**<0.0001**
LVEF (%)	23.58 ± 5.80	27.33 ± 5.25	25.03 ± 5.66	22.49 ± 4.47	20.68 ± 5.25	**<0.0001**
RVD_1 (mm)	40.62 ± 7.94	34.72 ± 5.22	37.50 ± 5.97	40.62 ± 6.22	46.91 ± 7.20	**<0.0001**
RV dysfunction grade (0–3, %)	32.1/22.67/33.29/11.33	100/0/0/0	39.0/59.3/1.7/0	0/48.1/51.4/0.6	0/0/66.1/33.9	**<0.0001**
TAPSE/PASP ratio (mm/mmHg)	0.40 ± 0.21	0.64 ± 0.26	0.46 ± 0.17	0.36 ± 0.12	0.27 ± 0.11	**<0.0001**
Mitral regurgitation (1–3, %)	24.8/40.7/34.5	45.1/38.2/16.7	25.0/45.4/29.7	19.7/44.8/35.6	13.0/37.6/49.5	**<0.0001**
Tricuspid regurgitation (1–3, %)	44.5/39.1/16.4	81.8/16.8/1.5	53.8/40.4/5.9	37.9/50.0/12.1	15.6/47.3/37.1	**<0.0001**
IVC (mm)	19.55 ± 5.75	16.18 ± 3.63	17.87 ± 5.02	19.20 ± 5.00	23.24 ± 5.79	**<0.0001**
Therapy						
ACEi/ARB (%)	78.5	84.8	80.8	78.6	72.5	**0.0008**
BB (%)	87.4	87.8	89.5	89.0	86.2	0.54
MRA (%)	76.7	74.5	73.3	79.7	79.0	0.13
Furosemide daily dose (mg)	80 (40; 125)	40 (40; 80)	60 (40; 120)	80 (40; 125)	100 (60; 165)	**<0.0001**
ICD any (%)	57.0	60.4	60.3	57.1	59.3	0.72
CRT any (%)	30.8	70.8	71.4	62.7	67.4	0.24
Follow-up						
Death (%)	320 (38.3%)	54 (26.5%)	62 (36.1%)	83 (45.4%)	121 (43.7%)	–
Urg. HTx (%)	105 (12.6%)	6 (2.9%)	16 (9.3%)	28 (15.3%)	55 (19.9%)	–
Norm. HTx %)	35 (4.2%)	5 (2.5%)	11 (6.4%)	10 (5.5%)	9 (3.3%)	
MCSi (%)	83 (9.9%)	15 (7.4%)	13 (7.6%)	18 (9.8%)	37 (13.4%)	–
Alive with no event (%)	293 (35.0%)	124 (60.8%)	70 (40.7%)	44 (24.0%)	55 (19.9%)	–

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BB, beta-blocker; BMI, body mass index; CAD, coronary artery disease; CRT, cardiac resynchronization therapy; eGFR, estimated glomerular filtration rate; Hb1Ac, glycated hemoglobin; HTx, heart transplantation; ICD, implantable cardioverter-defibrillator; IVC, inferior vena cava; LVEDD, left ventricular diameter in diastole; LVEF, left ventricular ejection fraction; MCSi, mechanical circulatory support implantation; MiR, mitral regurgitation; MLHFQ, Minnesota living with heart failure questionnaire; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; RV, right ventricular; RVD_1, right ventricle basal diameter in apical four chamber view; TriR, tricuspid regurgitation; RVGDs, RV global dysfunction score.
Significant p-values are in bold.

subgroup of 89 patients ($n = 36$, 10, 29 and 14 with preserved RV function, mild, moderate and severe RV dysfunction, respectively) underwent RV size and function assessment by nuclear imaging. In all cases, echocardiography and scintigraphy was performed within 48 h during stable clinical conditions (stable p.o. medication). The scintigraphic examination was a part of a broader research project performer in our hospital, we have strived to have balanced number of patients in all subgroups of RV function. Patients with preserved RV function had a RV-ejection fraction (RVEF) of 53.64% (±3.93%), patients with mild, moderate and severe RV dysfunction had a RVEF of 46.10% (±3.63%), 34.93% (±4.01%), and 27.57% (±2.96%), respectively (**Supplementary Figure S3A**). Although RVEF assessment showed a mild overlap between groups, the discrimination by echocardiography seems to be satisfactory.

Further, we have evaluated the RVD_1 measured by echocardiography and RV volume measured by nuclear imaging. Both parameters showed an acceptable degree of correlation—$r^2 = 0.76$, $p < 0.0001$ (**Supplementary Figure S3B**). Thus, although imprecise, echocardiography seems to be an acceptable tool for RV size and function assessment in daily clinical practice.

Combined parameter integrating both RV size and degree of dysfunction

As RV size contributes to an adverse outcome independently of RV dysfunction, we suggest it should be considered as a manifestation of RV dysfunction. We have developed a parameter called "RV global dysfunction score" (RVGDs) that integrates the information about both RV size and the degree of dysfunction. It was calculated as a product of RVD_1i and the

degree of RV dysfunction (1 for preserved RV function, 2 for mild RV dysfunction, 3 for moderate RV dysfunction and 4 for severe RV dysfunction), Figure 2.

This closely reflects a progressive increase in hazard ratio with RV function worsening (HR of 2.00 for mild RV dysfunction compared to preserved RV function, 2.99 for moderate RV dysfunction and 4.42 for severe RV dysfunction, see the paragraph RV size and function). We have further compared the prognostic power of RVGDs with RV dysfunction grade only using AUC and found out that RVGDs was significantly superior (Table 2).

RV global dysfunction score

The contribution of RV dilatation and RV dysfunction grade on outcome was analyzed more in detail. We have compared patients with better RV function but more dilated RV with those with worse RV function but smaller RV size. Patients were divided into four groups according to RVGDs (<20, 20–40, 40–60 and >60); these intuitive cut-off values tightly reflect the distribution of RVGDs (median value of 43.9, IQRs 20.16 and 68.44). A total of 204, 172, 183 and 277 patients were involved in the respective subgroups (Table 1).

With increasing RVGDs, the outcome of patients progressively deteriorated ($p < 0.0001$, Figure 3A). In the first subgroup (RVGDs <20), all patients had preserved RV function. In the second subgroup (RVGDs 20–40), patients with RVD_1i below median (≤18.9 mm/m^2 for this subgroup) and mild RV dysfunction had the same outcome as those with $RVD_1i > 18.9$ mm/m^2, but preserved RV function (Figure 3B). Similarly, in the third subgroup (RVGDs 40–60), patients with RVD_1i below median (≤19.8 mm/m^2 for this subgroup) and moderate RV dysfunction had similar outcome as those with $RVD_1i > 19.8$ mm/m^2 but mild RV dysfunction (Figure 3C). Finally, in the fourth subgroup (RVGDs >60), patients with RVD_1i below median (23.6 mm/m^2 for this subgroup) and severe RV dysfunction had similar outcome as those with $RVD_1i > 23.6$ mm/m^2 but moderate RV dysfunction (Figure 3D). In order to exclude that the impact of RV size was in fact caused by more severe tricuspid regurgitation or larger degree of pulmonary hypertension, we have performed Cox multivariable regression that revealed that RV global dysfunction score was associated with adverse outcome even when adjusted for tricuspid regurgitation severity and the degree of pulmonary hypertension (Table 3).

With increasing RVGDs, patients were older, more often males, had more severe LV dysfunction (lower LV-ejection fraction) and enlarged LV cavity, more severe mitral and tricuspid regurgitation, lower plasma sodium and higher BNP level, worse renal function and were more often diabetic. Nevertheless, RV global dysfunction score was associated with an adverse outcome even after the adjustment for all these variables (Table 4).

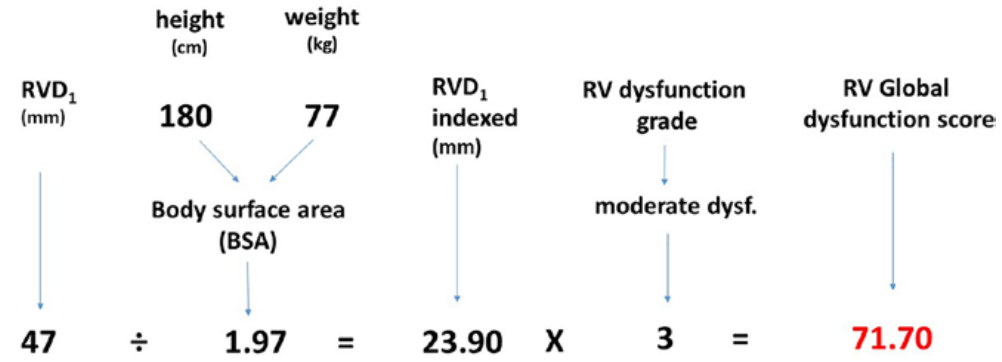

FIGURE 2
RV global dysfunction score. The calculation of RV global dysfunction score.

TABLE 2 Comparison of RV global dysfunction score with RV dysfunction grade only.

Time	AUC				
	RV global dysfunction	RV dysfunction grade	Delta AUC	95% CI	*p*
1st year	0.738	0.699	0.039	0.024; 0.055	**<0.0001**
2nd year	0.714	0.683	0.031	0.017; 0.044	**<0.0001**
3rd year	0.731	0.697	0.034	0.021; 0.047	**<0.0001**
4th year	0.737	0.703	0.034	0.021; 0.047	**<0.0001**

RV global dysfunction score was calculated as a product of RVD_1i (indexed to BSA) multiplied by a factor of 1–4 (1 for preserved RV function, 2—mild RV dysfunction, 3- moderate RV dysfunction, 4- severe RV dysfunction). The area under the curve (AUC) of the Cox proportional hazard regression models was compared at four different time points.
Significant *p*-values are in bold.

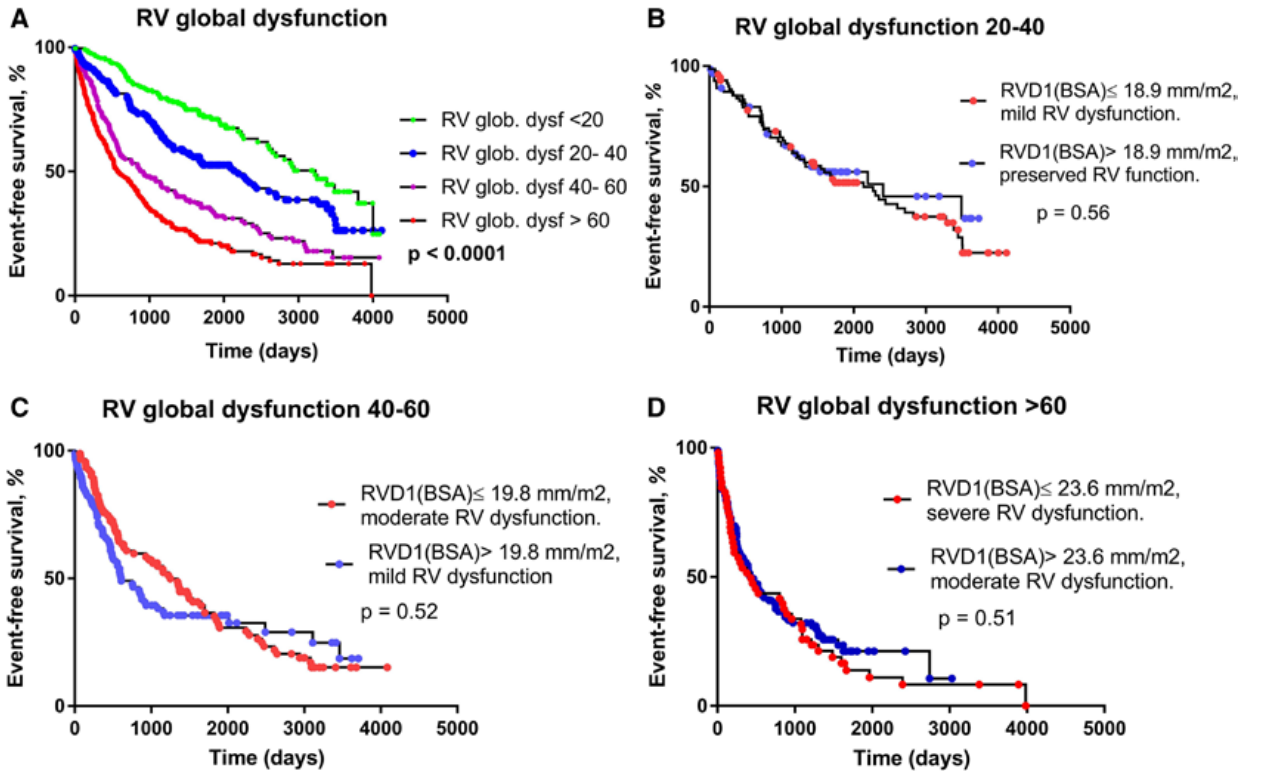

FIGURE 3
The relationship between RV global dysfunction score and prognosis. (**A**) Kaplan-Meier analysis of event-free survival according to RV global dysfunction score. (**B**) RV global dysfunction 20–40; RVD_1i median = 18.9 mm/m^2. $RVD_1i \leq 18.9$ mm/m^2 and mild RV dysfunction ($N = 84$), $RVD_1i > 18.9$ mm/m^2 and preserved RV function ($N = 67$). (**C**) RV global dysfunction 40–60; RVD_1i median = 19.8 mm/m^2. $RVD_1i \leq 19.8$ mm/m^2 and moderate RV dysfunction ($N = 91$), $RVD_1i > 19.8$ mm/m^2 and mild RV dysfunction ($N = 88$). D) RV global dysfunction >60; RVD_1i median = 23.6 mm/m^2. $RVD_1i \leq 23.6$ mm/m^2 and severe RV dysfunction ($N = 52$), RVD $RVD_1i > 23.6$ mm/m^2 and moderate RV dysfunction ($N = 99$).

TABLE 3 The impact of RV global dysfunction, pressure and volume overload on outcome.

	Univariable analysis			Multivariable analysis		
	HR	CI	*p*	HR	CI	*p*
RV global dysfunction, (100 units)	1.017	1.01–1.02	**<0.0001**	1.01	1.009–1.017	**<0.0001**
Tricuspid regurgitation severity, (1–3)	1.71	1.52–1.93	**<0.0001**	1.19	1.01–1.41	**0.04**
Estimated sPAP, (mmHg)	1.026	1.019–1.033	**<0.0001**	1.01	1.007–1.02	**0.0002**

Significant *p*-values are in bold.

TABLE 4 The impact of RV global dysfunction and other variables on outcome.

	Univariable analysis			Multivariable analysis		
	HR	CI	*p*	HR	CI	*p*
RV global dysfunction (100 units)	6.18	4.60–8.28	**<0.0001**	2.23	1.46–3.53	**0.0003**
Sex, (males vs. females)	1.87	1.44–2.47	**<0.0001**	1.51	1.13–2.05	**0.005**
LVEF, (%)	0.94	0.93–0.96	**<0.0001**	0.998	0.979–1.019	0.90
LVEDD, (mm)	1.02	1.02–1.04	**<0.0001**	1.01	1.0004–1.03	**0.04**
Mitral regurgitation, (1–3)	1.51	1.34–1.70	**<0.0001**	1.19	1.03–1.38	**0.02**
Tricuspid regurgitation, (1–3)	1.71	1.52–1.93	**<0.0001**	1.10	0.95–1.29	0.21
eGFR ($ml.min^{-1}.1.73\ m^{-2}$)	0.99	0.987–0.995	**<0.0001**	0.994	0.989–0.998	**0.005**
DM, (present vs. absent)	1.75	1.47–2.09	**<0.0001**	1.52	1.26–1.85	**<0.0001**
Na, (mmol/L)	0.91	0.89–0.93	**<0.0001**	0.96	0.94–0.98	**0.0005**
BNP, (100 ng/L)	1.07	1.06–1.08	**<0.0001**	1.05	1.03–1.06	**<0.0001**

Significant *p*-values are in bold.

Discussion

This study shows that RV dilatation in HFrEF patients independently adds to an adverse outcome and should be considered as a marker of impaired RV function per se. Integrating the information about RV size and degree of dysfunction into one score parameter reflects more accurately the degree of RV disease.

The assessment of RV function in HFrEF patients is extremely important as RV dysfunction was repeatedly shown to be independently associated with impaired survival (2, 3). Currently, much effort is spent to improve the assessment of RV function, which is difficult due to its complex geometry. Tricuspid annular plane systolic excursion (TAPSE) and TDI-derived tricuspid lateral annular systolic velocity (sm-TDi) are long used parameters, but they both assess RV shortening in the longitudinal plane only (15) and are RV geometry-dependent. RV fractional area change (FAC) reflecting a difference between end-diastolic and end-systolic RV areas offers a 2-dimensional evaluation of RV function. In recent years, RV strain has been introduced and shown to better characterize the degree of RV dysfunction (16). Nevertheless, all these parameters focus solely on the property of RV myocardium without taking RV size into account. RV size as well has been shown to have prognostic value in HFrEF patients (8). Current guidelines, however, consider RV size and function as separate entities (11).

RV dysfunction and dilatation are thought to have different pathophysiological background, which is likely the reason why they are evaluated as separate entities. In the situation of pressure overload (pulmonary artery hypertension, PAH) RV initially responds with homeometric remodeling characterized with preserved volume, concentric hypertrophy and normal or only slowly declining RV function. When this adaptive remodeling is exhausted, progressive RV dilatation occurs (heterometric remodeling) (17). In HFrEF patients, the etiology of RV dilatation is likely much more diverse and in many cases can be a direct consequence of underlying pathology (coronary artery disease, dilated cardiomyopathy). RV dilatation thus does not seem to be a final stage of RV disease, it occurs rather independently of RV dysfunction. In our study, we have shown that RV size *per se* is a specific manifestation of RV dysfunction; patients with lower degree of RV dysfunction but larger RV size had similar outcome as those with worse RV dysfunction but smaller RV size. Importantly, this phenomenon is independent of tricuspid regurgitation severity (volume overload) and degree of pulmonary hypertension (pressure overload). The negative prognostic impact of larger RV size is thus attributable neither to more severe tricuspid regurgitation nor pulmonary hypertension. Currently, the estimates of RV systolic function are being replaced by surrogates reflection RV-PA coupling that can be noninvasively estimated as the TAPSE/PASP ratio. Increased RV size leads to increased wall stress that is an important determinant of oxygen consumption (18). Increased oxygen demand can result in periods of ischemia, possibly triggering ventricular arrhythmias. Alternatively, increased oxygen demand results in lower RV contraction efficiency that may ultimately lead to RV pump failure and pump failure death. In patients with HFrEF and secondary pulmonary hypertension, RV dilatation was a predictor of unfavorable right ventricle-to-pulmonary artery coupling (19), which was associated with markedly worse mortality.

In our study we have demonstrated that the combined parameter integrating the information about both RV size and the degree of dysfunction provides improved information about the prognosis compared with the degree of RV dysfunction alone. To the best of our knowledge, it is the first study testing this concept. Our data suggest that rather than focusing on RV dysfunction grade only, "RV disease" is more complex and RV size needs to be taken into account as well. As it is a retrospective echocardiographic analysis (although using prospectively enrolled patients), we used parameters available in all patients (RVD_1, RV dysfunction grade assessed semi-quantitatively). Parameters assessing longitudinal RV function (TAPSE, Sm-TDi) are known to be reduced after cardiac surgery involving pericardial opening (20, 21). As a significant portion of patients (23.6%) have undergone previous cardiac surgery, using longitudinal parameters (TAPSE, Sm-TDi) to construct RV global dysfunction score seemed severely biased. Importantly, TAPSE/PASP ratio seems to be a better prognostic marker than crude RV dysfunction. Creating a combined score using RV size and TAPSE/PASP ratio (instead of RV dysfunction) might provide even better results than a score combining RV size and RV dysfunction. However, answering this question is not possible based on our data as TAPSE/PASP ratio was not available in a substantial portion of patients. It would most likely require a separate analysis of patients with/without the history of previous cardiac surgery. On the other hand, RVD_1 and RV dysfunction grade (assessed semi-quantitatively) are parameters that can be established with a reasonable degree of precision even in patients with very poor acoustic windows. Importantly, the coefficients used for the construction of RVGDs very closely reflect the increasing risk of an adverse outcome. RV function can be associated with renal dysfunction through increased central venous pressure and decreased renal perfusion. Although we have observed a loose association between eGFR and RV function (assessed by both semiquantitatively as well as by RV global dysfunction score, p for trend 0.02 and 0.01, $r^2 = 0.007$ and 0.008, respectively), RV function and eGFR were independently associated with impaired outcome. Therefore, RV function seems to be associated with prognosis regardless of renal function.

Naturally, a combination of different parameters reflecting RV function (FAC, RV strain) and size (RV end-diastolic or end-systolic volumes measured by 3D-echocardiography) might be superior to parameters chosen in the current study. Nevertheless, this is a proof-of-concept study showing that an integration of RV size and degree of dysfunction more accurately reflects the degree of RV disease. This new concept of RV evaluation should be further validated in other cohorts and it needs to be further investigated whether it is a general concept valid for other conditions associated with RV dysfunction (HFpEF, PAH).

Limitations

Apical 4-chamber view was used for RVD_1 measurement, RV global dysfunction score may lack generalizability if RVD_1 was assessed using RV-dedicated 4-chamber projection.

Only a part of echocardiograms were stored electronically and thus available for off-line analysis. Fractional area change (FAC) was not routinely measured in all patients, so the RV global dysfunction score based on quantitative parameters could have not been obtained. Similarly, LV/RV strain parameters were measured only in a minority of patients. Patients were treated not only conservatively (i.e., by optimal pharmacotherapy and ICD/CRT device implantation), but some of them underwent heart transplantation or implantation of mechanical circulatory support, which may bias outcome analysis. As the patients were enrolled between 2009 and 2016, none were treated with sacubitril-valsartan or SGLT2 inhibitors at the time of enrollment. Sacubitril-valsartan was first reimbursed for HFrEF patients in 2018 and SGLT2i in 2021 and virtually no patients with HFrEF were treated with these agents before the reimbursement. Although patients were prospectively enrolled, not all of them were followed in our hospital, so the information about changes in pharmacotherapy throughout the follow-up period is not available from all the patients. Similarly, data about cardiac decompensation were not available from all the patients, so it was not possible to analyze this endpoint. Our study cohort included rather young patients with more advanced HF; consequently, the results might not be fully applicable to patients with milder HF or to older patients.

Conclusion

RV dilatation should be considered to be a manifestation of RV dysfunction in HFrEF patients. A parameter integrating the information about both RV size and the degree of dysfunction provides superior prognostic assessment than the degree of RV dysfunction only.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Joint Ethics Committee of IKEM Thomayerova nemocnice (Thomayer Hospital), Vídeňská 800, 140 59 Praha 4 —Krč Czech Republic. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JB, designed the study; JB, KK and MK conducted the study, MT performed and analyzed scintigraphic substudy. PW, VM and JK performed the statistical evaluation, JK and VM supervized the entire project. All authors contributed to the article and approved the submitted version.

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Conflict of interest

JK is a member of Advisory Boards for Bayer, Boehringer Ingelheim, Daiichi Sankyo, Biosense Webster, Medtronic and St Jude Medical (Abbott). He has received speaker honoraria from the above-mentioned companies and from Biotronik, Mylan, Pfizer and Pro Med.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2023.1194174/full#supplementary-material

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Arrhythmias and laboratory abnormalities after an electrical accident: a single-center, retrospective study of 333 cases

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ORIGINAL PAPER

Arrhythmias and laboratory abnormalities after an electrical accident: a single-center, retrospective study of 333 cases

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Abstract

Background Even though electrical injuries are common in the emergency room, guidelines, consensus, and general recommendations for the management of these patients do not exist in Europe. Documented cases of delayed arrhythmias are rare and their connection with electrical injury has not been fully confirmed. We also use cardio-specific markers for the risk stratification of myocardial injury, but there is no significant study referring to their utility in this clinical situation. These reasons led us to retrospectively analyze all cases of electrical injuries over 23 years to determine the prevalence of cardiac arrhythmias (mainly malignant arrhythmias and delayed arrhythmias).

Methods We retrospectively searched all patients admitted to the University Hospital in Pilsen, CZ, with a diagnosis of electric injury (ICD diagnostic code T754) from 1997 to 2020. The hospital´s information system was used to research the injury; data were drawn from patient medical records.

Results We identified 333 cases of electrical injury in our hospital. Men accounted for about two-thirds, and women one-third. Children accounted for about one-third of cases. Most were low-voltage injuries (< 1000 V, 91.6%). All participants had an initial ECG, and 77.5% of patients had continuous ECG monitoring, usually lasting 24 h. Cardiac arrhythmias were noticed in 39 patients (11.7%). The most frequent arrhythmias were: ventricular fibrillation, sinus tachycardia, bradycardia and arrhythmia, atrial fibrillation, and supraventricular tachycardia. The ECG showed cardiac conduction abnormalities in 28 patients (8.1%), and ten patients (3%) had supraventricular or ventricular extrasystoles. In ten cases (3%), we found changes in ST segments and T waves on the initial ECG. Thirty-one patients (9.3%) suffered a loss of consciousness and 50 patients (15.02%) reported paresthesia. The most frequent ion disbalances were hypokalemia (18%) and hypocalcemia (3.3%). Patients with an ion disbalance had significantly more arrhythmias and newly diagnosed cardiac conduction abnormalities. Troponin levels (cTnI or hs-cTnT) were measured in 258 cases (77.48%) and found to be elevated above the 99th percentile in 19 cases (5.7%). Almost one-third of patients had burns of various degrees of seriousness, and 41 patients (12.3%) had concomitant traumatic injuries. Eleven patients underwent pre-hospital resuscitation, three died in the hospital, and another died as result of intracranial hemorrhage.

Conclusion All malignant arrhythmias occurred immediately after the electrical injury, delayed life-threatening arrhythmias were not observed, and no predictive factors of malignant arrhythmias were found. While elevations of cardiac troponins were observed sporadically, they did not appear helpful for risk stratification. In patients with arrhythmias, ion disbalance may be more critical. We concluded that asymptomatic, uninjured adult and pediatric patients with normal initial ECG findings do

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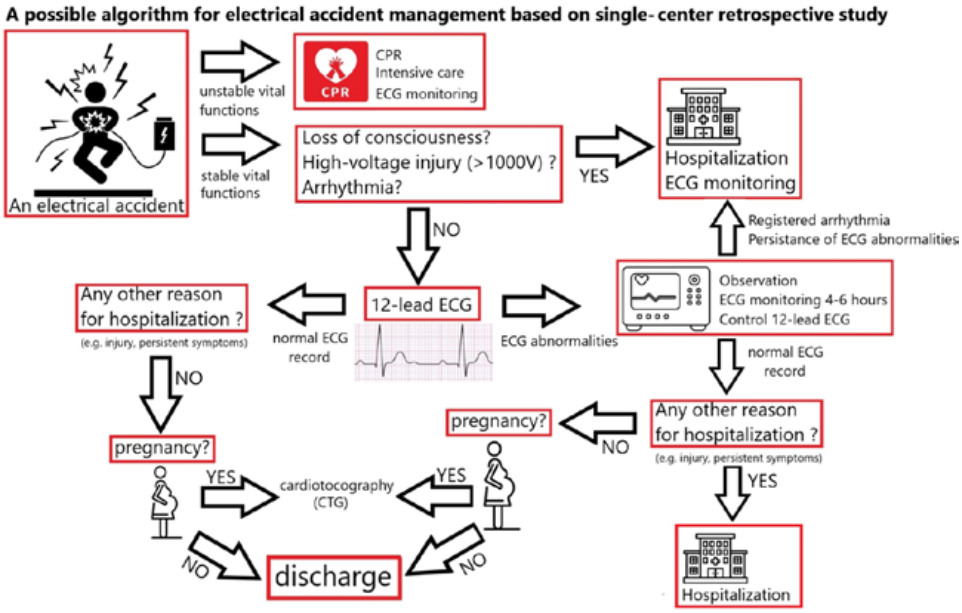
not need continuous ECG monitoring and may be discharged home. Recommendations for high-risk patients and patients with mild ECG abnormalities at admission are less obvious.

Graphical abstract



Keywords Electrical accident · Arrhythmia · Cardiac monitoring · Troponins · Ion disbalance

Introduction

Electrical injuries are very heterogeneous, ranging from minor skin burns to life-threatening injuries to internal organs. [1] The severity of injuries depends on the intensity of the electrical current (determined by the voltage of the source and resistance of the skin), type of current (direct or alternating), the pathway of the electrical current through the body, and duration of contact with the source of current. [2, 3]

Ventricular fibrillation is the most common cause of immediate death after an electrical injury. It can happen when the current reaches the heart within its vulnerable period. [3, 4] Another cause of death is asystole or respiratory arrest (secondary to paralysis of the central respiratory control system or due to paralysis of the respiratory muscles). [2] We can also observe non-lethal arrhythmias (e.g., sinus tachycardia, sinus bradycardia), isolated premature atrial or ventricular beats, bundle branch blocks, sinoatrial blocks, and various degrees of atrioventricular blocks after the electrical accident. [3]

The incidence of delayed malignant arrhythmias is extremely rare. Few documented cases can be found in the medical literature, but only two were reported with initial ECGs. [6, 7] However, fear of delayed arrhythmias is still a reason why patients are admitted to monitoring units, even if they have no risk factors, symptoms, or ECG abnormalities.

We analyzed data from all patients examined in the emergency room after accidental electrocution (AE) to determine the prevalence of cardiac arrhythmias (mainly malignant arrhythmias and delayed arrhythmias) and ECG abnormalities. Furthermore, we searched for significant laboratory abnormalities.

Subjects and methods

Study design and population

We retrospectively searched for all patients admitted to the University Hospital Pilsen, CZ, between 1997 and 2020, with ICD diagnostic code T754 (effects of electric current). We used the hospital information system to research and extract required data from patient's medical records. We did not apply any age limitations. We focused on these patient characteristics: age and sex of the patients, circumstances of the AE, type of current, source voltage, medical history and presenting symptoms (e.g., loss of consciousness, tingling or numbness of extremities, pain in the extremities, chest pain, palpitations, shortness of breath, dizziness, and others).

We looked for cardiac arrest, arrhythmias, ECG abnormalities, loss of consciousness, burns (and their severity), concomitant traumatic injuries, rhabdomyolysis, acute kidney injury, and other consequences of electrical injury. We also examined laboratory results, i.e., serum sodium, potassium, magnesium, calcium, phosphate, creatinine, cardiac troponin (cTnI or hs-cTnT), creatine kinase (CK), and myoglobin. Before 2015, cardiac Troponin I (cTnI) was used as a cardiospecific marker at the University Hospital Pilsen. Starting in 2015, this laboratory marker was replaced by high-sensitive cardiac Troponin T (hs-cTnT). This is why some subjects had data for cTnI and others for hs-cTnT.

Statistical analysis

All relevant data were recorded and edited in Microsoft Excel 2010. MedCalc software, version 19.4.6. (MedCalc, Ostend, Belgium) was used for statistical analysis. The frequencies of categorical variables are provided as absolute numbers and percentages, while continuous variables are presented by means, standard deviations or medians. The difference between the categorical variables was tested using the Chi-square test. The significance level (α) was set at 0.05.

Cardiac troponin I from samples between 2001 and 2015 was measured using an Abbott AxSym and Beckmann Coulter AccuTnI contemporary assay, and cardiac troponin T from samples between 2015 and 2020 was determined using a high-sensitive assay (fifth generation) on a Roche Cobas e602 analyzer.

Serum potassium levels < 3.8 or > 5.2 mmol/l were considered hypokalemia and hyperkalemia, respectively; serum calcium levels < 2.2 or > 2.6 mmol/l were considered hypocalcemia and hypercalcemia, respectively; serum magnesium levels < 0.8 or > 1.1 mmol/l were considered hypomagnesemia and hypermagnesemia, respectively. A normal myoglobin level was set < 86 ug/l for male and < 68 ug/l for female. A normal level of CK was set 0.3–3.3 ukat/l for male and 0.3–3.3 ukat/l for female.

Results

Patient characteristics

During the study period (23 years), 333 patients were admitted to the emergency room with the ICD T75.4 diagnosis (effects of electric current). Two-thirds were adults (155/74 males/females). The mean adult age was 34.84 years (range 19–85). There were 104 children (31.23%) in our sample, most were 1–5 years or 10–15 years. The main characteristics and medical history of patients are presented in Table 1.

Low-voltage injuries were by far the most common (< 1000 V, $n = 305$; 91.59%). Only 16 patients (4.80%) had high-voltage injuries. In 12 cases (3.60%), the source voltage was unknown. Direct current caused six injuries (1.80%), and lightning was suspected in one case. Forty-nine cases (14.71%) occurred at the workplace, Table 2.

Less than half of patients ($n = 143$; 42.94%) presented with symptoms at admission. The most common complaints were tingling and pain in the extremities, chest pain or pressure, palpitations, dizziness, headache, and weakness. All complaints are presented in Table 3.

Cardiopulmonary resuscitation (CPR)

Eleven patients (3.30%) had to be resuscitated immediately after AE. In 8 cases (2.40%), ventricular fibrillation was the cause of the cardiac arrest, and these patients were defibrillated with subsequent restoration of circulation. In one patient, defibrillation was followed by asystole required the use of a mechanical CPR device (LUCAS), after which blood circulation was restored. In two cases, CPR was performed by a bystander; on the first ECG recorded by EMTs, the injured were in sinus rhythm, and no other resuscitation was needed. In the last case, CPR was also performed by a bystander; on arrival, EMTs found the patient in sinus rhythm; however, the patient was dyspneic and had to be intubated and ventilated.

Four patients requiring CPR were associated with a high-voltage injury, and one was associated with a low-voltage injury. In the remaining cases requiring CPR, source voltage information was missing. Based on case circumstances, three appeared to be low-voltage sources. In summary, there were four high-voltage injuries, six low-voltage injuries, and one was unknown.

Three resuscitated patients died during hospitalization (one died on day 19, the second on day 5, and the third on day 74). The cause of death was post-hypoxic encephalopathy and brain edema. In all cases, patients were male and had likely suffered low-voltage injuries.

Arrhythmias and cardiac electrical injuries

Thirty-nine cases of cardiac arrhythmias/dysrhythmias, 10 cases of premature complexes, and 28 cases of cardiac conduction disorders were noticed. The most alarming were the 8 cases of ventricular fibrillation (Table 4). However, in one case, uncertainties remain about the cause of ventricular fibrillation. The patient was a 58-year-old man who suffered cardiac arrest while repairing a washing machine, but pathological Q and ST elevations were present on an initial ECG (the inferolateral location). According to echocardiography, left ventricular akinesia was present in the corresponding areas. Additionally, coronary angiography

Table 1 Characteristics of population

	All patients ($n=333$)	Adult patients ($n=229$)	Pediatric patients ($n=104$)
Age (years, mean ± SD)	25.96 ± 17.61	34.87 ± 13.5	6.33 ± 5.55
Age groups			
15–18 years	–	–	13 (12.50%)
10–15 years	–	–	22 (21.15%)
5–10 years	–	–	8 (7.69%)
1–5 years	–	–	58 (55.77%)
< 1 year	–	–	3 (2.88%)
Male/female	222/111 (66.67/33.33%)	155/74 (67.69/32.31%)	67/37 (64.42/35.58%)
Actual pregnancy	5 (1.50%)	5 (1.50%)	–
Medical history			
Structural heart disease	9 (2.70%)	6 (2.62%)	3 (2.88%)
Coronary heart disease	6 (1.80%)	6 (2.62%)	–
Heart failure	3 (0.90%)	3 (1.31%)	–
Cardiac surgery	3 (0.90%)	2 (0.87%)	1 (0.96%)
Arrhythmias	11 (3.30%)	10 (4.37%)	1 (0.96%)
Bundle branch blocks	8 (2.40%)	6 (2.62%)	2 (1.92%)
Atrioventricular blocks	3 (0.90%)	3 (1.31%)	–
History of palpitations	3 (0.90%)	3 (1.31%)	–
Pacemakers, ICDs	5 (1.50%)	5 (2.18%)	–
Arterial hypertension	28 (8.41%)	28 (12.23%)	–
Diabetes mellitus	7 (2.10%)	7 (3.06%)	–
Hyperlipidemia	29 (8.71%)	29 (12.66%)	–
Kidney disease	6 (1.80%)	3 (1.31%)	3 (2.88%)
Bronchial asthma/COPD	13 (3.90%)	10 (4.37%)	3 (2.88%)
Thyroid gland disease	8 (2.40%)	7 (3.06%)	1 (0.96%)
Malignancy	5 (1.50%)	5 (2.18%)	–
Stroke	1 (0.30%)	1 (0.44%)	–

SD standard deviation, *ICDs* implantable cardioverter-defibrillator, *COPD* chronic obstructive pulmonary disease

Table 2 Descriptions of electrical injuries

	All patients ($n=333$)	Adult patients ($n=229$)	Pediatric patients ($n=104$)
Voltage of the source			
Small (< 50 V)	2 (0.60%)	1 (0.44%)	1 (0.96%)
Low (50–1000 V)	303 (90.99%)	203 (88.65%)	100 (96.15%)
High (1000–52 000 V)	16 (4.80%)	13 (5.68%)	3 (2.88%)
Unknown	12 (3.60%)	12 (5.24%)	–
Lightning	1 (0.30%)	1 (0.44%)	–
Direct current	6 (1.80%)	6 (2.62%)	–
Suicide attempt	2 (0.60%)	2 (0.87%)	–
Workplace accident	49 (14.71%)	48 (20.96%)	1 (0.96%)

found multi-vessel disease, but no culprit lesion was found. Dynamic elevation of cardio-specific markers can be explained by acute coronary syndrome and previous resuscitation with a myocardial contusion. In the end, electricity-related marks on the right hand indicate an electrical injury. This was probably a coincidence between a subacute myocardial infarction and the AE.

Other observed arrhythmias were sinus tachycardia, sinus bradycardia, sinus arrhythmia, atrial fibrillation,

Table 3 Complaints at admission

	All patients ($n=333$)	Adult patients ($n=229$)	Pediatric patients ($n=104$)
Tingling in the extremities	50 (15.02%)	40 (17.47%)	10 (9.62%)
Chest pain/pressure	33 (9.91%)	27 (11.79%)	6 (5.77%)
Pain/burning in the extremities	23 (6.91%)	17 (7.42%)	6 (5.77%)
Palpitations	19 (5.71%)	19 (8.30%)	–
Dizziness	13 (3.90%)	11 (4.80%)	2 (1.92%)
Amnesia	13 (3.90%)	12 (5.24%)	1 (0.96%)
Headache	11 (3.30%)	7 (3.06%)	4 (3.85%)
Weakness, fatigue, faintness	10 (3.00%)	8 (3.49%)	2 (1.92%)
Nauseous	8 (2.40%)	5 (2.18%)	3 (2.88%)
Muscle cramp	5 (1.50%)	5 (2.18%)	–
Vomiting	4 (1.20%)	3 (1.31%)	1 (0.96%)
Dyspnea/shortness of breath	4 (1.20%)	2 (0.87%)	2 (1.92%)
Abdominal pain	3 (0.90%)	2 (0.87%)	1 (0.96%)
Cyanosis	2 (0.60%)	2 (0.87%)	–
Backache	2 (0.60%)	2 (0.87%)	–
Blurred vision	2 (0.60%)	1 (0.44%)	1 (0.96%)
Muscle twitching	2 (0.60%)	1 (0.44%)	1 (0.96%)
Weakness of the extremities	2 (0.60%)	–	2 (1.92%)
Immobility of the extremities	1 (0.30%)	–	1 (0.96%)
Numbness of the extremities	1 (0.30%)	–	1 (0.96%)
Speech disorder	1 (0.30%)	1 (0.44%)	–
Confusion	1 (0.30%)	1 (0.44%)	–

Table 4 Malignant arrhythmias

Age	Sex	Voltage	Year	Medical history	Trauma	Burns	Death
16	M	Low	2002	–	No	No	Yes
24	M	Unknown	2004	–	No	No	Yes
39	M	High	2000	paroxysmal atrial fibrillation, tick-borne encephalitis at age 38	Yes	3°	No
43	F	Low	2002	–	No	No	No
44	M	High	2005	rib fractures at age 19	No	3°	No
47	M	Low	2011	–	No	3°	No
50	M	Low	2012	–	No	No	No
58	M	Low	2013	concomitant subacute MI	No	No	Yes

M male, *F* female, *MI* myocardial infarction

supraventricular tachycardia that were terminated by verapamil, and an unspecified tachycardia terminated by amiodarone. In ten cases we found changes in the ST segment and T wave on the initial ECG. One patient with atrial fibrillation (of unknown duration) showed signs of heart failure at the time of admission. Echocardiography confirmed left ventricular systolic dysfunction (LVEF 15–20%). In four other patients, mild left ventricular systolic dysfunction (LVEF 40–50%) was also newly diagnosed. (Table 5).

Electrical injury

Altogether, 101 patients (30.33%) suffered burns of various degrees (second-degree burns were the most common). Concomitant traumatic injuries were found in 12.31% of cases. The most common injuries were fractures, traumatic brain injury and lacerated/incised wounds. We observed rhabdomyolysis in 5 severe cases and acute kidney injury in 4 cases.

Patients also showed neurological abnormalities (e.g., paresthesia, hyp/anesthesia muscle convulsion/spasm/fasciculation, autonomic dysfunction or speech disorder) and

Table 5 Arrhythmias and cardiac electrical injury

	All patients ($n=333$)	Adult patients ($n=229$)	Pediatric patients ($n=104$)
Arrhythmias/dysrhythmias	39 (11.71%)	36 (15.72%)	3 (2.88%)
Ventricular fibrillation	8 (2.40%)	7 (3.06%)	1 (0.96%)
Sinus tachycardia	14 (4.20%)	14 (6.11%)	–
Sinus bradycardia	11 (3.30%)	9 (3.93%)	2 (1.92%)
Sinus arrhythmia	2 (0.60%)	2 (0.87%)	–
Atrial fibrillation	4 (1.20%)	4 (1.75%)	–
SVT	1 (0.30%)	1 (0.44%)	–
Unspecified tachycardia	1 (0.30%)	1 (0.44%)	–
Premature complexes	10 (3.00%)	8 (3.49%)	2 (1.92%)
Supraventricular	4 (1.20%)	4 (1.75%)	–
Ventricular	5 (1.50%)	4 (1.75%)	1 (0.96%)
Unknown	1 (0.30%)	–	1 (0.96%)
Cardiac conduction abnormalities	26 (7.81%)	26 (11.35%)	–
LAH	2 (0.60%)	2 (0.87%)	–
IRBBB/ intermit. iRBBB	21 (6.31%)/2(0.60%)	21 (9.17%)/2 (0.87%)	normal finding
RBBB/ intermit. RBBB	1 (0.30%)/1(0.30%)	1 (0.44%)/1 (0.44%)	–
SAB (second degree)	1 (0.30%)	1 (0.44%)	–
AVB (first degree)	1 (0.30%)	1 (0.44%)	–
ECG changes of ST/T	10 (3.00%)	9 (3.93%)	1 (0.96%)
ST elevation	2 (0.60%)	1 (0.44%)	1 (0.96%)
ST depression	5 (1.50%)	5 (2.18%)	–
Negative T wave	3 (0.90%)	3 (1.31%)	normal finding
Cardiac decompensation	1 (0.30%)	1 (0.44%)	–
Newly dg. systolic dysfunction	5 (1.50%)	3 (1.31%)	2 (1.92%)

SVT supraventricular tachycardia, *iRBBB* incomplete right bundle branch block, *RBBB* complete right bundle branch block, *intermit.* intermittent, *SAB* sinoatrial block, *AVB* atrioventricular block, *dg.* diagnosed

31 patients reported a loss of consciousness. Three patients entered a vegetative state as a consequence of post-hypoxic encephalopathy or intracranial hemorrhage. In one case, the patient's neurological condition improved, and he returned home. Two other patients died within a year. (Table 6).

Clinical course and cardiac monitoring

Three hundred and thirty-three patients were examined in the emergency room after the AE, and all of them had an initial ECG. Five children were discharged home, two patients signed out against medical advice, one patient was hospitalized in another hospital, and the remaining 325 patients were admitted to various hospital departments. All pregnant women underwent cardiotocography (CTG) with normal findings.

Patients treated by EMTs at the scene of the accident had a 3-lead ECG record immediately and afterwards they had a 12-lead ECG record at the emergency room. Patients who came to the hospital themselves had a 12-lead ECG record at the emergency room. Afterwards, the patients were connected to a telemetry ECG monitoring system that records a 3-lead ECG. In some cases (especially incidents from earlier years), patients had repeated 12-lead ECGs during hospitalization.

The average length of hospital stays was 3.53 days, but this number is heavily influenced by long-term hospitalizations (patients with cardiac arrest, severe burns, or intracranial injuries). If we exclude hospitalizations longer than 30 days, the average hospital stay was 1.55 days. Ultimately, 316 patients were discharged home, and 13 were transferred to other hospitals (e.g., Prague Burn Center, intensive care units, and long-term treatment centers). The 30-day mortality was 0.60% and the 1-year mortality was 1.20%. (Table 7).

Biochemical analysis

Laboratory values were available for almost all patients. The average creatinine level was 91 µmol/l in adults and 52 µmol/l in children. Acute kidney injury was diagnosed in 4 cases (1.20%). Ion disbalance was found in 67 patients (20.12%), with hypokalemia being the most common ($n=60$; 18.02%). The frequencies of other ion abnormalities are shown in Table 8.

Table 6 Consequences of electrocution (except arrhythmias and cardiac electrical injuries)

	All patients ($n=333$)	Adult population ($n=229$)	Pediatric population ($n=104$)
Death	4 (1.20%)	3 (1.31%)	1 (0.96%)
Death due to electrocution	3 (0.90%)	2 (0.87%)	1 (0.96%)
Cardiopulmonary resuscitation	11 (3.30%)	10 (4.37%)	1 (0.96%)
Respiratory arrest/disorder	2 (0.60%)	1 (0.44%)	1 (0.96%)
Loss of consciousness	31 (9.31%)	26 (11.35%)	5 (4.81%)
Skin burns	101 (30.33%)	49 (21.40%)	52 (50.00%)
1st degree	11 (3.30%)	4 (1.75%)	7 (6.73%)
1st–2nd degree	6 (1.80%)	3 (1.31%)	3 (2.88%)
2nd degree	46 (13.81%)	16 (6.99%)	30 (28.85%)
2nd–3rd degree	6 (1.80%)	2 (0.87%)	4 (3.85%)
3rd degree	20 (6.01%)	14 (6.11%)	6 (5.77%)
4th degree	1 (0.30%)	1 (0.44%)	–
Unknown	8 (2.40%)	6 (2.62%)	2 (1.92%)
Small electricity-related marks	50 (15.02%)	27 (11.79%)	23 (22.12%)
Corneal burns	2 (0.60%)	2 (0.87%)	–
Respiratory tract burns	1 (0.30%)	1 (0.44%)	–
Concomitant traumatic injuries	41 (12.31%)	28 (12.23%)	13 (12.50%)
Fractures	14 (4.20%)	10 (4.37%)	4 (3.85%)
(Sub)luxations	3 (0.90%)	2 (0.87%)	1 (0.96%)
Lacerated/incised wounds	6 (1.80%)	5 (2.18%)	1 (0.96%)
Traumatic brain injury	11 (3.30%)	7 (3.06%)	4 (3.85%)
Lung contusion	4 (1.20%)	2 (0.87%)	2 (1.92%)
Pneumothorax	1 (0.30%)	1 (0.44%)	–
Kidney fissure	1 (0.30%)	1 (0.44%)	–
Spleen damage	1 (0.30%)	–	1 (0.96%)
Rhabdomyolysis	5 (1.50%)	4 (1.75%)	1 (0.96%)
Acute kidney injury	4 (1.20%)	3 (1.31%)	1 (0.96%)
Neurological abnormalities			
Paresthesia	50 (15.02%)	40 (17.47%)	10 (9.62%)
Hypoesthesia/anesthesia	2 (0.60%)	1 (0.44%)	1 (0.96%)
Convulsion/spasm/fasciculation	9 (2.70%)	8 (3.49%)	1 (0.96%)
Autonomic dysfunction	1 (0.30%)	–	1 (0.96%)
Speech disorder	1 (0.30%)	1 (0.44%)	–
Post-hypoxic encephalopathy Brain edema	3 (0.90%)	2 (0.87%)	1 (0.96%)
Vegetative state	3 (0.90%)	2 (0.87%)	1 (0.96%)

The level of creatine kinase (CK) was determined in 252 patients (75.68%) and was elevated in 106 cases (31.83%). The average level of CK was 5.6 ukat/l. Myoglobin level was measured in 233 patients (69.97%) and was elevated in 95 cases (28.53%). The average level of myoglobin was 316.64 ug/l. Cardiac troponin was measured in 258 cases (77.48%; cTnI in 171 cases and hs-cTnT in 87 cases), it was measured once in 184 patients, twice in 45 patients, and three or more times in 19 cases. Increased troponin levels above the 99th percentile were detected in 19 cases (5.71%; cTnI in 16 cases and hs-cTnT in 3 cases). The average level of TnI was 0.02 ug/l and the average level of TnT was 8.13 ng/l (in the first measurement).

We registered that 27.1% patients with ion disbalance and only 7.6% patients without ion disbalance had arrhythmia after AE (Fig. 1). This means that patients with ion disbalance (most often hypokalemia) had significantly more arrhythmias (significance level $P<0.0001$). The same situation applies to relationship between the incidence of ion disbalance and newly diagnosed cardiac conduction abnormalities (significance level $P<0.0232$). Newly diagnosed cardiac conduction abnormalities were

Table 7 Clinical course and cardiac monitoring

	All patients ($n=333$)	Adult population ($n=229$)	Pediatric population ($n=104$)
Hospitalization	326 (97.90%)	227 (99.13%)	99 (95.19%)
Refusal of hospitalization	4 (1.20%)	4 (1.75%)	–
Continuous ECG monitoring	258 (77.48%)	196 (85.59%)	62 (59.62%)
Duration of hospitalization (days. mean)	3.53	3.74	2.95
Without those longer than 30 days	1.55	1.64	1.34
Without those longer than 7 days	1.14	1.10	1.21
Discharge	310 (93.09%)	215 (93.89%)	95 (91.35%)
Transfer to another department	16 (4.80%)	14 (6.11%)	2 (1.92%)
Transfer to another hospital	13 (3.90%)	10 (4.37%)	3 (2.88%)
30-day mortality	2 (0.60%)	1 (0.44%)	1 (0.96%)
1-year mortality	4 (1.20%)	3 (1.31%)	1 (0.96%)

Table 8 Ion disbalances

	All patients ($n=333$)	Adult population ($n=229$)	Pediatric population ($n=104$)
Total	67 (20.12%)	58 (25.33%)	9 (8.65%)
Hypokalemia	60 (18.02%)	55 (24.02%)	5 (4.81%)
Hypocalcemia	11 (3.30%)	8 (3.49%)	3 (2.88%)
Hypomagnesaemia	8 (2.40%)	6 (2.62%)	2 (1.92%)
Hypophosphatemia	5 (1.50%)	5 (2.18%)	–
Hyponatremia	4 (1.20%)	4 (1.75%)	–
Hyperkalemia	4 (1.20%)	3 (1.31%)	1 (0.96%)
Hypercalcemia	3 (0.90%)	2 (0.87%)	1 (0.96%)
Hypermagnesemia	1 (0.30%)	1 (0.44%)	–
Hyperphosphatemia	1 (0.30%)	1 (0.44%)	–

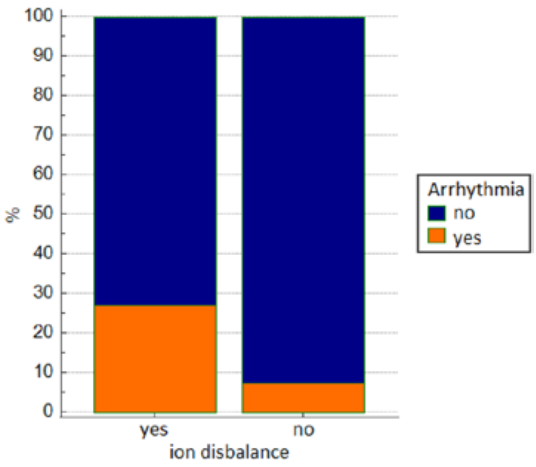


Fig. 1 relationship between the incidence of ion disbalance and arrhythmia

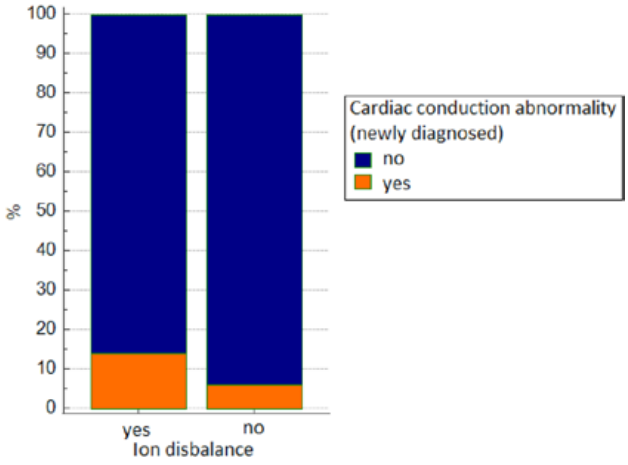


Fig. 2 relationship between the incidence of ion disbalance and newly diagnosed cardiac conduction abnormalities

seen in 14.3% of patients with ion disbalances but only 6.1% of patients without ion disbalances (Fig. 2).

To clarify these figures, the contingency tables that were the basis for creating the graphs are provided below.

	Patients with ion disbalances	Patients without ion disbalances
Patients with arrhythmia	19 (27.14%)	20 (7.60%)
Patients without arrhythmia	51 (72.86%)	243 (92.40%)
Patients with cardiac conduction abnormality	10 (14.29%)	16 (6.08%)
Patients without cardiac conduction abnormality	60 (85.71%)	247 (93.92%)

Pediatric subpopulation

Approximately one-third of subjects were under 18 years ($n=104$; 31.23%), the average age was 6.33 years. The distribution of particular age categories is shown in Table 1. The ratio of boys to girls was approximately the same among pediatric cases as in adult cases. High-voltage injuries ($n=3$; 2.88%) were rare in children, as was any history of serious diseases. The most common medical problems were: bronchial asthma, kidney disorders, and structural heart diseases. Fewer children reported symptoms at admission than adults ($n=24$; 23.08% vs. adult population $n=119$; 51.97%). This may be explained by the limited abilities of young children to express themselves. The most common complaints were tingling and pain in the extremities. Chest pain was less frequent in children ($n=6$; 5.77% vs. adults 11.79%) and no children reported palpitations. Only one child required cardiopulmonary resuscitation. Unfortunately, the child died on the fifth day of hospitalization. Loss of consciousness was also less frequent in pediatric cases ($n=5$; 4.81% vs. adults 11.35%). The incidence of the concomitant traumatic injuries was approximately same in children ($n=13$; 12.50% vs. adults 12.23%), but the incidence of burns was more than double ($n=52$; 50% vs. adults 21.40%). The number of detected arrhythmias was significantly lower in children ($n=3$; 2.88% vs. adults 15.72%). There was one case of ventricular fibrillation, two cases of sinus bradycardia, and two cases of premature complexes. No cardiac conduction abnormality was registered (vs. adults population 12.23%). A 15-year-old male with severe, extensive burns, spleen damage (with hemoperitoneum and the need for splenectomy), lung contusion, craniocerebral trauma, and a pelvic fracture had sinus bradycardia and diffuse ST elevations on the second day of hospitalization (at the time the potassium was 6.2 mmol/l). Ion disbalances were less common in children ($n=9$; 8.65% vs. adults 25.33%), the most common disbalance was hypokalemia ($n=5$; 4.81%). After the outpatient examination five children were discharged home, and 99 children were admitted to the hospital. Less than two-thirds of children had continuous ECG monitoring ($n=62$; 59.62% vs. adults 85.59%). Of the hospitalized children, 95 were discharged home, three were transferred to another hospital, and one died during hospitalization as a result of post-hypoxic encephalopathy and irreversible coma.

Discussion

Arrhythmias and ECG monitoring after electrical injury

We retrospectively analyzed 333 cases of accidental electrocution (AE). We observed that all life-threatening, malignant arrhythmias occurred immediately after the electrical injury. Other clinically relevant arrhythmias were rare, and the initial ECG was sufficient to diagnose them. Moreover, not all ECG findings should necessarily be evaluated as pathological (e.g., sinus tachycardia as a physiological response to pain/anxiety, incomplete RBBB in young patients, sporadic premature beats or non-specific ST/T changes). In addition, some ECG findings (e.g., sinus tachy/bradycardia, supraventricular tachycardia, premature complexes, cardiac conduction abnormalities or ST/T changes) may have been present before the electrical accident and they do not have to be the result of it. During ECG monitoring, we registered only one case of arrhythmia. It was paroxysm of atrial fibrillation that could be explained as a reaction to pain and severe burns (the same as sinus tachycardia present since admission) and did not require any treatment. No delayed malignant arrhythmia was observed.

According to the guidelines of the European Resuscitation Council (2015, Sect. 4), ECG monitoring is recommended after AE in all patients if they have a history of cardiorespiratory problems or have experienced a loss of consciousness, cardiac arrest, ECG abnormalities, or soft tissue damage or burns. It follows that ECG monitoring is not required when the patient is asymptomatic and the initial ECG is normal [16]. This recommendation is consistent with the results of our study (none of these patients had an arrhythmia during ECG monitoring).

Significantly, high-risk patients (with high-voltage injury, loss of consciousness, ECG abnormalities, soft tissue damage/burns, and a history of cardiovascular disease) were included in our study. Additionally, there was no new-onset arrhythmia during ECG monitoring (except for the previously described case of atrial fibrillation). Bailey et al. reached the same conclusion in prospective study published in 2017. One hundred and thirty-four patients with at least one risk factor (transthoracic current, tetany, loss of consciousness or voltage source ≥ 1000 V) were included in the study and none developed a potentially lethal late arrhythmia during the 24 h of cardiac monitoring. [9] On the other hand, our study registered a higher proportion of high-voltage electrical injuries in the group of patients with arrhythmias compared to those without arrhythmias. Post-treatment for these patients remains unclear, and high voltage should still be considered a risk factor.

Predictive factors of malignant arrhythmias

We observed eight cases of ventricular fibrillation in 7 men (87.5%) and 1 woman (12.5%). The percentage of men was slightly higher than the male–female ratio in the whole cohort. In 5 cases (62.5%), arrhythmias were induced by low-voltage and in 2 cases (25%) by high-voltage current. This was similar to the percentage of low-voltage injuries

vs. the percentage of high-voltage injuries in the overall cohort. The mean age of ventricular fibrillation patients was 40 years (range 16–58). Only one patient had a history of arrhythmia (paroxysmal atrial fibrillation). Medical histories of the other patients were entirely unremarkable (Table 4). We did not find any obvious predisposing factor for malignant arrhythmias, and the number of cases was insufficient for this purpose.

Delayed arrhythmias

Cases of delayed malignant arrhythmia are extremely rare. We found only six published cases in the medical literature. Moreover, some of them lack important information (e.g., an initial ECG). No delayed malignant arrhythmia has been reported in our study or in other larger recent studies. [3, 5, 8, 9, 15, 17, 20]

Few cases of delayed malignant arrhythmias have been published, and only two included an initial ECG. In 1987 Jensen et al. described three patients with delayed ventricular arrhythmias after electrical injury (onset 8–12 h after the accident). One patient developed recurrent ventricular fibrillation, another ventricular tachycardia, and one experienced numerous ventricular extrasystoles. [10] None had an initially recorded ECG after the electrical accident—if they had, it is unlikely that the ECG would have been normal.

In 1990, Sharma et al. published the case of a patient who, after a low-voltage injury, had a first-degree AV block and low voltage in the ECG at the time of admission. During cardiac monitoring, the AV block progressed to higher degrees over 2 h, ending to ventricular fibrillation requiring defibrillation. [6]

In 2001, Bailey et al. described a 16-year-old woman who was found dead 10 h after discharge from the hospital, where she had been treated for painful burns. No ECG was available, and the circumstances of her death were unclear, but we can suppose that a malignant arrhythmia was one possible cause. [11]

In 2015, Karataş et al. reported on a patient admitted to the hospital with a prolonged QTc interval (500 ms) and fragmented QRS complex. He developed pulseless ventricular tachycardia within 24 h. Follow-up ECG showed that the abnormalities had normalized within one month. [7]

Predictive value of the cardiac-specific biomarkers and the role of the ion disbalance

The predictive value of cardiac-specific biomarkers in risk stratification of myocardial injury after AE is still unclear. In 2010 Orak et al. analyzed the relationship between serum pro-brain natriuretic peptide (NT-proBNP), myoglobin, CK levels, cTnI, and morbidity and mortality after high-voltage electrical injuries. They found that NT-proBNP levels were higher in patients with arrhythmias, but CK-MB and cTnI levels were not. [12] Other studies also concluded that CK-MB was not a helpful marker for risk stratification of cardiac complications since it can be elevated due to muscle and soft tissue damage associated with the AE [13, 14]. Attention was then focused on the cardiac troponins, which are cardiac-specific and more sensitive. However, increased troponin levels were not usually noticed after an AE [8, 9, 15]. The most extensive retrospective study of AE, carried out by Pilecky et al., found CK elevation to be common, especially in patients with high-voltage injuries, but without any increase in CK-MB%. Significant elevation of cTnI was only detected in one patient, who had been resuscitated after ventricular fibrillation. [3]

We detected an elevation of CK in 31.83% of patients, myoglobin elevation in 28.53% of patients, and troponin elevation in 5.71% of patients. An elevation of CK and myoglobin was 5–6 times more common than troponin elevation, which can be explained by the extent of soft tissue damage. On the other hand, mild chronic elevation of troponin levels can be explained by the patient's medical history (arterial hypertension with possible myocardial hypertrophy, coronary artery disease, chronic heart failure, renal failure, etc.). Any dynamic increases are mainly consequence of the electrical injury (myocardial injury, AE-associated CPR with myocardial contusion, shock with myocardial hypoperfusion, etc.).

Patients with an ion disbalance (most often hypokalemia) had significantly more arrhythmias and newly diagnosed cardiac conduction abnormalities. This finding led us to ask whether an ion disbalance could be a risk factor for the development of arrhythmia following AE. Hypokalemia should be looked for in all patients with arrhythmias, premature complexes or cardiac conduction abnormalities and any ion disbalance should be normalized.

Pediatric subpopulation

There was a significantly lower incidence of arrhythmias and ST/T changes on initial ECGs in children compared to adults. Furthermore, no cardiac conduction abnormalities were registered. Only one case of malignant arrhythmia was reported and no delayed arrhythmias were detected. It follows that there is no need for a special pediatric approach to AEs compared to adults. In 2013, an evidence-based approach to electrical injuries in children was published. In cases involving stable, asymptomatic patients without concomitant traumatic injuries or burns outpatient management was recommended. For all others, inpatient management with laboratory examination, ECG and intravenous fluids was recommended [18]. The results and conclusion of our study agree with these recommendations.

Results of previous studies

The results of our study are consistent with previously published clinical studies. Only the incidence of ventricular fibrillation/tachycardia was higher than usually reported, but it is still a very small percentage of patients (2, 4%). We have no specific explanation for this fact.

In 2002, Blackwell et al. presented a study that differs from others in its prospective character. In the study, 212 patients after low-voltage AE were included in a 4-year follow-up. A new management protocol was developed and evaluated. It reduced the number of hospitalizations without any negative impact on mortality and morbidity [17].

In 2013 Searle et al. presented a retrospective study with 268 children and adults who were usually monitored for more than 12 h. On admission 66 patients had mild cardiac arrhythmias (sinus tachycardia or bradycardia, isolated extrasystoles), and none developed an arrhythmia requiring intervention. They also presented a standard protocol flowchart for patients with electrical injuries. Hospital-based monitoring was not recommended in asymptomatic, uninjured patients without ECG changes. [8] Three other studies (each involving more than 100 patients) were published with the same conclusions: asymptomatic patients without any risk factors and with a normal 12-lead ECG need no cardiac monitoring after an electrical injury. [5, 19, 20]

In 2016, Pawlik et al. retrospectively identified 240 patients who suffered an AE. They were monitored for an average of 4.25 h, no malignant arrhythmias were detected and the 90-day mortality was 0% [15]. Three years later, Pilecky et al. published the largest study on the risk of cardiac arrhythmias after AE. It was a retrospective, single-center study that included 480 patients. They registered 80 cases of arrhythmia (most often sinus tachycardia and bradycardia, with only one case of ventricular fibrillation before admission) and 92 ECG abnormalities (most often non-specific ST-T changes). According to the authors, routine ECG monitoring appears unnecessary, the 30-day mortality was 0%, and no late-onset malignant arrhythmias were observed [3].

Studies with high-risk patients were conducted by Bailey et al., who observed 134 patients with at least one risk factor (a transthoracic current, voltage source $\geq$ 1000 V, tetany > 1 s, any loss of consciousness). Although 11% of patients had mild ECG changes on admission, none required treatment, and no malignant arrhythmias occurred during 24-h monitoring period. Moreover, none of the patients had cardiac complications during the 1-year follow-up [9]. In 2018, Gille et al. studied 162 patients admitted to burn intensive care units. Arrhythmias were observed in 23 patients, including seven patients who required CPR (3 for ventricular fibrillation and 4 for asystole). Four patients developed self-limiting arrhythmias during hospitalization (one lasted more than 24 h after admission). [21]

Finally, there are studies focused on pediatric patients, and their results do not differ from those of adults in any meaningful way [22, 23]. In 1995, Bailey et al. described 151 cases of children with household AE. In 113 patients, cardiac monitoring was performed for 4 h (median), and no arrhythmias were observed. In conclusion, ECG monitoring did not appear necessary for children without risk factors after household electrical injuries [22]. Fifteen years later, Claudet et al. arrived at the same result. They analyzed 48 cases of children under 15 years following an AE, ten had risk factors (e.g., mainly wet skin or thoracic pain) and eight showed non-serious ECG abnormalities on admission. No delayed arrhythmias occurred. [23]

Limitations

The major limitations of our study are (1) the retrospective character of data collection, (2) inconsistent patient management and missing data, (3) unequal ratio of the low-voltage to high-voltage injuries, and (4) a change, during the study period, in the type troponin used to monitor cardiac injury and the method of its determination.

Data analyzed in the study were not primarily intended for research but represented routine medical documentation. Therefore, the quantity and quality of the information were not the same in all cases. For example, source voltage, circumstances surrounding the AE, biomarkers levels, and duration of ECG monitoring were unknown in some cases. The characteristics of the studied population also lack information regarding medication, especially antiarrhythmics and beta-blockers. Since high-voltage injuries were underrepresented, overall study results cannot be unconditionally extrapolated.

Unfortunately, continuous ECG monitoring was not performed in all patients. In a minority of patients, ECG data took from of periodic 12-lead ECGs recorded during hospitalization. It cannot be excluded that some non-serious arrhythmias may have occurred between recordings. The same situation could have occurred between the AE and the first ECG examination because not all patients went to the hospital immediately after the incident. Also, cardiac troponins levels were unavailable for every patient, and times from AE to blood sampling were variable. Another issue associated with cardiac troponins was that in 2015, we changed the type measured troponin from Troponin I (cTnI) to high-sensitive Troponin T (hs-cTnT). In general, hs-cTnT has a slightly higher sensitivity but, in specific situations, a lower specificity for diagnosing acute myocardial ischemia [24]. Due to the different diagnostic properties of the two molecules, the 99th percentiles are not comparable. Similarly, serum levels are different and independent, i.e., one level cannot be inferred from the other.

Although the number of patients enrolled in the study is relatively large compared to other studies, this number was still insufficient for general conclusions and globally applicable recommendations. A meta-analysis of all available studies would be needed for this purpose.

Conclusion

Our study suggests that routine ECG monitoring of all patients after a low-voltage electrical accident is unnecessary. All malignant arrhythmias occurred immediately after the electrical accident and delayed life-threatening arrhythmias were not observed. Considering the results of all previously published studies, it is clear that asymptomatic, uninjured patients with normal initial ECG findings do not need continuous ECG monitoring and may be discharged home. Recommendations for high-risk patients and patients with mild ECG abnormalities at admission are less obvious. The conclusions of individual studies of these patients are not consistent. Although, based on our results, even in these cases, new-onset of clinically relevant arrhythmias is unlikely. We found that the pediatric patients did not differ from adults, and there is no reason for special management. No predictive factors for malignant arrhythmias were found in this study. Elevations of cardiac troponins were sporadically observed and we did not observe any statically significant association of arrhythmias and elevated troponin levels. Determination of cardiac troponins is not necessary for all patients and should be indicated individually. In patients with arrhythmias or cardiac conduction abnormalities, prudent testing for ion disbalances is called for and adjusted as needed.

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Data availability The datasets generated and analyzed during the current study are available from corresponding author on reasonable request.

Declarations

Conflict of interest All authors declare that they have no conflicts of interest.

Ethical approval This retrospective study was performed in conformity with the Helsinki Declaration.

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FGF-23 is a biomarker of RV dysfunction and congestion in patients with HFrEF

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There is no biomarker reflecting right ventricular dysfunction in HFrEF patients used in clinical practice. We have aimed to look for a circulating marker of RV dysfunction employing a quantitative proteomic strategy. The Olink Proteomics Multiplex panels (Cardiovascular Disease II, III, Cardiometabolic, and Inflammation Target Panels) identified FGF-23 to be the most differentially abundant (more than 2.5-fold) in blood plasma of HF patients with severe RV dysfunction (n = 30) compared to those with preserved RV function (n = 31). A subsequent ELISA-based confirmatory analysis of circulating FGF-23 in a large cohort of patients (n = 344, 72.7% NYHA III/IV, LVEF 22.5%, 54.1% with moderate/severe RV dysfunction), followed by multivariable regression analysis, revealed that the plasma FGF-23 level was most significantly associated with RV dysfunction grade ($p = 0.0004$) and congestion in the systemic circulation ($p = 0.03$), but not with LV-ejection fraction ($p = 0.69$) or estimated glomerular filtration rate (eGFR, $p = 0.08$). FGF-23 was associated with the degree of RV dysfunction in both sub-cohorts (i.e. in patients with and without congestion, $p < 0.0001$). The association between FGF-23 and RV-dysfunction remained significant after the adjustment for BNP ($p = 0.01$). In contrast, when adjusted for BNP, FGF-23 was no longer associated with LV dysfunction ($p = 0.59$). The Cox proportional hazard model revealed that circulating FGF-23 was significantly associated with adverse outcomes even after adjusting for BNP, LVEF, RV dysfunction grade and eGFR. Circulating FGF-23 is thus a biomarker of right ventricular dysfunction in HFrEF patients regardless of congestion status.

Right ventricular dysfunction is a major complicating condition of heart failure (HF) and is associated with poor prognosis[1]. Unfortunately, no reliable biomarker reflecting RV dysfunction is currently available. Established biomarkers used in cardiovascular medicine for diagnostic and prognostic purposes capture distinct pathophysiological mechanisms; cardiac troponins reflect myocardial damage while natriuretic peptides mirror myocardial stress[2]. However, these biomarkers inform on both left ventricular (e.g. acute coronary syndrome) as well as right ventricular (e.g. pulmonary embolism) pathology[3,4].

Right ventricle is a low-pressure pump that operates in a relatively narrow zone of pressure changes and its dysfunction might be associated with the release of specific proteins that could be detectable in the circulation[5]. Biomarkers reflecting RV dysfunction could mirror HF progression, herald clinical worsening and increased risk of decompensation; such a biomarker could thus assist in tailoring appropriate HF therapy. RV function plays an especially important role in patients undergoing LV-mechanical circulatory support implantation[6], and a biomarker mirroring RV function could also assist in pre-LVAD or post-LVAD patient management.

The aim of the study was to identify a new circulating biomarker of RV dysfunction. Multiplexing proteomic analysis based on the proximity extension assay (Olink Proteomics, Uppsala, Sweden) with predefined panels of selected proteins was used to screen patient plasma samples. ELISA was used for confirmation in a large cohort of HF patients.

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Methods

Patients

Patients with stable HFrEF (LVEF < 40%) of at least 6 months duration were enrolled in the study between 2008 and 2011 in a prospectively defined registry. Patients enrolled in the study were those that were electively hospitalized at the Institute for Clinical and Experimental Medicine in Prague for transplant eligibility evaluation or device implantation. These patients were screened and those receiving stable and optimized medical therapy were enrolled. Patients had to have at least 6-month history of HFrEF and had to receive stable medical therapy for at least three months. Subjects with potentially reversible LV dysfunction (planned valve surgery, revascularization, or tachycardia-induced cardiomyopathy) were excluded. Upon study enrollment, patients completed a Minnesota Living with Heart Failure questionnaire (MLHFQ), underwent clinical assessment (physical examination), echocardiography (Vivid-7 and Vivid-9, General Electric, Milwaukee, Wisconsin) and blood sample collection.

Although only stable patients were enrolled, some of them showed signs of congestion in systemic or pulmonary circulation. Systemic congestion was present if patients had any of the following- enlarged jugular veins, lower limb edema, enlarged liver, positive hepatojugular reflux. Congestion in pulmonary circulation was assessed by auscultation or by chest X-ray evaluation by the attending physician. Patients with self-reported congestion were identified as those that responded to question 1 of MLHFQ with > 2 points (during the past month, has your heart problem prevented you from living as you wanted because it caused swelling in your ankles or legs?).

Left ventricular size and function were assessed according to published guidelines[7]. Right ventricular function was assessed semi-quantitatively (normal RV function, mild, moderate and severe RV dysfunction) in an apical 4-chamber view by using tricuspid annular systolic excursion (M-mode TAPSE) and tissue systolic velocity (Sm) with the following cutoffs: RVD0, normal: TAPSE > 20 mm, Sm > 12 cm/s; RVD1, mild impairment: TAPSE 16 to 20 mm, Sm 9 to 12 cm/s; RVD2, moderate: TAPSE 10 to 15 mm, Sm 6 to 9 cm/s; and RVD3, severe: TAPSE < 10 mm, Sm < 6 cm/s. In case of disagreement of criteria, qualitative visual estimation of RV motion in apical 4-chamber was also taken into account. In a subgroup of patients, RV function was assessed quantitatively as fractional area change (FAC)[8]. Patients were prospectively followed for a median of 3.17 years (IQRs 1.04, 8.05) for the occurrence of an adverse outcome that was defined as the combined endpoint of death, urgent heart transplantation, or ventricular assist device implantation. Due to the fact that time to non-urgent transplantation reflects donor availability rather than recipient's condition, patients who received a non-urgent heart transplant were censored as having no outcome event at the day of transplantation, as reported before[9]. In most cases, the information about the outcome was derived from internal records of the Institute for Clinical and Experimental Medicine (most patients were followed in our hospital). Whenever the information about the outcome was missing, National Institute of Health Information and Statistics was contacted. This institution maintains information about the living status of all Czech citizens.

All research was performed in accordance with relevant guidelines/regulations, the protocol was approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine and the Thomayer University Hospital, and all subjects signed an informed consent.

Discovery (Olink) cohort

A quantitative RV function assessment with FAC was performed in 122 HFrEF patients that were subsequently divided into quartiles according to the FAC. Subgroups of 31 patients with severe RV dysfunction (1st quartile of FAC) and 30 patients with preserved RV function (4th quartile of FAC) were used for Olink analysis together with 24 age, sex and body mass index—matched controls. Specimens were collected in EDTA–anticoagulated tubes (Vacuette, Greiner Bio-One, Austria), centrifuged at 2200 g for 10 min, collecting plasma which was stored at − 80 °C. Plasma samples were tested by the proximity extension assay using the Cardiovascular Disease II, Cardiovascular Disease III, Cardiometabolic, and Inflammation Target Panels (Olink Proteomics, Uppsala, Sweden, www.olink.com).

Confirmatory (ELISA) cohort

A subsequent cohort of 344 HF patients was used for ELISA-based verification analysis. FGF-23 levels were measured using the C-terminal human FGF-23 enzyme-linked immunosorbent assay (Immutopics, San Clemente, California). The interassay coefficients of variation were 11.8% at 29.3 relative units (RU)/ml and 5.6% at 285 RU/ml. BNP was measured by chemiluminescent microparticle immunoassay (CV 4.5%; Architect-BNP; Abbott).

Statistical evaluation

Olink: data were analyzed using R (version 4.1.0). Data were analyzed using packages *ggplot*, *multcomp* and *lmer*. Data were preprocessed according to Olink instructions. Briefly, all Cq values, which were higher than Cq values for negative control were replaced by values for negative control. Data were recalculated into relative quantities and log2 scaled to get data with normal distribution. The linear model was established for group comparison and contrast were used to find the difference between HFrEF patients and controls and between HFrEF patients with severe RV dysfunction and those with preserved RV function. FDR-adjusted p-value was used to determine statistical significance to eliminate multiple comparison error. Pearson correlation coefficient was calculated for FGF-23 abundances determined in Cardiovascular II and Inflammation panels. ELISA-based FGF-23 data analysis was performed using JMP 11 (SAS Institute Inc, Cary, NC). Data in tables and figures are presented as mean ± standard deviation, median with interquartile ranges (IQRs), or frequency (percent) as appropriate. Unpaired t-test or Mann–Whitney test were used to compare continuous variables between groups as appropriate. Cox´s univariable and multivariable model were used to test the effect of analyzed variables on prognosis.

Results

Discovery cohort (Olink proteomics multiplex panels)

In order to identify a circulating biomarker of RV dysfunction we determined relative abundances of 358 proteins in blood plasma samples of HF patients with severe RV dysfunction (n = 31), HF patients with preserved RV function (n = 30) and age/body size matched controls (n = 24) using Olink Target proteomic analysis. Olink Target is a biomarker platform that uses Proximity Extension Assay (PEA) technology combined with qPCR readout to determine relative abundances of selected proteins. Four most relevant Olink panels (Cardiovascular Disease II, Cardiovascular Disease III, Cardiometabolic, and Inflammation Target Panels were used; each of the panel analyzes 92 proteins with a minor overlap of proteins that are included in both panels. Altogether relative abundances of 358 proteins were determined using the four panels. Characteristics of patients and controls is given in Table 1 in the Online Supplement. NT-proBNP, BNP and Fibroblast growth factor-23 (FGF-23) were found to be the most differentially abundant proteins in HF patients compared to controls (Fig. 1A), adjusted $p < 0.0001$. A comparison between patients with preserved RV function and severe RV dysfunction showed FGF-23 to be the most differentially increased protein (> 2.6-fold, adjusted $p = 0.07$, unadjusted $p = 0.006$ Fig. 1B). The complete list of all analyzed proteins is given in Tables 2 and 3 in the Online supplement.

While the selected Olink panels included mostly unique sets of proteins, FGF-23 was captured in both the Cardiovascular II and Inflammation panels, which allowed us to correlate the FGF-23 measurements. Correlation between FGF-23 assessed in both panels was very good (r = 0.99). These results justified a subsequent ELISA-based verification of FGF-23 plasma levels in a larger cohort of patients.

Confirmatory cohort (ELISA-based FGF-23 analysis)

Patients

A cohort of 344 patients (84.9% males, 72.7% NYHA III/IV, LVEF 22.5%) with advanced HFrEF were enrolled in the study. A subgroup of 186 patients (54.1%) had moderate or severe RV dysfunction. Patients received a high degree of guideline-directed pharmacotherapy and device therapy—92.7% were treated with beta-blockers, 87.5% with ACEi/ARB and 56.7% with implantable cardioverter-defibrillator (ICD), Table 1. During a follow-up of 3.17 years (IQRs 1.04, 8.05), 247 patients (71.8%) experienced an adverse outcome (death, urgent heart transplantation, mechanical support implantation).

FGF-23 and RV dysfunction

Plasmatic FGF-23 concentration determined by ELISA in our cohort ranged from 26.33 to 4451 ng/l with a median of 146.62 ng/l (IQRs 85.64; 352.49). FGF-23 abundance was associated with increasing RV dysfunction grade (Fig. 2A). Regression analysis identified a significant association between FGF-23 and LV-ejection fraction, RV dysfunction grade, congestion (both in the systemic and pulmonary circulation), estimated glomerular filtration rate (eGFR) and glycated hemoglobin level (Hb1Ac, both $p < 0.05$). However, multivariable regression analysis identified that only RV dysfunction grade and congestion in the systemic circulation were most significantly associated with FGF-23 levels (Table 2). In order to investigate the relationship between congestion and RV dysfunction, we have divided the cohort according to the presence or absence of congestion in the systemic circulation (n = 84 and 260, respectively). In both subgroups, FGF-23 significantly increased with increasing RV dysfunction grade (Fig. 2B, C). Since the degree of congestion might vary over time, we investigated the impact of self-reported severity of congestion. Subjective perception of congestion was reported by 98 patients, 217 patients were without the subjective perception of congestion (information was missing in 29 patients). Similarly to patients with or without objective signs of congestion, FGF-23 progressively increased with increasing RV

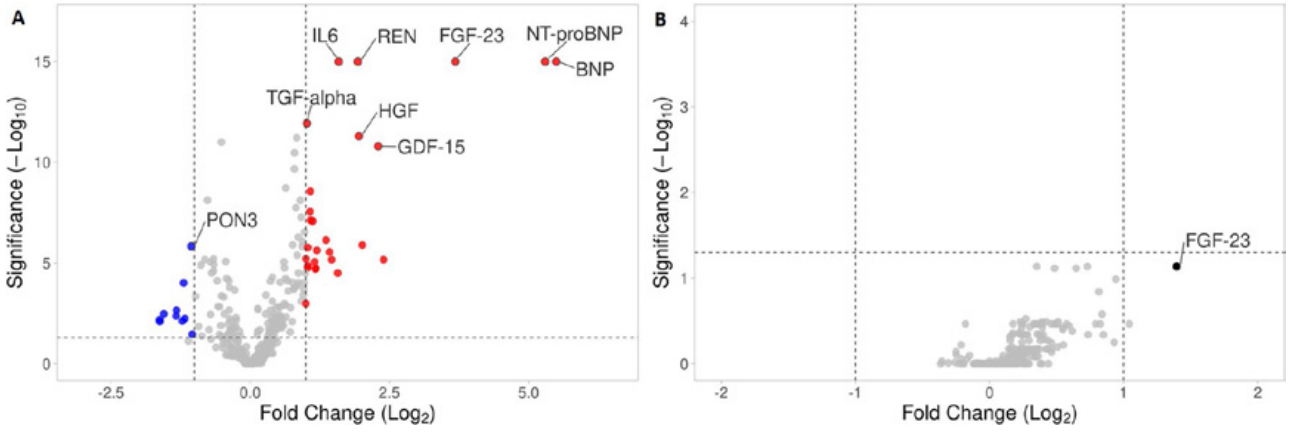

Figure 1. Olink proteomic analysis—Volcano plots showing relative abundances of plasmatic proteins. (**A**) A comparison between HFrEF patients and controls. NT-proBNP, BNP and FGF-23 were among the most markedly and significantly upregulated proteins in HFrEF patients while PON-3 (Serum paraoxonase/lactonase 3) was the most significantly downregulated (**B**) A comparison between HFrEF patients with and without RV dysfunction. FGF-23 was the most markedly upregulated in patients with HFrEF and severe RV dysfunction compared with those with HFrEF and preserved RV function.

	Whole cohort (n = 344)	Preserved RV function (n = 72)	Mild RV dysf (n = 86)	Moderate RV dysf (n = 131)	Severe RV dysf (n = 55)	P for trend
Age (years)	57.55 ± 10.36	56.06 ± 10.97	59.41 ± 8.98	57.67 ± 10.93	56.34 ± 9.93	0.96
Males (%)	84.9	70.8	81.4	90.8	94.5	**< 0.0001**
HF etiology (% CAD)	55.8	51.4	53.5	59.8	58.2	0.29
BMI (kg. m^{-2})	27.79 ± 4.77	28.71 ± 4.57	28.48 ± 4.95	26.85 ± 4.48	27.76 ± 5.15	**0.03**
NYHA (2–4,%)	27.3/66.3/6.4	31.9/66.7/1.4	33.7/59.3/7.0	25.2/65.6/9.2	16.4/78.2/5.5	**0.02**
BNP (ng. l^{-1})	568.3 (281.2; 1205.8)	225.9 (116.4; 456.2)	400.4 (228.6; 976.8)	743.6 (415.7; 1366.1)	994.1 (347.7; 1552.7)	**< 0.0001**
SBP (mmHg)	114.58 ± 18.85	121.25 ± 20.70	117.80 ± 19.80	111.03 ± 16.73	109.29 ± 16.37	**< 0.0001**
Hemoglobin (g. l^{-1})	140.57 ± 16.59	136.14 ± 14.60	141.98 ± 17.36	142.43 ± 16.48	139.71 ± 17.37	0.13
eGFR (ml. min^{-1} .1.73 m^{-2})	69.31 ± 21.88	74.44 ± 25.06	67.25 ± 19.86	69.29 ± 21.97	65.87 ± 19.46	0.06
Hb1Ac (mmol/mol)	49.28 ± 15.83	44.35 ± 11.26	48.06 ± 13.66	52.12 ± 19.34	50.93 ± 13.20	**0.002**
Cardiac morphology and function						
LVEDD (mm)	70.94 ± 9.19	69.47 ± 9.38	70.52 ± 9.90	71.22 ± 8.13	72.80 ± 10.03	**0.04**
LVEF (%)	22.52 ± 5.39	25.52 ± 5.66	23.72 ± 5.44	20.99 ± 4.47	20.36 ± 4.77	**< 0.0001**
RVD1 (mm)	39.26 ± 7.85	34.56 ± 6.02	36.40 ± 5.78	40.61 ± 6.36	46.79 ± 9.48	**< 0.0001**
MiR (1–3, %)	9.9/55.5/34.6	12.5/65.3/22.2	9.3/55.8/34.9	7.6/51.1/41.2	12.7/52.7/34.6	0.07
TriR (1–3, %)	32.1/55.3/12.6	58.3/40.3/1.4	37.6/57.7/4.7	23.1/61.5/15.4	9.4/56.6/34.0	**< 0.0001**
IVC (mm)	19.82 ± 5.79	16.74 ± 3.66	18.89 ± 5.00	20.07 ± 5.64	24.64 ± 6.45	**< 0.0001**
Quality of life						
MLHFQ sum	47.77 ± 22.18	42.37 ± 22.81	43.53 ± 21.98	50.75 ± 21.85	53.55 ± 20.45	**0.0007**
MLHFQ somatic	21.66 ± 9.82	19.66 ± 10.19	20.48 ± 9.81	22.56 ± 9.69	23.70 ± 9.27	**0.009**
MLHFQ emotional	8.35 ± 6.26	8.50 ± 6.74	7.38 ± 6.03	8.89 ± 6.14	8.40 ± 6.25	0.58
MLHFQ Q1 (swelling)	1.57 ± 1.88	1.05 ± 1.62	1.00 ± 1.55	1.86 ± 1.99	2.34 ± 1.93	**< 0.0001**
Comorbidities						
Diabetes (%)	162 (47.1)	17 (23.6)	33 (38.4)	78 (59.5)	34 (61.8)	**< 0.0001**
Stroke/TIA (%)	39 (11.3)	8 (11.1)	8 (9.3)	21 (16.0)	2 (3.6)	0.06
COPD (%)	44 (12.8)	10 (13.9)	9 (10.5)	19 (14.5)	6 (10.9)	0.79
Cancer (%)	9 (2.6)	3 (4.2)	1 (1.2)	4 (3.1)	1 (1.8)	0.63
Peripheral arterial disease (%)	35 (10.2)	5 (6.9)	9 (10.5)	16 (12.2)	5 (9.1)	0.67
Therapy						
ACEi/ARB (%)	87.5	88.9	91.9	87.8	78.2	0.13
ACEi/ARB dose (0–3, %)	12.5/48.5/31.1/7.9	11.1/43.1/33.3/12.5	8.1/50/32.6/9.3	12.2/52.6/30.6/4.6	21.8/43.5/27.5/7.2	0.55
BB (%)	92.7	88.9	95.4	92.4	94.6	0.44
BB dose (0–3, %)	7.3/50.2/29.8/12.7	11.1/34.2/38.4/16.3	5.6/47.1/34.7/12.6	7.6/56.4/23.8/12.2	5.4/60.0/25.5/9.1	0.06
MRA (%)	78.5	70.8	79.1	80.9	81.2	0.36
Furosemide daily dose (mg)	80 (40; 125)	60 (40; 80)	60 (40; 125)	80 (40; 125)	120 (60; 125)	**< 0.0001**
ICD any (%)	56.7	61.1	54.7	56.5	54.6	0.84
CRT any (%)	37.8	43.1	29.1	39.7	40	0.26
Outcome						
Death	164 (47.7%)	30 (41.7)	43 (50.0)	66 (50.4)	25 (45.5)	–
Urg. HTx (%)	49 (14.2%)	2 (2.8)	9 (10.5)	23 (17.6)	15 (27.3)	–
Norm. HTx (%)	20 (5.9%)	2 (2.8)	7 (8.1)	9 (6.9)	2 (3.6)	–
MCSi (%)	34 (9.9%)	5 (6.9)	4 (4.7)	15 (11.5)	10 (18.2)	–
Alive with no event	77 (22.4%)	33 (45.8)	23 (26.7)	18 (13.7)	3 (5.5)	–

Table 1. Patients characteristics. ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BB, beta-blocker; BMI, body mass index; BNP, B-type natriuretic peptide; CAD, coronary artery disease; CRT, cardiac resynchronization therapy; eGFR, estimated glomerular filtration rate; Hb1Ac, glycated hemoglobin; HTx, heart transplantation; ICD, implantable cardioverter-defibrillator; IVC, inferior vena cava; LVEDD, left ventricular diameter in diastole; LVEF, left ventricular ejection fraction; MCSi, mechanical circulatory support implantation; MiR, mitral regurgitation; MLHFQ, Minnesota living with heart failure questionnaire; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; RV, right ventricular; RVD_1, right ventricle basal diameter in apical four chamber view; TIA, transient ischemic attack; TriR, tricuspid regurgitation. Beta-blocker and angiotensin-converting enzyme inhibitor/angiotensin receptor blocker dose was evaluated as follows: 0-no dose, 1-low dose (≤ 33% of target dose), 2-intermediate dose (> 33% and ≤ 66% of target dose), and 3-high dose (> 66% of target dose). Significant values are in bold.

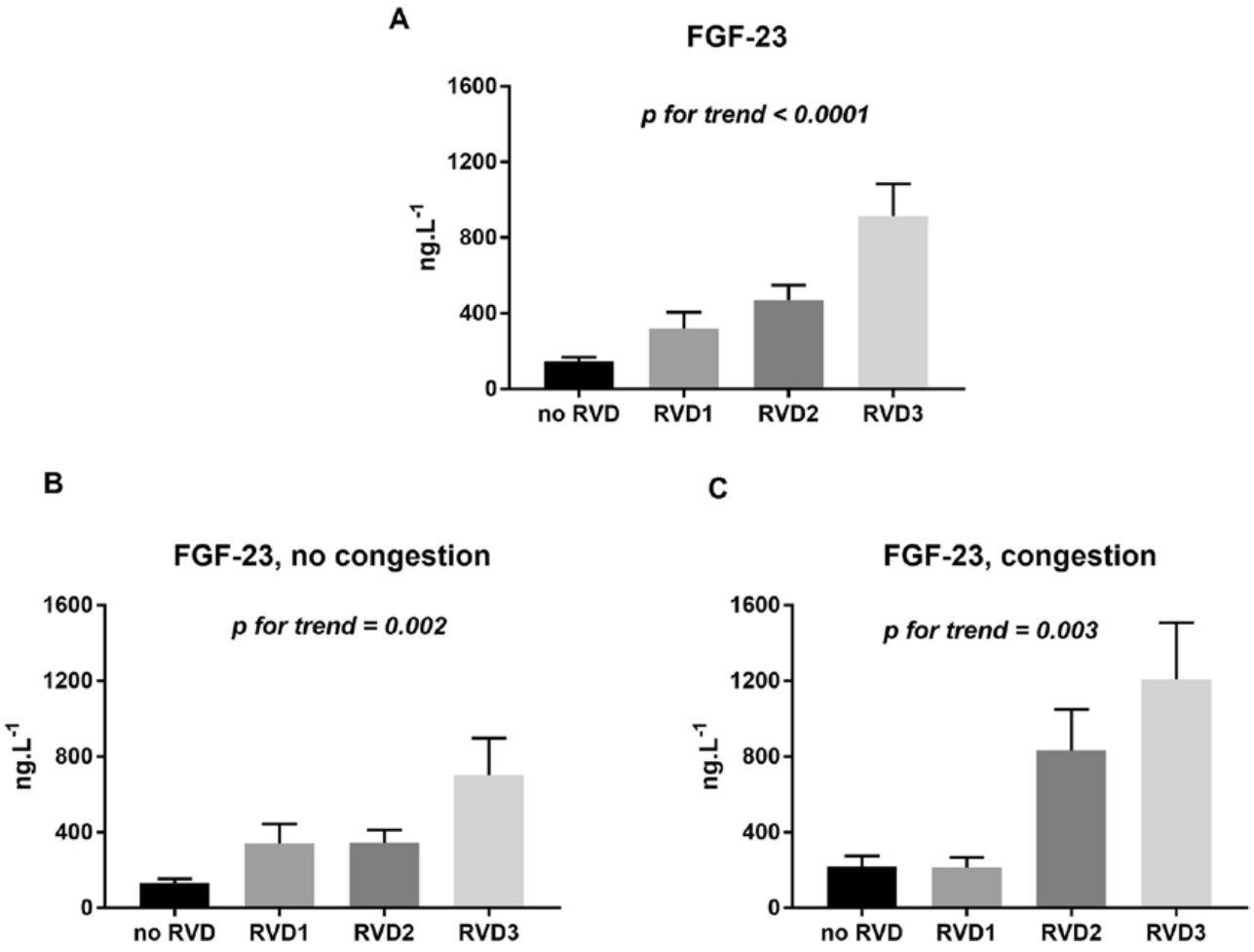

Figure 2. FGF-23 plasma levels determined by ELISA with respect to RV dysfunction grade. (**A**) the whole cohort, (**B**) patients without objective signs of congestion, (**C**) patients with objective signs of congestion Data are presented as mean ± SEM. noRVD—preserved RV function (n = 72), RVD1-mild RV dysfunction (n = 86), RVD2-moderate RV dysfunction (n = 131), RVD3-severe RV dysfunction (n = 55).

Variable	Univariable regression		Multivariable regression
	r^2	*p*	*p*
LVEF (%)	0.014	**0.03**	0.68
RV dysfunction grade (1–4)	0.07	**< 0.0001**	**0.0004**
Congestion in the pulmonary circulation (present vs. absent)	0.02	**0.008**	0.43
Congestion in the systemic circulation (present vs. absent)	0.04	**0.0003**	**0.03**
eGFR (ml. min^{-1} .1.73 m^{-2})	0.02	**0.02**	0.08
Hb1Ac (mmol/mol)	0.01	**0.04**	0.33

Table 2. Parameters associated with FGF-23 level. FGF-23, fibroblast growth factor 23; LVEF, left ventricular ejection fraction; RV, right ventricular; eGFR, estimated glomerular filtration rate; Hb1Ac, glycated hemoglobin. Significant values are in bold.

dysfunction grade independently of subjective perception of congestion (*p* for trend = 0.01 and 0.003, respectively, Fig. 1 and Table 4in the online supplement). Thus, plasmatic FGF-23 levels increased with worsening RV function regardless of congestion in the systemic circulation or subjective perception of congestion.

BNP and FGF-23 with respect to RV and LV dysfunction

BNP is an established biomarker reflecting myocardial wall stress. As patients with worse RV function had also worse LV function (lower LV-ejection fraction), we tried to clarify the informative role of both proteins (BNP,

FGF-23) with respect to LV and RV function. Both BNP and FGF-23 plasma levels significantly increased with worsening of RV function (Table 1, Fig. 2A).

Univariable analysis showed that both BNP and FGF-23 were associated with both left and right ventricular dysfunction (Table 3). When combined together, only BNP but not FGF-23 was associated with the degree of LV dysfunction, but both BNP and FGF-23 were associated with the degree of RV dysfunction. This suggests that BNP level is influenced by the degree of both LV and RV dysfunction, whereas FGF-23 abundance is driven specifically by the degree of RV dysfunction. FGF-23 can thus serve as a biomarker of RV dysfunction.

ROC curve analysis

As both BNP and FGF-23 were associated with RV dysfunction, we have analyzed the potential clinical value of FGF-23 compared to conventionally used BNP for the prediction of severe RV dysfunction. The area under the curve (AUC) for FGF-23 was 0.74 (95%, CI 0.69–0.78), which was marginally higher compared to BNP—0.69 (95% CI 0.64–0.74), $p = 0.29$. Based on ROC curve analysis, a BNP level of 500 ng/L was calculated as the optimal cut-off value for the identification of severe RV dysfunction. The positive predictive value of this cut-off concentration was 25.2% and the negative predictive value (NPV) 94.9%.

The introduction of FGF-23 significantly improved the identification of patients with severe RV dysfunction in our study. AUC of numerical product of BNP and FGF-23 was 0.75 (95%, CI 0.70–0.79), significantly higher that the AUC of BNP alone, $p = 0.02$, Fig. 3. FGF-23 level of 300 ng/L was identified as the optimal cut-off value to distinguish severe RV dysfunction. A combined parameter (BNP > 500 ng/L and FGF-23 > 300 ng/L) improved the positive predictive value for the prediction of severe RV dysfunction. Altogether 42.6% of patients with BNP > 500 ng/L and FGF-23 > 300 ng/L had a severe RV dysfunction; NPV of this combined parameter (91.4%) was comparable to NPV of BNP alone (94.9%).

Outcome analysis

Finally, the Cox proportional hazard model analysis including major confounders was performed to evaluate the impact of FGF-23 on prognosis of patients.

FGF-23 was significantly associated with adverse outcome even after adjustment for BNP, LV-ejection fraction, RV dysfunction grade and eGFR, Table 4.

	LV-ejection fraction		RV dysfunction grade	
	Univariable	Multivariable	Univariable	Multivariable
BNP	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
FGF-23	**0.03**	0.59	**< 0.0001**	**0.01**

Table 3. The relationship of BNP and FGF-23 with respect to LV and RV dysfunction. Only BNP but not FGF-23 was associated with the degree of LV dysfunction, but both BNP and FGF-23 were associated with the degree of RV dysfunction. BNP-B-type natriuretic peptide; FGF-23—fibroblast growth factor 23. Significant values are in bold.

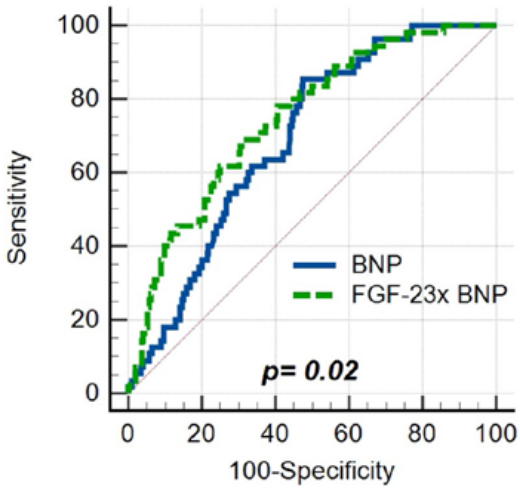

Figure 3. ROC curve analysis. AUC of the numerical product of BNP and FGF-23-0.75 (95%, CI 0.70–0.79) was significantly higher that the AUC of BNP alone – 0.69, (95% CI 0.64–0.74), $p = 0.02$.

Univariable analysis				Multivariable analysis		
	HR	95% CI	*p*	HR	95% CI	*p*
BNP (100 ng/L)	1.06	1.05–1.08	**<0.0001**	1.04	1.03–1.06	**<0.0001**
FGF-23 (100 ng/L)	1.04	1.03–1.06	**<0.0001**	1.02	1.002–1.03	**0.03**
LVEF (5%)	0.80	0.71–0.91	**0.0003**	1.03	0.89–1.18	0.67
RV dysf. grade (1–4)	1.70	1.49–1.94	**<0.0001**	1.49	1.28–1.73	**<0.0001**
eGFR (ml. min^{-1} .1.73 m^{-2})	0.99	0.98–0.993	**<0.0001**	0.99	0.98–0.99	**0.0003**

Table 4. Cox proportional hazard analysis. BNP, B-type natriuretic peptide; FGF-23, fibroblast growth factor 23; LVEF, left ventricular ejection fraction; RV, right ventricular; eGFR, estimated glomerular filtration rate; Hb1Ac, glycated hemoglobin. Significant values are in bold.

Discussion

Various biomarkers (troponins, natriuretic peptides)[10] are used for diagnostic and prognostic purposes in many cardiovascular clinical scenarios (acute coronary syndromes, HF, pulmonary embolism), but currently there is no biomarker specific for RV dysfunction. Proteomic analyses, including the targeted proximity extension-based assay used here, offer a powerful tool for identification of differentially abundant proteins. In our study, fibroblast growth factor-23 (FGF-23) was identified as the strongest upregulated protein in plasma of patients with severe RV dysfunction. Plasmatic FGF-23 level determined by ELISA correlated strongly with the degree of RV dysfunction independent of other possibly confounding variables including subjective perception or objective signs of congestion.

Right ventricle is a low-pressure pump that operates in a relatively narrow zone of pressure changes and its dysfunction might be associated with the release of specific proteins that could be detectable in the circulation[5]. FGF-23 is a 32 kDa secreted protein encoded by the gene *FGF23* located on chromosome 12[11]. FGF-23 acts as endocrine hormone via binding to its receptors FGFR and co-receptor klotho. FGF-23 is involved in phosphate homeostasis; it promotes renal phosphate excretion, decreases the synthesis of 1,25-dihydroxyvitamin D and decreases parathormon (PTH) synthesis[12]. FGF-23 is expressed mainly in osteocytes and osteoblasts in long bones[11]. However, FGF-23 mRNA and protein is also expressed in cardiac myocytes[13–16]. FGF-23 seems to play an important role in cardiac pathophysiology; in vitro it was shown to promote hypertrophy in isolated cardiac myocytes via FGF receptor-dependent activation of calcineurin-NFAT signaling pathway[13,17,18]. In the same study, intramyocardial or intravenous application of FGF-23 resulted in left-ventricular hypertrophy in mice[13]. FGF-23 stimulated proliferation, activation and collagen synthesis in cultured cardiac fibroblasts, while in isolated cardiac myocytes FGF-23 augmented the expression of pro-hypertrophic and pro-inflammatory genes[15].

Circulating levels of FGF-23 were shown to progressively rise with worsening renal function, which is believed to help to maintain serum phosphate levels in physiological ranges[19].

Nevertheless, in patients with HF, FGF-23 was not found to be associated with cardiorenal parameters[20]. We have also observed a weak but significant association between eGFR and FGF-23, but this association was no longer significant when adjusted for RV dysfunction. Previous studies have also reported an association between FGF-23 and LV-ejection fraction[21,22], which was also confirmed in our cohort. Our data suggest that the association between FGF-23 and LVEF is no longer significant when RV dysfunction is taken into account. To our best knowledge, our study is the first study showing the association between FGF-23 and right ventricular dysfunction. Moreover, conventionally used BNP seems to be associated with both LV and RV dysfunction whereas FGF-23 is specific for RV dysfunction only.

Previous studies have documented that plasmatic FGF-23 was also identified as one of the proteins most strongly associated with congestion[23]. It has been shown in experimental settings that peripheral venous congestion was sufficient to cause complex changes in the release of neurohormones and inflammatory mediators[24]. However, this study induced a peripheral congestion by inflating a venous pressure arm cuff. Although peripheral venous congestion mimics notable aspects of the HF congestion phenotype, congestion in the visceral compartment can be further modified by hypoxia and acidosis in enterocytes, increased gut permeability and inflammation and altered renal hemodynamics[24]. We have confirmed and further extended these findings; besides its association with congestion, FGF-23 is related to RV dysfunction regardless of congestion status.

In contrast to FGF-23, BNP (well established marker of HF) was found to reflect the degree of both LV and RV dysfunction. Both LV as well as RV cardiomyocytes are capable of BNP production with a rapid and significant dynamics[10]. Moreover, myocardial stress can reflect not only the degree of RV dysfunction, but RV enlargement as well[25].

The reasons why plasmatic FGF-23 levels increase in HFrEF patients is unclear and may include multiple mechanisms. Congestion in systemic circulation leads to visceral hypoxia and altered renal hemodynamics, so extra-cardiac production of FGF-23 is conceivable. However, since the association between FGF-23 and RV dysfunction was present even in patients with no overt congestion (or subjective perception of congestion) direct production of FGF-23 by diseased heart is possible as well. Although cardiomyocytes have been shown to be capable of FGF-23 production[14,16,26], a direct proof that the cardiac tissue or specifically the right ventricle is responsible for FGF-23 production is lacking. This remains to be elucidated by an analysis of fresh or fixed tissue samples or by FGF-23 measurement of blood samples derived from the coronary sinus.

The relationship between FGF-23 level and the degree of RV dysfunction is likely to be non-linear. In patients without congestion, subjects with mild and moderate dysfunction had comparable levels of FGF-23. Similarly,

in patients with congestion, subjects with preserved RV function and mild RV dysfunction had comparable levels of FGF-23. Nevertheless, FGF-23 level progressively increased with increasing degree of RV dysfunction grade in both subgroups.

Taken together, since we detected a strong association between FGF-23 and RV dysfunction that is independent of BNP levels and LV-ejection fraction, we propose that FGF-23 may serve as a novel biomarker of RV dysfunction.

Limitations

Patients were treated not only conservatively (i.e. by optimal pharmacotherapy and ICD/CRT device implantation), but some underwent also heart transplantation or implantation of mechanical circulatory support, which may bias outcome analysis. In addition, it is a single-centre study with a substantial predominance of male patients. Our study cohort included rather young patients with advanced HF but without multiple comorbidities. Consequently, the results might not be fully applicable to patients with milder HF or to older patients. RV dysfunction was assessed semiquantitatively, fractional area change (FAC) as a continuous variable that could be correlated with FGF-23 level was available only in a subgroup of patients. Patients in the confirmatory cohort were enrolled before ARNi and SGLT2i became used for HFrEF treatment. It remains to be investigated whether FGF-23 is a marker of RV dysfunction specific for HFrEF or if it reflects RV dysfunction in other clinical scenarios (HFpEF, pulmonary artery hypertension). The C-terminal human FGF-23 enzyme-linked immunosorbent assay was used in the study. This assay captures both intact (full length) as well as cleaved FGF-23. There are many conditions, however, that impact FGF-23 peptides separately[27]. Therefore, we are not able to distinguish an increased level of FGF-23 due to increased production from increased cleavage. For example iron deficiency, a common finding in HF, upregulates FGF-23 production but also increases cleavege which results in higher levels of c- terminal FGF-23 while the intact form of FGF-23 stays the same.

Conclusion

Circulating FGF-23 is a biomarker of right ventricular dysfunction in HFrEF patients regardless of congestion status.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

J.B., V.M. and J.P. designed the experiment, J.B., K.K. and M.K. enrolled the patients, M.K. and K.K. performed echocardiography, J.P. and P.J. performed the laboratory analysis. V.N. was responsible for statistical analysis, J.K. supervised the project. J.B., V.M. and J.P. wrote the manuscript, all authors reviewed the manuscript.

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Competing interests

Josef Kautzner is a member of Advisory Boards for Boehringer Ingelheim, Biosense Webster, Medtronic and St Jude Medical (Abbott). He has received speaker honoraria from the above-mentioned companies and from Biotronik, Cath Vision, Pfizer and Pro Med CS. Petr Jarolim received research support from Abbott Laboratories, Amgen, Inc., AstraZeneca, LP, Daiichi-Sankyo, Inc., GlaxoSmithKline, Merck & Co., Inc., Regeneron, Roche Diagnostics Corporation, and Siemens Healthineers. Remaining authors do not possess Competing Interest.

Additional information

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Original Article

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Endothelin type A receptor blockade attenuates aorto-caval fistula-induced heart failure in rats with angiotensin II-dependent hypertension

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Objective: Evaluation of the effect of endothelin type A (ET_A) receptor blockade on the course of volume-overload heart failure in rats with angiotensin II-dependent hypertension.

Methods: Ren-2 renin transgenic rats (TGR) were used as a model of hypertension. Heart failure was induced by creating an aorto-caval fistula (ACF). Selective ET_A receptor blockade was achieved by atrasentan. For comparison, other rat groups received trandolapril, an angiotensin-converting enzyme inhibitor (ACEi). Animals first underwent ACF creation and 2 weeks later the treatment with atrasentan or trandolapril, alone or combined, was applied; the follow-up period was 20 weeks.

Results: Eighteen days after creating ACF, untreated TGR began to die, and none was alive by day 79. Both atrasentan and trandolapril treatment improved the survival rate, ultimately to 56% (18 of 31 animals) and 69% (22 of 32 animals), respectively. Combined ACEi and ET_A receptor blockade improved the final survival rate to 52% (17 of 33 animals). The effects of the three treatment regimens on the survival rate did not significantly differ. All three treatment regimens suppressed the development of cardiac hypertrophy and lung congestion, decreased left ventricle (LV) end-diastolic volume and LV end-diastolic pressure, and improved LV systolic contractility in ACF TGR as compared with their untreated counterparts.

Conclusion: The treatment with ET_A receptor antagonist delays the onset of decompensation of volume-overload heart failure and improves the survival rate in hypertensive TGR with ACF-induced heart failure. However, the addition of ET_A receptor blockade did not enhance the beneficial effects beyond those obtained with standard treatment with ACEi alone.

Keywords: endothelin system, hypertension, Ren-2 renin transgenic rat, renin–angiotensin system, volume-overload heart failure

Abbreviations: ACE, angiotensin-converting enzyme; ACF, aorto-caval fistula; ACEi, angiotensin-converting enzyme inhibitor; ANG II, angiotensin II; ANG 1–7, angiotensin-(1–7); $(+dP/dt)_{max}$, maximum rates of pressure rise; $(-dP/dt)_{max}$, maximum rates of pressure fall; ESPVR, end-systolic pressure–volume relationship; ET_A, endothelin type A; ET-1, endothelin 1; HanSD, Hannover Sprague-Dawley rats; LV, left ventricle; LVEDP, left ventricle end-diastolic pressure; LVEDV, left ventricle end-diastolic volume; PRSW, preload recruitable stroke work; RAAS, renin–angiotensin–aldosterone system; RV, right ventricle; SNS, sympathetic nervous system; TGR, Ren-2 renin transgenic rats; TPR, total peripheral resistance

INTRODUCTION

Over the past 40 years, substantial progress has been made in the treatment of acute coronary syndromes. However, many surviving patients still develop substantial myocardial damage eventually leading to heart failure [1]. Heart failure has become a major public health problem [2,3]; despite the availability of multiple therapeutic measures and recent pharmacological advances, the prognosis remains bleak [2,4–7]. Inappropriately activated renin–angiotensin–aldosterone system (RAAS) is crucial for the progression of heart failure and blockade thereof has become a cornerstone component of the treatment. However, in the advanced phase of heart failure its effectiveness is limited [2,6–9], which was conspicuous in patients who had been hypertensive before the onset of

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heart failure [10–12]. Remarkably, in heart failure induced by volume overload, RAAS inhibition did not attenuate eccentric remodeling of the left ventricle (LV) or improve its systolic function [13–15]. Therefore, new therapeutic strategies for the treatment of heart failure are urgently needed and should be preceded by focused experimental studies [6,16].

It has long been proposed that persistent inappropriate activation of various neurohormonal systems underlies the progression of heart failure ('neurohormonal model of heart failure pathophysiology' [17–19]). More recently considerable attention was focused on the endothelin system and its most important peptide: endothelin-1 (ET-1) [20]. ET-1 via endothelin type A (ET_A) receptors induces vasoconstriction; activation of endothelin type B receptors leads to vasodilatation and natriuresis. Inappropriate activation of ET_A receptors is thought important in the pathophysiology of cardiovascular and renal diseases [20–25]. The endothelin system in the kidney and heart was shown to be markedly activated in animals with heart failure [26,27], and its prolonged upregulation proved maladaptive [22,24,25].

Therefore, the upregulated endothelin system might be an important target for therapeutic intervention in heart failure [22,24,25]. Indeed, Sakai *et al.* [28] reported that in heart failure post myocardial infarction long-term ET_A blockade improved the survival rate, an analogy to the improvement obtained with angiotensin-converting enzyme (ACE) inhibition which resulted in the introduction of angiotensin-converting enzyme inhibitor (ACEi) as a gold standard therapy of heart failure [29]. However, application of the endothelin system blockade yielded controversial results [21,22,30–32], and the effects in heart failure patients, admittedly receiving nonselective endothelin receptor antagonist (bosentan) or presumably selective ET_A antagonist (darusentan) were disappointing: early fluid retention actually leading to worsening of heart failure was a common finding [33–35]. In the landmark ENABLE study (Endothelin Antagonism with Bosentan and Lowering of Events) [36] endothelin receptor antagonist treatment was not recommended in heart failure patients; however, the pertinent experimental studies should continue [37]. Evidently, the effects of genuinely selective ET_A receptor blockade on the natural course of heart failure have not yet been evaluated [21–25]. The availability of orally active and indisputably selective ET_A receptor antagonist, atrasentan [21], enables exploration of this issue [21,22,25,38–41].

The rat model of volume overload induced by the creation of the aorto-caval fistula (ACF) reasonably well mimics human heart failure [13,15,42–47] and is officially recommended for preclinical studies [48,49]. The Ren-2 renin transgenic rat (TGR) model combines endogenous activation of the RAAS and hypertension [50,51], the two factors critical for the progression of heart failure [18,19,52,53]. We have shown that TGR with ACF exhibited markedly enhanced heart failure-related mortality [15,45,47]. Taking advantage of such suitable experimental research models and the availability of a highly selective ET_A receptor antagonist, we evaluated the effects of chronic atrasentan treatment on morbidity and mortality in ACF TGR.

To explore in more detail a possible role of interaction of the RAAS and endothelin system and sympathetic nervous system (SNS) [18,54–56] in the pathophysiology of ACF-induced heart failure, kidney tissue concentrations of angiotensin II (ANG II), ET-1 and norepinephrine were measured. In addition, in critical time-points of the experiments, we assessed the cardiac structure and function, using echocardiography and invasive pressure–volume analysis of the LV.

METHODS

Ethical approval, animals, heart failure model, and pharmacological therapeutic regimes

The studies were performed in accordance with guidelines and practices established by the Animal Care and Use Committee of the Institute for Clinical and Experimental Medicine, Prague, which accord with the European Convention on Animal Protection and Guidelines on Research Animal Use and approved by the Ministry of Health of the Czech Republic (project decision 26306/2020-4/OVZ). Heterozygous TGR were generated by breeding male homozygous TGR with female homozygous Hannover Sprague-Dawley (HanSD) rats. Male TGR and HanSD rats, at the initial age of 9 weeks, derived from several litters, were randomly assigned to experimental groups to make sure that the animals from a single litter did not prevail in any group. To obtain reliable data regarding the effects of two treatment regimens on the survival rate, high initial *n* values were used (not so for sham-operated animals) to enable a valid comparison of the long-term survival rate. Such required n values were established using the statistical power analysis method developed by Cohen [57].

Rats were anesthetized (tiletamine + zolazepam, Virbac SA, Carros Cedex, France, 8 mg/kg; and xylasine, Spofa, Czech Republic, 4 mg/kg intramuscularly) and heart failure was induced by volume overload caused by ACF created using needle technique as employed and validated by many investigators, including our own group [13,15,42–47,58,59].

Trandolapril (2 mg/l in drinking water; Gopten; Abbott, Prague, Czech Republic), was used to inhibit ACE because in our previous studies and here in preliminary experiments we demonstrated that at this dose the ACEi, trandolapril, provided maximal blockade of the RAAS and was well tolerated both by rats with ACF-induced heart failure and by sham-operated animals [15,47,59]. ET_A receptor blockade was achieved with atrasentan (5 mg/kg per day in drinking water; Abbott, Illinois, USA). The dose of atrasentan was adjusted weekly to actual water intake; such dosage was previously found to effectively block ET_A receptors [39,40].

Detailed experimental design

The whole experimental design of the study, with a presentation of the detailed time sequence of experimental maneuvers and different treatment regimes, is given in Fig. 1.

Series 1: Effects of treatment with endothelin type A receptor antagonist and angiotensin-converting enzyme inhibitor, alone or combined, on the survival rate and albuminuria

Animals underwent either sham-operation or ACF creation and were left without treatment for 2 weeks. At this time point (day 0) they were assigned to the following experimental groups:

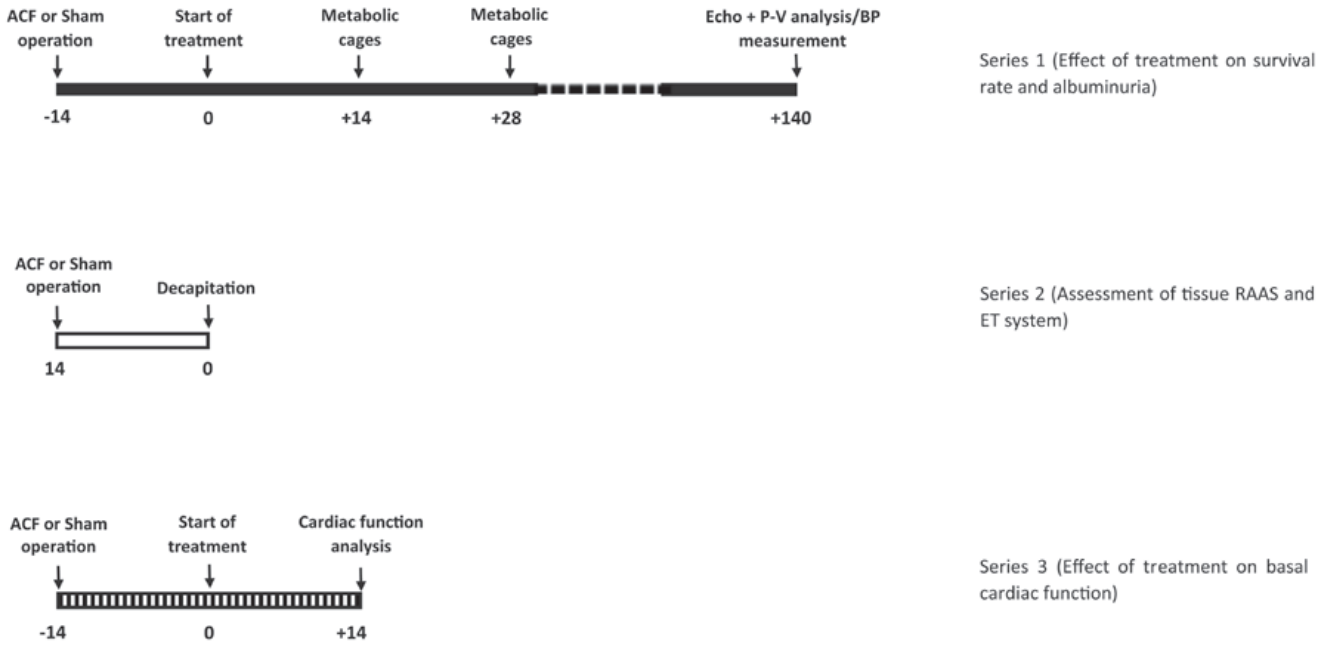

FIGURE 1 The experimental design of the whole study, delineating the time sequence of experimental maneuvers and different treatment regimes.

Group 1: Sham-operated HanSD rats + placebo (initial $n = 12$).
Group 2: Sham-operated TGR + placebo (initial $n = 14$).
Group 3: ACF TGR + placebo (i.e., untreated ACF TGR) (initial $n = 30$).
Group 4: ACF TGR + ET_A receptor antagonist (initial $n = 31$).
Group 5: ACF TGR + ACEi (initial $n = 32$).
Group 6: ACF TGR + ACEi + ET_A receptor antagonist (initial $n = 33$).

The follow-up period was 20 weeks. At the end of the experiment (on day +140), the survived rats were anesthetized and echocardiography was performed. Subsequently, LV functions were invasively assessed by employing pressure–volume analysis by techniques and protocols developed and validated for mice and rats by Pacher *et al.* [60]. This method was employed almost 10 years ago in our laboratory and it is routinely used in our studies evaluating cardiac functions in rats. Detailed descriptions can be found in numerous of our previous studies [15,47,61,62]. Briefly, rats were anesthetized with long-term anesthesia (thiopental sodium, 50 mg/kg, intraperitoneally, VAUB Pharma a.s., Roztoky, Czech Republic) commonly used for pressure–volume analysis [60]. Before the pressure–volume analysis, echocardiography was performed. Rats were intubated with a plastic cannula to ensure chest relaxation during the whole operation. The left jugular vein was cannulated for securing central venous access for solutions administration as required. A balloon catheter (LeMaitre Single Lumen Embolectomy Catheter, 2F, Burlington, Massachusetts, USA) was inserted under ultrasonic control via the right jugular vein to the vena cava inferior, below the diaphragm to maintain the best position for preload reduction. Just before the pressure–volume measurement of the LV, the conductance and pressure signals of the Millar pressure–volume catheter (Millar, 2F, Houston, Texas, USA) were calibrated using MPVS software (V2.2, Millar) according to the manufacturer's instructions. Functions of the LV were invasively assessed by a pressure–volume catheter introduced into the LV via the right carotid artery as described in previous studies [15,47,61,62]. For basal measurements, pancuronium (1 mg/kg, intravenously, Inresa Arzneimittel, Freiburg, Germany) was administered through the cannulated left jugular vein and rinsed with a bolus of saline to reduce noisiness in the signal caused by breathing. For effective determination of cardiac functions, the preload reductions were performed by slowly inflating the balloon catheter with aqua pour injection. Volume signal was calibrated by end-diastolic and end-systolic volume obtained shortly before invasive recordings. Data from pressure–volume loops were captured and analyzed in LabChart Pro software (ADInstruments, Bella Vista, New South Wales, Australia).

Series 2: Assessment of angiotensin II, endothelin-1, angiotensin 1–7, and norepinephrine levels and organ weights in the early phase after aorto-caval fistula-induced heart failure

Animals underwent either sham-operation or ACF creation and were left without treatment for 2 weeks and then were killed by decapitation. Whole kidney ANG II, angiotensin 1–7 (ANG 1–7) and norepinephrine levels, and ET-1 concentrations in the kidney cortex, kidney papilla, and lung tissue were measured, as described in our previous studies [38,39,45–47,51,63,64]. The following experimental groups ($n = 11$ each) were investigated:

Group 1: Sham-operated HanSD rats.
Group 2: Sham-operated TGR.
Group 3: ACF TGR.

Series 3: Effects of 2-week treatment with endothelin type A receptor antagonist and angiotensin-converting enzyme inhibitor, alone or combined, on basal cardiac function assessed by echocardiography and by pressure–volume analysis

Animals were prepared as described in series 1 and 2, and at week 0 the pharmacological treatment was applied for a period of 2 weeks. On day +14, the measurements were performed in the following groups:

Group 1: Sham-operated HanSD rats + placebo ($n = 7$).
Group 2: Sham-operated TGR + placebo (initial $n = 7$).
Group 3: ACF TGR + placebo (i.e., untreated ACF TGR) ($n = 10$).
Group 4: ACF TGR + ET_A receptor antagonist ($n = 9$).
Group 5: ACF TGR + ACEi ($n = 9$).
Group 6: ACF TGR + ACEi + ET_A receptor antagonist ($n = 9$).

Statistical analysis

Statistical analysis of the data was performed using GraphPad Prism software (Graph Pad Software, San Diego, California, USA). Comparison of survival curves was performed by log-rank (Mantel-Cox) test followed by Gehan-Breslow-Wilcoxon test. Statistical comparison of other results was made by Student's *t* test, Wilcoxon's signed-rank test for unpaired data, or one-way analysis of variance when appropriate. The values are expressed as the means ± standard error of the mean and n represents the number of animals. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Effects of treatment with endothelin type A receptor antagonist and angiotensin-converting enzyme inhibitor, alone or combined, on the survival rate and albuminuria

All sham-operated HanSD rats and TGR survived until the end of the study, and for clarity of presentation they are omitted from Fig. 2. As shown in Fig. 2a, untreated ACF TGR definitely began to die from day +14 (4 weeks after the creation of ACF), and by day +65 all the animals were dead. ET_A receptor antagonist and ACEi, applied alone, improved survival: the final rate was 56% (18 of 31 animals) and 69% (22 of 32 animals), respectively. With the combined treatment the final survival rate was 52% (17 of 33 animals). The three variants of treatment did not significantly differ in their effectiveness.

At the start of the treatment (2 weeks after sham-operation or creation of ACF), the sham-operated TGR showed about 35-fold higher albuminuria than observed in sham-operated HanSD rats (Fig. 2b). Significantly, the creation of ACF caused a significant about 65% decrease in albuminuria in TGR in this period. Albuminuria modestly but significantly increased throughout the study in sham-operated animals, in parallel with increasing age but, surprisingly, such age-dependent rise was relatively more pronounced in sham-operated HanSD rats. All three treatments reduced albuminuria in ACF TGR, but combined ACE and ET_A receptor blockade was the most effective. Remarkably, in ACF TGR receiving the combined treatment albuminuria was even 22-fold lower than in sham-operated HanSD rats (0.236 ± 0.02 vs. 5.14 ± 0.27 mg/24 h, $P < 0.05$).

Tissue angiotensin II, angiotensin 1–7, norepinephrine, and endothelin-1 levels and organ weights in the early phase after aorto-caval fistula-induced heart failure

Two weeks after the creation of ACF, TGR displayed a further increase in cardiac LV hypertrophy when compared with sham-operated TGR, and marked right ventricle (RV) hypertrophy (Table 1). In addition, ACF TGR displayed substantial lung congestion (increased wet lung weight) without significant differences in body, kidney, and liver weight.

Two weeks after the creation of ACF or sham-operation tissue concentrations of ANG II, ANG 1–7, norepinephrine, and ET-1 were as shown in Fig. 3. Sham-operated TGR showed higher kidney ANG II levels compared with sham-operated HanSD rats (Fig. 3a). Dissimilarly, kidney ANG 1–7 concentrations did not differ (Fig. 3b). Evidently, the intrarenal balance between the vasodilator and vasoconstrictor axes of the RAAS (expressed as the ratio of ANG 1–7 to ANG II) was shifted toward the vasoconstrictor axis. Kidney ANG II levels tended to be higher in ACF TGR (NS), however, the creation of ACF distinctly increased kidney ANG 1–7 levels. This marked increase resulted in a considerable increase in the ANG 1–7/ANG II ratio, up to the level found in sham-operated HanSD rats. There were no significant differences in kidney norepinephrine concentrations between experimental groups (Fig. 3c). Nor were there any significant between-group differences in the concentrations of ET-1 in the kidney cortex, kidney papilla, and lung tissue (Fig. 3d–f).

Effects of 2-weeks' treatment with endothelin type A receptor antagonist and angiotensin-converting enzyme inhibitor, alone or combined, on basal cardiac function assessed by echocardiography and by pressure–volume analysis

Sham-operated TGR displayed whole cardiac and LV hypertrophy as compared with sham-operated HanSD rats (Table 2). In TGR the hypertrophy was slightly but significantly greater than observed at week 0 (Table 1). Untreated ACF TGR displayed, again, bilateral cardiac hypertrophy that strikingly progressed over 2 weeks. The final increase above the values from week 0 (see Table 1) was by 27, 16, and 45% in the case of whole cardiac, LV, and RV hypertrophy, respectively. All treatment regimens substantially attenuated the degree of hypertrophy in ACF TGR; the concurrent lung weight decrease suggested attenuation of lung congestion.

Evaluation of cardiac structure and function by echocardiography showed that sham-operated TGR displayed higher LV anterior and posterior wall thickness and the LV relative wall thickness as compared with sham-operated HanSD rats (Table 3), showing effects of hypertension and

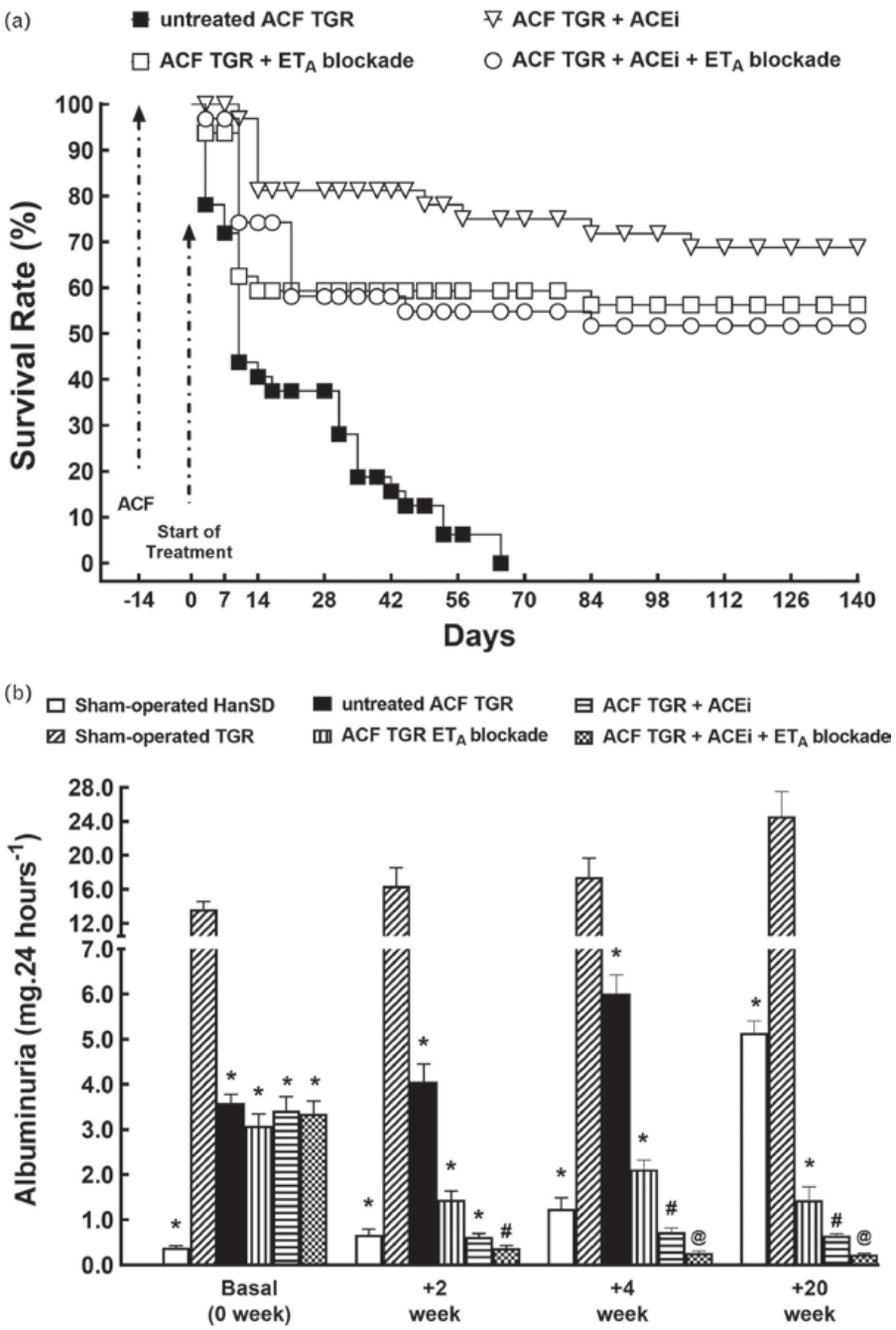

FIGURE 2 Effects of treatment on survival and albuminuria. The survival rate (a) and albuminuria (b) in sham-operated transgene-negative Hannover Sprague-Dawley rats, sham-operated heterozygous Ren-2 renin transgenic rats, and Ren-2 renin transgenic rats with aorto-caval fistula, treated with endothelin type A receptor antagonist, or with angiotensin-converting enzyme inhibitor, alone or combined. $^{*}P < 0.05$ versus sham-operated Ren-2 renin transgenic rats. $^{**}P < 0.05$ versus sham-operated Hannover Sprague-Dawley rats. $^{***}P < 0.05$ versus all other groups.

LV cardiac hypertrophy. Otherwise, there were no structural and functional LV changes or significant differences between sham-operated TGR and sham-operated HanSD rats. Nor were there any significant differences in RV parameters between sham-operated TGR and sham-operated HanSD rats. Untreated ACF TGR had increased stroke volume and cardiac output (a consequence of the shunt), strikingly increased LV and RV diameters, and decreased LV anterior and posterior wall thickness and LV relative wall thickness (indices of eccentric cardiac hypertrophy). In addition, untreated ACF TGR displayed decreased LV ejection fraction and LV fractional shortening as compared with sham-operated TGR (impairment of LV systolic function). In contrast, at this stage, untreated ACF TGR did not show any impairment of RV systolic function as seen from the normal RV ejection fraction. The treatment with ET$_A$ receptor antagonist alone or ACEi alone reduced both the LV and RV diameters but did not change the LV wall thickness and systolic function of the LV in ACF TGR. Notably, combined treatment with ACEi and ET$_A$ receptor antagonists did not

TABLE 1. Organ weights 2 weeks after the creation of the aorto-caval fistula or sham-operation, that is, before initiation of treatment protocols (week 0)

	Group		
	HanSD + water	TGR + water	ACF TGR + water
Tibia length (mm)	38.1 ± 0.3	37.9 ± 0.2	37.8 ± 0.3
Whole heart weight (mg)/tibia length (mm)	34.67 ± 0.64	43.54 ± 0.46*	54.26 ± 0.88**
LV weight (mg)/tibia length (mm)	24.91 ± 0.17	32.74 ± 0.51*	35.53 ± 0.62**
RV weight (mg)/tibia length (mm)	5.93 ± 0.16	6.31 ± 0.29	10.40 ± 0.48**
Lung weight (mg)/tibia length (mm)	44.41 ± 0.86	46.72 ± 1.08	78.79 ± 1.14**
Kidney weight (mg)/tibia length (mm)	37.01 ± 0.76	38.79 ± 0.69	40.08 ± 1.09
Liver weight (mg)/tibia length (mm)	410 ± 13	420 ± 18	421 ± 16

The values are the means ± SEM. ACF, aorto-caval fistula; HanSD, Hannover Sprague-Dawley rats; LV, left ventricle; RV, right ventricle; TGR, Ren-2 renin transgenic rats.
*$P < 0.05$ vs. sham-operated HanSD rats.
**$P < 0.05$ vs. TGR + water.

modify the LV and RV diameters but further decreased the LV anterior and posterior wall thickness and LV relative wall thickness and, unexpectedly, decreased RV ejection fraction.

On the evaluation of cardiac function by the invasive hemodynamics method (Figs. 4 and 5) sham-operated TGR showed, on one side, higher LV peak pressure (Fig. 4a), maximum rates of pressure rise $(+dP/dt)_{max}$ (Fig. 4d) and end-systolic pressure–volume relationship (ESPVR) (Fig. 5a) and of the total peripheral resistance (TPR) (Fig. 5d) and, on the other side, lower maximum rates of pressure fall $(-dP/dt)_{max}$ (Fig. 4e) and LV wall stress (Fig. 5f) as compared with sham-operated HanSD rats. These results are in line with the degree of hypertension and LV cardiac hypertrophy in sham-operated TGR. Untreated ACF TGR displayed significant decreases in LV peak pressure, $(+dP/dt)_{max}$, $(-dP/dt)_{max}$ (Fig. 4d and e), ESPVR, and preload recruitable stroke work (PRSW) (Fig. 5a and c), and increased LV relaxation constant tau (Fig. 4f) as compared with sham-operated TGR. This indicated impairment of load-dependent as well as load-independent LV systolic function and also of the LV diastolic function. Moreover, untreated ACF TGR showed a marked decrease in TPR (Fig. 5d), and particularly prominent increases in LV end-diastolic pressure (LVEDP), LV end-diastolic volume (LVEDV) (Fig. 4b and c), total power output (Fig. 5e) and LV wall stress (Fig. 5f), as compared with sham-operated TGR. Each of the three treatments decreased LVEDP, LVEDV, LV relaxation constant tau, and LV wall stress and increased $(+dP/dt)_{max}$ in ACF TGR but did not alter $(-dP/dt)_{max}$ and ESPVR. ET_A receptor blockade, applied alone or with ACEi, increased PRSW (Fig. 5c) or decreased end-diastolic pressure–volume relationship (Fig. 5b). Finally, only the combined treatment further decreased TPR in ACF TGR (Fig. 5d).

Effects of 20-weeks' treatment with endothelin type A receptor antagonist and angiotensin-converting enzyme inhibitor, alone or combined, on organ weights and cardiac function assessed by echocardiography and by pressure–volume analysis

Table 4 collects organ weights from animals that survived until the end of the study (22 weeks after sham-operation or creation of ACF and after 20-weeks' treatment). The values of whole, LV, and RV weights in the ACF TGR that were treated either with ET_A receptor antagonist alone or with ACEi alone were similar with those in untreated ACF TGR in the early phase (4 weeks after the creation of ACF). On the other hand, the extent of lung congestion was lower than observed in untreated ACF TGR (Table 2). Combined treatment with ACEi and ET_A receptor antagonist in ACF TGR significantly reduced the bilateral cardiac hypertrophy and lung congestion when compared with ACF TGR treated with ET_A receptor antagonist alone or with ACEi alone.

Table 5 presents an evaluation of cardiac structure and function by echocardiography, again, in the animals that survived until the end of the study. Irrespective of the treatment applied, all ACF TGR groups displayed increases in LV and RV diameters that were even greater than those measured in untreated ACF TGR in the early phase (4 weeks after induction of ACF, see Table 3). In addition, in each of ACF TGR treatment groups, the decreases in LV ejection fraction and LV fractional shortening were more pronounced than those observed in the early phase (see Table 3). Moreover, in contrast to the early phase, all ACF TGR groups exhibited impairment of RV systolic function. Significantly, the treatment regimens that included ET_A receptor blockade, alone and combined with ACEi, attenuated the increases in RV diameters and impairment of RV systolic function. Figs. 6 and 7 present an evaluation of cardiac function by the invasive hemodynamics method in animals that survived until the end of the study. All the values for sham-operated HanSD and sham-operated TGR were similar as observed in the early phase (see Figs. 4 and 5). All ACF TGR groups subjected to treatment showed similar load-sensitive values of systolic and diastolic function as well as of load-independent contractile function. Remarkably, the combined treatment with ACEi and ET_A receptor antagonist normalized LVEDP in ACF TGR (Fig. 6b) and brought them to levels observed in sham-operated TGR, however, without affecting LVEDV (Fig. 6c). Moreover, there were no significant differences in TPR, total power output and LV wall stress between ACF TGR groups subjected to different treatments (Fig. 7d–f).

DISCUSSION

We found that both ET_A receptor blockade and ACE inhibition substantially reduced the extremely high heart failure-related mortality in ACF TGR. This agrees with the

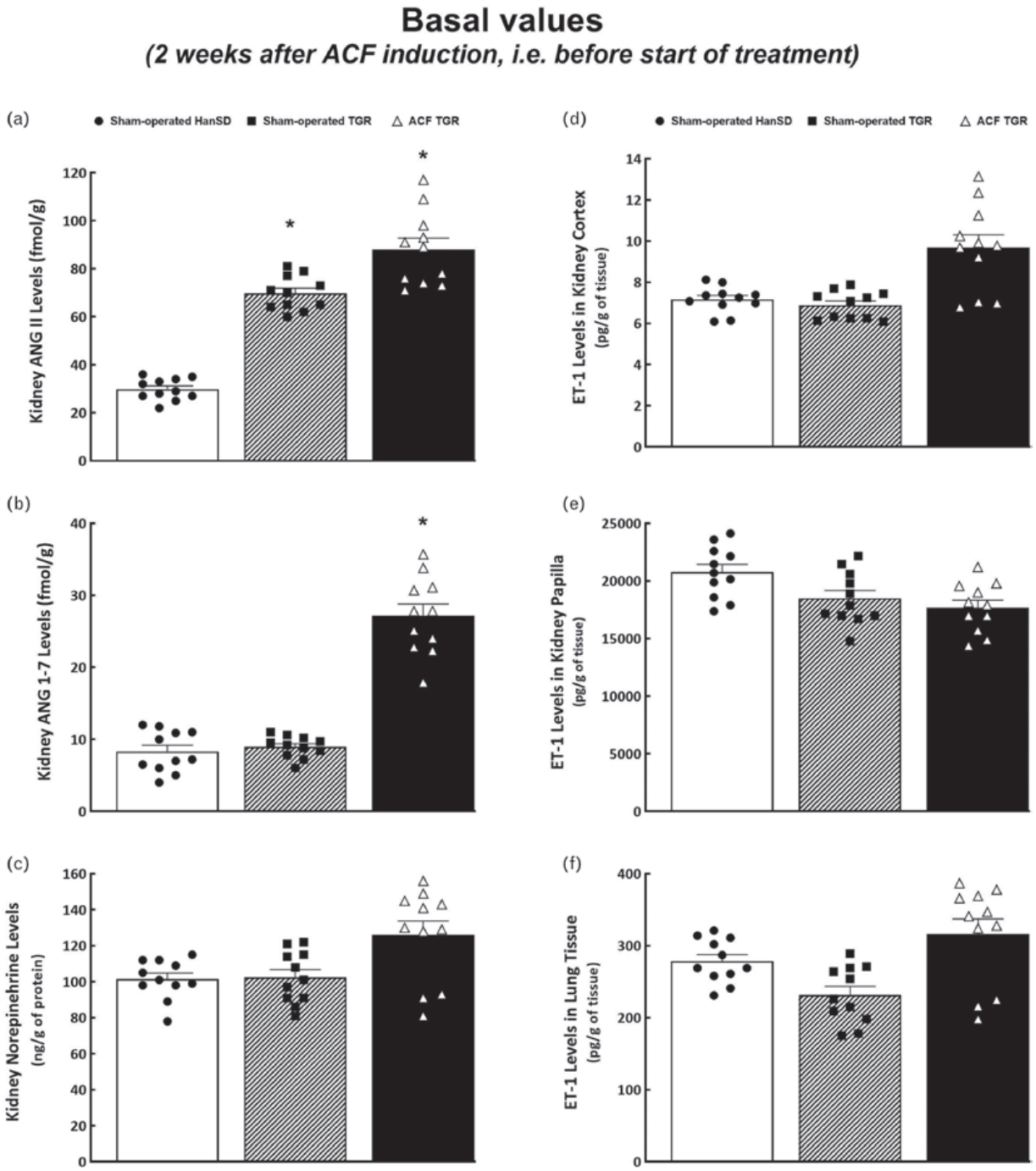

FIGURE 3 Tissue levels of angiotensin II (a), angiotensin 1–7 (b), norepinephrine (c), endothelin-1 (d) in kidney cortex, endothelin-1 in kidney papilla (e), and endothelin-1 in lung tissue (f) in sham-operated transgene-negative Hannover Sprague-Dawley rats, sham-operated heterozygous Ren-2 renin transgenic rats, and Ren-2 renin transgenic rats with aorto-caval fistula 2 weeks after the creation of the aorto-caval fistula or sham-operation. $^{*}P < 0.05$ versus sham-operated Hannover Sprague-Dawley rats.

proposal that the blockade of ET_A receptors might be an important target for therapeutic intervention in heart failure, at least in its volume-overload variant. A similar favorable action of ACEi (improvement of survival, attenuation of albuminuria, etc.) indicates that even though early activation of the vasoconstrictor/sodium retaining axis of the RAAS may be beneficial, its long-term pleiotropic actions are detrimental and contribute to the progression of heart failure.

Since both treatment regimens substantially delayed the heart failure-related morbidity and mortality in ACF TGR, we hypothesized that combined treatment with trandolapril and atrasentan should provide additive protective actions. However, this hypothesis has not been corroborated: the results of the combined therapy did not significantly differ from those of either single treatment. Therefore, one should consider individual aspects of heart failure-related morbidity as related to interaction at various levels of the RAAS and

TABLE 2. Organ weights 4 weeks after the creation of the aorto-caval fistula or sham-operation and after 2 weeks' treatment with endothelin type A receptor antagonist and angiotensin-converting enzyme inhibitor, alone or combined (week + 2)

	Group					
	HanSD + water	TGR + water	ACF TGR + water	ACF TGR + ACEi	ACF TGR + ET_A antagonist	ACF TGR + ACEi + ET_A antagonist
Tibia length (mm)	37.9 ± 0.3	38.1 ± 0.2	37.8 ± 0.2	38.1 ± 0.2	38.1 ± 0.3	37.9 ± 0.2
Whole heart weight (mg)/tibia length (mm)	37.21 ± 0.54	48.47 ± 0.98*	69.03 ± 1.18**	54.33 ± 1.15***	47.91 ± 0.75***	54.79 ± 2.04***
LV weight (mg)/tibia length (mm)	24.12 ± 0.11	35.12 ± 0.91*	41.09 ± 0.92**	33.96 ± 0.68***	30.81 ± 0.59***	35.06 ± 1.35***
RV weight (mg)/tibia length (mm)	7.28 ± 0.19	7.46 ± 0.25	15.09 ± 0.39**	12.05 ± 0.17***	9.92 ± 0.24***	10.75 ± 0.31***
Lung weight (mg)/tibia length (mm)	48.96 ± 1.06	48.52 ± 1.29	75.11 ± 1.38**	61.91 ± 1.27***	57.29 ± 1.29***	61.52 ± 1.33***
Kidney weight (mg)/tibia length (mm)	40.14 ± 0.41	42.19 ± 1.29	40.08 ± 1.09	38.92 ± 1.36	39.14 ± 1.08	38.99 ± 0.91
Liver weight (mg)/tibia length (mm)	437 ± 19	459 ± 17	445 ± 26	439 ± 26	444 ± 23	433 ± 27

The values are the means ± SEM. ACEi, angiotensin-converting enzyme inhibitor; ACF, aorto-caval fistula; ET_A, endothelin type A; HanSD, Hannover Sprague-Dawley rats; LV, left ventricle; RV, right ventricle; TGR, Ren-2 renin transgenic rats.
*$P < 0.05$ vs. sham-operated HanSD rats.
**$P < 0.05$ vs. TGR + water.
***$P < 0.05$ vs. ACF TGR + water.

endothelin systems [65,66]. ANG II can stimulate ET-1 release by various cell types [67–69] and vice versa, ET-1 stimulates ANG II formation [65,70]. Indeed, in TGR after 5/6 renal ablation (5/6 NX), RAAS inhibition decreased ET-1 levels similarly as observed in animals treated with ET_A receptor antagonist [38]. Evidently, some of the beneficial effects of the blockade of one system can also be partially mediated, indirectly, by inhibition of the other system.

Notably, the combined treatment exhibited more pronounced effect on albuminuria and was more effective in reducing bilateral cardiac hypertrophy and lung congestion. Since albuminuria and cardiac hypertrophy are independent risk factors for cardiovascular morbidity and mortality (including heart failure-related mortality) [3,7,10,64,71–73], greater beneficial effects of the combined treatment might provide additional protection. To make this conclusive, studies using long-term treatment protocols (e.g. 40 weeks) are required. Nevertheless, the present results strongly suggest a benefit of dual inhibition of the RAAS and ET_A system in chronic kidney disease, heart failure, and similar disorders. If so, understanding the underlying mechanism(s) is important.

To address this issue, we examined the effects of the three treatments (atrasentan, trandolapril, or both combined) on cardiac function after 2-weeks' treatment, at a time when untreated animals were showing high mortality. Untreated ACF TGR not only exhibited bilateral cardiac hypertrophy and prominent eccentric chamber remodeling but also an impairment of load-sensitive systolic and diastolic function and load-independent contractile function of the LV, which confirmed the view that the ACF-induced model of heart failure well represents heart failure with reduced ejection fraction elicited by chronic volume-overload insult [48,49,74].

TABLE 3. Echocardiographic analysis performed 4 weeks after the creation of the aorto-caval fistula or sham-operation and after 2 weeks' treatment with endothelin type A receptor antagonist and angiotensin-converting enzyme inhibitor, alone or combined (week + 2)

	Group					
	HanSD + water	TGR + water	ACF TGR + water	ACF TGR + ACEi	ACF TGR + ET_A antagonist	ACF TGR + ACEi + ET_A antagonist
Heart rate (s^{-1})	381 ± 16	379 ± 11	369 ± 9	368 ± 8	372 ± 9	377 ± 16
LV diastolic diameter (mm)	6.44 ± 0.12	6.14 ± 0.13	9.89 ± 0.21*	8.74 ± 0.21**	8.54 ± 0.23**	10.53 ± 0.23*
LV systolic diameter (mm)	3.32 ± 0.12	3. 07 ± 0.12	6.01 ± 0.22*	5.22 ± 0.17**	4.97 ± 0.22**	6.22 ± 0.22*
LV anterior wall thickness in diastole (mm)	2.04 ± 0.04	2.82 ± 0.04***	2.11 ± 0.05*	2.06 ± 0.04*	2.01 ± 0.03*	1.66 ± 0.05**
LV posterior wall thickness in diastole (mm)	2.18 ± 0.05	3.17 ± 0.08***	2.29 ± 0.08*	2.29 ± 0.05*	2.15 ± 0.05*	1.86 ± 0.04**
LV relative wall thickness	0.62 ± 0.02	1.05 ± 0.06***	0.42 ± 0.03*	0.44 ± 0.03*	0.51 ± 0.04*	0.35 ± 0.01*
LV ejection fraction (%)	79.2 ± 1.2	80.6 ± 0.9	65.2 ± 0.9*	64.8 ± 1.3*	70.9 ± 1.3*	68.9 ± 1.4*
LV fractional shortening (%)	50.5 ± 1.4	51.5 ± 0.7	38.8 ± 1.1*	39.3 ± 1.1*	42.2 ± 1.2*	41.1 ± 1.5*
LV stroke volume (μl)	149 ± 5.4	146 ± 7.9	384 ± 19*	334 ± 14*	303 ± 14*	373 ± 21*
Cardiac output (ml/min)	61.1 ± 1.5	59.8 ± 3.6	135.7 ± 5.6*	121.5 ± 6.6*	114.6 ± 4.4*	152.9 ± 8.8*
RV diastolic diameter (mm)	3.21 ± 0.07	3.16 ± 0.08	5.71 ± 0.33*	5.02 ± 0.21*	3.94 ± 0.09**	5.75 ± 0.22*
RV systolic diameter (mm)	3.05 ± 0.04	2.55 ± 0.11	5.04 ± 0.31*	3.56 ± 0.21**	3.49 ± 0.07**	5.31 ± 0.19*
RV ejection fraction (%)	57.7 ± 1.7	55.1 ± 2.1	51.1 ± 2.8	50.9 ± 2.4	52.1 ± 2.5	37.7 ± 2.1**

The values are the means ± SEM. ACEi, angiotensin-converting enzyme inhibitor; ACF, aorto-caval fistula; ET_A, endothelin type A; HanSD, Hannover Sprague-Dawley rats; LV, left ventricle; RV, right ventricle; TGR, Ren-2 renin transgenic rats.
*$P < 0.05$ vs. TGR + water.
**$P < 0.05$ vs. ACF TGR + water.
***$P < 0.05$ vs. sham-operated HanSD rats.

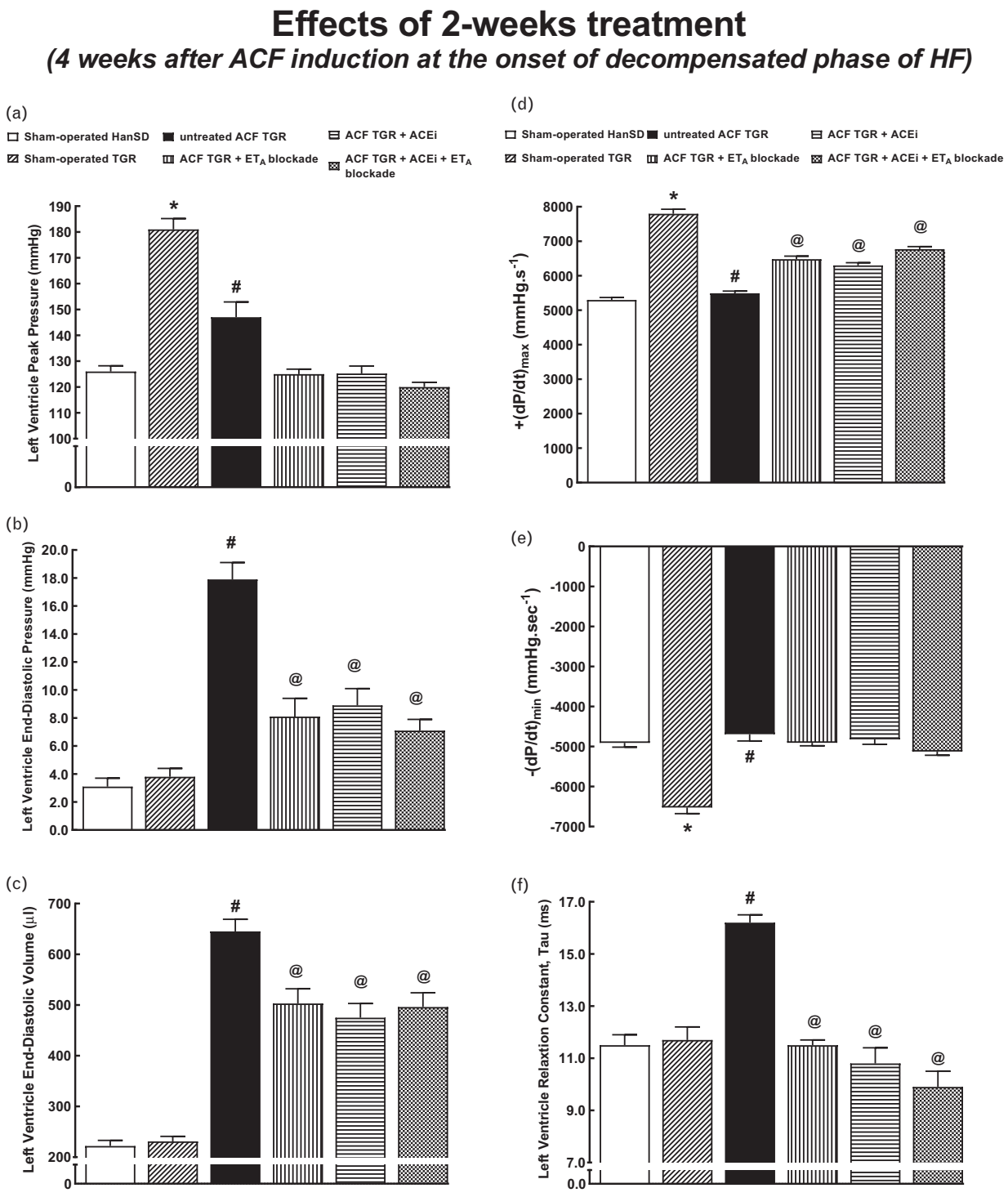

FIGURE 4 Left ventricle cardiac function assessment by invasive hemodynamic analysis performed 4 weeks after the creation of the aorto-caval fistula, that is, 2 weeks after initiation of treatments in sham-operated transgene-negative Hannover Sprague-Dawley rats, sham-operated heterozygous Ren-2 renin transgenic rats and Ren-2 renin transgenic rats with aorto-caval fistula, treated with either endothelin type A receptor antagonist alone or with angiotensin-converting enzyme inhibitor alone or with the combination of endothelin type A receptor antagonist and angiotensin-converting enzyme inhibitor. Left ventricle peak pressure (a), left ventricle end-diastolic pressure (b), left ventricle end-diastolic volume (c), maximum rates of pressure rise $(+dP/dt)_{max}$ (d), maximum rates of pressure fall $(-dP/dt)_{max}$ (e), left ventricle relaxation constant tau (f). $^{*}P<0.05$ sham-operated Ren-2 renin transgenic rats versus sham-operated Hannover Sprague-Dawley rats. $^{**}P<0.05$ untreated aorto-caval fistula Ren-2 renin transgenic rats versus sham-operated Ren-2 renin transgenic rats. $^{***}P<0.05$ treated aorto-caval fistula Ren-2 renin transgenic rats versus untreated aorto-caval fistula Ren-2 renin transgenic rats.

We hypothesized that the beneficial effects of the treatment regimens on long-term survival were preferentially mediated by cardiac mechanisms, in agreement with the recent evidence that 15 weeks' ACEi treatment significantly improved LV ejection fraction in ACF TGR [59]. Here we found that ACF TGR which survived until the end of the study and had been exposed to 20 weeks' treatment exhibited impaired load-dependent as well as load-

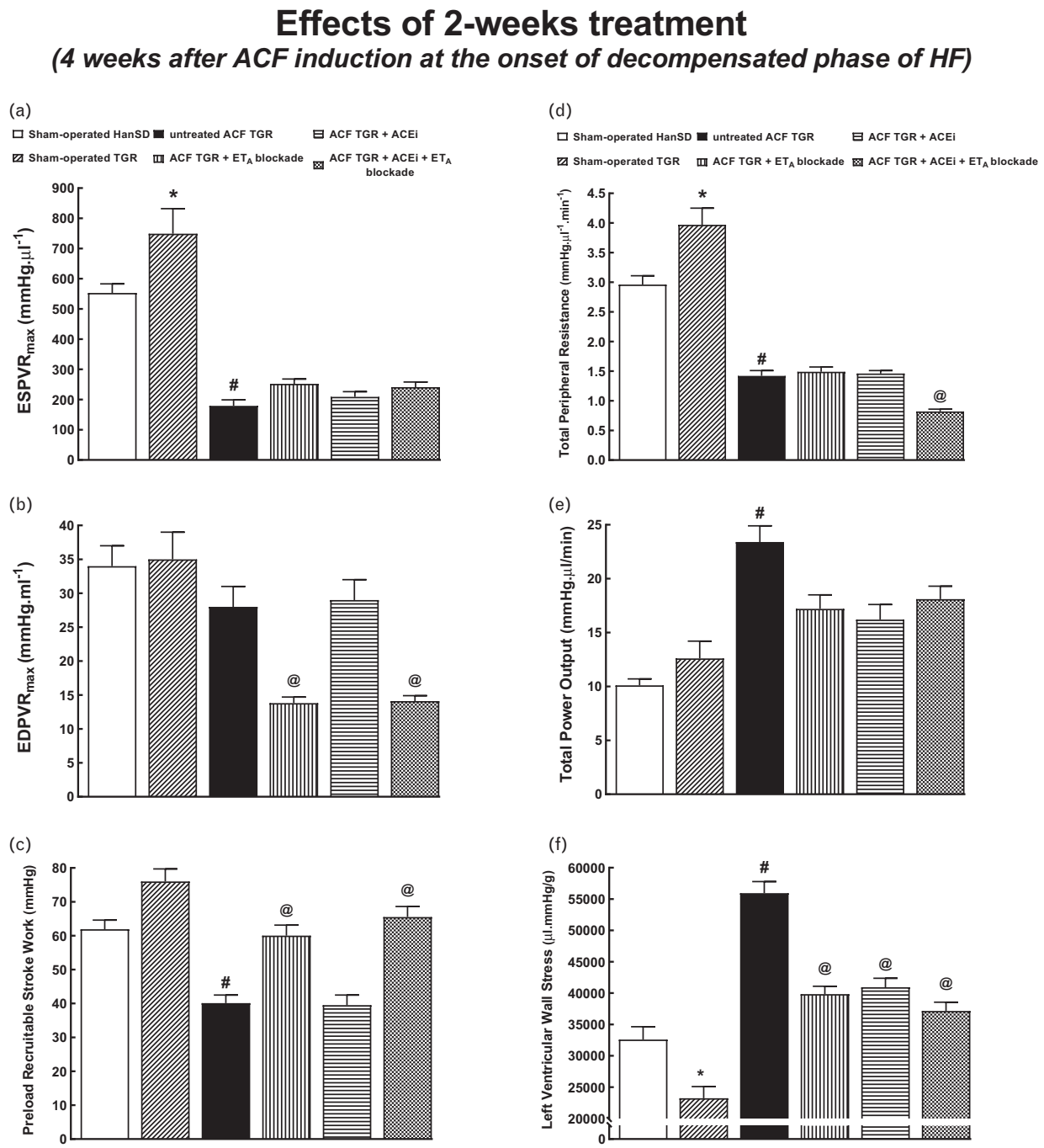

FIGURE 5 Left ventricle cardiac function assessment by invasive hemodynamic analysis performed 4 weeks after the creation of the aorto-caval fistula, that is, 2 weeks after initiation of treatments in sham-operated transgene-negative Hannover Sprague-Dawley rats, sham-operated heterozygous Ren-2 renin transgenic rats and Ren-2 renin transgenic rats with aorto-caval fistula, treated with either endothelin type A receptor antagonist alone or with angiotensin-converting enzyme inhibitor alone or with the combination of endothelin type A receptor antagonist and angiotensin-converting enzyme inhibitor. End-systolic pressure–volume relationship (a), end-diastolic pressure–volume relationship (b), preload recruitable stroke work (c), total peripheral resistance (d), total power output (e), and left ventricle wall stress (f). $^{*}P < 0.05$ sham-operated Ren-2 renin transgenic rats versus sham-operated Hannover Sprague-Dawley rats. $^{**}P < 0.05$ untreated aorto-caval fistula Ren-2 renin transgenic rats versus sham-operated Ren-2 renin transgenic rats. $^{***}P < 0.05$ treated aorto-caval fistula Ren-2 renin transgenic rats versus untreated aorto-caval fistula Ren-2 renin transgenic rats.

independent LV contractility. Moreover, irrespective of the treatment variant actually applied, they exhibited markedly increased LV wall stress, bilateral cardiac hypertrophy, and lung congestion. This could reflect a complete failure of our treatment regimens to improve cardiac morphology and function. However, it should be noticed that the data cannot be compared with those obtained at the same time from untreated ACF TGR because all of the latter died 10 weeks earlier. However, if we used for the comparison of the untreated ACF TGR in the early phase of heart failure (Table 2), we find that whole heart, LV, and RV weights and lung weights in the groups treated with either inhibitor for 20 weeks (see Table 4) are similar as in untreated ACF TGR, and with the combined treatment they are even lower.

TABLE 4. Organ weights determined 22 weeks after the creation of the aorto-caval fistula or sham-operation and after 20-weeks' treatment with endothelin type A receptor antagonist and angiotensin-converting enzyme inhibitor, alone or combined (week + 20)

	Group				
	HanSD + water	TGR + water	ACF TGR + ACEi	ACF TGR + ET_A antagonist	ACF TGR + ACEi + ET_A antagonist
Tibia length (mm)	43.6 ± 0.2	43.5 ± 0.3	43.9 ± 0.4	43.6 ± 0.3	43.4 ± 0.5
Whole heart weight (mg)/tibia length (mm)	32.44 ± 0.51	46.52 ± 0.99*	64.95 ± 1.09**	68.89 ± 0.53**	56.87 ± 0.49***
LV weight (mg)/tibia length (mm)	26.31 ± 0.21	33.96 ± 0.74*	39.11 ± 0.28**	40.37 ± 0.43**	34.48 ± 0.32***
RV weight (mg)/tibia length (mm)	7.44 ± 0.24	7.51 ± 0.18	15.27 ± 0.38**	16.58 ± 0.42**	12.09 ± 0.21***
Lung weight (mg)/tibia length (mm)	47.93 ± 1.09	47.66 ± 1.13	63.19 ± 1.21**	69.92 ± 2.27**	54.19 ± 0.68***
Kidney weight (mg)/tibia length (mm)	41.89 ± 1.02	41.56 ± 1.22	39.86 ± 1.17	41.89 ± 1.24	40.19 ± 0.97
Liver weight (mg)/tibia length (mm)	451 ± 17	441 ± 23	434 ± 29	438 ± 28	442 ± 25

The values are the means ± SEM. ACEi, angiotensin-converting enzyme inhibitor; ACF, aorto-caval fistula; ET_A, endothelin type A; HanSD, Hannover Sprague-Dawley rats; LV, left ventricle; RV, right ventricle; TGR, Ren-2 renin transgenic rats.
*$P < 0.05$ vs. sham-operated HanSD rats.
**$P < 0.05$ vs. TGR + water.
***$P < 0.05$ vs. ACF TGR + ACEi and vs. ACF TGR + ET_A antagonist.

Apparently, the eccentric cardiac remodeling and cardiac hypertrophy related to the enhanced cardiac output (blood recirculation through the fistula) progressed during the study. This was also supported by the data on cardiac function obtained by pressure–volume analysis: the respective values for ACF TGR treated for 20 weeks were similar to those found in untreated ACF TGR in the early phase after ACF-induced heart failure. Furthermore, the combined treatment reduced LVEDP to levels observed in sham-operated TGR.

Overall, each of the treatment regimens applied within the long-term protocol (20 weeks) improved cardiac morphology, systolic and diastolic function of the LV and reduced lung congestion. Most probably, the beneficial effects on the survival rate observed with atresan or trandolapril, alone or combined, are mostly mediated by cardiac mechanisms. This notion is further supported by the effectiveness of 2-weeks' treatment on cardiac morphology and function. At the onset of heart failure decompensation (when untreated rats were beginning to die) all treatment regimens substantially attenuated bilateral cardiac hypertrophy and lung congestion, reduced LVEDP, LVEDV, LV wall stress, and improved LV systolic contractility.

What was the degree of activation of the intrarenal neurohormonal systems in the earliest phase of ACF-induced heart failure? Two weeks after ACF creation, just before the treatment was initiated, ACF TGR did not display any significant increase in kidney ANG II levels. This suggests no substantial activation of the intrarenal vasoconstrictor/sodium retaining axis of the RAAS. In contrast, elevated kidney ANG 1–7 and increased ANG 1–7/ANG II ratio indicates activation of the intrarenal vasodilator/natriuretic axis of the RAAS. There was no increase in kidney norepinephrine concentration, that is, an absence of substantial activation of the intrarenal SNS. In addition, ACF TGR did not show increased concentrations of ET-1 in the renal cortex, papilla, and lung tissue, which suggests no substantial activation of the tissue endothelin system.

TABLE 5. Echocardiographic analysis performed 22 weeks after the creation of the aorto-caval fistula or sham-operation, and after 20 weeks' treatment with endothelin type A receptor antagonist and angiotensin-converting enzyme inhibitor, alone or combined (week + 20)

	Group				
	HanSD + water	TGR + water	ACF TGR + ACEi	ACF TGR + ET_A antagonist	ACF TGR + ACEi + ET_A antagonist
Heart rate (s^{-1})	376 ± 11	375 ± 11	368 ± 8	353 ± 11	347 ± 16
LV diastolic diameter (mm)	6.91 ± 0.21	7.01 ± 0.21	11.78 ± 0.33*	11.58 ± 0.39*	11.51 ± 0.17*
LV systolic diameter (mm)	3.74 ± 0.18	4.31 ± 0.39	7.77 ± 0.27*	7.85 ± 0.38*	7.69 ± 0.23*
LV anterior wall thickness in diastole (mm)	2.12 ± 0.05	3.08 ± 0.06**	2.03 ± 0.05*	2.16 ± 0.09*	1.84 ± 0.07*
LV posterior wall thickness in diastole (mm)	2.51 ± 0.06	3.12 ± 0.09**	2.05 ± 0.05*	2.19 ± 0.05*	1.94 ± 0.07*
LV relative wall thickness	0.71 ± 0.04	0.92 ± 0.04**	0.35 ± 0.02*	0.40 ± 0.03*	0.37 ± 0.03*
LV ejection fraction (%)	73.6 ± 1.7	72.5 ± 2.1	51.1 ± 1.6*	53.1 ± 2.3*	56.4 ± 1.9*
LV fractional shortening (%)	47.1 ± 1.5	46.1 ± 1.3	30.3 ± 0.7*	32.5 ± 1.5*	32.4 ± 1.3*
LV stroke volume (μl)	182 ± 10	178 ± 9	429 ± 21*	449 ± 31*	404 ± 28*
Cardiac output (ml/min)	64.3 ± 1.3	65.6 ± 3.2	162 ± 5.2*	157 ± 11*	149 ± 12*
RV diastolic diameter (mm)	3.09 ± 0.14	3.06 ± 0.09	6.69 ± 0.21*	5.38 ± 0.43***	5.61 ± 0.36***
RV systolic diameter (mm)	2.41 ± 0.11	2.39 ± 0.09	6.05 ± 0.12*	4.57 ± 0.28***	4.52 ± 0.27***
RV ejection fraction (%)	67.9 ± 3.9	65.7 ± 3.2	31.9 ± 1.1*	49.3 ± 2.7***	48.7 ± 2.2***

The values are the means ± SEM. ACEi, angiotensin-converting enzyme inhibitor; ACF, aorto-caval fistula; ET_A, endothelin type A; HanSD, Hannover Sprague-Dawley rats; LV, left ventricle; RV, right ventricle; TGR, Ren-2 renin transgenic rats.
*$P < 0.05$ vs. TGR + water.
**$P < 0.05$ vs. sham-operated HanSD rats.
***$P < 0.05$ vs. ACF TGR + ACEi.

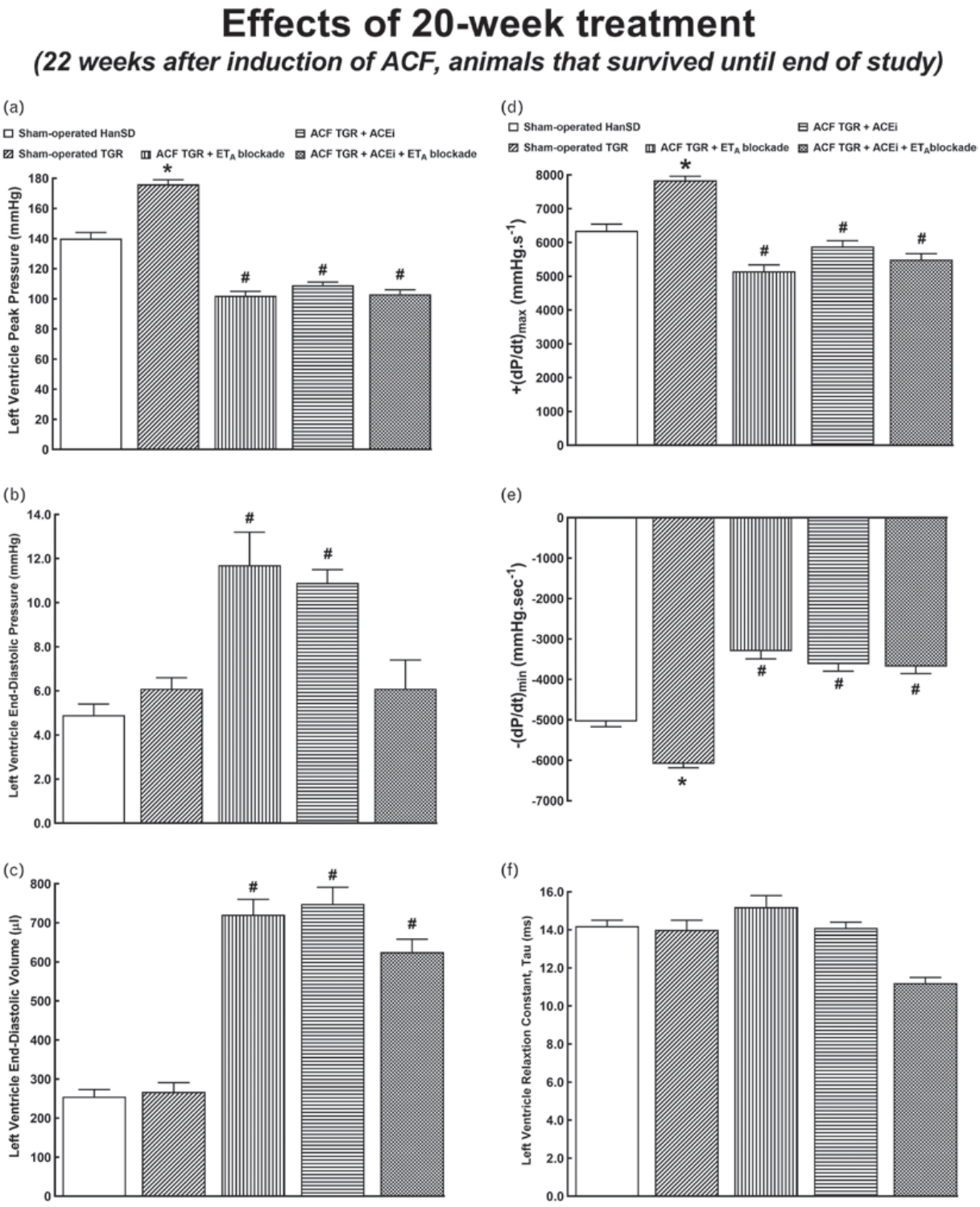

FIGURE 6 Part 1 of the left ventricle cardiac function assessment by invasive hemodynamic analysis performed 22 weeks after the creation of the aorto-caval fistula, that is, 20 weeks after initiation of treatments in sham-operated transgene-negative Hannover Sprague-Dawley rats, sham-operated heterozygous Ren-2 renin transgenic rats and Ren-2 renin transgenic rats with aorto-caval fistula, treated with either endothelin type A receptor antagonist alone or with angiotensin-converting enzyme inhibitor alone or with the combination of endothelin type A receptor antagonist and angiotensin-converting enzyme inhibitor. Left ventricle peak pressure (a), left ventricle end-diastolic pressure (b), left ventricle end-diastolic volume (c), maximum rates of pressure rise $(+dP/dt)_{max}$ (d), maximum rates of pressure fall $(-dP/dt)_{max}$ (e), left ventricle relaxation constant tau (f). $^{*}P < 0.05$ sham-operated Ren-2 renin transgenic rats versus sham-operated Hannover Sprague-Dawley rats. $^{***}P < 0.05$ treated aorto-caval fistula Ren-2 renin transgenic rats versus sham-operated Ren-2 renin transgenic rats.

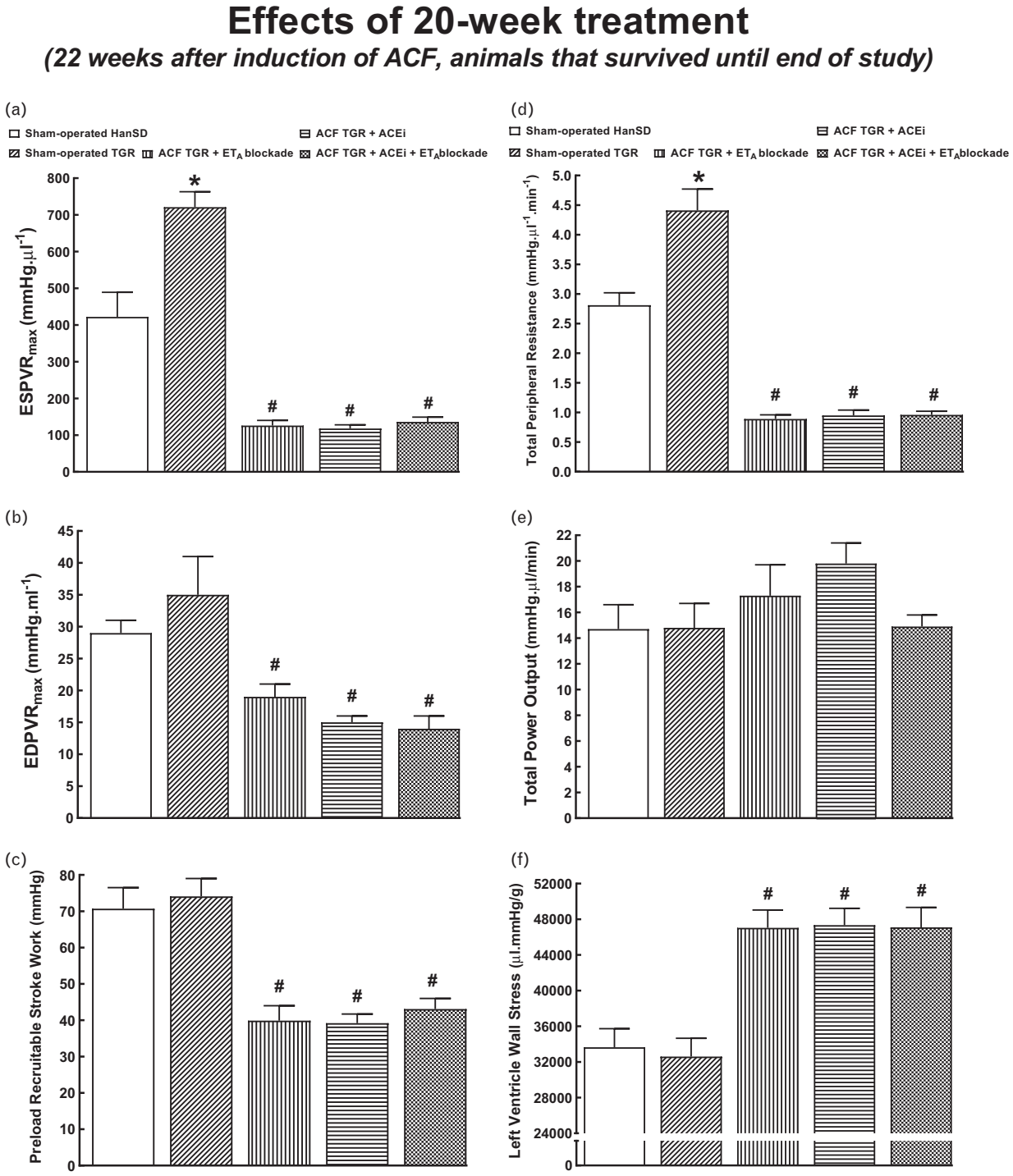

FIGURE 7 Part 2 of the left ventricle cardiac function assessment by invasive hemodynamic analysis performed 22 weeks after the creation of the aorto-caval fistula, that is, 20 weeks after initiation of treatments in sham-operated transgene-negative Hannover Sprague-Dawley rats, sham-operated heterozygous Ren-2 renin transgenic rats and Ren-2 renin transgenic rats with aorto-caval fistula, treated with either endothelin type A receptor antagonist alone or with angiotensin-converting enzyme inhibitor alone or with the combination of endothelin type A receptor antagonist and angiotensin-converting enzyme inhibitor. End-systolic pressure–volume relationship (a), end-diastolic pressure–volume relationship (b), preload recruitable stroke work (c), total peripheral resistance (d), total power output (e), and left ventricle wall stress (f). *$P < 0.05$ sham-operated Ren-2 renin transgenic rats versus sham-operated Hannover Sprague-Dawley rats. **$P < 0.05$ treated aorto-caval fistula Ren-2 renin transgenic rats versus sham-operated Ren-2 renin transgenic rats.

Evidently, in the very early phase of volume-overload heart failure, ACF TGR did not show intrarenal activation of the vasoconstrictor/sodium retaining axis of the RAAS, SNS, or endothelin system. However, there was a marked activation of the intrarenal vasodilator/natriuretic axis of the RAAS.

The evidence that kidney activity of RAAS, SNS, and endothelin system is not increased in ACF TGR seems incompatible with the neurohormonal theory of the pathophysiology of heart failure, which proposes that in heart failure the activity of the RAAS and SNS is increased and

compensates for the initial insult, even though in the long run such hyperactivity is known to be deleterious and critically contributes to the progression of heart failure [17–19,54–56]. In addition, the present results are at variance with our earlier report on an increased plasma and kidney RAAS and SNS activity in hypertensive rats [15,59,75]. However, this was so 5 weeks after the creation of ACF, in the phase of advanced heart failure decompensation, with 60% mortality (Fig. 5). All this accords with the neurohormonal concept that during the progression of heart failure the neurohormonal activity does increase and counteracts the cardiac function impairment (yet in the long run such inappropriate activation becomes extremely deleterious [17–19,54–56,76]). Briefly, in the very early phase of high-output heart failure, the sodium retaining activity of the RAAS is not apparent. However, in our ACF TGR, kidney ANG 1–7 and the ANG 1–7/ANG II ratio was increased. Evidently, the ACE2/ANG 1–7/Mas receptor axis of the RAAS, which counteracts the effects of the classical RAAS axis [77], was substantially activated. Taken together, since ANG 1–7 is the most important component of the ACE2/ANG 1–7/Mas axis, its activation was presumably the first compensatory event in response to ACF creation, preceding the activation of the vasoconstrictor/sodium retaining axis of the RAAS, SNS, and endothelin system. We believe that this counteracted the subsequently increased activity of the RAAS, SNS, and endothelin systems and attenuated their long-term deleterious influence, in agreement with the proposed role of ANG 1–7, particularly under conditions of elevated kidney ANG II levels [77,78], and with the recent evidence that the elevated ANG 1–7/ANG II ratio predicts a beneficial outcome of heart failure [79].

Limitations of the study

One limitation of the current study is the application of the ACF TGR model. On the other hand, its major advantage is that the rats are highly hypertensive and display marked systemic and intrarenal activation of the RAAS [50,51], thus they exhibit two most important risk factors for the progression of heart failure [18,19,52,53]. However, the model is sometimes regarded 'nonnatural' and the progression of heart failure is thought excessively accelerated. Moreover, the creation of the ACF is associated with a profound decrease in total peripheral resistance and a subsequent decrease in blood pressure: evidently, the initial increase in cardiac output cannot compensate the decrease in TPR. It must also be admitted that the ACF TGR model mimics heart failure dependent on chronic volume overload, a condition affecting only 5–7% of heart failure patients. Most of them suffer from severe mitral insufficiency which is resistant to treatment [3].

It is also admitted that all our series which evaluated cardiac functions were performed in long-term anesthetized and surgically stressed animals, which must have caused the increased activity of neurohormonal systems, particularly of the RAAS and SNS. Possibly, the resultant changes in neurohumoral and volume status could alter cardiac function to some extent, both in healthy animals and in those with ACF-induced heart failure. However, we demonstrated previously, in the initial comprehensive reference study and in our previous studies [15,47,61,62] that long-term anesthesia (isoflurane or barbiturates) and surgical procedures do not deteriorate the stability of animals. Therefore, despite such potential drawbacks the pressure–volume analyses are now accepted as a golden standard approach for the evaluation of cardiac function in mice and rats.

In conclusion, our results showed that, first, even in the absence of pronounced activation of the tissue endothelin system in the early phase of volume-overload heart failure, ET_A receptor blockade delays the onset of heart failure decompensation and improves the survival in ACF TGR. Second, the beneficial effects of each of the treatment regimens on long-term survival are most probably mediated by partial recovery of cardiac function, specifically by attenuation of bilateral cardiac hypertrophy, of lung congestion, by reducing LVEDP, LVEDV, LV wall stress, and also by improving LV systolic contractility. Third, the addition of ET_A receptor blockade did not increase the protection against heart failure-related mortality in ACF TGR beyond the improvement obtained with the treatment with ACEi alone.

On the whole, our results suggest that targeting the endothelin system should again be considered for the treatment of heart failure, at least of its volume-overload variant, and in individuals with background hypertension and enhanced RAAS activity.

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Conflicts of interest

There are no conflicts of interest.

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Pulmonary embolism-related refractory out-of-hospital cardiac arrest and extracorporeal cardiopulmonary resuscitation: Prague OHCA study post hoc analysis

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ORIGINAL SCIENTIFIC PAPER

Pulmonary embolism-related refractory out-of-hospital cardiac arrest and extracorporeal cardiopulmonary resuscitation: Prague OHCA study post hoc analysis

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Aims Refractory out-of-hospital cardiac arrest (r-OHCA) in patients with pulmonary embolism (PE) is associated with poor outcomes. The role of extracorporeal cardiopulmonary resuscitation (ECPR) in this patient group is uncertain. This study aims to analyse clinical course, outcomes, and the effect of an invasive procedure, including ECPR, in a randomized population.

Methods and results A post hoc analysis of a randomized controlled trial (Prague OHCA study) was conducted to evaluate the effect of ECPR vs. a standard approach in r-OHCA. A subgroup of patients with PE-related r-OHCA was identified, and procedural and outcome characteristics, including favourable neurological survival, organ donation, and complications, were compared to patients without PE. Pulmonary embolism was identified as a cause of r-OHCA in 24 of 256 (9.4%) enrolled patients. Patients with PE were more likely to be women [12/24 (50%) vs. 32/232 (13.8%); $P < 0.001$] and presented more frequently with an initial non-shockable rhythm [23/24 (95.8%) vs. 77/232 (33.2%); $P < 0.001$], as well as more severe acidosis at admission [median pH (interquartile range); 6.83 (6.75–6.88) vs. 6.98 (6.82–7.14); $P < 0.001$]. Their favourable 180-day neurological survival was significantly lower [2/24 (8.3%) vs. 66/232 (28.4%); $P = 0.049$], but the proportion of accepted organ donors was higher (16.7 vs. 4.7%, $P = 0.04$).

Conclusion Refractory out-of-hospital cardiac arrest due to PE has a different presentation and inferior outcomes compared to other causes but may represent an important source of organ donations. The ECPR method did not improve patient outcomes.

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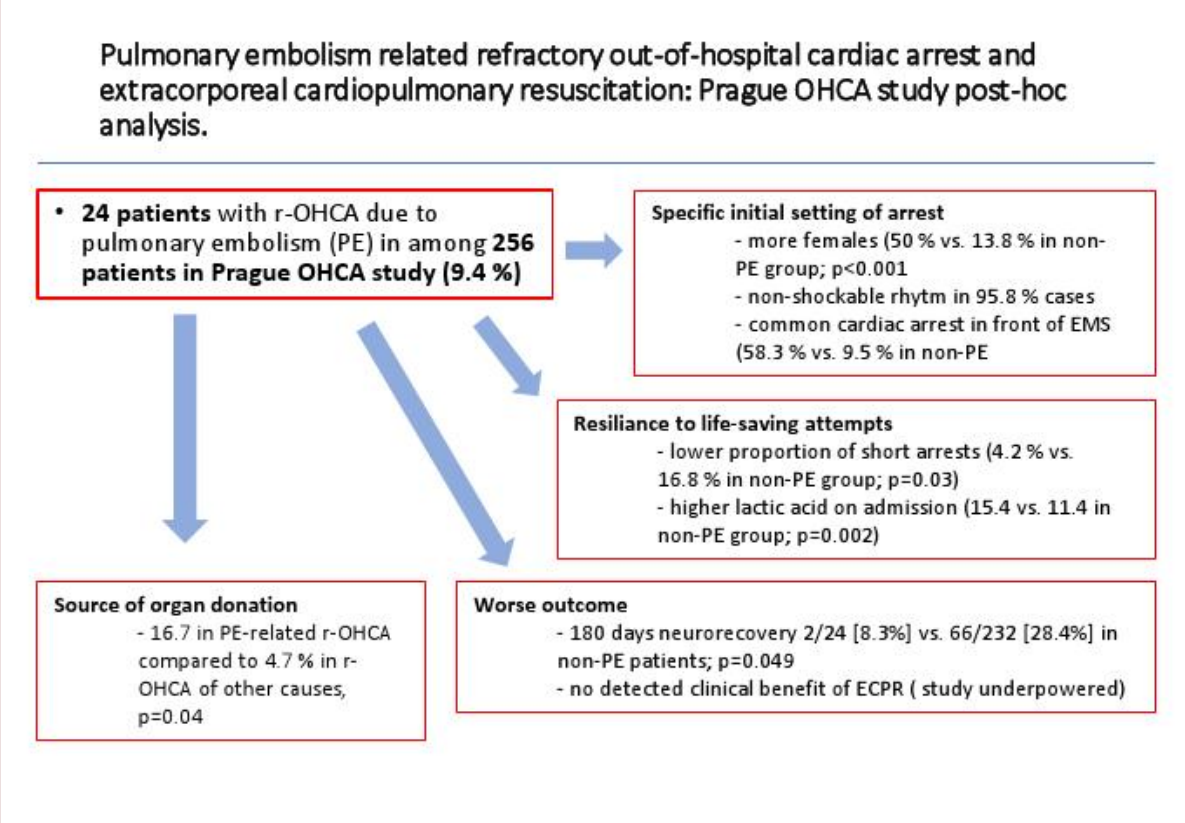
Graphical Abstract



Keywords pulmonary embolism • cardiac arrest • extracorporeal cardiopulmonary resuscitation • organ donorship

Introduction

Pulmonary embolism (PE) accounts for 2–7% of all out-of-hospital cardiac arrests (OHCA), with a high lethality rate of 65–95%.[1] Current advancements in cardiopulmonary resuscitation (CPR) in PE-related cardiac arrest (CA) consists of reperfusion attempts and consideration of selected patients for extracorporeal CPR (ECPR).[2] Data on ECPR in patients with PE-related CA, despite being promising, originate only from retrospective, non-randomized small studies.[3,4] Our study aimed to compare refractory OHCA (r-OHCA) caused by PE to other aetiologies regarding clinical course, outcome, and the effect of an invasive approach, including ECPR, in a randomized population.

Methods

Study design, population, and interventions

We present a post hoc analysis of the Prague OHCA study (NCT01511666), a randomized controlled trial in which patients with r-OHCA were randomized to invasive or standard treatment.[5] For a comprehensive description of the original study protocol, see *Supplementary Materials*. Briefly, patients in the invasive arm underwent intra-arrest transport to the hospital; those without ROSC on admission were indicated for ECPR, followed by invasive management. Patients given the standard care arm were managed on scene according to guidelines[2] and only transported if ROSC was achieved. Further post-resuscitation care in both arms complied with current recommendations.[2,6]

The present analysis identified a subgroup of patients with PE as the cause of r-OHCA. Baseline, procedural, and outcome characteristics were compared to other r-OHCA aetiologies.

Identification of pulmonary embolism

In the Prague OHCA study, the potential identification of PE as a cause of CA was thoroughly examined in each patient. Diagnosis of PE was confirmed with autopsy, computed tomography pulmonary angiography (CTPA), or invasive pulmonary angiography. However, in 7 of 256 patients (2.7%), the cause of CA was unidentified, and an autopsy was not performed.

Post hoc analysis outcomes and statistical analysis

The key outcome was 180-day survival with a good neurological outcome [cerebral performance category (CPC) scores 1 and 2]. Additional outcomes were cardiac and neurological recovery in 30 days. The current post hoc analysis also analysed the proportion of accepted organ donors and complications. The continuous data were assessed for normality using the Shapiro–Wilk test, and the data were reported using either means and standard deviations or medians and interquartile ranges. The Mann–Whitney and Fisher's exact tests were computed for numerical comparisons and the chi-square test for categorical comparisons. Statistical

Table 1 Baseline and procedural characteristics of patients with cardiac arrest due to pulmonary embolism and cardiac arrest of other cause: the proportion of complication

	PE CA cause	Other CA causes	*P*
Age (years)	63 (54–69.5)	57.5 (47–65)	0.05
Sex			
Female	12 (50%)	32 (13.8%)	**<0.001**
Male	12 (50%)	200 (86.2%)	
Medical history			
Hypertension	4 (30.8%)	85 (47.8%)	0.27
Coronary artery disease	1 (8.3%)	33 (18.9%)	0.7
Chronic heart failure	1 (7.7%)	15 (8.7%)	1.0
Diabetes	3 (25%)	33 (18.9%)	0.7
Chronic kidney disease	0 (0%)	5 (2.9%)	1.0
COPD	1 (7.7%)	9 (5.3%)	0.53
ICD implanted	0 (0%)	3 (1.6%)	1.0
Location of cardiac arrest			
Home	7 (29.2%)	69 (29.7%)	**<0.001**
Public place	2 (8.3%)	121 (52.2%)	
EMS vehicle	14 (58.3%)	22 (9.5%)	
Health facility	1 (4.2%)	1 (0.4%)	
Workplace	0 (0%)	19 (8.2%)	
First observed rhythm			
VF/VT	1 (4.2%)	155 (66.8%)	**<0.001**
Asystole	8 (33.3%)	47 (20.3%)	
PEA	15 (62.5%)	30 (12.9%)	
Bystander CPR	24 (100%)	228 (98.3%)	
Time from collapse to EMS arrival (min)	8 (5.5–11.8)	9 (7–11)	0.83
Time from collapse to ACLS (physician arrival) (min)	6 (0–11.5)	11 (8–13.5)	**0.001**
Number of epinephrine doses pre-hospitally (mg)	5 (4–6)	4 (4–5)	0.22
Number of defibrillations	0 (0–0)	3 (1–6)	**<0.001**
Intermitent ROSC	11 (45.8%)	75 (32.3%)	0.26
Pre-hospital hypothermia used	3 (12.5%)	30 (12.9%)	1.0
Time to hospital admission (min)	40 (34.5–57.8)	54 (46–64)	1.0
Time to hospital admission (min)	40 (34.5–57.8)	54 (46–64)	**0.01**
Declared dead	9 (37.5%)	66 (28.4%)	0.46
Pre-hospitally	5 (55.6%)	41 (62.1%)	0.73
Within 1 h from admission	4 (44.4%)	25 (37.9%)	
Time of CPR (time from collapse to death/ROSC or ECLS) (min)	50.5 (40.5–67.5)	53 (35.5–68.5)	0.99
Time of CPR subgroups			
<30 min	1 (4.2%)	39 (16.8%)	
≥30 and <45 min	7 (29.2%)	45 (19.4%)	**0.03**
≥45 min	16 (66.7%)	148 (63.8%)	
Hyper-invasive arm randomization	12 (50%)	112 (48.3%)	0.46
ECLS			
ECLS implanted	9 (37.5%)	86 (37.1%)	1
Time to ECLS (min) (*from collapse to ECLS*)	51 (46.8–65.5)	61 (56–70)	0.42
Time of implantation (min)(*door to ECLS*)	10 (8.8–17.3)	13 (10–16)	0.09
Laboratory values on admission			
pH	6.83 (6.75–6.88)	6.98 (6.82–7.14)	**< 0.001**
pH ≤ 6.85 and CPC 1 + 2	1 (5.6%)	9 (4.9%)	**1.0**

Continued

Table 1 *Continued*

	PE CA cause	Other CA causes	P
pH ≤ 6.80 and CPC 1 + 2	0 (0%)	5 (2.7%)	1.0
Lactate (mmol/L)	15.5 (11.7–19)	11,4 (8.1–14.7)	**0.002**
Immediate cause of death			
Refractory arrest	10 (45.5%)	70 (42.9%)	0.14
Brain death	7 (31.8%)	23 (14.1%)	
MODS	2 (9.1%)	50 (30.7%)	
Cardiogenic shock	1 (4.5%)	13 (8%)	
Unknown	1 (4.5%)	4 (2.5%)	
Bleeding	1 (4.5%)	3 (1.8%)	
Organ donorship			
Accepted	4 (16.7%)	11 (4.7%)	**0.04**
Proportion of complications			
Bleeding — any	53.3%	22.4%	**0.01**
Fatal	25%	5.3%	0.18
Intracranial haemorrhage	12.5%	2.37%	
Organ lacerations	18.2%	1.5%	**0.002**
Ischaemic gut	26.7%	34.5%	0.78
Technical	0 (0%)	3 (1.3%)	1.0

ACLS, advanced cardiovascular life support; CA, cardiac arrest; COPD, chronic obstructive pulmonary disease; CPC, cerebral performance category; CPR, cardiopulmonary resuscitation; ECLS, extracorporeal life support; EMS, emergency medical service; ICD, implantable cardioverter-defibrillator; MODS, multiple organ dysfunction syndrome; PE, pulmonary embolism; PEA, pulseless electrical activity; ROSC, return of spontaneous circulation; VF, ventricular fibrillation; VT, ventriculart tachycardia.

significance was set at $P < 0.05$. MedCalc® Statistical Software (version 19.7 233; MedCalc Software Ltd, Belgium; 2021) was used for all analyses.

Results

Baseline characteristics of patients with PE-related r-OHCA

Pulmonary embolism was identified as the cause of CA in 24 (9.4%) of 256 enrolled patients. *Table 1* highlights differences between the PE-related r-OHCA group and other causes of r-OHCA, including a higher proportion of females, more frequent occurrence of CA after emergency medical service (EMS) arrival, and non-shockable initial rhythm in the PE-related group. Both groups had long CPR duration (>50 min), severe acidosis, and high lactate levels on admission. However, the PE-related group had a significantly lower proportion of CPR duration below <30 min and higher lactic acid levels.

Outcome of patients with PE-related CA and organ donorship

The key outcome of favourable neurological survival at 180 days and the additional outcome of cardiac recovery at 30 days were significantly lower in the PE-related group, compared to non-PE group as shown in *Table 2*. There was no difference in the key outcome for PE patients between the invasive and standard arm [2/12 (16.7%) vs. 0/12 (0%); $P = 0.24$]. In patients with PE, a significantly higher proportion of accepted organ donors was observed compared to the non-PE group [4/24 (16.7%) vs. 20/232 (4.7%); $P = 0.04$].

Table 2 Comparison of cardiac arrest outcomes between pulmonary embolism caused cardiac arrest and cardiac arrest of other cause

	PE CA cause	Other PE cause	P
Key outcome			
Survival with CPC at 180 days			
1 or 2	2 (8.3%)	66 (28.4%)	**0.049**
>3	22 (91.7%)	166 (71.6%)	
Additional outcomes			
Neurorecovery at 30 days			
Yes	2 (16.7%)	60 (25.9%)	0.08
No	22 (91.7%)	172 (74.1%)	
Cardiac recovery at 30 days			
Yes	4 (16.7%)	95 (40.9%)	**0.03**
No	20 (7.3%)	137 (59.1%)	

CA, cardiac arrest; CPC, cerebral performance category; PE, pulmonary embolism.

Diagnostics and treatment of PE-related CA complications

The final diagnosis of PE was confirmed in 4 (16.7%) patients by invasive pulmonary angiography, in 7/24 (29.2%) by CTPA and in 13/24 (54.2%) by autopsy.

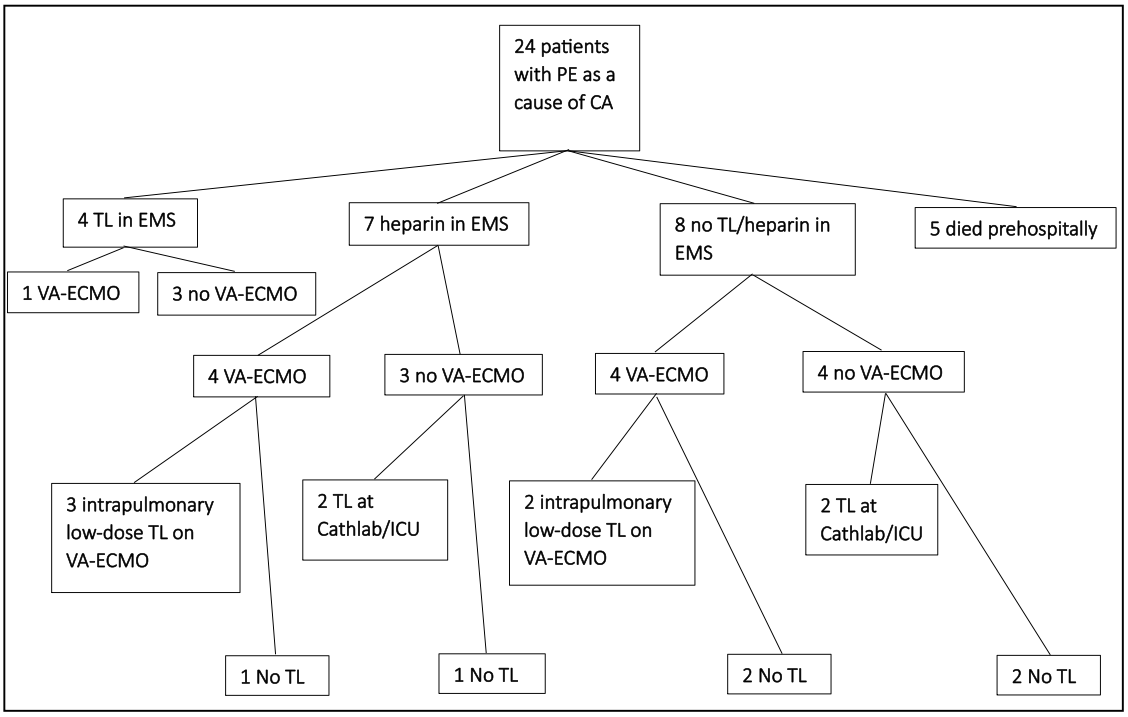


Figure 1 Flowchart of anticoagulant/thrombolytic therapy and venoarterial extracorporeal membrane oxygenation. CA, cardiac arrest; EMS, emergency medical service; ICU, intensive care unit; PE, pulmonary embolism; TL, thrombolysis; VA-ECMO. venoarterial extracorporeal membrane oxygenation.

Thrombolysis (TL) was used in 11/24 patients (46%), and no patient underwent surgical thrombectomy. The relationship between TL/heparin and venoarterial extracorporeal membrane oxygenation (VA-ECMO) treatment is illustrated in *Figure 1*.

Patients with r-OHCA due to PE had a higher incidence of bleeding complications, including two fatal cases. Additionally, the rate of organ laceration was higher in this group. In PE patients on VA-ECMO, 5 of 9 (55.6%) experienced bleeding complications (e.g. pulmonary haemorrhage, ECMO cannula haemorrhage, and intracranial bleeding).

Discussion

The population of PE-related refractory CA differed from non-PE-related disease. Higher female representation, which is in sharp contrast to the parental study that comprised mostly male patients (86.2%), broadens the data, showing a higher incidence of more severe forms of PE in females compared to males.[7] The prevailing occurrence of CA during the initial management by EMS crews, most likely caused by calling the EMS because of symptoms preceding CA, led to significantly shorter ACLS onset and transport time. The negligible representation of patients with short duration of CPR (4.2%) and severe metabolic derangement at admission illustrate its resilience to life-saving attempts, which contributed to poor outcome.

The significantly worse outcome in patients with PE-related r-OHCA is consistent with previous findings on CA due to PE.[8] Conversely, our data showed a significantly higher proportion of accepted organ donors within the PE subpopulation.

The limited number of patients with PE and the study's post hoc design made a meaningful evaluation of a mortality benefit in the invasive arm impossible. However, the occurrence of only survivors in the invasive arm is complemented by the current recommendation to consider ECPR in highly selected patients with PE-related r-OHCA.[2,8] These results are the only available data on using ECPR in PE-related r-OHCA derived from a randomized population. Current evidence comes from observational data[9] and non-randomized studies with general high-risk PE patients[10,11] or mixed patient populations with PE after CA with ROSC or patients with in-hospital CA without prolonged periods of CPR.[12]

The evidence about TL treatment under VA-ECMO is insufficient and derived from small retrospective studies ($n = 13–23$ patients) that combine patients with shock with those after CPR.[13,14] These studies suggest a possible benefit in these 35 patients or at least in selected patients when using intra-pulmonary TL[14] despite a 30% rate of major bleeding. Although the rate of major bleeding was similar in our trial, it did not generate any survivors. Administration of anticoagulant and thrombolytic drugs combined with prolonged CPR/ECPR explains the significantly higher rate of bleeding in patients with PE yet underscores the questionable benefit of advanced invasive measures in this high-risk population.

Limitations

Because the PE-related CA analysis was not pre-specified, it may have been influenced by confounding. Other limitations are that the study was conducted at a single center and the sample size of patients with PE was small, although comparable with other series.

Conclusion

Pulmonary embolism-related r-OHCA has different manifestations than other aetiologies. Patients with r-OHCA due to PE have inferior

outcomes, but they might expand the organ donor pool. Evaluation of an invasive approach, including the ECPR procedure, was underpowered in our study to detect eventual clinical benefits. Explorations of study could serve as a basis for further research.

Supplementary material

Supplementary material is available at *European Heart Journal: Acute Cardiovascular Care* online.

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Conflict of interest: Dr. Belohlavek reported receiving lecture honoraria from Maquet Company. No other authors reported disclosures.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Outcomes of patients with myocardial infarction and cardiogenic shock treated with culprit vessel-only versus multivessel primary PCI

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Original Article

Outcomes of patients with myocardial infarction and cardiogenic shock treated with culprit vessel-only versus multivessel primary PCI

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ABSTRACT

Introduction and objectives: Multivessel primary percutaneous coronary intervention (pPCI) is still often used in patients with ST-elevation myocardial infarction (STEMI) and cardiogenic shock (CS). The study aimed to compare the characteristics and prognosis of patients with CS-STEMI and multivessel coronary disease (MVD) treated with culprit vessel-only pPCI or multivessel-pPCI during the initial procedure.
Material and methods: From 2016 to 2020, 23,703 primary PCI patients with STEMI were included in a national all-comers registry of cardiovascular interventions. Of them, 1,213 (5.1%) patients had CS and MVD at admission to the hospital. Initially, 921 (75.9%) patients were treated with culprit vessel (CV)-pPCI and 292 (24.1%) with multivessel (MV)-pPCI.
Results: Patients with 3-vessel disease and left main disease had a higher probability of being treated with MV-pPCI than patients with 2-vessel disease and patients without left main disease (28.5% vs. 18.6%; $p < 0.001$ and 37.7% vs. 20.6%; $p < 0.001$). Intra-aortic balloon pump, extracorporeal membrane oxygenation (ECMO), and other mechanical circulatory support systems were more often used in patients with MV-pPCI. Thirty (30)-day and 1-year all-cause mortality rates were similar in the CV-pPCI and MV-pPCI groups (odds ratio, 1.01; 95% confidence interval [CI] 0.77 to 1.32; $p = 0.937$ and 1.1; 95% CI 0.84 to 1.44; $p = 0.477$). The presence of 3-vessel disease and the use of ECMO were the strongest adjusted predictors of 30-day and 1-year mortality.
Conclusions: Our data from an extensive all-comers registry suggests that selective use of MV-pPCI does not increase the all-cause mortality rate in patients with CS-STEMI and MVD compared to CV-pPCI.

Abbreviations: pPCI, primary percutaneous coronary intervention; AMI, acute myocardial infarction; STEMI, ST-elevation myocardial infarction; CS, cardiogenic shock; MVD, multivessel coronary disease; CABG, coronary artery bypass grafting; NRCI, National Registry of Cardiovascular Interventions; CV-pPCI, culprit vessel-only primary percutaneous coronary intervention; MV-pPCI, multivessel primary percutaneous coronary intervention; IABP, intra-aortic balloon pump; ECMO, extracorporeal membrane oxygenation; MCS, mechanical circulatory support.

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1. Introduction

Cardiogenic shock (CS) is the leading cause of in-hospital death in patients with acute myocardial infarction (AMI)[1]. The incidence of CS complicating AMI is between 3–13%[2,3]. This means that approximately 40,000 to 50,000 CS patients are treated in the USA and approximately 60,000–70,000 in Europe per year[4]. Unfortunately, the thirty-day mortality remains high even in the primary percutaneous coronary intervention (pPCI) era at nearly 40% and approaches 50% at one year at least[5,6]. Since CABG should always be considered in patients with CS-AMI and multivessel coronary disease (MVD), the pPCI is much more often used in patients with acute myocardial infarction with ST-segment elevation (STEMI). The reason is that pPCI achieves reperfusion faster, and with improvements in PCI techniques, it can be successfully performed in most patients with MVD[7]. Data from the SHOCK trial[8,9] and an analysis of the Korean Acute Myocardial Infarction Registry[10] suggest that multivessel revascularization at the time of primary PCI was associated with better outcomes in patients with STEMI and cardiogenic shock compared with culprit vessel revascularization only. Conversely, the Culprit Lesion Only PCI Versus Multivessel PCI in Cardiogenic Shock (CULPRIT-SHOCK) trial, so far the largest randomized CS trial (published 18 years after the SHOCK trial) demonstrated that a culprit vessel-only strategy (CV-pPCI) was superior to immediate multivessel PCI (MV-pPCI) for patients with CS and multivessel coronary artery disease (MVD)[11]. This finding changed the guidelines in favor of CV-pPCI[7,12]. Nonetheless, multivessel PCI is still often used in these patients[13].

Using data from the National Registry of Cardiovascular Interventions (NRCI), National Registry of Paid Health Services, and Registry of Death Records, our study aimed to compare the characteristics and prognosis of patients with CS-STEMI and MVD treated with culprit vessel-only pPCI vs. multivessel PCI during the initial procedure.

2. Material and Methods

2.1. The National Registry of Cardiovascular Interventions (NRCI)

The NRCI is a prospective multicenter registry that collects data on all PCIs performed in all PCI centers in the Czech Republic since 2005. The NRCI is part of the National Health Information System. In recent years approx. In total, 20,000–25,000 records have been entered into the NRCI register annually. Every PCI performed in the Czech Republic, including selected clinical data, detailed data on indications for PCI, and procedural information, must be, in accordance with applicable law, consecutively entered into the NRCI. Data are subsequently correlated with the Registry of Death Records to ascertain short-term and long-term mortality[14,15]. All coronary and noncoronary interventional procedures are entered into the NRCI. Data regarding the use of the intra-aortic balloon pump (IABP), extracorporeal membrane oxygenation (ECMO), and other mechanical circulatory support systems (MCS) was obtained from the National Registry of Paid Health Services.

2.2. Patients and Definitions

Our analysis of the NRCI was performed using consecutive patients with STEMI treated with primary PCI who presented to the catheterization laboratory with cardiogenic shock or developed CS during PCI. Cardiogenic shock was diagnosed using the generally accepted definition if the patient with AMI had systolic blood pressure <90 mmHg or the use of catecholamines to maintain a systolic blood pressure of ≥90 mmHg was necessary, clinical signs of pulmonary congestion, and signs of impaired organ perfusion with at least one of the following manifestations: altered mental status, cold and clammy skin, and limbs, oliguria with a urine output of <30 ml per hour, or an arterial lactate level >2.0 mmol per liter. All patients also had to have multivessel coronary artery disease, defined as ≥70% stenosis in at least two coronary arteries. Patients with mechanical complications from AMI were excluded. All pPCI procedures were performed in high-volume PCI centers with non-stop service and at least 150 pPCI per year.

We compared baseline and procedural characteristics and 30-day and 1-year mortality among patients treated with either CV-pPCI or MV-pPCI. Culprit vessel-pPCI was defined as pPCI of only one major coronary artery or its branches, which was considered to be the cause of MI by the physician during the initial procedure. Multivessel-pPCI meant pPCI of at least two major coronary arteries or their branches during the initial procedure for STEMI with CS. The decision to perform CV-pPCI or MV-pPCI was completely up to the physician's discretion. Predictors of short- and long-term mortality were evaluated.

2.3. Statistical analysis

Continuous variables (age) were presented using arithmetic means with standard deviation (SD) for normally distributed variables. Categorical parameters were summarized using frequency tables with absolute and relative frequencies. Categorical variables were compared between treatment groups using Fisher's exact test, and continuous variables (age) were compared using the Mann-Whitney test. A p-value of <0.05 was considered statistically significant. Univariate and multivariate regression analyses were used to compare 30-day and 1-year mortality predictors. Only predictors with a p-value <0.05 in univariate entered the multivariate analysis. All analyses were performed with SPSS version 24.0.0.1 (IBM Corporation, Armonk, New York).

3. Results

3.1. Baseline patient and procedural characteristics

From January 1, 2016, to December 31, 2020, 23,703 primary PCI patients with STEMI were included in the NRCI. This period was chosen to utilize standardized registry data. A total of 1,213 (5.1%) patients had CS and MVD at admission to the hospital. Initially, 921 (75.9%) patients were treated with CV-pPCI and 292 (24.1%) with MV-pPCI. Thirty (30)-day and 1-year mortality was 50.5% vs. 51.4% and 59.0% vs. 61.3% in CV-pPCI and MV-pPCI groups. In total, 64 (21.9%) patients in MV-pPCI had 100% stenoses in two vessels.

Table 1 shows the baseline clinical characteristics of patients with CS-STEMI and MVD treated either with CV-pPCI or MV-pPCI. CV-pPCI was preferred over MV-pPCI in all patients, both men and women, although women were more often treated with CV-pPCI than men (79.8% vs. 74.6%; $p < 0.001$). Culprit vessel-pPCI and MV-pPCI patients did not differ regarding age, previous PCI and CABG, chronic kidney disease, cardiopulmonary resuscitation, and artificial lung ventilation at admission.

Culprit vessel-pPCI, compared to MV-pPCI patients, had the same occurrence of anterior myocardial infarction, time delay to reperfusion, and thrombolysis in myocardial infarction (TIMI) flow 0 before PCI (Table 2). Patients with MV-pPCI were significantly more likely to have 3-vessel or left main disease. Post-procedural TIMI flow 3 in the culprit artery was achieved more often in patients with CV-pPCI (76.8% vs. 66.8%; $p < 0.001$). Intra-aortic balloon pump, ECMO, and other MCS were more often used in patients with MV-pPCI.

Table 1
Characteristics of the patients at baseline.

	All patients N (total %)	CV-pPCI N (%)	MV-pPCI N (%)	p
Total	1213 (100)	921 (75.9)	292 (24.1)	-
Male	896 (73.9)	668 (74.6)	228 (25.4)	**< 0.001**
Age years (mean ± SD)	68 ± 11.4	68.1 ± 11.2	66.2 ± 11.4	0.780
<40	10 (0.8)	7 (70.0)	3 (30.0)	0.125
40–49	62 (5.1)	39 (62.9)	23 (37.1)	
50–59	196 (16.2)	144 (73.5)	52 (26.5)	
60–69	405 (33.4)	313 (77.3)	92 (22.7)	
70–79	342 (28.2)	260 (76.0)	82 (24.0)	
≥80	198 (16.3)	158 (79.8)	40 (20.2)	
Previous PCI	183 (15.1)	148 (80.9)	35 (19.1)	0.890
Previous CABG	71 (5.9)	57 (80.3)	14 (19.7)	0.376
Chronic kidney disease/failure	87 (7.2)	63 (72.4)	24 (27.6)	0.426
After CPR	728 (60.0)	556 (76.4)	172 (23.6)	0.657
Artificial lung ventilation	821 (67.7)	615 (74.9)	206 (25.1)	0.227

PCI, percutaneous coronary intervention; CV-pPCI, culprit vessel only primary PCI; MV-pPCI, multivessel primary PCI; N, number; SD, standard deviation; CABG, coronary artery bypass grafting; CPR, cardiopulmonary resuscitation; Mann-Whitney test with p-value was used for continuous variables (age). Categorical parameters (others) are expressed as absolute numbers (percentages) and compared using Fisher's exact test.

3.2. Predictors of 30-day and 1-year all-cause mortality

Based on the results of univariate logistic regression analysis, 30-day, and 1-year all-cause mortality was similar in the CV-pPCI and MV-pPCI groups (odds ratio [OR], 1.01; 95% confidence interval [CI] 0.77 to 1.32; p = 0.937 and 1.1; 0.84 to 1.44; p = 0.477, respectively). The predictors of 30-day and 1-year mortality among all patients with CS-STEMI and MVD were age above 70 years (OR, 1.48; 95% CI 1.1 to 1.99; p = 0.009 and 1.6; 1.18 to 2.16; p = 0.002), presence of chronic kidney disease or failure (1.58; 1.01 to 2.49; p = 0.047 and 1.86; 1.15 to 3.02; p = 0.012), artificial lung ventilation (1.34; 1.05 to 10.71; p = 0.019 and 1.3; 1.02 to 1.66; p = 0.036), 3-vessel disease (1.59; 1.26 to 2.00; p < 0.001 and 1.64; 1.30 to 2.06; p < 0.001), left main disease (1.4; 1.05 to 1.88; p = 0.022 and 1.5; 1.11 to 2.02; p = 0.008) and use of extracorporeal membrane oxygenation (ECMO) on the same day as pPCI (1.74; 1.07 to 2.82; p = 0.024 and 1.64; 1.00 to 2.68; p = 0.050). Post-procedural TIMI flow 3 (0.36; 0.23 to 0.56, p < 0.001 and 0.54; 0.35 to 0.82, p = 0.004) and inferior or posterior MI localization (0.63; 0.49 to 0.82; p < 0.001 and 0.61; 0.47 to 0.78; p < 0.001) increased the probability of survival (Fig. 1). Using multivariate logistic regression analysis, the presence of 3-vessel disease was a strong independent predictor of 30-day and 1-year mortality in patients with CS-STEMI and MVD treated with pPCI (OR 1.60; 95% CI 1.27 to 2.03; p < 0.001 and 1.64; 1.30 to 2.07; p < 0.001, respectively). The other strong independent predictor of 30-day and 1-year mortality was the use of ECMO on the same day as pPCI (OR 1.83; 95% CI 1.12 to 2.98; p = 0.016 and 1.70; 95% CI 1.03 to 2.81; p = 0.037) (Table 3).

4. Discussion

Using data from the all-comers national registries, this study evaluated the characteristics and prognosis of patients with STEMI, CS, and MVD treated with culprit vessel-only pPCI or multivessel PCI during the initial procedure. We suggest that the selective use of MV-pPCI does not increase the mortality rate in patients with CS-STEMI and MVD compared to CV-pPCI.

The treatment for patients with STEMI and MVD is under continuous debate and is very different depending on whether the patient is in CS. Studies published in the previous decade in patients with STEMI and MVD without CS proved that complete revascularization of all significant coronary lesions improves the prognosis of the patients[16-23]. Current European Society of Cardiology (ESC), American College of Cardiology (ACC), and Japanese guidelines, recommend PCI on culprit lesions during the initial procedure and PCI or CABG for non-culprit stenoses using a staged procedure during hospitalization or within 40 days of the index myocardial infarction[7,12,24,25]. Performing PCI on all significant stenoses during the initial procedure can be done on stable patients with non-complex lesions suitable for uncomplicated, low-risk PCI[12,16]. The question remains, how to recognize non-culprit lesions that may cause major adverse cardiac events in the future. Some authors recommend using the angiographic severity of the stenosis as an indicator (≥70% diameter stenosis). Others emphasize the role of functional hemodynamic testing (fractional flow reserve and similar methods), intravascular imaging (optical coherence tomography, intravascular ultrasound, near-infrared spectroscopy), positron emission tomography, nuclear magnetic resonance, computer tomography or non-invasive testing like single photon computer tomography, or exercise echocardiography[26-33]. Effective pharmacotherapeutic stabilization and even regression of atherosclerotic plaques must also be considered[34-36]. The situation for patients with STEMI and cardiogenic shock is different from those who are hemodynamically stable. On the one hand, treatment of all ischemic lesions during initial primary PCI may improve perfusion of the myocardium, thus increasing heart contractility; on the other hand, any possible complication, including the relatively frequent troponin elevations that occurs during non-culprit PCI can lead to critical clinical consequences and progression of shock. Multivessel PCI also prolongs procedural times and can lead to contrast-induced nephropathy. Significant vasoconstriction often occurs in STEMI, especially in CS patients, where catecholamines are frequently used. This can lead to overestimation of coronary stenoses and their treatment by inappropriate PCI[24,37,38]. These are the probable explanations for the results seen in the CULPRIT-SHOCK trial, in which patients with STEMI or non-STEMI (NSTEMI) with cardiogenic shock were randomized to culprit lesion-only PCI or immediate PCI of all obstructive lesions (i.e., those with >70% stenosis of the diameter)[11]. In the multivessel PCI group, recanalization of chronic total occlusions was performed when possible, and complete revascularization was achieved in 81% of patients. In the culprit lesion-only PCI group, staged revascularization was performed in 17.7% of the patients. At 30 days, the primary endpoint (i.e., death or severe renal failure leading to renal replacement therapy) was higher with immediate multivessel PCI than with culprit lesion-only PCI. The results were similar for death from any cause and were consistent across the pre-specified subgroups. At one year, the mortality did not differ significantly between the two

Table 2
Procedural characteristics.

	All patients N (total %)	CV-pPCI N (%)	MV-pPCI N (%)	p
MI location				
Anterior	640 (52.8)	468 (73.1)	172 (26.9)	0.671
Inferior/posterior	401 (33.1)	335 (83.5)	66 (16.5)	
Lateral	95 (7.8)	65 (68.4)	30 (31.6)	
Not known/LBBB	77 (6.3)	53 (68.8)	24 (31.2)	-
Time from symptom onset to PCI				
<2 hr (<120 min)	62 (5.1)	48 (77.4)	14 (22.6)	0.722
2–3 hr (120–179 min)	21 (1.7)	15 (71.4)	6 (28.6)	
3–4 hr (180–239 min)	19 (1.6)	13 (68.4)	6 (31.6)	
4–8 hr (240–479 min)	118 (9.7)	95 (80.5)	23 (19.5)	
>8 hr (≥480 min)	949 (78.2)	725 (76.4)	224 (23.6)	
Not known	44 (3.6)	25 (56.8)	19 (43.2)	-
No. of diseased vessels *				
1	0 (0.0)	0 (0.0)	0 (0.0)	-
2	547 (45.1)	445 (81.4)	102 (18.6)	**<0.001**
3	666 (54.9)	476 (71.5)	190 (28.5)	
Left main stenosis >50%	239 (19.7)	149 (62.3)	90 (37.7)	**<0.001**
TIMI flow before PCI				
0	768 (63.3)	581 (75.7)	187 (24.3)	0.675
1	131 (10.8)	96 (73.3)	35 (26.7)	
2	168 (13.8)	128 (76.2)	40 (23.8)	
3	146 (12.0)	116 (79.5)	30 (20.5)	
TIMI flow after PCI				
0	110 (9.1)	63 (57.3)	47 (42.7)	**< 0.001**
1	56 (4.6)	41 (73.2)	15 (26.8)	
2	145 (12.0)	110 (75.9)	35 (24.1)	
3	902 (74.4)	707 (78.4)	195 (21.6)	
Procedures				
IABP the same day as PCI	78 (6.4%)	47 (5.1%)	31 (10.6%)	**0.001**
ECMO on the same day as PCI	80 (6.6%)	49 (5.3%)	31 (10.6%)	**0.003**
MCS - short/medium term the same day as PCI	11 (0.9%)	5 (0.5%)	6 (2.1%)	**0.028**
MCS - long-term the same day as PCI	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
IABP from 1-30 days after PCI	9 (0.7%)	6 (0.7%)	3 (1.0%)	0.456
ECMO from 1-30 days after PCI	15 (1.2%)	8 (0.9%)	7 (2.4%)	0.062
MCS - short/medium term from 1-30 days after PCI	4 (0.3%)	1 (0.1%)	3 (1.0%)	**0.045**
MCS - long-term from 1-30 days after PCI	3 (0.2%)	1 (0.1%)	2 (0.7%)	0.146
Complications				
Vessel complications requiring surgery	5 (0.4)	2 (0.2)	3 (1.0)	0.094
Severe bleeding	3 (0.2)	2 (0.2)	1 (0.3)	0.563
Part of the year				
Spring	316 (26.1)	245 (77.5)	71 (22.5)	0.790
Summer	299 (24.6)	231 (77.3)	68 (22.7)	
Autumn	293 (24.2)	206 (70.3)	87 (29.7)	
Winter	305 (25.1)	239 (78.4)	66 (21.6)	
Part of the day (in time of PCI)				
8:00-16:00 (working hours)	74 (6.1)	55 (74.3)	19 (25.7)	0.957
16:00-8:00 (not working hours)	83 (6.8)	62 (74.7)	21 (25.3)	
Not known	1056 (87.1)	804 (76.1)	252 (23.9)	-
Day in the week (in time of PCI)				
Monday	161 (13.3)	116 (72)	45 (28)	0.864
Tuesday	195 (16.1)	150 (76.9)	45 (23.1)	
Wednesday	151 (12.4)	119 (78.8)	32 (21.2)	
Thursday	174 (14.3)	133 (76.4)	41 (23.6)	
Friday	192 (15.8)	148 (77.1)	44 (22.9)	
Saturday	173 (14.3)	131 (75.7)	42 (24.3)	
Sunday	167 (13.8)	124 (74.3)	43 (25.7)	

PCI, percutaneous coronary intervention; CV-pPCI, culprit vessel only primary PCI; MV-pPCI, multivessel primary PCI; MI, myocardial infarction; TIMI, Thrombolysis in Myocardial Infarction; IABP, intra-aortic balloon pump; ECMO, extracorporeal membrane oxygenation; MCS, mechanical circulatory support.
Categorical parameters are expressed as absolute numbers (percentage) and compared using Fisher's exact test. * Definition variable.

groups[39]. The CULPRIT-SHOCK trial provided clear evidence that a culprit lesion-only PCI strategy is preferred over initial multivessel PCI for patients with cardiogenic shock[11]. Multivessel PCI should not be performed on a routine basis but can be considered in some patients[7,12,24,37,40].

Using data from a national all-comers registry, we tried to analyze the differences between CV-pPCI and MV-pPCI during the initial intervention in patients with CS-STEMI. The incidence of CS and MVD among patients with STEMI treated with pPCI was 5.1% in our registry, which is similar to other data sources[40,41]. Since the analysis included the years 2016–2020, the interventional treatment of CS-STEMI was mostly influenced by the ESC STEMI guidelines published in 2017 and by ESC Revascularization guidelines published in 2018[7,42]. The ESC STEMI guidelines state that immediate PCI is indicated for patients with cardiogenic shock if coronary anatomy is suitable (class I) and complete revascularization during the index procedure should be considered (class IIa). However, after the results of CULPRIT-SHOCK were published, the ESC Revascularization guidelines postulated that in cardiogenic shock, routine revascularization of

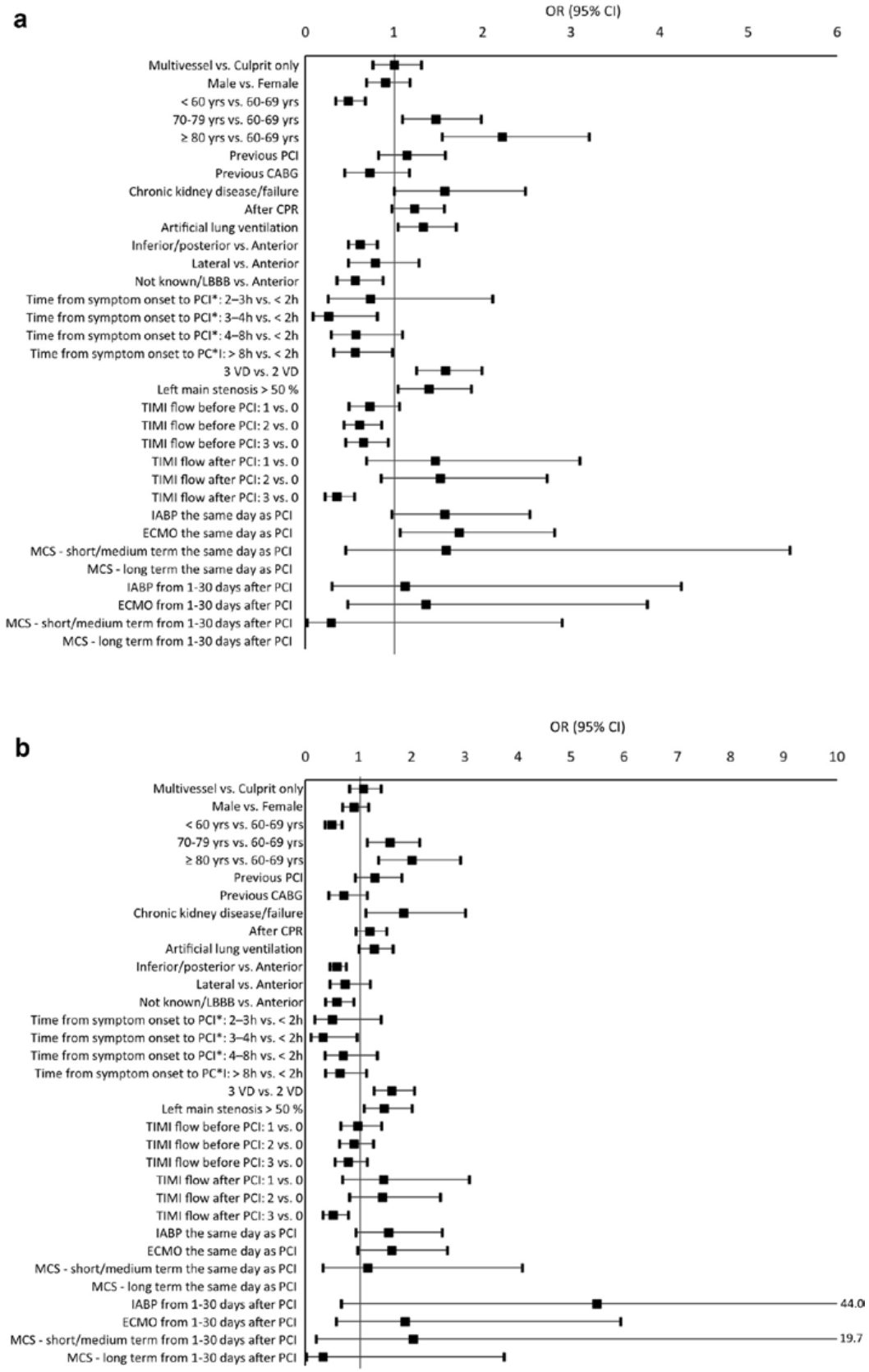

Figure 1. a Predictors of 30-day all-cause mortality. Calculated by univariate logistic regression analysis. OR, odds ratio; CI, confidence interval; yrs., years; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; CPR, cardiopulmonary resuscitation; MI, myocardial infarction; LBBB, left bundle branch block; TIMI, Thrombolysis in Myocardial Infarction; IABP, intra-aortic balloon pump; ECMO, extracorporeal membrane oxygenation; MCS, mechanical circulatory support. b Predictors of 1-year all-cause mortality. Calculated by univariate logistic regression analysis. OR, odds ratio; CI, confidence interval; yrs., years; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; CPR, cardiopulmonary resuscitation; MI, myocardial infarction; LBBB, left bundle branch block; TIMI, Thrombolysis in Myocardial Infarction; IABP, intra-aortic balloon pump; ECMO, extracorporeal membrane oxygenation; MCS, mechanical circulatory support.

Table 3
Predictors of 30-day and 1-year all-cause mortality (multivariate logistic regression analysis).

Predictor	30-days mortality		1-year mortality	
	OR (95% IS)	p	OR (95% IS)	p
Primary PCI	0.90 (0.68; 1.18)	0.439	0.99 (0.75; 1.30)	0.923
Gender	1.16 (0.89; 1.51)	0.273	1.15 (0.88; 1.51)	0.292
3VD vs. 2VD	1.60 (1.27; 2.03)	**<0.001**	1.64 (1.30; 2.07)	**<0.001**
Left main stenosis > 50%	1.01 (1.00; 1.03)	0.139	1.02 (1.00; 1.04)	0.101
IABP the same day as PCI	1.48 (0.91; 2.40)	0.110	1.45 (0.88; 2.40)	0.147
ECMO on the same day as PCI	1.83 (1.12; 2.98)	**0.016**	1.70 (1.03; 2.81)	**0.037**

PCI, percutaneous coronary intervention; 3VD, 3-vessel disease; 2VD, 2-vessel disease; IABP, intra-aortic balloon pump; ECMO, extracorporeal membrane oxygenation. Values OR > 1 mean category of the predictor, which is concerning mortality riskier than reference category. Values OR < 1 mean category, and compared with the reference category was less risky. P-values <0.05 are statistically significant; confidence interval does not include the value of 1.

non–infarct-related artery (non-IRA) lesions is not recommended during primary PCI (class III). Some specific angiographic scenarios, such as subtotal non-culprit lesions with reduced TIMI flow or multiple possible culprit lesions, may benefit from immediate multivessel PCI. However, this should be considered on an individual basis[40]. We were surprised that, despite these recommendations, the percentage of MV-pPCI in the Czech all-comers registry had risen from 19.17% in 2016 to 30.74% in 2020. This trend will need further evaluation and discussion within the national interventional community. Data from the Polish Registry Of Acute Coronary Syndromes (PL-ACS) of patients with AMI complicated with CS and treated with PCI between 2008 and 2019 showed more frequent use of MV-pPCI than CV-pPCI (54.2% vs. 45.8%)[13]. CV-pPCI and MV-pPCI patients did not differ in most baseline clinical and procedural characteristics. Patients with MV-pPCI were likelier to have significant 3-vessel or left main disease. TIMI flow 3 in the culprit artery was achieved more often in patients undergoing CV-pPCI than MV-PCI, which was contrary to PL-ACS data. We presume that in routine clinical practice, physicians finish the procedure if TIMI flow 3 is achieved in the culprit lesion; if not, they try to treat the other coronary vessels. Thrombolysis in MI flow 3 after pPCI was achieved in 74.4% of our study population, which is similar to the Polish registry and lower than in the CULPRIT-SHOCK trial[11,13]. The difference can be explained by the selection of patients in the randomized trial. Intra-aortic balloon pump, ECMO, and other MCS were only used in 16.3% of our patients, which is comparable to the PL-ACS registry but less often than in the CULPRIT-SHOCK trial[11,13]. IABP, ECMO, and other MCS were more often used in patients with MV-pPCI. Bleeding was rarely reported in NRCI, and we consider these data underestimated and irrelevant. Different seasons of the year, day of the week, or pPCI performed during working or non-working hours did not affect the choice of interventional strategy (Table 2); the same was true in the subanalysis of the CULPRIT-SHOCK trial[43].

Thirty-day and 1-year mortality were 50.5% vs. 51.4% and 59.0% vs. 61.3% in CV-pPCI and MV-pPCI groups in our all-comers registry. This is consistent with data from other trials and registries[11,13,38,40,44-48]. The mortality was similar in the CV-pPCI and MV-pPCI groups (odds ratio, 0.99; 95% CI 0.76 to 1.29; p = 0.937 and 0.91; 0.69 to 1.19; p = 0.477, respectively). As we do not have sufficient data about the severity of CS in both groups and IABP, ECMO, and other MCS were more often used in patients with MV-pPCI, the mortality may also be affected. On the other hand, we currently do not have any data demonstrating the role of IABP, ECMO, or other MCS on the overall mortality of patients with AMI and CS.[49] Using univariate logistic regression analyses, the positive predictors of mortality among all patients with CS-STEMI and MVD were age above 70 years, chronic kidney disease or failure, mechanical ventilation, 3-vessel, left main disease, and use of ECMO. Thrombolysis in MI flow 3 at the end of pPCI, as well as an inferior or posterior myocardial infarction increased the probability of survival. Other risk factors for adverse prognosis such as biomarkers (glucose, creatinine, cystatin C, lactate, interleukin-6, brain natriuretic peptide) and markers of hemodynamic instability (pulmonary capillary wedge pressure, left ventricular end-diastolic pressure) were not followed in the registry[40,50-52]. The IABP-SHOCK II risk score, which is the only CS risk score with both internal and external validation, could not be calculated from registry data[53]. Based on a multivariate logistic regression analysis, the presence of 3-vessel disease and the use of ECMO were the strongest adjusted predictors of 30-day and 1-year all-cause mortality in our patients.

5. Study Limitations

Our study analyzed all-comers registries, and these types of studies always have limitations. On the other side, the registry was unique, complex, consistent with applicable law (i.e., all patient data are required to be entered into the registry), and involved consecutively treated patients. Some data regarding prognostic risk factors in patients with CS, such as biomarkers or markers of hemodynamic instability, were not followed in our registry. Likewise, we did not have data on the severity of CS, use of catecholamines, and prevalence of bleeding. The higher use of IABP, ECMO, and other MCS in the MV-pPCI group may have influenced the study results.

6. Conclusions

Our data from a large all-comers registry suggests that selective use of MV-pPCI does not increase the mortality rate in patients with CS-STEMI and MVD compared to CV-pPCI.

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Conflict of interest

The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

Author contributions

O. Hlinomaz contributed to writing - original draft preparation, writing - reviewing and editing, and visualization. Z. Motovska contributed to conceptualization, methodology, writing - reviewing and editing, and supervision. P. Kala, M. Hromadka, J. Precek, J. Mrozek, P. Cervinka, J. Kettner, J. Matejka, A. Zohor, J. Bis contributed to data curation. J. Jarkovsky contributed to formal analysis and data curation. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki.

Informed Consent Statement

Informed consent for performing PCI was obtained. Data from patients treated with PCI must be, in accordance with applicable law, entered into the national registry.

Impact on Daily Practice

Selective use of multivessel primary PCI does not increase the 30-day and 1-year mortality in patients with STEMI, CS, and MVD compared to CV-pPCI. The predictors of mortality were age above 70 years, presence of chronic kidney disease or failure, artificial lung ventilation, 3-vessel, left main disease, and use of ECMO. TIMI flow 3 at the end of primary PCI and inferior myocardial infarction increased the probability of survival.

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Appendix A. Supplementary data

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Myocardial native T_1 mapping and extracellular volume quantification in asymptomatic female carriers of Duchenne muscular dystrophy gene mutations

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Abstract

Background Female carriers of dystrophin gene mutations (DMD-FC) were previously considered non-manifesting, but in recent decades, cardiomyopathy associated with muscular dystrophy and myocardial fibrosis has been described. Our study aimed to assess prospectively myocardial fibrosis in asymptomatic DMD-FC compared to a sex-matched control group (CG) with similar age distribution using native T_1 mapping and extracellular volume (ECV) quantification by cardiovascular magnetic resonance (CMR) imaging.

Materials and methods 38 DMD-FC with verified genetic mutation and 22 healthy volunteers were included. Using CMR, native T_1 relaxation time and ECV quantification were determined in each group. Late gadolinium enhancement (LGE) was assessed in all cases.

Results There were 38 DMD-FC (mean age 39.1 ± 8.8 years) and 22 healthy volunteers (mean age 39.9 ± 12.6 years) imagined by CMR. The mean global native T_1 relaxation time was similar for DMD-FC and CG (1005.1 ± 26.3 ms vs. 1003.5 ± 25.0 ms; p-value = 0.81). Likewise, the mean global ECV value was also similar between the groups (27.92 ± 2.02% vs. 27.10 ± 2.89%; p-value = 0.20). The segmental analysis of mean ECV values according to the American Heart Association classification did not show any differences between DMD-FC and CG. There was a non-significant trend towards higher mean ECV values of DMD-FC in the inferior and inferolateral segments of the myocardium (p-value = 0.075 and 0.070 respectively).

Conclusion There were no statistically significant differences in the mean global and segmental native T_1 relaxation times and the mean global or segmental ECV values. There was a trend towards higher segmental mean ECV values of DMD-FC in the inferior and inferolateral walls of the myocardium.

Keywords Cardiac magnetic resonance, Duchenne muscular dystrophy, Native T_1 mapping, Extracellular volume quantification, Late gadolinium enhancement

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Introduction

Duchenne (DMD) and Becker (BMD) muscular dystrophies are X-linked recessive disorders caused by mutations in the dystrophin gene. Progressive muscular wasting, weakness, respiratory failure, and cardiovascular diseases are caused by pathogenic variants in DMD patients [1, 2]. It has been found that cardiac complications play a relevant role in muscular dystrophies [3].

The most common form of cardiac involvement in muscular dystrophy is dilated cardiomyopathy [4, 5], presenting as an age-related progression of left ventricular (LV) dysfunction and myocardial fibrosis, which can be detected by late gadolinium enhancement (LGE) cardiovascular magnetic resonance (CMR) imaging [6].

Female carriers of Duchenne muscular dystrophy gene mutations (DMD- healthy patients) were previously considered non-manifesting [5]. However, in recent decades, it has become evident that DMD-FC can be affected similarly, albeit more mildly, than DMD patients (affected males) [7–10]. DMD-FC can present cardiac involvement, such as cardiomyopathy, associated with muscular dystrophy [11, 12]. Although DMD-FC are usually asymptomatic, they also can be affected, similarly to DMD patients, by myocardial fibrosis. A well-established technique for assessing myocardial fibrosis is late gadolinium enhancement (LGE). However, it is limited for identifying interstitial diffuse fibrotic changes in the myocardium [13]. Since it may precede the development of LV dysfunction [11], its assessment is crucial in DMD-FC.

Therefore, other CMR-based methods, such as native T_1 mapping and extracellular volume (ECV) quantification, might be more suitable for detecting diffuse myocardial fibrosis, as already-published studies in DMD/BMD patients have shown [14–18]. Also, there have been a few published reports about mildly elevated native T_1 relaxation time or elevated ECV values in DMD/BMD-FC [11, 19, 20].

This study aims to assess prospectively myocardial fibrosis in asymptomatic DMD-FC compared to a sex-matched control group (CG) with similar age distribution using native T_1 mapping and ECV quantification by CMR. Prior to now, there had been no direct comparison to CG.

Materials and methods

The demographically similar study population included 38 DMD-FC with verified genetic mutation and 22 healthy volunteers. The most common mutation was the deletion of 45–52 exons. The asymptomatic DMD-FC were defined based on clinical examination, and all completed the prepared questionnaire.

All eligible subjects who fulfilled the inclusion criteria (age over 18 years, signed informed consent, absence of CMR contraindications, and cardiovascular pathology besides dystrophic cardiomyopathies) were enrolled. The volunteers had no pathological findings on CMR, no anamnesis of cardiac disease, and no other pathological tests. Exclusion criteria for both groups included renal insufficiency (estimated glomerular filtration rate < 30 mL/min/1.73 m^2), CMR contraindications, or limited life expectancy.

The hematocrit was obtained on the same day that CMR was performed. Following the Declaration of Helsinki (2000) of the World Medical Association, the Faculty Hospital St. Anne's Ethics Committee (reference number 55 V/2016) approved the study.

CMR acquisition

CMR was performed using a 1.5T scanner (Ingenia, Philips Medical Systems, Best, The Netherlands) according to our standard protocol. It was equipped with 5-element posterior and 32-element anterior phased-array receiver coils allowing for parallel acquisition techniques in the supine position with repeated breath-hold. Functional imaging using balanced turbo field echo (b-TFE) cine sequences included four-chamber, two-chamber, and LV outflow tract long-axis views and a short-axis (SAX) stack. Typical acquisition parameters were: field of view 320×280 mm, reconstruction matrix 256, slice thickness 8 mm, acquisition voxel size 1.7×1.7×8.0 mm, repetition time ≈ 3.2 ms, echo time ≈ 1.6 ms, and SENSE factor 1.7.

LGE images in all long-axis views and the SAX view were acquired 10 min after an intravenous bolus of 0.2 mmol/kg of the gadolinium-based contrast agent gadobutrol (Gadovist, Bayer-Schering Pharma, Germany) using an inversion recovery (IR-TFE) sequence, and, if uncertain, by phase-sensitive inversion recovery TFE (PSIR-TFE). Both two- and three-dimensional acquisitions were performed in mid-diastole. Typical parameters for the IR-TFE sequence were field of view 320×320 mm, reconstruction matrix 288, voxel size 1.6×1.7×10 mm, repetition time ≈ 4.1 ms, echo time ≈ 1.2 ms, and SENSE factor 2.5.

T_1 mapping was performed as described previously [17] using a Modified Look-Locker Inversion recovery sequence (MOLLI) with a 5(3)3 scheme to measure native T_1 (pre-contrast) and a 4(1)3(1)2 scheme for T_1 post-gadolinium (15 min after contrast agent administration). MOLLI sequences were acquired at the mid-ventricular level in the SAX plane using typical imaging parameters (field of view 300×300 mm, reconstruction matrix 256, slice thickness 10 mm, acquisition voxel size 2.00×2.00×10.00 mm, time to repetition ≈ 2.2 ms, echo time ≈ 1.1 ms, flip angle 35°, SENSE factor 2).

MR data analysis

Two experienced readers (MLMP, TH) used cvi42 (release 5.13.9, Circle Cardiovascular Imaging, Calgary, Canada) to construct the native T_1 and post-gadolinium T_1 maps. They manually contoured the epi- and endocardial walls in the mid-ventricular slice of SAX using 10% of the myocardial wall as border cutting in both the native and the post-gadolinium images. Then, a motion correction algorithm (integrated into cvi42) was applied to register the images, and the software calculated pixel-by-pixel maps from these images. The ECV quantification was calculated according to (1-hematocrit) (1/T1myo,post – 1/T1myo,native)/(1/T1blood,post – 1/T1blood,native) for each segment, and the mean global ECV value was the average of the values in those segments [21]. The acronyms of the mentioned formula represent: the native and post-gadolinium T_1 values of the myocardium/blood before and after the application of the contrast agent.

Wall motion abnormalities were assessed qualitatively (visually) by an experienced cardiologist (RP) with 29 years of experience and a radiologist (VF) with 38 years of experience with CMR. LV functional and morphological parameters were calculated from the SAX stack using the summation-of-disc method following the recommendations on post-processing evaluation from the Society for Cardiovascular Magnetic Resonance (SCMR) [22].

The radiologist (VF) and the cardiologist (RP) employed a semi-quantitative approach to determine the presence of LGE according to the American Heart Association (AHA) 17-segment model [23] in the IntelliSpace Portal workspace (version 11, Philips Healthcare). LGE was defined as positive if the visual enhancement was higher than the mean signal intensity of the reference myocardium, a remote or unaffected myocardial region within the same patient. A DMD-FC with LGE in at least one myocardial segment was considered LGE-positive. If no enhancement was observed, the DMD-FC was identified as LGE-negative.

Statistics

Variables in both groups were compared using the Student's t-test for unpaired data. The normal distribution was checked by the Kolmogorov-Smirnov test and a visual inspection of histograms; in a case when the Student's t-test revealed no difference, this meant the Kolmogorov-Smirnov test was also used to check the identity of distributions of quantitative variables in both groups. The data are presented as mean ± standard deviation (SD).

The power of the tests was considered for native T_1 relaxation time and ECV values to determine the magnitude of the effect that could be identified as statistically significant by the Student's t-test. The following assumptions were used: $\alpha = 0.05$; power = 0.8 (0.6, respectively); normal distribution; standard deviation corresponding with the actual distribution in the CG. As the nature of our study was explorative, we neither assumed nor performed any multiple testing corrections, which would increase the risk of type II error and decrease the power of the test.

The interobserver agreement of both native and post-gadolinium global T_1 relaxation time was assessed with the intraclass correlation coefficient (ICC, two-way mixed-effects model), which was determined from eight randomly selected cases analysed by two readers (MLMP, TH).

All analyses were performed using Statistica (version 14.0. TIBCO Software Inc., 2020) and in R (v4.2.1) with RStudio IDE (v2022.7.1.554, RStudio, PBC) software. The value of $\alpha = 0.05$ was used as a threshold for statistical significance throughout the analyses.

Results

38 DMD-FC (mean age 39.1 ± 8.8 years) and 22 healthy volunteers (mean age 39.9 ± 12.6 years) were included. There was no statistically significant difference between the LVEF of DMD-FC (65.7 ± 5.5%) and CG (68.4 ± 6.57%; p = 0.09). 5% of asymptomatic DMD-FC of our cohort had small hypokinesia of the apex and the inferolateral wall of LV. Also, 20% of DMD-FC had LGE that was detected on the inferolateral wall (4 DMD-FC) and inferior wall (3 DMD-FC) of LV, while LGE was absent in the CG.

The mean global native T_1 relaxation time was similar for DMD-FC and CG (1005.1 ± 26.3 ms vs. 1003.5 ± 25 ms; p-value = 0.81) (Fig. 1), as well as the mean global ECV value (27.92 ± 2.02% vs. 27.09 ± 2.89%; p-value = 0.20) (Fig. 2). The representative native and post-gadolinium T_1 maps are presented in Fig. 3 for DMD-FC and Fig. 4 for healthy volunteer. No statistically significant differences were detected between DMD-FC with LGE positive and LGE negative in the mean ECV segmental values. The ECV segmental quantification analysis, according to the AHA classification, did not show any differences for DMD-FC vs. CG. Although statistically non-significant, there was a trend towards higher mean ECV values in the inferior (p = 0.075) and inferolateral (p = 0.070) segments of the myocardium in DMD-FC. The detailed results are shown in Table 1. When the identical distribution of measured variables in DMD-FC and in CG was checked by Kolmogorov-Smirnov test, it yielded a p-value > 0.10 in all cases, including mean global and segmental T_1 relaxation times and ECV values, age and LVEF.

Regarding the global native T_1 relaxation time, the sample size was sufficiently large to statistically prove a difference of 19 ms with a power of 80% and a difference of 15 ms with a power of 60%. In the case of ECV values, the sample size enabled the discrimination of values

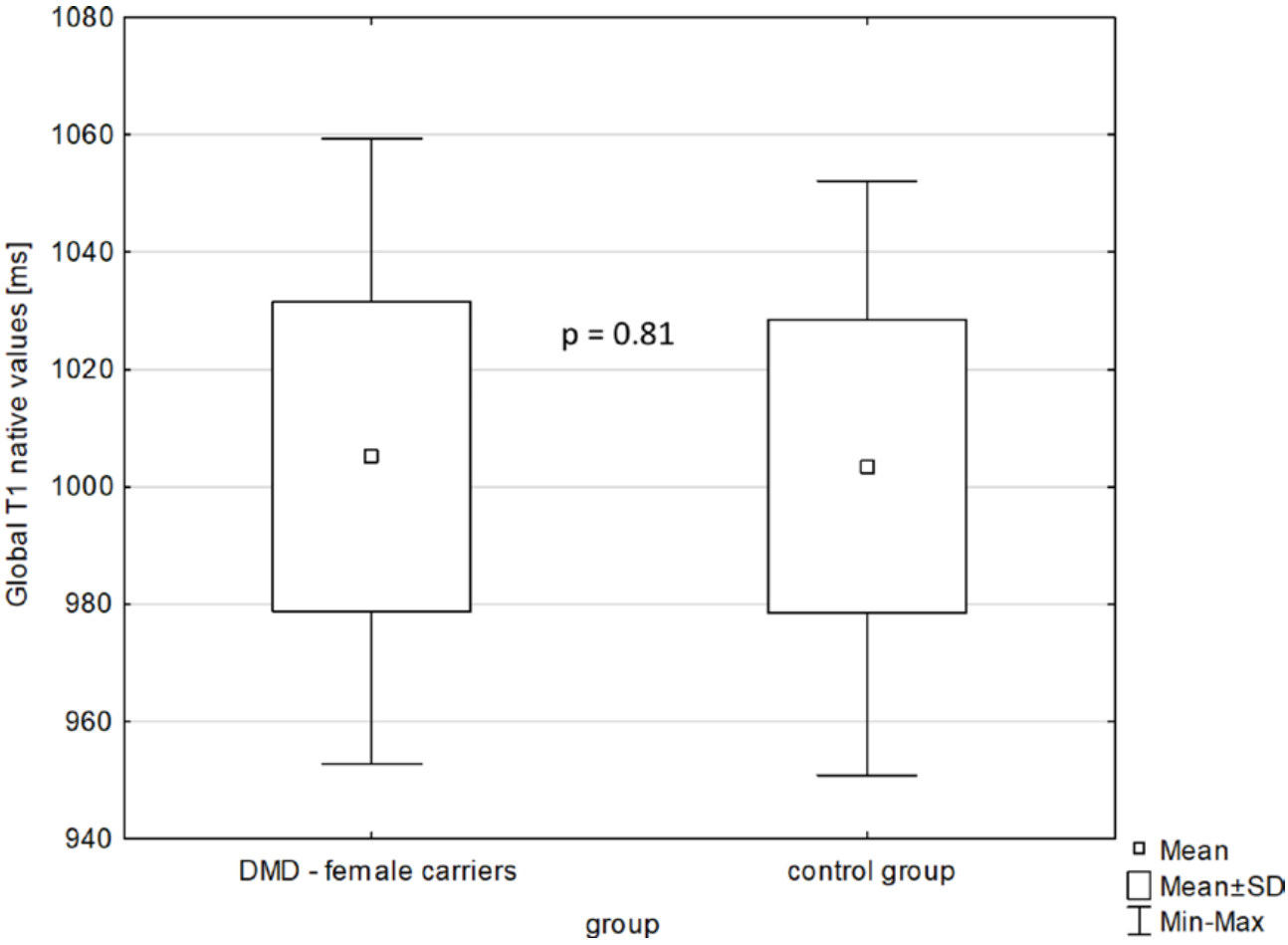

Fig. 1 The mean global native T_1 relaxation time values [ms] in DMD-FC and CG.DMD- FC = Female carriers of Duchenne muscular dystrophy gene mutations; CG = Control group

differing by 2.3% with a power of 80% and a difference of 1.8% with a power of 60%.

The values of ICC were 0.980 (95% CI: 0.921 to 0.995) for mean global native T_1 and 0.976 (95% CI: 0.906 to 0.994) for mean global T_1 post-gadolinium relaxation times, showing excellent interobserver reliability.

Discussion

There were no statistically significant differences in the mean global and segmental native T_1 relaxation times or the mean global and segmental ECV values in asymptomatic DMD-FC with normal LVEF compared to sex -matched CG with similar age distribution. Regarding the segmental values, although statistically non-significant, there was a trend towards higher mean ECV values in the inferior and inferolateral segments of the myocardium in DMD-FC in our study. This may be potentially of concern, as similar segments are affected as in DMD patients; however it should be noted that the magnitude of those differences is much smaller than of those observed between DMD patients and CG, as is evident in various cited studies [15, 16] including ours [17], which correspond to the typically affected regions in the muscular dystrophies.

Cardiac involvement, such as cardiomyopathy and the presence of LGE in typical localization, was recently described in DMD-FC [11, 12]. To date, there have been only 3 published works focused on native T_1 mapping and ECV quantification in DMD/BMD-FC [11, 19, 20], of which only one used a case-control design.

The first of the studies describes diffuse myocardial fibrosis assessed by ECV quantification in DMD/BMD-FC of gene mutations for muscular dystrophies [20]. Analysed were 5 DMD/BMD-FC ranging from 43.0 to 51.7 years. It showed elevated global ECV values of 37.4% in DMD/BMD-FC and BMD/DMD/myotonic dystrophic patients regardless of the presence of LGE. All 5 screened DMD/BMD-FC were older than DMD-FC in our study. Moreover, one of them had reduced LVEF, 2 of them were LGE positive, and there was also a combination of DMD and BMD-FC.

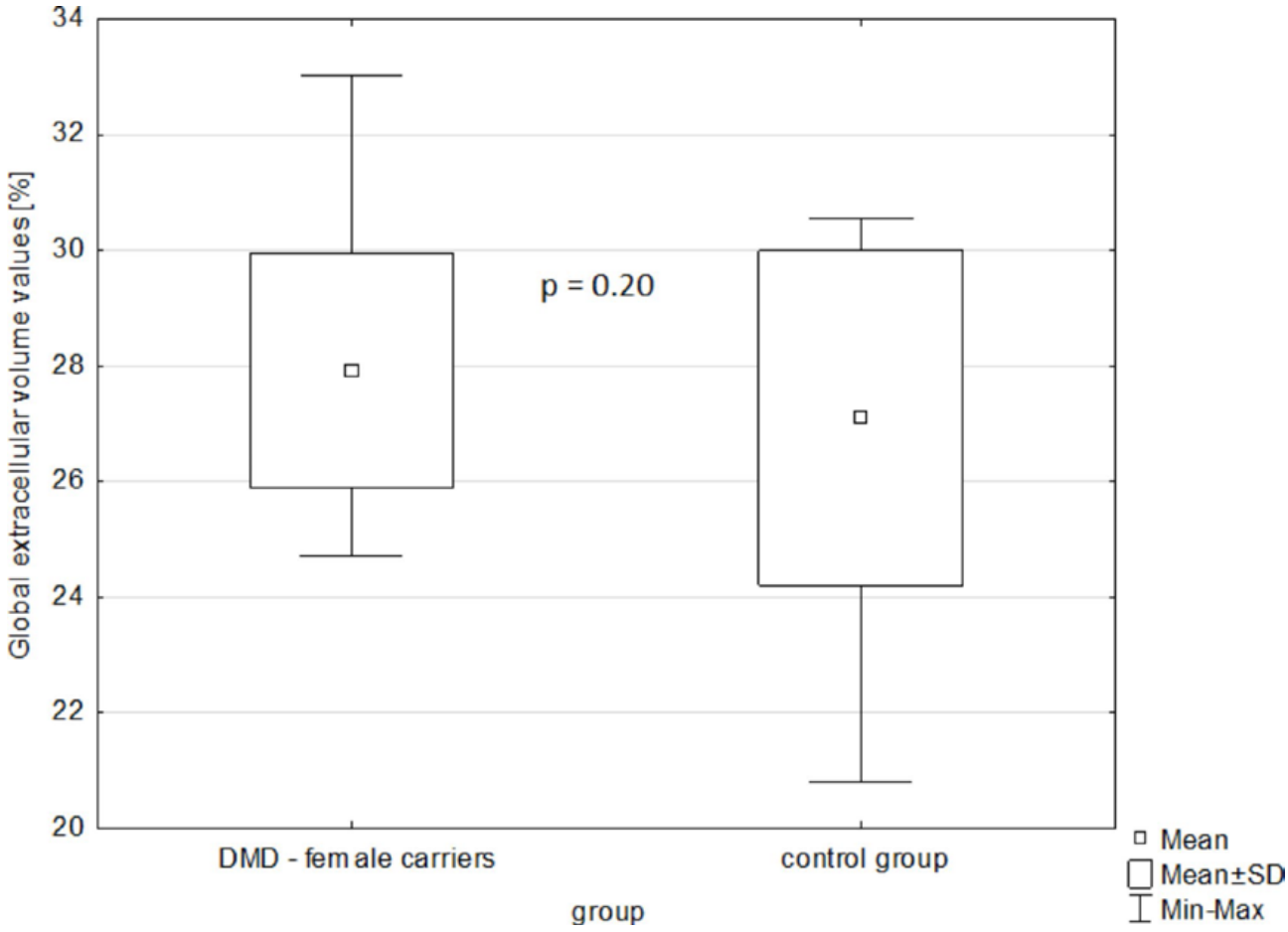

Fig. 2 The mean global extracellular volume values [%] in DMD-FC and CG.DMD- FC = Female carriers of Duchenne muscular dystrophy gene mutations; CG = Control group

The second one [11] also reported mildly abnormal ECV values in DMD-FC.

The last study compared the DMD-FC to non-carrier female relatives and CG and found higher native T_1 relaxation time in DMD/BMD-FC compared to non-carriers and CG [19]; however this study did not assess the ECV quantification or regional differences in native T_1 relaxation time. While our study had sufficient power to reveal the differences in global native T_1 relaxation time with a size comparable to [19] and employed study subjects of a comparable age, it did not confirm the increase in native T_1 relaxation time found in this study.

The main contribution of our study compared to the mentioned studies [11, 12, 19, 20] is the evaluation of the diffuse fibrotic changes of the myocardium using native T_1 mapping and ECV quantification in a much larger cohort of DMD-FC, and with regards to CG. Moreover, we focused on the assessment of more than just the presence of LGE in the typical location or the described mildly elevated value of ECV or global native T_1 relaxation time. This is the first study that compares global and segmental native T_1 and ECV values between DMD-FC and CG in a case/control design.

LGE can aid in detecting the extent and the location of myocardial fibrosis. Additionally LGE can uncover incipient heart disease by its presence [14, 24]. Whereas native T_1 mapping and ECV quantification allow for the measurement of diffuse fibrosis that foregoes the mentioned LGE. These methods are also useful in detecting myocardial inflammation or any alteration of the extracellular space [14, 25].

Contrary to DMD-FC, many more studies have assessed native T_1 mapping and quantified ECV in DMD patients [16, 26, 27]. Although LGE positive DMD patients exhibited an increased global native T_1 relaxation time compared to CG, there was no difference between LGE negative DMD patients and CG [27]. Moreover, DMD patients had a significantly increased lateral myocardial native T_1 relaxation time compared to CG [28].

These results align with other authors who detected increased native T_1 relaxation time and ECV values in DMD patients. ECV quantification was regardless the

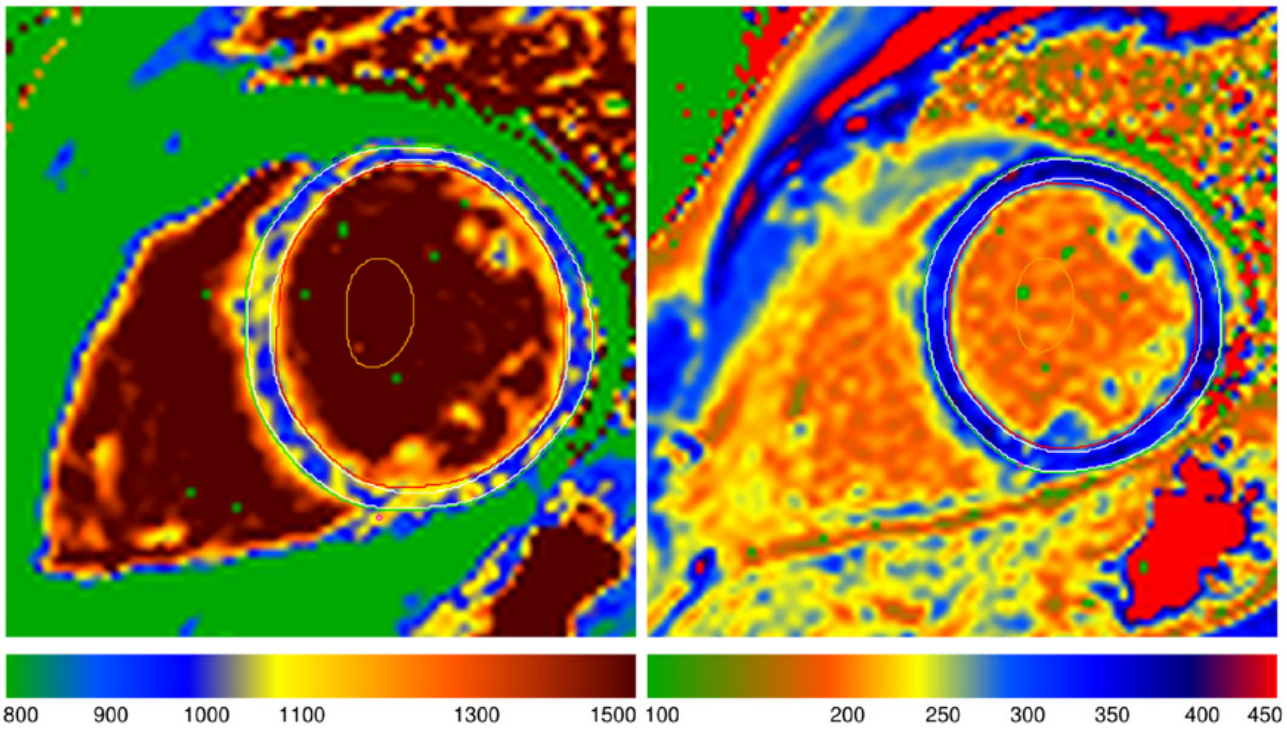

Fig. 3 A representative picture of the native and post-gadolinium T_1 map of DMD-FC. DMD-FC - Female carriers of Duchenne muscular dystrophy gene mutations

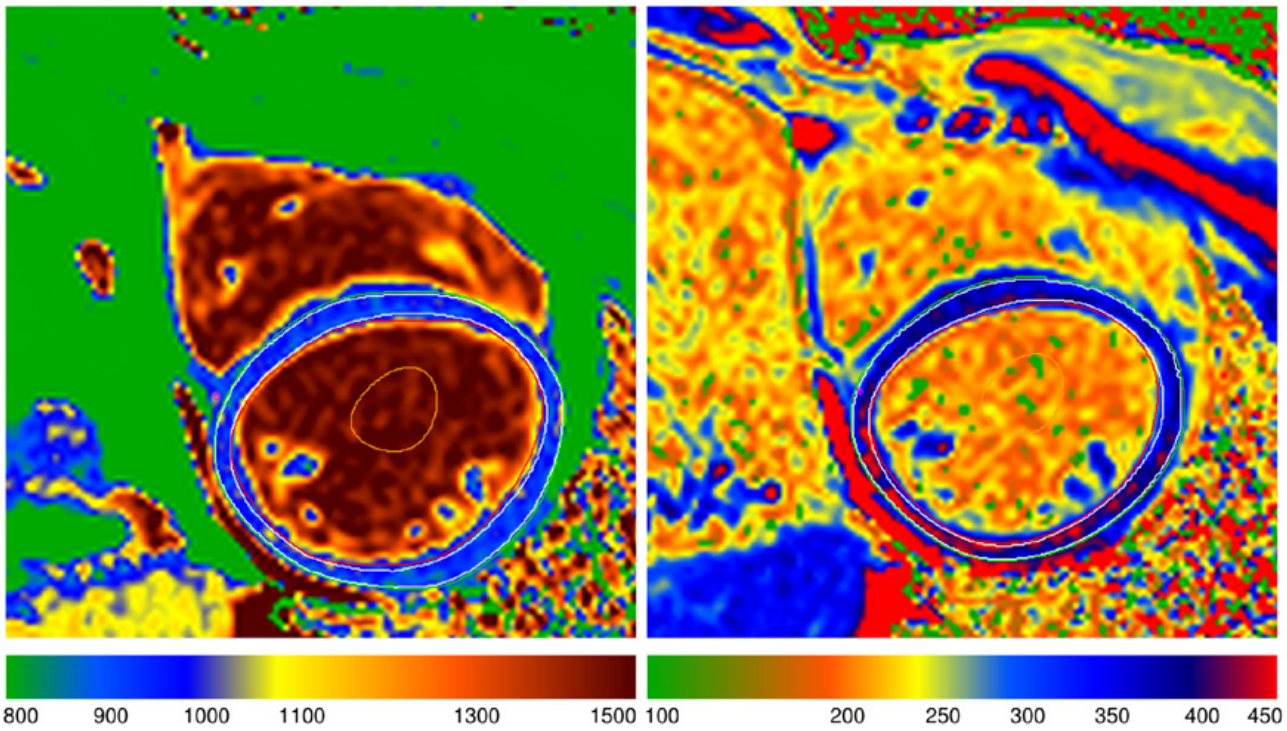

Fig. 4 A representative picture of the native and post-gadolinium T_1 map of a healthy volunteer

Table 1 Comparison of mean global and segmental native T_1 relaxation time and mean global and segmental ECV values and other parameters between DMD-FC and the CG

Parameter	DMD-FC (n = 38)	CG (n = 22)	P-value
Age [years]	39 ± 8.8	39.9 ± 12.6	0.76
BMI [kg/m^2]	23.8 ± 3.4	23.17 ± 3.9	0.64
LVEF [%]	65.6 ± 5.5	68.4 ± 6.57	0.33
Global native T_1 relaxation time [ms]	1005.1 ± 26.3	1003.5 ± 25.0	0.81
Native T_1 relaxation time [ms]: anterior segment	997.3 ± 33.7	1006.7 ± 35.9	0.31
Native T_1 relaxation time [ms]: anteroseptal segment	1001.5 ± 35.2	994.9 ± 26.9	0.45
Native T_1 relaxation time [ms]: inferoseptal segment	1013.8 ± 26.0	1004.5 ± 30.8	0.22
Native T_1 relaxation time [ms]: inferior segment	1008.7 ± 40.1	1003.5 ± 31.9	0.60
Native T_1 relaxation time [ms]: inferolateral segment	1012.5 ± 32.4	1012.4 ± 36.7	0.99
Native T_1 relaxation time [ms]: anterolateral segment	992.8 ± 32.0	1004.7 ± 37.0	0.19
Global ECV value [%]	27.93 ± 2.02	27.10 ± 2.89	0.20
ECV value: anterior segment	27.86 ± 2.35	26.90 ± 2.66	0.16
ECV value: anteroseptal segment	27.72 ± 2.10	27.62 ± 3.17	0.88
ECV value: inferoseptal segment	28.41 ± 2.21	27.73 ± 3.25	0.34
ECV value: inferior segment	28.26 ± 2.62	26.79 ± 3.65	0.075
ECV value: inferolateral segment	27.83 ± 2.94	26.30 ± 3.34	0.070
ECV value: anterolateral segment	27.63 ± 2.30	27.02 ± 3.12	0.39

Parameters are shown as mean ± standard deviation

BMI = body mass index; ECV = extracellular volume; DMD- FC = Female carriers of Duchenne muscular dystrophy gene mutations; CG = Control group; LVEF = left ventricle ejection fraction

ability to differentiate LGE negative DMD patients and CG [29].

Both native T_1 mapping and ECV quantification can identify the differences and regional abnormalities on the inferolateral wall of the LV between DMD patients and CG with or without LGE [21, 30–33]. Additionally, increased native T_1 relaxation time and reduced LVEF was reported in LGE positive DMD patients, evidencing a relationship between native T_1 relaxation time and LV function [28].

Based on our previous studies, all global LV strains and tissue Doppler parameters in asymptomatic DMD-FC were decreased compared to CG [34, 35]. Despite a preserved LVEF, subclinical changes in the LV systolic function were discovered in DMD-FC, including decreased global LV strains. However, their mean global and segmental native T_1 relaxation times and mean global and segmental ECV values were similar to sex- matched CG with similar age distribution. Furthermore, 20% of our DMD-FC cohort had LGE on the inferolateral wall of LV, which is typical localization for the extracellular volume expansion described in DMD patients [36, 37].

While the differences in native T_1 relaxation time and ECV quantification in symptomatic DMD patients are already known, our DMD-FC cohort did not have any cardiac symptoms.

Limitations

This work has some limitations. Some of them are related to the general limitations of native T_1 mapping [4, 38]. It is also limited by its sample size due to the rare occurrence of DMD disease. It is a single centre study. All the subjects involved in this study, were analysed under the same conditions: the images were acquired with the same equipment and protocol, and the analyses were assessed similarly. The healthy volunteers were not tightly selected, as they were considered healthy because of their lack of CMR-based evidence of cardiac problems. Any possible bias affected all the subjects equally. Although the interobserver agreement was excellent, it was determined only for the mean global native T_1 relaxation time and mean global ECV values. Future research is essential to validate the whole process's reproducibility.

Conclusion

The mean global and segmental native T_1 relaxation times and mean global and segmental ECV values in asymptomatic DMD-FC with normal LVEF were similar to CG, as there was no statistically significant difference in any of the CMR-assessed parameters. Although non-significant, there was a trend towards higher mean ECV values of DMD-FC in inferior and inferolateral segments of the myocardium, which should be subjected to prospective assessment.

Abbreviations

AHA	American heart association
BMD	Becker muscular dystrophy
b-TFE	balanced - turbo field echo
CI	Confidence interval
CG	Control group
CMR	Cardiac magnetic resonance
DMD	Duchenne muscular dystrophy

DMD-FC	Female carriers of Duchenne muscular dystrophy gene mutations
ECV	Extracellular volume
EDV	End-diastolic volume
ESV	End-systolic volume
ICC	Intraclass correlation
IR-TFE	Inversion-recovery turbo field echo
LGE	Late gadolinium enhancement
LV	Left ventricle
LVEF	Ejection fraction of left ventricle
PSIR	Phase-sensitive inversion recovery
SAX	Short axis
SCMR	Society for Cardiovascular Magnetic Resonance
SD	Standard deviation
T1myo,post	post-gadolinium T_1 values of myocardium
T1myo,native	native T_1 values of myocardium
T1blood,post	post-gadolinium T_1 values of blood
T1blood,native	native T_1 values of blood

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13023-023-02899-9.

Supplementary Material 1

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Not applicable.

Author's contribution
RP, VK, and MP were the main contributors to the study; they also designed the study. The main role of LM was to write the manuscript. LJ and LO contributed to patient recruitment and inclusion, VF and TH supervised the CMR examinations, and VF and LO performed the clinical data analysis. MMP and TH performed the CMR data analysis, and JM performed the statistical analysis. All authors read, critically reviewed, and approved the final manuscript.

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Data Availability
The datasets analysed during the current study are available from the corresponding author upon reasonable request. Data are located in controlled access data storage REDCap at St. Anne's University Hospital (https://redcap.fnusa.cz/redcap/).

Declarations

Ethics approval and consent to participate
The study was performed in accordance with the Declaration of Helsinki (2000) of the World Medical Association. Ethics Committee of the Faculty Hospital St. Anne's (reference number 55 V/2016) approved the study. Written informed consent was obtained from the subjects and/or their legally authorised representative.

Consent for publication
no person´s personal data are published.

Competing interests
The authors declare that they have no competing interests.

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Secretoneurin levels are higher in dilated cardiomyopathy than in ischaemic cardiomyopathy: preliminary results

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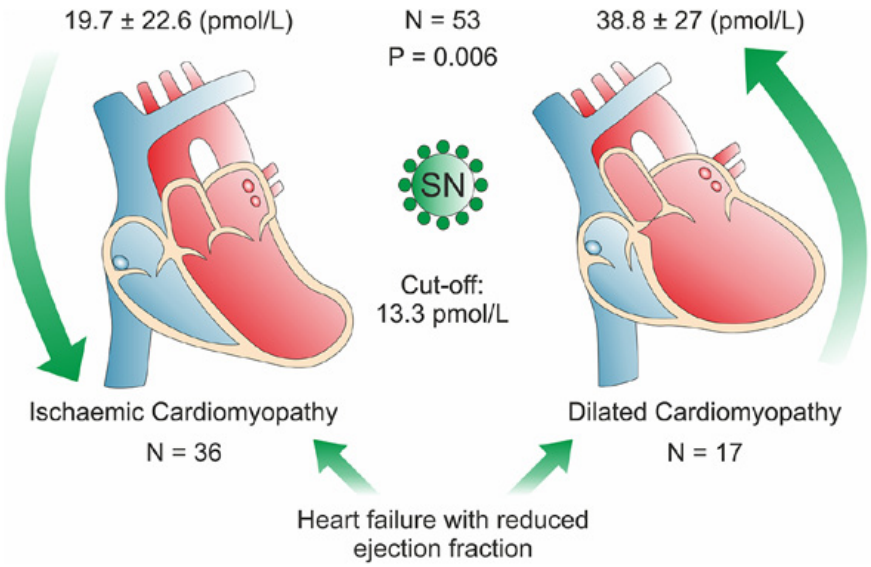
Background: Secretoneurin (SN) is a neuropeptide with potential utility as a biomarker of cardiovascular episodes. The main effect of SN is mediated through its inhibition of calmodulin-dependent kinase II (CaMKII), which influences calcium handling. We aimed to associate the levels of SN in plasma with different causes of heart failure.
Methods: We prospectively enrolled consecutive patients with ischaemic (ICM) and dilated (DCM) cardiomyopathy from the outpatient heart failure clinic and healthy individuals. SN was analysed from venous blood by use of the ELISA method. SN plasma levels were compared in DCM, ICM and healthy individuals with non-parametric tests.
Results: A total of 53 patients (81.1% male, 18.9% female; mean age 67.9 ± 12.6 years) and 34 healthy individuals (38% male, 62% female) were included in the analysis. Plasma SN levels were significantly higher in the dilated cardiomyopathy (38.8 ± 27 pmol/L) as compared with the ischaemic cardiomyopathy (19.7 ± 22.6 pmol/L) group ($P = 0.006$). There was no significant difference between females vs. males (27.1 ± 23 vs. 25.5 ± 26.2 pmol/L, P = NS). Plasma SN levels allowed DCM and ICM to be differentiated with 88% sensitivity and 61% specificity ($P = 0.007$), the cut of value is 13.3 pmol/L. Plasma SN levels differed significantly between healthy volunteers and both ICM ($P < 0.0001$) and DCM ($P = 0.049$). Plasma SN levels did not differ according to age and were not associated with comorbidities, left ventricular ejection fraction, heart failure medication, troponin, creatinine, or natriuretic peptide plasma levels.
Conclusion: Plasma secretoneurin levels differed significantly in DCM vs. ICM, being higher in the former. Based on plasma SN levels, discrimination between DCM and ICM might be possible. Healthy individuals produce higher SN plasma levels than stable HFrEF patients.

KEYWORDS

secretoneurin, heart failure, CaMKII, dilated cardiomyopathy, ischaemic cardiomyopathy



GRAPHICAL ABSTRACT

Introduction

Secretoneurin (SN) is a 33–amino acid neuropeptide from the chromogranin peptide family. SN may be a novel biomarker with potential use in cardiovascular medicine (1). Its pathway differs from those of the most often measured natriuretic peptides and troponin. SN's main effects are most likely transmitted by calmodulin-dependent kinase II (CaMKII). However, other cellular pathways may play a role (2). Since CaMKII is one of the regulators of calcium handling in the cell, it may enhance protective effects of SN in the diseased myocardium (3). CaMKII inhibition in the myocardium improves contraction and suppresses proneness to arrhythmia by diminishing calcium leakage from the sarcoplasmic reticulum (3). Calcium is crucial to myocardial excitation-contraction coupling and intracellular signalling. Since in heart failure patients, the calcium handling is known to be disrupted, the SN plasma levels may entail prognostic information (4).

Of note, in the recent sub-analysis of the GISSI-HF trial in patients with chronic heart failure, SN concentrations were associated with mortality, even after adjusting for multiple factors (5). SN levels were also weakly associated with admission to the hospital for cardiovascular reasons (5).

We already know that SN levels may contain prognostic information in heart failure (HF) patients. Therefore, we aim to address possible differences in the SN levels associated mainly with the HF aetiology.

Materials and methods

Patients

For this study, we prospectively enrolled 53 consecutive patients from the heart failure outpatient clinic of University Hospital Ostrava from August 2022 to January 2023 and 34 heathy volunteers in October 2023. The study sample comprised only patients with a reduced ejection fraction of two causes (ischaemic cardiomyopathy, dilated cardiomyopathy). All patients were in stabilized condition without recent heart failure decompensation. All patients with dilated cardiomyopathy have no coronary artery disease (no coronary artery stenosis $\geq$ 70% or 50% of the common trunk of the left coronary artery). Acute myocardial infarction and/or coronary artery by-pass grafting surgery within three months before enrolment were exclusion criteria.

The healthy volunteers were without any treated disease. Most importantly, no cardiac disease was present, defined by the absence of clinical symptoms, normal echocardiography, and normal plasma levels of markers of cardiac injury at the time of blood sampling. This study was approved by the Institutional Review Board of University Hospital Ostrava (Nr. 526/2022) and conducted in accordance with the Helsinki Declaration. All patients have signed informed consent.

Secretoneurin analysis

Blood sampling was performed in patients with chronic stable heart failure during outpatient visits. SN was analysed from venous

blood by use of the ELISA method (CardiNor AS, Oslo, Norway) (6). The blood was drawn into the lithium-heparin tubes, plasma was separated and frozen to −70°C, only one defrosting cycle was allowed. The intra-assay coefficients of variation for SN were lower than 5% and inter-assay coefficient lower than 10%. The level of determination (LoD) for the CardiNor SN is 5,1 pmol/L, The level of quantification (LoQ) is 7,6 pmol/L. Analytical range is 11,8–299,2 pmol/L.

Statistics

Continuous variables are expressed as mean ± standard deviation or as median and interquartile range when indicated and compared by the Mann–Whitney *U* test since the data were non-normally distributed according to both the Kolmogorov-Smirnov and Levene's tests. Categorical variables are expressed as percentages and compared by the chi-square test, Fisher's exact test, or logistic regression, as appropriate. Correlations between SN levels and other biomarkers were examined by Spearman correlation. The difference between plasma SN levels in ICM vs. DCM or healthy individuals was examined by Kruskal-Wallis or Mann–Whitney *U* tests. Receiver operating characteristics analysis was performed for SN discriminative abilities related to the cause of heart failure. A two-tailed $\alpha < 0.05$ was considered statistically significant. All analyses were performed using IBM SPSS for Mac version 23 (IBM, Armonk, USA).

TABLE 1 Baseline characteristics of the study population, ischemic (ICM) vs. Dilated cardiomyopathy (DCM).

	Total population	ICM	DCM	*P* value
	N = 53	*N* = 37	*N* = 16	
Age (years)	67.9 ± 12.6	69.9 ± 11.6	65.7 ± 14.6	0.574
Males (%)	81.1	83.7	70.6	0.260
Secretoneurin	25.8 ± 25.4 22.5 (45.3)	19.7 ± 22.6 13.3 (38.5)	38.8 ± 27 34.4 (27.2)	0.006
Body weight (kg)	77.1 ± 27.3	73 ± 31	85.6 ± 15.4	0.194
Body height (cm)	172.8 ± 10.8	173.3 ± 10.6	171.8 ± 11.4	0.760
Body mass index (kg/m^2)	27.7 ± 4.5	27 ± 3.9	29.4 ± 5.5	0.084
LV ejection fraction (%)	29.7 ± 6.6	30.8 ± 6.5	27.2 ± 6.3	0.066
Hypertension (%)	86.2	54	29.4	0.052
AF (%)	8.5	8.1	5.9	1.0
Dyslipidaemia (%)	79.3	54	17.6	0.003
Diabetes mellitus (%)	34.6	32.4	35.3	1.0
Previous stroke/ TIA (%)	9.6	16.2	5.8	0.193
NYHA class II (%)	57.7	59.4	47	0.111
NYHA class III (%)	34.6	32.4	35.3	0.128
ACEi (%)	34.8	29.7	29.4	1.0
sGLT2i (%)	56.5	51.3	41.2	0.527
Betablockers (%)	87	70.3	82.3	0.647
MRCA (%)	77.8	62.2	70.6	1.0
Amiodaron (%)	37.8	26.7	35.3	0.497
ARNI (%)	51.1	40.5	50	1.0
NT-pro-BNP	2876.2 ± 4709 1,376 (2885)	3346.9 ± 5539.4 1402.7 (3927)	1990.2 ± 2407 1,000 (2504.6)	0.681
Creatinine	118.3 ± 66.8	103.1 ± 33.1	101.6	0.037
Potassium	3.78 ± 1.4	3.5 ± 1.7	4.3 ± 0.43	0.115
Total calcium	1,59 ± 1.26	1.66 ± 1.26	1.48 ± 1.3	0.617
hs-TnI	526.78 ± 2112	761.7 ± 2597.9	86 ± 136.4	0.474
CRP	8.3 ± 13.8	9.7 ± 15.8	5.4 ± 7.7	0.955

Indices are shown as mean ± standard deviation or percentages for categorical variables and compared for ICM and DCM. As an alternative median and interquartile range is shown when indicated—SN, NT-pro-BNP. ACEi, angiotensin-converting enzyme inhibitor; AF, atrial fibrillation; ARNI, angiotensin receptor/neprilysin inhibitor; CRP, C- reactive protein; hs-TnI, high sensitivity Troponine I; LV, left ventricle; MRCA, mineralocorticoid receptor antagonist; NT-pro-BNP, N-terminal pro brain natriuretic peptide; NYHA, New York heart association; sGLT2i, sodium-glucose cotransporter 2 inhibitors; TIA, transient ischemic attack.

Results

A total of 53 HFrEF patients (81.1% male, 18.9% female) aged 67.9 ± 12.6 with a BMI of 27.7 ± 4.4 and 34 healthy individuals (38% male, 62% female) were included in the analysis. Mean SN values (pmol/L) according to the cause of heart failure were 19.7 ± 22.6 for ischaemic cardiomyopathy (*N* = 36), 38.8 ± 27 for dilated cardiomyopathy (*N* = 17) (7); the median and IQR were as follows ICM: 13.3(29.6), DCM: 34.4 (27.2) pmol/L. Mean SN values for health individuals were 50.7 ± 15.3 pmol/L. The mean left ventricular ejection fraction (LV EF) in HFrEF patients was 29.7 ± 6.6. Comorbidities, anthropometric factors, medication, mean levels of biomarkers and plasma electrolytes are depicted in Table 1.

SN plasma levels differed significantly in the dilated cardiomyopathy (DCM) as compared with the ischemic cardiomyopathy (ICM) group (*P* = 0.006, Figure 1), irrespective

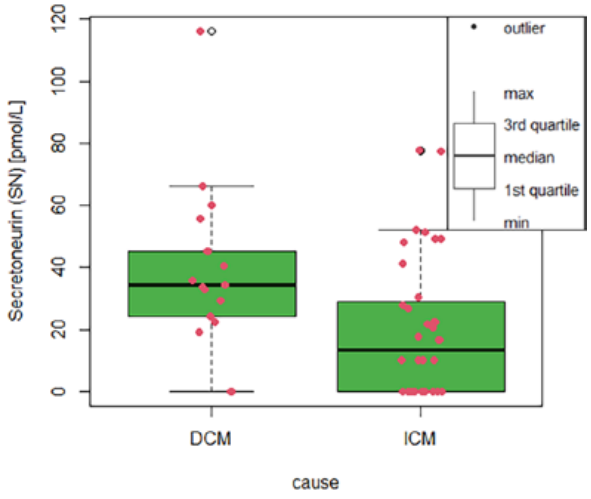


FIGURE 1
Box-plot with whiskers displaying differences in SN plasma level between ICM and DCM. The red dots stand for individual measurement value. Secretoneurin (SN) plasma levels in pmol/L according to the cause of heart failure; ICM, ischaemic cardiomyopathy; DCM, dilated cardiomyopathy.

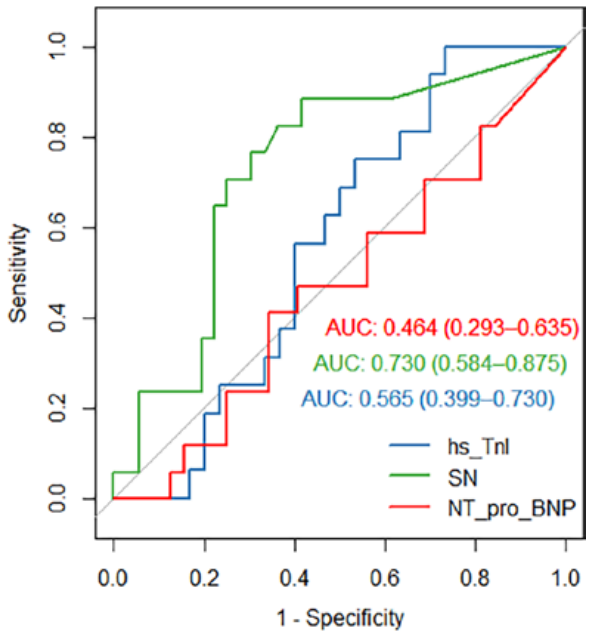


FIGURE 2
Receiver operating curve (ROC) for secretoneurin (SN, green curve), high-sensitivity troponin I (hs-TnI, blue curve) and N-terminal-pro natriuretic peptide (NT-pro-BNP, red curve) differentiating dilated cardiomyopathy from ischaemic cardiomyopathy (SN, AUC 0.73, 95% CI 0.58–0.87, $P = 0.007$; TnI, AUC 0.56, 95% CI 0.4–0.73, P = NS;NT-pro-BNP, AUC 0.44, 95% CI 0.27–0.62, P = NS).

TABLE 2 Baseline characteristics of the healthy volunteers.

	Healthy volunteers	Males	Females	*P* value
	N = 34	*N* = 13	*N* = 21	
Age (years)	31 ± 7.1	30.8 ± 7.1	29.9 ± 6.9	0.596
Secretoneurin	50.7 ± 16.3	50.7 ± 15.3	50.7 ± 16.3	0.64
Body weight (kg)	69 ± 14	69 ± 14	67 ± 14	0.001
Body height (cm)	171.8 ± 8.9	171.8 ± 8.9	170.8 ± 8.9	<0.001
Body mass index (kg/m^2)	23.2 ± 3.6	23.1 ± 3.6	23 ± 3.6	0.477
NT-pro-BNP	55.5 ± 29.2	55.5 ± 29.2	57.5 ± 30	0.066
Creatinine	74.1 ± 12.45	74.1.1 ± 12.45	71.9 ± 10.6	<0.001
Natrium	138.2 ± 1.75	138.2 ± 1.75	138.2 ± 1.76	0.208
Potassium	4.1 ± 0.29	4.1 ± 0.29	4.1 ± 0.3	0.03
Ca^{2+} (ionized)	1.17 ± 0.1	1.2 ± 0.1	1.17 ± 0.1	0.519
Total calcium	2.48 ± 0.13	2.48 ± 0.11	2.48 ± 0.13	0.079
Mg^{2+}	0.79 ± 0.1	0.79 ± 0.1	0.79 ± 0.05	0.341
hs-TnI	8.1 ± 29.4	8.1 ± 29.42	8.6 ± 31	0.195
Hs-CRP	1.8 ± 2.36	1.8 ± 2.4	2 ± 2.4	0.026
IL-6	3.1 ± 2.6	3.1 ± 2.6	3.2 ± 2.8	0.440
Leukocytes	7.52 ± 1.7	7.5 ± 1.7	7.5 ± 1.6	0.201
Thrombocytes	280 ± 59.1	280 ± 59.1	281.8 ± 59	0.013

Indices are shown as mean ± standard deviation and compared for males and females. Ca^{2+}, ionized calcium; Hs-TnI, high-sensitivity Troponine I; IL-6, interleukin 6; Mg^{2+}, ionized magnesium; NT-pro-BNP, N-terminal-pro-brain natriuretic peptide.

of NYHA class, heart failure pharmacotherapy and LV EF. In the multivariable analysis, SN still differed significantly between ICM and DCM, even after entering LV EF, age, heart failure treatment and hypertension/gender as covariates in the regression model. On the contrary, LV EF did not differ significantly between the DCM and the ICM group (27.2 ± 6.3 vs. 30.8 ± 6.5, P = NS). The presence/absence of outliers in both groups did not change the test result, what means that the significance or non-significance of the test was not different; the outliers stayed in the analysis. Coronary artery by-pass grafting surgery was done 24.1% of the ICM patients, all of them more than one year before the SN plasma levels sampling. There was no difference between SN plasma levels in females and males, respectively (27.1 ± 23 vs. 25.5 ± 26.2 pmol/L, P = NS); the median and IQR for females/males 26.8 (49.8), 22.4 (41.3) pmol/L, respectively. The ICM vs. DCM group differ in Dyslipidaemia ($P = 0.003$) and creatinine level ($P = 0.036$). The groups did not differ in age, anthropometric factors, diabetes, cerebrovascular accidents, NYHA class, LV EF, analytes, or HF medication (Table 1). Moreover, SN levels were able to discriminate dilated cardiomyopathy from ischemic cardiomyopathy as causation with 88% sensitivity and 61% specificity (AUC 0.73, 95% CI 0.58–0.87, $P = 0.007$, Figure 2), the cut-off value is 13.3 pmol/L. Of note, plasma SN did not vary according to age and was not associated with comorbidities, left ventricular ejection fraction, troponin, creatinine, or natriuretic peptide levels in plasma. There were also no significant correlations between SN and N-terminal pro natriuretic peptide (NT-pro-BNP), high-sensitivity troponin (hs-TnI), creatinine, natrium, total calcium or potassium plasma levels.

There is also no significant difference between total calcium between ICM and DCM (Table 1). However, there is a significant difference in calcium levels between the whole HFrEF group and the healthy individuals ($P = 0.0001$).

Our healthy volunteers ($N = 34$, 62% females, Table 2) have SN plasma levels of 50.7 ± 15.3 pmol/L, which was significantly higher than in both DCM ($P = 0.049$) and ICM ($P < 0.0001$) or HFrEF (ICM/DCM combined), $P < 0.0001$. Males and females in the healthy individuals group differed in anthropometric parameters, plasma creatinine level, hs-CRP, and thrombocyte count (Table 2). We have not observed myocardial injury in any of the healthy individuals (Table 2). Our healthy volunteers were also significantly younger ($P < 0.0001$) and differed in the BMI ($P < 0.0001$), being lower in the healthy individuals as compared to the HFrEF group.

SN plasma levels in healthy individuals were not associated with total or ionized calcium, hs-CRP, IL-6, pH, hs-TnI, NT-pro-BNP, or albumin level. Plasma levels of SN did not vary according to the age, sex, anthropometric parameters, thrombocytes, haemoglobin, or leukocytes in the healthy individuals (P = NS). Potassium and ionized magnesium levels were borderline non-significant when associated to the SN plasma levels ($P = 0.064$, $P = 0.065$, respectively).

Discussion

Main findings

The main findings of our preliminary analysis can be summarized as follows:

(1) We found plasma SN levels to be significantly higher in patients with dilated cardiomyopathy than in patients with ischaemic cardiomyopathy.
(2) Plasma SN levels were able to differentiate between DCM and ICM with 88% sensitivity and 61% specificity with a cut-off value 13.3 pmol/L.
(3) Plasma SN levels differed significantly in healthy volunteers as compared to ICM or DCM, healthy volunteers produced higher plasma SN.
(4) Plasma SN levels did not differ according to age or sex and were not associated with comorbidities, left ventricular ejection fraction, troponin, creatinine, or natriuretic peptide levels in plasma.

SN takes part in many processes, namely: apoptosis, immune response, inflammation/chemotaxis, endothelium relaxation, calcium handling, arrhythmogenesis, and cell cycle regulation (2). The broad range of biological effects, independent from classical overload markers such as N-terminal pro-brain natriuretic peptide (NT-pro-BNP) or troponin, suggests that SN is a potentially useful biomarker in cardiology (8). SN has been studied in catecholaminergic polymorphic ventricular arrhythmia, in which it was moderately elevated irrespective of arrhythmogenic episodes (8). SN seems to be a marker reflecting particular cellular mechanism of specific disease than the clinical episodes itself. This pattern of differentiating a type of a disease (ICM vs. DCM), we have observed also in our analysis.

In patients after coronary artery bypass graft (CABG) surgery, SN levels were significantly higher in non-survivors as compared with survivors (173 vs. 143 pmol/L) (9).

The cut-off value for increased mortality risk is >204 pmol/L in patients with aortic stenosis (10). Moreover, in critically ill patients in the intensive care unit, SN was able to predict mortality on top of classical risk factors (11).

In our study, SN levels were somewhat lower than those observed in patients with CABG, critically ill patients, or patients with aortic stenosis before replacement, most of whom were in advanced heart failure or dying (9–11). The reason for lower SN levels in our trial might be the stable state of most of our heart failure patients, who are not comparable to critically ill patients, patients after coronary bypass surgery or younger healthy individuals. On the contrary, in the stable condition of the ICM and/or DCM HFrEF patients are producing even significantly less SN than the healthy individuals in our analysis. Of note, our healthy volunteers were significantly younger but with comparable body size to the HF group.

Moreover, all the previous studies predicted hard clinical endpoints as all-cause or cardiovascular mortality in severe disease states in a completely different patient population compared to our study, differentiating the cause of the disease in stable heart failure.

There are two trials from the same study group evaluating SN in acute and chronic heart failure. In acute heart failure, SN levels were closely associated with mortality. Moreover, SN reclassified patients to their correct risk strata on top of other predictors of mortality (3). In more recent trial in patients with chronic heart failure, SN concentrations were also able to stratify the patients to favourable and poor prognosis on multivariable analysis (5, 12). The SN levels in the chronic HF trial (42;35–62.8 pmol/L) were also very close to our own observations (5, 12).

None of these trials however analysed different SN levels according to the aetiology of HF, ischemic aetiology was only used as an adjusting factor to predict mortality in chronic HF (5, 12). In addition, these trials did not include their own healthy individual subgroup to compare the SN plasma levels with the HF population. Consequently, it is more complicated to draw any conclusion on basal SN secretion patterns in a particular disease specific subgroup of patients. The common denominator of SN elevation in critically ill patients (3, 8–11) is probably tissue ischemia since the studied population otherwise differed in multiple factors. On the contrary, the basal secretion in the fully compensated state may be specific to the underlying disease.

The discriminative capacity of SN with AUC around 0.7 may seem low, though the confidence interval is quite narrow (0.5–0.8). We also must keep in mind that our whole cohort constituted of patients with HFrEF, and no other biomarker has been able to discriminate between the causes of heart failure (ROC curve for hs-TnI and NT-pro BNP are shown in Figure 2). In general, the main goal of biomarkers in heart failure is to provide information regarding the magnitude of risk, and they can also be used to monitor the effects of changes in treatment and detect subclinical disease (13). Although in many of these applications is the utility of SN not clear at the present time, we hope to provide information in the near future on the development of plasma SN levels through time and in response to changes in heart failure treatment. It is becoming clearer, that SN has the capacity to risk stratify and predict the mortality risk in patients with acute and chronic heart failure (3, 5, 12). It is less evident however, whether SN will be able to reflect treatment changes in HF or HF progression. Based on our results SN may also differentiate the ICM vs. DCM cause of heart failure.

We may only speculate that the higher plasma SN levels in DCM, as compared with those in ICM represent more molecularly advanced heart failure and/or impaired calcium handling. Whether higher plasma levels in our study will also predict higher mortality or more frequent heart failure hospitalizations will be revealed in the follow-up. The other, somewhat contradictory, explanation may be that higher plasma SN levels in DCM may be a surrogate for cardiomyocyte's regenerative activity.

Potentially, the SN discriminative capacity (ICM vs. DCM) may be used in the initial heart failure differential diagnosis if confirmed by more extensive trials from different investigator groups.

However, up to now, every elevation of plasma SN levels has led to a worse prognosis in different disease states (8–12). Though all the predictive capacity was limited to acute or critical patient status.

The possible explanation for different SN plasma levels will probably be at the cellular and subcellular levels. In a study with patients with advanced heart failure, ICM and DCM have significantly different molecular profiles (14). DCM (non-ischemic) samples have 32 differentially expressed profiles, and ICM samples have 185 differentially expressed proteins compared to non-failing heart samples (14). The most enriched proteins in ICM are serum amyloid A1, lipopolysaccharide-binding protein, and activated protein C, which are biomarkers associated with coronary artery disease (15, 16). Conversely, non-ischemic enriched proteins are primarily involved in the mitochondrial membrane respiratory chain (14). Also, the extracellular matrix (ECM), a critical component interacting with cells and modulating tissue functions, is different in ICM and DCM (non-ischemic) (14). In DCM, ECM has predominantly interstitial collagen deposition; in ICM, fibrotic replacement is more likely (17). Most interestingly, specific abnormalities in calcium handling have been demonstrated depending on the etiology of HF. ICM is associated with a decreased rate of calcium uptake into the sarcoplasmic reticulum (SR), while DCM is associated with a decreased rate of calcium release from the SR (18).

In our trial, there was no difference in calcium levels between ICM and DCM. However, both groups were hypocalcaemic as compared to healthy individuals. Severe hypocalcaemia may even be the sole cause of heart failure (19). Moreover, in patients after myocardial infarction, low serum calcium may result in a higher mortality rate (20). Extracellular calcium levels may, however not reflect the cytosolic free calcium and related calcium handling in the sarcoplasmic reticulum (21).

All these factors and many unknowns may influence the plasmatic levels of SN in DCM vs. ICM.

Also, our trial confirmed the independence of plasma SN levels from troponin I and NT-pro-BNP levels in plasma, both in HFrEF ICM/DCM patients and healthy individuals. Our results are, at this point, hypothesis-generating at best. Plasma SN levels must be associated with clinical events to show its predictive capacity. Since we have not collected enough clinical events yet, we cannot state whether SN has or has not the prognostic capacity in HFrEF patients. To capture the relationship between calcium handling and SN, the association of ionized calcium plasma levels may be sampled and correlated with SN plasma levels in future trials.

Limitations

Our sample was small and unbalanced for sex, with male predominance in the heart failure group and female predominance in the healthy volunteer group. Moreover, there were more patients with ischemic cardiomyopathy than with dilated cardiomyopathy. These results cannot be extrapolated to different patient population (different cardiomyopathies) or acute state of the heart failure.

Conclusion

Plasma secretoneurin levels differed significantly in DCM vs. ICM, being higher in the former. Moreover, discrimination between DCM and ICM might be possible based on plasma SN levels. Healthy individuals produce higher SN plasma levels than stable HFrEF patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors upon reasonable request and in compliance with the General Data Protection Rule (GDPR).

Ethics statement

The studies involving humans were approved by Etická komise FN Ostrava (eticka.komise@fno.cz). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JP: Conceptualization, Data curation, Formal analysis, Supervision, Writing – original draft, Writing – review & editing. JD: Conceptualization, Investigation, Methodology, Resources, Writing – review & editing. ML: Conceptualization, Investigation, Methodology, Project administration, Resources, Writing – review & editing. DS: Methodology, Project administration, Supervision, Validation, Writing – review & editing. ZŠ: Conceptualization, Funding acquisition, Methodology, Project administration, Writing – review & editing. NC: Investigation, Methodology, Project administration, Resources, Writing – review & editing. PŠ: Investigation, Methodology, Writing – review & editing. LE: Investigation, Methodology, Validation, Writing – review & editing. DP: Data curation, Formal analysis, Visualization, Writing – review & editing. JV: Project administration, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Original articles

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SIMULTANEOUS BIVENTRICULAR PRESSURE-VOLUME ANALYSIS IN RATS

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The pressure-volume (PV) analysis is used for an accurate assessment of load-independent cardiac function and is important for the study of cardiovascular diseases and various therapeutic modalities. PV analysis is often performed on one of the ventricles, or on both ventricles but sequentially. Since both ventricles interact with each other and their functions are mutually interdependent, especially in various disease conditions such as pulmonary hypertension or heart failure, it is important to quantify the function of both ventricles at the same time. Therefore, our aim was to describe a standardized protocol for simultaneous right (RV) and left (LV) ventricle of PV analysis, including an especially controllable preload reduction manoeuver. Our second aim was to test whether simultaneous catheterization of both LV and RV is necessary for the determination of biventricular PV relationship compared to sequential measurement of both ventricles separately. In this article, we showed the feasibility and the value of simultaneous biventricular PV analysis in the measurement of contractility parameters (end-systolic pressure-volume relationship (ESPVR), ventricular pressure over time (dP/dt)max, divided by end-diastolic volume (dP/dt max-EDV)) with a comparison to the sequential measurement of the RV and LV ventricles separately. We described in detail the protocol for simultaneous biventricular PV analysis in rats using a pair of conductance-micromanometer catheters with a preload-reducing manoeuver using balloon catheter inflation in the inferior *vena cava*. We also described technical tips and show examples of PV loop data obtained in normotensive and hypertensive rats, with and without heart failure due to volume overload. This protocol could be useful for scientists studying hemodynamics and cardiac contractility in various models of cardiovascular diseases with a focus on biventricular differences and ventricular interdependence.

Key words: *heart, hemodynamics, ventricular interaction, pressure-volume analysis, right ventricular function, end-diastolic volume, hypertension, heart failure*

INTRODUCTION

An exact assessment of cardiac function is crucial for studies of cardiovascular disease and pulmonary-vascular preclinical research. The cardiac ventricles serve as hemodynamic pumps that generate changes of intracavitary pressure and volume. For global, load-independent assessment of chamber performance, it is necessary to assess both pressure and volume simultaneously. Pressure-volume (PV) relations were conceptualized by Sagawa *et al.* in studies of canine ventricular function and provided a powerful approach for quantification cardiac function (1-3). The development of conductance catheter with a micromanometer probe for instant PV registration allowed Kass and others to perform PV loop analysis in humans (4-6). Miniaturization of PV catheters also made cardiac function analysis possible in rodents (7-10). Invasive PV analysis is currently considered as the 'gold standard' in the evaluation of systolic and diastolic function of the left (LV) and right ventricle (RV) in rodent animal models (11).

Left and right ventricular functions are often studied separately (12). However, both ventricles and their functions are closely related. The ventricles share the interventricular septum and therefore LV contraction has profound effects on contractile function of RV due to systolic ventricular interaction (13). Both ventricles also share and compete for the same pericardial space and therefore changes in the end-diastolic volume (EDV) of one ventricle has an effect on the EDV and pressure of the other ventricle (diastolic ventricular interaction) (13-15). Several studies have reported the implementation of biventricular PV analysis in the assessment of cardiac function. However, these studies have only measured the left ventricle (LV) and right ventricle (RV) sequentially, rather than simultaneously (8, 16). Sequential measurement of LV and RV bring several disadvantages compared to simultaneous measurement, including longer surgery time, increased risk of bleeding during the sequential introduction of catheters into LV and RV, and/or different heart rate during different time measurements resulting in altered contractility, which can be a limitation for studies aimed at comparing LV and RV function. For precise quantification of ventricular function, it is therefore ideal to obtain biventricular assessment of systolic and diastolic functions from simultaneous data acquisition (13, 16) during the same transient manoeuvers such as preload reduction and afterload increase. The biventricular

PV analysis is a useful tool for studying such interventricular relations which helps to understand diseases associated with dysfunction of the RV and LV, such as acute pulmonary embolism, pulmonary arterial hypertension and/or left heart failure (17-19). Such an approach may also be a tool for an understanding of the mechanisms responsible for progression of left heart failure into biventricular disease (19, 20).

For PV data acquisition, it is superior to use a closed-chest rather than an open-chest approach to avoid pericardiotomy and hemorrhage (21). Closed-chest catheterization of experimental animals is also similar to human heart hemodynamic studies (22). Closed-chest catheterizations of LV and especially of RV in small rodents, together with preload reduction for contractility assessment, are technically challenging and many experimental studies do not describe them in sufficient detail.

Measuring load-independent parameters of contractility is one of the main advantages of PV analysis. The end-systolic pressure-volume relationship (ESPVR) and the first derivate of ventricular pressure over time (dP/dt max), divided by end-diastolic volume (dP/dt max-EDV) are load-independent systolic function parameters. To examine load-independent properties such as ESPVR and dP/dt max-EDV of the LV and RV, a family of PV loops need to be generated by preload reduction (23, 24). ESPVR is defined as an index of end-systolic elastance that provides important information on the contractile function and represents the maximum pressure developed by the ventricle at any given volume (23-26). Contrary to ESPVR, which due to marginal preload reductions could be curvilinear, dP/dt max-EDV is linear, and its slope is a sensitive load-independent measure of left ventricular contractile performance (24, 27). Nevertheless, both ESPVR and dP/dt max-EDV parameters are viewed as a change in ventricular contractility widely used in many experimental and clinical studies (24, 27-30).

Therefore, the aim of this study was to develop and describe a standardized protocol for simultaneous RV and LV catheterization, including an especially controllable preload reduction manoeuver, necessary for determining the contractility function parameters. The second aim was to test whether simultaneous catheterization of both LV and RV is necessary for the determination of biventricular PV relationship compared to sequential catheterization and following measurement of both ventricles separately.

MATERIAL AND METHODS

Animals

The study was performed in accordance with relevant regulations and approved by the Animal Ethics Committee of IKEM and, consequently, by the Ministry of Health of the Czech Republic (#12468/2021-5/OVZ).

All animals were housed in transparent plastic cages for four animals at room under stable 12 hours light to 12 hours dark conditions with temperature (22 ± 1ºC) and humidity (40%). Rats were fed a normal protein diet (0.45% NaCl, 19–21% protein) manufactured by SEMED (Prague, Czech Republic) and had free access to tap water. All animals used in this study were bred at the Center for Experimental Medicine of this Institute from stock animals supplied by the Max Delbruck Center for Molecular Medicine in Berlin, Germany. Transgenic (Ren-2 gene) hypertensive rats (TGR) were generated by breeding male homozygous TGR with female homozygous Hannover Sprague-Dawley (HanSD) rats as described in the original study (31). HanSD rats served as normotensive controls. Eight-week male TGR rats were anaesthetized using ketamine and midazolam applied intraperitoneally (Calypsol, Gedeon Richter, Budapest, Hungary, 160 mg/kg and Midazolam, Kalcex, Riga, Latvia, 160 mg/kg) and underwent the aorto-caval fistula (ACF/sham) procedure (by 18-gauge needle), which was described previously by Garcia and Diebold (32). This procedure is routinely performed in our laboratory, and the technical details were reported in our previous studies (33-35). The sham-operated control rats underwent opening and closing of the abdominal cavity. Both control and experimental animals were given the post-operative analgesic meloxicam (1–2 mg/kg/day, subcutaneously for 2–3 days). Three weeks after surgery, the simultaneous biventricular PV analysis was performed. After the operation, all rats were euthanized with an overdose of intravenous thiopental (200 mg/kg, VAUB Pharma a.s., Roztoky, Czech Republic).

Experimental protocol

The surgical technique of catheter insertion is important to maintain the animal in the basal state without excessive stress, bleeding or damage to nerves, vessels and surrounding tissue. Before catheter insertion, it is important to carefully prepare the vessels. It is necessary to separate the veins more carefully than the larger arteries due to the smaller composition of muscle and endothelial layers. Catheterization of the right carotid artery, right jugular, left femoral vein and tracheostomy is needed to maintain biventricular PV analysis.

Pressure calibration

PV catheters have pressure and conductance sensors, which need to be pre-soaked in saline at body temperature for 30 minutes and calibrated slightly before the insertion of the catheter. Pressure sensors are usually calibrated by MPVS Ultra unit hardware and software which are able to adequately correct the offset value of pressure. Other known methods for extra correction of pressure signals are pressure gauge kits or Delta-Cal Electronic Pressure Simulator (Transonic, Ithaca, NY, USA).

Volume calibration

The catheter conductance sensors must be calibrated before insertion of catheter according to the manufacturer's instructions to avoid signal noise. After the experiment, the conductance signal needs to be calibrated to absolute volume before evaluation of collected data. There are several options on how to calibrate the conductance signal to absolute volume, and each method has its advantages and limitations. In this study, we used cuvette calibration unit with wells of precise volumes placed in a prewarmed water (37ºC) bath. Heparinized warm blood of the animal is taken from the LV and RV separately after *in vivo* experiment and pipetted into a calibration cuvette immediately after the surgery. The tip of the catheter from the LV or RV is pushed through to the catheter holder and placed into each well with heparinized blood. All sensors must be submerged, remaining immersed in blood, stable for at least 10 seconds in each well (5 or more wells are sufficient). These data are collected in volume channels in LabChart Pro software (ADInstruments, Dunedin, New Zealand) in relative volume units (RVU). The conductance output of the catheter tip from the wells are then correlated with the known volumes to develop a calibration equation that converts data from RVUs into units of true volume (µl). The obtained volume signals are underestimated compared to values measured by MRI due to parallel conductance, which refers to the conductivity of the myocardium that surrounds the RV and LV blood pool. The contribution of parallel conductance to measurement imprecision is affected by chamber geometry and is lower in dilated hearts. The option of employing alpha calibration as an

adjunct to saline and cuvette calibration is feasible. This approach takes into consideration the homogeneity of blood, which is determined by Baan's equation, through an independent method of volume or flow estimation (28, 29).

Anesthesia, ventilation and preparations

Prior to the operation, prepare all the necessary surgical instruments and devices (*Figs. 1* and *2*). Anesthetize the rat with

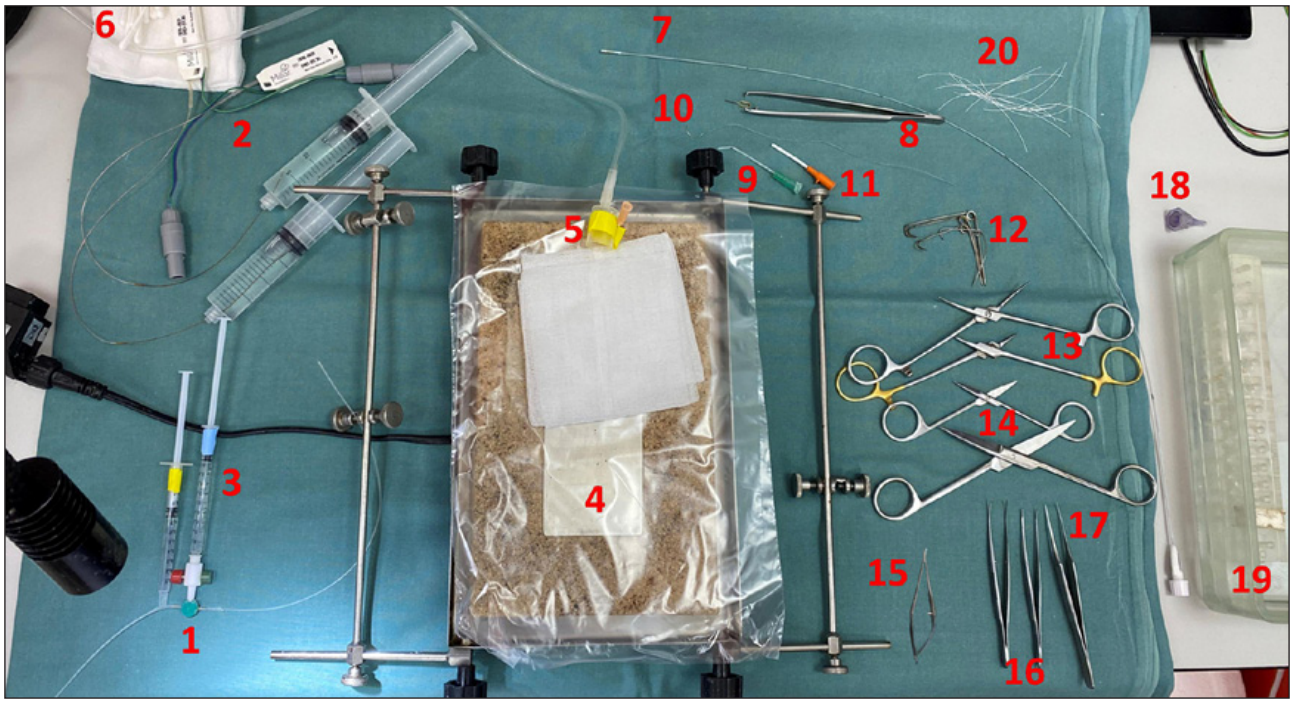

Fig. 1. Surgical instruments required for biventricular PV analysis. 1 - Catheter with stopcock for intravenous administration; 2 - Two PV catheters (Model SPR869, Millar, Houston, TX, USA), 3 - Injection with saline or other substance for intravenous administration, 4 - Heated pad, 5 - Oxygen supply tube, 6 - Gauze and cotton swabs, 7 - Balloon catheter (LeMaitre Single Lumen Embolectomy catheter, 2F, Burlington, MA, USA), 8 - Micro clamp applying forceps with clamp, 9 - Bent introducer for right ventricle catheterization, 10 - Suture for fixation of endotracheal cannula to the skin, 11 - Endotracheal cannula, 12 - Tissue holders, 13 - Pean forceps, 14 - Scissors for microdissection and scissors for skin dissection, 15 - curved microscissors, 16 - Tissue blunt curved forceps, 17 - Sharp vessel forceps, 18 - Catheter holder for cuvette calibration, 19 - Cuvettes blood calibration with bath, 20 - Suture for vessel ligation.

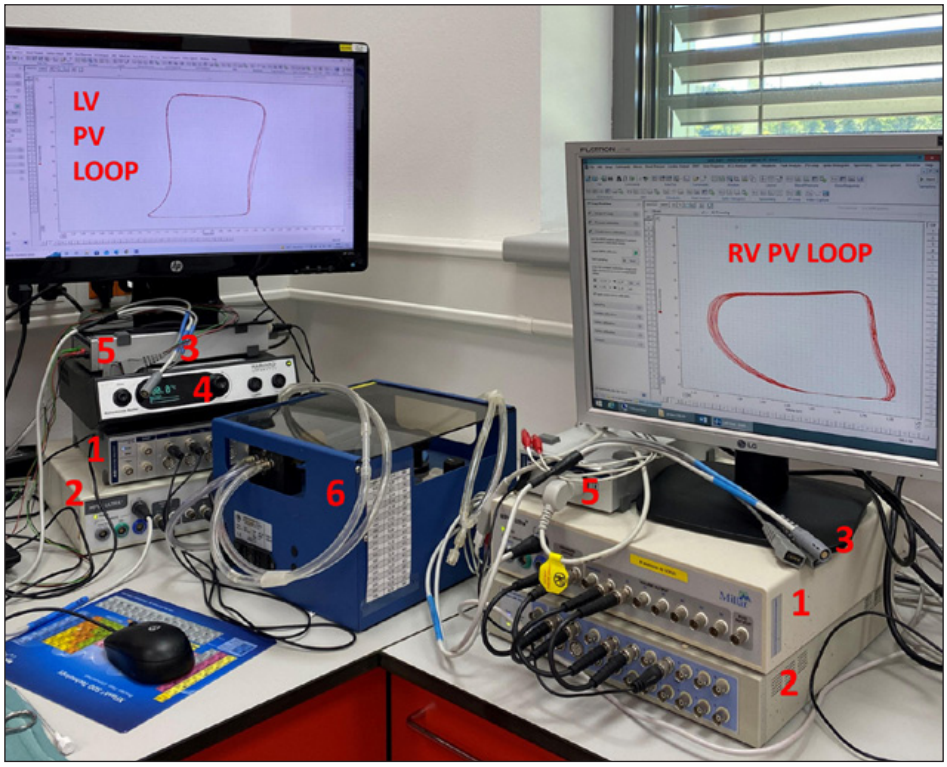

Fig. 2. Complete technological setup for biventricular PV analysis. 1 - MPVS Ultra PV unit (Millar, Houston, TX, USA), 2 - PowerLab (ADInstruments, Dunedin, New Zealand), 3 - MPVS Ultra cables for catheter connecting, 4 - Hemothermic monitoring system (Harvard Apparatus, Holliston, MA, USA), 5 - Bio Amp with ECG leads (Model FE231, ADInstruments), 6 – Electronic ventilator (Ugo Basile, Gemonio, Italy).

long-term anesthesia (for example thiopental, 80 mg/kg, intraperitoneal, VAUB Pharma a.s., Roztoky, Czech Republic), commonly used for PV analysis and prepare the rat for surgical procedure. Weigh the animal, pull the tongue out of its mouth, shave the neck, chest and groin on both sides. Place anesthetized rat on a heating pad in supine position, turning its head to the surgeon. Attach the ECG leads to the animal (Bio Amp, ADInstruments, Dunedin, New Zealand), insert the thermometer (Harvard Apparatus, Holliston, MA, USA) into the anus, and attach the oximeter flap (Nonin Model 2500A VET, Nonin Medical, Plymouth, MN, USA) to the rat's hind paw. Perform a tracheostomy to ensure chest relaxation and facilitate respiration during the whole operation. Start with an incision in the ventral midline cervical line from manubrium sterni to the hyoid bone. Bluntly dissect cervical tissue and expose sternohyoid and sternocleidomastoid muscles. Split midline of the fascia overlying the sternohyoid and retract muscles laterally to expose the trachea. Isolate the trachea out of laryngeal nerve and place 3–0 silk suture under the trachea. Make midline incision caudal to the thyroid gland and cut half of the one annular ligament between tracheal rings with small scissor. Introduce endotracheal cannula to the trachea and further in a caudal direction. Attach endotracheal cannula to the skin with 3–0 silk suture to ensure a stable position of the cannula throughout the operation (*Fig. 3*). Attach the canula to the ventilator (Ugo Basile, Gemonio, Italy), before muscle relaxant (pancuronium) administration as described in data acquisition section or during any part of the operation in case of irregular or weak breathing during the operation. Set the ventilation settings based on the animal's weight using the following formulas:
respiration rate (RR, min^{-1}) = $53.5 \times M^{-0.26}$;
tidal volume (V_t, ml) = $6.2\ M^{1.01}$ (M = animal mass/kg) (7).

Securing central venous access

The intravenous administration of saline is necessary during the operation to allow for sufficient hydration, replacement of blood loss, and to administer vasoactive drugs to test the circulation when needed. Start with an incision above the left clavicle in the area where the respiratory movement of the jugular vein is clearly visible. In this site, extend the tracheostomy incision on the skin laterally approximately 20 mm towards the direction of the acromial end of the left clavicle. Make an incision using tissue scissors and forceps in the area where the respiratory movement of the jugular vein was identified. Free the submandibular gland using blunt dissection and visualize the jugular vein. Dissect the left external jugular vein with blunt scissors and isolate the distal part of the vessel where the diameter is widest. Tighten the suture on the vein cranially and hold the end of the suture to the needle holder. Prepare two untightened sutures caudally. Cut a small incision that will fit the cannula with microscissors and gently stretch the left jugular vein with pean in the rostral direction. Insert the cannula with saline into the jugular vein, and tighten the caudal nodes on the vessel with the inserted cannula. To maintain fluid homeostasis throughout the operation, set the rate on the intravenous infusion pump of saline solution to 1 ml/kg/h.

Placing preload reduction balloon catheter into central vein

To facilitate femoral catheterization, position the rat with the heating pad on the side with the left paw facing the surgeon (be careful not to pull out the inserted cannulas). Continue insertion of the balloon catheter (LeMaitre Single Lumen Embolectomy Catheter, 2F, Burlington, MA, USA) to the left femoral vein.

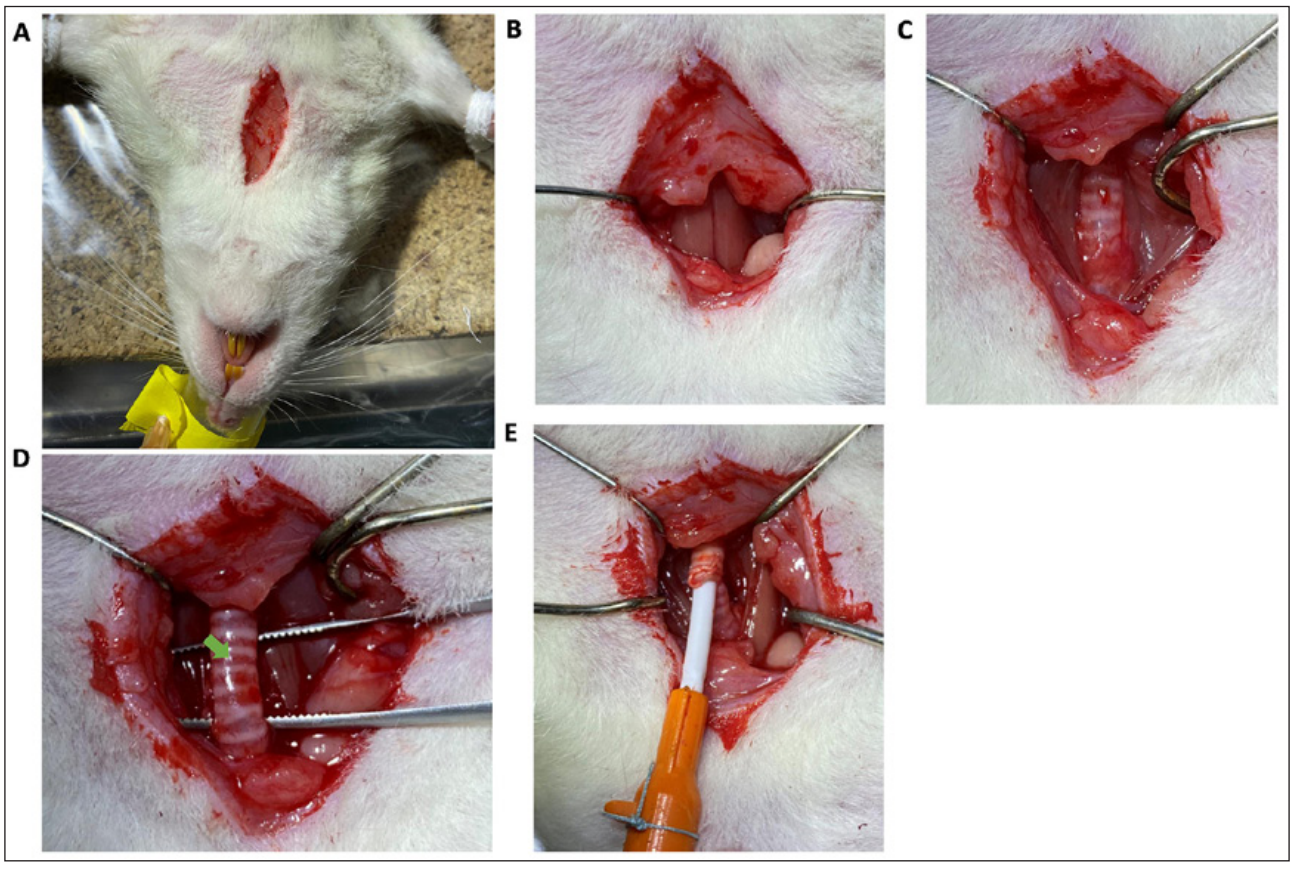

Fig. 3. Detailed surgical steps during tracheostomy. Follows A-E. Green arrow - site in annular ligament where the trachea is cut between cartilaginous rings.

Stretch and fasten the left leg of the rat laterally to the pad (this step will help introduce the catheter through bifurcation to the vena cava inferior) and cut the skin in the groin area approximately 25 mm to the shape of a cross (*Fig. 4A*). Gently extend the area and tissues, separate the connective tissues using blunt scissors and expose the femoral vein and artery (*Fig. 4B*). Use blunt forceps to isolate approximately 15 mm of the femoral vein from the artery and femoral nerve (*Fig. 4E*). Make one distal and two rostral knots around the femoral vein. Tighten the distal knot, and carefully hang up the knot on the pean in the direction of the stretched left paw. To avoid bleeding, clamp the femoral vein behind the prepared proximal knot. Beware of bleeding from the profundal femoris vein, part of femoral vein bifurcation (tightening the suture around the profundal femoris vein or using the extra clamp is recommended). Under the microscope, (Leica S9i, 40× zoom, Wetzlar, Deutschland) cut the femoral vein with microscissors, insert a balloon catheter into the femoral vein and push the catheter behind proximal knots. Insertion of the balloon catheter into a vein is critical, as the vessel can easily rupture should excessive pressure push the catheter against the wall of vessel. Rotate the catheter in both directions slowly while removing and inserting the catheter into the vessel to simplify the insertion of the catheter. Release the clamp and subsequently move the balloon catheter further, quickly tightening the prepared proximal knots. Insert the catheter further through the bifurcation into the inferior vena cava and then tighten the second proximal node. Insert the balloon catheter from the femoral vein to the vena cava inferior under echocardiography navigation (probe 10S, 5–11.5 MHz, Vivid 7 Dimension, GE Healthcare, Chicago, IL, USA) just below the diaphragm to maintain the best position for preload reductions (*Fig. 4G*). It is important to put a balloon catheter slightly below the diaphragm, but not too close because the diaphragm can be shifted by inflation of the balloon catheter with saline. The catheter in the heart can therefore be moved to the wrong position. Cover the wound with a pair of gauze to avoid heat loss.

Placing pressure-volume catheter into the left ventricle via the right carotid artery

Connect the PV catheter (Model SPR869, Millar, Houston, TX, USA) 30 min before using the MPVS ultra unit (Millar, Houston, TX, USA) connected to PowerLab (ADInstruments, Dunedin, New Zealand), and to PC with data-acquisition LabChart Pro software (ADInstruments, Dunedin, New Zeland). Calibrate the pressure and conductance (volume) sensors of the Millar PV catheter using MPVS software (V2.2, Millar, Houston, TX, USA), according to the manufacturer's instructions just before introducing the catheter to the vessel. Use tissue holders and extend the wound created during tracheostomy. Extend sternohyoid, sternomastoideus, and omohyoid muscles with tissue holders. Right carotid artery pulsation will be visible between those three muscles. Dissect the omohyoid muscle longitudinally with blunt scissors and expose the right carotid artery. Gently separate the vagus nerve and isolate an approximately 12 mm section of the carotid artery. Tighten the rostral suture on the carotid artery, and put the end of the suture in a needle holder. Prepare two untightened caudal sutures below the carotid artery. Elevate and pull one of the untightened caudal sutures with a needle holder. Before making an incision, ensure that no blood flows into the isolated part of the carotid artery. Using a microscope and microscissors, make an incision on the most cranial part of the carotid artery. Adjust the incision to fit the catheter and introduce the catheter into the carotid artery. It is possible to put the PV catheter to the created incision on the carotid artery freely, but it is also possible to use sharp vessel forceps (for example in the case of a twisted vessel). Avoid touching the catheter sensors with forceps or other metallic instruments as it may lead to loss of calibration or permanent sensor damage. Tie the suture on the cranial part of the

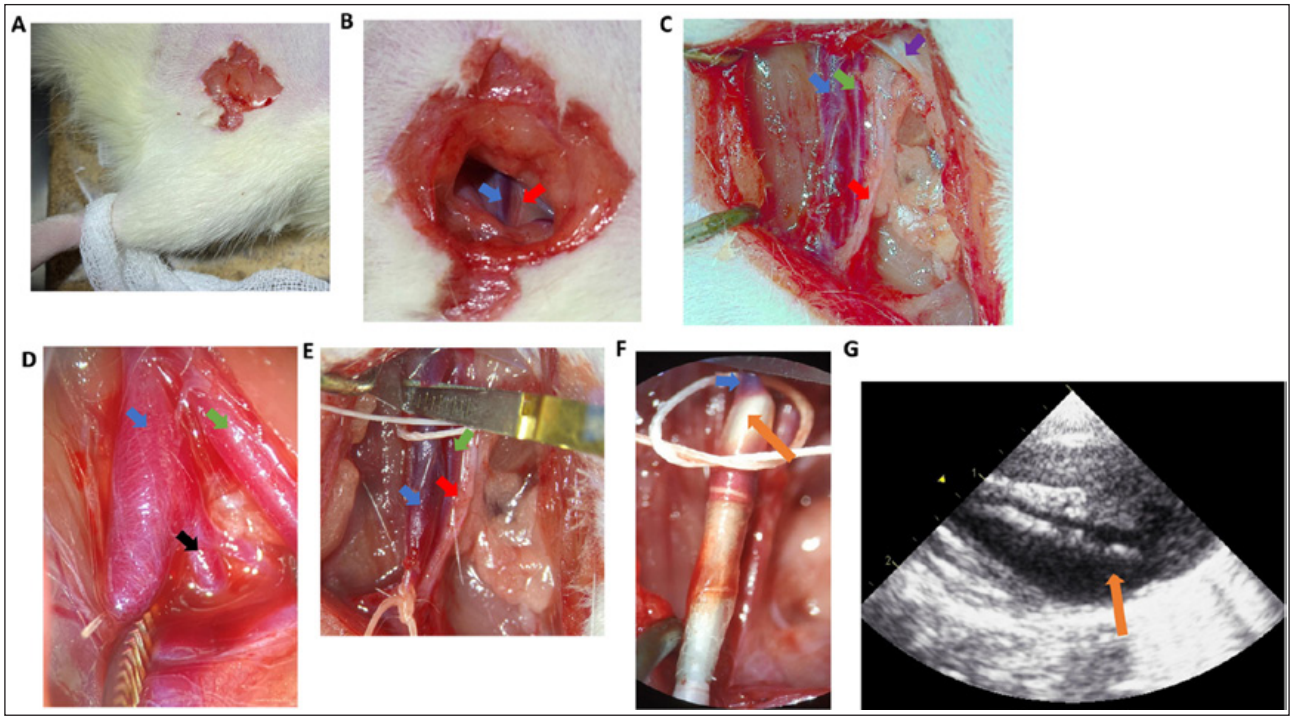

Fig. 4. Insertion of embolectomy balloon catheter to vena cava inferior *via* the femoral vein approach. Follows A-G: Blue arrows point to the femoral vein; green arrows point to the femoral artery; red arrows point to the femoral nerve; purple arrow points to the ligament inguinal; black arrow points to profundal femoris vein; orange arrow points to balloon catheter. (G): Ultrasonic view of *vena cava* with introduced balloon catheter bellow diaphragm.

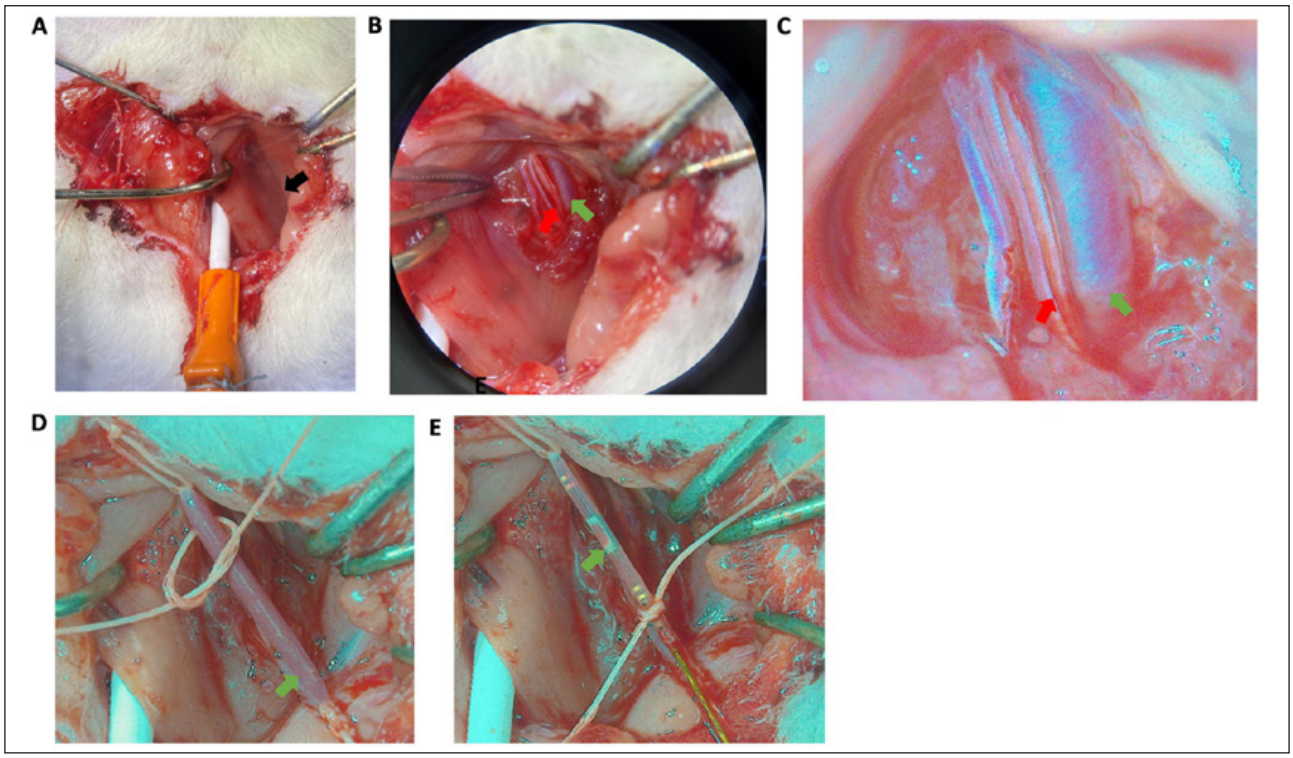

Fig. 5. Detailed surgical steps of PV catheter insertion into the right carotid artery. Follows A-E: Green arrows point to the left carotid artery; red arrows point to the vagus nerve; black arrow points to a site between 3 muscles (sternohyoid, sternomastoideus, and omohyoid muscle) below which right common carotid artery is located.

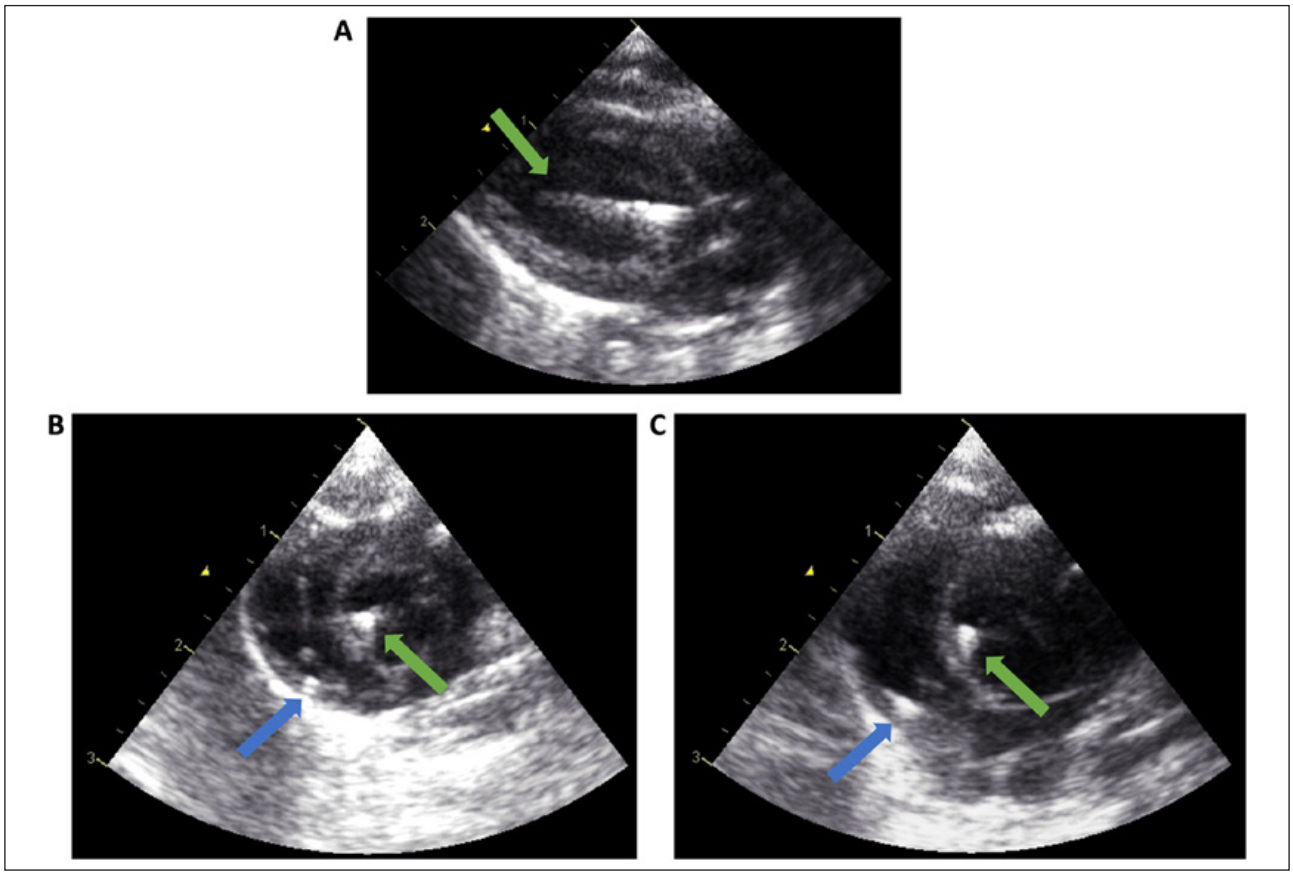

Fig. 6. Echocardiography images of PV catheters in the left and right ventricle. (A): PV catheter in the left ventricle (green arrow) in parasternal long-axis; (B): PV catheter in left (green arrow) and right ventricle (blue arrow) in parasternal short axis. (C): PV catheter in the left (green arrow) and right ventricle (blue arrow) in apical 4 chamber view.

catheter with the carotid artery (behind PV sensors). Release the needle holder with caudal suture and push the catheter forward into the aorta and tie the caudal suture on the catheter and the artery (*Fig. 5*). In this position, it is possible to measure pressure records from the carotid or after moving the catheter, aortic pressures (for example, it can be used later to calculate total peripheral resistance). Under transthoracic echocardiographic guidance, move the catheter forward through the aortic arch above the aortic valve. Visualize the catheter in the aorta in parasternal long-axis view and move the catheter to the LV (*Fig. 6*). The utilization of echocardiography guidance during catheter insertion exhibits substantial advantages, primarily concerning safety measures during the introduction of the catheter into the ventricle at an adequate position to generate optimal PV signals. It is critical to avoid any disruptions and signal noise that may arise from catheter-to-wall or papillary muscle contact, underscoring the significance of positioning precision when introducing the catheter *via* echocardiography guidance. Echocardiographic navigation also reduces the risk of the penetration of LV free wall, interventricular septum, or other damage of the heart. Release the needle holder with rostral suture to left catheter freely in the heart and check echocardiographically if the catheter is still in the correct position, fully in the LV, without touching the wall. Check the shape of the PV loop in LabChart software. If an echocardiogram is not available, the position of the catheter in the heart can be estimated using pressure and PV loop recordings from the LV.

Placing pressure-volume catheter into the right ventricle via the right jugular vein

Before the right jugular vein catheterization, create a bent introducer (*Fig. 7*) and calibrate the volume and pressure of the

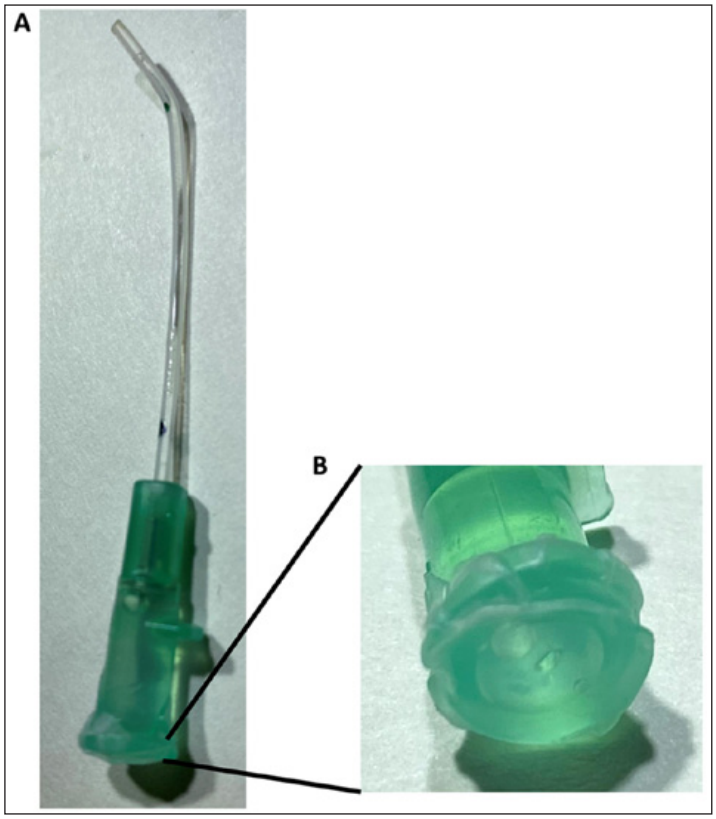

Fig. 7. The introducer for right ventricle catheterization. Creation of bent introducer (A) is needed for simple catheterization of the right ventricle. Make a mark using permanent marker at 40 mm from the tip of the catheter (Introcan Safety 18G, Braun, Melsungen, Germany). This mark will also be showing position of bended tip in the heart. Bend the catheter tube (on the side of mark) and needle with needle holder approximately 10 mm from the tip at 45 - degree angle and dip bent part of catheter into boiling water for 10 seconds. Remove needle from catheter and cover lever lock plug with 10×10 mm piece of parafilm (Bemis, Neenah, WI, USA) to avoid blood loss. Using a needle make small incision which will closely fit to pressure-volume catheter (B).

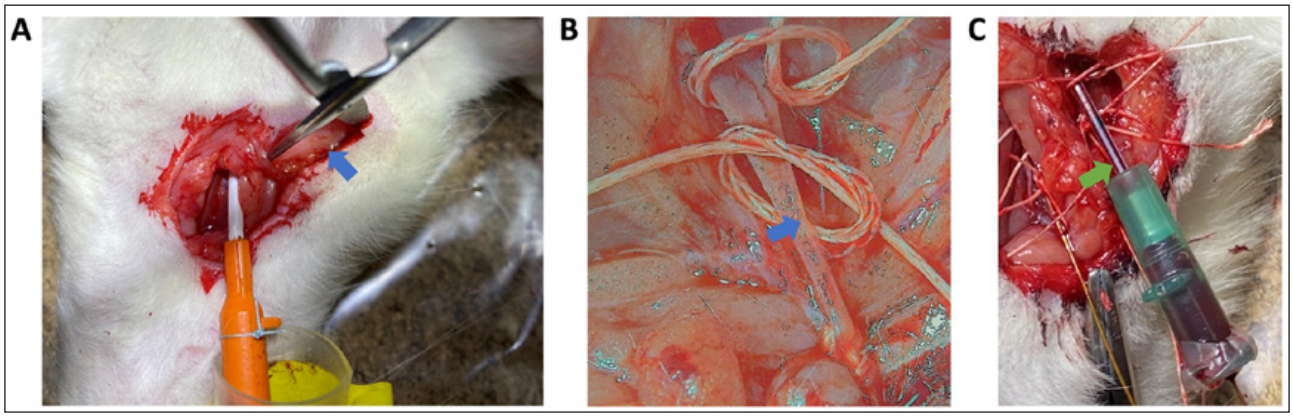

Fig. 8. Detailed surgical steps of PV catheter insertion to the right jugular vein *via* curved introducer. Follows A-C. (A): blue arrow - site in which the jugular vein is located during blunt dissection; (B): site on jugular vein where the small incision which fits introducer dimensions is created with microscissors; (C): green arrow - PV catheter inside of introducer clothed with parafilm to prevent blood loss.

second catheter in same manner as in the first PV catheter. Expand tracheostomy incision laterally in the direction of the acromial end of the right clavicle. Make the same dissection as in the left jugular vein with blunt scissors and isolate the distal part of the vessel where the diameter of the vessel is widest. Tighten the suture on the vein proximally, put the end of the

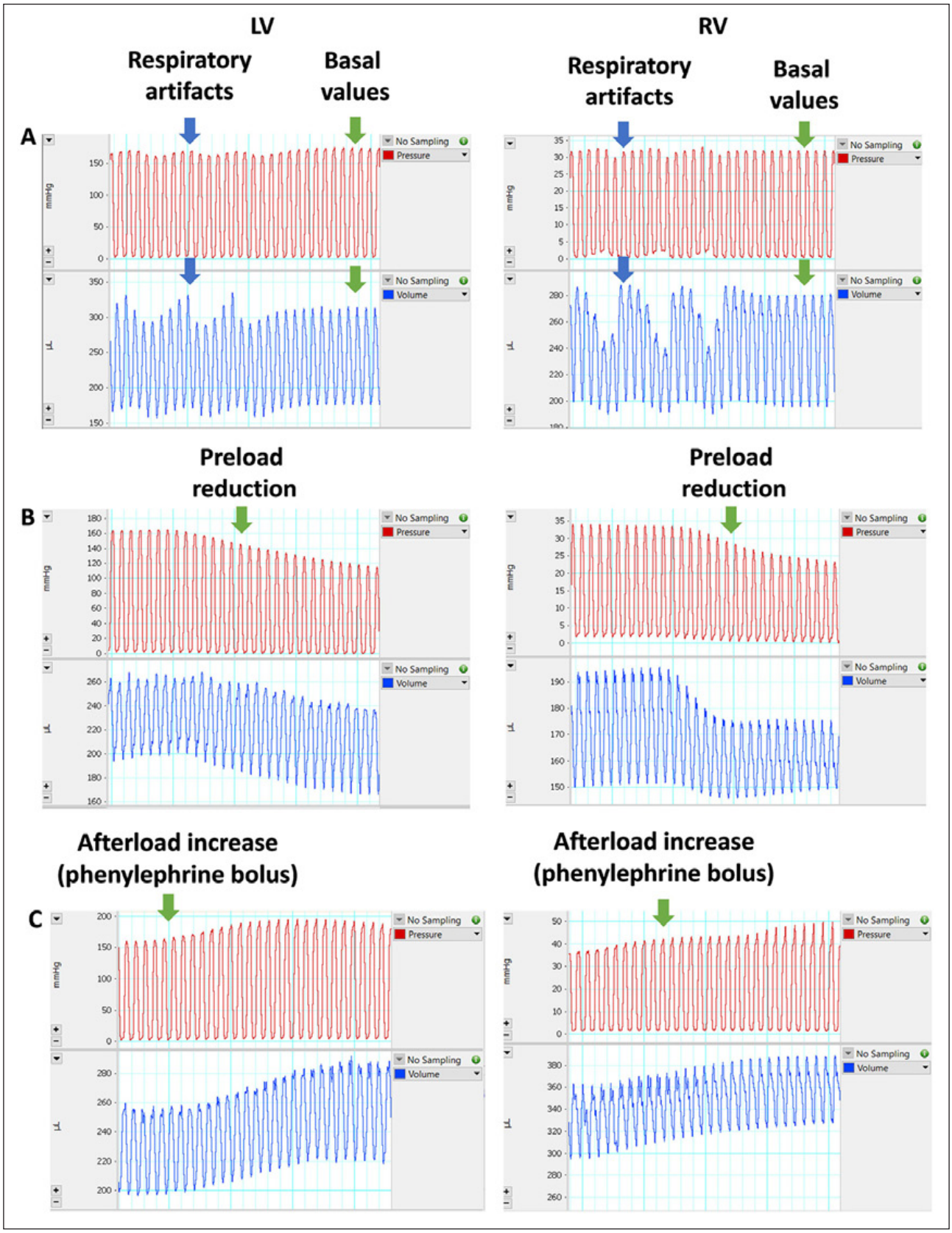

Fig. 9. Pressure and volume data of both ventricles (left side - LV, right side - RV) measured in transgenic (Ren-2) hypertensive rat. On the x axis is time (s).

suture to the needle holder and prepare the next two untightened sutures below the vein. Fill the catheter introducer with saline to avoid air embolism and incise the jugular vein enough to insert created introducer. Lift the cut part of the vessel with sharp vessel forceps. Insert the introducer to the jugular vein and tighten the suture on the vein and the introducer. Slowly move the introducer further into the vena cava superior with a mark of catheter pointing to the right side (*Fig. 7*). The resistance during insertion of the catheter is usually caused by the vein going under the pectoral muscle. Moving the introducer forward while the bent tip is directed to the left side will reduce resistance and simplify the insertion. When the proximal mark of the introducer is close to the incision created in the jugular vein, turn the introducer with the mark pointing upwards. At this point, the tip of the introducer is facing toward the right atrium, and saline solution inside the introducer is visibly pulsating due to the contraction of the heart. Insert the PV catheter to the pre-created small incision in parafilm attached to the introducer and easily push catheter forward through curvature and right atrium to the RV. Pull back the introducer slightly while carefully pushing forward the PV catheter (*Fig. 8C*). Observe systolic pressure and PV loop in the LabChart software. The introducer (*Fig. 7*) is designed so that the PV catheter falls immediately into the RV. The position of the catheter in the RV may change slightly after connecting the ventilator to the endotracheal tube or by re-positioning the animal. In any case, checking the midline position of the PV catheter in the RV by echocardiography is more difficult compared to the control of the PV catheter in the LV, but still possible in the apical 4 chamber projection or the parasternal short axis (*Fig. 6*). Secondly, check the correct position of PV catheter on the pressure and volume channel in Labchart software and place it so that its position does not cause PV loop artifacts arising from contact with the ventricular wall.

Data acquisition

1. Set respiratory rate on ventilator (Ugo Basile, Gemonio, Italy) according to the weight of the animal and attach the tube of ventilator to the tracheal cannula.
2. Administer pancuronium intravenously (1 mg/kg, Inresa Arznemittel, Freiburg, Germany) through cannulated left jugular vein and rinse with a bolus of saline to reduce noisiness in PV signal caused by spontaneous breathing during data acquisition.
3. 10 minutes after catheter insertion and relaxation, briefly turn off the ventilator to disable breathing.
4. Make a quick note about basal measurement in Labchart software on both computers connected *via* MPVS ultra unit to both catheters in the same time and record basal values in both ventricles for 10 seconds in apneic pause (*Fig. 9A*).

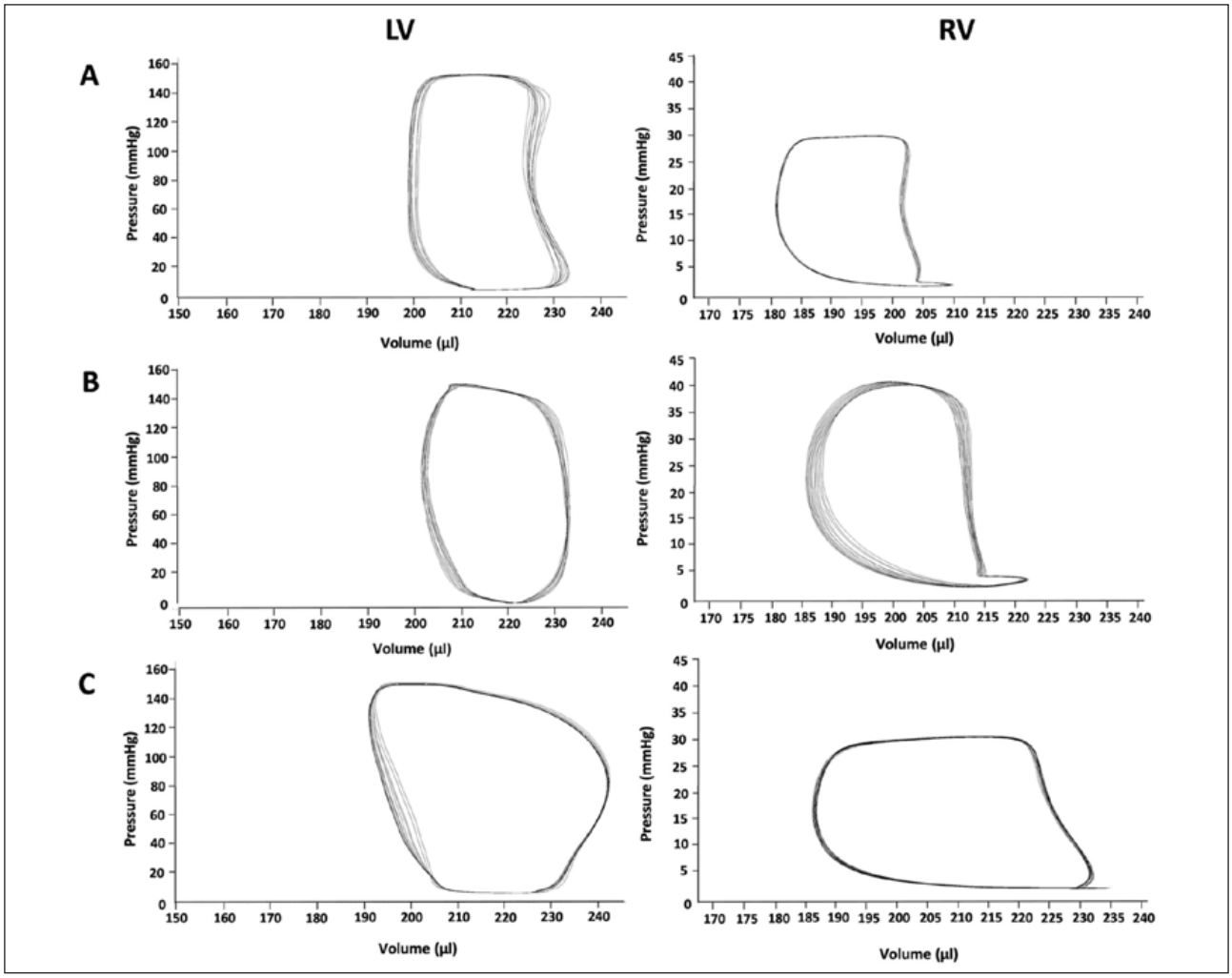

Fig. 10. PV loops in closed-chest, open chest with intact pericardium, open chest with pericardiotomy, measured in one normotensive rat (HanSD) simultaneously in left (left side) and right ventricle (right side). (A): Closed chest; (B): Open chest values, (C): Open chest after pericardiotomy. Note immediate dilatation of RV upon removal of pericardial and mediastinal constraint forces.

5. Repeat measurement 4 times with a 30-second pause between measurements to maintain normal physiological functions.

6. Make a note about preload reduction measurement in LabChart software on both computers and again turn off the ventilator to stop breathing. Reduce preload by slowly inflating the (LeMaitre Single Lumen Embolectomy Catheter, 2F, Burlington, MA, USA) balloon catheter with aqua pour injection with the maximum volume of 0.5 ml. PV loops in the LV and RV should be fluently reduced in pressure and volume signals (*Fig. 9B*). Try to optimize the position of the catheters under echocardiography guidance to maintain the best shape of loops without any artifacts (*Fig. 10*). Extra ventricular beats are especially common at the beginning of preload reduction, or rapid inappropriate decrease of volume when the PV catheter is placed too close to the ventricular wall and the LeMaitre embolectomy catheter too close to the diaphragm.

7. Record 4 decent decreasing PV loops to determine cardiac functions effectively (*Fig. 11*).

8. To enable the extension of the PV loop in the opposite direction of the reduced preload, increase afterload by administering sympathomimetic agents intravenously, such as phenylephrine at a dosage of 20 – 30 µg/kg + bolus. Once maximum pressure is achieved, immediately reduce preload to allow for continued measurement of PV loops and an accurate determination of cardiac contractility. Repeat this measurement 4 times with 30 second breaks between measurements (*Fig. 9C*).

9. After all necessary measurements, perform salt calibration by administration of 30% saline solution intravenously and rinse with a bolus of normal saline. Check in Labchart software for a shift of volume while the pressure should remain unchanged. Repeat these measurements 4 times with 30-second breaks between measurements.

10. Wait a few minutes for blood salinity to normalize. After completion of all interventions and measurements, administer a lethal injection of anesthesia intravenously (thiopental, 200 mg/kg, VAUB Pharma a.s., Roztoky, Czech Republic), open the chest and take fresh blood (into the heparinized syringe to eliminate instantaneous clotting) from LV. Use an introducer for taking the blood from RV.

11. Pipette the collected blood into the calibration cuvettes (Millar, Houston, TX, USA) and calibrate volume sensors of both PV catheters in Labchart.

Data analysis

Data were analyzed offline in LabChart Pro software (ADInstruments, Dunedin, New Zealand). Contractility was

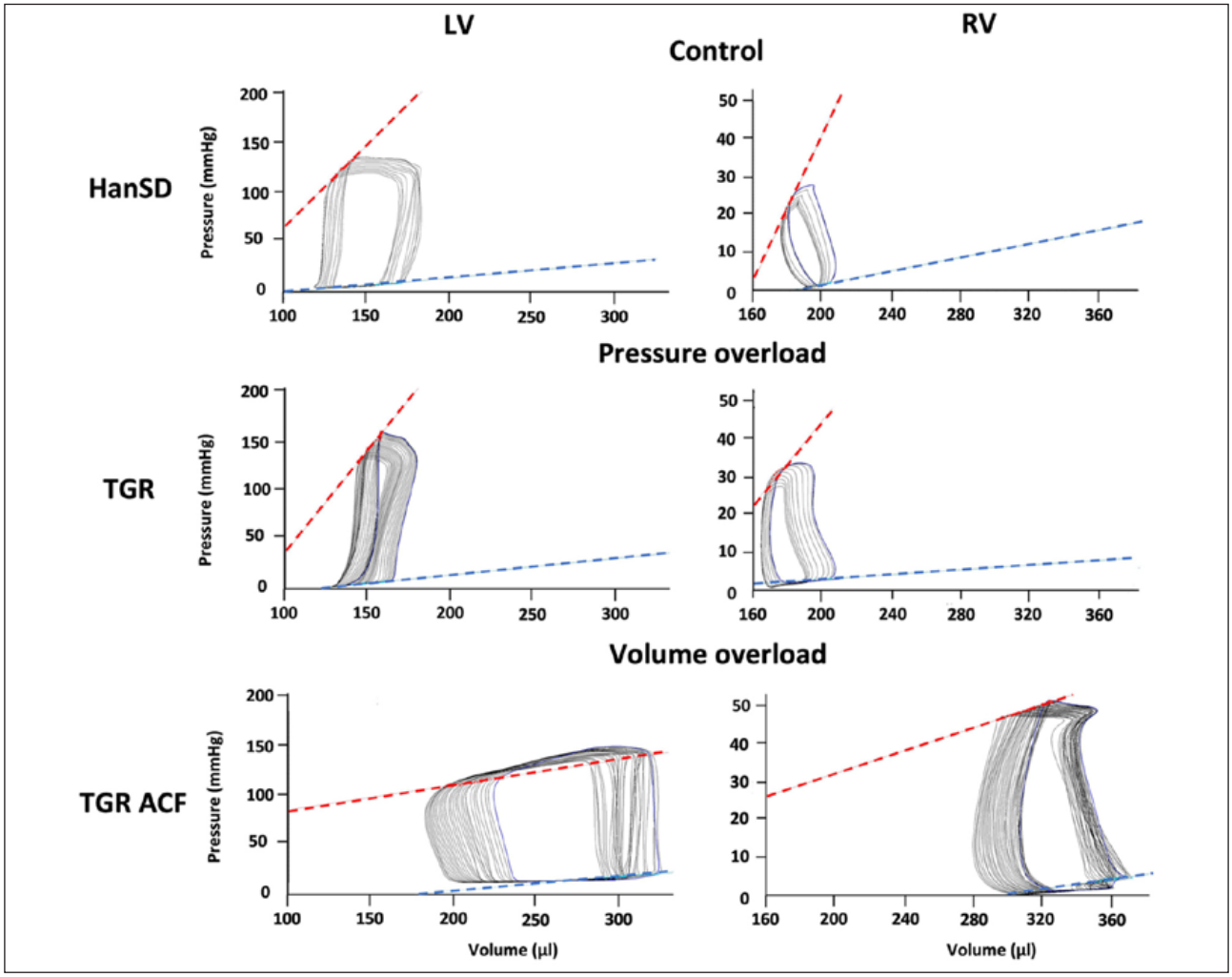

Fig. 11. Representative PV loops of both ventricles in rat models of hypertension and heart failure. Red dashed line - slope of end-systolic pressure-volume relationship; blue dashed line - end-diastolic pressure-volume relationship, visualized during occlusion; LV, left ventricle; RV, right ventricle; HanSD, normotensive rats; TGR, transgenic (Ren-2) hypertensive rats; TGR ACF, the model of heart failure induced by aorto-caval fistula (volume overload) in transgenic (Ren-2) hypertensive rats, 3 weeks after the creation of aorto-caval fistula.

quantified based on the results of load-independent systolic function parameters dP/dt max-EDV and the maximal slope of ESPVR, also referred to as end-systolic elastance (Ees) obtained by occlusion of the inferior vena cava.

Ees was obtained from the PV loops and was based on the formula:

Pes = Ees* (Ves – V0),

where Pes is end-systolic pressure, Ves refers to the end-systolic volume and V0 is volume axis intercept (30).

All analyzed PV data were without any artifacts and for evaluation of contractility parameters. PV loops were selected from data obtained by inferior vena cava occlusion without preload reduction limit values, *i.e.* not below 90 mmHg due to the avoidance of curvilinear response of contractility parameter ESPVR caused by limiting perfusion to the heart.

Statistical analysis of the data were performed using GraphPad Prism software v9.4.1 (Graph Pad Software, San Diego, CA, USA). Data of contractility parameters (ESPVR, dP/dt max-EDV) were plotted by simple linear regression. Statistical analysis of data presented in *Table 1* was performed by a two-tailed unpaired Student's t test. Values are expressed as mean ±SD. The values of p below 0.05 were considered statistically significant.

RESULTS

Simultaneous measurement of contractility by parameter ESPVR between LV and RV showed a significant correlation when measured at multiple time points in one animal ($p<0.01$, R2=0.591, *Fig. 12A*) and a large group of animals ($p<0.05$, R2=0.214, *Fig. 12C*). Sequentially measured data from LV and RV showed no correlation between LV and RV in ESPVR. Moreover, the simultaneous measurement between LV and RV showed a significant correlation in dP/dt max-EDV when measured at multiple time points in one animal ($p<0.05$, R2=0.432, *Fig. 12B*), but not among the large group of animals ($p=0.736$, R2=0.006, *Fig. 12D*). Similarly to ESPVR, sequentially measured data from LV and RV showed no correlation between LV and RV in dP/dt max-EDV systolic function parameter.

Data from PV analysis differ between strains with different conditions (*Table 1*). TGR rats had significantly higher ESP and contractility parameter ESPVR in LV. Moreover, TGR rats had increased stroke work in RV compared to HanSD control rats. TGR ACF rats also had significantly increased EDV, and ESV due to volume overload than TGR without ACF. In TGR ACF rats, ESP, and systolic function parameters PRSW and dP/dt max-EDV was decreased in LV, whereas ESPVR was decreased in LV and also in RV compared to TGR group (*Table 1*).

DISCUSSION

In this article, we describe the methodological protocol and technical tips for simultaneous biventricular assessment of cardiac function using PV analysis and provide reference values in various rat strains and pathologic conditions (*Table 1*). We are also proposing original femoral vein approach of preload reduction in vena cava inferior by balloon catheter, which allowed us to monitor the contractility of the RV and LV simultaneously during a single occlusion. In a similar way, we

Table 1. Reference values from PV analysis of various experimental rat models.

Strain	**HanSD**	**TGR**	**TGR ACF**
Condition	**Control**	**Pressure overload**	**Pressure-volume overload**
n	**7**	**9**	**15**
Left ventricle			
SW (mmHg*µl)	8199±2756	9127±3489	10321±2667
EDV (µl)	261±82	266±51	336±76$^{@}$
ESV (µl)	181±60	192±42	244±64$^{@}$
ESP (mmHg)	141±9	168±12*	147±14$^{@}$
EDP (mmHg)	5.6±2.7	6.4±2.7	9.3±6.1
Heart rate (bpm)	362±35	381±25	392±20
ESPVR (mmHg* µl)	1.17±0.58	2.05±0.93*	0.52±0.22$^{@}$
EDPVR (mmHg* µl)	0.17±0.13	0.1±0.05	0.07±0.05
PRSW (mmHg* µl)	95±63	92±26	50±24$^{@}$
dP/dt ma±-EDV (mmHg s^{-1} $µl^{-1}$)	51±40	60±40	28±12$^{@}$
Right ventricle			
SW (mmHg*µl)	1085±493	1675±497*	2623±873$^{@}$
EDV (µl)	273±85	303±28	370±99$^{@}$
ESV (µl)	224±69	232±35	276±82
ESP (mmHg)	32±4	35±4	42±6$^{@}$
EDP (mmHg)	2.5±0.7	3.2±1.8	4.2±1.9
Heart rate (bpm)	364±22	379±24	393±21
EDPVR (mmHg* µl)	0.39±0.19	0.49±0.19	0.21±0.11$^{@}$
ESPVR (mmHg* µl)	0.09±0.05	0.06±0.05	0.05±0.03
PRSW (mmHg* µl)	16.8±10.2	20.2±10	15.5±11.7
dP/dt ma±-EDV (mmHg s^{-1} $µl^{-1}$)	17.6±10	14.1±5.4	10.4±6.1

HanSD, normotensive rats; TGR, transgenic (Ren-2 gene) hypertensive rats; SW, stroke work; ESV, minimum volume (end-systolic volume); ESP, maximum pressure; EDP, end-diastolic pressure; ESPVR, end-systolic pressure-volume relationship; EDPVR, end-diastolic pressure-volume relationship; PRSW, preload recruitable stroke work; dP/dt max-EDV, relation between the peak of the first derivative of ventricular pressure (dP/dt max) and end-diastolic volume. Values are means ±SD; *$p<0.05$ TGR vs. HanSD; $^{@}p<0.05$ TGR ACF vs. TGR.

tested a phenylephrine bolus administration as a method to increase afterload in controllable fashion (38).

Due to continuous ventricular interactions, simultaneous assessment of both cardiac ventricles provides greater insight into pathophysiology than univentricular PV analysis. This method provides more detailed data about ventricular interdependence and can help to understand the pathophysiology of several cardiovascular diseases, in which interventricular changes play an important role. Before the experiment, we made a series of measurements where we compared both catheters in the ventricles of the heart simultaneously against a sequential measurement and we did not observe any electrical interference and PV data together with captured RV and LV loops were unchanged. The noise would probably occur if we used catheters connected to one system, but we used two PV systems separately connected to two separate computers (*Fig. 2*, *Fig. 13*). Therefore, we demonstrated that both ventricles can be analyzed simultaneously using two MPVS systems and did not find any electrical interference between the two conductance catheters simultaneously in LV and RV, which could affect the data (39).

Several studies were focused on systolic ventricular interaction, some of which used PV analysis (15, 19, 40-43). However, whether simultaneous biventricular PV analysis is more reliable than sequentially measured RV and LV for study of relation between LV-RV ventricular function was not tested before. During preload reduction, the PV loop can reach pressures under ~50–75 mmHg, at which point limiting perfusion to the heart occurs, resulting in this curvilinear response. In order to prevent this curvilinear response, we evaluated PV loops, when preload was reduced to above 90 mmHg of ESP. In this way, we tried to collect data so that the PV curve was linear rather than curvilinear. Despite curvilinear or linear properties, the ESPVR parameter is widely used for the estimation of contractility in small animals (10, 23, 44). Moreover, we also evaluated systolic function parameter dP/dt max-EDV, which reliably reflects the intrinsic inotropic state of the LV and appears to be even more reliable than parameter ESPVR (24, 27-30).

Our results showed a higher degree of correlation in systolic function parameters (ESPVR, dP/dt max-EDV) when measured simultaneously compared to the sequential measurement. In the case of ESPVR, we confirmed this greater correlation in simultaneous biventricular PV analysis compared to sequential PV analysis in both time-varying (*Fig. 12A*) and animal varying data (*Fig. 12C*). Moreover, in the case of the dP/dt max-EDV parameter, the correlation between RV and LV was significantly more pronounced than sequential in time-varying data in one animal (*Fig. 12B*), but not among large groups of animals, probably due to the different pathological state of animals (TGR with ACF and sham rats) (*Fig. 12D*). Despite that, our data confirmed that the relationship between RV and LV systolic function is significantly more pronounced when both catheters are in both ventricles simultaneously compared to sequential measurement. This significant relationship between RV and LV systolic function was not confirmed in the case of sequential measurement, during which only one catheter was in the heart at a time. It is important to note that PV loops from RV and LV measured in basal conditions did not differ significantly between simultaneous and sequential measurements (*Fig. 14*). Although the method of simultaneous and sequential measurements of RV and LV are similar in the shape of PV loops, for the assessment of systolic function between RV and LV simultaneous biventricular PV analysis is more suitable.

Besides that, we did not test the specific causes of the differences between simultaneous biventricular and sequential measurements, but we can speculate why this difference

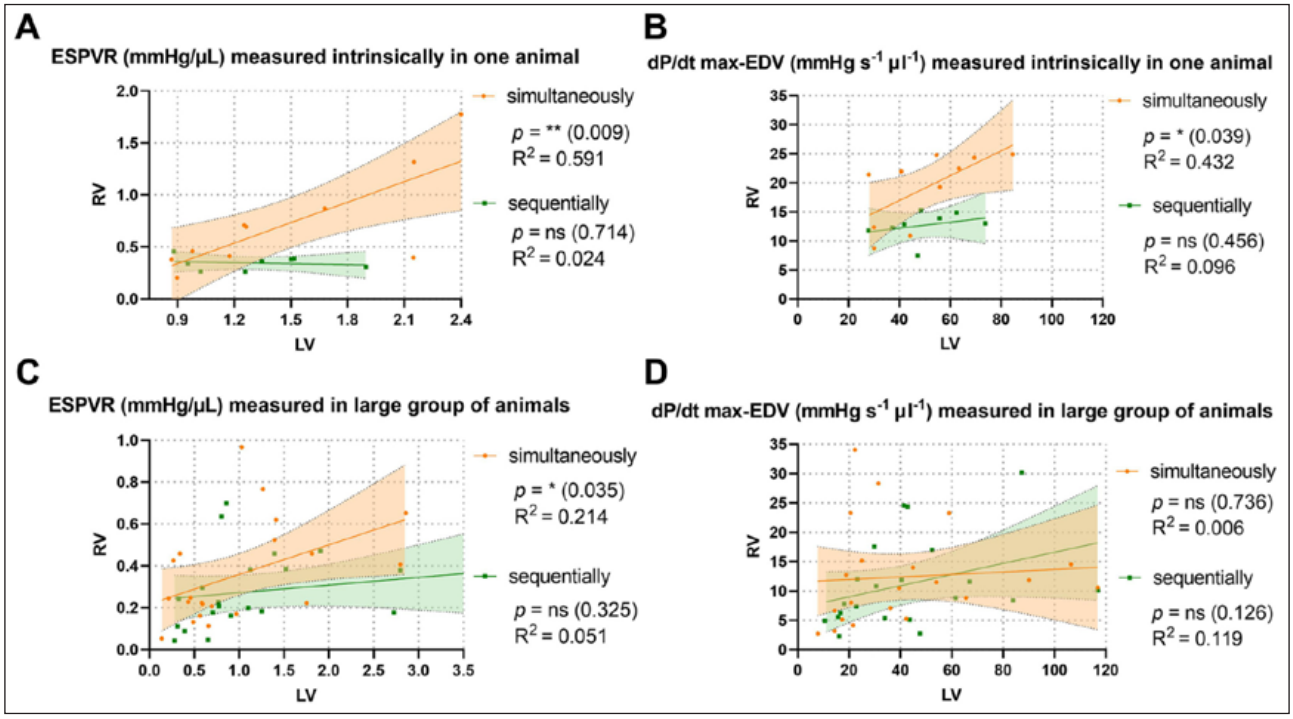

Fig. 12. Relation of contractility between right and left ventricle measured by simultaneous (biventricular) and sequential (ventricles measured separately) pressure-volume analysis. (A, C): systolic function parameter ESPVR - end-systolic pressure-volume relationship; (C, D): systolic function parameter dP/dt max-EDV - relation between the peak of the first derivative of ventricular pressure (dP/dt max) and end-diastolic volume. (A, B): Data measured intrinsically in one normotensive (HanSD) rat during preload reducing manoeuver, volume expansion and after pericardiotomy. (C, D): Data measured in 21 hypertensive rats (TGR) with (ACF) and without volume overload (sham) during preload reducing manoeuver.

occurred. Based on the literature and several studies, we can consider that the change in contractility between simultaneous and sequential measurements could have occurred due to different timings of the PV measurement in the ventricle, due to different HR (45), or due to increased bleeding (46), which could have occurred during repeated removal and insertion of the catheter. The difference could also have occurred due to changes in body temperature (46), different anesthesia metabolic state (7), redox state or the amount of intravenously administered physiological solution (47).

Only a few previous studies attempted closed-chest assessment of the biventricular PV relationship in rodents (8, 16). In an adult normotensive rat, it is difficult to introduce a catheter into the RV, even for an experienced surgeon, and the success rate of catheter insertion to the RV according to our previous experience is about 40–60%. There are previous studies that describe catheterization techniques for how to approach RV or pulmonary artery in closed-chest rats (38, 48), but LV was not simultaneously studied. In this protocol, we used a curved polyethylene introducer with a parafilm membrane which allowed us to increase the success rate and reduce surgical complications during RV catheter insertion.

Another critical step is the correct placement of the PV catheter into the LV or RV cavity. The interventricular septum or free ventricular wall, especially of the RV, is very susceptible to penetration and ensuing tamponade. This risk can be minimized by placing the catheter into the apex of the heart under transthoracic echocardiography navigation and by checking the waveform in software. Such coordination in movement prevents incorrect values from being measured caused by the wrong position of the catheter in the heart and helps to reduce the number of animals necessary to conduct the study (38, 48). Moreover, we did not observe any change in the PV loop or hemodynamics after the insertion of the catheter into the RV. We also tested the given protocol with 3–4 hours of data collection and we did not observe significant changes in hemodynamics. In addition to the relatively short monitoring of LV and RV pressure and volume, long-term catheterization methods such as telemetric measurements of pressures in the LV and RV are also known, but it has been generally

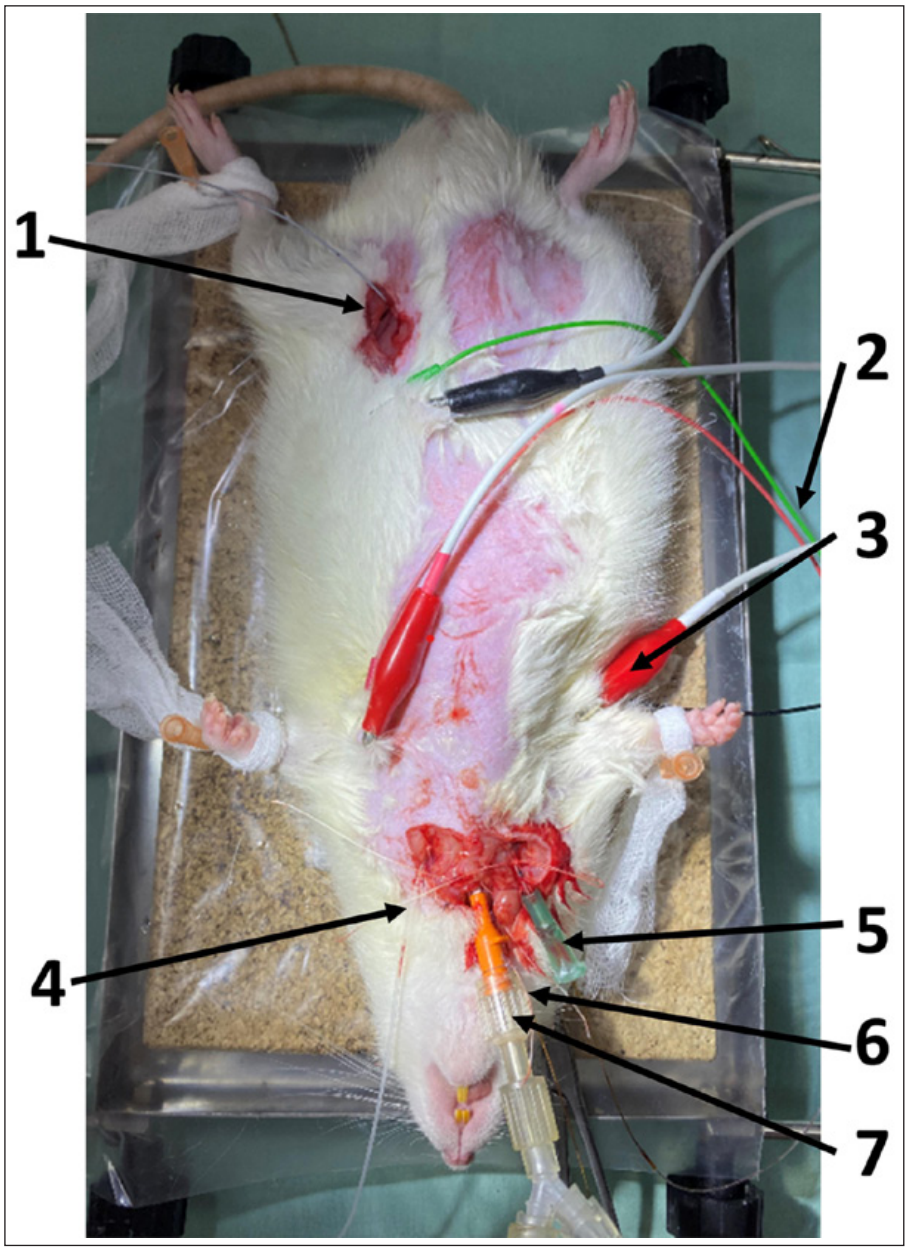

Fig. 13. Experimental rat during biventricular PV analysis. (1): Balloon catheter introduced to vena cava inferior *via* femoral vein approach; (2): ECG leads from computer connected with signals from left ventricle; (3): ECG leads from a computer connected with signals from right ventricle; (4): Catheter for intravenous administration inserted into the left external jugular vein; (5): Introducer with the PV catheter in the right external jugular vein, pushed forward to the right ventricle; (6): PV catheter in the right common carotid artery, pushed forward to the left ventricle; (7): Tube from ventilator connected to endotracheal cannula.

considered that placement of the pressure catheter *via* the carotid and through the aortic valve is not chronically viable due to the risk of valve damage (49). However, Sato and colleagues were able to monitor LV function telemetrically *via* the right carotid approach and reported that all observed hemodynamic parameters were essentially stable during the 2-week observation with no histological damage (50). Some studies have also focused on chronic telemetric monitoring of the RV, but with catheterization through the apex of the RV, which causes damage (51). An interesting vascular approach with preserved right ventricular integrity for pulmonary artery pressures using telemetric monitoring was published by Schreiber *et al.* (52). Simultaneous LV and RV telemetry has not been published according to the previous literature, but in the case of biventricular PV analysis, we did not notice any significant changes in hemodynamic parameters.

In articles describing PV analysis, volume calibration is often insufficiently described. Variations in calibration procedures may explain the heterogeneity of results across studies and researchers. A combination of saline/cuvette calibration is considered by many authors a reliable method for volume calibration despite potential risks because of volume loading and contractility changes. This method has its limits in changing contractility (negative inotropic effect) and volume loading changes by administered hypertonic saline. Despite its limits, this method, in combination with cuvette calibration, accurately determines parallel conductance for dual-field conductance catheters (10, 53, 54).

Volume calibration by 2D echocardiography using Simpson's method or Teichholtz equation of LV volume, can be used for PV protocols in LV that have preserved shape and geometry (9, 33, 55). However, these methods are not adequate for LV with regional wall abnormalities/aneurysms or for RV. Gross approximation of RV volume can be performed by the monoplane ellipsoid approximation method (8, 56) but is still affected by imprecision. 3D volumetric methods, such as cardiac MRI, SPECT, CT, or 3D echocardiography are the gold standards for measurements of RV volume in patients. Despite of difficulty in estimating RV volume in small animals, alternative methods of non-invasive measurement of RV volume are emerging (57). Compared to the MRI method, saline calibration underestimated ventricular volume. However, both techniques are reliably used for right ventricular volume estimation in rodents (58).

Pacher and colleagues preferred the establishment of LV volume calibration using the flow-velocity probe, placed on descending thoracic aorta multiplied by a measure of cross-sectional diameter or using a flow probe (open chest approach, Transonic, Ithaca, NY, USA) that provided flow without requiring area assessment. However, this invasive open chest approach at the end of the experiment could not be suitable for the experimental models with serious stages of cardiovascular diseases, or with many performed interventions, which could reduce the chance of the animal's survival to the end stage of the PV analysis (7).

All these studies used conductance catheters which need the above-mentioned calibrations. However, there is also the option to use an admittance catheter using the admittance method to avoid any of these calibrations. The admittance method was first described in 2007 by Wei *et al.*, (59) and comparative studies (36, 60) have shown that the admittance method and admittance catheters (Transonic, Ithaca, NY, USA) are comparable in accuracy to conductance catheters. This method was validated in studies using PV analysis, including biventricular PV analysis in mice by Potus *et al.* and Tabima *et al.* (16, 61).

However, these studies have only described and measured basal values, and have not included the acquisition of critical parameters of systolic function or any preload reduction maneuvers. We managed to measure several useful reference values in rodent hypertension and experimental model of heart failure which are comparable with quantitative outcomes from other studies (*Table 1*). The animal protocol conditions (such as anesthesia used, age of the animals, *etc.*) differ between ours and the studies with which we compared the reference values, and therefore these comparisons should be taken with caution. Pacher

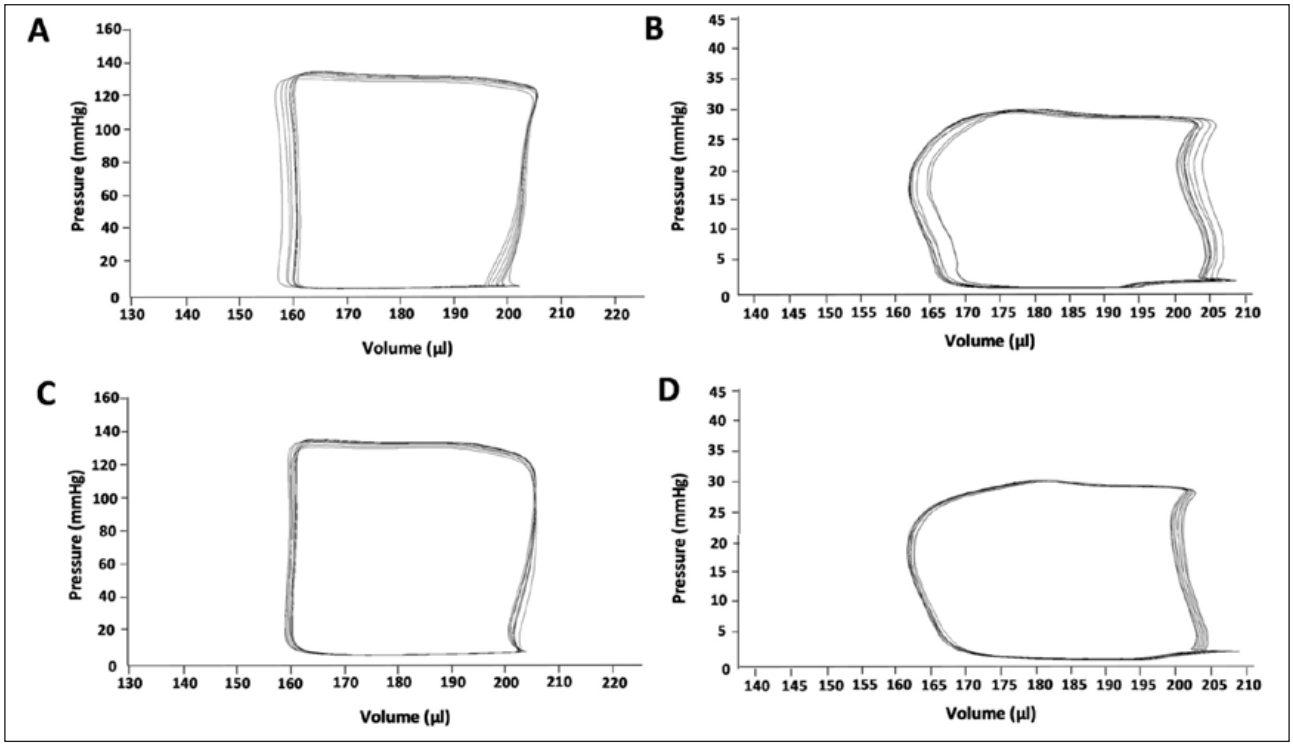

Fig. 14. PV loops measured sequentially (A) and simultaneously (B) in left (LV) and right ventricle (RV) in one normotensive rat (HanSD). Between A and B is no significant difference.

and colleagues summarized measured reference values from left ventricular PV analysis from different rat strains for five years (7). Our measured values of maximum pressures (ESP, 141±9 vs. 113–142 mmHg), end-diastolic pressures (EDP, 5.6±2.7 vs. 1–7 mmHg), and the most reliable contractility parameter PRSW (95±63 vs. 50–140 mmHg) matched their data. We also compared our data with other studies which were focused on biventricular PV analysis. Many of our measured parameters (EDP, ESP, and PRSW), between normotensive HanSD rats and rats with aorto-caval fistula matched Havlenova *et al.* (8). Our data of pressure also agree with the results of Deten and colleagues from normotensive Long Evans rats from the LV (ESP 150±4, EDP 5±1 mmHg) and RV (ESP 34±1, EDP 4 mmHg), in which they also compared the pressures between the sexes (38).

Our results from the LV of HanSD control, TGR control, and TGR ACF rats are in match with data collected by Kala *et al.* in 2021 (55), or Thitiwuthikiat *et al.* observed in the pressure-overloaded heart by transverse aortic constriction (62). TGR rats had similarly increased LV ESP compared to HanSD rats and created ACF significantly reduced LV ESP due to volume overload. In ACF rats, we also observed increased EDV and ESV, which are also changed in a similar manner in the unique murine model of heart failure in Tgαq*44 (63). Moreover, we observed decreased contractility, which was reflected in the reduced parameters of PRSW, and ESPVR, not only in LV but also in RV, in TGR ACF rats compared to TGR control. A similar impact of ACF in the RV, however not in TGR, but HanSD model was also observed by Havlenova *et al.* (8).

In conclusion, in this article, we described a detailed methodological procedure for the assessment of cardiac functions of both ventricles and reported reference values and PV loops of normotensive, hypertensive, and volume-overloaded rats. We also provided a novel femoral vein approach of precisely-controlled occlusion of the inferior vena cava in rats which, to our best knowledge, has not been previously described. Moreover, our data showed that simultaneous catheterization of both LV and RV is more suitable for the determination of biventricular PV relationship compared to sequential measurement of both ventricles separately. This protocol could be helpful to scientists with a focus on biventricular interactions, differences, and for the determination of detailed hemodynamic and contractility parameters in various experimental models of cardiovascular diseases.

Since each method has its strengths, we are also aware of the limitations of this study. The conductance system uses a mathematically ideal cylinder to calculate the volume and therefore this method has a certain bias for the LV and especially RV, which is also increased by the deformation of LV and RV geometry due to pressure and volume-overload (29). The next limitation belongs to the cuvette calibration method, whereas potential fluid extravasation or fibrosis in our listed disease models may influence parallel conductance. Another is the limitation of anesthesia, thus our results and results from other authors could differ from ours measured and shown in this article. Nonetheless, even considering the aforementioned limitations, we are convinced that this study has an important place in the determination of LV, and RV contractility and can contribute to the knowledge of diseases where interventricular dependencies play an important role.

Abbreviations: dP/dt max-EDV, relation between the peak of the first derivative of ventricular pressure and end-diastolic volume; EDPVR, end-diastolic pressure-volume relationship; ESPVR, end-systolic pressure-volume relationship; EDV, end-diastolic volume; ESP, end-systolic pressure; ESV, end-systolic volume; LV, left ventricle; PRSW, preload recruitable stroke work; RR, respiration rate; RV, right ventricle; RVU, relative volume unit; SW, stroke work; V_t, tidal volume.

Author contributions: All experiments were performed at Experimental Medicine Center, Institute for Clinical and Experimental Medicine in Prague. M.M. and V.M designed research; M.M. and P.K. performed experiments; M.M. acquired and analyzed data; M.M. P.K. and V.M. prepared figures; M.M. and V.M. prepared manuscript; M.M., P.K., and V.M. revised manuscript.

All authors approved the final version of the manuscript and agreed to be accountable for all aspects in ensuring questions related to the accuracy or integrity of any part of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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