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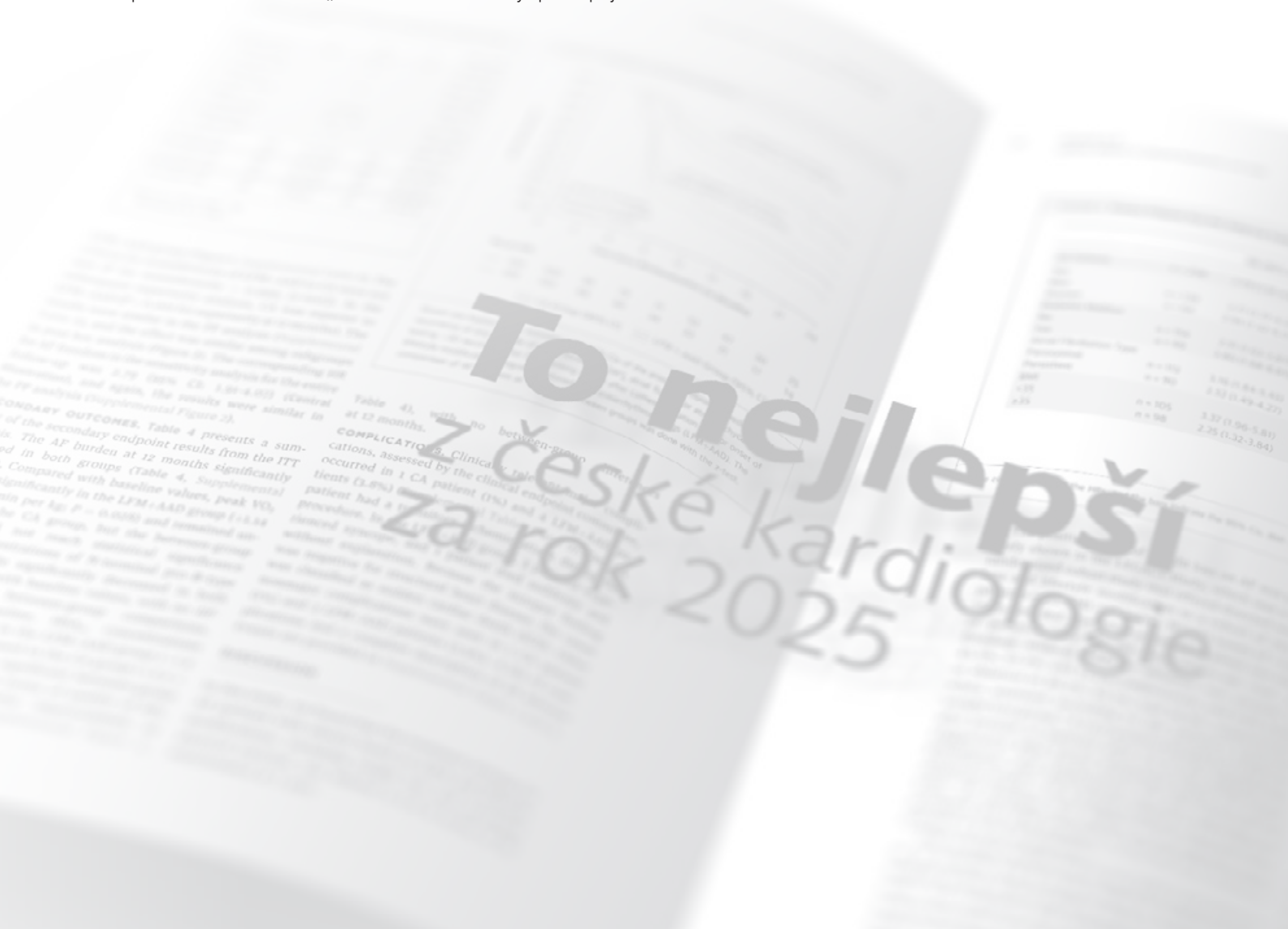
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Catheter Ablation vs Lifestyle Modification With Antiarrhythmic Drugs to Treat Atrial Fibrillation

PRAGUE-25 Trial

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ABSTRACT

BACKGROUND Obesity is an important risk factor for atrial fibrillation (AF). Nonrandomized studies have shown that weight loss and increased physical activity are associated with AF reduction.

OBJECTIVES The goal of this study was to assess whether treatment based on lifestyle modification (LFM; directed weight loss and physical exercise) in combination with antiarrhythmic drugs (AADs) is noninferior to catheter ablation (CA) in patients with AF and obesity.

METHODS In a randomized multicenter noninferiority trial, we enrolled patients with paroxysmal or persistent AF and a body mass index (BMI) of 30–40 kg/m². Patients were randomized to the CA vs LFM+AAD groups in a 1:1 ratio. Seven-day electrocardiographic Holter recordings were performed every 3 months. The primary endpoint was AF freedom during the 12 months after randomization (ie, absence of any AF episode lasting >30 s; the blanking period was 3 months). Secondary endpoints included AF burden, peak oxygen uptake during cardiopulmonary exercise testing, changes in metabolic parameters, and quality of life as assessed with the Atrial Fibrillation Effect on Quality of Life (AFEQT) questionnaire, all compared between randomization and 12 months.

RESULTS A total of 212 patients were enrolled and randomized. Nine patients withdrew consent, leaving 203 patients for the final analysis; 100 patients were allocated to the CA group and 103 to the LFM+AAD group (overall age 60 ± 9 years, 31.5% female, BMI 34.9 ± 3.0 kg/m², 55.7% with paroxysmal AF); the mean follow-up time was 23.5 months. The percentage of patients with AF freedom at 12 months was 73.0% (95% CI: 64.3%–81.7%) in the CA group and 34.6% (95% CI: 25.3%–43.9%) in the LFM+AAD group ($P_{\text{noninferiority}} = 0.99$, $P_{\text{superiority}} < 0.001$). Weight change (−6.4 ± 7.9 kg vs −0.35 ± 4.8 kg; $P < 0.001$) and decreased HbA_{1c} were more significant in the LFM+AAD group than in the CA group.

CONCLUSIONS Despite important metabolic improvements associated with LFM, CA was superior to LFM combined with AADs in improving freedom from AF at 1 year in patients with AF and obesity. (JACC. 2025;86:18–28)

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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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Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia and affects an estimated 60 million people worldwide.¹ Epidemiologic studies have demonstrated that obesity is one of the strongest predictors of developing AF.² An interindividual difference in body mass index (BMI) of 5 kg/m² is associated with a 19% to 29% higher incidence of AF.³

Catheter ablation (CA) has been documented as the most effective treatment of AF. CABANA (Catheter Ablation vs Anti-arrhythmic Drug Therapy for Atrial Fibrillation Trial) (the largest randomized controlled trial [RCT] that compared CA and antiarrhythmic drugs [AADs] for AF treatment) reported that 36.4% of ablation patients and 59.2% of drug-therapy patients had experienced a recurrence of AF within 12 months.⁴

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Obesity was very prevalent among patients in RCTs that assessed the effect of CA. The median BMI in the CABANA trial was 30 kg/m².⁵ A median BMI of 30 kg/m² was reported in the EARLY-AF trial, which compared cryoablation with AADs,⁶ and a mean BMI of 28.5 kg/m² was reported in the ADVENT (Pulsed Field or Conventional Thermal Ablation for Paroxysmal Atrial Fibrillation) trial, which compared thermal with pulsed-field ablation.⁷ In AF patients with BMI ≥27, directed weight loss and other lifestyle modifications (LFMs), it has been shown that including increasing physical activity, have been associated with improved SR maintenance.⁸ In the nonrandomized LEGACY (Long-Term Effect of Goal-Directed Weight Management in an Atrial Fibrillation Cohort: A Long-Term Follow-Up Study), weight losses >10% and 3% to 9% of body weight resulted in AF freedom without AADs or CA in 45.5% and 22.2% of patients, respectively.⁸ However, in trials comparing CA with medical treatment, no weight reduction or other LFM programs were part of the medical arms, even though almost one-half of the studied population had a BMI ≥30 kg/m². Based on the positive results of LFM on sinus rhythm (SR) maintenance, we hypothesized that LFM can significantly augment the treatment effects of AADs in obese AF patients. Therefore, we initiated an RCT with the main question of whether the combination of LFM+AAD can serve as a noninferior alternative to CA in AF patients with obesity.

METHODS

TRIAL DESIGN. The PRAGUE-25 (Catheter Ablation vs. AADs and Risk Factor Modification; [NCT04011800](#))

trial is a randomized, multicenter, investigator-initiated, noninferiority trial. The trial was conducted in 5 centers in the Czech Republic (listed in the [Supplemental Appendix](#)). The study was approved by the Ethics Committee at the University Hospital Kralovske Vinohrady (EK-VP/34/0/2020) and the ethics committees of all participating centers. All patients signed informed consents before enrollment. The details of the protocol were published previously⁹ and are provided in the [Supplemental Material](#). Data collection and analyses were performed by the Institute for Biostatistical Analyses (Masaryk University, Brno, Czech Republic) and Charles University (Prague, Czech Republic). The first author prepared the article, and all authors have confirmed the accuracy of the data and approved the article. The trial was supported by a peer-reviewed research grant from the Czech Health Research Council, Czech Republic: NU21-02-00388.

PATIENT POPULATION AND RANDOMIZATION. Patients with symptomatic AF who met all of the following criteria were eligible: symptomatic AF (paroxysmal, persistent, or long-standing persistent), BMI 30–40 kg/m², and signed informed consent.

The most important exclusion criteria were a history of tachycardia-induced cardiomyopathy, BMI >40 kg/m², left ventricular ejection fraction ≤40%, untreated coronary artery disease, left atrial size >60 mm, indication for surgical treatment of obesity, age ≥75 years, contraindication for AADs, and significant limitations that could affect physical activity. Further inclusion and exclusion criteria are detailed in the [Supplemental Appendix](#). At the baseline visit, all patients in both groups were informed, in detail, about the risk of obesity as well as other risk factors as they related to AF. Eligible patients meeting the inclusion criteria were randomized in a 1:1 ratio to the catheter ablation (CA) group or the LFM plus AADs (LFM+AAD) group. Randomization was done using a Web-based system in permuted blocks, with stratification according to BMI, sex, and AF type.

BASELINE EXAMINATIONS. After randomization, all patients underwent: 1) a baseline cardiopulmonary exercise test (CPET) ([Supplemental Appendix](#)); 2) echocardiography; 3) quality of life analysis (using the Atrial Fibrillation Effect on Quality of Life questionnaire [AFEQT]); 4) blood biochemistry; and 5) a baseline 7-day electrocardiographic (ECG) Holter recording. All baseline examinations were done within 4 weeks of randomization.

ABBREVIATIONS AND ACRONYMS

AAD = antiarrhythmic drug
AF = atrial fibrillation
BMI = body mass index
CA = catheter ablation
CPET = cardiopulmonary exercise test
ECG = electrocardiography
GLP-1 = glucagon-like peptide 1
ITT = intention-to-treat
LFM = lifestyle modification
PP = per-protocol
RCT = randomized controlled trial
SCD = sudden cardiac death
SR = sinus rhythm

TRIAL PROCEDURES. CA group. Patients in the CA group underwent an CA procedure within 6 weeks of randomization. The CA protocol is described in the [Supplemental Appendix](#). The first 3 months after CA were considered to be the blanking period. AADs or cardioversions were allowed in the CA group only during the blanking period; all AADs had to be stopped before the end of the 3-month blanking period. The reinitiation of AADs after the blanking period was allowed only in cases of arrhythmia recurrence and was considered to be treatment failure.

LFM + AAD group. The full LFM+AAD program was started within 4 weeks after randomization. LFM was not managed by treating cardiologists, but by teams of dietary specialists and physiotherapists. The initial patient consultations with nutritional specialists and physiotherapists occurred during the 4 weeks after randomization. In addition to regular in-person and telephone consultations, patients were encouraged to record their calorie intake and exercise activity with the use of the OBEFIS mobile phone application. Besides reducing calorie intake, patients were instructed and repeatedly encouraged to decrease alcohol consumption (but complete abstinence was not required). All patients had a consultation with a physiotherapist, who prepared individual exercise programs based on the CPET results. Patients monitored their heart rate during exercise with the use of a fitness band. Details regarding LFM are provided in the [Supplemental Appendix](#). The initiation of antiarrhythmic medication in LFM+AAD patients commenced within 4 weeks of randomization, with the dosage up-titration during the first 3 months after randomization. The choice of AAD for each patient was determined according to local practice; amiodarone was available as a third-line choice only after previous failure of class IC AADs, and, in contrast to the CA group, the use of AADs was allowed during the whole follow-up. Similarly, as in the CA patients, the first 3 months after the start of the LFM program were considered to be the blanking period. Blood pressure, lipids, and diabetes were managed similarly in both groups by the study's cardiologists or the patients' diabetologists according to current guidelines.

OUTPATIENT FOLLOW-UP AND HOLTER MONITORING. Starting from the day of CA or the start of the full LFM+AAD program (ie, day 0 [DO]), follow-up visits were performed at 3, 6, 9, and 12 months and then every 6 months. At the 3-month follow-up visit, all patients in AF (ie, both groups) underwent electrical cardioversion. In the first 3 months after DO, which

were considered to be the blanking period in both groups, AF recurrences were not considered as an endpoint.

Seven-day Holter recordings were performed every 3 months during the first year and every 6 months thereafter. Evaluations of Holter recordings were blinded; all Holter recordings were evaluated by an organization outside of the study without access to patient information (Medical Data Transfer).

Starting from DO, outpatient visits were also performed every 3 months in the first year and every 6 months thereafter. During each outpatient visit, all new urgent or unscheduled visits due to AF or other cardiovascular reasons, other important changes in the health status, and all changes in medication, with a special focus on antiarrhythmic medication, were recorded. During the 12-month follow-up, echocardiography, quality of life evaluation, biochemistry, and CPET were repeated.

TRIAL ENDPOINTS. The primary endpoint (AF freedom) was the absence of any atrial tachyarrhythmia (AF, atrial flutter, or atrial tachycardia) lasting >30 seconds during the 1 year following the blanking period. Evaluation of arrhythmia recurrence was conducted with the use of 7-day Holter recording analyses, ECGs conducted during outpatient visits, and, if applicable, documented AF at unplanned patient visits (patient-reported palpitation without documented AF was not considered to be an AF recurrence).

The secondary endpoints were changes in AF burden (expressed as a percentage of time in AF during Holter recording), peak VO_2 max during CPET, AFEQT scores, and prespecified metabolic parameters (body weight, lipid levels, and glycated hemoglobin [HbA_{1c}] levels) between baseline and the 12-month visit.

STATISTICAL CONSIDERATIONS AND POWER CALCULATION. In a meta-analysis of 24 RCTs comparing CA, AADs, and placebo, the overall success rate of AADs, defined as the disappearance of arrhythmia during follow-up, was 52% (95% CI: 47%–57%).¹⁰ In the CABANA and STOP-AF (Cryoballoon Catheter Ablation in Antiarrhythmic Drug Naive Paroxysmal Atrial Fibrillation) trials, the 1-year AF freedoms in the medication (AAD) groups were 47.1%, and 45.0%, respectively.^{5,11} Several nonrandomized studies documented the positive effect of LFM on AF freedom. In the LEGACY study, 45.5% of patients who achieved >10% weight loss and 22.2% of patients who achieved 3% to 9% weight loss remained AF free without AADs or ablation. Therefore, an additional effect of 20% was expected for weight loss and physical exercise; we assumed an absence of any

atrial tachyarrhythmia in 65% of patients in the LFM+AAD group at 12 months after randomization.

Concerning the efficacy of CA, AF freedom was present in 63.6% at 12 months in the CABANA trial.⁵ In a meta-analysis of RCTs comparing CAs to AADs, the single-procedure success rate of CA without AADs was 57% (95% CI: 50%-64%).¹⁰ The median BMI in most published studies on CA for AF was ~30 kg/m². Because the minimum BMI in our study was 30 kg/m², and because the effect of CA decreases with increased BMI, the expected 1-year AF freedom in the CA group in our study was 60%.

The first step in selecting the noninferiority margin was to estimate the minimum acceptable retention of CA over placebo. No studies comparing CA and AADs had a placebo arm. In studies comparing AADs and placebo, the overall success rate (generally defined as the disappearance of arrhythmia during follow-up) in 8 placebo treatment arms was 24.9% (95% CI: 15%-34%).¹⁰ A noninferiority margin of 12% was selected, which was less than the lower band of the 95% CI, or one-half of the treatment effect of placebo. If expressed as an OR, it corresponds to 1.65, which is less than the most liberal margin of 1.92 required by regulatory guidelines. Details on the statistical considerations of treatment effect and power calculation are described in the [Supplemental Appendix](#). The sample size for the noninferiority analysis was estimated with the use of a 1-sided z-test. The sample size calculation assumed 80% power, 2.5% 1-sided alpha, and a noninferiority margin of 12%. Using these assumptions, 202 patients (101 in each group) would need to be enrolled to demonstrate noninferiority. With an expected drop-out rate of 5%, 212 patients were initially planned. Because the analysis of the primary endpoint was planned for 1 year after randomization, all patients had to have at least 1 year of follow-up after randomization.

STATISTICAL ANALYSIS. Analyses of the primary and secondary endpoints were based on the intention-to-treat (ITT) and per-protocol (PP) principles. If the noninferiority criterion for the primary endpoint was not satisfied, then superiority was evaluated. Standard descriptive statistical methods were used in the analysis. Categorical data were summarized with the use of absolute and relative frequencies, whereas continuous data were described with the use of either mean $\pm$ SD or median (Q1-Q3), depending on data distribution. Kaplan-Meier estimations and Cox proportional hazards regression models were used for data visualization and analysis, with the treatment group included as a covariate. The

TABLE 1 Baseline Characteristics

	CA Group (n = 100)	LFM+AAD Group (n = 103)
Age, y	60 $\pm$ 8	60 $\pm$ 9
Male	68 (68.0)	71 (68.9)
Body weight, kg	110 $\pm$ 15	109 $\pm$ 17
Body mass index, kg/m ²		
Mean	35.0 $\pm$ 2.9	34.9 $\pm$ 3.2
30.0–32.9	30 (30.0)	35 (34.0)
33.0–35.9	33 (33.0)	30 (29.1)
$\geq$ 36.0	37 (37.0)	38 (36.9)
AF type		
Paroxysmal	56 (56.0)	57 (55.3)
Persistent	39 (39.0)	41 (39.8)
Long-standing persistent	5 (5.0)	5 (4.9)
Medical history		
Heart failure	13 (13.0)	11 (10.7)
Hypertension	84 (84.0)	86 (83.5)
Diabetes mellitus	19 (19.0)	30 (29.1)
Stroke/TIA	3 (3.0)	3 (2.9)
Coronary artery disease	8 (8.0)	6 (5.8)
History of myocardial infarction	3 (3.0)	1 (1.0)
CHA ₂ DS ₂ -VASc score		
Mean	2.0 $\pm$ 1.2	2.0 $\pm$ 1.2
0	7 (7.0)	9 (8.7)
1	33 (33.0)	29 (28.2)
2	27 (27.0)	30 (29.1)
3	20 (20.0)	21 (20.4)
$>$ 3	12 (12)	14 (13.6)
History of electrical cardioversion	36 (36.0)	47 (45.6)
Pacemaker	3 (3.0)	1 (1.0)
Baseline medication		
AADs	59 (59.0)	69 (67.0)
Oral anticoagulants	90 (90.0)	92 (89.3)
BBs	75 (75.0)	86 (83.5)
No. of antihypertensives including BBs	2.3 $\pm$ 1.3	2.3 $\pm$ 1.2
Statins	50 (50.0)	51 (49.5)
Fibrates	5 (5.0)	5 (4.9)
Ezetimib	4 (4.0)	2 (1.9)
Antidiabetics	18 (18.0)	27 (26.2)
Echocardiography		
Left atrium diameter, mm	45.0 $\pm$ 6.2	45.6 $\pm$ 5.7
LVEF, %	59.2 $\pm$ 8.3	61.2 $\pm$ 5.6

Values are shown as n (%) or mean $\pm$ SD. P values were calculated with the use of Fisher test or Mann-Whitney U-test.

AAD = antiarrhythmic drug; BB = beta-blocker; CA = catheter ablation; LFM = lifestyle modification; LVEF = left ventricular ejection fraction; TIA = transient ischemic attack.

noninferiority of LFM+AAD was assessed with the use of a 1-sided z-test based on differences in 12-month AF-free survival proportions, with a noninferiority margin of 12%. Subsequently, the superiority of CA was evaluated with the use of a 1-sided z-test. Secondary endpoints were evaluated by means of the Mann-Whitney test to assess the statistical significance of differences between the 2 groups. In

TABLE 2 Weight Change (kg) During Follow-Up

	n	CA	n	LFM+AAD	Mean Difference (95% CI)	P Value
Baseline visit	100	109.5 ± 14.6	103	109.3 ± 17.2	0.27 (–4.15 to 4.68)	0.581
3-month visit	100	109.2 ± 14.5	102	106.2 ± 18.2	3.00 (–1.57 to 7.56)	0.073
6-month visit	100	109.1 ± 14.4	100	104.5 ± 18.9	4.60 (–0.08 to 9.28)	0.016 ^a
9-month visit	100	109.4 ± 15.2	100	103.3 ± 18.6	6.14 (1.41–10.87)	0.004 ^a
12-month visit	100	109.2 ± 15.5	100	103.1 ± 19.0	6.11 (1.26–10.95)	0.004 ^a
18-month visit	70	108.1 ± 14.9	73	102.5 ± 18.0	5.60 (0.15–11.06)	0.016 ^a
24-month visit	46	108.3 ± 13.9	57	101.0 ± 19.2	7.27 (0.81–13.74)	0.008 ^a

Values are mean ± SD unless otherwise indicated. ^aP < 0.05.
Abbreviations as in [Table 1](#).

addition, the paired Wilcoxon signed rank test was used to evaluate the statistical significance of changes within each group between baseline and 12-month values.

Because the planned statistical analyses did not include adjustments for multiplicity when evaluating secondary endpoints or subgroups, the results are presented as point estimates with 95% CIs. The widths of the CIs have not been adjusted for multiplicity and should not be used to infer definitive treatment effects. All statistical analyses were performed with the use of SPSS software version 29.0.1.0 and R version 4.2.2.

RESULTS

PATIENTS AND FOLLOW-UP. From May 2021 to November 2023, 212 patients were randomized (CONSORT diagram in [Supplemental Figure 1](#)). Nine patients withdrew consent within the first month after randomization, so 203 patients (60 ± 9 years of age, 31.5% female, BMI 34.9 ± 3.0 kg/m², and 55.7% with paroxysmal AF) entered the study and were analyzed. One hundred were randomized to the CA group and 103 to the LFM+AAD group. The baseline characteristics were balanced between the 2 groups ([Table 1](#), [Supplemental Table 1](#)). During the study course, 26 patients underwent cross-over (1 from CA to LFM+AAD and 25 from LFM+AAD to CA), but only 3 (1 CA and 2 LFM+AAD) crossovers occurred before the primary endpoint had been reached. Details are shown on the study flow chart provided in [Supplemental Figure 1](#). The mean follow-ups were 23.3 ± 8.5 months in the CA group and 23.6 ± 9.2 months in the LFM+AAD group. Adherence to Holter monitoring and CPET examinations are presented in [Supplemental Table 2](#).

TREATMENT CHARACTERISTICS. In the CA group, 48 patients underwent radiofrequency and 51 pulsed-field ablations within 6 weeks of randomization. All patients underwent pulmonary vein isolation, and 35

patients (35.4%) with nonparoxysmal AF received additional ablation lesions. Further information about the ablations is provided in [Supplemental Table 3](#). During follow-up, 7 patients (7%) underwent a redo ablation (and 1 patient had 2 redo ablations) because of arrhythmia recurrence. At the 12-month visit, 16 CA patients (16%) were on AADs because of documented AF recurrences ([Table 2](#)). Compared with baseline values, nonsignificant body weight reduction was present in CA patients at 12 months (–0.35 ± 7.78 kg) and at 24 months (–0.08 ± 5.96 kg) ([Table 2](#)).

In the LFM+AAD group, there was a significant reduction in body weight at 12 months compared with baseline (–6.37 ± 7.94 kg; P < 0.001), and that weight loss was maintained through the 24-month follow-up (–6.29 ± 8.80 kg; P < 0.001) ([Table 2](#), [Supplemental Table 4](#)). Liraglutide or semaglutide was briefly (6 ± 4 months) used by 15 patients (14.6%). At 3 months, AADs were used in 97 LFM+AAD patients (95.1%); most patients were on propafenone or flecainide. The use of AADs over time is presented in [Table 3](#). Owing to palpitations, 2 LFM+AAD patients underwent ablation before any documented occurrence of AF. Based on the questionnaire administered during the last study visit in the LFM+AAD group, 66 patients (64.1%) reported decreased alcohol consumption, 79 (76.7%) reported an increase in physical activity, and 17 (16.5%) were being treated for obstructive sleep apnea ([Supplemental Table 5](#)). Sixty-eight patients (66%) regularly used the OBEFIS mobile app to monitor their physical activity and to communicate with a dietary specialist. Details on laboratory values and medications in patients with diabetes in both groups at the 12-month visit are presented in [Supplemental Tables 6 and 7](#).

PRIMARY OUTCOME. In the ITT analysis, arrhythmia-free survival at 12 months was present in 73.0% (95% CI: 64.3%–81.7%) of patients in the CA group and 34.6% (95% CI: 25.3%–43.9%) of patients in the

TABLE 3 Proportion of Patients Using Antiarrhythmic Drugs During Follow-Up

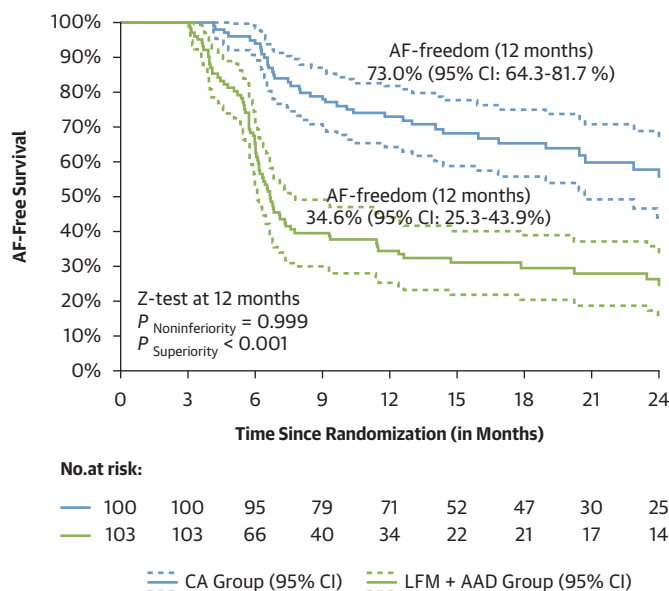
	n	CA	n	LFM+AAD
3-month visit	100	3 (3.0)	102	97 (95.1)
Propafenone		0		46 (47.4)
Dose, mg/d		–		404 ± 134
Flecainide		0		26 (26.8)
Dose, mg/d		–		148 ± 61
Sotalol		3 (100.0)		3 (3.1)
Dose, mg/d		107 ± 46		187 ± 122
Amiodarone		0		22 (22.7)
Dose, mg/d		–		186 ± 32
6-month visit	100	10 (10.0)	100	88 (88.0)
9-month visit	100	14 (14.0)	100	77 (77.0)
12-month visit	100	16 (16.0)	100	66 (66.0)
18-month visit	70	15 (21.4)	73	47 (64.4)
24-month visit	46	7 (15.2)	57	37 (64.9)

Values are n (%) or mean ± SD.
Abbreviations as in Table 1.

LFM+AAD group (Figure 1, Supplemental Table 8). The criteria for noninferiority of LFM+AAD to CA were not met (P for noninferiority = 0.999; [z-test]). In the subsequent superiority analysis, CA was superior to LFM+AAD ($P < 0.001$ for superiority at 12 months). The results were similar in the PP analysis (Supplemental Table 8), and the effect was similar among subgroups in post hoc analysis (Figure 2). The corresponding HR for AF freedom in the sensitivity analysis for the entire follow-up was 2.79 (95% CI: 1.91–4.07) (Central Illustration), and again, the results were similar in the PP analysis (Supplemental Figure 2).

SECONDARY OUTCOMES. Table 4 presents a summary of the secondary endpoint results from the ITT analysis. The AF burden at 12 months significantly decreased in both groups (Table 4, Supplemental Figure 4). Compared with baseline values, peak VO_2 increased significantly in the LFM+AAD group ($+1.14 \pm 3.90$ mL/min per kg; $P = 0.028$) and remained unchanged in the CA group, but the between-group difference did not reach statistical significance (Table 4). Concentrations of N-terminal pro-B-type natriuretic peptide significantly decreased in both groups compared with baseline values, with no differences in the between-group comparisons. Compared with baseline, HbA_{1c} concentrations significantly decreased in the LFM+AAD group (-1.4 ± 4.8 mmol/L) and increased in the CA group ($+2.5 \pm 10.5$ mmol/L) resulting in significant between-group difference at 12 months. In terms of quality of life, both groups reported similar improvements as assessed by the AFEQT (Supplemental Figure 5,

FIGURE 1 Estimates of the Primary Endpoint



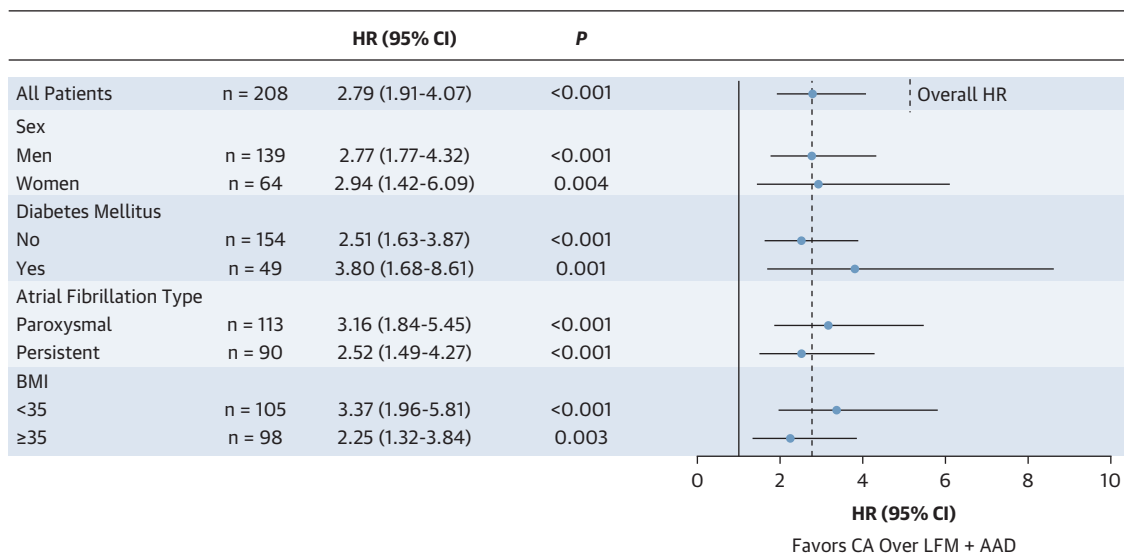
Shown are Kaplan-Meier estimates and 95% CIs of the primary endpoint (freedom from recurrence of any episode of atrial fibrillation [AF], atrial flutter, or atrial tachycardia lasting >30 seconds) after the blanking period after catheter ablation (CA) or onset of lifestyle modification program combined with antiarrhythmic drugs (LFM+AAD). The comparison of AF freedom at 12 months between groups was done with the z-test.

Table 4), with no between-group differences at 12 months.

COMPLICATIONS. Clinically relevant major complications, assessed by the clinical endpoint committee, occurred in 1 CA patient (1%) and 4 LFM+AAD patients (3.8%) (Supplemental Tables 3 and 9). One CA patient had a transitory ischemic attack during the procedure. In the LFM+AAD group, 3 patients experienced syncope, and 1 patient died suddenly and without explanation. Because the autopsy finding was negative for structural heart disease, the cause was classified as sudden cardiac death (SCD). Other nonmajor complications were seen in 1 CA patient (1%) and 3 LFM+AAD patients (2.9%). A list of complications and a complete description of all adverse events are provided in Supplemental Tables 3 and 9.

DISCUSSION

In this study, we found that the treatment strategy for AF patients with obesity based on AADs and lifestyle modifications, including weight loss and increased physical exercise, was inferior to CA in terms of SR maintenance at 1 year.

FIGURE 2 Primary Endpoint Post Hoc Subgroup Analysis (Intention to Treat)

The circles represent the HRs, and the bars indicate the 95% CIs. BMI = body mass index.

The positive effect of weight loss on AF was previously shown in the LEGACY study, which was a non-randomized cohort study that offered directed weight loss and lifestyle modification to a cohort of 355 AF patients with BMI ≥ 27 kg/m². Weight losses of $\geq 10\%$, 3% to 9%, and $\leq 3\%$ were achieved in about one-third of patients each. At the final follow-up visit, AF freedom without AADs or ablation was present in 45.5%, 22.2%, and 13.4%, respectively, and with AADs or ablation in 86.2%, 65.5%, and 36.9%, respectively, when assessed according to the patient's achieved weight loss group.⁸ A very positive benefit for LFM was also present in patients after CA in the ARREST-AF (Aggressive Risk Factor Reduction Study for Atrial Fibrillation and Implications for the Outcome of Ablation) study.¹² In a design similar to the LEGACY trial, AF freedom was higher in 61 patients who accepted the LFM program vs the 88 who declined (32.9% vs 9.7% of patients).

The average weight loss in our LFM+AAD patients at 12 months was 6.37 kg, which is similar to the middle group of the LEGACY study (−6.0 kg). However, the LEGACY and ARREST-AF studies were nonrandomized, and in the ARREST-AF study, patients were divided according to their acceptance of the LFM program; patients who declined formed the control group. The absence of randomization could have significantly biased their results because motivated patients who accepted the LFM program could

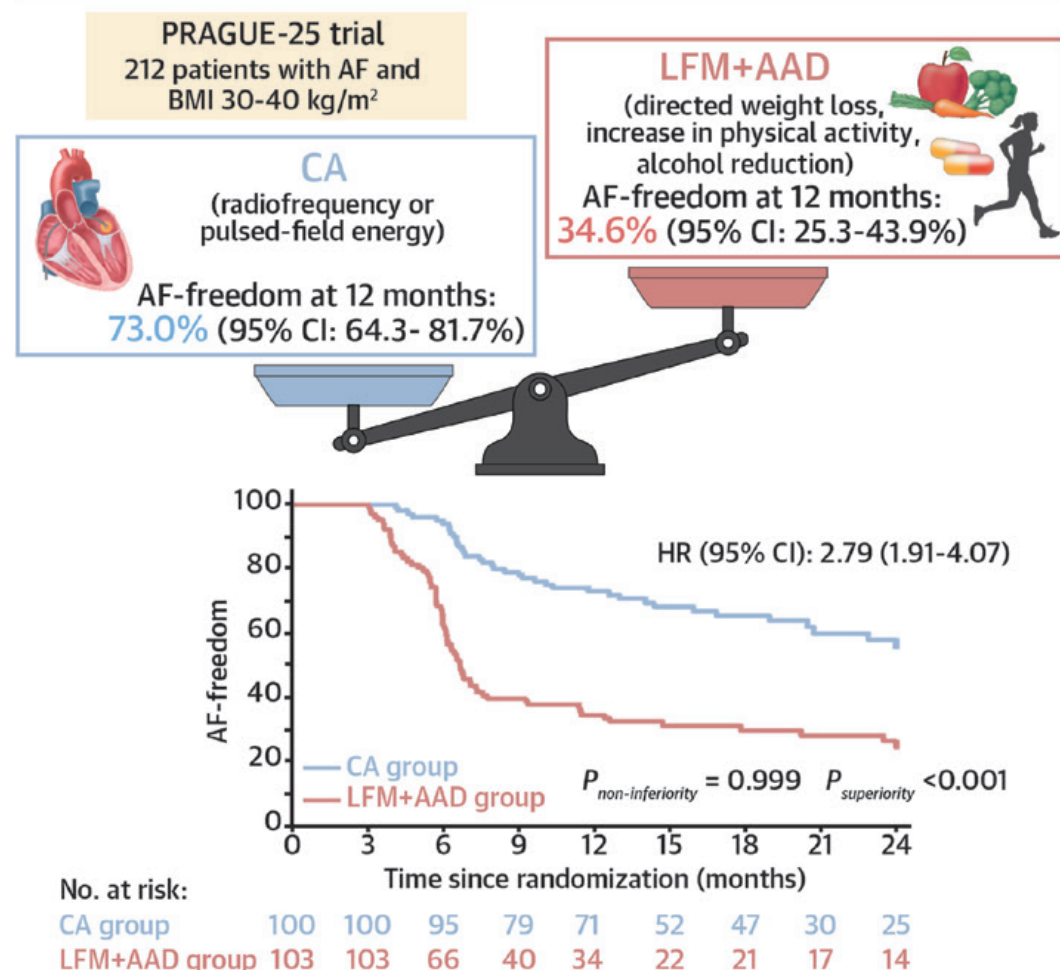
differ significantly in areas (well-being, drug adherence, etc) other than weight loss.

The results of the ARREST-AF study were challenged by the recent SORT-AF (SORT - AF Supervised Obesity Reduction Trial for AF Ablation Patients) trial. That trial randomized 133 AF patients with BMIs of 30 to 40 kg/m² after CA to either a weight-reduction program or standard care. AF burden after ablation decreased significantly in both groups, but there was no significant difference in AF burden between the weight loss and control groups 12 months after the CA.¹³ It is noteworthy that compared with the ARREST-AF study, weight loss in the SORT-AF study was only modest (−4.6 $\pm$ 8.6 kg, or 3.91% of the initial body weight).¹³

The weight loss achieved in our LFM+AAD patients was modest as well. Greater weight reduction would definitely be expected if glucagon-like peptide 1 (GLP-1) agonists had been used. In meta-analyses of studies with liraglutide, semaglutide, and tirzepatide, the resulting weight loss was −4.5 kg with >1.8 mg liraglutide, −9.9 kg with >2.4 mg semaglutide, and −15.3% of the initial body weight with tirzepatide.^{14,15} Unfortunately, our LFM+AAD group did not reach the planned weight loss of 10% of body weight, which is an important study limitation. Greater weight loss would most likely have led to better outcomes regarding SR maintenance. On the other hand, the weight loss achieved at 12 months was

CENTRAL ILLUSTRATION Summary of the Results

Catheter Ablation (CA) vs. Lifestyle Modification with Antiarrhythmic Drugs (LFM+AAD) to Treat Atrial Fibrillation (AF)



Despite important metabolic improvements associated with LFM, CA was superior to LFM combined with AADs in improving AF-freedom at one year in people with obesity.

Osmancik P, et al. JACC. 2025;86(1):18-28.

maintained at 24 months in our study, which is in contrast to GLP-1 studies, in which treatment-related weight loss was not maintained after GLP-1 was stopped. Moreover, with regard to previous studies with GLP-1 agonists, despite the clear and significant effect on body weight, no significant effect on AF was seen in the meta-analysis of 7 RCTs that assessed the use of GLP-1 agonists on the incidence of AF (OR: 0.81; 95% CI: 0.78-1.15).¹⁶

In our study, 1-year AF freedom in the LFM+AAD group (34.6%) was lower than expected. In the

CABANA trial, AF freedom was 40.8% in patients on AADs at 12 months.⁴ In the 2 most recent RCTs on CA, AF freedom on AADs at 12 months was present in 45% (STOP-AF trial) and 32.2% (EARLY-AF trial).^{6,11} Recently, Ornelas-Lorendo et al compared the effectiveness of Class 1C and III AADs in obese (BMI ≥ 30 kg/m²) and nonobese AF patients. Nonresponse to Class 1C AADs in patients with obesity was significantly higher than those without obesity.¹⁷ Explanations for the lower-than-expected AF freedom in our study may be related to the lower

TABLE 4 Secondary Endpoints: AF Burden, VO₂ max, Metabolic Parameters, and AFEQT at Baseline and 12 Months

	CA Group		LFM-AAD Group		Between-Group Differences (95% CI)	P Value
	Baseline	12 Months	Baseline	12 Months		
AF burden, %	31.1 ± 42.6	12.1 ± 31.2 ^a	35.9 ± 44.1	22.1 ± 37.2 ^b	−4.8 (−17.3 to 7.7)	0.17
VO ₂ max, mL/kg/min	17.90 ± 4.57	18.05 ± 4.74	19.09 ± 5.07	20.38 ± 5.81 ^c	−0.98 (−2.02 to 0.07)	0.13
HbA _{1c} , mmol/L	39.6 ± 9.8	41.6 ± 11.2 ^d	40.7 ± 7.1	39.6 ± 8.0 ^e	3.9 (1.4–6.3)	<0.001 ^f
Total cholesterol, mmol/L	4.52 ± 1.12	4.28 ± 0.96	4.46 ± 1.13	4.29 ± 1.10	−0.05 (−0.32 to 0.22)	0.79
Triglycerides, mmol/L	1.86 ± 1.20	1.71 ± 1.29	1.79 ± 0.87	1.50 ± 0.73 ^e	0.15 (−0.11 to 0.41)	0.08
CRP, mmol/L	4.59 ± 4.82	3.77 ± 4.31	4.34 ± 4.18	3.67 ± 3.87 ^b	−0.12 (−1.50 to 1.25)	0.40
NT-pro BNP, pg/mL	506 ± 566	284 ± 463 ^a	495 ± 548	342 ± 412 ^b	−64 (−222 to 94)	0.21
AFEQT	68.6 ± 19.9	86.2 ± 14.3 ^a	72.7 ± 19.0	85.4 ± 15.4 ^e	4.3 (−0.8 to 9.4)	0.14

^aP < 0.001. ^bP < 0.01. ^cP < 0.05. ^dP < 0.05. ^eP < 0.001. ^fP < 0.05.

AFEQT = Atrial Fibrillation Effect on Quality-of-life Questionnaire.

effectiveness of Class 1C AADs (used in 74% of our patients) combined with the less-than-expected (moderate) weight reduction in our patients. Finally, the presence of manifest AF may indicate a more advanced stage of the disease, which may limit the treatment efficacy of preventive programs.

With regard to the efficacy of CA in our study, despite the higher BMIs of our patients, the 1-year AF freedom (73.0%) in our CA patients was similar to the 1-year AF freedom after CA reported in other recent trials, such as CABANA (63.6%), STOP-AF (74.6%), and ADVENT (72%).^{4,7,11}

The low rate of complications in our study (1 transient ischemic attack [1%]) highlights the steadily increasing safety profile of CA. In a large series of >153,000 patients, obesity was associated with a significant risk of vascular access complications, but with only a nonsignificant trend toward other ablation-related complications.¹⁸ The risk of vascular access complications can be reduced by periprocedural ultrasound guidance,¹⁹ which was used on all patients in the present study. Similarly low rates of complications during CA in overweight and obese patients were also seen in the recent SORT-AF trial (0.75% of patients).¹³

In contrast, major drug-related adverse events (AEs) were present in 4 patients on AADs (3.8%). The rates of complications in previous studies comparing CA and AADs were similar. Still, the types of major AEs differed between groups,^{20,21} and AEs associated with AADs were usually less clinically relevant. Our study included 1 sudden unexplained death that occurred in the LFM+AAD group in a patient being treated with propafenone. It is well established that AF is associated with increased general mortality, including SCD.²² The use of AADs has been known for some time to be associated with a higher risk of SCD, although the risk is highest in patients with heart

failure or coronary artery disease (CAD).²³ Although both of these conditions were exclusion criteria in our study, unrecognized CAD or latent channelopathy can be present in apparent healthy AF patients. Moreover, the risk of SCD in AF patients on Class 1C AADs is higher even in the absence of CAD and was described decades ago.²⁴ AADs are still commonly used to treat symptomatic AF; however, the risk associated with AADs should be considered before treatment onset.

The LFM+AAD group nonetheless experienced a significant decrease in body weight, an improvement in metabolic parameters, and an improvement in physical performance during CPET. At 12 months, LFM-AAD patients experienced a significant decrease in HbA_{1c} concentrations and body weight compared with CA patients. In the long term, higher HbA_{1c} concentrations are associated with increased risk of cardiovascular events and cardiovascular mortality,²⁵ as well as an increased risk of AF.²⁶ Increased body weight is associated with a higher risk of heart failure, cardiovascular events, and all-cause mortality.^{27,28} Weight reduction is associated with improved outcomes, especially if cardiorespiratory fitness was part of the weight reduction program.²⁹ Given the strong association between these positive metabolic changes and enhanced cardiovascular outcomes, the reduced effectiveness of the LFM+AAD strategy relative to AF freedom should not dissuade obese patients from pursuing weight loss and LFM.

Compared with baseline values, the LFM program was associated with an increase in peak VO₂ at 12 months. An inverse association between decreased cardiorespiratory fitness and cardiovascular events over the long term has been described previously.³⁰ As was shown, significant improvements in cardiovascular outcomes occur even with small changes in peak VO₂.²⁹ Despite lower AF freedom in the

LFM+AAD group, the quality of life improvements were similar in both arms, which emphasizes the positive subjective perception of weight loss and, probably, the increase in physical capacity. Although the main goal of the study was to compare AF freedom at 1 year, the results should be viewed and interpreted in the context of the aforementioned improvements in the LFM+AAD group, and the positive metabolic changes in the LFM+AAD group should not be underestimated.

STUDY LIMITATIONS. GLP-1 agonists were not systematically used in the LFM+AAD group. The planned target of a 10% reduction in body weight was not achieved. Because the effect of weight loss on AF freedom in nonrandomized studies was related to achieved weight loss, greater weight loss in our patients would probably have been associated with better SR maintenance. Furthermore, the positive effect of weight loss on AF could appear later on, ie, the effect might not have been fully present during the follow-up of our study. Arrhythmia recurrences were monitored by means of 7-day ECG Holter recordings; implantable loop recorders were not used. Our power analysis anticipated AF freedom in 60% of CA and 65% of LFM+AAD patients; the study was not adequately powered to consider other acceptable scenarios of AF freedom for noninferiority (eg, 55% vs 60%). The LFM+AAD group was targeted toward dedicated weight reduction (which also included reduced alcohol consumption) and increased physical activity; other risk factors, such as tobacco use, obstructive sleep apnea, and more strict blood pressure control, were managed as part of standard care

by the study's cardiologists. Exercise activity was not meticulously monitored.

CONCLUSIONS

Despite the significant positive benefits of LFM programs, CA was superior to LFM combined with AADs in terms of AF freedom at 1 year. Therefore, the referral to CA in this population should not be delayed until the patient loses weight. Nonetheless, patients with obesity should not be discouraged from losing weight, because several other significant benefits are associated with weight reduction.

DATA AVAILABILITY. The data supporting this study's findings are available from the corresponding author (Dr Osmancik) on reasonable request (eg, for meta-analyses).

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KEY WORDS antiarrhythmic drug, atrial fibrillation, catheter ablation, lifestyle modification, obesity

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

L. Monzo et al.

Growth Differentiation Factor-15 Is Associated With Congestion-Related Anorexia and Weight Loss in Advanced Heart Failure

JACC: Heart Failure
Impact Factor: 11,8



ORIGINAL RESEARCH

HEART FAILURE

Growth Differentiation Factor-15 Is Associated With Congestion-Related Anorexia and Weight Loss in Advanced Heart Failure



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ABSTRACT

BACKGROUND Growth differentiation factor (GDF)-15 is a pleiotropic cytokine that is associated with appetite-suppressing effects and weight loss in patients with malignancy.

OBJECTIVES This study aims to investigate the relationships between GDF-15 levels, anorexia, cachexia, and clinical outcomes in patients with advanced heart failure with reduced ejection fraction (HFrEF).

METHODS In this observational, retrospective analysis, a total of 344 patients with advanced HFrEF (age 58 ± 10 years, 85% male, 67% NYHA functional class III), underwent clinical and echocardiographic examination, body composition evaluation by skinfolds and dual-energy x-ray absorptiometry, circulating metabolite assessment, Minnesota Living with Heart Failure Questionnaire, and right heart catheterization.

RESULTS The median GDF-15 level was 1,503 ng/L (Q1-Q3: 955-2,332 ng/L) (reference range: $<1,200$ ng/L). Higher GDF-15 levels were associated with more prevalent anorexia and cachexia. Patients with higher GDF-15 had increased circulating free fatty acids and beta-hydroxybutyrate, lower albumin, cholesterol, and insulin/glucagon ratio, consistent with a catabolic state. Patients with higher GDF-15 had worse congestion and more severe right ventricular dysfunction. In multivariable Cox analysis, elevated GDF-15 was independently associated with risk of the combined endpoint of death, urgent transplantation, or left ventricular assist device implantation, even after adjusting for coexisting anorexia and cachexia (T3 vs T1 HR: 2.31 [95% CI: 1.47-3.66]; $P < 0.001$).

CONCLUSIONS In patients with advanced HFrEF, elevated circulating GDF-15 levels are associated with a higher prevalence of anorexia and cachexia, right ventricular dysfunction, and congestion, as well as an independently increased risk of adverse events. Further studies are warranted to determine whether therapies altering GDF-15 signaling pathways can affect metabolic status and clinical outcomes in advanced HFrEF. (JACC Heart Fail. 2025;13:315-329) © 2025 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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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****BMI** = body mass index**BW** = body weight**DEXA** = dual-energy x-ray
absorptiometry**GDF** = growth differentiation
factor**HF** = heart failure**HFREF** = heart failure with
reduced ejection fraction**LVAD** = left ventricular assist
device**MLHFQ** = Minnesota Living
With Heart Failure
Questionnaire

Growth differentiation factor (GDF)-15 is a pleiotropic cytokine from the transforming growth factor beta superfamily. Under normal conditions, GDF-15 is expressed and secreted at low levels by various cell types.¹ However, its expression increases in response to stressors such as inflammation, senescence, or heightened ambient reactive oxygen species, playing a key role in the cellular stress response program.¹ Blood concentrations of GDF-15 are markedly elevated in chronic diseases such as heart failure (HF), kidney failure, pulmonary diseases, and cancer, and they are predictive of poor survival in these conditions.^{2–5} Most of these diseases are also asso-

ciated with body wasting due to enhanced catabolism and diminished caloric intake.^{5–8}

Recent basic science research suggests that GDF-15 could be directly involved in the negative energy balance in chronic diseases due to its appetite-suppressing and catabolic effects. Central anorectic actions of GDF-15 are mediated through interactions with the glial cell line-derived factor-family receptor alpha-like (GFRAL) in the brain.^{9–11} In experimental settings, a reduction of GDF-15 signaling using a monoclonal antibody prevented anorexia and weight loss in cancer or multiorgan failure models.^{12,13} It remains unknown whether GDF-15 could play an active role in development of anorexia and cachexia that accompanies HF.

We hypothesized that GDF-15 is associated with anorexia and history of weight loss in HF. We also wanted to examine how GDF-15 links to previously identified risk factors for HF-related anorexia, such as chronic splanchnic congestion or right heart dysfunction.^{14,15} To test these hypothesis, we investigated the relationships between GDF-15 levels, anorexia, and outcomes in a large cohort of thoroughly phenotyped patients with advanced heart failure with reduced ejection fraction (HFREF) participating in a prospective observational study.

METHODS

STUDY POPULATION. We performed a retrospective analysis of prospectively collected data. The cohort prospectively included consecutive patients with chronic (>6 months) symptomatic HFREF (left ventricular ejection fraction [LVEF] ≤40%) electively hospitalized for consideration of advanced therapies at the IKEM (Institute for Clinical and Experimental Medicine) in Prague (Czech Republic) from January 2008 to June 2011. To avoid the influence of acute

cardiac and noncardiac conditions potentially influencing GDF-15 values, patients with acute ischemia, uncontrolled cardiac arrhythmia, hemodynamic instability needing inotropes or mechanical circulatory support, active malignancy, endocrine disease, and chronic or acute infection were excluded. Patients with reversible cardiac dysfunction were also excluded. Patients with hypervolemia on admission (based on clinical examination) were enrolled into the study after diuresis and reaching normovolemia (central venous pressure <10 mm Hg), if possible. The local ethics committee approved the protocol. All patients signed informed consent with the procedures and with participation in this research study.

STUDY PROTOCOL. After signing informed consent, all patients (N = 344) underwent history review, physical examination (including body weight measurement), echocardiography, electrocardiogram, and blood sampling with circulating metabolite assessment. Most patients (n = 314) completed the Minnesota Living With Heart Failure Questionnaire (MLHFQ). Some patients also underwent right heart catheterization (n = 172) (as part of the diagnostic work-up) and body composition assessment by dual-energy x-ray absorptiometry (DEXA) (n = 150) and the skinfold method (n = 321) as a part of research study examining substrate metabolism in HF. All investigations were performed in a post-absorptive state following an overnight fasting.

The MLHFQ is a self-administered disease-specific questionnaire for patients with HF¹⁶ comprising 21 items rated on 6-point Likert scales representing different degrees of impact of HF on health-related quality of life (HRQoL) from 0 (none) to 5 (very much). It provides a total score that range from 0 to 105 (best to worst HRQoL). Anorexia severity was graded according to the individual answer to the question 11 of the MLHFQ (MLHFQ-Q11: “Did your HF prevent you from living as you wanted during the past month by making you eat less of the foods you like?”). MELD-XI (Model for End-stage Liver Disease excluding INR) score¹⁷ was calculated in the subset of patients with available bilirubin concentration (n = 68).

ECHOCARDIOGRAPHY AND INVASIVE MEASURES. The echocardiographic examination was performed on all patients using standard, commercially available ultrasound equipment (Vivid 7, General Electric Medical Systems) with a 2.5-MHz phased-array transducer. Cardiac chambers’ function and dimensions were measured according to contemporary recommendations.¹⁸ Mitral and tricuspid regurgitation was assessed semiquantitatively and expressed in

4 grades (absent/trivial, mild, moderate, and severe). Right ventricular (RV) function was evaluated in the apical 4-chamber view by using tricuspid annular systolic excursion (TAPSE) or tissue Doppler-derived tricuspid lateral annular systolic velocity (S').¹⁹ Right ventricular dysfunction (RVD) was quantified (0 to 3) with the following cutoffs: RVD0 (normal), TAPSE >20 mm, and S' >12 cm/s; RVD1 (mild impairment), TAPSE 16 to 20 mm, and S' 9 to 12 cm/s; RVD2 (moderate impairment), TAPSE 10 to 15 mm, and S' 6 to 8 cm/s; and RVD3 (severe impairment), TAPSE <10 mm, and S' <6 cm/s. In case of disagreement of criteria, qualitative visual estimation of RV motion in apical 4-chamber was also taken into account.

Right heart catheterization was performed according to the current recommendation using a 7Fr balloon-tipped triple-lumen Swan-Ganz catheter (Braun Melsungen AG) inserted via the right internal jugular vein.²⁰ Pressure waveforms were recorded and annotated by an invasive hemodynamic module (Mac-Lab, GE Healthcare).

BODY WEIGHT AND BODY COMPOSITION ASSESSMENT.

Body weight (BW) was measured on all patients by using an electronic scale (HBF-510W). Antecedent weight 6 months before the evaluation was carefully ascertained by subjects' historic recollection and by review of available medical records, as done previously.^{15,21} Four-site skinfold thickness (triplicate measurement in nondominant subscapular, bicipital, tricipital, and suprailiac areas) was measured by using Best's caliper with controlled grip strength. Skinfold sum was converted into total body fat by using established age- and sex-specific formulas,²² and then reported as percentage of total body composition by using the Siri equation: body fat percentage = $(4.95/\text{body density}) - 4.50 \times 100$.²³ In a subset of subjects enrolled in the study, body composition was also measured by using DEXA (Lunar prodigy, General Electric Healthcare) to externally validate results. Body fat proportion according to DEXA and the skinfold method showed good agreement ($r = 0.748$; $P < 0.001$). Cardiac cachexia was defined by the presence of significant unintentional loss of edema-free body weight (>5% in the past 6 months).²⁴

GDF-15 AND OTHER LABORATORY ASSAYS. Blood samples were consistently collected from a peripheral vein between 6 and 7 AM, immediately chilled on ice, and centrifuged within 20 minutes at a force of $800 \times g$ at a temperature of 4 °C. Plasma was collected, distributed into aliquots, and frozen at -80 °C. Plasma GDF-15 concentrations were

measured in the Biomarker Research and Clinical Trials Laboratory at Brigham and Women's Hospital in Boston. Patient specimens were diluted 4 times and 50- μ L aliquots were tested in duplicates using the Quantikine Human GDF-15 Immunoassay (R and D Systems Inc) (reportable range: 94–6,000 ng/L; reference range <1,200 ng/L; total imprecision: 8.3% at 162 ng/L, 7.6% at 414 ng/L, and 12.0% at 797 ng/L).

The B-type natriuretic peptide (BNP) concentrations were measured using a chemiluminescent immunoassay (Architect BNP, Abbott Laboratories) (long-term analytical coefficient of variation [CV] 4.5%). Beta-hydroxybutyrate (beta-OHB; normal value: 0–74 μ mol/L, CV [intra-assay]: 5%) and free fatty acids (FFAs; reference value men: 0.1–0.6 mmol/L; reference value women: 0.1–0.45 mmol/L; CV [intra-assay]: 1.5%) were measured using a commercially available enzymatic assays (Autokit 3-HB and NEFA-HR, Wako Chemicals). The homeostatic model assessment for insulin resistance was calculated using the formula: $[\text{glucose (nmol/L)} \times \text{insulin } (\mu\text{U/mL}) / 22.5]$.¹⁵ Fasting glucose ≥ 126 mg/dL or use of antidiabetic medication defined diabetes mellitus. Insulin concentration was determined using Insulin IRMA kit (Beckman Coulter; normal value: 2.1–22.0 mIU/L; CV [intra-assay]: 4%) and glucagon using Glucagon RIA kit (Millipore; normal value [fasting]: 50–150 pg/mL; CV [intra-assay]: 4%–7%). Estimated glomerular filtration rate (eGFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

STATISTICAL ANALYSIS. Data are shown as mean $\pm$ SD or median (Q1–Q3) for continuous variables (according to distribution) and total count (n) with proportion (%) for categorical variables. One-way analysis of variance and Kruskal-Wallis tests were used to compare continuous variables between groups depending on the normality of the distribution, and the chi square test was used for categorical variables. Normality was assessed using the Shapiro-Wilks test. Variables not normally distributed were log-transformed before the analysis. To assess the association of independent variables with continuous (ie, GDF-15), ordinal (ie, MLHFQ question 11), or categorical (ie, cachexia) dependent variables, separate multiple linear, multinomial logistic or binomial logistic regression analyses were conducted, respectively. All regression analyses were age- and sex-adjusted. The assumption of log-linearity for independent continuous variables in binomial and multinomial regression analysis was assessed using restricted cubic splines with 3 knots, according to Harrell's rule.²⁵ Wald test was used to evaluate the

linearity assumptions. The continuous variables were then categorized as needed to satisfy the model's assumptions.

Cox proportional hazards regression, adjusted for potential confounders (age, sex, LVEF, body mass index [BMI], systolic blood pressure, eGFR, hemoglobin concentration, and ischemic HF etiology) was used to analyze the association of GDF-15 concentration with the adverse outcome, defined as a combined endpoint of death, urgent transplantation, or left ventricular assist device (LVAD) implantation without heart transplantation. Because time to nonurgent transplantation reflects donor availability rather than recipient's condition, those patients were censored as having no outcome at the day of transplantation, as previously proposed.¹⁵ Kaplan-Meier curves with the log-rank statistics were used to show the outcome. The 2-tailed significance level was set at $P < 0.05$. All analyses were performed using JMP pro 17.0 statistical software (SAS Institute, Inc).

RESULTS

POPULATION CHARACTERISTICS. A total of 344 consecutive HFrEF patients referred to our center for evaluation of advanced HF therapies (cardiac device implantation, heart transplantation, or LVAD implantation) were enrolled in this prospective observational study. Median GDF-15 level was 1,503 ng/L (Q1-Q3: 955-2,332 ng/L). The I-NEED-HELP (inotropes, NYHA functional class, end-organ dysfunction, defibrillator shocks, hospitalizations, low blood pressure, prognostic medication) criteria²⁶ available in our data set, including very low LVEF (mean: 22%; 24% of the population with LVEF $< 20\%$), elevated natriuretic peptides (median BNP: 568 ng/L), presence of end-organ dysfunction (eGFR < 45 mL/min/m²: 15%), low systolic blood pressure (< 100 mm Hg: 17%), advanced NYHA functional class III/IV (73%), and the requirement of high doses of diuretic agents (mean daily oral dose of furosemide: 96 ± 81 mg), were consistent with an advanced HF population (Supplemental Table 1). Based on the distribution in the overall population, GDF-15 tertiles were created with cutoffs of the following: 1st tertile (T1): $< 1,100$ ng/L ($n = 113$); 2nd tertile (T2): 1,100-1,900 ng/L ($n = 114$); and 3rd tertile (T3): $> 1,900$ ng/L ($n = 117$). Baseline population characteristics according to GDF-15 tertiles are presented in Table 1.

GDF-15 AND ITS CORRELATES. Patients in the higher GDF-15 tertile were older, more likely to be men, had more severe impairment of HRQoL as indicated by higher MLHFQ scores, and were more often diagnosed with ischemic HF etiology (all $P < 0.01$).

Additionally, they exhibited a higher prevalence of comorbidities such as diabetes mellitus, hypertension, atrial fibrillation, and chronic kidney disease (all $P < 0.05$) (Table 1), each of which differently impacted circulating GDF-15 levels (stronger association for eGFR) (Supplemental Table 1). Although patients in the highest tertile did not exhibit significant differences in BMI and BW compared to other tertiles, they had higher scores in the anorexia severity score, greater unintentional body weight lost in the preceding 6 months, and higher prevalence of cachexia, along with a lower percentage of body fat (fat/lean ratio), as measured by both DEXA and the skinfold method (all $P < 0.01$) (Figure 1). No significant differences in baseline characteristics were found between patients who did or did not have DEXA (Supplemental Table 2).

By tertiles of GDF-15, patients did not differ in LVEF or resting cardiac output but displayed worse RV function. Additionally, patients with higher GDF-15 concentrations had more systemic congestion (higher BNP and right atrial pressure [RAP], larger inferior vena cava), increased RAP/pulmonary artery wedge pressure ratio, and more impaired kidney (lower eGFR) and liver (higher MELD-XI score) function (all $P < 0.01$) (Figures 2 and 3A), indicating a close association between GDF-15, RV failure, and splanchnic congestion.

From a metabolic perspective, patients with higher GDF-15 levels were more likely to have diabetes; higher glucagon, lower albumin, and cholesterol levels, along with more marked alterations in humoral regulators of liver ketogenesis (insulin/glucagon ratio) and increased markers of lipolysis (FFA, beta-OHB) (all $P < 0.01$) (Figure 3B).

ASSOCIATION BETWEEN CIRCULATING GDF-15 AND MLHFQ. Patients with higher circulating levels of GDF-15 showed strong positive correlations with more marked signs of congestion (question 1), symptoms of anorexia (question 11), and a higher burden of HF-related hospitalizations (question 14) (all $P < 0.001$). In the psychological domain, increased levels of GDF-15 were associated with higher anxiety (question 19; $P < 0.001$) but did not have a significant link to depression (question 21; $P = 0.528$) (Supplemental Table 3).

DETERMINANTS OF ANOREXIA AND ITS ASSOCIATION WITH GDF-15. Anorexia (score ≥ 1 at MLHFQ-Q11) was reported by more than two-thirds ($n = 220$; 70%) of the study population. In the sex- and age-adjusted analysis, higher anorexia severity score was associated with more marked symptoms, increased natriuretic peptides, higher circulating GDF-15, worse

TABLE 1 Baseline Characteristics of the Population According to Circulating GDF-15 Tertiles

	Tertile 1 (n = 113)	Tertile 2 (n = 114)	Tertile 3 (n = 117)	P Value
GDF-15, ng/L	807 (664-953)	1,494 (1,395-1,677)	2,725 (2,295-3,460)	NA
Clinical				
Age, y	53 ± 10	59 ± 10	61 ± 10	<0.001
Male	85 (75)	97 (85)	110 (94)	<0.001
MLHFQ score ^a	43.4 ± 22.7	46.7 ± 21.5	52.6 ± 21.6	0.009
HF ischemic etiology	50 (44)	64 (56)	78 (67)	0.003
HF duration, y	11 (8-15)	11 (7-19)	10 (6-19)	0.831
NYHA functional class				0.016
II	42 (37)	25 (22)	27 (23)	
III-IV	71 (63)	89 (78)	90 (77)	
Diabetes mellitus	14 (12)	38 (33)	59 (50)	<0.001
Hypertension	44 (39)	53 (46)	68 (58)	0.013
Atrial fibrillation	10 (9)	23 (20)	26 (22)	0.015
COPD	11 (10)	13 (11)	20 (17)	0.214
CKD, eGFR <60 mL/min/1.73 m ²	11 (10)	43 (38)	70 (60)	<0.001
Body mass index, kg/m ²	28.2 ± 4.4	27.9 ± 5.2	27.3 ± 4.7	0.302
Body weight, kg	85.7 ± 15.3	85.7 ± 17.9	84.4 ± 16.1	0.788
Body weight change in 6 mo, kg	0 (−2 to 4)	0 (−7 to 2)	−3 (−12 to 2)	<0.001
Cachexia ^b	17 (16)	39 (35)	49 (44)	<0.001
DEXA fat mass, kg ^c	29.8 ± 10.9	25.8 ± 10.8	26.7 ± 11.0	0.178
DEXA lean mass, kg ^c	52.6 ± 11.3	54.6 ± 9.6	55.6 ± 8.6	0.320
DEXA fat/lean mass ratio	0.59 ± 0.24	0.48 ± 0.21	0.47 ± 0.16	0.011
DEXA BMC mass, kg ^c	3.1 ± 0.6	3.1 ± 0.7	3.2 ± 0.6	0.993
Body fat (skinfolts), % ^c	28.2 ± 10.5	24.9 ± 10.2	22.5 ± 8.9	<0.001
Anorexia score ^d	1 (0-3)	2 (0-3)	3 (1-4)	<0.001
MELD-XI score ^e	9.5 ± 3.4	11.0 ± 3.1	16.8 ± 4.2	<0.001
Laboratory values				
Hemoglobin, g/L	142 ± 15	143 ± 16	136 ± 18	0.005
Albumin, g/L	39.5 ± 3.9	38.9 ± 3.8	37.3 ± 4.2	<0.001
eGFR, mL/min/1.73 m ²	84 ± 17	68 ± 18	57 ± 21	<0.001
HbA _{1c} , mmol/mol	42 (39-46)	46 (40-50)	49 (43-58)	<0.001
HOMA-IR index	2.2 (1.3-3.8)	2.4 (1.6-4.3)	2.3 (1.4-3.6)	0.345
Insulin/glucagon ratio	0.13 (0.09-0.19)	0.11 (0.06-0.17)	0.08 (0.05-0.12)	<0.001
Insulin, mIU/L	9.1 (5.6-14.7)	8.9 (6.4-15.0)	8.2 (5.7-13.3)	0.369
Glucagon, pg/mL	76.0 ± 20.7	97.0 ± 43.9	117.3 ± 60.3	<0.001
BNP, ng/L	331 (145-642)	497 (285-990)	1,133 (612-2,060)	<0.001
Triacylglycerols, mmol/L	1.8 ± 0.9	1.7 ± 0.9	1.4 ± 1.2	0.025
Total cholesterol, mmol/L	4.7 ± 0.9	4.4 ± 0.9	3.8 ± 1.1	<0.001
Free fatty acids, mmol/L	0.46 ± 0.22	0.57 ± 0.29	0.62 ± 0.30	<0.001
Beta-hydroxybutyrate, μmol/L	46 (26-98)	65 (36-151)	122 (41-248)	<0.001
Echocardiography^c				
LV end-diastolic diameter, mm	70 ± 9	72 ± 9	70 ± 9	0.198
LV ejection fraction, %	23 ± 5	22 ± 5	22 ± 6	0.234
E/e' ratio	13 ± 5	16 ± 8	17 ± 9	0.012
TAPSE, mm	18 ± 5	16 ± 5	15 ± 4	<0.001
RV dysfunction	74 (76)	79 (88)	88 (91)	0.008
RV dysfunction grade				<0.001
0	38 (34)	22 (19)	12 (10)	
1	33 (29)	30 (27)	23 (20)	
2	33 (29)	42 (36)	56 (48)	
3	9 (8)	20 (18)	26 (22)	
Mitral regurgitation ≥ moderate	38 (35)	47 (42)	34 (29)	0.150
Tricuspid regurgitation ≥ moderate	22 (20)	34 (31)	54 (46)	<0.001
Inferior vena cava, mm	18 ± 5	19 ± 5	22 ± 6	<0.001

Continued on the next page

	Tertile 1 (n = 113)	Tertile 2 (n = 114)	Tertile 3 (n = 117)	P Value
Hemodynamics ^c				
RAP, mm Hg	7.3 ± 5.4	7.4 ± 4.6	13.3 ± 6.2	<0.001
Mean PA pressure, mm Hg	31.9 ± 12.5	32.3 ± 12.2	37.6 ± 9.8	0.014
PAWP, mm Hg	21.8 ± 9.8	21.3 ± 8.9	25.9 ± 6.9	0.006
RAP/PAWP ratio	0.33 ± 0.25	0.34 ± 0.19	0.51 ± 0.25	<0.001
Cardiac output, L/min	4.1 ± 1.0	3.8 ± 0.7	4.1 ± 1.2	0.327
PVR, WU	2.2 (1.6-3.3)	2.4 (1.8-4.1)	2.6 (1.8-4.1)	0.289
Treatment				
Furosemide	100 (89)	110 (96)	110 (94)	0.317
ACEI or ARB	107 (95)	103 (90)	90 (77)	<0.001
MRA	87 (77)	92 (81)	92 (79)	0.791
Beta-blocker	106 (94)	109 (96)	104 (89)	0.125
Triple therapy (ACEI-ARB/MRA/beta-blocker)	79 (70)	79 (69)	62 (53)	0.010
OAD/insulin	22 (19)/4 (4)	43 (38)/14 (12)	58 (50)/25 (21)	<0.001
Devices				
ICD	61 (54)	65 (57)	70 (60)	
CRT	38 (34)	46 (40)	47 (40)	

Values are HR (95% CI), mean ± SD, and n (%), unless otherwise indicated. ^aRange 0-100; higher scores indicate poorer health related quality of life. Performed in 314 patients.

^bCachexia was defined as an unintentional edema-free weight loss of >5% in the past 6 months. ^cDEXA was performed in 150 patients; the skinfold method was performed in 321 patients; MELD-XI was computable in 68 patients; echocardiography was performed in 344 patients; and right heart catheterization was performed in 172 patients.

^dAnorexia score was quantified by MLHFQ question 11 ("Did your heart failure prevent you from living as you wanted during the past month by making you eat less of the foods you like?") on a scale 0-5.

ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; BMC = bone mineral content; BNP = brain natriuretic peptide; CKD = chronic kidney disease; COPD = chronic obstructive pulmonary disease; CRT = cardiac resynchronization therapy; DEXA = dual-energy x-ray absorptiometry; E = early diastolic transmitral flow velocity; e' = mean early diastolic relaxation velocity at mitral annular position; eGFR = estimated glomerular filtration rate; GDF = growth differentiation factor; HbA_{1c} = glycated hemoglobin; HF = heart failure; HOMA-IR = homeostasis model assessment of insulin resistance; ICD = implantable cardioverter-defibrillator; LV = left ventricle; MELD-XI = Model for End-stage Liver Disease excluding INR; MLHFQ = Minnesota Living With Heart Failure Questionnaire; MRA = mineralocorticoid receptor antagonist; NA = not applicable; OAD = oral antidiabetic drugs; PA = pulmonary artery; PAWP = pulmonary artery wedge pressure; PVR = pulmonary vascular resistance; RAP = right atrial pressure; RV = right ventricular; TAPSE = tricuspid annular plane systolic excursion.

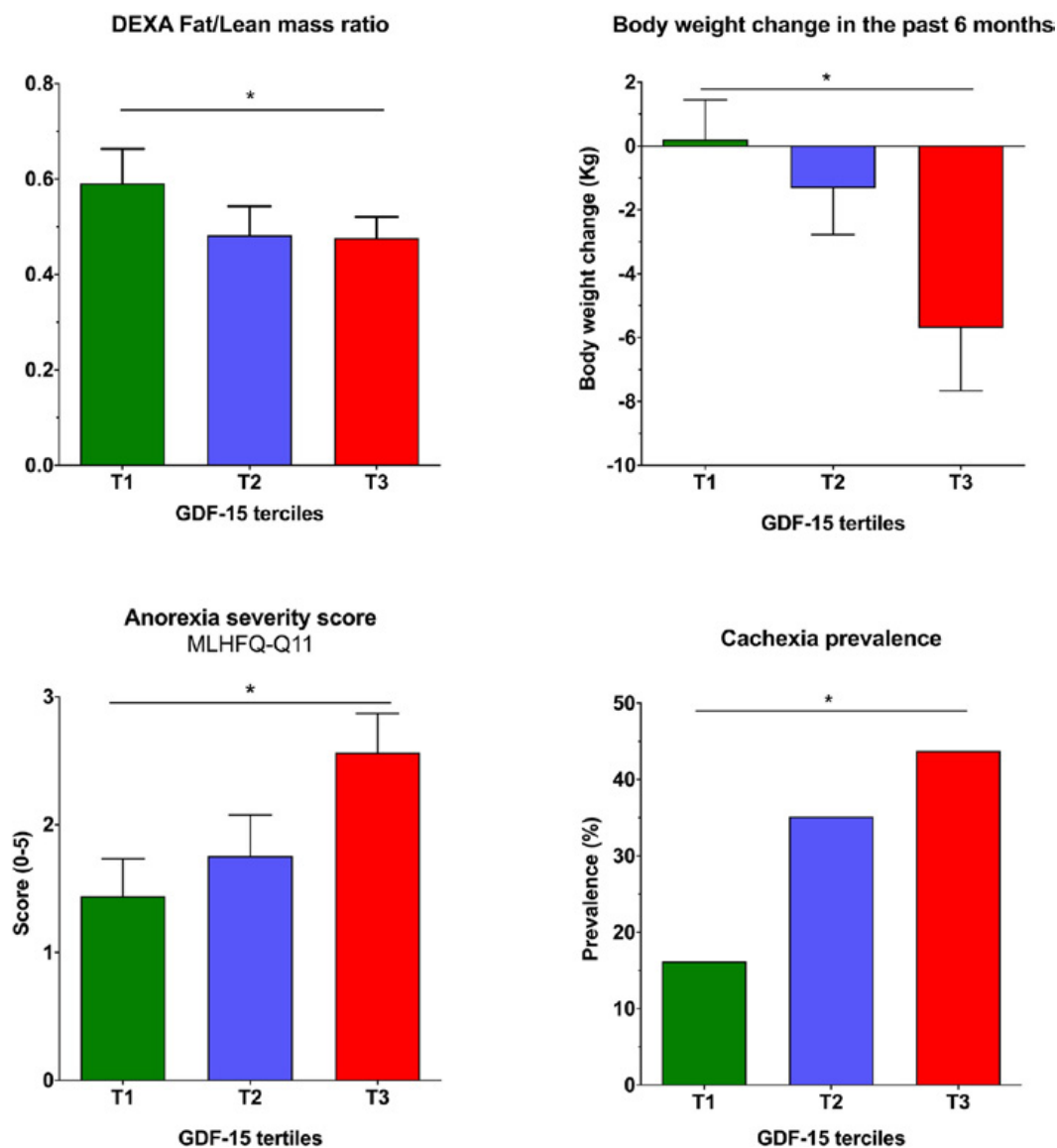
renal function, and higher prevalence and severity of RV dysfunction (Supplemental Table 4). From a metabolic perspective, more marked symptoms of anorexia were associated with diabetic status and impaired glycemic control, along with increased beta-OHB levels. In the multivariable regression analysis, higher GDF-15 concentrations and the presence of RV dysfunction were identified as the only independent predictors of anorexia severity (Supplemental Table 4).

DETERMINANTS OF CACHEXIA AND ITS ASSOCIATION WITH GDF-15. One third of the study population (n = 105; 32%) experienced unintentional, edema-free BW loss >5% in the past 6 months, compatible with a diagnosis of cardiac cachexia. The presence of cachexia was associated with anorexia severity (MLHFQ-Q11 score ≥3; OR: 2.837 [95% CI: 1.717-4.686]; *P* < 0.001), along with several metabolic and structural changes indicative of a catabolic state, including lower albumin levels, along with marked reductions in anthropometric parameters (BMI <25 kg/m², BW, and body fat) (Supplemental Table 5). Furthermore, cachexia was strongly associated with an increase in natriuretic peptides

concentration and circulating GDF-15, along with a more severe RV dysfunction. In the multivariable regression analysis, lower BMI and albumin and higher circulating GDF-15 levels were independently associated to an increased probability of having cachexia (Supplemental Table 5). Excluding anthropometric measures typically associated with an unintentional BW loss (BMI, BW, body fat), high circulating GDF-15, albumin levels, and increased severity of RV dysfunction emerged as independent predictors of cachexia (Supplemental Table 5).

OUTCOMES. During a median follow-up of 1,163 days (Q1-Q3: 384-2,945 days), there were 247 composite events including 165 deaths, 33 LVAD implantations, and 49 urgent cardiac transplantations. Nonurgent cardiac transplantation was not included in the composite and occurred in 20 cases (6%). Patients in the highest tertile of GDF-15 showed a heightened risk of adverse clinical events compared to those in the lower tertile (T3 vs T1: HR: 3.79 [95% CI: 2.71-5.31]; *P* < 0.001) in univariate Cox regression analysis (Figure 4). Increased anorexia score severity and cachexia were also linked to poorer survival outcomes in univariate Cox regression analysis. Patients

FIGURE 1 Fat/Lean Mass Ratio, Body Weight Change, Anorexia Severity, and Cachexia Prevalence by GDF-15 Tertiles

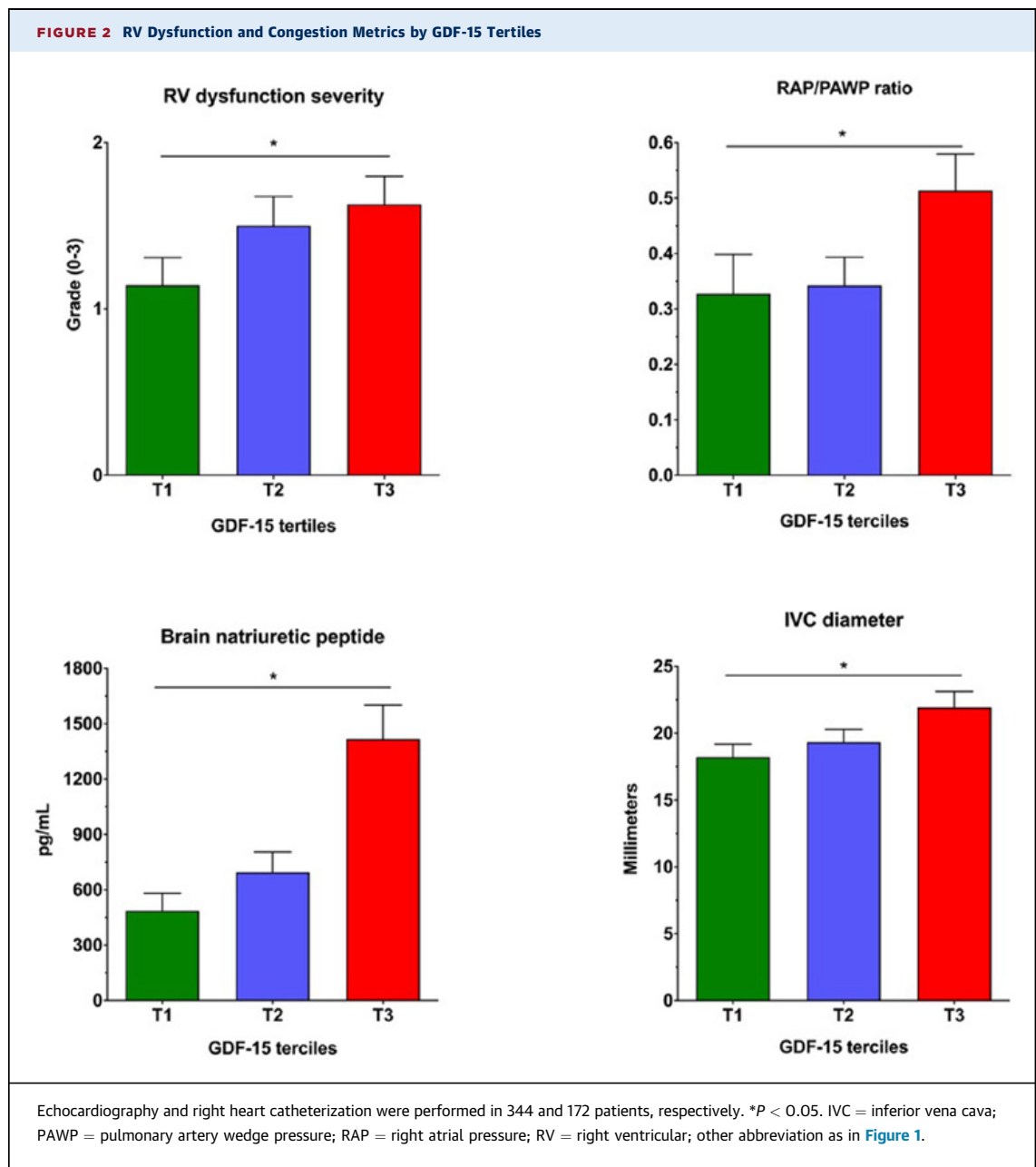


Minnesota Living With Heart Failure Questionnaire (MLHFQ) and dual-energy x-ray absorptiometry (DEXA) scans were performed in 314 and 150 patients, respectively. * $P < 0.05$. GDF = growth differentiation factor; Q11 = question 11.

with more pronounced anorexia symptoms according to MLHFQ question 11 score (none = 0; low = 1-3; high = 4-5) exhibited an increased risk of adverse clinical events compared to those reporting no anorexia symptoms (HR: 1.78 [95% CI: 1.29-2.47]; $P < 0.001$) (Supplemental Figure 1A). Similarly, patients diagnosed with cachexia had a worse prognosis than those without (HR: 1.41 [95% CI: 1.08-1.85]; $P = 0.010$) (Supplemental Figure 1B). Sensitivity

analysis using as endpoint a composite of death or urgent cardiac transplantation (ie, excluding LVAD implantation to avoid potential referral bias to our tertiary center) showed similar results (GDF-15, T3 vs T1 HR: 4.32 [95% CI: 2.98-6.26]; $P < 0.001$; anorexia HR: 1.39 [95% CI: 1.02-1.90]; $P = 0.038$; cachexia HR: 1.43 [95% CI: 1.08-1.91]; $P = 0.013$).

In multivariable Cox analysis, elevated GDF-15 was independently associated with increased risk for

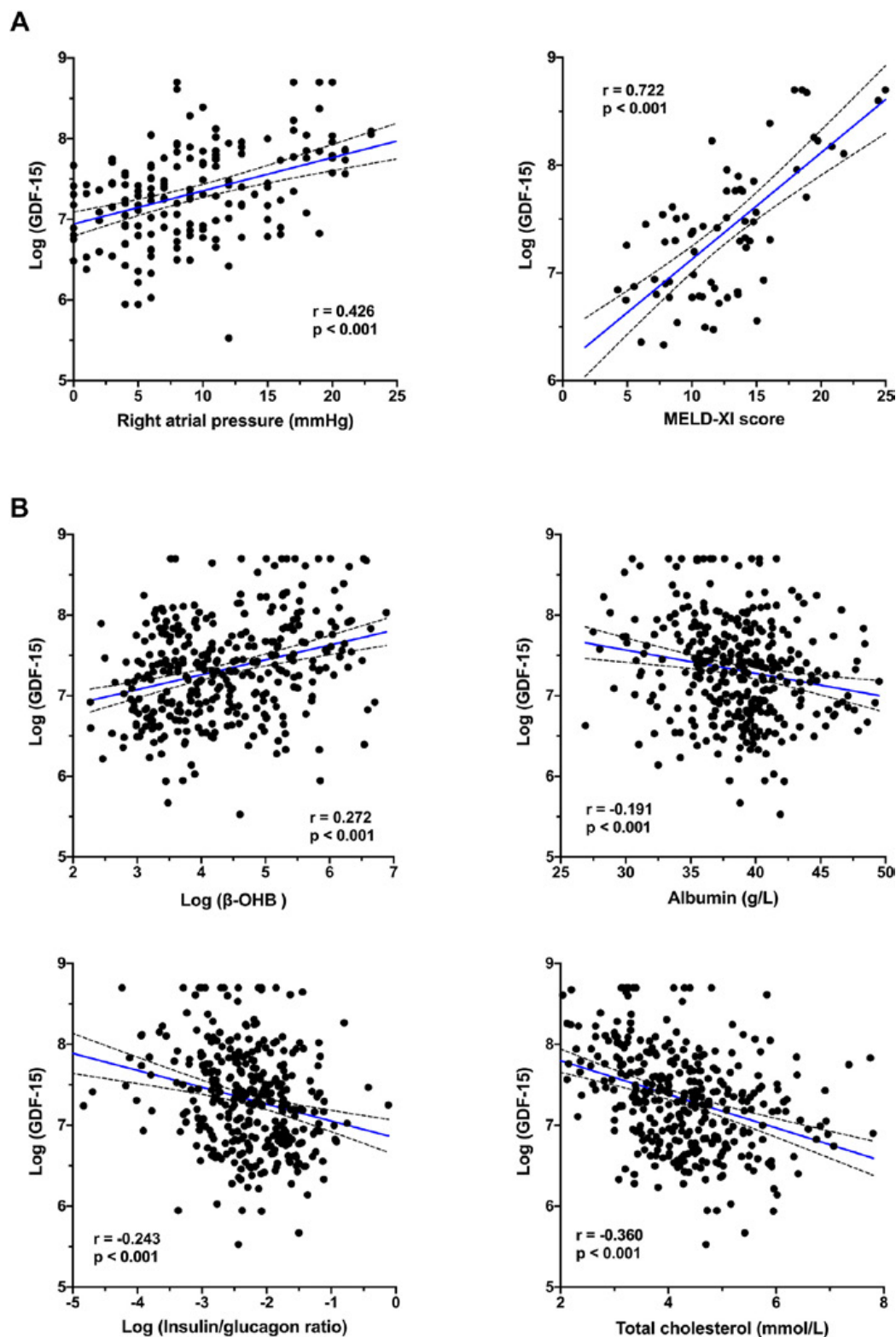


adverse outcomes, even after adjusting for coexisting anorexia and cachexia (T3 vs T1 HR: 2.31 [95% CI: 1.47-3.66]; $P < 0.001$). When RV dysfunction, a well-known determinant of anorexia-cachexia syndrome¹⁴ and a strong driver of adverse outcomes,¹⁵ is included in the model, both the presence of RV dysfunction (HR: 1.86 [95% CI: 1.11-3.13]; $P = 0.019$) and increased circulating GDF-15 (T3 vs T1 HR: 2.20 [95% CI: 1.34-3.62]; $P = 0.002$) were retained as independent predictors of adverse outcome.

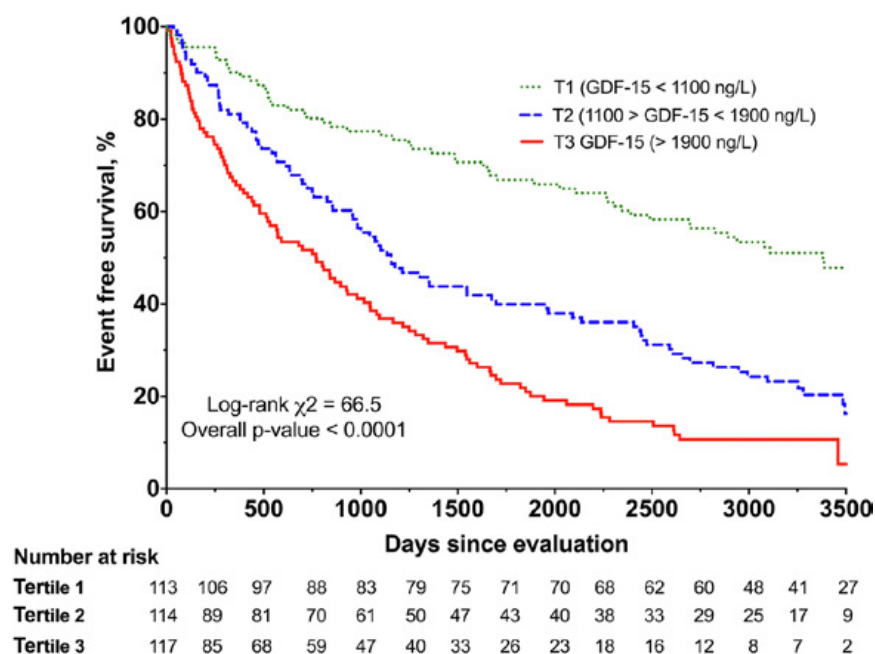
DISCUSSION

In this study, we observed that elevated circulating levels of GDF-15 were associated with increased severity of anorexia and a higher prevalence of cachexia in patients with advanced HF $\ddot{r}$ EF. RV dysfunction and systemic congestion were associated with GDF-15 levels and were significantly related to anorexia and cachexia (Central Illustration), suggesting that there are 2 possible mechanistic

FIGURE 3 Correlation of GDF-15



(A) RAP and liver dysfunction. (B) Metabolic parameters. RAP and MELD-XI were available in 170 and 68 patients, respectively. MELD-XI = Model for End-stage Liver Disease excluding INR; other abbreviations as in [Figures 1 and 2](#).

FIGURE 4 Clinical Outcomes According to GDF-15 Tertiles

Kaplan-Meier curves for the composite outcome of death, urgent transplantation, or left ventricular assist device implantation without heart transplantation stratified by tertiles of circulating GDF-15. T = tertile; other abbreviation as in Figure 1.

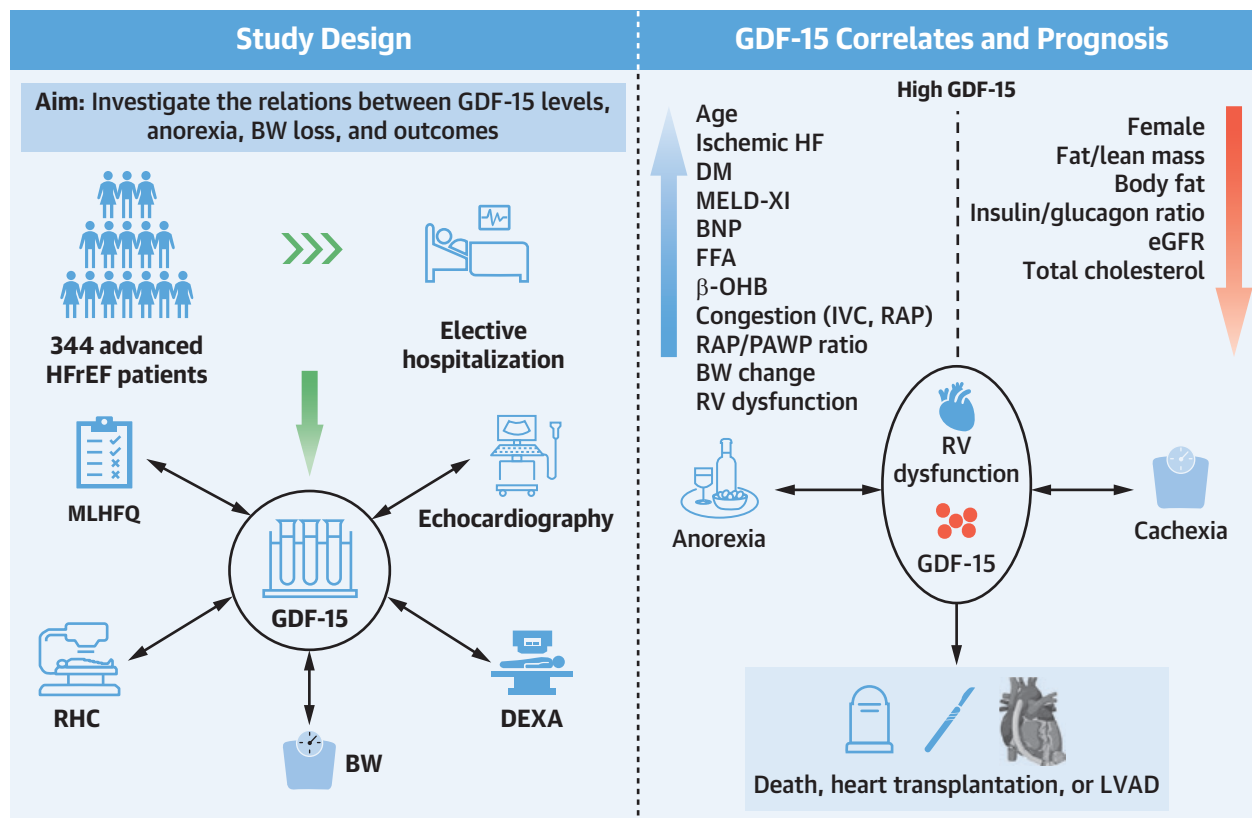
pathways—1 direct hemodynamic and another indirect neurohumoral, mediated by GDF-15—that may link systemic congestion to anorexia in advanced HF (Supplemental Figure 2). Our data support the concept that pharmacologic interference with GDF-15 signaling can improve nutritional status in advanced HF and prevent the progression of cardiac cachexia.

Although anorexia and cachexia are 2 well-recognized findings in cancer,²⁷ they can also complicate other chronic diseases, including HF.²⁸ In patients with advanced HF, the prevalence of anorexia and cachexia is high, with a strong negative impact on the quality of life and survival, as again confirmed in this study.^{29,30} To emphasize the clinical importance of wasting in HF patients, it was recently proposed to modify the acronym summarizing indications for advanced therapies referral (I-NEED-HELP) to include a C for cachexia, resulting in C-I-NEED-HELP.³¹ The mechanisms through which HF may lead to malnutrition are complex, involving a combination of neurohormonal, metabolic, and

immunological factors, all contributing to an imbalance between anabolic and catabolic processes.³²

Experimental and human data suggest that GDF-15 plays a crucial role in the development of cancer-related anorexia and weight loss^{33,34} and is associated with poor survival.⁵ Moreover, GDF-15 has recently been shown to play a pivotal role in the nausea associated with pregnancy.³⁵ GDF-15 impacts energy metabolism by binding to its receptor GFRAL, which is selectively expressed in the brain neurons of the area postrema and the nucleus of the solitary tract, which are important brainstem regions involved in appetite and weight regulation.⁹ Through this interaction, GDF-15 activate neuronal pathways that are perceived as aversive,³⁶ thereby suppressing food intake and mediating the central anorectic effect.³⁷ Experimental data have shown that the administration of recombinant GDF-15 can trigger conditioned taste aversion in mice.³⁸ Additionally, the GDF-15/GFRAL signaling pathway also induces the expression of genes involved in lipid metabolism and activates the peripheral sympathetic axis.¹³

CENTRAL ILLUSTRATION Design and Key Findings



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In the right panel, double-headed arrows show that circulating growth differentiation factor (GDF)-15 and right ventricular (RV) dysfunction are independently associated with anorexia and cachexia. Variables listed next to the upward or downward arrow are positively or negatively associated with higher GDF-15 levels, respectively. Parts of the figure were created using Biorender.com. β-OHB = beta-hydroxybutyrate; BNP = brain natriuretic peptide; BW = body weight; DEXA = dual-energy x-ray absorptiometry; DM = diabetes mellitus; eGFR = estimated glomerular filtration rate; FFA = free fatty acids; HF = heart failure; HFrEF = heart failure with reduced ejection fraction; IVC = inferior vena cava; LVAD = left ventricular assist device; MELD-XI = Model for End-stage Liver Disease excluding INR; PAWP = pulmonary artery wedge pressure; RAP = right atrial pressure; RHC = right heart catheterization.

These mechanisms elicit a lipolytic overdrive in adipose tissue, resulting in BW reduction independent of anorexia.^{9,13}

In our study, GDF-15 had a stronger association with fat mass than with lean mass. This finding may reflect recent cachexia, where fat tissue catabolism, likely accelerated by lipolytic activity of highly increased natriuretic peptides,³⁹ precedes the breakdown of lean tissue. This hypothesis is supported by previous evidence indicating that the loss of fat mass, along with appendicular muscle mass, is closely associated with adverse outcomes and may serve as an early indicator for detecting cardiac cachexia.^{15,40,41}

Our data suggest that the effects of GDF-15 in HF may be similar to those observed in patients with malignancies.²⁷ In particular, we propose that the combination of a strong central anorectic effects of GDF-15, along with nutrient malabsorption due to RV failure and consequent bowel congestion, could lead together to malnutrition and wasting syndrome in patients with advanced HF. Circulating GDF-15 is elevated in patients with HF⁴² and correlates with disease severity and prognosis.⁴³ We found that elevated circulating levels of GDF-15 were associated with more severe RVD and worse congestion as evidenced by elevated BNP levels, more pronounced edema severity (MLHFQ question 1), enlarged inferior

vena cava dimension, raised central venous pressure, and more impaired function of liver and kidneys. These findings suggest that RV failure and the ensuing congestion could be key factors in driving the development of HF-related anorexia/cachexia, potentially triggering an increase in circulating GDF-15 levels, which then acts centrally to reduce appetite. In other studies in patients with chronic HF, GDF-15 was also associated with increased severity of congestion.⁴⁴ Similarly, GDF-15 levels were markedly increased in acute HF patients, and rapidly declined with decongestion, supporting the enhancing effect of hypervolemia on GDF-15 levels.⁴⁵ Likewise, mechanical left ventricular (LV) unloading and improved LV end-organ perfusion from LVAD implantation in end-stage HF also significantly rapidly reduced plasma GDF-15 levels, along with improvements in kidney and liver function. This study also showed that peripheral tissues, rather than the heart itself, are the primary source of GDF-15 in HF patients.⁴⁶

Venous congestion could also directly impact on the development of cardiac cachexia in HF.^{15,47} RV failure results in splanchnic congestion, visceral organs edema with increased bowel wall thickness, and, ultimately, malabsorption.¹⁴ Intestinal congestion can also induce appetite loss and postprandial satiety,¹⁴ further worsening the reduction in food intake. Mesenteric venous congestion can facilitate the development of cardiac cachexia by promoting increased bowel permeability, leading to bacterial translocation and subsequent release of endotoxins into the bloodstream,⁴⁸ stimulating immune activation and the production of proinflammatory cytokines.⁴⁹ Although the precise mechanisms linking hemodynamic congestion to elevated GDF-15 remain unclear, GDF-15 production in this context may serve as a protective mechanism by increasing intestinal mucin secretion, thereby counteracting alterations in the bowel barrier and preventing bacterial translocation.⁵⁰ Liver distress due to splanchnic congestion may also be as a primary source of GDF-15 in HF patients,⁵¹ as supported by the strong positive correlation between GDF-15 levels and MELD-XI scores (Supplemental Figure 2).

Inhibition of the GDF-15/GFRAL signaling in brainstem neurons holds promising therapeutic potential to mitigate the occurrence of appetite and weight loss in patients with HF. Building on previous cancer research where preclinical studies showed that antibody-mediated inhibition of GDF-15/GFRAL pathway effectively mitigated tumor-induced weight loss,^{13,52,53} similar experimental studies were conducted in HF models. In a rat model of monocrotaline-induced RV

dysfunction, neutralization of GDF-15 with a selective monoclonal antibody prevented experimentally induced anorexia and weight loss, and preserved lean and fat mass.¹²

This study contributes human data to the existing experimental evidence, providing biological plausibility for testing novel therapies aimed at antagonizing GDF-15 pathways in chronic HFrEF. Our findings indicate that GDF-15 levels were not significantly associated with static anthropometric measures such as BMI but were instead correlated with changes in BW over time. Consequently, the potential selection of patients for GDF-15-lowering therapies should focus on the history of weight loss, rather than on BMI or absolute weight. Future studies in advanced HF might include GDF-15 as a core variable. This approach could enhance our understanding of GDF-15 role in HF, especially when combined with data on downstream effects such as anorexia, appetite, and energetic balance, and possibly guide the development of more effective treatments.

Some treatments that improve outcomes in chronic HF, such as valsartan and sacubitril-valsartan,^{54,55} do not significantly alter circulating levels of GDF-15, even though they enhance cardiovascular outcomes. In contrast, ivabradine has been shown to reduce GDF-15 concentrations,⁵⁶ whereas metabolic drugs such as empagliflozin have been found to increase these levels in ambulatory HF patients.⁵⁷ This may suggest that the GDF-15 response to guideline-directed medical therapy is not uniform and may reflect diverse mechanisms how these drugs work. Future studies on GDF-15 modulation might benefit from focusing on patients with advanced HF for whom the association between GDF-15 levels and clinical events is likely to be most pronounced.⁵⁸

Recently, a novel monoclonal antibody therapy (ponsegromab), designed to selectively bind and inactivate GDF-15, was shown to increase weight gain, enhance physical activity, and reduce anorexia, cachexia symptoms, and nausea in a dose-dependent manner among cancer patients with elevated GDF-15 levels,⁵⁹ confirming its key role in the development of anorexia and cachexia. Ponsegromab is currently being evaluated in a clinical trial (GARDEN TIMI 74 [A Study of Ponsegromab in People With Heart Failure]; [NCT05492500](https://clinicaltrials.gov/ct2/show/study/NCT05492500)) to assess its potential to improve HrQOL and 6-minute walk distance in patients with advanced HF. Our findings suggest that antianorexic and anticatabolic effects of GDF-15 antagonism may be beneficial beyond traditional neurohormonal and cardiorenal mechanisms in advanced HFrEF with wasting.

STUDY LIMITATIONS. The observational design of this study limits its ability to make inferences toward causality. We did not collect information on dietary intake, leaving us unable to determine whether patients with anorexia/cachexia experienced deficiencies in energy or protein intake. Potential unmeasured confounders, such as dietary habits,⁶⁰ physical activity levels,⁶¹ proinflammatory states,⁶² and genetic predispositions,⁶³ may also have influenced the observed relationship between GDF-15 levels and anorexia. There are more specific instruments for the evaluation of anorexia than those we used,^{64,65} and more detailed nutritional surveys in advanced HF patients are warranted in the future. Our data set lacked the granularity to precisely assess all the I-NEED-HELP markers of advanced HF.²⁶ Nevertheless, the available I-NEED-HELP criteria and other common parameters used to estimate HF severity (eg, high events rate, severe LV remodeling) were consistent with the advanced stage of the HF patients studied. Our population mainly comprises middle-age, White males with advanced HFrEF. This imbalance was likely caused by both HF demographics in the explored age strata (ie, <65 years of age) and selection bias (patients referred or enrolled in a single tertiary center). We did not identify significant interaction effects between sex and the association of GDF-15 with anorexia, cachexia, and clinical outcomes (all $P > 0.10$); however, additional data are needed to confirm these findings. Additionally, caution is advised when extending these results to patients with different types of HF, including heart failure with preserved ejection fraction (HFpEF). HFpEF is more commonly associated with an obesity phenotype, which contrasts sharply with the cachexia often observed in advanced HFrEF. BW data were not consistently collected throughout the follow-up period of the study, limiting our ability to analyze the impact of GDF-15 levels on prospective weight changes. Finally, we do not have specific DEXA data on the regional distribution of body composition, preventing us from analyzing changes in appendicular muscle mass in the limbs, which has been associated with adverse outcomes in patients with HF.^{15,40,41}

CONCLUSIONS

In patients with advanced HFrEF, elevated circulating GDF-15 levels were associated with a higher prevalence of anorexia and cachexia along with increased risk for adverse events. The central and peripheral effects of GDF-15 on energy metabolism,

potentially mediated by RV failure and congestion, could play a crucial role in the development of HF-related anorexia and cachexia. These findings support future study of novel interventions targeting of GDF-15 as a strategy to prevent anorexia and weight loss in patients with chronic advanced HFrEF.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Increased circulating levels of GDF-15 were associated with more severe anorexia and a higher incidence of cachexia in patients with advanced HFrEF, as well as an increased risk of adverse events. The impact of GDF-15 levels on energy metabolism, possibly influenced by RV failure and fluid overload, suggests a connection between systemic congestion and the onset of cardiac anorexia and cachexia in advanced stages of HF.

TRANSLATIONAL OUTLOOK: Our findings provide biological plausibility for testing novel therapies that target the inhibition of GDF-15/GFRAL signaling in brainstem neurons, aiming to mitigate appetite loss and weight reduction in advanced HFrEF.

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KEY WORDS anorexia, cachexia, growth differentiation factor-15, heart failure, right ventricular dysfunction

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

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Biomarkers of RV Dysfunction in HFrEF Identified by Direct Tissue Proteomics: Extracellular Proteins Fibromodulin and Fibulin-5

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Biomarkers of RV Dysfunction in HFrEF Identified by Direct Tissue Proteomics: Extracellular Proteins Fibromodulin and Fibulin-5

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BACKGROUND: Right ventricular dysfunction (RVD) is common in patients with heart failure with reduced ejection fraction, and it is associated with poor prognosis. However, no biomarker reflecting RVD is available for routine clinical use.

METHODS: Proteomic analysis of myocardium from the left ventricle and right ventricle (RV) of patients with heart failure with reduced ejection fraction with (n=10) and without RVD (n=10) who underwent heart transplantation was performed. Concentrations of 2 ECM (extracellular matrix) proteins with the highest myocardial upregulation in RVD, FMOD (fibromodulin) and FBLN5 (fibulin-5), were assayed in the blood and tested in a separate cohort of patients with heart failure with reduced ejection fraction (n=232) to test for the association of the 2 proteins with RV function and long-term outcomes.

RESULTS: Multivariable linear regression revealed that plasma concentrations of both FMOD and FBLN5 were significantly associated with RV function regardless of the RV function assessment method. No association of FMOD or FBLN5 with left ventricular dysfunction, cardiac index, body mass index, diabetes status, or kidney function was found. Plasma levels of FMOD and FBLN5 were significantly associated with patient outcomes ($P=0.005$; $P=0.004$). Area under the curve analysis showed that the addition of FBLN5 or FMOD to RV function assessment had a significantly higher area under the curve after 4 years of follow-up (0.653 and 0.631, respectively) compared with RV function alone (0.570; $P<0.05$ for both). Similarly, the combination of MAGGIC (Meta-Analysis Global Group in Chronic Heart Failure) score, FBLN5, and FMOD had a significantly larger area under the curve (0.669) than the combination of MAGGIC score+RVD grade (0.572; $P=0.02$). The Kaplan-Meier analysis demonstrated that patients with the elevation of both FMOD and FBLN5 (ie, FMOD >64 ng/mL and FMOD >27 ng/mL) had a worse prognosis than those with the elevation of either FBLN5 or FMOD ($P=0.03$) demonstrating the additive prognostic value of both proteins.

CONCLUSIONS: Our study proposes that circulating levels of FMOD and FBLN5 may serve as new biomarkers of RVD in patients with heart failure with reduced ejection fraction.

Key Words: biomarkers ■ extracellular matrix ■ fibromodulin ■ fibrosis ■ heart failure

Right ventricular dysfunction (RVD) is a major complicating condition of heart failure (HF) with both preserved and reduced ejection fractions and is associated with poor prognosis.^{1–3} Although no specific RVD-targeted therapy is currently available, RVD is commonly present in advanced HF and may herald clinical worsening

with the need for therapy intensification or timely indication of heart transplantation. Development of severe RVD may preclude the use of long-term left ventricular (LV)-assist devices and, thus, considerably impacts long-term survival.⁴

The right ventricle (RV) function is currently assessed mainly by imaging, and echocardiography

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WHAT IS NEW?

- Heart failure–associated changes in myocardial protein expression can be identified by proteomics.
- Differentially expressed myocardial proteins can be used as disease markers, pending their altered expression in myocardium translates into their changed concentration in blood.
- ECM (extracellular matrix) proteins FMOD (fibromodulin) and FBLN5 (fibulin-5) are significantly upregulated in the myocardium and in plasma in patients with heart failure with reduced ejection fraction with right ventricular dysfunction.

WHAT ARE THE CLINICAL IMPLICATIONS?

- Currently, there is no specific circulating biomarker available for routine clinical use to recognize specifically right ventricular dysfunction in the context of heart failure with reduced ejection fraction.
- FMOD and FBLN5 plasma levels are strongly associated with right ventricular dysfunction and bring improved information about prognosis, which makes them promising biomarkers of right ventricular dysfunction.

Nonstandard Abbreviations and Acronyms

AUC	area under the curve
BGLN	biglycan
ECM	extracellular matrix
FBLN5	fibulin-5
FMOD	fibromodulin
HF	heart failure
HFrEF	heart failure with reduced ejection fraction
LV	left ventricle
LVEF	left ventricular ejection fraction
MFAP4	microfibril-associated glycoprotein 4
MRC2	mannose receptor C-type 2
MYH10	myosin 10
NRI	net reclassification improvement
RV	right ventricle
RVD	right ventricular dysfunction
THBS4	thrombospondin 4
UCHL1	ubiquitin carboxyl-terminal hydrolase isoenzyme L1
VCAN	versican

is the most commonly used method. There is no specific circulating biomarker available in routine clinical use to recognize specifically RVD in the context of HF with reduced ejection fraction (HFrEF). Identification of such a biomarker could add to echocardiographic RV evaluation, better risk-stratify patients, and

help with further decision-making in advanced HFrEF management.

RVD may result from or translate into specific proteome changes in both ventricles. The proteins with altered expression can be used as disease markers, pending their altered expression in myocardium translates into altered concentration in blood. Such proteins can be either passively released from the myocardium as a result of tissue leakage caused by cell death or injury or actively secreted from the cells to the interstitial fluid. To look for such potential markers of RVD in patients with advanced HF, we have used proteomic analysis of myocardium from both ventricles of patients with HFrEF with and without RVD. Two proteins of ECM (extracellular matrix) showing the most marked upregulation in myocardial tissue were further assessed in plasma; their plasmatic levels were tested for the relationship with RV function and outcome.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request. The mass spectrometry data have been made publicly available by deposition to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD043768.

All methods are described in detail in the [Supplemental Material](#).

Patients

Subjects with symptomatic but hemodynamically stable HFrEF (LV ejection fraction [LVEF] <40%) that had a duration of at least 6 months were enrolled in the study.^{1,5} Those with potentially reversible LV dysfunction (planned valve surgery, revascularization, or tachycardia-induced cardiomyopathy) were excluded. 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 (IKEM), and all subjects signed an informed consent.

Patients Cohort 1 (Proteomics Cohort)

A cohort of 122 consecutive patients who underwent heart transplantation without preceding use of an LV-assist device was divided according to the RV function measured by fractional area change determined by a single experienced echocardiographer before heart transplantation. A subgroup of 10 HFrEF male patients with severe RVD (first quartile of fractional area change), and 10 body size, cause, age, and comorbidity-matched HFrEF male patients with preserved RV function (fourth quartile of fractional area change) were used for proteomic analysis. In addition, 10 age and body size-matched subjects without a clinical history of HF and with normal LV and RV functions that were refused as heart donors due to hemodynamic instability or moderate coronary lesions were added to the analysis as a proxy for nonfailing controls. Patient data for the proteomics cohort are provided in [Table S1](#).

Patients Cohort 2 (Clinical Cohort)

A group of 232 patients with HFrEF that were referred to IKEM for evaluation of advanced therapies, and 65 non-HF controls were used for this analysis. Patients were prospectively enrolled between 2017 and 2020 and underwent complex in-hospital evaluation, including echocardiography, right heart catheterization, and blood sampling. Patients were prospectively followed for the occurrence of an adverse outcome that was defined as the combined end point of death, urgent heart transplantation, or ventricular assist device implantation. Because the time to nonurgent transplantation reflects donor availability rather than the recipient's condition, patients who received a nonurgent heart transplant were censored as having no outcome event on the day of transplantation. The control group consisted of 65 subjects (36 subjects without evidence of coronary artery disease or HF evaluated for the presence of patent foramen ovale; 29 subjects were healthy hospital employees).

RESULTS

Proteomic Analysis

Myocardial samples of LV and RV from 10 patients with HFrEF and severe RVD, 10 patients with HFrEF and preserved RV function (noRVD), and 10 control individuals were subjected to quantitative proteomic analysis. Because RVD may be also associated with molecular changes in the LV, and with respect to their different ontology, wall thickness, working pressures, physiology, and pathology, we analyzed separately both RVs and

LVs. Despite the matching for age, body, size, HF cause, and comorbidities, patients with severe RVD had shorter duration of HF, lower LVEF, and higher NTproBNP (all suggesting more rapid HF progression) than those with preserved RV function. The description of patients and controls is given in [Table S1](#).

The label-free quantification proteomic analyses of RV and LV resulted in the identification of 3477 and 3633 protein groups, respectively. Supervised discrimination analysis (sparse partial least squares discrimination analysis), of the proteomic data from RV and LV clearly separated nonfailing (control) hearts from the failing hearts and, albeit less completely, also samples from patients with RVD (red) and patients without RVD (green) in both ventricles (Figure 1). This incomplete separation of RVD and noRVD proteomic profiles reflects rather mild changes in the myocardial proteome of patients with HFrEF with RVD and without RVD. In fact, we identified only 12 and 6 differentially expressed proteins (>2 -fold; $P<0.05$) in the RV and LV of patients with RVD, respectively, compared with patients without RVD (Figure 2). None of the 18 RVD-associated changes in protein expression was identified simultaneously in both ventricles. Table 1 lists the identified differentially expressed proteins. A list of all proteins identified in the proteomic analysis is available as a [Supplemental Dataset](#). The mass spectrometry data have been made publicly available by deposition to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD043768.

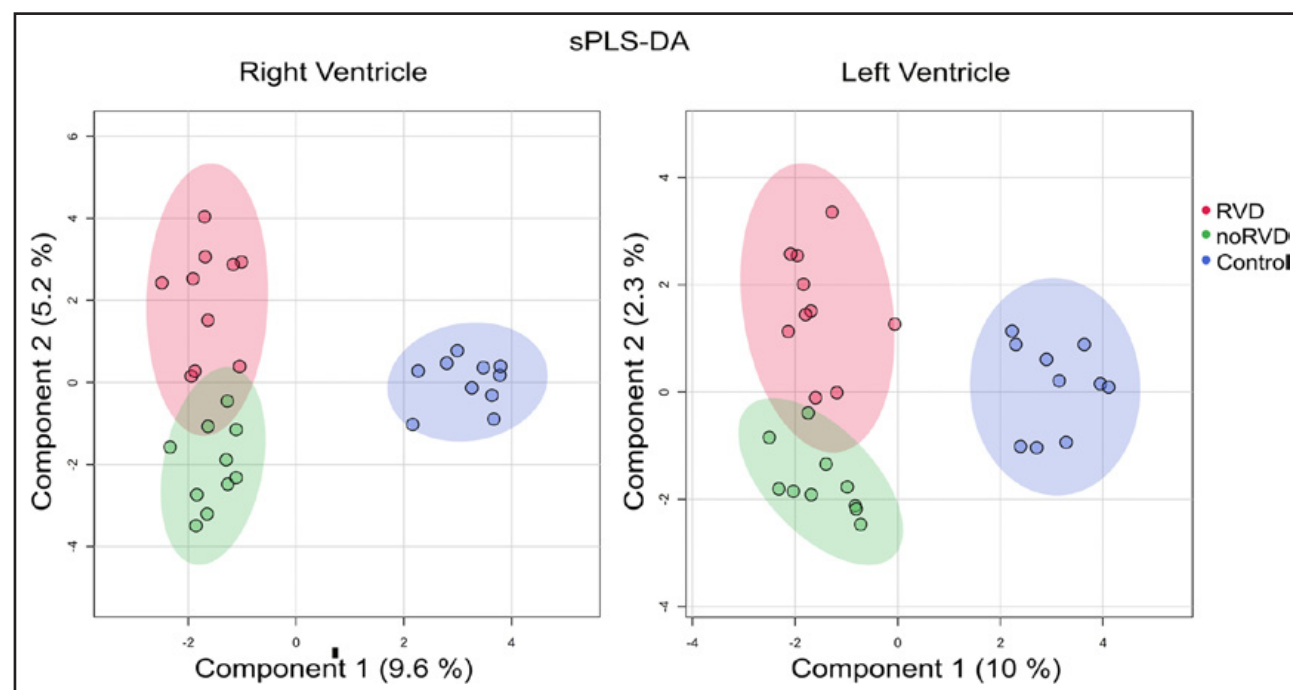


Figure 1. Sparse least squares discrimination analysis (sPLS-DA) of the quantitative proteomic data.

Results of the separate analyses of the right and left ventricles are shown. RVD indicates right ventricular dysfunction.

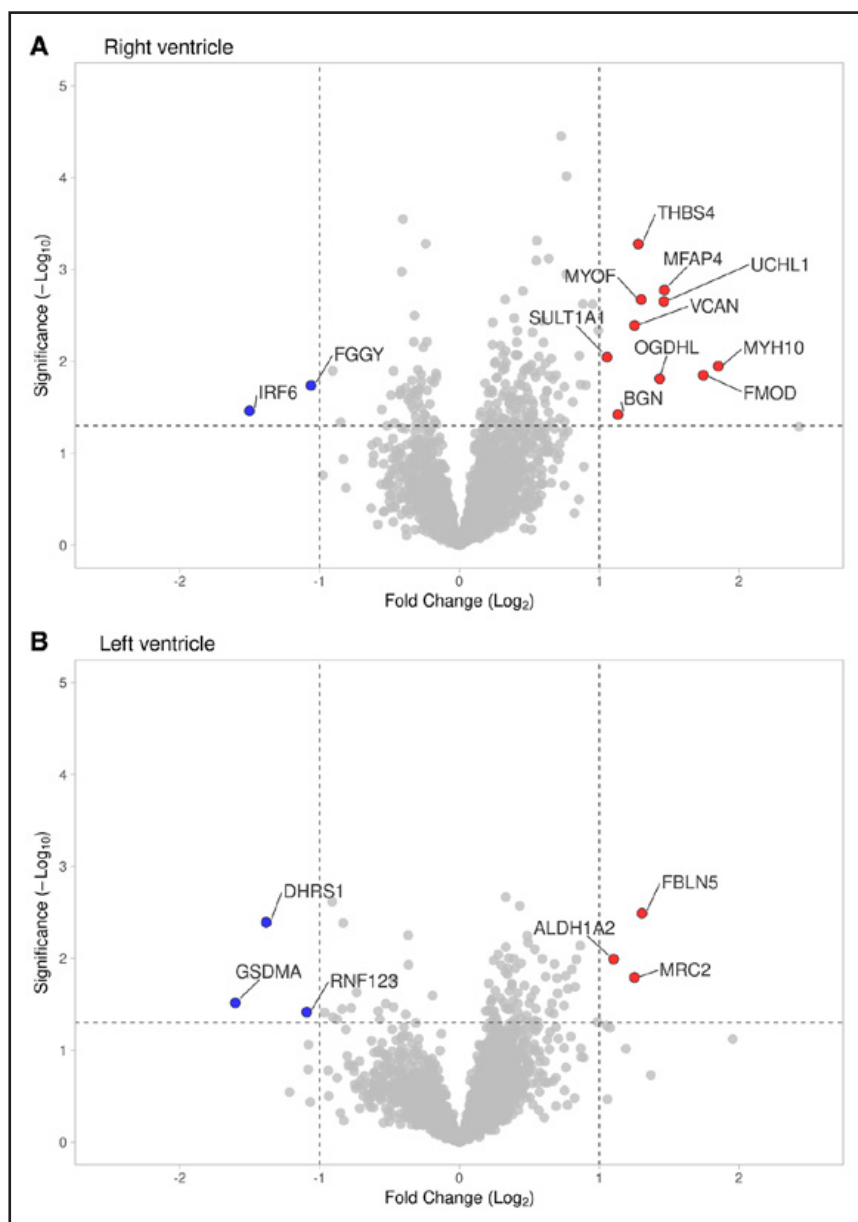


Figure 2. Label-free quantification proteomic analyses of ventricular myocardium of patients with heart failure with reduced ejection fraction with right ventricular dysfunction (RVD) and without RVD (noRVD). Proteins upregulated in RVD vs noRVD are shown in red and downregulated in blue. Dotted lines indicate the thresholds for a 2-fold change in expression (vertical) and statistical significance ($P_{\text{adjusted}}=0.05$; horizontal). Gene names are shown instead of the full-length protein names. **A**, Protein expression changes in the right ventricle. **B**, Protein expression changes in the left ventricle.

Bioinformatic Analysis-Pathway Enrichment

Because the proteome changes were limited and the numbers of the differentially expressed (>2 -fold) proteins were low, gene ontology pathway analysis revealed significant enrichment only among the proteins upregulated in RV (Figure S1). “ECM organization” ($P_{\text{adjusted}} < 0.001$) was the process with the highest number of matched proteins.

In agreement with the gene ontology enrichment analysis, 5 of the 10 proteins that were markedly upregulated in the RV in patients with HFrEF with RVD (FMOD [fibromodulin], MFAP4 [microfibril-associated glycoprotein 4], VCAN [versican], BGLN [biglycan], and THBS4 [thrombospondin 4]) are ECM proteins annotated as “secreted,” “extracellular space,” and “ECM” according to the UniProt database. Similarly, the protein most markedly upregulated in the LV of patients with HFrEF with RVD, FBLN5 (fibulin-5), is also

annotated as secreted/ECM protein. In addition, 3 other proteins upregulated in the hearts of patients with RVD, namely, MYH10 (myosin-10), UCHL1 (ubiquitin carboxyl-terminal hydrolase isoenzyme L1), and MRC2 (C-type mannose receptor 2), are known to participate in the process of ECM remodeling as well, despite their intracellular localization.^{6–8} The results of our proteomic analysis, thus, clearly pointed toward pronounced ECM remodeling as a hallmark of RVD in patients with HFrEF.

Confirmation of the Proteomic Data in Myocardial Tissue

The differential myocardial expression of 6 proteins discovered using our proteomic approach was subsequently verified using specific antibodies. The same

Table 1. Proteins Differentially Expressed in Both Ventricles of Patients With HFrEF With RVD Compared With Patients With HFrEF Without RVD

Accession	Gene name	Protein name	Unique peptides	RVD/noRVD fold change	P value
Differential expression in the right ventricle of patients with RVD					
Upregulated proteins					
P35580	MYH10	Myosin-10	38	3.61	0.0113
Q06828	FMOD	Fibromodulin	6	3.35	0.0141
P55083	MFAP4	Microfibril-associated glycoprotein 4	4	2.76	0.0017
P09936	UCHL1	Ubiquitin carboxyl-terminal hydrolase isozyme L1	15	2.76	0.0022
Q9ULD0	OGDHL	2-oxoglutarate dehydrogenase-like, mitochondrial	13	2.70	0.0154
Q9NZM1	MYOF	Myoferlin	36	2.46	0.0021
P35443	THBS4	Thrombospondin 4	15	2.43	0.0005
P13611	VCAN	Versican core protein	8	2.38	0.0041
P21810	BGLN	Biglycan	8	2.19	0.0380
P50225	SULT1A1	Sulfotransferase 1A1	6	2.08	0.0090
Downregulated proteins					
O14896	IRF6	Interferon regulatory factor 6	6	0.35	0.0346
Q96C11	FGGY	FGGY carbohydrate kinase domain-containing protein	3	0.48	0.0183
Differential expression in the left ventricle of patients with RVD					
Upregulated proteins					
Q9UBX5	FBLN5	Fibulin-5	6	2.47	0.0032
Q9UBG0	MRC2	Mannose receptor C-type 2	11	2.38	0.0162
O94788	ALDH1A2	Retinal dehydrogenase 2	13	2.15	0.0102
Downregulated proteins					
Q96QA5	GSDMA	Gasdermin-A	9	0.33	0.0305
Q96LJ7	DHRS1	Dehydrogenase/reductase SDR family member 1	4	0.38	0.0041
Q5XPI4	RN123	E3 ubiquitin-protein ligase RNF123	12	0.47	0.0385

HFrEF indicates heart failure with reduced ejection fraction; and RVD, right ventricular dysfunction.

samples were used for both the proteomic and confirmatory analyses. The results of the confirmatory analysis were fully in agreement with the proteomic analysis, and the expression of FMOD, MFAP4, UCHL1, MYOF, and VCAN was confirmed to be significantly higher in the RV of HFrEF patients with RVD, while the expression of FBLN5 was significantly increased in the LV (in HFrEF patients with RVD). All the proteins were also significantly upregulated relative to the respective ventricles of nonfailing controls (Figure 3).

Secreted/ECM Myocardial Proteins in Blood Plasma

Five of 10 proteins upregulated in the RV of patients with RVD are secreted proteins, components of ECM, and we reasoned that secreted proteins could serve as circulating biomarkers. FMOD was the most markedly (3.3-fold according to the proteomic analysis) upregulated ECM protein in the RV of patients with HFrEF with RVD. FBLN5 was the most strongly upregulated protein (2.5-fold) in the LV of patients with HFrEF with RVD. Looking for RVD markers, we, therefore, asked whether the marked myocardial upregulation of these secreted/ECM proteins would be mirrored in the blood plasma of

patients with HFrEF. Proteins from both ventricles were considered because both ventricles may be the source of (potentially distinct) information on RVD.

FMOD and FBLN5 in the Blood Plasma

Using specific sandwich ELISA, we first determined plasma concentration of FMOD and FBLN5 in plasma samples of the 20 HFrEF individuals (collected at the time of the transplantation) represented in the proteomic analysis (the cohort 1) and healthy male volunteers of comparable age ($n=10$). Both FMOD and FBLN5 were detectable in the plasma of all analyzed groups (ie, controls, patients with HFrEF with preserved RV function, and patients with HFrEF with severe RVD).

Controls showed the lowest level of both FMOD (17.58 ± 3.84 ng/mL) and FBLN5 (54.32 ± 15.78 ng/mL), followed by patients with HFrEF with preserved RV function (22.19 ± 8.18 ng/mL for FMOD and 71.63 ± 23.61 ng/mL for FBLN5). Patients with HFrEF with RVD showed the highest concentrations for both proteins (27.14 ± 8.86 from FMOD and 96.32 ± 48.08 ng/mL for FBLN5; Figure S2). In agreement with the increased myocardial expression, the plasma concentration of both proteins in the RVD group was higher compared

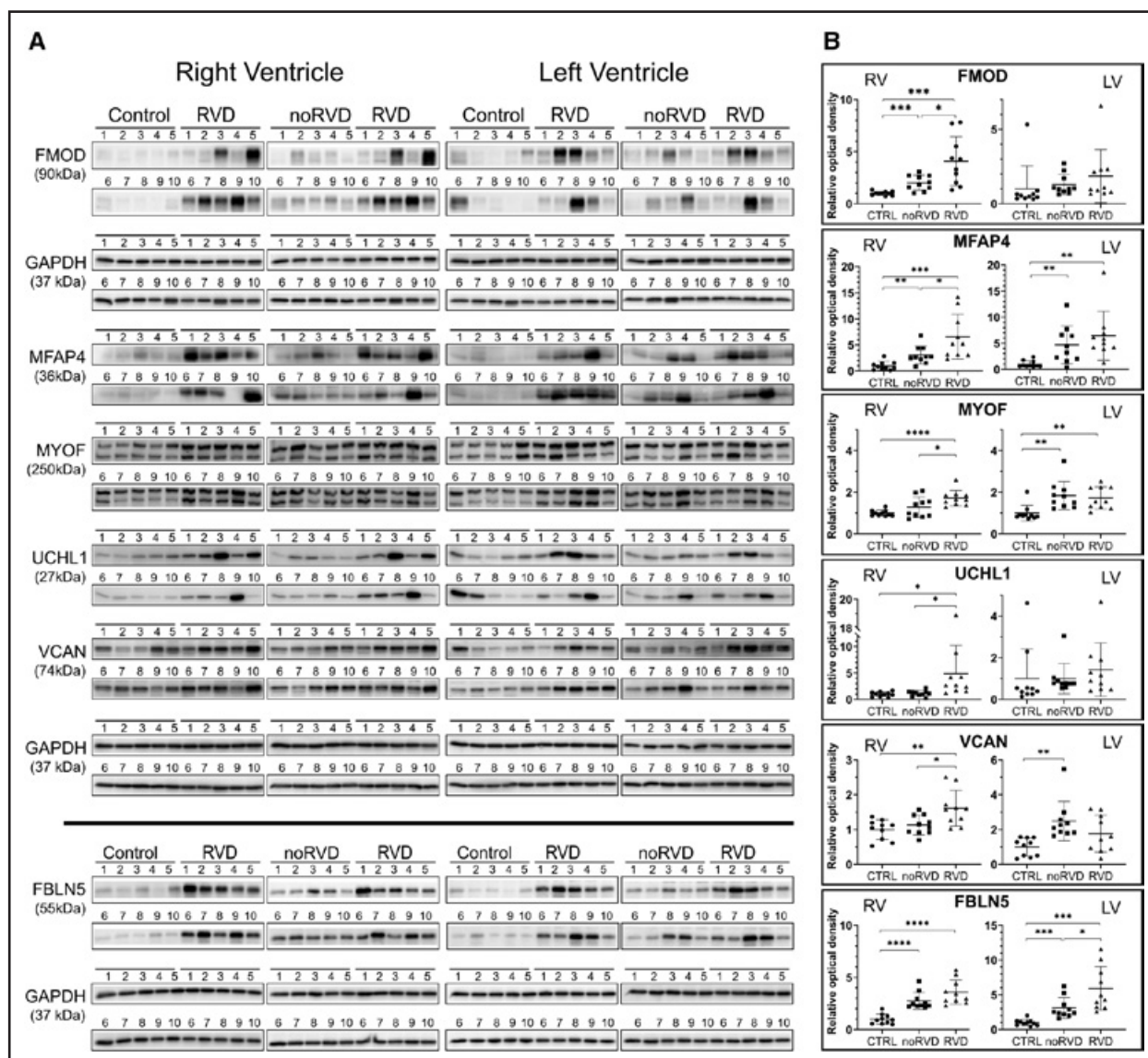


Figure 3. Confirmation of differential expression by Western blotting.

A, Relative protein expression was determined in both ventricles of all the patients included in the proteomic analysis (right ventricular dysfunction [RVD] and noRVD). To obtain also information on the background expression, nonfailing donor heart samples were included (control [CTRL]). Right ventricular (RV) and left ventricle (LV) samples were analyzed separately; GAPDH was used as a loading control. **B**, Densitometry of the Western blots. The relative optical density of the bands normalized to the CTRLs is plotted. Statistical significance is indicated where reached. Gene names are shown instead of full protein names FMOD (fibromodulin), MFAP4 (microfibril-associated glycoprotein 4), VCAN (versican), MYOF (myoferlin), UCHL1 (ubiquitin carboxyl-terminal hydrolase isoenzyme L1), and FBLN5 (fibulin-5).

with controls ($P=0.005$ for FMOD; $P=0.017$ for FBLN5) and tended to be numerically higher compared with the noRVD group as well. Considering the limited size of the original cohort 1, we evaluated the plasma concentration of FMOD and FBLN5 in a large cohort of well-characterized patients with advanced HF (cohort 2).

The Utility of FBLN5 and FMOD in Clinical Scenarios

We tested the utility of FMOD and FBLN5 plasma concentrations in a cohort of 232 patients with HFrEF

and 65 controls. Patients (aged 56.75 ± 10.32 years; 89.2% men) presented with advanced HF (86.2% New York Heart Association III/IV and mean LVEF 23.3%). Significant number of patients ($n=142$; 61.7%) had moderate or severe RVD (details in Table 2). FMOD and FBLN5 plasma levels progressively and significantly increased with worsening RV function ($P_{\text{for trend}} < 0.0001$ for both proteins). When we analyzed only patients with HFrEF, both proteins still showed a significant association with RVD although it was less significant for FBLN5 ($P_{\text{for trend}} < 0.0001$ for FMOD; $P_{\text{for trend}} = 0.037$ for FBLN5).

Table 2. Clinical Cohort Patient Characteristics

	Controls (n=65)	Patients with HFrEF (n=232)	P value (controls vs HFrEF)	HFrEF, preserved RV function (n=47)	HFrEF, mild RVD (n=43)	HFrEF, moderate RVD (n=96)	HFrEF, severe RVD (n=46)	P value (trend in HFrEF)
Age, y	52.88±13.09	56.75±10.32	0.01	59.05±8.54	57.08±9.84	56.22±10.34	55.32±12.24	0.07
Males, %	75.4	89.2	0.007	84.4	93.0	88.5	93.5	0.30
HF cause (% CAD)	n/a	39.7	n/a	57.8	34.9	37.7	30.4	0.01
NYHA (1–4, %)	65/0/0/0 (100/0/0/0)	0/32/136/64 (0/13.8/58.6/27.6)	<0.0001	0/12/30/5 (0/25.5/63.8/10.6)	0/8/25/10 (0/18.6/58.1/23.3)	0/10/55/31 (0/10.4/57.3/32.3)	0/2/26/18 (0/4.3/56.5/39.1)	<0.0001
BMI	27.86±5.13	27.69±4.53	0.84	28.25±5.70	27.33±4.35	28.11±4.02	26.34±4.08	0.14
BNP, ng/L	21.9 (8–39.6)	1016 (495–1879)	<0.0001	653.3 (262.2–1298)	961 (490.5–1944.3)	995.2 (536.1–1778.0)	1823.7 (867.9–2817.4)	<0.0001
SBP, mm Hg	124.9±19.1	110.2±16.5	<0.0001	112.2±16.4	113.1±18.2	108.9±16.0	106.9±14.2	0.06
Hemoglobin, g/L	139.5±18.4	138.2±19.2	0.71	134.2±20.4	139.0±19.5	140.2±19.3	136.8±17.1	0.38
eGFR, mL/min per 1.73 m ²	90.6±12.6	64.8±23.4	<0.0001	59.7±22.1	66.1±22.6	66.2±24.9	65.0±22.7	0.26
Echocardiography								
LVEDD, mm	49.47±4.39	71.85±9.52	<0.0001	71.07±11.29	74.09±9.80	71.01±8.85	72.57±8.72	0.93
LVEF, %	60.49±4.58	21.39±5.39	<0.0001	23.88±7.04	22.15±3.99	21.54±4.73	17.77±4.19	<0.0001
TAPSE, mm	24.12±4.98	15.11±4.07	<0.0001	19.20±3.01	16.63±3.50	14.11±3.24	11.81±3.40	<0.0001
Sm-TDI, mm	12.85±1.91	8.20±2.33	<0.0001	10.20±1.68	8.78±1.91	7.83±2.10	6.67±2.32	<0.0001
RVD grade (0–3)	36/0/0/0 (100/0/0/0)	47/43/96/46 (20.3/18.7/41.2/20)	<0.0001	47/0/0/0 (100/0/0/0)	0/43/0/0 (0/100/0/0)	0/0/96/0 (0/0/100/0)	0/0/0/46 (0/0/0/100)	<0.0001
MiR (0–3), %	3/30/3/0 (8.3/83.3/8.3/0)	43/71/72/46 (18.5/30.6/31.0/19.8)	<0.0001	14/12/12/9 (29.8/25.5/25.5/19.2)	5/17/17/4 (11.6/39.5/39.5/9.4)	17/26/32/21 (17.7/27.1/33.2/22.0)	7/16/11/12 (15.2/34.8/23.9/26.1)	0.33
TriR (0–3), %	33/11/1/0 (92.0/3.0/3.0/0)	72/78/51/31 (31.0/33.6/22.0/13.4)	<0.0001	22/14/6/5 (46.8/29.8/12.8/10.6)	17/16/8/2 (39.5/37.2/18.6/4.7)	27/31/24/14 (28.1/32.3/25.0/14.6)	6/16/14/10 (13.0/34.9/30.4/21.7)	0.007
IVC, mm	15.94±4.26	22.63±6.11	<0.0001	19.80±5.79	20.36±7.32	23.32±5.14	26.13±5.10	<0.0001
Hemodynamics								
RAP, mm Hg	n/a	9.5±5.6	n/a	6.76±4.44	9.05±5.18	10.53±5.74	10.89±5.75	<0.0001
mPAP, mm Hg	n/a	34.3±10.2	n/a	30.11±11.41	33.67±11.53	36.16±9.12	35.70±8.61	0.002
PCWP, mm Hg	n/a	22.9±8.1	n/a	19.18±9.21	21.93±8.85	24.12±6.69	25.41±7.68	<0.0001
CI, L/min per m ²	n/a	1.8±0.4	n/a	2.07±0.54	1.91±0.47	1.74±0.34	1.79±0.51	0.0003
Therapy								
ACEi/ARB/ ARNi, %	28.1	68.1	<0.0001	71.1	76.7	67.7	56.5	0.10
BB, %	12.5	83.6	<0.0001	88.9	86.1	80.2	82.6	0.27
MRA, %	6.25	87.1	<0.0001	82.2	88.4	85.4	93.5	0.20
Furosemide daily dose, mg	0 (0–0)	125 (66.9–250)	<0.0001	80 (60–193.8)	125 (77.5–250)	90 (60–155)	80 (40–208.8)	0.47
ICD any, %	0	87.5	<0.0001	88.9	95.4	87.5	80.4	0.12
CRT any, %	0	45.3	<0.0001	42.2	51.2	45.8	43.5	0.97
Outcome								
Death, %	0 (0%)	51 (22.0)	<0.0001	12 (25.5)	13 (30.2)	20 (20.8)	6 (13.0)	n/a
Urg. HTx, %	0 (0%)	61 (26.3)	<0.0001	8 (17.0)	10 (23.3)	25 (26.1)	18 (39.1)	n/a
Norm. HTx, %	0 (0%)	8 (3.4)	<0.0001	2 (4.3)	1 (2.3)	27 (28.1)	1 (2.2)	n/a
MCSi, %	0 (0%)	67 (28.9)	<0.0001	10 (21.3)	12 (27.9)	4 (4.2)	18 (39.1)	n/a
Alive with no event, %	65 (100%)	45 (19.4)	<0.0001	15 (31.9)	7 (16.3)	20 (20.8)	3 (6.6)	n/a

Data are presented as means±SD or as median (with interquartile ranges) as appropriate. Laboratory parameters and echocardiography were available for only a sub-group of 36 control subjects. Hemodynamic data were not available for any control subjects. ACEi indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARNi, angiotensin receptor/neprilysin inhibitor; BB, β -blocker; BMI, body mass index; BNP, B-type natriuretic peptide; CAD, coronary artery disease; CI, cardiac index; CRT, cardiac resynchronization therapy; eGFR, estimated glomerular filtration rate; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; HTx, heart transplantation; ICD, implantable cardioverter defibrillator; IVC, inferior vena cava; LVEDD, left ventricular diameter in end-diastole; LVEF, left ventricular ejection fraction; MCSi, mechanical circulatory support implantation; MiR, mitral regurgitation; mPAP, mean pulmonary artery pressure; MRA, mineralocorticoid receptor antagonist; n/a, not available; NYHA, New York Heart Association; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; RV, right ventricular; RVD, right ventricular dysfunction; SBP, systemic blood pressure; Sm-TDI, systolic motion of RV basal segment assessed by tissue Doppler imaging; TAPSE, tricuspid annular plane systolic excursion; and TriR, tricuspid regurgitation.

Regression Analysis

Despite the positive association of both FBLN5 and FMOD with RVD grade, we strived for a more robust analysis to answer the question of whether FBLN5 and FMOD are specifically associated with RVD or whether the observed association is due to possible confounders. We have performed multivariable regression analysis including most relevant confounders (LVEF, age, sex, body mass index, estimated glomerular filtration rate, the presence of diabetes, cardiac index, and pulmonary vascular resistance). RV function was assessed in 5 different ways (RVD grade, tricuspid annular plane systolic excursion, systolic motion of RV basal segment assessed by tissue Doppler imaging, pulmonary artery pulsatility index, and central venous pressure/pulmonary capillary wedge pressure ratio). Both FBLN5 and FMOD consistently showed significant association with RV function regardless of how RV function was assessed (Table 3). Besides the RV function, FBLN5 showed a consistent association with pulmonary vascular resistance and age. FMOD was strongly and significantly associated with RV function only.

FMOD and FBLN5 as Predictors of Adverse Outcome in HFrEF

During a follow-up of a median period of 4.4 (interquartile range 3.6–5.0) years, 179 patients (77.2%) in our cohort experienced an adverse outcome, defined as death/urgent heart transplantation/implantation of

mechanical circulatory support. The Cox proportion hazard model revealed that both FMOD and FBLN5 were significantly associated with patient outcome, with a hazard ratio of 1.20 (95% CI, 1.05–1.37) per 1 SD of FMOD plasma concentration ($P=0.005$) and a hazard ratio of 1.30 (95% CI, 1.12–1.51) per 1 SD of FBLN5 plasma concentration ($P=0.0004$).

Clinical Value of FMOD and FBLN5 Plasma Levels in Addition to RV Function Assessment

As both candidate markers FMOD and FBLN5 were associated with patient outcome and showed a significant association with RV function, we asked whether any of these proteins could improve the prognostic stratification of patients beyond RV function assessment by echocardiography. When adjusted for RVD grade, FMOD was no longer a significant predictor of outcome in Cox regression analysis (hazard ratio, 1.29 [95% CI, 0.98–1.30] for each SD of FMOD concentration; $P=0.08$). In contrast, FBLN5 remained significantly associated with outcome even when adjusted for RVD grade (hazard ratio, 1.30 [95% CI, 1.12–1.51] for each SD of FBLN5 concentration; $P=0.0006$).

Both proteins, however, showed a significantly higher area under the curve (AUC) when combined with RVD grade at a single time point of 4 years (176 of a total of 179 outcome events were reached at this time point; Table 4).

We further used the net reclassification improvement (NRI) index that attempts to quantify how well a

Table 3. Regression Analysis

	FBLN5	FMOD	FBLN5	FMOD	FBLN5	FMOD	FBLN5	FMOD	FBLN5	FMOD
RV dysf. grade	0.171*	0.363†	NI	NI	NI	NI	NI	NI	NI	NI
TAPSE	NI	NI	1.915‡	0.272†	NI	NI	NI	NI	NI	NI
Sm-TDI	NI	NI	NI	NI	0.144*	0.272†	NI	NI	NI	NI
PAPi	NI	NI	NI	NI	NI	NI	0.249†	0.352†	NI	NI
CVP/PCWP ratio	NI	NI	NI	NI	NI	NI	NI	NI	0.185§	0.299†
LVEF	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
eGFR	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
DM	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
BMI	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Age, y	0.239§	NS	0.209§	NS	0.178*	NS	0.194§	NS	0.197§	NS
Sex	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PVR	0.232§	NS	0.239§	NS	0.234§	NS	0.229§	NS	0.216§	NS
CI	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Multivariable regression was used to determine factors associated with the FMOD and FBLN5 levels. Due to the nonnormal distribution, ln-transformed FMOD and FBLN5 levels were used. Standardized β is reported. BMI indicates body mass index; CI, cardiac index; CVP, central venous pressure; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FBLN5, fibulin-5; FMOD, fibromodulin; LVEF, left ventricular ejection fraction; NI, the variable was not included in the model; NS, not significant; PAPi, pulmonary artery pulsatility index; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RV, right ventricular; Sm-TDI, systolic motion of RV basal segment assessed by tissue Doppler imaging; and TAPSE, tricuspid annular plane systolic excursion.

* $P<0.05$.

† $P<0.001$.

‡ $P=0.057$.

§ $P<0.01$.

Table 4. The Comparison of RV Function Assessment/MAGGIC Score With Addition of FMOD and FBLN5

AUC				NRI		
RV dysf. grade (95% CI)	RV dysf. grade+FMOD (95% CI)	Delta AUC (95% CI)	P value	NRI (95% CI)	NRI+ (95% CI)	NRI− (95% CI)
0.570 (0.476 to 0.664)	0.631 (0.532 to 0.729)	0.06 (0.06 to 0.115)	0.028	0.20 (0.10 to 0.42)	0.02 (−0.06 to 0.05)	0.18 (0.09 to 0.36)
	RV dysf. grade+FBLN5					
	0.653 (0.548 to 0.759)	0.083 (0.01 to 0.166)	0.049	0.41 (0.13 to 0.63)	0.17 (0.08 to 0.31)	0.24 (−0.02 to 0.35)
	RV dysf. grade+FMOD+FBLN5					
	0.662 (0.538 to 0.718)	0.092 (0.06 to 0.172)	0.020	0.22 (0.15 to 0.42)	0.03 (0.02 to 0.05)	0.20 (0.11 to 0.32)
MAGGIC	MAGGIC+FMOD					
0.579 (0.465 to 0.692)	0.658 (0.546 to 0.769)	0.079 (0.024 to 0.134)	0.005	0.37 (0.06 to 0.80)	0.12 (0.01 to 0.21)	0.25 (0.02 to 0.61)
	MAGGIC+FBLN5					
	0.650 (0.541 to 0.759)	0.071 (−0.005 to 0.147)	0.072	0.33 (0.12 to 0.65)	0.15 (0.08 to 0.26)	0.18 (−0.04 to 0.45)
	MAGGIC+FMOD+FBLN5					
	0.669 (0.558 to 0.781)	0.090 (0.032 to 0.144)	0.002	0.39 (0.08 to 0.84)	0.15 (0.03 to 0.19)	0.28 (0.09 to 0.58)
MAGGIC+RV dysf. grade	MAGGIC+FMOD					
0.572 (0.461 to 0.683)	0.658 (0.546 to 0.769)	0.086 (0.004 to 0.14)	0.040	0.02 (−0.27 to 0.19)	−0.03 (−0.26 to 0.08)	0.06 (−0.20 to 0.19)
	MAGGIC+FBLN5					
	0.650 (0.541 to 0.759)	0.078 (−0.012 to 0.162)	0.091	0.02 (−0.27 to 0.28)	0.0001 (−0.14 to 0.15)	0.02 (−0.23 to 0.14)
	MAGGIC+FMOD+FBLN5					
	0.669 (0.558 to 0.781)	0.097 (0.014 to 0.181)	0.020	0.11 (−0.36 to 0.54)	−0.007 (−0.22 to 0.09)	0.11 (−0.137 to 0.452)

The addition of both FBLN5 and FMOD to semiquantitative RV function assessment by echocardiography (normal RV function, mild, moderate, and severe RV dysfunction) leads to an improvement in AUC compared with RV function assessment only. NRI was significant for the overall score (NRI) but the reclassification of patients with events into the high-risk group (NRI+), and the low-risk group (NRI−) was different for FBLN5 and FMOD, respectively. Adding FMOD to the MAGGIC score showed a significantly higher AUC; the addition of FBLN5 to MAGGIC was not statistically significant. However, both FMOD and FBLN5 significantly improved the overall NRI score and the reclassification of patients with events into the high-risk group (NRI+). The reclassification of patients without an event into the low-risk group (NRI−) was significant for FMOD only. The addition of FMOD or FMOD+FBLN5 (but not FBLN5 alone) to the MAGGIC score showed a significantly larger AUC than the combination of MAGGIC+RV dysfunction grade. However, no significant improvement in NRI was observed. AUC indicates area under the curve; FBLN5, fibulin-5; FMOD, fibromodulin; MAGGIC, Meta-Analysis Global Group in Chronic Heart Failure; NRI, net reclassification improvement; and RV, right ventricular.

new model reclassifies subjects, either appropriately or inappropriately, compared with an old model.⁹ FBLN5 markedly improved the continuous NRI, with a significant improvement in event NRI (NRI+), but without a change in nonevent NRI (NRI−). FMOD markedly improved continuous NRI and nonevent NRI (NRI−) without a significant improvement of event NRI (NRI+; Table 4).

Adding FMOD and FBLN5 to MAGGIC Score Improves Long-Term Prediction

In clinical practice, the prognosis of patients with HF is often evaluated by composite scoring systems that help to guide therapeutic decisions. MAGGIC score is one of those and encompasses various patient characteristics, degree of LV dysfunction (LVEF), HF pharmacotherapy, and comorbidities.¹⁰ We evaluated whether plasma concentrations of FMOD or FBLN5 add any additional prognostic information beyond the MAGGIC score (Table 4). Adding plasma FMOD to the MAGGIC score showed a significantly higher AUC after 4 years ($P=0.005$). In addition to that, FMOD correctly reclassified both

patients with events into a more high-risk group (NRI+) and those without events into the low-risk group (NRI−). Adding plasma FBLN5 to the MAGGIC score numerically increased AUC; however, the change was not statistically significant ($P=0.07$).

Finally, we analyzed whether FBLN5 and FMOD added to the MAGGIC score can outperform MAGGIC together with RV function assessment. The addition of FMOD or FMOD+FBLN5 (but not FBLN5 alone) to the MAGGIC score resulted in a significantly larger AUC after 4 years than the combination of MAGGIC+RV grade. Nevertheless, no significant improvement in NRI was observed (Table 4).

A Combined Prognostic Role of FMOD and FBLN5

As both FMOD and FBLN5 plasma levels were found to be significantly associated with outcome, we analyzed the prognostic role of both proteins individually and when combined together.

Patients were divided according to the mean plasma concentration of both proteins (ie, >27 ng/mL for FMOD

and >64 ng/mL for FBLN5). Those with elevated concentrations of either FMOD or FBLN5 had worse event-free survival than the rest of the cohort (Figure 4A and 4B). When combined together, patients with an elevated concentration of either biomarker had significantly worse outcomes than those with low levels of both biomarkers. Similarly, patients with an elevation of both biomarkers had significantly worse outcomes than those with only 1 elevated biomarker (Figure 4C).

DISCUSSION

RVD is a commonly accompanying condition in patients with HFrEF and is associated with poor prognosis.^{3,11} Unfortunately, no specific biomarker of RVD in the HF context is available for routine clinical use. In our study, we have shown that highly sensitive and unbiased proteomic methods can assist with the identification of candidate marker proteins and that circulating concentrations of ECM proteins FMOD and FBLN5 may serve as indicators of RVD and predict the outcome of patients with HFrEF.

RVD is associated with progressive changes in the tissue composition of RV with cardiomyocyte hypertrophy, stress-induced changes in sarcomeric proteins, inflammatory cell infiltration, and changes in ECM.³

However, RVD may also specifically affect the composition of the LV. Our proteomic approach has identified distinct sets of 12 proteins differentially expressed in the RV and 6 proteins differentially expressed in the LV of patients with HFrEF with severe RVD. Among 13 upregulated proteins, 6 are components of ECM (FMOD, FBLN5, VCAN, BGLN, MFAP4, and THBS4). Three additional upregulated proteins, namely, UCHL1, MYH10, and MRC2 are intracellular but contribute to ECM remodeling.^{6–8}

Our results, thus, suggest that RVD in patients with HFrEF is associated with significant alterations in ECM composition in both ventricles. Propagation of ECM and the presence of regenerative, interstitial, and perivascular cardiac fibrosis are key pathological features of HF contributing to systolic and diastolic impairment,^{12,13} including RVD,^{14,15} and have been proposed as a potential therapeutic target.^{16,17}

In patients with HFrEF with RVD, myocardial FMOD and FBLN5 were among the most markedly upregulated proteins, but their upregulation was ventricle-specific (FMOD being significantly upregulated in RV whereas FBLN5 in LV).

FMOD is a leucine-rich proteoglycan that binds collagen and regulates its cross-linking and collagen fibril

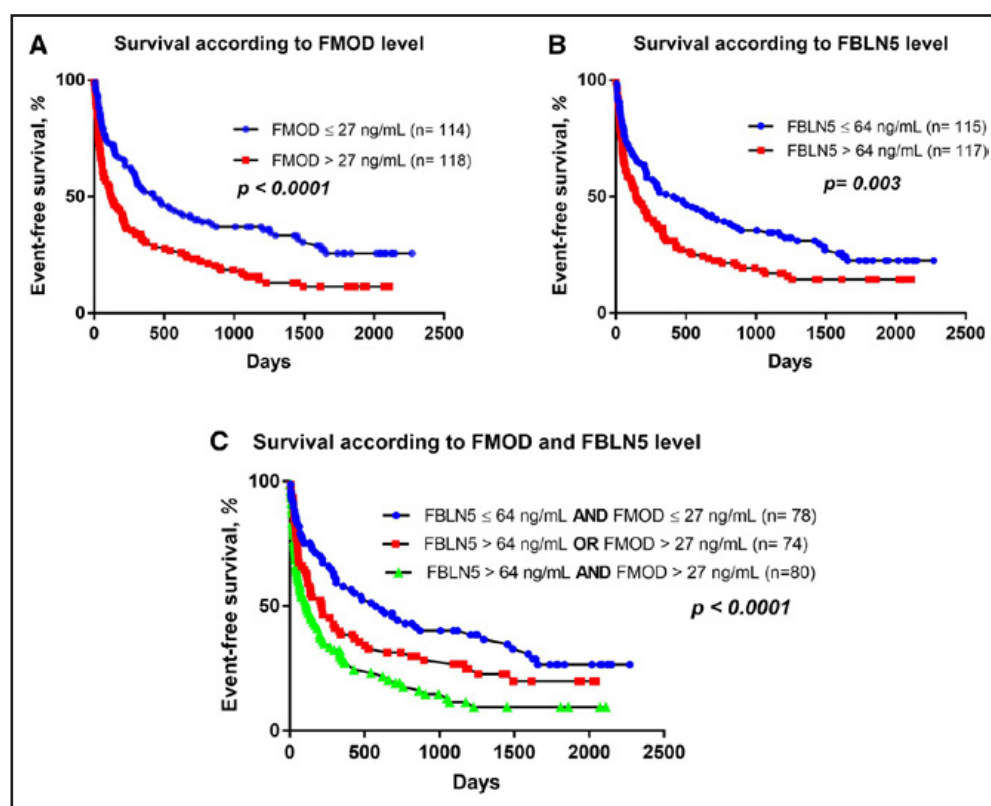


Figure 4. Survival analysis according to the plasma levels of FMOD (fibromodulin) and FBLN5 (fibulin-5).

Patients were divided according to the mean plasma concentrations of FMOD and FBLN5. **A**, Kaplan-Meier analysis showing that patients with FMOD plasma concentration >27 ng/mL had worse outcomes than the rest of the cohort. **B**, Kaplan-Meier analysis showing that patients with FBLN5 concentration >64 ng/mL had worse outcomes than the rest of the cohort. **C**, Kaplan-Meier analysis showing the additive prognostic effect when both markers were elevated.

organization.¹⁸ In vitro, both cardiomyocytes and fibroblasts can produce FMOD upon proinflammatory stimuli, suggesting that both cell types may participate in the cardiac ECM remodeling.¹⁹ FMOD together with ECM proteoglycans BGLN and VCAN (both also upregulated in our study) was previously found to be upregulated in the LV in patients with ischemic HFrEF.²⁰ Transcriptomic profiling showed an increased expression of the *FMOD* gene in RVD due to pulmonary hypertension.²¹ FMOD protein upregulation has been recently reported in failing RV in patients with pulmonary arterial hypertension.¹⁴

Along with collagen fibrils, elastic fibers are another important component of ECM. Formed mainly by proteins elastin and fibrillin, elastic fibers provide tissues, especially in lungs, skin, and blood vessels, with elasticity²² and play a role in wound healing, especially in the inflammation phase of the process.^{22,23} FBLN5 associates with tropoelastin and assists its deposition into fibrillin-based microfibrils, forming the elastic fibers.²² FBLN5 is essential for elastogenesis and is involved in several pathologies including cutis laxa.^{24,25} Expression of FBLN5 is, similarly to collagen fibrillogenesis, regulated by TGF- β (transforming growth factor beta) signaling.^{23,26} Akin to FMOD, FBLN5 was previously found to be upregulated in LV of patients with ischemic HFrEF.²⁰

FMOD and FBLN5 as Biomarkers of RVD

Our initial proteomic analysis (comparing HFrEF patients with severe RVD versus those with preserved RV function) revealed higher FBLN5 and FMOD levels in LV and RV tissues, respectively.

This finding might be biased, however, by lower LVEF and more rapid progression of HF in the severe RVD subgroup of the proteomic cohort. Nevertheless, analysis of plasma concentrations of FMOD and FBLN5 performed on a larger and independent cohort of patients demonstrated a significant association between the plasma level of both proteins and the degree of RVD.

To the best of our knowledge, this is the first study demonstrating the association between both FMOD and FBLN5, and RV function. As we have used both noninvasive (RVD grade, tricuspid annular plane systolic excursion, and systolic motion of RV basal segment assessed by tissue Doppler imaging) as well as invasive measures of RV function (pulmonary artery pulsatility index and central venous pressure/pulmonary capillary wedge pressure ratio) and received almost identical results, we consider the association between both FMOD and FBLN5, and RV function particularly robust. While FMOD was specific for RVD only, FBLN5 showed also a significant association with RV afterload (assessed as pulmonary vascular resistance) and age. This suggests that compared with FMOD, FBLN5 probably mirrors slightly different pathophysiological processes; RVD might be also a result of chronically elevated RV afterload. However, there was

no association of FMOD or FBLN5 plasma levels with LVEF, cardiac output, body mass index, diabetes status, or kidney function. In addition, plasma levels of both proteins were significantly associated with patient outcomes in the Cox proportional hazard model, suggesting that these proteins mirror pathophysiologically relevant processes with clinical significance.

The prognosis of patients with HFrEF is driven by various factors (degree of HF itself, quality of HF treatment, and comorbidities).⁵ All these factors are included in scoring systems used for prognostic stratification (ie, MAGGIC score).¹⁰ Although the RV function is strongly associated with prognosis in patients with HFrEF as well, it is missing in the MAGGIC score, which is likely due to the lack of a universal single parameter describing RV function. The addition of FMOD to MAGGIC score significantly increased the AUC compared with MAGGIC alone, and the combination of FMOD or FMOD+FBLN5 together with MAGGIC score outperformed the combination of MAGGIC score together with RVD. This suggests that FMOD and FBLN5 do not merely reflect RVD but integrate other (possibly distinct) prognostically important processes. This concept is further supported by the fact that the prognostic role of FMOD and FBLN5 is additive. The additivity of plasmatic concentrations of FMOD and FBLN5 may be attributed to the distinct molecular roles of each protein in the heart (ie, collagen deposition/cross-linking for FMOD and elastic fiber formation for FBLN5) and to a different intensity of the processes in the respective ventricles. However, it should be pointed out that in addition to the heart, FMOD and FBLN5 are expressed in various tissues that may contribute to the plasma pool of both proteins. Some of those tissues are affected by HFrEF (lungs, kidney, liver, or spleen). Vascular ECM remodeling, profibrotic changes, or other molecular alterations in these tissues may, thus, theoretically increase the FBLN5 and FMOD plasma concentrations and contribute to the prognostic value of these proteins in patients with HFrEF. These properties make FMOD and FBLN5 promising blood biomarkers integrating multiple HF-related processes in addition to RVD.

Limitations

Despite we have matched patients taken for the proteomic analysis (patients cohort 1) with respect to age, body size, HF cause, and comorbidities, those with severe RVD had also markedly lower LVEF and shorter duration of HF suggesting faster HF progression. Although the regression analysis performed on a larger and independent group of patients with HFrEF suggested a robust association between FMOD/FBLN5 and RV function, the potential association of FMOD/FBLN5 with other pathological processes associated with more rapid HF progression (and, thus, more profound RVD) cannot be completely excluded.

We cannot conclude whether the observed FMOD and FBLN5 upregulation in the myocardium of patients with HFrEF with RVD could be attributed to cardiomyocytes, fibroblasts, or other cell types present in the myocardium. Similarly, we cannot determine whether the myocardium is the only tissue responsible for the increased circulating concentrations of the proposed biomarkers.

Our study cohort is considerably male-predominant and included rather young patients with advanced HF reflecting patients referred to our Institute. Consequently, the generalizability of our results to women and to older patients with less advanced HF should be further tested. Most patients in our study were treated by optimal pharmacotherapy/implantable cardioverter defibrillator/cardiac resynchronization therapy device implantation, but some of them underwent heart transplantation or implantation of mechanical circulatory support, which may bias the outcome analysis. As the patients were enrolled before the SGLT2i (sodium/glucose cotransporter 2 inhibitors) era, none were treated with these agents. Although patients were prospectively enrolled, not all of them were followed in our hospital; therefore, the information about changes in pharmacotherapy throughout the follow-up period was not available from all the patients. Similarly, data about further cardiac decompensation events were not available from all the patients, so it was not possible to analyze this end point. As we have strived for maximal applicability of our results, we have included all patients with HF regardless of cause. However, differences in ECM remodeling may exist depending on the HF cause.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Supplemental Methods
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Transpulmonary Proteome Gradients Identify Pathways Involved in Pulmonary Vascular Disease Due To Heart Failure

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ORIGINAL ARTICLE



Transpulmonary Proteome Gradients Identify Pathways Involved in Pulmonary Vascular Disease Due To Heart Failure

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BACKGROUND: Some, but not all, patients with heart failure (HF) develop pulmonary vascular disease (PVD), which contributes to poor prognosis. Mechanisms leading to PVD in HF are poorly understood. We aimed to analyze transpulmonary gradients of proteins consumed or elaborated across the lungs to identify mediators of PVD by unbiased proteomics.

METHODS: Overall, 21 controls and 160 patients with HF with reduced ejection fraction underwent pulmonary artery catheterization with blood sampling from postcapillary (wedged balloon) and precapillary (unwedged) position to obtain transpulmonary gradients. The samples from controls and HF from the highest (Q4, n=40) and lowest quartile (Q1, n=40) of pulmonary vascular resistance (PVR) were analyzed using the proteomic proximity extension assay (Olink) of 275 proteins. Venous blood concentrations or transpulmonary gradients were analyzed to identify biomarkers or potential mediators of PVD.

RESULTS: Comparison of Q1 and Q4 of PVR identified PSP-D (pulmonary surfactant-associated protein D) as a marker of PVD. Examination of gradients across the lungs in high PVR HF revealed significant uptake of 18 proteins, mostly associated with inflammation (chemokines, oncostatin-M, MMP9 [matrix metalloproteinase 9]) or with TGF (transforming growth factor)/activin pathway (GDF2 [growth differentiation factor 2]/BMP9 [bone morphogenic protein 9]), and release of 5 proteins, notably IL (interleukin) 6 and IL33. In contrast, these protein gradients were negligible in controls and low PVR patients with HF. Active pulmonary release of IL6 contributed to systemic elevation of IL6 and correlated with right ventricular function.

CONCLUSIONS: The lungs of patients with HF with high PVR display abnormal uptake and release of proinflammatory cytokines from IL6/gp130 family (IL6, IL33, oncostatin-M), along with increased transpulmonary uptake of GDF2/BMP9. The study shows that proteins orchestrating inflammation or pulmonary vessel remodeling in group 1 pulmonary hypertension, are also operating in patients with PVD due to HF.

REGISTRATION: URL: <https://www.clinicaltrials.gov>; Unique identifier: NCT06331208.

Key Words: heart failure ■ hypertension, pulmonary ■ inflammation ■ proteomics ■ vascular disease

Some, but not all, patients with left-sided heart failure (HF) develop pulmonary vascular disease (PVD), which increases right ventricular (RV) afterload and promotes transition to biventricular HF, with poor prognosis.^{1–3} PVD is defined hemodynamically by an increase

in pulmonary vascular resistance (PVR). Histopathologic studies have shown that the morphological substrate of PVD is an intimal thickening in pulmonary venules and arterioles, with an absence of plexiform lesions typical for group 1 pulmonary hypertension (G1 PH).^{3,4} The

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WHAT IS NEW?

- Some patients with heart failure (HF) develop pulmonary vascular disease (PVD), characterized by an increase in pulmonary vascular resistance. Transpulmonary gradients of proteins consumed or elaborated across the lungs of these patients may identify proteins causally involved in PVD development.
- This is the first study that systematically characterized the transpulmonary proteome in HF-related PVD using proteomics and sampling across the pulmonary vascular bed.
- Patients with HF with PVD had significant uptake of mediators associated with inflammation (chemokines, oncostatin-M, MMP9 [matrix metalloproteinase 9]), TGF (transforming growth factor)/activin pathway (GDF2 [growth differentiation factor 2]/BMP9 [bone morphogenetic protein 9]) and release of interleukin 6 and 33. These protein gradients were negligible in controls or in patients with HF without PVD.

WHAT ARE THE CLINICAL IMPLICATIONS?

- The data support the observations that in considerable number of patients with HF and pulmonary hypertension, there is a phenotype overlap between G1 and G2 pulmonary hypertension.
- The study raises a new hypothesis that PVD in HF is caused by vascular inflammation and structural vessel remodeling, in addition to vasoconstriction; proteins identified could serve as targets to treat or prevent HF-related PVD.

mechanisms and signaling pathways involved in the development of PVD are poorly understood. This may explain in part why conventional pulmonary vasodilators, used for G1 PH, are not effective in patients with group 2 PH (G2 PH) due to underlying HF.^{3,5–7}

PVD is more commonly present in patients with more advanced HF. Detailed analyses of transpulmonary arteriovenous differences provide unique mechanistic insight to evaluate production and consumption of proteins or metabolites across the lung circulation.^{8,9} In this approach, precapillary blood is sampled from the pulmonary artery (PA), and postcapillary blood is obtained from wedged PA balloon catheter.^{10,11} Here, we combined such sampling techniques with targeted proteomics by proximity extension assay,¹² and compared patients with HF with and without PVD to healthy controls without HF or PVD.¹

We reasoned, that net lung uptake of a regulatory protein is reflecting either cleavage, transendothelial transport or receptor-mediated removal, while net lung release reflect active secretion of the protein into pulmonary vascular tree.¹³ If a transpulmonary gradient of a protein differs between patients with markedly different PVR, it implies that the protein may be mechanistically linked to PVD. To test these hypotheses and to

Nonstandard Abbreviations and Acronyms

ADM	proadrenomedullin
BMP9	bone morphogenetic protein 9
BNP	B-type natriuretic peptide
CCL	C-C motif chemokine
CXCL10	C-X-C motif chemokine 10
FDR	false discovery rate
FGF	fibroblast growth factor
G1 PH	group 1 pulmonary hypertension
G2 PH	group 2 pulmonary hypertension
GDF2	growth differentiation factor 2
HF	heart failure
HFrEF	HF with reduced ejection fraction
IGFBP7	insulin-like growth factor-binding protein
IL	interleukin
MCP3	monocyte chemotactic protein 3
MMP9	matrix metalloproteinase 9
NPX	normalized protein expression
NTproBNP	N-terminal pro-B-type natriuretic peptide
OSM	oncostatin M
PA	pulmonary artery
PON3	paraoxonase 3
PSP-D	pulmonary surfactant-associated protein D
PVD	pulmonary vascular disease
PVR	pulmonary vascular resistance
TGFA	transforming growth factor alpha
TNF	tumor necrosis factor
TNFRSF13B	tumor necrosis factor receptor superfamily member 13B
TR	transferrin receptor protein

identify biomarkers or putative mediators of PVD in HF, we conducted a cross-sectional comparative study of controls and patients with HF with reduced ejection fraction (HFrEF) with or without PVD, using top-performing targeted proteomic technology.¹⁴

METHODS

Patient Cohorts and Procedures

The data that support the findings of this study are available from the corresponding author on reasonable request. The study examined consecutive patients with chronic (duration of >6 months) symptomatic HFrEF (left ventricular ejection fraction ≤40%) electively hospitalized for consideration of advanced therapies at the Institute for Clinical and Experimental Medicine in Prague, Czech Republic. Patients with acute ischemia, uncontrolled cardiac arrhythmia, and hemodynamic instability requiring inotropes or mechanical circulatory support,

reversible cardiac dysfunction, active malignancy, recent pulmonary embolism, endocrine disease, chronic or acute infection were excluded. Patients with hypervolemia on admission (based on clinical examination, increased body weight, or measured central venous pressure) were enrolled into the study after diuresis and reaching normovolemia (central venous pressure <10 mmHg or return of body weight to normal with disappearance of clinical signs of congestion). The study complied with Declaration of Helsinki, and the local ethics committee approved the protocol.

All subjects signed informed consent to participate in this research study. Patients with HF underwent history review, physical examination, echocardiography, ECG, Kansas City Cardiomyopathy Questionnaire, and morning (6–7 AM) peripheral venous blood sampling in the postabsorptive state after an overnight fast. Control subjects were patients of the same hospital (Institute for Clinical and Experimental Medicine) who underwent diagnostic right heart catheterization to rule out cardiac disease ($n=5$) or subjects who underwent right heart catheterization as part of closure of patent foramen ovale ($n=16$), and normal echocardiography and hemodynamic findings were demonstrated.

Right heart catheterization was performed according to current recommendations using a 7F balloon-tipped triple-lumen Swan-Ganz catheter (Braun AG, Germany) inserted via the right internal jugular vein (patients with HF) or femoral vein (controls referred for patent foramen ovale closure). Hemodynamic zero was calibrated at mid-thoracic level. Pressure waveforms were recorded over several respiratory cycles and annotated by an invasive hemodynamic module (Mac-Lab, GE Healthcare) as recommended.¹⁵ Cardiac output was measured by thermodilution as an average of at least 3 measurements (of 5 in patient with atrial fibrillation). PVR was calculated as transpulmonary pressure gradient (mean PA pressure–PA wedge pressure), divided by cardiac output and was expressed in Wood units. During right heart catheterization, blood samples were obtained from the tip of free-floating catheter in PA with a deflated balloon (pulmonary arterial sample) and from a wedged PA catheter (pulmonary venous sample), after discarding the initial 5 mL of aspirated blood. Wedged position was verified by fluoroscopy oximetry, whereby only samples with $O_2\text{sat} > 90\%$ were considered as representative of pulmonary venous blood¹¹ ($n=160$). Cost considerations necessitated a more focused analysis for proteomic assays, so we identified patients with HFrEF and PVD as those subjects in the top quartile of PVR distribution (high PVR HF group, $n=40$), and we then contrasted them to subjects with HFrEF in the bottom quartile of PVR distribution (low PVR HF group, $n=40$). The patient disposition and flow are shown in the CONSORT (Consolidated Standards of Reporting Trials) diagram (Figure S1).

Targeted Proteomic Analysis

Blood samples from both sampling sites were handled in the same way and were placed into EDTA tubes and centrifuged within 20 minutes at a force of $800\times g$ at a temperature of 4°C , distributed into aliquots, and frozen at -80°C until analysis. The Olink Target 96 Inflammation, Cardiovascular II and III panels (Olink Proteomics AB, Uppsala, Sweden) were used to measure proteins according to the manufacturer's instructions. The Olink protocol utilizes the Proximity Extension Assay technology, which allows for the simultaneous analysis of 92

analytes in small sample ($<10\ \mu\text{L}$) volumes.^{12,16} Briefly, pairs of oligonucleotide-labeled antibody probes specifically bind to their respective target proteins. When these probes come close to each other, the oligonucleotides hybridize, and proximity-dependent DNA polymerization occurs. This generates a unique polymerase chain reaction target sequence. The resulting DNA sequence is then detected and quantified using a microfluidic real-time polymerase chain reaction instrument (Biomark HD, Fluidigm). To ensure data quality and account for variation between runs, internal controls, including an extension control and an inter-plate control were used for normalization. The final output of the assay is presented as normalized protein expression (NPX) values, which are on a log₂-scale. Higher NPX values indicate higher protein expression. Detailed assay validation data, such as detection limits and intra-assay and inter-assay precisions is on manufacturer's website (www.olink.com). The list of proteins in all used OLINK panels is in Table S1.

Other Biochemical Analyses

Routine biochemistry was analyzed by the Abbott Architect ci1600 analyzer. Estimated glomerular filtration rate was calculated by the Chronic Kidney Disease Epidemiology Collaboration equation. The BNP (B-type natriuretic peptide) concentrations were measured by using microparticle immunoassay (Architect BNP; Abbott Laboratories, Chicago, IL; long-term analytical coefficient of variation 4.5%). Circulating IL (interleukin) 6 was verified by Elecsys IL6 method (ECLIA by Roche Diagnostics, Mannheim, Germany; long-term analytical coefficient of variation 2.6%).

Statistical Analysis

Data were analyzed by using JMP18 software (SAS Institute Inc, Cary, NC). Data are presented as means $\pm$ SD or medians (interquartile ranges), where appropriate. Differences between groups were tested by using the χ^2 test, unpaired Student t test, or ANOVA. The statistical significance of PA concentrations (to identify markers) or transpulmonary fold-change gradients (to identify putative mediators) was tested using unpaired and paired t test, respectively. A 2-sided P value of <0.05 was considered statistically significant for all data besides the proteome. For proteomic analysis results, P value was adjusted for false discovery rate (FDR) using Benjamini-Hochberg procedure. In OLINK proteomic analysis, we obtained complete NPX values from all tested proteins in all samples. The transpulmonary gradients were calculated as pulmonary postcapillary concentration (wedged PA catheter sampling) minus precapillary concentration (unwedged PA sampling). Differences in NPX (log₂-fold change) were plotted against FDR-adjusted P values to obtain Volcano plots performed separately in control, high PVR HF, and low PVR HF groups. Protein-protein interaction network analysis was performed using STRING (Search Tool for Retrieval of Interacting Genes/Proteins) version 12.

RESULTS

Of the 160 patients with HF (median PVR, 2.88 [interquartile range, 1.90–3.92 Wood units]; Figure S2A), we performed transpulmonary proteomic analyses on those with low PVR (bottom quartile Q1, $n=40$, $\text{PVR} < 1.90$

Wood units) and high PVR (top quartile, Q4, $n=40$, $PVR>3.92$ Wood units). Patients with HF were most often middle-aged males with typical characteristics of severely symptomatic HFrEF, whereas HF-free controls had similar anthropometric characteristics, but were slightly younger and more often female (Table 1).

Patients with HF had severely dilated and dysfunctional left ventricles, often with dysfunctional right ventricle, pulmonary hypertension (mean $PA>20$ mmHg in 86%), and reduced cardiac output (Table 2). Patients with HF and high or low PVR has similar anthropometry, age, sex, HF duration, HF cause or HF therapy, but patients with PVD were more symptomatic (higher New York Heart Association class, lower Kansas City Cardiomyopathy Questionnaire scores), and had more severely elevated BNP, CRP, and IL6 levels compared with patients with HF and no PVD, along with more significant mitral regurgitation, higher PA pressures, and more severely reduced cardiac output. Importantly, high PVR patients had identical right atrial pressure and had only marginally (mean difference, 3.1 mmHg) higher PA wedge pressure, indicating that compensation and volume status were relatively well-matched.

Proteomic Biomarkers of HF or PVD

First, we conducted targeted analysis of the proteome in unwedged PA blood, and compared all subjects with HF (high and low PVR together) to controls. A heat map of NPX of HF versus controls showed clear separation of

both groups (Figure S3A). With FDR-adjusted $P<0.05$ and cutoff of NPX $\log_2$ -fold-change >2 or <0.5 , we identified 33 proteins upregulated and 5 downregulated in HF (Table S2; Figure S3B). Upregulated proteins consisted of previously known markers of HF (NTproBNP [N-terminal pro-B-type natriuretic peptide], BNP, GDF15, FGF [fibroblast growth factor] 21, FGF23; Table S1), inflammatory mediators (IL6, IL8, TNF [tumor necrosis factor] receptor family members), chemokines (CCL [C-C motif chemokine] 3, CCL15, CCL20), and neurohormones (ACE [angiotensin-converting enzyme] 2, IGFBP7 [insulin-like growth factor-binding protein], ADM [proadrenomedullin], renin). One of the downregulated proteins in HF was the reactive oxygen species defense protein PON3 (paraoxonase 3).

Next, we compared the proteome of unwedged PA blood in patients with HF and high or low PVR to identify circulating biomarkers of PVD. We identified only 3 proteins that were significantly (FDR-adjusted $P<0.05$) different in patients with HF and high PVR: PSP-D (pulmonary surfactant-associated protein D), chemokine CCL3, and TNF receptor superfamily member TNFRSF13B (tumor necrosis factor receptor superfamily member 13B).

Transpulmonary Proteomics: Putative Mediators of HF-Related PVD

As a next step, we analyzed transpulmonary protein gradients by subtracting concentrations in pulmonary venous blood (wedged PA sampling) from PA blood (unwedged

Table 1. Baseline Characteristics

	Controls (n=21)	HF (n=80)	P value	HF low PVR (n=40)	HF high PVR (n=40)	P value
Age, y	49.5±10.6	56.4±7.9	0.010	55.0±7.6	57.7±8.2	0.133
Sex (male)	62%	87%	0.010	93%	83%	0.171
BMI, kg·m ⁻²	27.7±5.2	27.3±4.4	0.76	28.2±4.4	26.4±4.2	0.063
HF duration, y	...	5.6±5.9	...	6.1±5.9	5.2±6.0	0.46
HF cause, CAD	...	40%	...	40%	40%	0.99
NYHA class	1.4±0.6	3.0±0.6	<0.0001	2.8±0.5	3.2±0.5	0.006
KCCQ, TSS	78±23	55±24	0.0001	63±23	47±24	0.007
Diabetes	0%	31%	0.0002	30%	33%	0.81
eGFR, mL·min ⁻¹ ·1.73 m ⁻²	1.52±0.17	1.06±0.36	<0.0001	1.13±0.37	0.99±0.34	0.071
BNP, ng·L ⁻¹ *	17 (9–44)	1153 (432–2143)	<0.0001	914 (236–1862)	1519 (963–2384)	0.011
CRP, mg·L ⁻¹ *	2.7 (0.9–4.5)	5.7 (2.2–11.7)	0.001	4.5 (1.7–9.5)	7.4 (2.6–13.7)	0.056
IL6, ng·L ⁻¹ *	0.7 (0.6–0.8)	4.8 (2.2–12.2)	<0.0001	2.6 (0.8–11.7)	8.0 (3.9–13.3)	0.026
Furosemide mg/d	...	172±123	...	184±127	159±119	0.38
ACE or ARB or ARNi	42%	65%	0.04	72%	57%	0.11
MRA	10%	92%	<0.0001	97%	87%	0.16
BB	16%	80%	<0.0001	85%	75%	0.27

Values are means (±SD) tested by *t* test or median (IQR) by Wilcoxon test. ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; ARNi, angiotensin receptor-neprilysin inhibitor; BB, beta-blocker; BMI, body mass index; BNP, B-type natriuretic peptide; CAD, coronary artery disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HF, heart failure; IL, interleukin; IQR, interquartile range; KCCQ, Kansas City Cardiomyopathy Questionnaire; MRA, mineralocorticoid receptor inhibitor; NYHA, New York Heart Association; PVR, pulmonary vascular resistance; and TSS, total symptom score.

*Median (IQR) by Wilcoxon test.

Table 2. Echocardiography and Invasive Hemodynamics

	Controls (n=21)	HF (n=80)	P value	HF low PVR (n=40)	HF high PVR (n=40)	P value
LVED diameter, mm	49.5±3.3	70.5±8.4	<0.0001	71±8.4	69±8.6	0.40
LV ejection fraction, %	59.7±5.2	21.3±6.7	<0.0001	21.2±4.2	21.4±8.6	0.86
LAVI, mL·m ⁻²	29.6±10	64.4±20.7	<0.0001	60.6±21.9	68.3±19.0	0.128
Mitral reg. grade, (1–4)	1.0±0.5	2.5±1.0	<0.0001	2.2±1.0	2.9±1.0	0.006
RV diameter 1, mm	35±4.6	46.3±7.0	<0.0001	45±7.7	47.6±6.0	0.10
RV dysfunction grade (0–4)	0±0	2.5±1.2	<0.0001	2.4±1.4	2.7±1.0	0.28
Tricuspid reg. grade (1–4)	1.0±0.2	2.2±1.0	<0.0001	2.1±1.1	2.3±0.9	0.34
TAPSE, mm	24.2±5.9	15.3±4.1	<0.0001	15.6±3.9	15.0±4.3	0.53
Inf. vena cava diam., mm	15.9±4.3	22.9±6.7	<0.0001	23.3±7.5	22.6±5.9	0.64
Mean blood pressure, mm Hg	96±13	86±10	0.005	87±9.4	86±10	0.71
Right atrial pressure, mm Hg	4.8±3.2	8.7±5.0	<0.0001	8.6±5.0	8.7±5.1	0.94
PA mean pressure, mm Hg	15.4±5.6	33.4±10.8	<0.0001	26.5±7.5	40.4±9.1	<0.0001
PA wedge pressure, mm Hg	7.6±3.8	21.7±7.1	<0.0001	20.2±7.3	23.3±6.7	0.04
Cardiac output, L·min ⁻¹	5.8±1.7	3.7±1.1	<0.0001	4.4±1.0	3.0±0.7	<0.0001
PA compliance, mL·mmHg ⁻¹	5.2±1.7	2.2±1.6	<0.0001	3.2±1.7	1.3±0.4	<0.0001
PVR, w.u.	1.38±0.61	3.66±2.58	<0.0001	1.45±0.36	5.86±1.87	<0.0001
Transpulm. gradient, mm Hg	7.8±3.8	11.7±6.43	0.0007	6.3±2.0	17.1±4.5	<0.0001
PA elastance, mm Hg·mL ⁻¹	0.39±0.09	1.21±0.67	<0.0001	0.72±0.28	1.69±0.60	<0.0001
PA sat O ₂ , %	73±4	59±10	<0.0001	64±8	54±10	<0.0001
Wedge PA sat O ₂ , %	97±1	96±3	0.14	96±3	97±3	0.19
Cardiac index, L·m ⁻²	2.88±0.74	1.82±0.51	<0.0001	2.1±0.5	1.5±0.3	<0.0001
Heart rate, min ⁻¹	75±15	80±14	0.22	76±14	83±13	0.26

Values are means±SD, *P* values: tested by *t* test. HF indicates heart failure; LAVI, left atrial volume index; LV, left ventricular; LVED, left ventricular end-diastolic; PA, pulmonary artery; PVR, pulmonary vascular resistance; RV, right ventricle; sat, saturation; TAPSE, tricuspid annular plane systolic excursion; and w.u., Wood units.

PA sampling), assuming that proteins involved in pathogenesis of PVD (ie, putative mediators) are the proteins either taken up or elaborated across the pulmonary circulation.

Results are presented in Table 3 and also as Volcano plots (Figure 1A through 1C). In controls, only 1 protein (the chemokine CCL19) displayed significant transpulmonary gradient (net uptake), which was of marginal significance (FDR-adjusted $P < 0.05$; Figure 1A). In patients with HF and low PVR, we identified only 5 proteins with significant transpulmonary gradient—CCL19, OSM (oncostatin-M), and 2 other chemokines (Figure 1B).

In contrast, patients with HF and high PVR displayed 23 proteins with significant transpulmonary gradients, including 18 proteins that were consumed and 5 proteins being released from the lung into circulation (Figure 1C), mostly inflammatory cytokines and chemokines. The highest lung uptake was observed for chemokine CCL19, cytokine OSM, BNP, BMP9 (bone morphogenetic protein 9) aka GDF2 (growth differentiation factor 2), TGFA (transforming growth factor α), TR (transferrin receptor), and MMP9 (matrix metalloproteinase 9; Figure 2). The highest lung release was observed for IL6 and IL33, and the chemokines MCP3 (monocyte chemoattractant protein 3), CCL4, and CXCL10 (C-X-C motif chemokine 10). Protein ontology analysis using STRING

demonstrated central roles of IL6, OSM, and CCL4 in patients with HF and high PVR (Figure S4).

Distribution analyses in the most relevant hits excluded transpulmonary differences being driven by outlying values (Figure 2). Because several proteins (IL6, CCL3) are present in both OLINK panels (inflammation and Cardiovascular Panel II), we were able to assess reproducibility of the method. As shown in Figure S1D, results of both IL6 measurements by OLINK correlated very well ($r = 0.99$), and both IL6 dots in the Volcano plots in Figure 1C were close to each other. We further validated BNP results by ELISA measurements and observed excellent correlation for BNP ($r = 0.97$, $P < 0.0001$), and acceptable correlation for IL6 ($r = 0.67$, $P < 0.001$).

There was also significantly increased IL6 release from lungs of patients with HF with high PVR based on ELISA measurements (Figure 3A), and systemic IL6 concentration by ELISA correlated with RV tricuspid annulus plane systolic excursion, indicating the association of IL6 levels with poorer RV function (Figure 3B).

DISCUSSION

This is the first study to systematically characterize the transpulmonary proteome in HF-related PVD. The

Table 3. Significant Transpulmonary Gradients of Proteins

Protein	UniProt ID	NPX K2-K1 (log2FC)	P value adjusted	Net pulmonary kinetics
Controls				
CCL19	Q99731	−0.13565	0.040787	Taken up
Heart failure with low PVR				
OSM	P13725	−0.22244	0.024964	Taken up
CCL19	Q99731	−0.16323	0.024964	Taken up
MMP9	P14780	−0.15822	0.046083	Taken up
CCL4	P13236	0.116997	0.046083	Released
CXCL10	P02778	0.123701	0.046083	Released
Heart failure with high PVR				
OSM	P13725	−0.38186	0.000815	Taken up
MMP9	P14780	−0.31003	0.006239	Taken up
CCL19	Q99731	−0.27428	0.000001	Taken up
BNP	P16860	−0.21109	0.000298	Taken up
TR (TFCR)	P02786	−0.19005	0.000271	Taken up
IL2RB	P14784	−0.16617	0.038841	Taken up
TGFA	P01135	−0.16067	0.013826	Taken up
GDF2 (BMP9)	Q9UK05	−0.15676	0.003769	Taken up
CCL28	Q9NRJ3	−0.12256	0.001523	Taken up
GT (FABP6)	P51161	−0.11972	0.033794	Taken up
MERTK	Q12866	−0.09758	0.033794	Taken up
FABP2	P12104	−0.09427	0.024708	Taken up
CCL25	O15444	−0.08313	0.042691	Taken up
KIM1 (HAVCR1)	Q96D42	−0.08123	0.030517	Taken up
TF (TFR)	P02786	−0.07098	0.036286	Taken up
SCF (KITLG)*	P21583	−0.06656	0.042691	Taken up
TIE2 (TEK)	Q02763	−0.05807	0.041734	Taken up
PRELP	P51888	−0.0393	0.030517	Taken up
CCL4	P13236	0.127964	0.041734	Released
MCP3 (CCL7)	P80098	0.132624	0.030517	Released
CXCL10	P02778	0.198088	0.006899	Released
IL6*	P05231	0.224349	6.59×10 ^{−5}	Released
IL6**	P05231	0.249581	0.000483	Released
IL33	O95760	0.306348	0.000142	Released

Newer abbreviations are in brackets (from web <https://olink.com/products/olink-target-96>, in [Supplemental Material](#)). For UniProt ID, see [Supplemental Material](#). BNP indicates B-type natriuretic peptide; CCL, C-C motif chemokine; CXCL10, C-X-C motif chemokine 10; FABP2, fatty acid-binding protein, intestinal; GDF2 (BMP9), growth differentiation factor 2 (bone morphogenetic protein 9); GT (FABP6), gastrotropin (fatty acid-binding protein 6); IL, interleukin; IL2RB, IL2 receptor subunit beta; KIM1 (HAVCR1), kidney injury molecule 1 (hepatitis A virus cellular receptor 1); MCP3 (CCL7), monocyte chemoattractant protein 3 (C-C motif chemokine 7); MERTK, tyrosine-protein kinase Mer; MMP9, matrix metalloproteinase 9; NPX, normalized protein expression; OSM, oncostatin-M; PRELP, prolargin; PVR, pulmonary vascular resistance; SCF (KITLG), stem cell factor (Kit ligand); TF (TFR), transferrin receptor protein 1; TGFA, transforming growth factor alpha; TIE2 (TEK), angiopoietin-1 receptor; and TR (TFCR), transferrin receptor protein 1.

*In Olink Cardiovascular Panel II.

**In Olink panel inflammation.

principal findings are that transpulmonary proteomic signatures of patients with PVD strongly differ from controls and from patients with HF and lower PVR, particularly for

gradients of inflammatory chemokines, cytokines from IL6 superfamily, and proteins that are involved in pulmonary vascular remodeling typical for G1 PH. This study supports the observations that in a considerable number of patients, there is a phenotype overlap between G1 and G2 PH.^{17,18} The data raise a new hypothesis that PVD in HF is caused by vascular inflammation and structural vessel remodeling, in addition to vasoconstriction, which may be important therapeutic targets to treat or prevent PVD in HF.

PVD in HF adds a hemodynamic load in excess of that transmitted passively across the lungs from left atrial hypertension. Increases in PVR in HF are independently associated with increased mortality,^{1,19} and impose an additional load to RV and promote progression of HF,³ while also contributing to gas exchange abnormalities and pathological increases in lung congestion.²⁰ Increase in PVR in G2 PH develops in part due to vasoconstriction, as it is at least partly reversible with pulmonary vasodilators in most patients,¹⁷ by the direct effect of congestion on pulmonary vessels,^{2,21} and through chronic structural alterations of pulmonary vasculature, such as vessel thickening and rarefaction.^{3,4,6}

Using a biomarker approach comparing mixed venous blood samples of patients with high and low PVR, we identified PSP-D as a biomarker associated with high PVR in HF. Elevated PSP-D in HF likely reflects a stressed or disrupted alveolar-capillary interface,³ as plasma levels of PSP-D are known to be elevated for weeks after resolution of cardiogenic pulmonary edema.^{22,23} Because transpulmonary gradients were net zero, elevated PSD-D is likely a biomarker of previous episodes of congestion, rather than actively contributing to PVD pathophysiology, at least at steady state and in the compensated state as evaluated herein.

Analyses of transpulmonary proteomic gradients provided several novel findings. The results indicate that pulmonary vascular homeostasis in healthy subjects is characterized by almost zero transpulmonary differences of regulatory proteins. In patients with HF, with increasing PVR we observed transpulmonary gradients of several proinflammatory cytokines and also proteins linked to vascular remodeling. Inflammation has been associated with G1 PH,²⁴ but we show here that it is also associated with G2 PH, alongside other noninflammatory mechanisms shared with G1 PH. Importantly, we studied HF subjects after intravenous decongestion, so patients with and without PVD had identical central venous pressure so the effect of congestion in our study was effectively mitigated, and observed transpulmonary gradients are representative of chronic processes of PVD.

Inflammatory Proteins Associated With PVD

Specifically, elevated PVR in HF was associated with net lung release of IL6. Patients with HF, and particularly

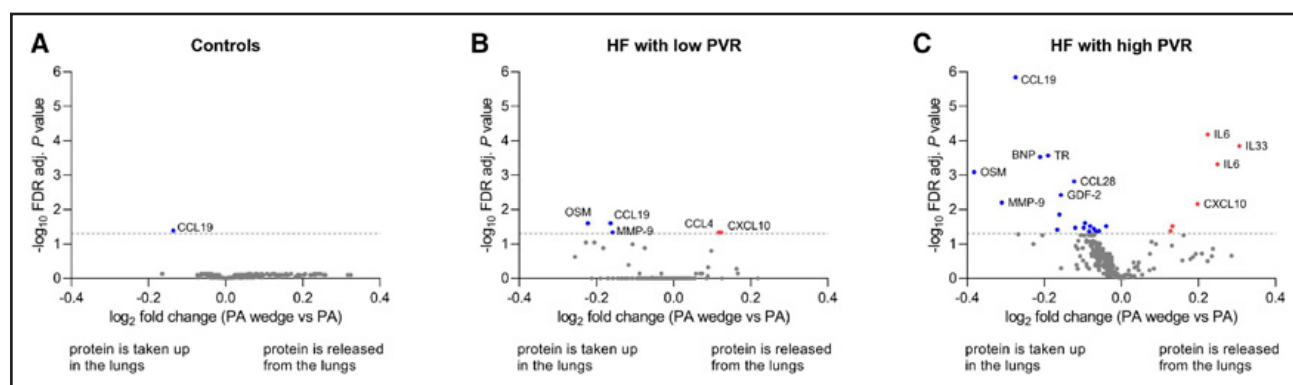


Figure 1. Transpulmonary proteome gradients in heart failure (HF)-related pulmonary vascular disease.

Volcano plots displaying proteins taken up in the lungs (blue dots) or released from the lungs (red dots) in control subjects (**A**, $n=21$), in patients with HF with low pulmonary vascular resistance (PVR; **B**, $n=40$) and in HF with high PVR (**C**, $n=40$). Transpulmonary differences were calculated as postcapillary (pulmonary artery [PA] wedged blood) minus precapillary concentrations (PA). BNP indicates B-type natriuretic peptide; CCL, C-C motif chemokine; CXCL10, C-X-C motif chemokine 10; FDR, false discovery rate; GDF2, growth differentiation factor 2; IL, interleukin; MMP9, matrix metalloproteinase 9; OSM, oncostatin-M; and TR, transferrin receptor.

those with PVD, had greater systemic inflammation from venous blood samples, as reflected by IL6 and CRP. Our transpulmonary data suggest that the lungs themselves are a source of inflammatory mediators in HF with PVD. Positive IL6 transpulmonary gradient in patients with HF comprised $\approx 8\%$ of systemic venous blood IL6 levels, indicating that pulmonary IL6 production contributes considerably to the circulating pool of IL6.

Our findings strongly support causal link between IL6 and pulmonary hypertension, that was already suggested in patients with pulmonary arterial hypertension²⁵ and in a PH-heart failure with preserved ejection fraction experimental model.²⁶ IL6 or other inflammatory mediators may originate from pulmonary vessels, from perivascular inflammatory infiltrates, as observed in pulmonary arterial hypertension,²⁷ or from extravascular cells such as alveolar myofibroblasts responding to capillary stress failure,²⁸ or from pulmonary interstitial inflammation corresponding to ground glass opacities present in some G2 PH or group 3 PH patients.¹⁷ Lung IL6 release may reflect an upstream vascular injury,²⁹ but it also possible that IL6 directly contributes to PVD and this question is being actively investigated in clinical trials. In the TRANSFORM-UK trial (A Therapeutic Open Label Study of Tocilizumab in the Treatment of Pulmonary Arterial Hypertension) in G1 PH, the IL6 receptor antagonist tocilizumab reduced CRP levels but did not reduce PVR, and actually increased IL6 levels.²⁹ The HERMES (Effects of Ziltivekimab Versus Placebo on Morbidity and Mortality in Patients With Heart Failure With Mildly Reduced or Preserved Ejection Fraction and Systemic Inflammation) and ATHENA (A Research Study Looking Into How Ziltivekimab Works Compared to Placebo in Participants With Heart Failure and Inflammation) trials are currently testing the effects of the IL6 targeting antibody ziltivekimab on reducing worsening HF or

cardiovascular death, as well as functional outcomes in patients with heart failure with preserved ejection fraction and systemic inflammation (URL: <https://www.clinicaltrials.gov>; Unique identifiers: NCT05636176 and NCT06200207). It may be that ziltivekimab works in part through the reduction of pulmonary vascular inflammation and has effects on pulmonary vascular load or right heart function.

Another inflammatory cytokine secreted from lungs with high PVR is IL33, an alarmin previously implicated in hyperproliferative response of pulmonary vessels in hypoxic PH.³⁰ Interestingly, the receptor for IL33 is ST2 protein, and circulating soluble ST2 protein levels are associated with proinflammatory comorbidities, RV pressure overload, and dysfunction in HF,²³ probably reflecting increased IL33 lung release.³¹ The lungs of patients with HF and high PVR also secreted chemokine CXCL10, which impairs pulmonary angiogenesis,³² and is elevated in HF,³³ as well as chemokine CCL4 (formerly MIP 1 β [macrophage inflammatory protein 1 β]), also known to be upregulated in the lungs of individuals with PH.³²

Several proteins were highly consumed in the high PVR lungs (ie, with negative gradient), including OSM. OSM is a pleiotropic cytokine belonging to IL6 family that signals via GP130 coreceptor and regulates inflammation and endothelial IL6 production. In an experimental model, lung OSM administration induces recruitment of inflammatory cells into the lungs, leading to a profibrotic gene expression footprint, collagen deposition, and pathological correlates characteristic of human interstitial lung disease or lung changes due to systemic scleroderma.³⁴ It was shown that OSM induces IL6 secretion from endothelial cells³⁵ and smooth muscle cells,³⁶ and also stimulates pulmonary release of IL33.^{37,38} Therefore, the increased transpulmonary uptake of OSM observed here in high PVR lungs could be a primary upstream event, leading to pulmonary inflammation and release

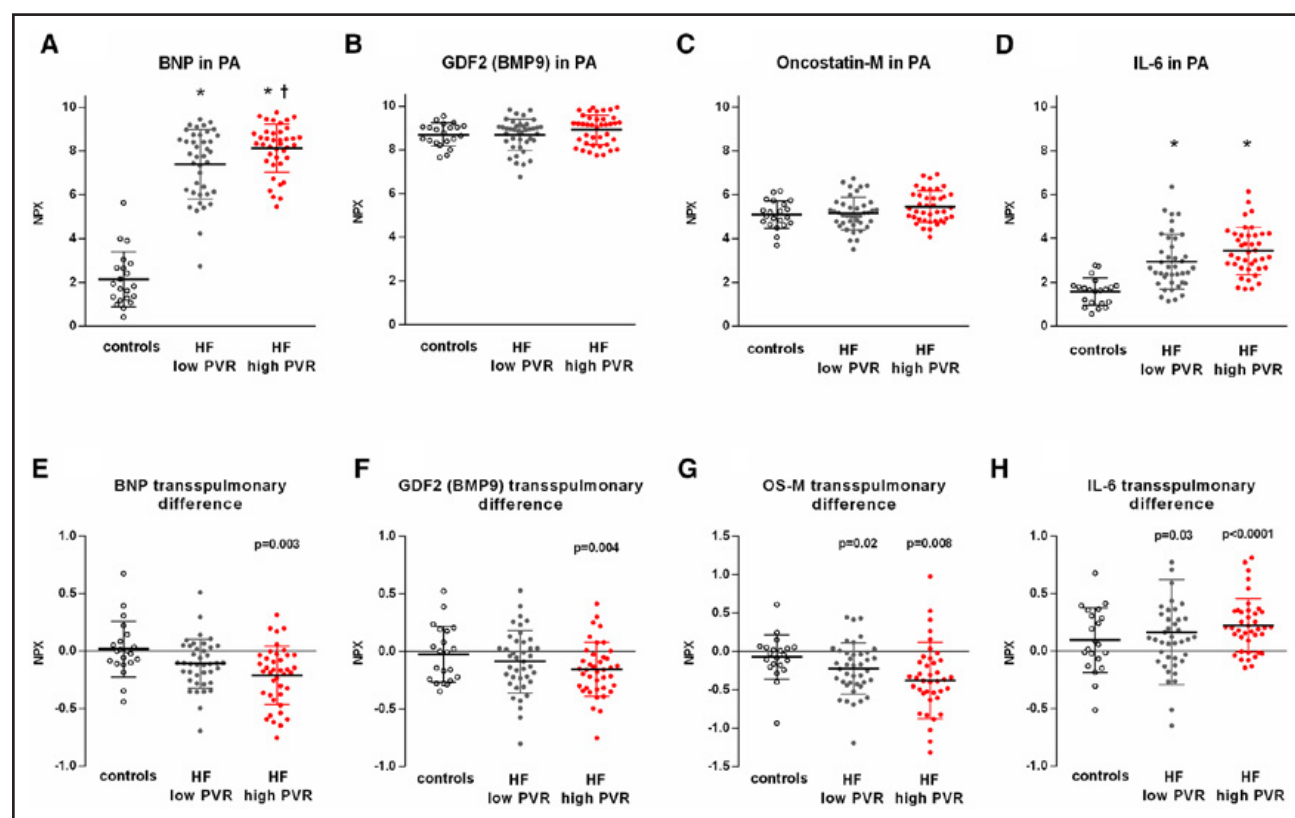


Figure 2. Individual patient values of key identified proteins.

A through **D**, Individual pulmonary artery (PA) concentrations in controls (open dots, $n=21$), in heart failure (HF) patients with low pulmonary vascular resistance (PVR; grey dots, $n=40$) and in HF with high PVR (red dots, $n=40$) of BNP (B-type natriuretic peptide, ANOVA $P<0.0001$, **A**), GDF2 (growth differentiation factor 2, aka BMP9 [bone morphogenic protein 9], ANOVA $P=0.24$, **B**), OS-M (oncostatin-M, ANOVA $P=0.10$, **C**), and IL (interleukin, ANOVA $P<0.0001$, **D**). The values (means $\pm$ SD) are from Proximity Extension Assay Olink assay and expressed as normalized protein expression (NPX) units, Tukey post hoc test <0.05 vs controls (*) or vs HF low PVR (†). **E** and **F**, Transpulmonary gradients of individual patients. P values in **E** through **H** are false discovery rate-adjusted and represent significant difference from net zero gradient (null hypothesis).

of other secondary cytokines, such as IL6 and IL33. OSM could, therefore, also serve as a target to treat PVD. A monoclonal antibody against OSM vixarelimab is currently being tested for pulmonary fibrotic disease (URL: <https://www.clinicaltrials.gov>; Unique identifier: NCT05785624), and the present data suggest that effects on PVD should be examined as well.

Increased CCL19 and MMP9 uptake in the high PVR group may reflect leukocyte recruitment, inflammation-triggered ECM (extracellular matrix) remodeling with degradation of elastin, endothelial to mesenchymal transition, and thickening of pulmonary vascular walls.^{39,40} Similarly, chemokine CCL28 displayed increased uptake in the high PVR group, and was previously identified as a marker of PH in patients with chronic obstructive pulmonary disease.⁴¹

Noninflammatory Proteins Associated With PVD

Interestingly, we observed enhanced lung uptake of GDF2 (also known as BMP9) in high PVR group. GDF2 is produced in the liver, and is one of key ligands of BMPR2

(bone morphogenic protein receptor 2), mutations of which are the most common cause of hereditary G1 PH.^{5,42} Acquired deficiency of GDF2 due to liver dysfunction is causally linked to the development of portopulmonary hypertension⁴³ and hepatopulmonary syndrome.⁴⁴ We cannot make inferences as to whether enhanced uptake of GDF2 in the high PVR group was causally related to high PVR, or it represents a compensatory response to antagonize profibrotic TGF/activin signaling.⁵ However, the present results suggest that TGF/activin/BMP signaling pathway, which drives structural remodeling of pulmonary vessels in G1 PH, is also part of G2 PH pathophysiology, and could be a drug target. The activin receptor ligand trap sotatercept successfully prevented pulmonary remodeling in experimental model of PVD due to HF⁴⁵ and is currently clinically tested in patients with heart failure with preserved ejection fraction with significant precapillary PH in the CADENCE trial (A Study of Sotatercept for the Treatment of Cpc-PH Due to HFpEF; URL: <https://www.clinicaltrials.gov>; Unique identifier: NCT04945460).

Finally, we observed enhanced uptake of BNP across the lungs in patients with HF and high PVR.

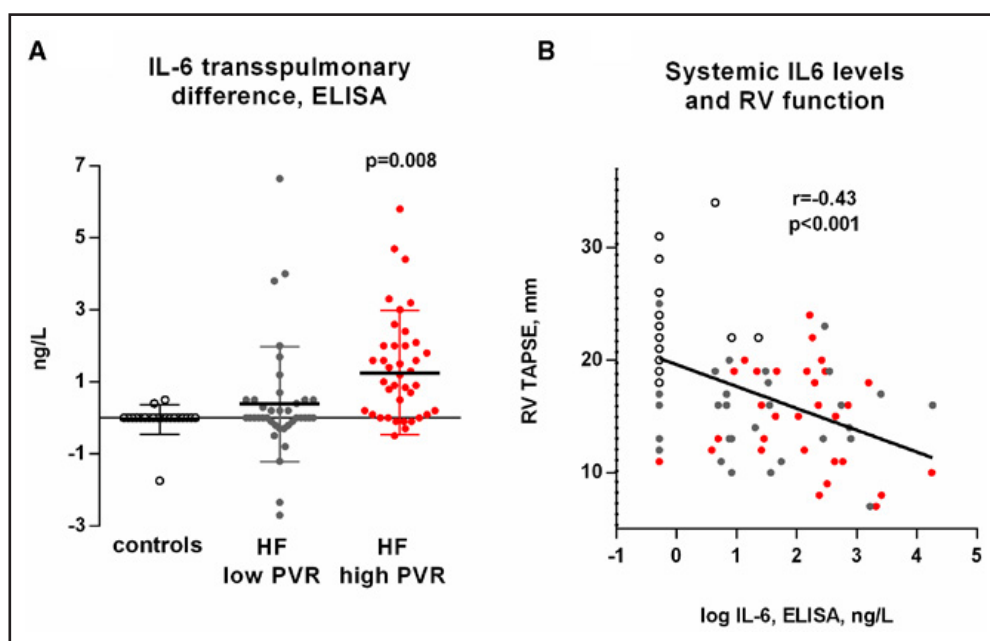


Figure 3. ELISA-validated pulmonary release of IL (interleukin) 6 and relation to right ventricular (RV) function.

A, Transpulmonary release of IL6 measured by ELISA and expressed as ng/L. **B**, Relation of RV function (tricuspid annulus plane systolic excursion [TAPSE]) with pulmonary artery concentration of IL6, correlation coefficient, and linear regression line. HF indicates heart failure; and PVR, pulmonary vascular resistance.

This likely corresponds to enhanced contra-regulatory vasodilatation via cGMP pathway in high PVR HF subjects¹⁰ which may explain why inhibition of BNP degradation by neprilysin inhibition improves PVR in HFrEF.⁴⁶

Limitations

This study must be interpreted in the context of several limitations. The study was prospective but cross-sectional, limiting the ability to reach conclusion regarding causality. The sample size was relatively small, involved mostly patients with advanced HFrEF, and the number of measured proteins was restricted. Due to budget constraints, we compared 2 outlying quartiles rather than all patients with HF, yet both HF groups were balanced in terms of relative congestion, comorbidities, age, and anthropometrics. Notably, Proximity Extension Assay technology uses similar optimized double antibody detection like conventional sandwich ELISA, but offers larger sensitivity and range - so the position of ELISA as a gold standard is questionable, though we did confirm findings for IL6 and BNP by ELISA. Proximity Extension Assay technology does not report absolute protein concentration, but this was not an obstacle for the design of our study which looked at relative changes of the analytes across the lungs, providing internal normalization. Proteins that were more taken up or more released in patients with higher PVR we considered as putative mediators of PVD, assuming greater mechanistic link to PVD than proteins with zero transpulmonary

gradient; however, no formal statistical mediation analyses were performed.

Conclusions

In summary, the lungs of patients with HF, particularly those with high PVR, display abnormal uptake and release of inflammatory chemokines and cytokines from IL6 family, along with increased pulmonary uptake of OSM and GDF2/BMP9, a mediator of lung inflammation and a ligand of the BMPR2 receptor involved in the pathogenesis of G1 PH, respectively. These data provide novel insights into the mechanisms underlying development of PVD in HF, and call for further evaluation in clinical trials targeting these pathways to improve outcomes in patients with PVD due to HF.

ARTICLE INFORMATION

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Disclosures

Dr Melenovsky has served as a consultant for Novo Nordisk, Merck, and Bayer and is receiving research support from Regeneron. Dr Jarolim has received

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Supplemental Material

Tables S1 and S2

Figures S1–S4

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M. Chovanec et al.

Pressure-Volume Analysis Demonstrates Short- and Long-Term Hemodynamic Effects of Atrioventricular Interval Modulation Therapy in Hypertension

JACC: Clinical Electrophysiology
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ORIGINAL RESEARCH

Pressure-Volume Analysis Demonstrates Short- and Long-Term Hemodynamic Effects of Atrioventricular Interval Modulation Therapy in Hypertension

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ABSTRACT

BACKGROUND Prior studies show that atrioventricular interval modulation (AVIM) therapy provides immediate, substantial, and sustained clinically meaningful reductions in ambulatory and office systolic blood pressures (SBPs) in hypertensive patients with pacemakers.

OBJECTIVES The goal of this study was to assess the acute and chronic mechanisms by which AVIM therapy reduces blood pressure and assess the impact of lead location on these mechanisms.

METHODS Acute hemodynamic effects were assessed by invasive pressure-volume (PV) analysis in hypertensive subjects (N = 16) with intact atrioventricular (AV) conduction. The impact of standard right ventricular pacing (RVp) vs left bundle branch area pacing (LBBAp) was also evaluated. Chronic effects of AVIM therapy were assessed by using noninvasive PV analysis with data from a subgroup of subjects (n = 32) from the MODERATO II study (NCT02837445).

RESULTS Acutely studied subjects had average office SBP of 144 ± 25 mm Hg despite antihypertensive medications. AVIM therapy significantly reduced end-diastolic volume (-12.6 ± 14.0 mL with RVp [$P = 0.003$]; -18.6 ± 16.2 mL with LBBAp [$P < 0.001$]) and effective arterial elastance (-0.23 ± 0.39 mm Hg/mL with RVp [$P = 0.032$]; -0.31 ± 0.55 mm Hg/mL with LBBAp [$P = 0.037$]), leading to significant SBP reductions (-17.1 ± 10.1 mm Hg with RVp [$P < 0.001$]; -19.2 ± 12.6 mm Hg with LBBAp [$P < 0.001$]). Neither left ventricular (LV) end-systolic nor end-diastolic properties were significantly affected by AVIM therapy at either pacing site. Noninvasive PV analysis showed similar reductions in SBP (-11.7 ± 21.8 mm Hg; $P = 0.023$) and reductions of end-diastolic volume (-21.5 ± 19.2 mL; $P = 0.005$ vs control) indicative of reverse remodeling.

CONCLUSIONS AVIM therapy acutely reduces SBP by decreasing preload and effective arterial elastance unrelated to lead location without affecting LV end-systolic or end-diastolic function. Chronic AVIM therapy reduces SBP and end-diastolic volume indicative of favorable reverse LV remodeling. (JACC Clin Electrophysiol. 2025;■:■-■)

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ABBREVIATIONS
AND ACRONYMS

aSBP = ambulatory systolic blood pressure
AV = atrioventricular
AVIM = atrioventricular interval modulation
CSP = conduction system pacing
DBP = diastolic blood pressure
E_a = arterial elastance
EDV = end-diastolic volume
E_{es} = end-systolic elastance
EF = ejection fraction
ESV = end-systolic volume
ESPVR = end-systolic pressure-volume relationship
HTN = hypertension
LBBA = left bundle branch area
LV = left ventricular
oSBP = office systolic blood pressure
PV = pressure-volume
RV = right ventricular
SBP = systolic blood pressure
V_o = volume axis intercept of ESPVR

More than 1 million individuals undergo implantation or replacement of a pacemaker every year; approximately 70% of these individuals have coexisting hypertension (HTN).¹ The high prevalence of HTN in this group is representative of the elderly pacemaker population and is accompanied by other significant cardiovascular comorbidities such as arterial disease, stroke, heart failure, and atrial fibrillation.² Not surprisingly, the majority of pacemaker recipients have isolated systolic HTN, a difficult-to-treat form of HTN.³ Consistent with other populations of patients with HTN, there is a high rate of uncontrolled HTN in the pacemaker population despite pharmacologic therapy.⁴ Accordingly, an algorithm to treat HTN contained in an implanted pulse generator offers an attractive risk-benefit profile in patients who are already clinically indicated for a pacemaker. In the MODERATO I and II studies,^{5,6} atrioventricular (AV) interval modulation (AVIM) therapy consisting of a sequence of variably timed short and longer AV intervals immediately and substantially reduced systolic blood pressure (SBP) with a durable effect over years of therapy delivery (**Central Illustration**).

The long-term efficacy and safety of AVIM therapy as a treatment for uncontrolled HTN in patients indicated for a dual-chamber pacemaker are currently being investigated in the BACKBEAT (Bradycardia Pacemaker With AV Interval Modulation for Blood Pressure Treatment) Investigational Device Exemption Study (NCT06059638). In this study, AVIM therapy, incorporated into Astra and Azure dual-chamber pacemakers (Medtronic) through a RAMware download, delivers a repeating sequence of 8 to 13 beats with short A-V intervals (20-80 milliseconds), followed by 1 to 3 beats with longer A-V intervals (100-180 milliseconds). The mechanisms by which AVIM therapy reduces blood pressure include reducing ventricular preload volume during the short

A-V intervals (decreasing left ventricular [LV] pressure generation according to the Frank-Starling mechanism) and modulating arterial resistance by intermittent exposure of the baroreceptors to increased blood pressure pulses approximating baseline during the longer A-V intervals. Assessments of the acute and chronic effects of AVIM therapy on ventricular preload, ventricular function, ventricular structure, and ventricular-vascular coupling are fundamental to understanding how to optimize the long-term benefits and minimize potential adverse effects of AVIM therapy. It is important to note that prior studies showing proof of concept, and supporting the efficacy and safety of AVIM therapy, were conducted before the widespread adoption of conduction system pacing (CSP) involving the placement of the ventricular lead in the left bundle branch area (LBBA). How CSP modifies and influences the effects of AVIM therapy is currently unknown, but a comprehensive understanding can be provided through ventricular pressure-volume (PV) analysis. PV analysis through invasive means is most suited to elucidating the acute effects of AVIM therapy, whereas noninvasive PV analysis is most suited for studying the chronic effects.

The primary purpose of the current study was to provide a comprehensive hemodynamic assessment of the acute and chronic effects of AVIM therapy using PV analysis. Hemodynamic assessments were made using 2 different patient cohorts: acute data from a cohort of patients scheduled to undergo pacemaker implantation and chronic data from subjects enrolled in the MODERATO II study (NCT02837445). A secondary assessment analyzed the impact of right ventricular (RV) pacing vs CSP on these effects.

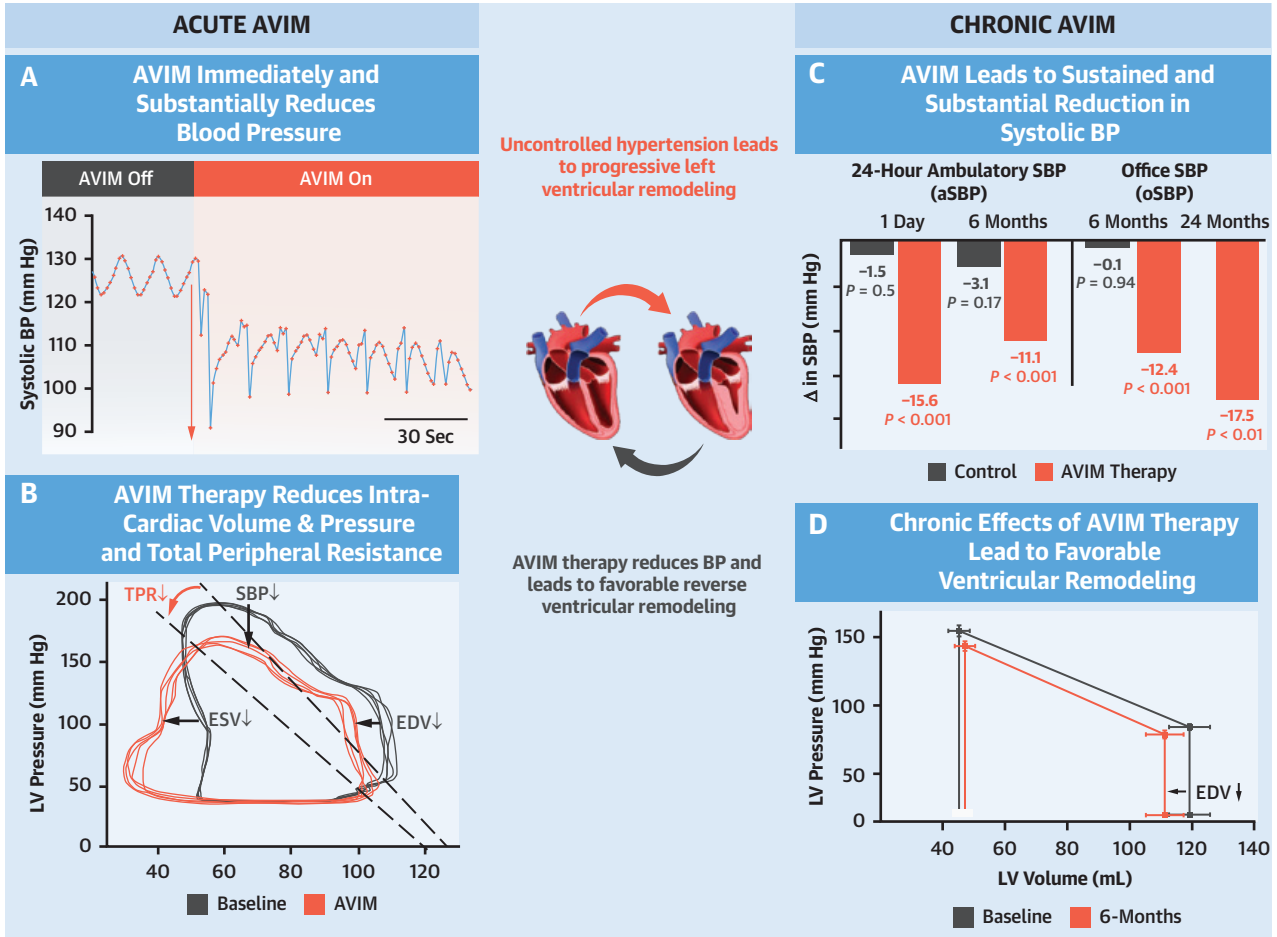
METHODS

ACUTE EFFECTS OF AVIM THERAPY ON BLOOD PRESSURE AND LV FUNCTION WITH DIFFERENT LEAD LOCATIONS. The acute hemodynamic effects of AVIM therapy were assessed at Na Homolce Hospital using PV analysis in 16 subjects with HTN

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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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CENTRAL ILLUSTRATION Acute and Chronic Effects of Atrioventricular Interval Modulation Therapy**AVIM Therapy Uses a Dual-Chamber Pacemaker to Deliver Repeated Sequences of Short and Longer AV Intervals**

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(A) Immediate and substantial reduction in systolic blood pressure (SBP) is seen upon activation of atrioventricular (AV) interval modulation (AVIM) therapy. Each orange dot reflects a measure of SBP during a single cardiac cycle.¹⁵ (B) Pressure-volume loop shows a reduction in SBP, end-systolic volume (ESV), end-diastolic volume (EDV), and total peripheral resistance (TPR) during delivery of AVIM therapy. (C) Results of the MODERATO II study exhibit a sustained as well as a substantial reduction in both ambulatory SBP (aSBP) and office SBP (oSBP) at 6 and 24 months, respectively.⁵ (D) Chronic noninvasive pressure-volume loop data showing the effects of AVIM therapy and favorable ventricular remodeling at 6 months, with reductions in left ventricular (LV) volumes and pressures.

scheduled to undergo pacemaker implantation with placement of LBBA leads. Subjects without intrinsic 1:1 AV conduction were excluded from this acute study to allow for each subject to serve as their own control. The study was approved by the ethics committee, and all patients provided written informed consent.

After placing the right atrial lead in a standard fashion, 2 ventricular pacing leads were temporarily placed: one in a standard non-LBBA position (in the right ventricle) and a second in the LBBA confirmed using standard methods. The leads were then connected to a pacemaker capable of delivering AVIM therapy. The pacemaker was programmed to pace the

atrium at a rate of approximately 5 beats/min above the intrinsic sinus rate and allow intrinsic AV conduction.

PV loops were measured under 5 pacing conditions, allowing for 5-minute stabilization periods at each setting: 1) atrial pacing alone (with intrinsic conduction); 2) AV sequential (DDD) pacing with AV delay = 130 milliseconds; 3) return to baseline atrial pacing; 4) AVIM therapy pacing; and 5) final return to atrial pacing. The second lead was then connected to the pacemaker and the sequence repeated (Supplemental Figure 1). The order in which AVIM vs AV sequential pacing, and the order in which lead location (right ventricle vs LBBA) were tested in this sequence, were randomly varied to prevent systematic bias. After completion of the protocol, the RV lead was removed, leaving the LBBA lead for use in the permanent pacemaker system. PV loops were calibrated and analyzed as detailed in the following sections.

ACUTE INVASIVE PV LOOPS. PV loops were obtained by using a previously described conductance catheter (CD Leycom).⁷ In brief, a pigtail catheter with a solid-state pressure sensor in the middle of an array of 12 equally spaced electrodes (10 mm spacing) was placed in the left ventricle. A high-frequency alternating electrical current of known amperage was passed between the most proximal and most distal electrodes, creating an electric field within the left ventricle. Voltage drops measured between successive pairs of electrodes are inversely proportional to the cross-sectional LV area at the level of the electrode pair; segmental volumes were estimated by the product of the cross-sectional area and the known, fixed distance between electrodes. Total estimated ventricular volume was calculated by summing the segmental volumes of each electrode pair within the LV chamber. The raw time-varying signal was calibrated by determining a gain factor (α) and an offset factor (ie, the parallel conductance), which were determined based on preprocedural echocardiographic measurements of LV ejection fraction (EF) and end-diastolic volume (EDV).⁸

After calibration, PV loops were analyzed to yield EDV and end-systolic volume (ESV) and end-diastolic and end-systolic pressures. LV end-systolic PV relationships (ESPVRs) were determined from single-beat methods from which end-systolic elastance (E_{es} ; a measure of LV contractility) and V_0 (the ESPVR volume axis intercept) were determined.^{9,10} Similarly, end-diastolic PV relationships were also determined from single-beat methods.^{11,12} Effective arterial elastance (E_a) was determined as the ratio

TABLE 1 Demographic Characteristics of Subjects in the Acute Invasive PV Loop Study (N = 16)

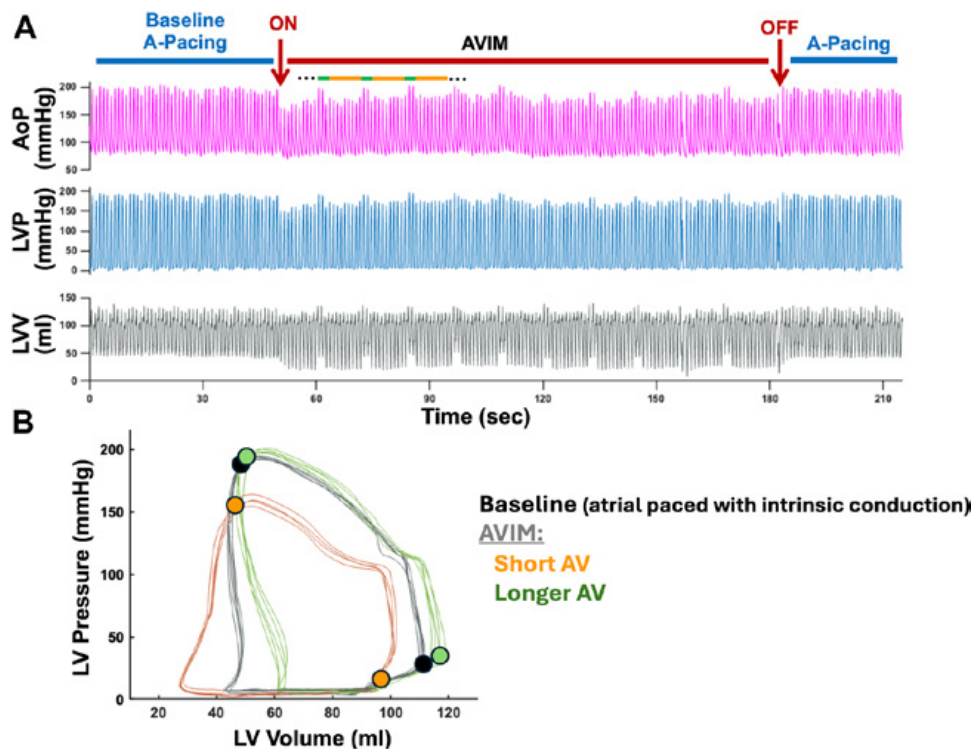
Age, y	71.7 ± 10.4
Male	7 (44)
Height, cm	168.6 ± 10.2
Weight, kg	80.8 ± 16.6
LVEF, %	63.4 ± 4.9
Pacemaker indication ^a	
Sick sinus syndrome	19
AV block I	1
AV block II	7
AV block III	0
Other	1
Blood pressure	
Office SBP, mm Hg	144.0 ± 25.0
Office DBP, mm Hg	70.7 ± 11.9
Office heart rate, beats/min	70.5 ± 16.7
Medication use, no.	2.3 ± 1.1
Potassium-sparing diuretic	9
Beta-blocker	4
ACE inhibitor	13
ARB	0
CCB	7
Centrally acting agent	3
Medical history	
Diabetes	4
Coronary artery disease	4
Prior atrial fibrillation	2
Stroke	1

Values are mean ± SD or n (%) unless otherwise indicated. ^aSubjects may have more than one indication for a pacemaker implant.

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; AV = atrioventricular; CCB = calcium-channel blocker; DBP = diastolic blood pressure; LVEF = left ventricular ejection fraction; PV = pressure-volume; SBP = systolic blood pressure.

between end-systolic pressure and stroke volume. All beats chosen for analysis were obtained at end-expiration.

CHRONIC (6-MONTH) EFFECTS OF AVIM THERAPY ON BLOOD PRESSURE, LV FUNCTION, AND LV STRUCTURE. The chronic effects of AVIM therapy on noninvasive PV loops were assessed by comparing 6-month follow-up data vs baseline data obtained from subjects in the MODERATO II study. Details of the inclusion criteria, follow-up measurements, and study endpoints have been previously described in detail.⁵ In brief, MODERATO II was a prospective, multicenter (13 European centers), double-blind study investigating the efficacy of therapy in subjects with uncontrolled HTN and an indication for implantation or replacement of a dual-chamber pacemaker. Subjects were required to be taking a stable antihypertensive medical regimen for HTN for at least 6 weeks, with average daytime ambulatory

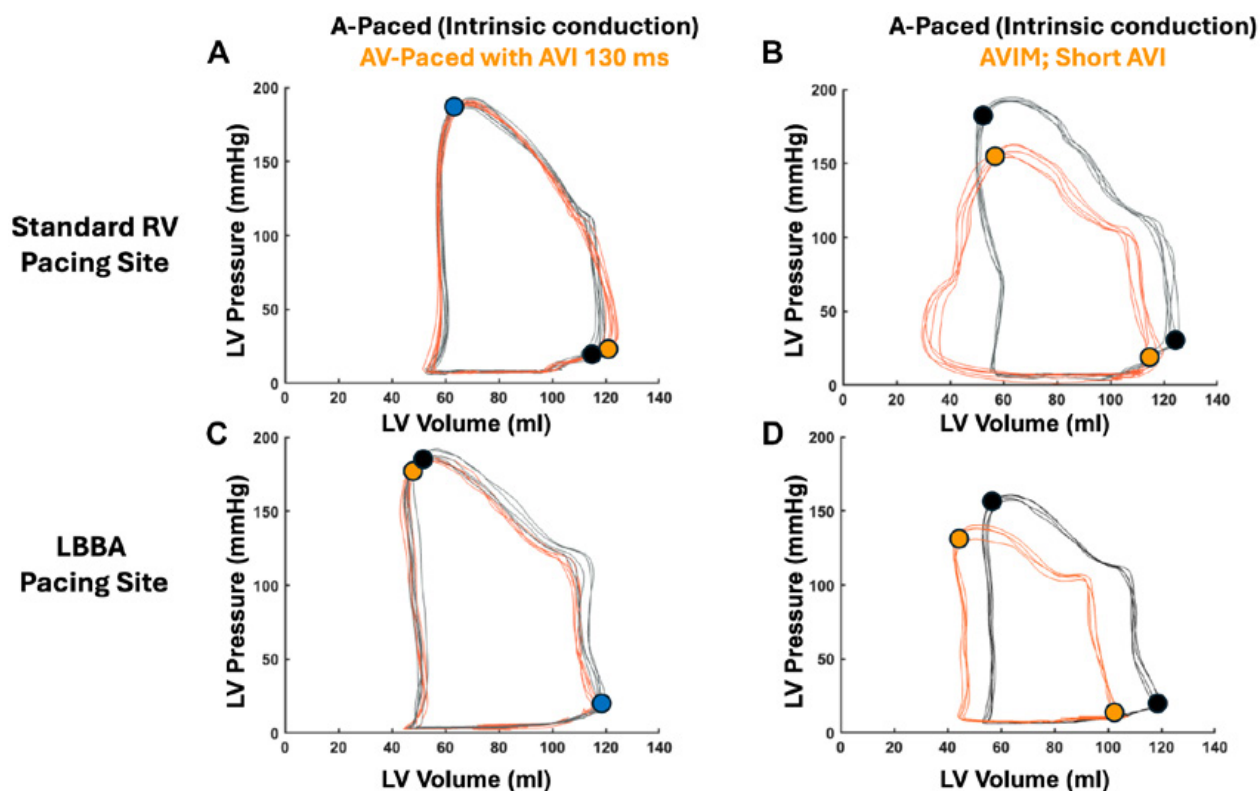
FIGURE 1 Example of Hemodynamic Response to AVIM Therapy

(A) Continuous left ventricular (LV) pressure (LVP), LV volume (LVM), and aortic pressure (AoP) tracings during atrial pacing (A-Pacing) with intrinsic conduction, during atrioventricular (AV) interval modulation (AVIM) therapy, and during resumption of A-Pacing. In this example, AVIM therapy consisted of 10 beats with a short AV interval (orange bars) and 2 beats with a longer AV interval (green bars). (B) Concomitantly measured pressure-volume loops from intrinsic AV conduction beat during A-Pacing (black), from a long AV interval beat during AVIM therapy (green), and from a short AV interval beat during AVIM therapy (orange). As detailed in the text, the loops measured during longer AV interval beats were similar to those during normal A-Pacing with intrinsic conduction. Loops measured during short AV interval beats had reduced preload and, accordingly, generated less pressure. End-diastolic and end-systolic points of each curve are shown by the dots at the bottom right and top left of each loop, respectively.

systolic blood pressure (sBP) ≥ 130 mm Hg and office systolic blood pressure (oSBP) ≥ 140 mm Hg. Subjects with LV EF $< 50\%$ and/or symptoms of heart failure (NYHA functional class II or higher) were excluded, and both groups continued with prerandomization medical therapy. Follow-up was conducted at 1, 3, and 6 months after randomization for assessment of primary and secondary endpoints. A subgroup of subjects ($n = 32$) agreed to be evaluated after 12 months of AVIM therapy. Echocardiographic images and blood pressure measurements were collected at each time point with AVIM therapy turned off and on (in both groups) so that both the sonographer and the echocardiographic core laboratory were blinded to group assignment.

NONINVASIVE PV ANALYSIS. As described previously,^{13,14} noninvasive PV loops were constructed from 3 points on the PV plane estimated from the brachial SBP and diastolic blood pressure (DBP), LV EDV, and ESV. LV end-diastolic pressure was estimated from Doppler measures of E and e' waves: LV end-diastolic pressure $\approx 11.96 + 0.596 \cdot E/e'$. E_a , a measure of ventricular afterload imposed by the arterial system, was quantified by dividing the estimated LV end-systolic pressure by stroke volume.

STATISTICAL ANALYSIS. For the acute study, an average of volume and pressure parameters from 3 to 5 cardiac cycles with intrinsic AV conduction was compared vs the average obtained using the specific

FIGURE 2 Example Pressure-Volume Loops During Pacing and AVIM Therapy

Invasive pressure-volume loops comparing ventricular mechanics during steady pacing from the atrium (with intrinsic conduction), pacing from a standard right ventricular (RV) site (A and B), and pacing from the left bundle branch area (LBBA) (C and D). Panels A and C compare loops measured during steady A-Pacing with intrinsic AV conduction vs those measured during AV sequential pacing with an AV interval (AVI) of 130 milliseconds from RV and LBBA sites, respectively. Panels B and D compare loops measured during steady A-Pacing with intrinsic AV conduction vs those measured during the short AVI beats (50 milliseconds) during AVIM therapy from RV and LBBA sites. End-diastolic and end-systolic points of each curve are shown by the dots at the bottom right and top left of each loop (note: blue dots indicate points where the pressure-volume points of 2 loops on a particular graph are coincident). Abbreviations as in [Figure 1](#).

pacing modality and lead position. Sixteen subjects had a full set of data comparing baseline vs AVIM therapy in both lead positions, and 14 of the 16 subjects had a full set of data comparing baseline vs standard pacing. For the chronic study, all patients who had paired baseline and 6-month data ($n = 32$ [11 control and 21 treatment]) were included in the primary analysis of 6-month results. For an additional supplemental analysis of longer term effects, all patients who had data at 6 months and 12 months and received AVIM therapy for a full 12 months were included ($n = 14$). For all cohorts, descriptive statistics (means, medians, SDs, and percentages) were used to summarize findings.

Data are presented as mean $\pm$ SD, and comparisons were made with paired two-sided Student *t*-tests. *P* values <0.05 were considered statistically significant. No corrections were made for multiple comparisons. A sample size of 16 subjects enabled the

detection of a >10 mm Hg reduction in pressure with 80% power ($P = 0.05$).

Evaluation of the long-term effects of AVIM therapy included all subjects with paired data at baseline and 6 months (32 subjects), allowing comparison of changes in the evaluated parameters using paired Student *t*-tests. Descriptive statistics were used.

RESULTS

ACUTE EFFECTS OF AVIM THERAPY ON BLOOD PRESSURE AND LV FUNCTION FROM DIFFERENT LEAD LOCATIONS. Baseline characteristics of subjects who participated in the acute invasive PV loop study are summarized in [Table 1](#). Patients averaged 71 years of age, had an average oSBP of 144 mm Hg despite taking an average of 2.3 antihypertensive medications, had normal LV function, were receiving a first-time pacemaker implant (ie, no replacement

devices), and had numerous comorbid conditions typical of the pacemaker patient population.

Figure 1A shows tracings of aortic pressure, LV pressure, LV volume, and simultaneously measured PV loops (Figure 1B) during atrial pacing with intrinsic conduction, with the onset of AVIM therapy and with cessation of AVIM therapy and resumption of atrial pacing with intrinsic conduction. In this example, compared with baseline, SBP decreased by approximately 23 mm Hg on the 10 beats with a short AV delay during AVIM therapy. On the 2 beats with longer AV delays, SBP approximates the pressures seen during baseline atrial pacing.

Comparable effects are also noted in the LV volume signal: reduced EDV during the short AV delay beats and EDVs approximating baseline values during beats with a longer AV delay. The effects are clearly evident on the corresponding PV loops (Figure 1B). It is worth noting that as seen on the time-domain plots, the blood pressure reduction with initiation of AVIM therapy is immediate, substantial, and stable, returning to baseline without an overshoot upon resumption of intrinsic conduction. This observation (lack of overshoot) indicates that during AVIM therapy, there is no baroreflex activation in response to the significant reduction of blood pressure during the short AV interval, as would typically be expected to occur without the autonomic nervous system modulation achieved through the intermittent introduction of the longer AV interval.¹²

The impact of lead location on AV sequential pacing and AVIM therapy is shown as an example in the PV loops of Figure 2. Figures 2A and 2C present examples of PV loops with atrial pacing (with intrinsic AV conduction) compared with AV sequential pacing (with a 130-millisecond AV interval) with standard RV lead location (Figure 2A) and LBBA lead position (Figure 2C). There is no significant impact on the PV loops between atrial and ventricular pacing with either lead location. Detailed comparisons of the atrial pacing (with intrinsic AV conduction) to RV pacing (with both standard and LBBA lead locations) on LV chamber properties are summarized in Table 2 (n = 14). Except for a nonsignificant approximately 3 mm Hg average reduction of aortic SBP with LBBA pacing, there was no significant impact of ventricular pacing at either site on ventricular pressures, volumes, contractility (indexed by single beat estimates of E_{es} and V_o), stroke work, or PV area, which correlates to myocardial oxygen consumption.

PV loops measured during beats with a short AV interval are similarly affected with standard and LBBA pacing locations. In both cases, preload and

TABLE 2 Hemodynamic and PV Loop Parameters Derived From Steady-State Pacing Comparing A-Pacing vs RV Pacing With Lead Position Either in a Standard Location or With LBBA Location: Results From the Acute Hemodynamic Study (n = 14)

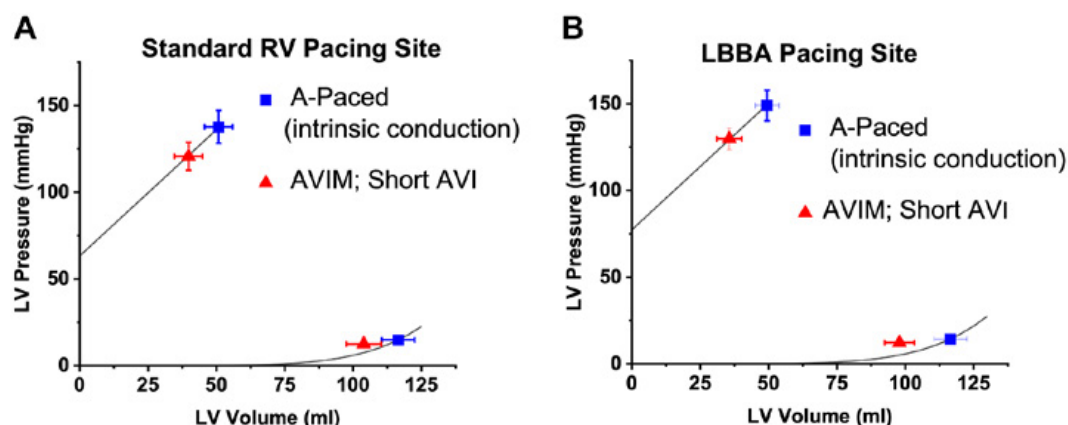
	A-Pacing	RV Pacing	Difference	P Value
Standard RV pacing site				
Aortic SBP, mm Hg	136.4 ± 35.6	134.7 ± 36.8	−1.7 ± 5.1	0.236
EDV, mL	125.6 ± 22.8	124.0 ± 26.3	−1.6 ± 16.8	0.721
ESV, mL	51.7 ± 18.2	53.8 ± 19.3	2.1 ± 12.0	0.528
EDP, mm Hg	9.6 ± 5.5	9.9 ± 5.5	0.3 ± 2.4	0.659
E_{es} , mm Hg/mL	1.03 ± 0.31	1.05 ± 0.32	0.02 ± 0.2	0.696
V_o , mL	−76.6 ± 36.2	−74.4 ± 42.8	2.3 ± 22.9	0.714
E_a , mm Hg/mL	1.94 ± 0.93	1.98 ± 1.0	0.04 ± 0.45	0.726
SW, mm Hg · mL	9,440 ± 3,336	9,398 ± 4,303	−42 ± 1692	0.927
PVA, mm Hg · mL	17,900 ± 6,782	17,593 ± 8,295	−1,564 ± 5,554	0.727
LBBA pacing				
Aortic SBP, mm Hg	138.5 ± 28.6	135.5 ± 27.1	−3.0 ± 4.1	0.016
EDV, mL	122.0 ± 22.3	121.4 ± 24.7	−0.6 ± 17.9	0.895
ESV, mL	53.2 ± 21.8	54.9 ± 23.2	1.6 ± 8.8	0.499
EDP, mm Hg	9.8 ± 4.2	9.5 ± 4.9	−0.3 ± 2.9	0.718
E_{es} , mm Hg/mL	1.31 ± 0.59	1.31 ± 0.63	−0.01 ± 0.4	0.945
V_o , mL	−44.2 ± 94.1	−46.6 ± 72.9	−2.4 ± 45.5	0.805
E_a , mm Hg/mL	2.06 ± 0.74	2.04 ± 0.86	−0.03 ± 0.47	0.823
SW, mm Hg · mL	9,291 ± 2,496	8,606 ± 3,006	−685 ± 1,729	0.162
PVA, mm Hg · mL	15,704 ± 6,902	14,807 ± 5,728	−897 ± 2,887	0.266

A-Pacing = atrial pacing; E_a = effective arterial elastance; EDV = end-diastolic volume; EDP = end-diastolic pressure; ESV = end-systolic pressure; E_{es} = end-systolic elastance, which is the slope of the end-systolic pressure-volume relationship; LBBA = left bundle branch area; PVA = pressure-volume area; RV = right ventricular; SW = stroke work; V_o = volume axis intercept of end-systolic pressure-volume relationship; other abbreviations in Table 1.

TABLE 3 Hemodynamic and PV Loop Parameters on the Short AV Delay Beats During AVIM Pacing Compared With Steady-State Beats During A-Pacing: Results From the Acute Hemodynamic Study

	Steady-State A-Pacing	AVIM	Change	P Value
RV pacing AVIM				
Aortic BP, mm Hg	137.7 ± 37.7	120.7 ± 31.7	−17.1 ± 10.1	<0.001
EDV, mL	116.5 ± 23.9	103.9 ± 25.6	−12.6 ± 14.0	0.003
ESV, mL	50.8 ± 20.5	39.8 ± 20.4	−11.0 ± 11.4	0.002
EDP, mm Hg	14.8 ± 5.6	12.4 ± 5.8	−2.3 ± 3.9	0.031
E_{es} , mL/mm Hg	1.48 ± 0.48	1.46 ± 0.63	−0.02 ± 0.41	0.858
V_o , mL	−42.1 ± 31.7	−44.9 ± 33.8	−2.8 ± 15.6	0.481
E_a , mL/mm Hg	2.14 ± 0.67	1.91 ± 0.71	−0.23 ± 0.39	0.032
SW, mm Hg · mL	8,066 ± 3,176	6,470 ± 3,360	−1596 ± 1934	0.005
PVA, mm Hg · mL	14,258 ± 6,006	11,138 ± 5,044	−3120 ± 2918	<0.001
LBBA pacing AVIM				
Aortic SBP, mm Hg	149.0 ± 35.4	129.8 ± 25.5	−19.2 ± 12.6	<0.001
EDV, mL	116.5 ± 23.9	97.9 ± 21.7	−18.6 ± 16.2	<0.001
ESV, mL	49.6 ± 17.5	35.6 ± 18.2	−14.1 ± 14.6	0.002
EDP, mm Hg	14.3 ± 5.7	12.2 ± 5.3	−2.13 ± 3.9	0.045
E_{es} , mL/mm Hg	1.47 ± 0.36	1.49 ± 0.39	0.02 ± 0.28	0.795
V_o , mL	−54.3 ± 29.6	−52.4 ± 31.5	1.88 ± 18.0	0.682
E_a , mL/mm Hg	2.32 ± 0.68	2.01 ± 0.50	−0.31 ± 0.55	0.037
SW, mm Hg · mL	9,077 ± 4,102	7,207 ± 3,667	−1,870 ± 2,051	0.002
PVA, mm Hg · mL	16,705 ± 7,378	12,589 ± 5,865	−4,116 ± 3,297	<0.001

Abbreviations as in Tables 1 and 2.

FIGURE 3 Average End-Systolic and End-Diastolic Pressure-Volume Points From A-Pacing and RV Pacing From the Acute Study

Plots of average ($\pm$ SE of the estimate) end-systolic and end-diastolic pressure-volume points measured during steady state A-Pacing with the corresponding points during the short AVI pacing using a standard RV pacing site (A) or LBBA pacing site (B). End-systolic pressure-volume relationship and end-diastolic pressure-volume relationships are derived from single-beat methods applied only to the data obtained during the respective A-Pacing. Other abbreviations as in [Figures 1 and 2](#).

pressure generation are significantly decreased, as shown in [Figures 2B and 2D](#). Average results are summarized in [Table 3](#) ($n = 16$). Independent of lead location, the hemodynamic effects of AVIM therapy are comparable in all parameters, including the almost 20% reduction in SBP. Importantly, similar to AV sequential pacing, delivery of short AV intervals and AVIM therapy from either the RV or LBBA locations had no significant impact on LV contractility (indexed by E_{es} and V_o).

To further summarize this key finding visually, we plotted the average end-systolic and end-diastolic PV points measured during steady-state atrial pacing with the corresponding points during the short AV interval pacing with the 2 different sites of ventricular pacing ([Figure 3](#)). The ESPVR and end-diastolic PV relationship displayed on these graphs are derived from single beat methods applied during atrial pacing. As seen in the graphs, on average, AVIM therapy reduces preload volume and pressure and end-systolic volume and pressure generation, without a significant change in LV contractility. In addition, there was a significant reduction in E_a , indicative of a reduction in total peripheral resistance and ventricular afterload. Finally, stroke work and PV area were significantly reduced by approximately 20% with the short AV delay, signifying a reduction in myocardial oxygen consumption; this

effect was observed without a significant impact on stroke volume.

CHRONIC (6-MONTH) EFFECTS OF AVIM THERAPY ON BLOOD PRESSURE AND LV FUNCTION.

From the original cohort of subjects who participated in the MODERATO II study, echocardiograms were available for 32 subjects: 11 from the control arm and 21 from the treatment arm. Nineteen (60%) subjects received the Moderato system as a new implant and 13 (40%) as a replacement device. There were no significant differences between treatment and control arm subjects in terms of baseline characteristics ([Table 4](#)). The cohort was 56% male, had normal LV EF ($59.4\% \pm 5.9\%$), an average oSBP of 151.9 ± 14.3 mm Hg, and aSBP of 136.4 ± 11.1 mm Hg despite being prescribed an average of 3 antihypertensive medications. Notably, isolated systolic HTN was present in approximately 88% of subjects.

Group-averaged noninvasive PV loops at baseline and after 6 months' follow-up are shown in [Figure 4](#); quantitative details are provided in [Table 5](#). In patients receiving AVIM therapy, SBP decreased by 11.7 mm Hg (158.2 ± 19.1 mm Hg vs 146.5 ± 16.6 mm Hg; $P = 0.02$), and EDV was lowered by 7.5 mL (118.1 ± 30.3 mL vs 110.6 ± 27.9 mL; $P = 0.096$) when comparing baseline vs 6-month follow up. This indicates a leftward shift of the end-diastolic

pressure volume relationship (ie, reverse remodeling) (**Central Illustration**). Concomitantly, LVEF decreased by $4.3\% \pm 6.1\%$ (from $63.7\% \pm 6.4\%$ to $59.4\% \pm 6.2\%$; $P = 0.004$).

As further summarized in **Table 5** and evident in **Figure 4**, while SBP decreased by 11.7 ± 21.8 mm Hg ($P = 0.023$), end-systolic volume did not change significantly, signifying a small reduction in LV contractility. Estimated end-diastolic pressure did not change (18.9 ± 3.4 vs 18.5 ± 2.3 mm Hg; $P =$ not significant). In control subjects, there was no change in blood pressure, and LV EDV increased significantly (111.0 ± 12.4 mL vs 125.0 ± 19.8 mL; $P = 0.03$), indicating progressive remodeling. The change in EDV was significantly different between the control and treatment groups ($P = 0.005$). There were no changes in any of the other hemodynamic parameters assessed, and, importantly, the changes observed occurred with minimal change in antihypertensive therapy in either group (**Table 6**).

A subset of treatment subjects had echocardiographic images that were suitable for analysis at 12-month follow-up ($n = 14$). As summarized in **Supplemental Table 1**, all of the hemodynamic benefits observed at 6 months were maintained in this subgroup, including the reductions in oSBP and EDV.

DISCUSSION

The current study examined the acute and chronic effects of AVIM therapy on LV size and function via PV analyses. In the acute setting, PV loops measured with the conductance catheter confirmed that the main mechanism of blood pressure reduction is a reduction in LV preload volume. Prior findings showing that AVIM therapy reduces blood pressure without increasing ventricular afterload as indexed by E_a ¹⁵ were also reconfirmed. This is significant in that in contrast to AVIM therapy, with simple short AV pacing without intermittently interspersed beats with longer AV intervals, reductions in blood pressure are counteracted by acute activation of the sympathetic system; it was therefore inferred that AVIM reduces blood pressure without activation of the sympathetic nervous system. As also shown in our subjects with normal EF, there was no acute impact of RV pacing on LV contractility, neither from a standard RV site nor from LBBA. Acute AVIM therapy was equally effective at reducing blood pressure with pacing delivered using a standard RV lead location or an LBBA site. Because CSP using leads in the LBBA continues to gain prominence clinically, these data provide important details on the

TABLE 4 Baseline Demographic Characteristics of Subjects Included in the Chronic (6-Month Follow-Up) Noninvasive PV loop Substudy of the MODERATO II Study

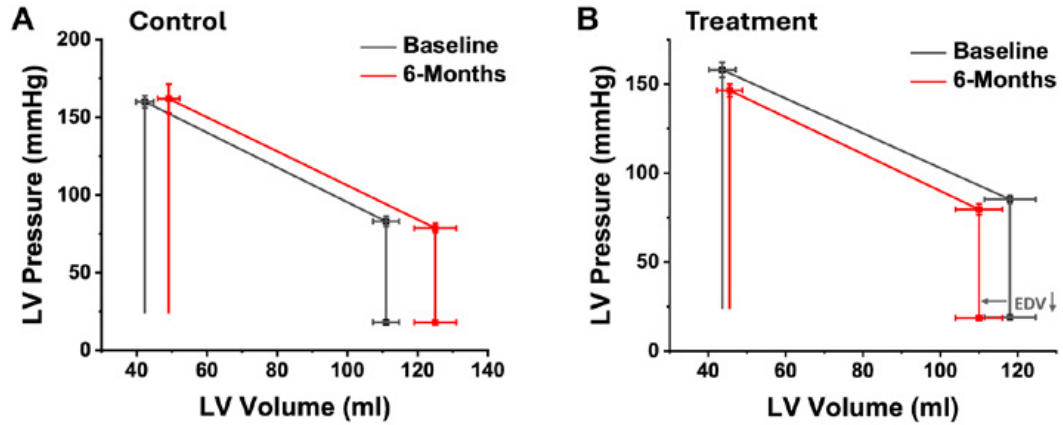
	All (N = 32)	Control (n = 11)	Treatment (n = 21)	P Value
Age, y	73.2 $\pm$ 9.0	73.4 $\pm$ 9.8	73.0 $\pm$ 8.8	0.927
Male sex	18 (56)	7 (64)	11 (52)	0.712
Height, cm	168.4 $\pm$ 9.8	165.9 $\pm$ 8.1	169.7 $\pm$ 10.6	0.311
Weight, kg	85.3 $\pm$ 17.2	84.7 $\pm$ 19.2	85.6 $\pm$ 16.5	0.898
LVEF, %	59.4 $\pm$ 5.9	57.5 $\pm$ 3.9	60.3 $\pm$ 6.6	0.210
Pacemaker				
New implant	19 (59)	7 (64)	12 (57)	1.000
Replacement	13 (41)	4 (36)	9 (43)	
Indication				
Sick sinus syndrome	9 (28)	4 (36)	5 (24)	0.681
Bradycardia	6 (19)	2 (18)	4 (19)	1.000
AV block I	5 (16)	2 (18)	3 (14)	1.000
AV block II	12 (38)	3 (27)	9 (43)	0.465
AV block III	3 (9)	1 (9)	2 (10)	1.000
Other	2 (6)	1 (9)	1 (5)	1.000
Baseline blood pressures				
Office SBP, mm Hg	151.9 $\pm$ 14.3	152.0 $\pm$ 16.0	151.9 $\pm$ 13.7	0.993
Office DBP, mm Hg	80.3 $\pm$ 11.7	77.0 $\pm$ 13.5	82.0 $\pm$ 10.6	0.256
Office heart rate, beats/min	64.8 $\pm$ 9.1	63.7 $\pm$ 9.5	65.3 $\pm$ 9.2	0.645
aSBP, mm Hg	136.4 $\pm$ 11.1	137.5 $\pm$ 14.1	136.4 $\pm$ 9.6	0.795
aDBP, mm Hg	73.8 $\pm$ 6.2	74.8 $\pm$ 5.9	73.3 $\pm$ 6.4	0.529
aHR, beats/min	71.9 $\pm$ 9.2	67.9 $\pm$ 9.9	73.9 $\pm$ 8.3	0.078
Isolated systolic HTN	28 (88)	8 (73)	20 (95)	0.110
Medications, n	3.0 $\pm$ 1.4	2.7 $\pm$ 1.3	3.2 $\pm$ 1.5	0.399
Loop diuretic	18 (56)	5 (45)	13 (62)	0.465
Potassium-sparing diuretic	3 (9)	1 (9)	2 (10)	1.000
Beta-blocker	11 (34)	2 (18)	9 (43)	0.248
ACE inhibitor	20 (63)	9 (82)	11 (52)	0.140
ARB	11 (34)	2 (18)	9 (43)	0.250
CCB	20 (63)	6 (55)	14 (67)	0.703
Alpha-agonist	3 (9)	1 (9)	2 (10)	1.000
Centrally acting agent	3 (9)	2 (18)	1 (5)	0.266
Medical history				
Diabetes	12 (38)	3 (27)	9 (43)	0.465
Prior atrial fibrillation	5 (16)	3 (27)	2 (10)	0.310
Coronary artery disease	12 (38)	5 (45)	7 (33)	0.703
Stroke	1 (3)	0 (0)	1 (5)	1.000

Values are mean $\pm$ SD or n (%) unless otherwise indicated.

aSBP = average 24-hour ambulatory systolic blood pressure; aDBP = average 24-hour ambulatory diastolic blood pressure; aHR = average 24-hour heart rate; HTN = hypertension; other abbreviations as in **Tables 1 and 2**.

acute hemodynamic effects of LBBA pacing as related to delivery of AVIM therapy.

In the acute study using invasive PV measurements, we found that LV contractility in subjects with normal EF was not significantly impaired by RV pacing, whether from a standard RV region or from LBBA compared with atrial pacing. This was the case for both standard pacing and pacing with short AV intervals during AVIM therapy. With longer term exposure, there was an approximately 4% (absolute) reduction in LV EF. Noninvasive PV loops suggest that there may be a reduction in LV contractility as

FIGURE 4 Noninvasive Pressure-Volume Loops During Long-Term Follow-Up

Noninvasively estimated group-averaged pressure-volume loops comparing baseline vs 6 months' follow-up in control (A) vs treatment (B) subjects. Over the follow-up period, the left ventricular (LV) end-diastolic volume increased in the control subjects (remodeling), whereas end-diastolic volume decreased in the treatment subjects (reverse remodeling). [Table 5](#) provides details on the quantitative analysis.

LV pressure generation decreased by 11.7 mm Hg, whereas end-systolic volume remained the same. Importantly, the changes in contractility occurred with a significant reduction in LV EDV and no increase in estimated LV EDP, indicating beneficial

reverse ventricular remodeling, and this suggests that the reduction of contractility is not clinically meaningful. Comparing the effects in the AVIM therapy treatment group vs those of the control group, which represents the natural progression of adverse remodeling (progressive LV dilatation), underscores the beneficial impact of AVIM therapy on cardiac remodeling.

Using a noninvasive approach to PV analysis, long-term AVIM therapy exerts beneficial effects on LV size. As shown in the control group of the MODERATO II study, LV EDV increased during the 6-

TABLE 5 Summary of PV Loops Hemodynamics of Subjects Participating in the Chronic Noninvasive PV Loop Substudy

	Baseline	6 Months	Change	P Value	
				Baseline vs 6 Months	Treatment vs Control
Treatment (n = 21)					
Office SBP, mm Hg	158.2 ± 19.1	146.5 ± 16.6	−11.7 ± 21.8	0.023	0.147
Office DBP, mm Hg	85.2 ± 10.3	79.6 ± 13.8	−5.6 ± 14.2	0.087	0.800
EDV, mL	118.1 ± 30.3	110.6 ± 27.9	−7.5 ± 19.8	0.096	0.005
ESV, mL	43.6 ± 15.9	45.5 ± 15.1	1.9 ± 10.9	0.431	0.233
EF, %	63.7 ± 6.4	59.4 ± 6.2	−4.3 ± 6.1	0.004	0.175
E/e'	11.7 ± 5.7	11.0 ± 3.9	−0.71 ± 3.9	0.415	0.916
eEDP, mm Hg	18.9 ± 3.4	18.5 ± 2.3	−0.42 ± 2.3	0.414	0.915
E _a , mm Hg/mL	2.0 ± 0.6	2.1 ± 0.5	0.10 ± 0.53	0.424	0.359
Control (n = 11)					
Office SBP, mm Hg	160.2 ± 12.8	162.5 ± 30.9	2.2 ± 30.8	0.814	
Office DBP, mm Hg	83.1 ± 11.2	78.7 ± 9.8	−4.4 ± 7.8	0.090	
EDV, mL	111.0 ± 12.4	125.0 ± 19.8	14.0 ± 18.1	0.028	
ESV, mL	42.3 ± 8.3	49.1 ± 10.4	6.8 ± 10.9	0.064	
EF, %	62.1 ± 4.8	60.9 ± 4.0	−1.2 ± 6.2	0.547	
E/e'	10.5 ± 2.3	10.0 ± 4.1	−0.54 ± 5.24	0.741	
eEDP, mm Hg	18.2 ± 1.4	17.9 ± 2.4	−0.32 ± 3.12	0.547	
E _a , mm Hg/mL	2.1 ± 0.3	2.0 ± 0.7	−0.11 ± 0.7	0.612	

EF = ejection fraction; E/e' = ratio of Doppler-derived peak E and e' waves; other abbreviations as in [Tables 2 and 4](#).

TABLE 6 Number of Medication Changes (Any Increase or Decrease in Dose) Between Baseline and 6-Month Follow-Up by Study Group and Drug Class (for All Subjects Included in the Chronic PV Loop Study)

	Treatment		Control	
Subjects with a change in medications	3 (14%)		2 (18%)	
	Increase	Decrease	Increase	Decrease
Diuretic	3	2	1	1
ACE inhibitor	0	1	1	1
ARB	1	1	0	1
Beta-blocker	1	1	0	0
Potassium-sparing diuretics	1	0	0	0
CCB	0	1	1	0
Sum of all changes	6	6	3	2

Abbreviations as in [Table 1](#).

month follow-up period. Such a change is referred to as ventricular remodeling and is, in part, a consequence of progressively increased wall stress imposed on the myocardium with chronic pressure overload, one of the adverse consequences of persistent uncontrolled HTN. In the MODERATO II treatment group, remodeling was not only prevented but was reversed, a phenomenon known as reverse remodeling (**Central Illustration**). The primary focus of the MODERATO II study was to examine the impact of AVIM therapy on blood pressure and safety. The study showed an average 12.4 mm Hg reduction in oSBP and a 11.1 mm Hg reduction in aSBP despite nearly constant antihypertensive therapies with no safety concerns. The effects on oSBP were maintained throughout 24 months of follow-up. The current analysis extends these findings by looking at the short- and long-term mechanisms associated with the clinical effects seen.

STUDY LIMITATIONS. In the acute studies of invasive studies of PV loops, E_{es} and V_o were determined by the widely used single-beat method.^{9,10} A more accurate means of assessing these parameters involves use of transient inferior vena cava occlusion. This would require obtaining additional vascular access and result in longer procedure times; it was therefore deemed unjustifiable for the purposes of the current study. Furthermore, many of the relevant findings are evident by examining the configuration of the PV loops without the need to resort to evaluation of the PV relations. For example, it is worth noting the minimal changes in the contour of the PV loop comparing atrial vs ventricular pacing from either the RV or LBBA (**Figures 2A and 2C**). Nevertheless, it could be that a more accurate means of assessing E_{es} and V_o would have been more sensitive in detecting pacing site-related changes on contractility. In addition, the impact of 6-month exposure to AVIM therapy on LV size and function are from a subset of MODERATO II subjects with technically suitable echocardiographic images, resulting in an unbalanced number of control and treatment patients. Nevertheless, there were no significant differences in baseline characteristics between the included control and treatment subjects. The chronic study relied on noninvasive assessments of PV loops based on echocardiographic measurement of volumes, which are subject to variability due to different angles of the imaging plane. Finally, in the chronic setting, we examined AVIM delivered only through

RV pacing; delivery of AVIM through LBBA pacing may have obviated the small reduction in LV EF and contractility noted by noninvasive PV analysis.

CONCLUSIONS

In this acute study using invasive PV analysis, we showed that the predominant mechanism by which AVIM therapy reduces blood pressure is reduction of LV preload and afterload without compensatory sympathetic activation. AVIM therapy is equally effective in the acute setting whether delivered from a standard RV lead location or from LBBA (CSP). Furthermore, in these subjects with normal EF, RV pacing from either a standard lead location or LBBA did not have a significant acute effect on LV contractility compared with that measured during atrial pacing with intrinsic conduction. In a separate cohort, noninvasive PV analysis revealed that long-term AVIM therapy not only prevented but reversed the progressive increase of LV volume seen in the control group (ie, AVIM therapy induced ventricular reverse remodeling). Data from these acute and chronic PV loop studies support the mechanism of action and show a favorable impact of AVIM therapy on cardiovascular hemodynamics. The ongoing BACKBEAT pivotal study will include patients receiving AVIM through both RV pacing and CSP to further elucidate the long-term impact of pacing site on AVIM therapy and LV function.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: A large proportion of patients with a pacemaker have HTN that is poorly controlled despite medical therapy. With increased duration of exposure to high blood pressures, myocardial mass increases and the LV chamber increases in size, a process called remodeling. AVIM therapy is a pacemaker-based approach that reduces blood pressure through delivery of repeating sequences of short and longer AV intervals. Over a 6-month period, the blood pressure reductions achieved with delivery of AVIM

therapy were statistically and clinically significant, sustained in nature, and reversed LV chamber enlargement, a process called “reverse” remodeling.

TRANSLATIONAL OUTLOOK: An ongoing prospective, randomized, double blind, pivotal study (the BACKBEAT trial) is investigating the safety and efficacy of AVIM therapy as a treatment for HTN in patients with a guideline indication for a dual-chamber pacemaker.

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KEY WORDS atrioventricular interval modulation, hypertension, pacemaker

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

P. Stojadinovic et al.

Acute durability of cavotricuspid isthmus block after pulsed electric field ablation: randomized comparison of two pentaspline catheter configurations (SECTION trial)

Europace
Impact Factor: 7,5



Acute durability of cavotricuspid isthmus block after pulsed electric field ablation: randomized comparison of two pentaspline catheter configurations (SECTION trial)

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Aims

Cavotricuspid isthmus (CTI) ablation is commonly performed alongside catheter ablation of atrial fibrillation (AF). However, the acute efficacy of the CTI ablation using the pentaspline catheter and pulsed electric field (PEF) energy has not been systematically evaluated. This randomized study assessed the acute efficacy and extent of haemolysis associated with CTI ablation when performed using two different configurations of the pentaspline catheter.

Methods and results

A total of 178 patients (age 65 ± 10 years, 66% of males) undergoing PEF ablation of the CTI in conjunction with AF ablation were randomly assigned to receive ablation using either the basket configuration ($n = 95$) or the flower configuration ($n = 83$) of the pentaspline catheter. The CTI ablation was performed before left atrial ablation. It was guided by intracardiac echocardiography, and bidirectional block was confirmed by pacing manoeuvres. Venous blood samples to assess haemolytic biomarkers were collected before and immediately after the CTI ablation. The groups were broadly comparable in baseline characteristics. The flower group demonstrated superior procedural efficiency, with fewer applications required to achieve a CTI block (3.4 ± 3.1 vs. 8.0 ± 4.1 , $P < 0.001$), a shorter time to block (96 ± 289 vs. 177 ± 192 s, $P < 0.001$), and fewer total applications (10.1 ± 3.4 vs. 13.3 ± 5.1 , $P < 0.001$). Acute reconduction occurred in 20% of cases overall, but was significantly lower in the flower group (6% vs. 32%, $P < 0.001$; hazard ratio: 0.14, 95% confidence interval: 0.06–0.40). Haemolysis was notably lower in the flower group, with significantly less post-procedural free haemoglobin (154 ± 112 vs. 210 ± 115 mg/L, $P < 0.001$). One case of transient ST elevations occurred in the flower group without clinical consequence.

Conclusion

Pulsed electric field ablation of the CTI using the flower configuration of the pentaspline catheter demonstrated higher acute efficacy in achieving CTI block and a more favourable safety profile regarding haemolysis compared to the basket configuration. This is likely due to the larger footprint and improved tissue contact of all electrodes, minimizing the leakage of PEF energy into the blood pool.

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Graphical Abstract

PULSED ELECTRIC FIELD ABLATION OF CAVOTRICUSPID ISTHMUS

POPULATION



178 patients
AF ablation and
concomitant CTI ablation



Four high-volume EP
centres in Czechia

INTERVENTION

- **CTI** prior to LA ablation
- **Pentaspine** catheter
- **ICE** guided procedure

1:1 randomization

Basket
configuration
N = 95 pts



Flower
configuration
N = 83 pts



Bidirectional CTI block confirmed using
pacing manoeuvres.

RESULTS

- **Flower** superior to basket configuration:
 - Fewer applications
 - Less hemolysis
 - Lower reconnection rate
- No additional value of adenosine testing
- One asymptomatic ST elevation in flower configuration

CONCLUSION

**FLOWER CONFIGURATION
SHOULD BE PREFERRED
APPROACH!**

Keywords

Catheter ablation • Atrial flutter • Pulsed electric field

What's new?

1. Pulsed electric field ablation using the pentaspine catheter is a safe, fast, and effective method for cavotricuspid isthmus (CTI) ablation.
2. The flower configuration exhibited superior performance, requiring fewer applications, which resulted in less haemolysis and lower acute reconnection rates.
3. Most acute reconductions occurred within the first 30 min of the waiting period.
4. Adenosine testing for dormant conduction on CTI did not provide any additional value.

Introduction

Catheter ablation is a well-established treatment strategy for rhythm control in symptomatic patients with atrial fibrillation (AF), with pulmonary vein (PV) isolation (PVI) being the cornerstone of the ablation procedure.¹⁻³ In addition to PVI, cavotricuspid isthmus (CTI) ablation is often performed as a concomitant procedure, especially in patients with documented or inducible typical atrial flutter or as part of a more extensive ablation strategy.⁴

Traditionally, CTI ablation has utilized radiofrequency (RF) energy, demonstrating excellent acute success rates and long-term durability.^{5,6} However, the introduction of pulsed electric field (PEF) ablation as a non-thermal and tissue-selective modality has emerged as a promising alternative to conventional energy sources.⁷⁻¹¹ Despite the increasing interest in PEF ablation, its application to CTI ablation is still relatively

new, and data regarding the acute durability of CTI block achieved with PEF energy are currently limited.

This study aimed to systematically evaluate the acute durability of the CTI block when ablation was performed using PEF energy. We specifically compared two configurations, the basket and flower, of the pentaspine catheter (Farawave, Boston Scientific Inc.). We focused on their relative efficacy in achieving acute CTI block and their association with procedure-related adverse events, such as haemolysis and coronary vasospasm.

Additionally, the study investigated the incidence and timing of reconnection on CTI during the procedural waiting period, allowing us to identify the optimal waiting time for assessing acute lesion durability. We also examined the role of adenosine administration in unmasking dormant conduction, which may provide insight into the necessity of routine adenosine testing in this context.

Methods

Study population and study design

A multicentre, randomized study was conducted at four high-volume centres in Czechia with the routine use of intracardiac echocardiography (ICE). Patients with symptomatic AF scheduled for ablation by PEF energy were eligible if they had either (i) paroxysmal AF and documented typical atrial flutter or (ii) persistent AF with planned CTI ablation as a part of a complex procedure. Exclusion criteria were a left atrial (LA) diameter of >65 mm (measured in the parasternal long-axis view), a history of CTI ablation, a history of cardiac valve surgery, significant valvular disease, and an age of <18 years. Simple randomization was used to allocate the patients into two groups in a 1:1 fashion, with basket or flower catheter configurations used to create the CTI block.

Ethical committee approval

The protocol has been approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital in Prague, reg. number 28518/24; A-24-24. The trial was registered in the [ClinicalTrials.gov](https://clinicaltrials.gov) Registry, reg. number NCT06691074 (accessible at clinicaltrials.gov). All patients provided written informed consent before the procedure.

Catheter ablation procedure

Procedures were performed under general anaesthesia or deep analgo-sedation, using propofol and fentanyl, with uninterrupted anticoagulation. After obtaining vascular access, heparin was administered to maintain an activated clotting time level of 350 s. A decapolar catheter was introduced into the coronary sinus (CS), while a duodecapolar catheter was placed in the right atrium around the tricuspid annulus. A single transseptal puncture was performed under the ICE guidance (AcuNav, Siemens). After obtaining the LA access, the 31 mm pentaspline ablation catheter was pulled back into the right atrium. Patients presenting with AF or other atrial arrhythmias at the beginning of the procedure were cardioverted.

Before initiating PEF ablation on the CTI, sublingual nitrates were administered as two nitroglycerin sprays, each at 0.3 mg. Nitroglycerin was not administered to patients with hypotension (systolic blood pressure < 90 mmHg). The CTI ablation was performed during regular atrial pacing from the proximal CS at a cycle length of 600 ms. Sequential applications of PEF energy were delivered in an overlapping manner (at least one-half of the catheter dimension) from the tricuspid annulus to the inferior cava vein under ICE guidance with careful monitoring of catheter-tissue contact. Three applications were deployed at a single site, each consisting of a train of five packets of pulses over 2.5 s. During basket ablation at the CTI, the terminal J-shaped portion of the wire remained outside the catheter. The fluoroscopic and corresponding ICE images of the pentaspline catheter positioned at the CTI are depicted in *Figure 1* in both basket and flower configurations. If an acute block could not be achieved using the study-allocated catheter configuration, the operators were allowed to employ an alternative configuration at their discretion to achieve the acute block. After demonstrating a bidirectional CTI block with standard pacing manoeuvres

(differential pacing from the low lateral right atrium and proximal CS), the surface electrocardiogram was analysed across all 12 leads to check for any ST-segment elevation.

The LA procedure was then conducted during regular atrial pacing from the proximal CS. Any recovery of conduction over the CTI and the corresponding time since the last ablation on the CTI were recorded. Dormant conduction over the CTI was assessed using an intravenous bolus of adenosine (12 mg, or 18 mg in patients weighing over 100 kg) during continuous atrial pacing immediately after finishing the CTI lesion. This testing was repeated at the end of the procedure. If necessary, additional PEF applications were delivered on the CTI at the operator's discretion.

The total waiting time and the number of PEF applications on the CTI were documented. The waiting time for CTI block durability was not pre-specified, as the duration of the LA procedure in the meantime ensured a reasonable time for CTI reconnection. In addition, the time taken for the LA procedure using the Farapulse system is highly predictable, so no difference in waiting time between groups was expected.

Pulsed electric field ablation in the left atrium

After the CTI ablation, the LA was accessed through a pre-punctured hole in the septum. Pulmonary vein isolation was performed with a pentaspline catheter under ICE guidance. The catheter was positioned sequentially at the ostia of each PV, with eight applications used per vein, with four in a basket configuration and four in a flower configuration. Additional applications at the PV ostia or antra were used as needed. Pulmonary vein isolation was confirmed by pacing and sensing from the PVs, demonstrating the exit and entry block. Ablation beyond PVI, including posterior wall, anterior wall, or lateral mitral isthmus, was performed at the operator's discretion based on the extent of LA disease, and spontaneous initiation or inducibility of AF/atrial tachycardia. The catheter was used in the flower or basket configuration to ensure a broad, overlapping lesion set.

Plasmatic biomarkers

Venous blood samples were collected to assess plasma biomarkers of haemolysis [free haemoglobin (fHb), lactate dehydrogenase (LDH), and

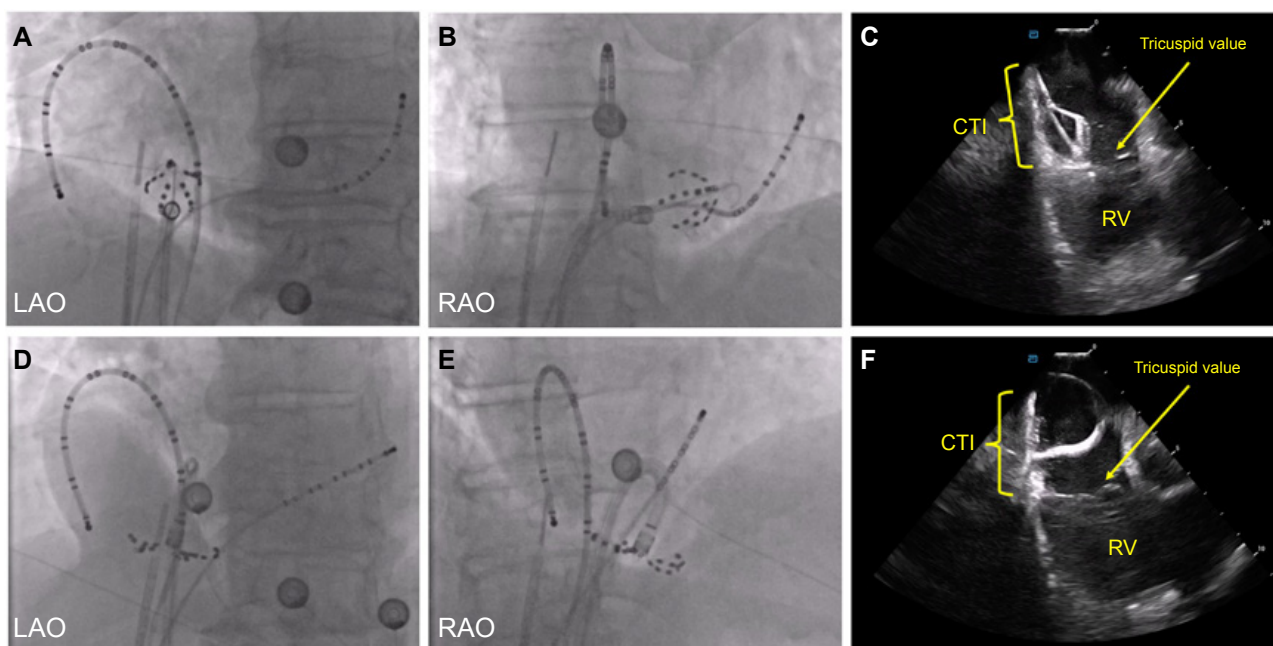


Figure 1 Position of the pentaspline catheter at the CTI. The pentaspline catheter is shown in basket configuration in LAO and RAO view (A and B) and in flower configuration (D and E) with corresponding examples of ICE imaging (C and F). Corresponding video files (mp4) are available in the [Supplementary material](#). CTI, cavotricuspid isthmus; RV, right ventricle.

Table 1 Baseline characteristics

	All N = 178	Basket group N = 95	Flower group N = 83
Females	61 (34%)	25 (26%)	36 (43%)
Age (years)	65 (10)	65 (10)	66 (10)
BMI (kg/m ²)	30.3 (5.2)	30.2 (5.3)	30.4 (5.1)
AF history (months)	40 (43)	36 (38)	44 (48)
History of typical flutter	38 (21%)	21 (22%)	17 (20%)
Previous AF ablation	21 (12%)	14 (15%)	7 (8.4%)
Arterial hypertension	121 (68%)	70 (74%)	51 (61%)
Diabetes	34 (19%)	13 (14%)	21 (25%)
Coronary artery disease	17 (9.6%)	12 (13%)	5 (6.0%)
eGFR < 60 mL/min (CKD-EPI)	19 (11%)	11 (12%)	8 (9.6%)
CHA ₂ DS ₂ -VASc	2.57 (1.59)	2.43 (1.60)	2.73 (1.58)
Echocardiography			
LVEF (%)	56 (11)	55 (11)	56 (12)
LAVi (mL/m ²)	46 (16)	46 (15)	46 (17)
LAd (mm)	45 (7)	45 (7)	46 (7)
TAPSE (mm)	22.0 (5.2)	22.5 (4.6)	21.5 (5.9)
S' (cm/s)	11.4 (4.8)	11.5 (5.1)	11.2 (4.3)
RA area (cm ²)	21 (6)	22 (6)	21 (6)
RV d1 (mm)	39.0 (5.7)	39.3 (6.6)	38.5 (4.4)
Rhythm on admission			
Sinus rhythm	127 (71%)	68 (72%)	59 (71%)
AF	42 (24%)	24 (25%)	18 (22%)
Typical flutter	5 (2.8%)	2 (2.1%)	3 (3.6%)
Atrial tachycardia	4 (2.2%)	1 (1.1%)	3 (3.6%)

Values are mean (standard deviation) or count (%).

AF, atrial fibrillation; BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; LAd, left atrial diameter; LAVi, left atrial volume index; LVEF, left ventricular ejection fraction; RA, right atrium; RVD1, right ventricle diameter; TAPSE, tricuspid annulus systolic excursion.

total bilirubin]. Samples were taken at two time points: before the procedure and after CTI isolation and before LA ablation.

Statistical analysis

While no pilot data were available for comparing different Farawave configurations on CTI, we estimated a reconnection-free rate of 80% in the basket configuration based on our clinical experience. For flower configuration, we expected an improvement of the reconnection-free rate to 95%. Such a hypothesis can be tested with a two-sided alpha of 0.05 and a power of 0.80 in a study with 76 patients in each of two arms.

All statistical analyses, based on the intention-to-treat principle, were conducted using R (The R Foundation for Statistical Computing, Vienna, Austria). Continuous variables are presented as means $\pm$ standard deviations or medians with interquartile range (IQR). Categorical variables are displayed as counts with percentages. Groups were compared using the Mann–Whitney *U* test, Pearson's χ^2 , and Fisher's exact test as appropriate. Kaplan–Meier plots with the log-rank test and a Cox proportional hazards regression model were used to compare the risk of acute CTI reconduction in randomized study groups, as well as in subgroups according to gender. Time to reconduction was used as the time-to-event variable, and hazard ratios with 95% confidence intervals were estimated for each analysis. The Cox model was also used to explore the interaction between gender and the randomization group, testing for effect modification. A *P* < 0.05 was considered significant throughout the study.

Results

Between September 2024 and May 2025, 178 patients were enrolled, and all completed the study protocol. A total of 95 patients underwent CTI ablation using the basket configuration, while 83 patients were treated with the flower configuration. Baseline characteristics listed in Table 1 were broadly comparable between the two groups, although the flower group had a higher proportion of female patients (43% vs. 26%, *P* = 0.02) and a greater prevalence of diabetes (25% vs. 14%, *P* = 0.049). Other demographic and echocardiographic parameters, including age, CHA₂DS₂-VASc score, LA volume index, and ejection fraction, showed no significant differences between groups.

Procedural data are provided in Table 2. In terms of procedural efficiency, the flower group was superior to the basket group, requiring fewer applications to achieve conduction block (3.4 ± 3.1 vs. 8.0 ± 4.1 , *P* < 0.001), shorter time to CTI block (96 ± 289 vs. 177 ± 192 s, *P* < 0.001), and fewer total applications (10.1 ± 3.4 vs. 13.3 ± 5.1 , *P* < 0.001). Cross-over occurred in two patients (both from the basket group) due to an inability to achieve the CTI block with the allocated catheter configuration. The CTI anatomy, including isthmus length and the presence of a pouch, was comparable between both groups. Furthermore, other procedural metrics, such as fluoroscopy time and total PEF pulses, were similar for both groups.

Table 2 Procedural characteristics

	All N = 178	Basket group N = 95	Flower group N = 83	P-value
Hospital stay (days)	2.57 (1.99)	2.51 (1.73)	2.64 (2.27)	0.6
Procedural skin-to-skin time (min)	82 (27)	82 (27)	81 (28)	0.7
Fluoroscopy time (min)	11.3 (4.9)	10.8 (5.0)	11.9 (4.9)	0.2
Number of applications until the CTI block	5.9 (4.3)	8.0 (4.1)	3.4 (3.1)	<0.001
Time to CTI block (min)	2.3 (4.1)	2.9 (3.2)	1.6 (4.9)	<0.001
Reconduction	35 (20%)	30 (32%)	5 (6.0%)	<0.001
Time to CTI reconduction (min)	18 (18)	15 (16)	33 (20)	0.10
Waiting time for CTI reconduction (min) ^a	45 (18)	44 (17)	46 (19)	0.9
CTI length (mm)	37 (9)	36 (10)	37 (9)	0.5
Pouch on CTI	14 (7.9%)	9 (9.5%)	5 (6.0%)	0.4
Total number of CTI applications	11.8 (4.7)	13.3 (5.1)	10.1 (3.4)	<0.001
Total number of applications	75 (18)	77 (18)	72 (18)	0.05
AADs at discharge				0.8
No AADs	88 (49%)	45 (47%)	43 (52%)	
Ic AADs	47 (26%)	26 (27%)	21 (25%)	
Amiodarone	43 (24%)	24 (25%)	19 (23%)	
Posterior wall ablation	133 (75%)	70 (74%)	63 (77%)	0.6
Anterior wall ablation	65 (37%)	34 (36%)	31 (38%)	0.8
Mitral isthmus ablation	15 (8.4%)	9 (9.5%)	6 (7.2%)	0.6

Values are mean (standard deviation) or count (%). Bold/italicized font indicate the significant P-values (<0.05).

AADs, antiarrhythmic drugs; CTI, cavotricuspid isthmus.

^aAssessed in patients without reconduction.

Acute reconductions at the CTI occurred in 35 of 178 (20%) patients. Most reconduction events on the CTI occurred during the first 30 min (Figure 2A), with nearly 50% of reconductions within the first 10 min of waiting time (Figure 2B).

The median waiting time was virtually identical in the flower and basket groups: 44 (IQR: 33–56) minutes and 43 (IQR: 33–55) minutes, respectively. Cavotricuspid isthmus reconductions were significantly less frequent in the flower group compared to the basket group [5/83 (6%) vs. 30/95 (32%), HR: 0.15, 95% CI: 0.06–0.40, $P < 0.001$]. Kaplan–Meier survival curves illustrating freedom from CTI reconduction during the waiting time are shown in Figure 3. In patients with reconduction, the median time to CTI reconduction was 9 (IQR: 5–21) minutes in the basket group and 34 (IQR: 33–45) minutes in the flower group, respectively. Notably, adenosine administration conducted early after CTI ablation and at the end of the procedure did not reveal dormant conduction across the CTI in any patient.

Cox regression analysis showed that the flower configuration compared to the basket configuration had a reduced risk of CTI reconduction, consistent across both genders (P for interaction = 0.29), and gender had no independent effect on reconduction (adjusted $P = 0.6$).

Adjunctive ablation beyond PVI was performed with comparable extent in study groups: posterior wall ablation (77% flower vs. 74% basket, $P = 0.6$), anterior wall ablation (38% vs. 36%, $P = 0.8$), and mitral isthmus ablation (7.2% vs. 9.5%, $P = 0.6$). At the time of discharge, approximately half of the patients in both groups used antiarrhythmic drugs.

Regarding haemolysis (Table 3), the basket group showed significantly higher levels of free haemoglobin after CTI ablation (210 ± 115 vs. 154 ± 112 mg/L, $P < 0.001$) and a greater absolute increase in free haemoglobin (113 ± 107 vs. 76 ± 106 mg/L, $P = 0.002$). Lactate dehydrogenase, bilirubin, and creatinine levels were comparable between

the groups. Increments of free haemoglobin per application were only non-significantly higher (by 32%) in the basket vs. the flower group (9.6 vs. 7.3 mg/L). Changes in haemolytic markers during the periprocedural period are displayed in Figure 4. Transient ST elevations were observed only in one patient from the flower group who did not receive prophylactic nitroglycerin due to baseline hypotension. This event did not require further treatment or investigation, and it did not prolong the hospital stay. No other major clinical complications occurred.

Discussion

To the best of our knowledge, this is the first study to systematically evaluate the safety and efficacy of PEF ablation of the CTI using the pentaspline catheter in both flower and basket configurations. Pulsed electric field ablation was a highly effective and safe method for CTI ablation, with reasonable acute durability of conduction block across the CTI. These results suggest that PEF is a viable alternative to traditional thermal ablation techniques for treating CTI-dependent atrial flutter, especially when CTI ablation is performed concomitantly with the ablation of AF.

One of the key contributions of our study is the comparative analysis of two catheter configurations: the flower configuration and the basket configuration. The flower configuration consistently outperformed the basket configuration across several parameters. Specifically, it achieved CTI block with fewer applications, demonstrated reduced haemolysis, and showed a lower acute reconduction rate in both male and female patient subgroups. These findings suggest that the flower configuration may facilitate more effective and homogeneous lesion formation in the CTI region due to more expansive and conformal tissue contact.

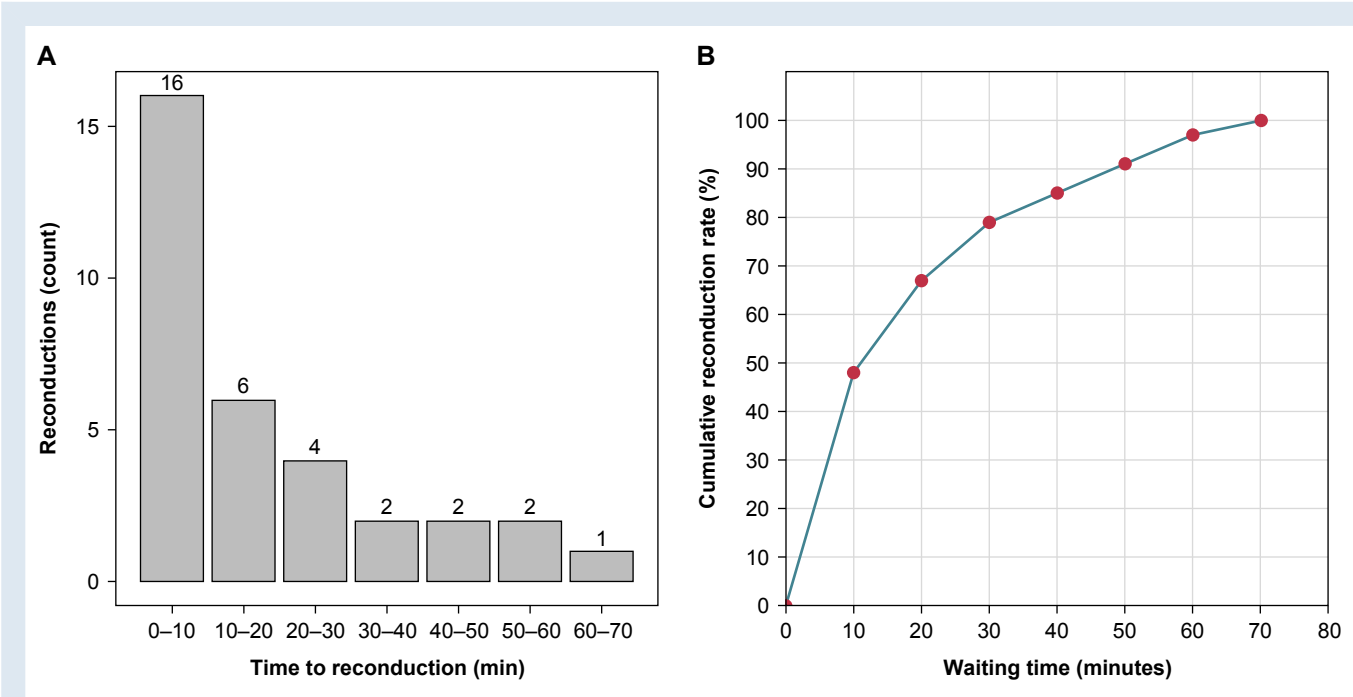


Figure 2 Temporal distribution and cumulative CTI reconduction rates during the waiting period. A graph shows a decline in reconduction rates during the waiting period. Half of them occurred during the first 10 min (A). Note that during a waiting period of 10 and 30 min, ~50% and 80% of acute CTI reconductions were detected, respectively. Longer waiting times and potential re-ablation may enhance the long-term durability of the CTI block (B).

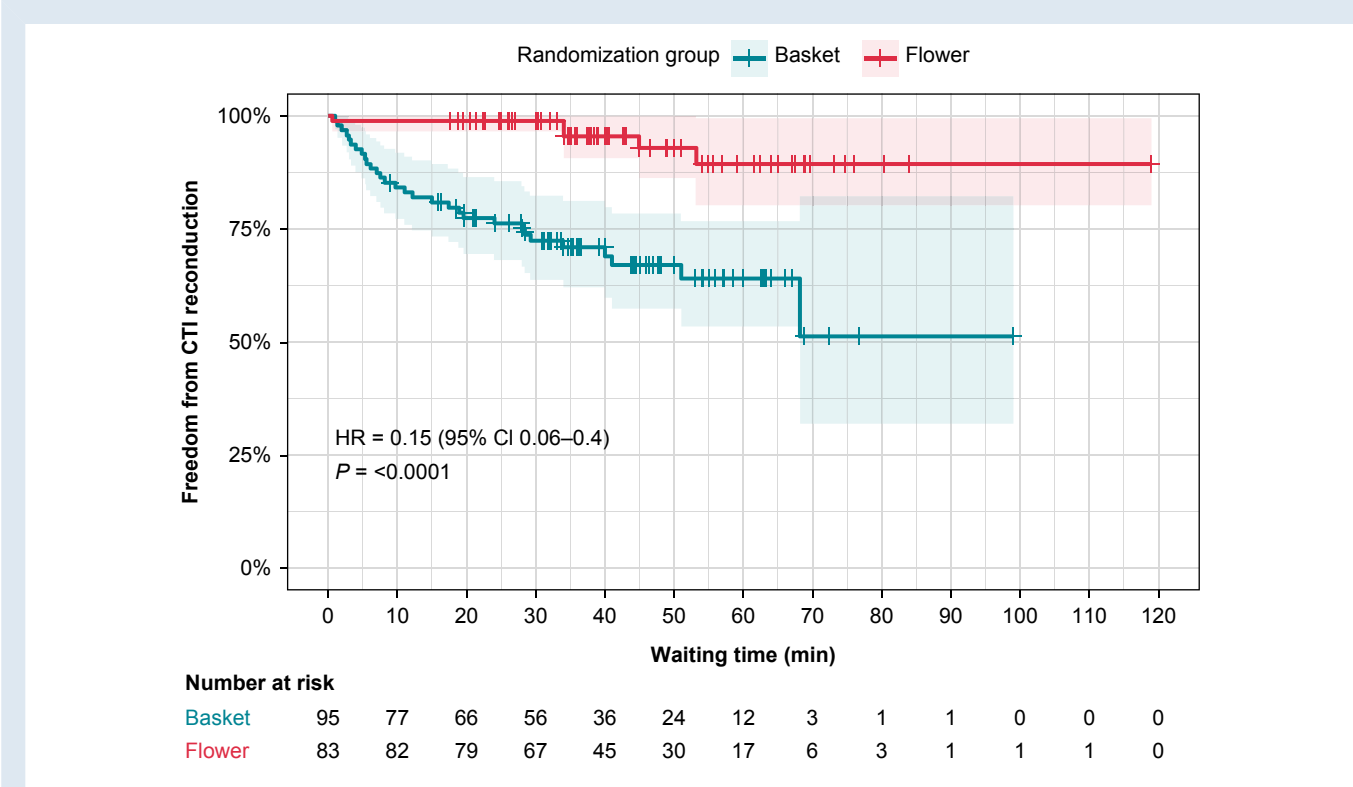


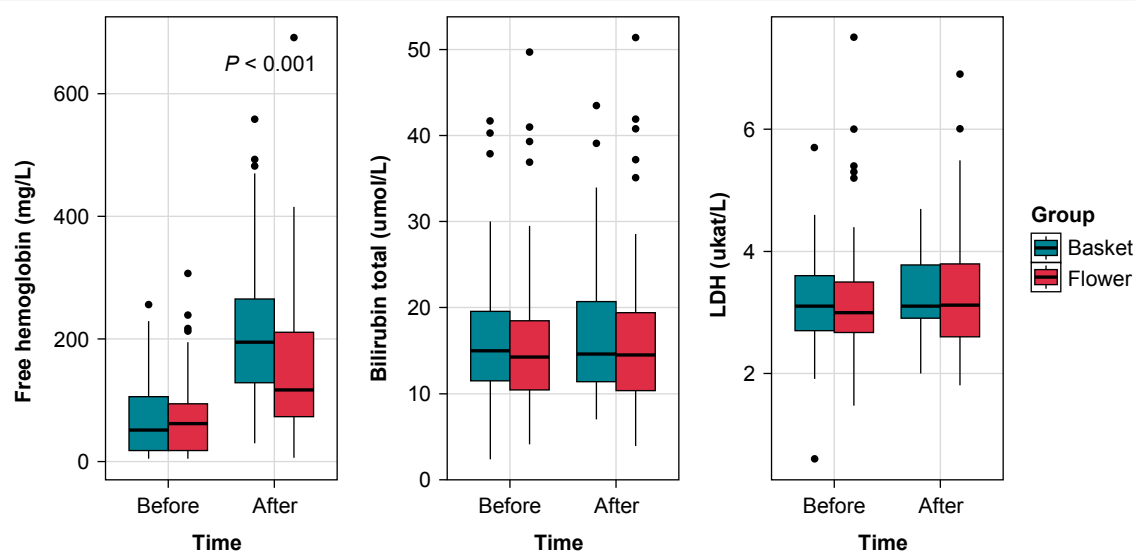
Figure 3 Freedom from CTI reconduction during the waiting time. Kaplan–Meier survival curves illustrating freedom from CTI reconduction during the waiting time dichotomized by catheter configuration (basket vs. flower). P-values are for long-rank test.

Table 3 Haemolytic markers at baseline and after the CTI ablation

	All N = 178	Basket group N = 95	Flower group N = 83	P-value
Free Hb baseline (mg/L)	72 (63)	73 (63)	71 (63)	0.9
Free Hb after ablation (mg/L)	182 (117)	210 (115)	154 (112)	<0.001
Free Hb difference (mg/L)	96 (108)	113 (107)	76 (106)	0.002
Free Hb difference per application (mg/L)	8.6 (9.5)	9.6 (10.2)	7.3 (8.7)	0.10
LDH baseline (µkat/L)	3.16 (0.80)	3.13 (0.70)	3.20 (0.91)	0.8
LDH after ablation (µkat/L)	3.29 (0.80)	3.28 (0.62)	3.31 (0.96)	0.5
Bilirubin baseline (µmol/L)	16 (8)	16 (7)	16 (8)	0.5
Bilirubin after ablation (µmol/L)	17 (8)	17 (7)	16 (9)	0.4

Values are means (SD). Bold/italicized font indicate the significant P-values (<0.05).

Hb, haemoglobin; LDH, lactate dehydrogenase.

**Figure 4** Haemolytic markers. Procedural (CTI ablation-related) changes in plasma biomarkers of haemolysis, categorized by study groups.

Our data provide valuable insights into the optimal strategy for CTI ablation using PEF. We found that most acute reconduction events occurred within the first 30 min after the initial ablation. This observation aligns with previous studies that have utilized RF energy.^{12,13} Importantly, if the waiting time were limited to just 10 min, approximately half of these reconduction cases would have been missed, which may compromise the long-term success of the procedure. This highlights the necessity of a sufficient waiting period, ideally 20–30 min after CTI ablation, to confirm a durable block. Additionally, performing CTI ablation at the beginning of the procedure proved practical and efficient, as it allowed for adequate waiting time without increasing the total procedure duration. The utility of a waiting period to detect residual connections after PEF PVI has been demonstrated in another study.¹⁴

Adenosine testing has been extensively employed after PVI or CTI ablation using RF energy to unmask dormant conduction.^{15,16} This technique enhances the durability of lesion sets by identifying transient reconductions, thereby reducing waiting time and allowing for additional ablation during the index procedure.¹⁷ However, in the context of PEF of CTI, our observations showed no evidence of reconduction

following adenosine administration. This suggests that the non-thermal mechanism of PEF may differ from the RF lesion formation mechanism. Based on these findings, we propose that adenosine should not be routinely used following CTI ablation with PEF, and its role in this context should be re-evaluated.

Our results should be considered in light of the fact that we routinely use ICE in AF ablation procedures. Intracardiac echocardiography plays a crucial role in providing real-time visualization of the catheter-tissue interface, making it a significant contributor to the success and safety of the procedure.^{18,19} It allowed us to confirm the appropriate contact of the pentaspline catheter with the CTI, especially in the area behind the Eustachian ridge, as well as the accurate positioning of the catheter to create overlapping lesions. In addition, ICE imaging can prevent excessive prolapse of the splines into the tricuspid valve annulus, which could lead to suboptimal lesion formation or increase the risk of valvular or ventricular complications. Therefore, our study likely provided the lowest estimate of CTI reconduction rates for centres that are not using ICE navigation.

From a safety perspective, the low incidence of adverse events reinforces the favourable safety profile of PEF in CTI ablation. Only one

case of transient ST elevations, probably due to coronary artery spasm, was observed in a patient in the flower group who had not been pretreated with nitroglycerin due to baseline hypotension.

A study by Nies et al.²⁰ demonstrated that haemolysis associated with *in vitro* PEF ablation is both dose- and contact-dependent. They found that the absence of catheter contact resulted in a threefold increase in free haemoglobin levels. In contrast, our findings indicate that the significantly higher haemolysis observed in the basket vs. flower group is primarily due to the greater number of applications required rather than differences in catheter-tissue contact. This is supported by a non-significantly higher increment of free haemoglobin per application in the basket vs. the flower group. The absence of a difference comparable to that in the experimental study remains speculative. In part, this can be explained by the presence of non-zero contact (at least two splines) in the case of a basket configuration and occasional suboptimum contact of the catheter in a flower configuration. None of the study patients experienced severe acute kidney injury (AKI) after the procedure. This observation is consistent with previous studies, which have shown that AKI after PEF ablation is a relatively rare event.²¹

Overall, the results from this study indicate that, although the pentaspline catheter was primarily designed for PVI, its acute efficacy and safety profile in flower configuration also support its use for CTI ablation to prevent the typical flutter in selected patients undergoing PEF ablation of AF.

Study limitations

This study has several limitations. Coronary angiography was not performed during the ablation procedures, which means that minor or asymptomatic coronary artery spasms may have been missed. We also did not systematically collect data on nitroglycerin premedication. Not providing this treatment to hypotensive patients as per the study protocol may not be an optimal strategy. We did not assess the clinical outcomes of patients as the randomized study protocol was followed only until the moment of establishing the first CTI block. Any further CTI and LA ablations were at the discretion of the operator, and the subsequent clinical endpoints primarily included recurrent AF and non-CTI-dependent atrial tachycardias. We did not use a dedicated 3D mapping system as it was not commercially available at the time of the study. The slight imbalance in group sizes resulted from simple randomization without stratification or blocking. Although baseline characteristics in both study groups were broadly comparable, we cannot completely rule out the possibility that minor differences may have affected the results. Additionally, randomization was not stratified by operator or centre, which may have introduced variability in procedural technique and outcomes. No screening log was maintained; therefore, data on non-eligible patients are unavailable, which limits the assessment of the study's external validity. From a technical perspective, recrossing the interatrial septum via a pre-punctured transseptal hole without leaving a wire in the LA may be challenging, particularly in centres without experience with ICE. The PR intervals before and after ablation were not systematically assessed. However, no cases of even first-degree atrioventricular block were reported as complications of the procedure. Importantly, the findings of this study may not be generalizable to other PEF catheter platforms, as procedural characteristics, energy delivery parameters, and catheter design may differ between systems. Finally, we were unable to evaluate the impact of both ablation strategies on the extent of myocardial necrosis because the early troponin level is not representative, while the delayed level is predominantly influenced by the extent of LA ablation.

Conclusion

Cavotricuspid isthmus ablation using PEF energy with the flower configuration of the pentaspline catheter has been found to be effective and safe in patients who have been pretreated with nitroglycerin.

The flower configuration outperformed the basket configuration by requiring fewer applications and resulting in a lower haemolysis and acute reconduction rates, which provides potential clinical benefits. A waiting period of 20–30 min for acute CTI reconduction appeared relevant, while adenosine testing did not provide additional value. The findings of the study support considering the flower configuration as a preferable approach and provide an important guide for standardizing PEF-based CTI ablation.

Supplementary material

Supplementary material is available at [Europace](https://eurpub.oxfordjournals.org/) online.

Authors' contributions

- (1) Substantial contributions to the conception and design or the acquisition, analysis, or interpretation of the data: P.S. and D.W.
- (2) Substantial contributions to the drafting of the articles or critical revision for important intellectual content: P.S., A.B., Š.H., J.P., N.V., D.W., E.B., P.Š., J.H., P.P., R.Č., and J.K.
- (3) Final approval of the version to be published: P.S., D.W., and J.K.
- (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved: P.S.

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Conflict of interest: J.K. reports personal fees from Biosense Webster, Boston Scientific, GE Healthcare, Medtronic, and St Jude Medical (Abbott) for participation in scientific advisory boards and has received speaker honoraria from Biosense Webster, Biotronik, Boston Scientific, Medtronic, ProMed CS, St Jude Medical (Abbott), and Viatrix. P.P. has received speaker honoraria from St Jude Medical (Abbott) and Biosense Webster and has served as a consultant for Biotronik, Medtronic, and Boston Scientific. The remaining authors have no disclosures.

Data availability

Data are available from the authors on request.

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L. Pleva et al.

Comparing the Efficacy of Sirolimus and Paclitaxel-Eluting Balloon Catheters in the Treatment of Coronary In-Stent Restenosis:
A Prospective Randomized Study (TIS 2 Study)

Circulation: Cardiovascular Interventions
Impact Factor: 7,4



ORIGINAL ARTICLE

Comparing the Efficacy of Sirolimus and Paclitaxel-Eluting Balloon Catheters in the Treatment of Coronary In-Stent Restenosis: A Prospective Randomized Study (TIS 2 Study)

Leos Pleva , MD, PhD; Pavel Kukla , MD; Tomas Kovarnik , MD, PhD; Jana Zapletalova, MSc, PhD

BACKGROUND: Current therapy for in-stent restenosis (ISR) is based on drug-eluting stents (DES) or drug-eluting balloon catheters. This prospective randomized study compared the efficacy of a novel sirolimus-eluting balloon (SEB) catheter to that of a paclitaxel-eluting balloon (PEB) catheter for the treatment of bare-metal stent (BMS-ISR) or DES-ISR.

METHODS: A total of 145 patients with 158 BMS or DES-ISR lesions were randomly assigned to the treatment with either SEB or PEB. The in-segment late lumen loss at 12 months, the 12-month incidence of binary ISR, and major adverse cardiac events (cardiac death, nonfatal acute myocardial infarction, or target lesion revascularization) were compared between groups.

RESULTS: The noninferiority of SEB compared with PEB in the treatment of BMS/DES-ISR with respect to late lumen loss was not demonstrated (Δ late lumen loss, -0.024 mm [95% CI, -0.277 to 0.229]; for a noninferiority margin of 0.20 mm), except in the post hoc subanalysis for the BMS-ISR group (-0.203 mm [95% CI, -0.584 to 0.178]). No significant differences in the incidence of repeated binary ISR (31.6% versus 30.4%, $P=0.906$) or 12-month major adverse cardiac events (31% for both; $P>0.999$) between the SEB and PEB groups were observed.

CONCLUSIONS: The noninferiority of SEB relative to PEB in the treatment of BMS/DES-ISR with respect to late lumen loss was not confirmed.

REGISTRATION: URL: <https://www.clinicaltrials.gov>; Unique identifier: NCT03667313.

GRAPHIC ABSTRACT: A [graphic abstract](#) is available for this article.

Key Words: angioplasty, balloon, coronary ■ coronary restenosis ■ paclitaxel ■ sirolimus

See Editorial by Paradies and Alfonso

Current therapy for in-stent restenosis (ISR) is based on drug-eluting stents (DESs) or drug-eluting balloon (DEB) catheters. To date, paclitaxel is the preferred drug for balloon coating (paclitaxel-eluting balloon [PEB] catheter) due to its irreversible binding to the microtubes, resulting in long persistence in the vascular cells.¹ Sirolimus and its analogues reversibly bind to the FK-506 binding protein 12, forming a complex with the

mammalian target of rapamycin, thus blocking cell cycle progression at the juncture of the G1 and S phases.²

In contrast to paclitaxel, sirolimus must be continuously released for several weeks to effectively inhibit neointimal proliferation.^{2,3} Moreover, in our previous TIS 1 study (Treatment of In-Stent Restenosis), we demonstrated that in BMS-ISR treatment, iopromide-coated PEB yielded significantly lower 12-month late lumen

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WHAT IS KNOWN

- Paclitaxel-eluting balloon has been used in the treatment of in-stent restenosis.
- However, the efficacy of sirolimus-eluting balloon in treating this condition remains unclear.

WHAT THE STUDY ADDS

- We evaluated the efficacy of sirolimus-eluting balloon and paclitaxel-eluting balloon in the treatment of bare-metal stent/drug-eluting stents-in-stent restenosis.
- The noninferiority of sirolimus-eluting balloon compared with paclitaxel-eluting balloon in treating in-stent restenosis with respect to 12-month late lumen loss was not demonstrated.
- The incidence of 12-month major adverse cardiac events and binary restenosis did not differ between the 2 groups.

Nonstandard Abbreviations and Acronyms

BMS	bare-metal stent
DEB	drug-eluting balloon
DES	drug-eluting stent
DS	diameter stenosis
ISR	in-stent restenosis
LLL	late lumen loss
MACE	major adverse cardiac events
MLD	minimum lumen diameter
PEB	paclitaxel-eluting balloon
TIS 1	Treatment of In-Stent Restenosis
TLR	target lesion revascularization

loss (LLL) compared with everolimus-eluting stents ($P=0.0004$). However, no differences in 12-month minimum lumen diameter (MLD; $P=0.481$), diameter stenosis (%DS; $P=0.816$) or repeated binary ISR ($P=0.078$) were found.⁴

In the case of DES, limus drugs are more effective in the inhibition of neointimal hyperplasia compared with paclitaxel.^{1,2} However, paclitaxel is still used in DEB due to its easier binding to the balloon surface and better pharmacokinetics. Sirolimus-encapsulating technologies can be used for coating balloon catheters and provide sufficient drug transfer to the vessel wall. We hypothesized that a novel sirolimus-eluting DEB (with phospholipid-encapsulated nanoparticles) may be at least as effective as PEB.

The objective of our prospective randomized study, TIS 2 (Treatment of In-Stent Restenosis 2; URL: <https://www.clinicaltrials.gov>; Unique identifier: NCT03667313), was to compare (with statistical evidence of noninferiority)

the efficacy of a novel DEB with sirolimus (MagicTouch; SEB with Nanoluté coating) with the standard iopromide-coated PEB (Sequent Please Neo) for the treatment of restenosis in bare-metal (BMS-ISR) or drug-eluting stents (DES-ISR).

METHODS

Data Availability

Anonymized data have been made publicly available at the LabArchives repository and can be accessed at: https://mynotebook.labarchives.com/share_attachment/TIS%25202%2520study/Ny44MDAwMDAwMDAwMDAwMDF8NTk2NzY5LzYtOC9UcmVITm9kZS85NjAxODEzNjF8MTkuNzk5OTk5OTk5OTk5OTk3.

Patients

Between January 2019 and May 2022, a total of 145 patients with ISR were randomly assigned to the treatment with SEB or PEB at 2 sites in the Czech Republic (University Hospital Ostrava and General University Hospital in Prague).

The study included adult patients (≥ 18 years of age) with BMS-ISR or DES-ISR ($\geq 70\%$ DS by visual estimation or 50% to 69% DS with documented evidence of ischemia). Patients with acute coronary syndromes caused by ISR were also included. In the case of ST-segment-elevation myocardial infarction and complete stent occlusion, they were included only if significant residual ISR was evident following thrombus aspiration. To exclude mechanical causes of ISR, an X-ray postprocessing system (CLEARstent; Siemens, Forchheim, Germany) was used. The principal exclusion criteria were as follows: concomitant diseases carrying expected survival of < 12 months or limiting the possibility of follow-up coronary angiography.

The in-segment LLL at 12 months, as measured by quantitative coronary angiography was the primary end point of the presented study. Secondary end points included the incidence of binary ISR ($\geq 50\%$ DS) and the overall incidence of 12-month major adverse cardiac events (MACE; cardiac death, nonfatal acute myocardial infarction, or target lesion revascularization [TLR]).

The study protocol complied with the Declaration of Helsinki and was approved by the Ethics Committee of University Hospital Ostrava, Czech Republic. The study was registered at ClinicalTrials.gov (URL: <https://www.clinicaltrials.gov>; Unique identifier: NCT03667313) on the November 23, 2018. Written informed consent was obtained from each patient before enrollment in the study.

Study Devices

The MagicTouch balloon catheter (Concept Medical, Miami, FL) is a SEB with phospholipid-encapsulated sirolimus nanoparticles (Nanoluté coating). The size of drug nanoparticles is about 100–300 nm, with a sirolimus concentration on the balloon surface of 1.27 mg/mm². The recommended inflation time is 60 seconds.

In the iopromide-coated PEB Sequent Please Neo (B. Braun, Melsungen, Germany), paclitaxel (3 $\mu\text{g}/\text{mm}^2$) is bound via the hydrophilic contrast agent iopromide (Paccocath),

increasing paclitaxel solubility and vascular wall penetration. The recommended inflation time is 30 seconds.

Randomization

Patients were randomized in a 1:1 manner to treatment with SEB or PEB. Randomization (using random permuted blocks) was stratified according to the type of original stent (BMS versus DES-ISR). The investigators and patients were not blinded to the treatment allocation. However, clinical events and angiographic measurements were recorded by an independent investigator who was blinded to avoid any bias.

Interventions

PCI was performed under standard conditions. After lesion preparation (semi-compliant or scoring balloon predilation), the DEB was inflated for 60 seconds (SEB) or 30 seconds (PEB). The implantation of an additional bailout stent was allowed in cases of in-segment suboptimal results (>30%) or dissection (>type B).⁵ The patients received dual antiplatelet therapy for 6 months.⁵

Follow-up

Clinical follow-up was performed at 6 and 12 months, and angiographic follow-up at 12 months (± 2 months), unless required earlier. Any death that was not clearly due to noncardiac causes was considered cardiac-related. The fourth universal definition by the ESC⁶ was used for the definition of myocardial infarction. Stent thrombosis, MACE, and target lesion failure (cardiovascular death, target vessel myocardial infarction, or ischemia-driven TLR) were defined according to the Academic Research Consortium criteria.⁷

Angiographic Follow-Up

The same imaging projections as in the periprocedural angiography were used for the follow-up coronary angiography. American College of Cardiology/American Heart Association criteria⁸ and the Mehran classification were used to assess the lesion type and ISR.⁹ Lesions were evaluated in the in-segment section (± 5 mm from the proximal and distal edges of the stent), and the following parameters were measured: MLD, reference lumen diameter, acute gain, lesion length, %DS, and LLL (LLL = $\text{MLD}_{\text{after intervention}} - \text{MLD}_{\text{control}}$); binary restenosis was defined as repeated narrowing of the lumen $\geq 50\%$ (DS $\geq 50\%$).⁷

Sample Size and Statistical Analysis

This noninferiority study was based on our previous TIS study,⁴ with a 12-month LLL of 0.09 (± 0.44) mm in the PEB arm. Using a noninferiority margin of 0.20 mm as the clinically significant difference between groups, an α error of 5%, and 90% power, the required sample size was 124. Accounting for a 15% dropout rate, the cohort size was set at 145 patients. The evaluation was performed on an intention-to-treat basis.

Continuous variables with normal distribution are presented as mean $\pm$ SD and compared using the Student *t* test. Nonnormal variables are reported as median (range) and analyzed with the Mann-Whitney *U* test. Categorical variables are expressed as absolute and relative frequencies (%) and compared using the χ^2 test. Odds ratios are reported with 95% CIs, with $P < 0.05$

Table 1. Baseline Characteristics

	SEB	PEB	P value
Patients, n	72	73	...
ISR lesions, n	79	79	...
Demographic and clinical parameters			
Male, n	56 (77.8%)	59 (80.8%)	0.651*
Female, n	16 (22.2%)	14 (19.2%)	
Age, y	69.7 $\pm$ 9.6†	68.0 $\pm$ 10.6†	0.319#
Body mass index, kg/m ²	27.8 (20.6–37.8)§	28.4 (20.6–38.6)§	0.523
EF LK, %	50.0 (25–68)§	50.0 (25–65)§	0.705
Ever smoked	34 (47.2%)	38 (52.1%)	0.505*
Diabetes	32 (44.4%)	26 (35.6%)	0.278*
Renal insufficiency	11 (15.3%)	6 (8.2%)	0.197*
CABG	10 (13.9%)	7 (9.6%)	0.421*
Previous MI	48 (66.7%)	50 (68.5%)	0.814*
Baseline PCI			
STEMI/NSTEMI	41 (56.9%)	47 (64.4%)	0.410*
2VD/3VD	53 (73.6%)	45 (61.6%)	0.177*
B2/C lesion	59 (74.7%)	60 (75.9%)	0.854*
Lesion localization			
LM	2 (2.5%)	2 (2.5%)	0.367*
LAD	25 (31.6%)	27 (34.2%)	
LCx/OM	14 (17.2%)	23 (29.1%)	
RCA	36 (45.6%)	25 (31.6%)	
SVG	2 (2.5%)	2 (2.5%)	
Previous stent diameter, mm	3.25 (2.25–5.0)§	3.25 (2.25–5.0)§	0.476
Previous stent length, mm	24.0 (12–66)§	24.0 (8–76)§	0.831
In-stent restenosis			
BMS-ISR	36 (45.6%)	39 (49.4%)	0.633*
DES-ISR	43 (54.4%)	40 (50.6%)	
Time to ISR, d	1224 (64–6913)§	1069 (62–5765)§	0.612
UAP/NSTEMI	26 (36.1%)	24 (32.9%)	0.750*
STEMI	3 (4.2%)	2 (2.7%)	
Stable AP	40 (55.6%)	41 (56.2%)	
Silent	4 (5.6%)	6 (8.2%)	
Repeated ISR	10 (12.7%)	9 (11.4%)	0.807*
Thrombus	10 (12.7%)	12 (15.2%)	0.646*
Ostial ISR	4 (5.1%)	5 (6.3%)	1.000*
Bifurcation	9 (11.4%)	25 (31.6%)	0.002*
Type of ISR			
I (focal, all)	21 (26.6%)	37 (46.8%)	0.008*
II (diffuse)	43 (54.4%)	21 (26.6%)	0.0004*
III (proliferative)	5 (6.3%)	6 (7.6%)	0.755*
IV (occlusion)	10 (12.7%)	15 (19.0%)	0.276*
Nonfocal (II–IV)	58 (73.4%)	42 (53.2%)	0.008*
Periprocedural parameters			
Cutting predilation	66 (83.5%)	58 (73.4%)	0.121

(Continued)

Table 1. Continued

	SEB	PEB	P value
ISR; DEB diameter	3.5 (2.5–4.0)§	3.5 (2.25–4.0)§	0.651¶
ISR; DEB length	20.0 (15–70)§	20.0 (10–65)§	0.265¶
Dilation pressure, atm	12 (12–26)§	12 (12–28)§	0.865¶
Second DEB	13 (16.5%)	12 (15.2%)	0.827*
DEB per lesion	1.23±0.55†	1.15±0.46†	0.445‡
Bailout stent implantation	18 (22.8%)	18 (22.8%)	1.000*
Duration of DAPT, mo	6 (2–8)§	6 (2–9)§	0.752¶

Qualitative data are given as n (%). 2VD indicates 2 vessel disease; 3VD, 3 vessel disease; AP, angina pectoris; atm, atmosphere; BMS, bare-metal stent; CABG, coronary artery bypass grafting; DAPT, dual antiplatelet therapy; DEB, drug-eluting balloon; DES, drug-eluting stent; EF LK, ejection fraction of left ventricle; ISR, in-stent restenosis; LAD, left anterior descending; LCx, left circumflex artery; LM, left main; MI, myocardial infarction; NSTEMI, non-ST-segment-elevation myocardial infarction; OM, obtuse marginal; PCI, percutaneous coronary intervention; PEB, paclitaxel-eluting balloon; RCA, right coronary artery; SEB, sirolimus-eluting balloon; STEMI, ST-segment-elevation myocardial infarction; SVG, saphenous vein graft; and UAP, unstable angina pectoris.

*P value: significance of χ^2 test.

†Quantitative data are given as mean (±SD).

‡P value: significance of Student 2-sample *t* test.

§Quantitative data are given as median (range: min-max).

¶P value: significance of Mann-Whitney *U* test.

considered significant. The 95% CI for the difference in LLL between study arms was compared with the noninferiority margin. Time-to-event data were analyzed with Kaplan-Meier curves and the log-rank test. The cumulative frequency distribution of LLL was assessed using the Kolmogorov-Smirnov test.

RESULTS

A total of 145 patients with 158 ISR lesions were included in the study. Basic demographic, clinical and angiographic data are presented in Table 1.

The representation of BMS- and DES-ISR was the same in both treatment arms ($P=0.633$). The SEB group had a significantly higher frequency of nonfocal (diffuse+proliferative+occlusive) ISR than focal ISR ($P=0.008$). Bifurcation ISR lesions were more common in the PEB group ($P=0.002$). Periprocedurally, patients treated with SEB achieved significantly lower residual stenosis (post-PCI %DS) compared with PEB ($P=0.025$).

A 12-month angiographic follow-up was performed in 91.7% of patients in the SEB group and 89.0% of patients in the PEB group ($P=0.592$). After 12 months, no statistically significant difference in the measured angiographic parameters between the 2 groups was observed (Table 2). However, the primary end point of our study was to evaluate whether SEB is noninferior to PEB. The difference in Δ LLL between the SEB and PEB groups was -0.024 mm (95% CI, -0.277 to 0.229). The

upper bound of the 2-sided 95% CI of LLL difference exceeded the noninferiority margin (0.20 mm); in effect, the noninferiority of SEB was not demonstrated (Table 3; Figure 1).

Nonetheless, there were no significant differences in the incidence of repeated binary ISR ($P=0.906$) or 12-month MACE ($P>0.999$) between the groups (Table 3). The 2 groups did not differ in clinical event-free survival either (Figure 2).

A post hoc subanalysis separately evaluating the treatment of BMS and DES-ISR with SEB and PEB was performed. Data for both subgroups are shown in Table 4.

The noninferiority of SEB versus PEB treatment was demonstrated only in the BMS-ISR subgroup (Δ LLL -0.203 mm; 95% CI, -0.584 to 0.178); but not in the DES-ISR subgroup (Δ LLL 0.110 mm; 95% CI, -0.233 to 0.453 ; Table 3; Figure 1).

In terms of secondary end points, no difference was observed in the incidence of recurrent binary restenosis ($P=0.949$ and >0.999) or 12-month MACE ($P=0.884$ and 0.926) in the 2 subgroups (Table 5).

After recruitment, the significantly higher incidence of nonfocal ISR in the SEB group ($P=0.008$) led to an additional post hoc subanalysis. The noninferiority of SEB compared with PEB in LLL was not demonstrated in either the focal or nonfocal ISR subgroups (Table 3).

Table 2. Baseline, Postprocedural, and 12-Month Follow-Up Quantitative Control Angiography Parameters

	SEB	PEB	P value
Preprocedural parameters; ISR			
RefD, mm	2.68 (1.87–3.63)*	2.69 (1.78–3.85)*	0.918†
MLD, mm	0.53 (0–1.61)*	0.70 (0–2.10)*	0.664†
%DS	77 (51–100)*	76 (35–100)*	0.708†
ISR length	12.6 (4.7–54.5)*	10.9 (3.7–51.3)*	0.130†
Postprocedural parameters; post re-PCI			
RefD, mm	2.90 (2.15–3.87)*	2.92 (1.98–3.79)*	0.663†
MLD, mm	2.34 (1.57–3.19)*	2.22 (1.36–3.20)*	0.079†
Acute gain, mm	1.76 (0.63–2.87)*	1.48 (0.40–3.20)*	0.078†
%DS	21 (1–40)*	24 (6–41)*	0.025†
12 months follow-up parameters			
RefD, mm	2.83 (1.89–3.90)*	2.86 (1.86–4.30)*	0.922†
MLD, mm	1.85 (0–3.58)*	1.63 (0–2.89)*	0.463†
%DS	32 (0–100)*	36 (8–100)*	0.270†
Binary re-ISR	25 (31.6%)	24 (30.4%)	0.906‡

Qualitative data are given as n (%). DS indicates diameter stenosis; ISR, in-stent restenosis; MLD, minimal lumen diameter; PCI, percutaneous coronary intervention; PEB, paclitaxel-eluting balloon; RefD, reference lumen diameter; and SEB, sirolimus-eluting balloon.

*Quantitative data are given as median (range: min-max).

†P value: significance of Mann-Whitney *U* test.

‡P value: significance of χ^2 test.

Table 3. Late Lumen Loss Difference

	SEB	PEB	Delta	95% CI	Noninferiority margin (0–20 mm)
LLL, mm					
All ISR	0.596±0.83	0.62±0.781	−0.024	−0.227 to 0.229	>0.20
BMS-ISR	0.411±0.856	0.614±0.802	−0.203	−0.584 to 0.178	<0.20
DES-ISR	0.736±0.799	0.626±0.771	0.110	−0.233 to 0.453	>0.20
Focal ISR	0.451±0.776	0.500±0.560	−0.049	−0.415 to 0.317	>0.20
Nonfocal ISR	0.694±0.853	0.738±0.901	−0.044	−0.416 to 0.328	>0.20

Quantitative data are given as mean (±SD). BMS indicates bare metal stent; DES, drug eluting stent; ISR, in-stent restenosis; LLL, late lumen loss; PEB, paclitaxel-eluting balloon; and SEB, sirolimus-eluting balloon.

DISCUSSION

Our prospective randomized study compared the efficacy of a novel SEB with sirolimus encapsulated in phospholipid nanoparticles to iopromide-coated PEB in the therapy of BMS or DES-ISR.

Sirolimus delivery to the balloon surface is challenging due to limited tissue uptake and short retention of limus drugs. Drug-encapsulation technologies, such as phospholipid-encapsulated sirolimus nanoparticles (Nanoluté coating), have shown promise for efficient vessel wall transfer with high tissue concentration and minimal systemic leakage. Lemos et al³ reported that 56% of sirolimus is released within 40 to 60 seconds of balloon inflation, with persistence in the vessel wall for 15 to 30 days. There are also other ways of applying sirolimus to the surface of the balloon catheter: the Selution SLR balloon catheter (Med Alliance, Nyon, Switzerland) uses micro-reservoirs in a biodegradable polymer as a carrier of sirolimus, while the SeQuent Please SCB (B. Braun) uses crystalline sirolimus (4 µg/mm²) bound by butylated hydroxytoluene.¹⁰

Due to different pharmacokinetics, sirolimus requires a longer balloon insufflation time (60 seconds) compared with paclitaxel (30 seconds), potentially contributing to a significantly lower postprocedural %DS in the SEB arm ($P=0.025$), although the acute gain was similar.

Several registries evaluated the efficacy of SEB with Nanoluté coating that included both de novo and ISR lesions. The Nanoluté registry followed 408 patients (45% of them with ISR) treated with SEB. The 24-month MACE occurred in 4.2% of cases and that of the ISR subgroup in 5.5%, respectively.¹¹

In the largest registry, EASTBOURNE (All-Comers Sirolimus-Coated Balloon European Registry), which included 2123 patients (44% of them with ISR) treated with SEB. TLR was more common in patients treated for ISR compared with de novo lesions (RR, 1.90 [95% CI, 1.13–3.19]). ISR was also an independent risk factor for the need for repeat TLR (odds ratio, 5.5 [95% CI, 3.38–8.88]).¹²

Recently, some randomized trials comparing Nanoluté-coated SEB and PEB have been published. The TRANSFORM I trial (A Randomized, Double-Blind, Placebo-Controlled, Phase 3 Study of Navitoclax in Combination With Ruxolitinib Versus Ruxolitinib in Subjects With Myelofibrosis) compared SEB with iopromide-PEB in the treatment of de novo small vessel disease. Similar to our study, they did not confirm the noninferiority of SEB in 6-month net lumen gain (opposite of LLL; net gain=MLD_{6M}−MLD_{baseline}; p for noninferiority=0.173).¹³

In addition, comparisons of other SEB versus PEB in the treatment of ISR have been made. In their DES-ISR study, Han et al¹⁴ demonstrated that crystalline-SEB was

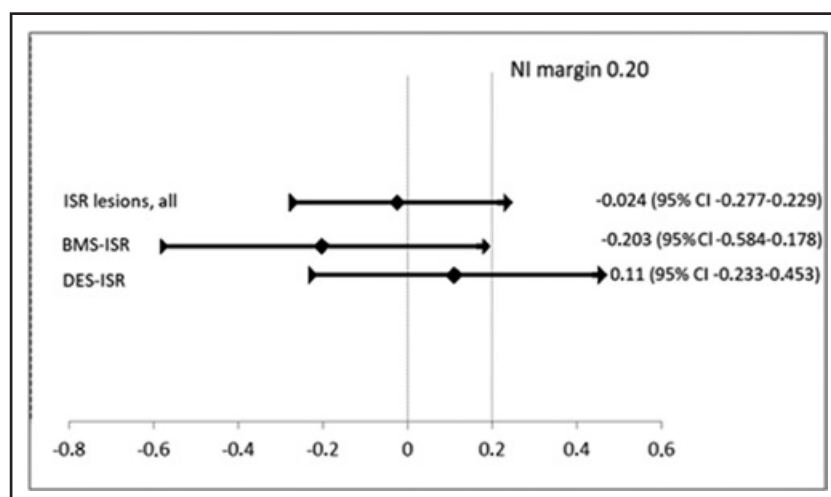


Figure 1. The 2-sided 95% CI for the difference between treatments and the noninferiority (NI) margin. BMS indicates bare metal stent; DES, drug eluting stent; and ISR, in-stent restenosis.

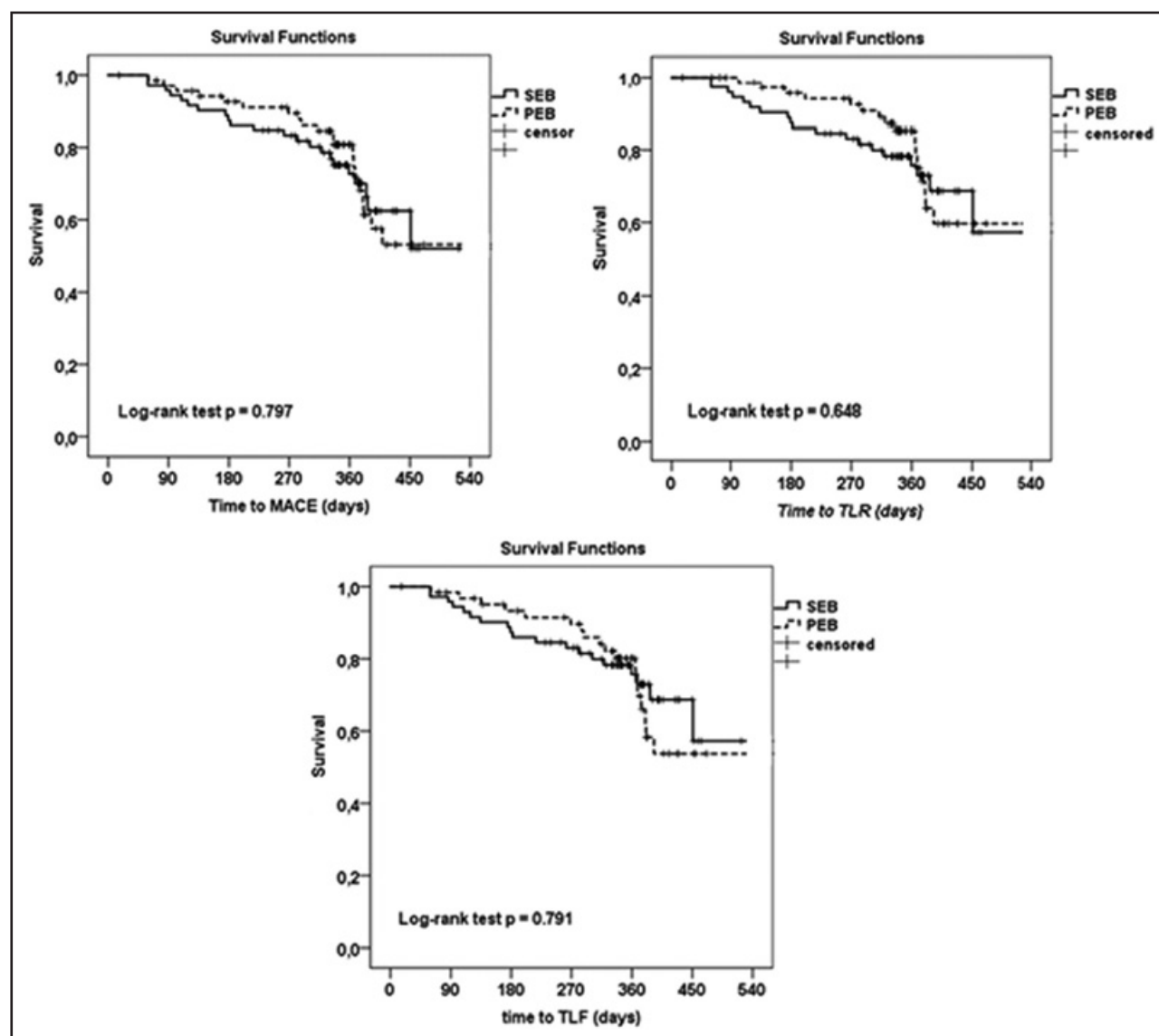


Figure 2. Kaplan-Meier analysis of event-free survival.

MACE indicates major adverse cardiac events; PEB, paclitaxel-eluting balloon; SEB, sirolimus-eluting balloon; TLF, target lesion failure; and TLR, target lesion revascularization.

noninferior to iopromide-PEB at 9 months LLL (Δ LLL, 0.05 mm [95% CI, -0.0523 to 0.1476]; with noninferiority margin of 0.20 mm). The incidence of 12-month target lesion failure and patient-oriented composite end points was similar in both groups.

A combined analysis of 2 parallel studies (Malaysian and German-Swiss studies) also compared the efficacy of crystalline-SEB with iopromide-PEB in the treatment of DES-ISR. The study demonstrated noninferiority of SEB at the primary end point, 6-month LLL ($P=0.794$). However, the follow-up time in their research was shorter, and the chosen noninferiority margin of 0.35 mm was higher than in our study. Similar to our study, the 2 groups did not differ in the 12-month incidence of MACE ($P>0.99$).¹⁵

Differences between these 2 studies and our study include different SEB coating (phospholipid-encapsulated

versus crystalline) and concentrations (1.27 mg/mm² versus 4 μ g/mm²); both these factors likely (similar to PEB) affect the SEB efficacy and require further analysis.

In our study, a 12-month angiographic follow-up was used to account for neointimal hyperplasia that continues beyond 6 months, potentially yielding more accurate findings.

Compared with BMS-ISR, the incidence of DES-ISR is lower, but the treatment of the remaining DES-ISR is more challenging due to its more complex underlying causes and is associated with poorer long-term outcomes.¹⁶ Despite this fact, we included both BMS- and DES-ISR patients in our study to be as close to real-world clinical practice as possible (with regard not only to developed countries). Patients' 1:1 randomization to SEB or PEB arms was stratified by the type of the original stent

Table 4. Subgroup Analysis

BMS-ISR	SEB	PEB	P value
Patients/lesions, n	35/36	37/39	
Preprocedural parameters; ISR			
RefD, mm	2.64 (2.04–3.63)*	2.56 (1.78–3.62)*	0.446†
MLD, mm	0.70 (0–1.50)*	0.71 (0–1.31)*	0.885†
%DS	74.0 (51–100)*	74.5 (52–100)*	0.859†
ISR length	12.6 (6.3–36.9)*	12.5 (4.0–44.3)*	0.674†
Postprocedural parameters; post re-PCI			
RefD, mm	2.88 (2.20–3.57)*	2.83 (1.98–3.79)*	0.768†
Acute gain, mm	1.48 (0.63–2.87)*	1.44 (0.43–3.20)*	0.419†
MLD, mm	2.29 (1.67–2.87)*	2.19 (1.36–3.20)*	0.293†
%DS	21 (1–40)*	25.4 (7–39)*	0.205†
Twelve months follow-up parameters			
RefD, mm	2.65 (1.89–3.90)*	2.82 (1.98–4.30)*	0.953†
MLD, mm	2.00 (0–3.14)*	1.87 (0–2.89)*	0.438†
%DS	29 (0–100)*	34 (8–100)*	0.419†
Binary re-ISR	9 (25.0)	10 (25.6)	0.949‡
DES-ISR			
Patients/lesions, n	37/43	36/40	
Preprocedural parameters; ISR			
RefD (mm)	2.72 (1.87–3.47)*	2.83 (1.90–3.85)*	0.427†
MLD (mm)	0.53 (0–1.61)*	0.68 (0–2.10)*	0.768†
%DS	80 (53–100)*	78 (35–100)*	0.915†
ISR length	11.5 (4.69–54.5)*	9.96 (3.68–51.3)*	0.100†
Postprocedural parameters; post re-PCI			
RefD (mm)	2.91 (2.15–3.87)*	3.02 (2.19–3.71)*	0.828†
Acute gain (mm)	1.81 (0.81–2.83)*	1.62 (0.40–2.89)*	0.180†
MLD (mm)	2.36 (1.57–3.19)*	2.30 (1.53–3.15)*	0.182†
%DS	20 (5–33)*	24 (6–41)*	0.033†
Twelve months follow-up parameters			
RefD, mm	2.91 (1.96–3.78)*	2.95 (1.96–3.94)*	0.760†
MLD, mm	1.81 (0–3.58)*	1.60 (0–2.77)*	0.755†
%DS	32 (3–100)*	38 (15–100)*	0.371†
Binary re-ISR	16 (37.2)	14 (35.0)	1.000‡

Qualitative data are given as n (%). BMS indicates bare metal-stent; DES, drug-eluting stent; DS, diameter stenosis; ISR, in-stent restenosis; MLD, minimal lumen diameter; PCI, percutaneous coronary intervention; PEB, paclitaxel-eluting balloon; RefD, reference lumen diameter; and SEB, sirolimus-eluting balloon.

*Quantitative data are given as median (range: min–max).

†P value: significance of Mann-Whitney U test.

‡P value: significance of χ^2 test.

implant (BMS or DES-ISR) to achieve the best balance of treatment allocation. A post hoc subanalysis confirmed the differential outcomes of DEB in BMS- and DES-ISR treatment. Only the BMS-ISR subgroup exhibited noninferiority of SEB versus PEB for the primary end point, 12-month LLL. However, it should be noted that the results of these subgroup analyses are merely exploratory in nature.

Compared with studies using DEB for de novo lesions treatment, a higher incidence of bailout stenting was noted in our study, mainly due to a higher rate of

Table 5. Twelve-Months Clinical Follow-Up

	SEB	PEB	
	n (%)	n (%)	P
MACE	22 (30.6)	23 (31.5)	0.858
Cardiovascular death	0 (0)	2 (2.7)	0.497
MI	3 (4.2)	2 (2.7)	1.000
TLR	19 (26.4)	19 (26.0)	1.000
Noncardiovascular death	0 (0)	1 (1.4)	1.000
TLF	19 (26.4)	20 (27.4)	0.891
Stent thrombosis	2 (2.5)	1 (1.3)	1.0002
2nd MACE	4 (5.6)	0 (0)	0.057

Qualitative data are given as n (%). P value: significance of χ^2 test. MACE indicates major adverse cardiac events; MI, myocardial infarction; PEB, paclitaxel-eluting balloon; SEB, sirolimus-eluting balloon; TLF, target lesion failure; and TLR, target lesion revascularization.

in-segment dissection (>type B), possibly arising due to the aggressive ISR lesion predilatation.

The 12-month in-segment LLL served as the primary end point. The use of these surrogate angiographic end points makes it possible to reduce the required file size while maintaining adequate statistical power.^{7,17} Several clinical studies have shown a correlation, especially between LLL and %DS and the incidence of TLR.^{7,17}

Intravascular imaging allows more accurate assessment of coronary ISR. However, many ISR studies continue to use angiographic primary end points. To facilitate a comparison between those studies and our earlier TIS 1 study, we have adopted a similar approach. To exclude the main mechanical causes of ISR, an X-ray postprocessing system was used.

Recent ESC guidelines advise against DEB use in DES-ISR.¹⁸ Nevertheless, this amendment is based on earlier meta-analyses comparing PEB and DES efficacy.¹⁶ Further studies are needed to evaluate SEB efficacy with novel DES.

In their meta-analysis comparing SEB with PEB in the treatment of both de novo and ISR lesions, Sedhom et al¹⁹ found no significant difference in the incidence of clinically driven TLR and other clinical outcomes, including MACE, cardiovascular death, or target vessel myocardial infarction. A subsequent subgroup analysis revealed no significant differences between the studies in regard to de novo and ISR lesions. However, the use of SEB was associated with a significantly higher rate of binary ISR, LLL, and a lower MLD compared with PEB. In addition, late lumen enlargement was more frequently observed in the PEB group compared with the SEB group.

Limitations

Several limitations of our study should be mentioned. Neither the patients nor the investigators were blinded as to the chosen method of treatment. However, the 12-month quantitative coronary angiography was evaluated by a blinded, independent investigator. The noninferiority margin

of 0.2 mm, based on previous DEB versus DES studies, reflects clinically significant LLL differences but may require further validation for DEB therapies, particularly between different drugs. The study included both BMS- and DES-ISR patients, despite the fact that the cause of DES-ISR is more complex and the treatment more challenging. The use of intravascular imaging could enhance understanding of ISR mechanisms and outcomes. Moreover, this study did not have sufficient statistical power to detect significant differences in clinical end points (ie, MACE).

Conclusions

This prospective randomized study compared the efficacy of novel SEB with that of PEB for the therapy of BMS or DES-ISR. We did not confirm the noninferiority of SEB compared with PEB in the treatment of BMS/DES-ISR with respect to LLL. The noninferiority of SEB was proven only in BMS-ISR patients.

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Disclosures

None.

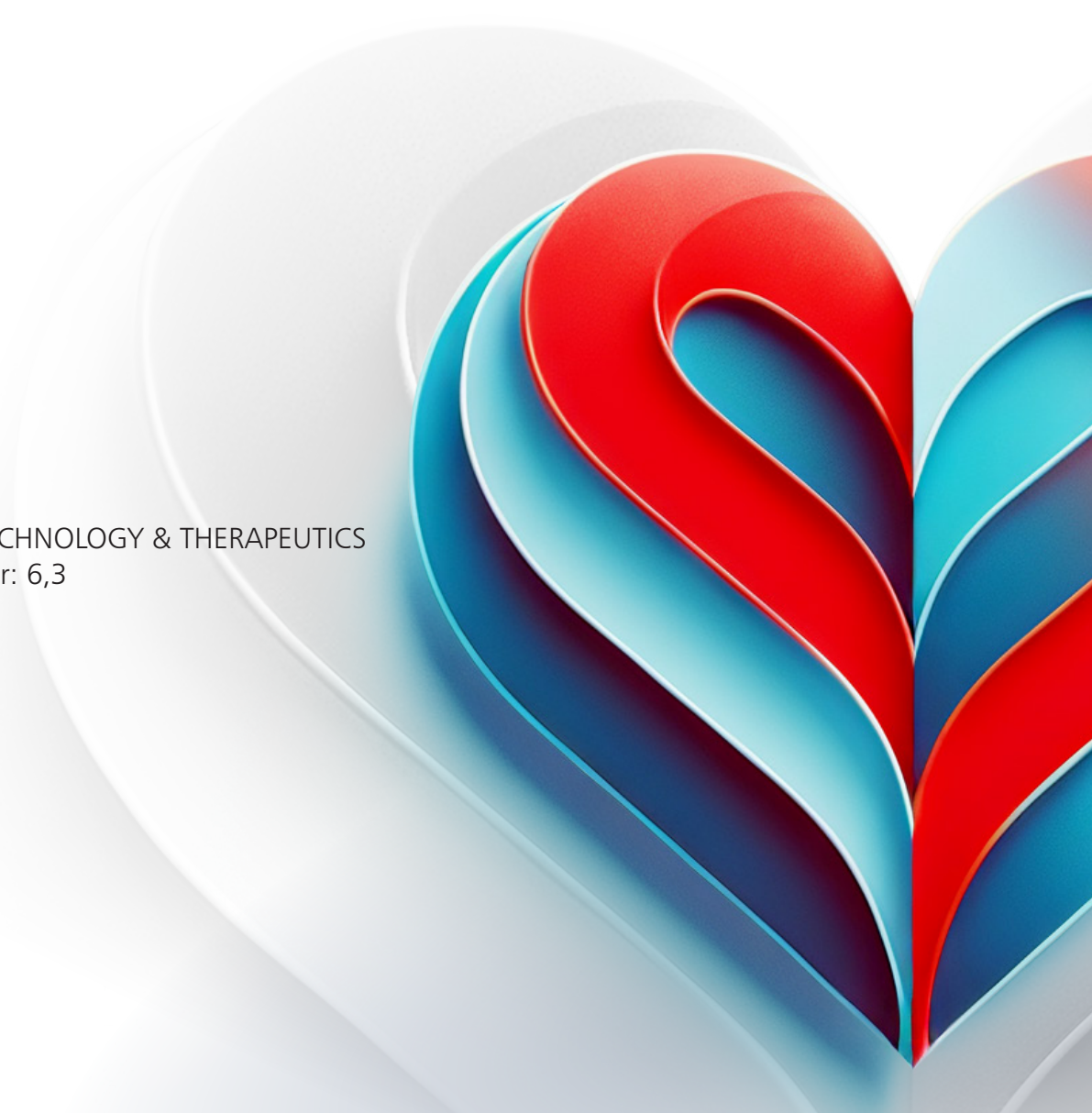
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AI-Guided Cardiac Computer Tomography in Type 1 Diabetes Patients with Low Coronary Artery Calcium Score

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ORIGINAL ARTICLE

AI-Guided Cardiac Computer Tomography in Type 1 Diabetes Patients with Low Coronary Artery Calcium Score

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Abstract

Objective: Cardiovascular risk stratification based on traditional risk factors lacks precision at the individual level. While coronary artery calcium (CAC) scoring enhances risk prediction by detecting calcified atherosclerotic plaques, it may underestimate risk in individuals with noncalcified plaques—a pattern common in younger type 1 diabetes (T1D) patients. Understanding the prevalence of noncalcified atherosclerosis in T1D is crucial for developing more effective screening strategies. Therefore, this study aimed to assess the burden of clinically significant atherosclerosis in T1D patients with CAC <100 using artificial intelligence (AI)-guided quantitative coronary computed tomographic angiography (AI-QCT).

Methods: This study enrolled T1D patients aged ≥30 years with disease duration ≥10 years and no manifest or symptomatic atherosclerotic cardiovascular disease (ASCVD). CAC and carotid ultrasound were assessed in all participants. AI-QCT was performed in patients with CAC 0 and at least one plaque in the carotid arteries or those with CAC 1–99.

Results: Among the 167 participants (mean age 52 ± 10 years; 44% women; T1D duration 29 ± 11 years), 93 (56%) had CAC = 0, 46 (28%) had CAC 1–99, 8 (5%) had CAC 100–299, and 20 (12%) had CAC ≥300. AI-QCT was performed in a subset of 52 patients. Only 11 (21%) had no evidence of coronary artery disease. Significant coronary stenosis was identified in 17% of patients, and 30 (73%) presented with at least one high-risk plaque. Compared with CAC-based risk categories, AI-QCT reclassified 58% of patients, and 21% compared with the STENO1 risk categories. There was only fair agreement between AI-QCT and CAC ($\kappa = 0.25$), and a slight agreement between AI-QCT and STENO1 risk categories ($\kappa = 0.02$).

Conclusion: AI-QCT may reveal subclinical atherosclerotic burden and high-risk features that remain undetected by traditional risk models or CAC. These findings challenge the assumption that a low CAC score equates to a low cardiovascular risk in T1D.

Keywords: AI-QCT, ASCVD risk, atherosclerosis, CCTA, risk stratification, type 1 diabetes.

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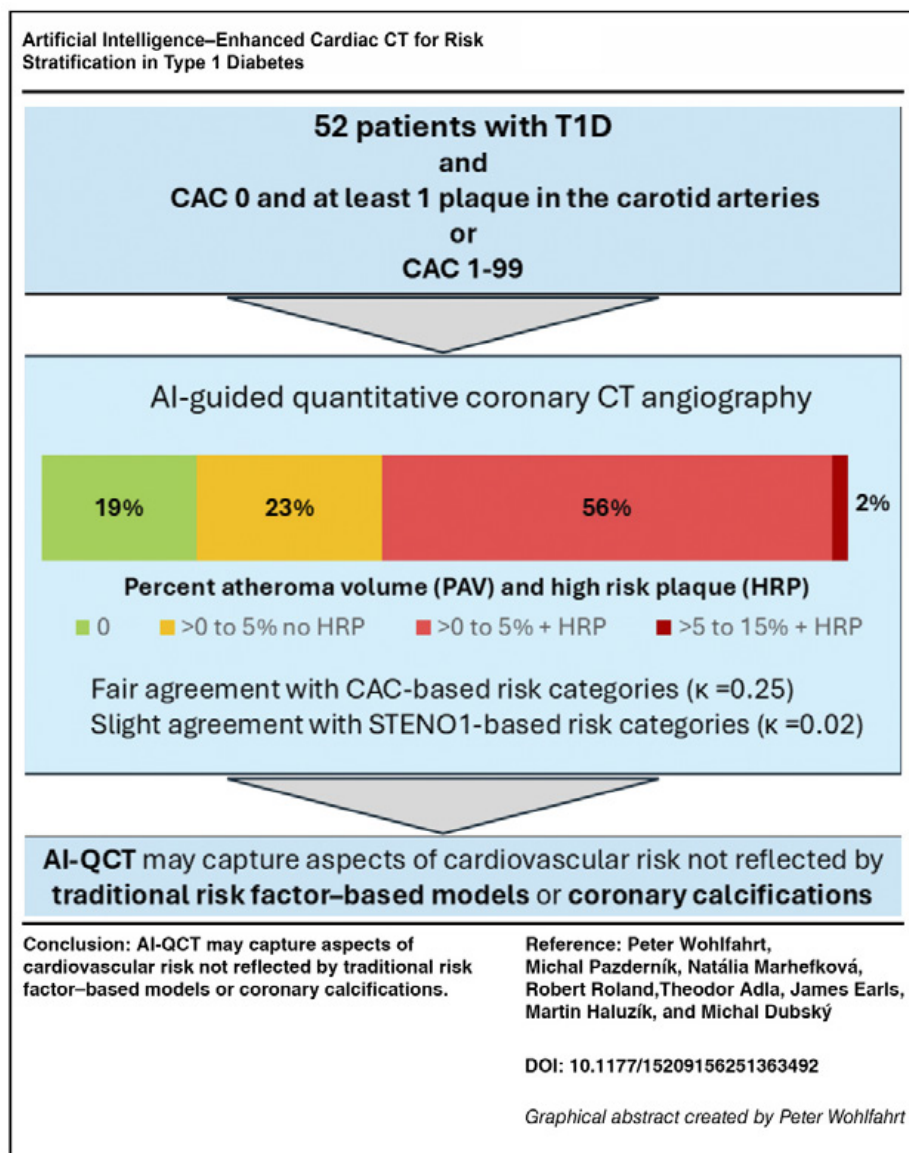
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Introduction

Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of death in individuals with type 1 diabetes (T1D). Compared with the general population, men with T1D face an almost fourfold increase in ASCVD risk, while women face an eightfold increase, resulting in a reduced life expectancy of at least 11 years.¹ Due to the early onset of T1D, ASCVD risk exceeds that of individuals with type 2 diabetes (T2D) and often manifests at a younger age. Notably, ASCVD risk in T1D spans a broad spectrum from low to very high, necessitating accurate individual-level risk stratification to guide the intensity of preventive interventions. However, since T1D accounts for only 5%–10% of all diabetes cases, ASCVD risk stratification in this population is less developed than in T2D.

Traditional risk factors have been used to predict ASCVD risk in T1D. While these models can predict the risk on a population level, they often misclassify patients on the individual level. Consequently, current guidelines assign only a Class IIB

recommendation, mainly due to the lack of supporting evidence.² The STENO1 risk engine, a commonly used tool in T1D, incorporates traditional risk factors such as age, sex, diabetes duration, glycemic control (HbA1c), systolic blood pressure, low-density lipoprotein (LDL) cholesterol, glomerular filtration rate, albuminuria, smoking status, and physical activity.

Recently, the Lancet Commission proposed a fundamental shift in the understanding of coronary artery disease, moving away from a paradigm centered on late-stage epicardial obstruction and myocardial ischemia, toward early detection of nonobstructive atherosclerotic plaque and proactive prevention.³ Detecting subclinical atherosclerosis offers a promising approach for personalizing preventive strategies. Among noninvasive tools, coronary artery calcium (CAC) scoring is widely regarded as a sensitive method for risk stratification in T2D.⁴ Primary prevention strategies, such as intensive LDL cholesterol lowering and the initiation of aspirin therapy in patients with CAC >100, have been recommended.⁵ However, CAC scoring is limited to identifying advanced calcified plaques and fails to detect early-stage, noncalcified atherosclerosis. Studies

have shown that 15%–20% of patients with acute coronary syndromes present with a CAC score of 0, emphasizing the need to assess noncalcified plaques for effective risk stratification.^{6,7}

Coronary computed tomographic angiography (CCTA) allows visualization of both calcified and noncalcified plaques. Originally used to detect significant coronary artery stenoses, CCTA has since evolved into a tool capable of characterizing coronary plaque morphology and composition, thus improving ASCVD risk assessment.

One such prognostic feature is nonobstructive coronary artery disease (CAD), which, despite lacking hemodynamic significance, is associated with a 1.5 to 7.5-fold increased risk of major adverse cardiovascular events (MACE) compared with normal CCTA findings.^{8–10} Another hallmark of ASCVD is the presence of high-risk plaques (HRPs), defined as low-density noncalcified plaques with positive remodeling.^{11–13} HRPs are strongly associated with MACE risk, increasing the risk by a factor of 9.2.¹⁴ In addition to qualitative assessments, CCTA allows for the quantification of coronary plaque burden through percent atheroma volume (PAV)—a metric calculated as the ratio of plaque volume to total vessel volume.¹⁵ PAV offers a comprehensive evaluation of disease severity. The SCOT-HEART study identified total plaque burden as the strongest predictor of myocardial infarction risk.¹⁶

Artificial intelligence (AI)-guided quantitative coronary computed tomography angiography (AI-QCT) further refines plaque detection and characterization, and has been shown to predict MACE independently of traditional clinical risk factors and CAC scores. Furthermore, in predicting cardiovascular outcomes, AI-QCT analysis is superior to human expert interpretation.¹⁵

These advances underscore the utility of AI-QCT in diagnosing coronary stenosis and improving ASCVD risk stratification, thereby supporting more personalized prevention strategies. Given the high prevalence of noncalcified plaques in younger T1D patients, we hypothesized that CCTA could uncover subclinical coronary atherosclerosis in patients classified as low-to-moderate risk by CAC. However, studies on the prevalence of noncalcified plaques in the T1D population are lacking, which limits the widespread application of AI-QCT as a screening strategy. Pilot data on the prevalence of AI-QCT-derived prognostic markers in the T1D population are necessary to inform the design of a sufficiently powered study.

Thus, the primary aim of this study was to assess the prevalence of clinically significant AI-QCT features in T1D patients with CAC <100. In addition, we sought to explore the association between conventional clinical risk factors and AI-QCT findings to refine patient selection for advanced imaging.

Methods

Study design

As part of an effort to improve risk stratification in T1D, we established a prospective registry titled CORDIA. This registry enrolls T1D patients followed at a large tertiary center. Inclusion criteria include an age of 30 years or greater and a duration of T1D of 10 years or longer. Exclusion criteria are a history of CAD, peripheral arterial disease, or stroke; symptoms suggestive of CAD or heart failure (e.g.,

chest pain, dyspnea); and target organ damage defined as estimated glomerular filtration rate <60 mL/min/1.73 m², albumin-to-creatinine ratio >30 mg/g, or microvascular complications in three distinct organ systems (e.g., microalbuminuria, retinopathy, neuropathy).

The Ethics Committees of IKEM and Thomayer's Hospital approved the study protocol. All patients provided written informed consent. The study adhered to all applicable regulatory requirements, ICH-GCP guidelines, and the Declaration of Helsinki.

Data availability statement

Anonymized data are available from the corresponding author upon reasonable request.

Study protocol

Stage 1: CAC. All participants underwent clinical examination, laboratory assessment, CAC scoring, and carotid ultrasound to detect atherosclerotic changes. Atherosclerotic plaque in the carotid arteries was defined by the intima-media thickening ≥ 1.5 mm. The 10-year ASCVD risk was calculated using the STENO1 risk engine using the online calculator (www.sdcc.dk/T1riskengine).¹⁷ According to ESC guidelines, STENO1 risk categories were defined as low (<5%), moderate (5 to <10%), high (10 to <20%), and very high ($\geq 20\%$).²

Stage 2: CCTA. Because a substantial proportion of T1D patients are at low risk and may not benefit from CCTA examinations, we excluded patients with CAC 0 and no atherosclerotic plaque in the carotid arteries from this step. Thus, only patients with CAC scores of 1–99 and those with CAC = 0 and at least one carotid plaque were referred for CCTA. Imaging was performed using a ≥ 64 -slice scanner (Siemens Somatom Sensation64, Forchheim, Germany).

Stage 3: AI-QCT. CCTA images were analyzed using FDA-cleared AI-based software (Clearly Inc.).¹⁸ This platform employs convolutional neural networks for image quality assessment, coronary segmentation and labeling, lumen wall evaluation, vessel contouring, and plaque characterization. Several prior studies have supported the method's validity.^{15,18–21}

The software identifies centerlines, lumen boundaries, and outer vessel walls across multiple image phases, selecting the four most optimal datasets for analysis. Coronary segments with a diameter >1.5 mm were included. Plaques were defined as any tissue >1 mm² within the coronary artery wall, distinct from the vessel lumen, perivascular fat, or adjacent tissues. Stenoses were classified according to the CAD-RADS 2.0 guidelines.²²

Plaque volumes were measured and categorized by Hounsfield unit (HU) values: low-density noncalcified plaque (<30 HU), noncalcified plaque (–30 to 350 HU), and calcified plaque (>350 HU). PAV was computed as plaque volume relative to total vessel volume. PAV staging was as follows: Stage 0 (PAV = 0), Stage 1 (>0%–5%), Stage 2 (>5%–15%), and Stage 3 (>15%).^{15,23}

Arterial remodeling was calculated as lesion diameter/reference diameter. Positive remodeling was defined as a ratio

≥1.1. The coexistence of low-density non-calcified plaque and positive remodeling defined HRP.¹⁰

Risk stratification based on AI-QCT

Risk categories were defined based on prior literature.^{14,15} Patients with PAV = 0 were classified as low risk; PAV >0%–5% without HRP as moderate risk; and PAV >0%–5% with HRP or PAV >5% as high risk.

Statistical analysis

Continuous variables are presented as means with standard deviations (SD) or medians with interquartile ranges (IQR), as appropriate. Categorical variables are summarized as counts and percentages.

Differences across the four CAC categories were assessed using one-way ANOVA, the Kruskal–Wallis test, or the Chi-square test, depending on data distribution and measurement level. Post hoc comparisons between groups were conducted with Bonferroni correction.

For comparisons between the two CAC groups, we used the Student's *t*-test for normally distributed continuous variables, the Mann–Whitney U test for non-normally distributed continuous variables, and the Chi-square test for categorical variables. Spearman correlation was applied to evaluate associations between atherosclerotic burden and clinical variables.

To compare cardiovascular risk classification between the STENO-1 model and AI-QCT, we calculated the proportion of patients with discordant risk categories. Agreement between STENO-1, CAD-RADS 2, and AI-QCT risk estimates was assessed using linearly weighted Cohen's kappa. To enable

direct comparison, we harmonized risk categories across methods by grouping patients with minimal-to-moderate non-obstructive and moderate stenosis into a single “moderate-risk” category. Similarly, patients with a STENO-1 estimated risk >10% were classified as “high-risk.”

All analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY). Two-sided *P*-values <0.05 were considered statistically significant.

Results

Stage 1: CAC

Between January 2023 and July 2024, we examined 167 patients with T1D (mean age 51.6 ± 9.5 years; 44.3% women; mean T1D duration 29.1 ± 10.6 years). CAC scores were distributed as follows: 93 (55.7%) had CAC 0, 46 patients (27.5%) CAC 1–99, 8 (4.8%) CAC 100–299, and 20 (12.0%) CAC ≥300. Patient characteristics by CAC groups are shown in Supplementary Table S1. Individuals with a higher CAC were older, had longer DM duration, were more often male, and had a higher intensity of lipid-lowering therapy as compared with individuals with a CAC of 0.

Stage 2: CCTA

To further evaluate CAD prevalence in individuals categorized as having low-moderate ASCVD risk by CAC (CAC <100), we performed the CCTA examination. For this examination, we selected 52 patients with CAC 0 and at least one atherosclerotic plaque in the carotid arteries as evaluated by carotid sonography (*n* = 19), or patients with CAC 1–99 (*n* = 33). Demographic characteristics of these

TABLE 1. DEMOGRAPHICS BY CORONARY ARTERY CALCIUM SCORE

	All n = 52	CAC = 0 n = 19	CAC 1–99 n = 33	P
Age, yrs	54 ± 7	55 ± 7	54 ± 7	0.49
Female gender, <i>n</i> (%)	21 (40)	13 (68)	8 (24)	0.003
Systolic BP, mmHg	130 ± 13	127 ± 13	131 ± 13	0.28
Diastolic BP, mmHg	77 ± 9	74 ± 9	79 ± 8	0.07
Heart rate, min ⁻¹	78 ± 10	78 ± 8	78 ± 11	0.85
BMI, kg m ⁻²	27.5 ± 4.1	26.5 ± 4.6	28.1 ± 3.8	0.17
DM duration, years	31 ± 11	31 ± 8	30 ± 13	0.79
HbA1c, mmol/mol	54 ± 10	52 ± 9	55 ± 10	0.40
Total cholesterol, mmol/L	4.6 ± 0.6	4.9 ± 0.6	4.4 ± 0.6	0.009
HDL cholesterol, mmol/L	1.6 ± 0.4	1.9 ± 0.4	1.5 ± 0.4	<0.001
LDL cholesterol, mmol/L	2.5 ± 0.5	2.6 ± 0.6	2.5 ± 0.5	0.42
Triglycerides, mmol/L	0.8 (0.6–1.0)	0.7 (0.5–0.8)	0.9 (0.7–1.1)	0.30
Lp(a), nmol/L	15 (3–37)	12 (8–23)	18 (3–41)	0.55
Apo A1, g/L	1.8 ± 0.3	1.9 ± 0.3	1.6 ± 0.3	0.002
Apo B, g/L	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.46
Apo CIII, mg/L	134 ± 43	140 ± 45	131 ± 42	0.90
Statin therapy, <i>n</i> (%)	30 (58)	12 (63)	18 (55)	0.58
Statin dose*, mg	10 (0–20)	10 (0–10)	10 (0–20)	0.89
Smoker/Ex-smoker, (%)	1 (2)/4 (8)	0 (0)/1 (5)	1 (3)/3 (10)	0.51
NT-proBNP, ng/L	57 (29–95)	76 (48–100)	51 (25–74)	0.40
Creatinine, μmol/L	85 ± 15	87 ± 17	85 ± 13	0.67
Urea, mmol/L	6.1 ± 1.3	6.2 ± 1.5	6.0 ± 1.2	0.57
Albumin/creatinine, g/mol	0.8 (0.5–1.4)	0.7 (0.4–0.9)	0.9 (0.5–1.7)	0.15

*Atorvastatin equivalent dose.

CAC, coronary artery calcium, BP, blood pressure, BMI, body mass index, DM, diabetes, HDL, high density lipoprotein, LDL, low density lipoprotein.

52 patients are shown in Table 1. The mean age of these 52 patients was 54 ± 7 years, with 21 (40.4%) being women, and a mean duration of T1D of 31 ± 11 years. According to the STENO1 engine, four patients (7.7%) were categorized as moderate risk (ASCVD risk 5 to <10%), 27 (51.9%) high risk (ASCVD risk 10 to <20%), and 21 (40.4%) very high risk (ASCVD risk $\geq 20\%$).

According to the CADS-RADS2 classification, 11 patients (21.2%) had an absence of CAD (Stage 0), while 17 (32.7%) had minimal nonobstructive CAD (Stage 1), 14 (26.9%) had mild-nonobstructive CAD (Stage 2), 1 (1.9%) had moderate stenosis (Stage 3), and 9 (17.3%) had severe stenosis (Stage 4A).

The CCTA-based CADS-RADS2 classification reclassified CAC-based categories. Only 58% of patients with CAC 0 had no CAD, while 11% had severe stenosis. Among patients with CAC 1 to 99, 21% had severe coronary stenosis (Table 2). As evaluated by linear weighted kappa ($\kappa = 0.45$), there was only a moderate agreement between CADS-RADS2 and CAC risk categories.

Stage 3: AI-QCT

The AI-QCT analysis of PAV revealed that 41 (78.8%) patients had PAV levels in the >0%–5% range, and 1 (1.9%) patient had PAV in the 5%–15% range. Of these 42 patients with PAV >0, 30 (71.4%) had at least one HRP, with eight patients having two HRPs and four patients having three HRPs. Figure 1 illustrates an example of CCTA and AI-QCT analysis in a patient with CAC 0.

Compared with CAC, the AI-QCT examination reclassified six patients with CAC 0 and 24 patients with CAC 1–99 as high-risk, totaling 57.7% of reclassified patients (Table 3). As evaluated by linear weighted kappa ($\kappa = 0.25$), there was only a fair agreement between AI-QCT and CAC risk categories.

The AI-QCT also reclassified patients across the STENO1 risk categories. Among those initially categorized as STENO1 moderate risk ($n = 4$), two patients exhibited a PAV in the >0%–5% range along with HRP, warranting reclassification into a higher-risk category. Within the STENO1 high-risk group ($n = 27$), six patients (22.2%) had a PAV of 0, as did three patients (14.3%) in the STENO1 very high-risk group

($n = 21$), indicating potential overestimation of risk in these individuals. Overall, AI-QCT reclassified two moderate-risk patients into a higher-risk group and nine high- or very high-risk patients into a lower-risk group, resulting in a total reclassification rate of 21.2% (Table 3). The linear weighted kappa between STENO1 and AI-QCT risk stratification was 0.02, indicating only slight agreement between methods. This divergence in risk stratification suggests that AI-QCT may capture aspects of cardiovascular risk that are not reflected in traditional risk factor-based models.

Clinical factors associated with AI-QCT variables

To identify patients who may benefit most from AI-QCT, we evaluated the associations between clinical risk factors and AI-QCT-derived measures of coronary atherosclerosis. However, there was no association between PAV and age, DM duration, LDL, or Apo-B level (Table 4). Furthermore, the STENO1 risk engine was not associated with any features of the coronary atherosclerosis burden. Only HDL cholesterol and ApoA1 levels were associated with PAV and noncalcified plaque volume. The negative association between age and both noncalcified and low-density plaque volume presumably reflected a higher prevalence of these early-stage plaques in younger patients.

Discussion

This study highlights the utility of AI-based CCTA evaluation in refining ASCVD risk in T1D patients with low-to-moderate ASCVD risk as defined by CAC. We found that only 21% of patients with CAC <100 have an absence of CAD, while 17% have significant coronary artery stenosis, and 58% have at least one high-risk plaque. Overall, AI-QCT showed only slight agreement with STENO1 risk categories, suggesting that AI-QCT may capture aspects of cardiovascular risk not reflected by traditional risk factor-based models. Furthermore, our findings highlight the substantial burden of noncalcified atherosclerosis in T1D patients that remains undetected by CAC, challenging the prevailing assumption that a low CAC score equates to low cardiovascular risk in this population.

TABLE 2. CADS-RADS2 CATEGORIES BY STENO1 RISK CATEGORIES (PANEL A) AND CORONARY ARTERY CALCIUM SCORE (PANEL B)

		CADS-RADS2				
		Absence of CAD	Minimal nonobstructive	Mild nonobstructive	Moderate stenosis	Severe stenosis
Panel A						
STENO1						
5 to <10%	1	1	2	0	0	
10 to <20%	6	10	8	0	3	
>20%	4	6	4	1	6	
Linear weighted kappa 0.03 (95% CI: −0.03 to 0.06).						
Panel B						
CAC						
0	11	5	1	0	2	
>0 to 99	0	12	13	1	7	
Linear weighted kappa 0.45 (95% CI: 0.26 to 0.64).						

Color denotes risk—green: low, orange: moderate, red: high to very high.

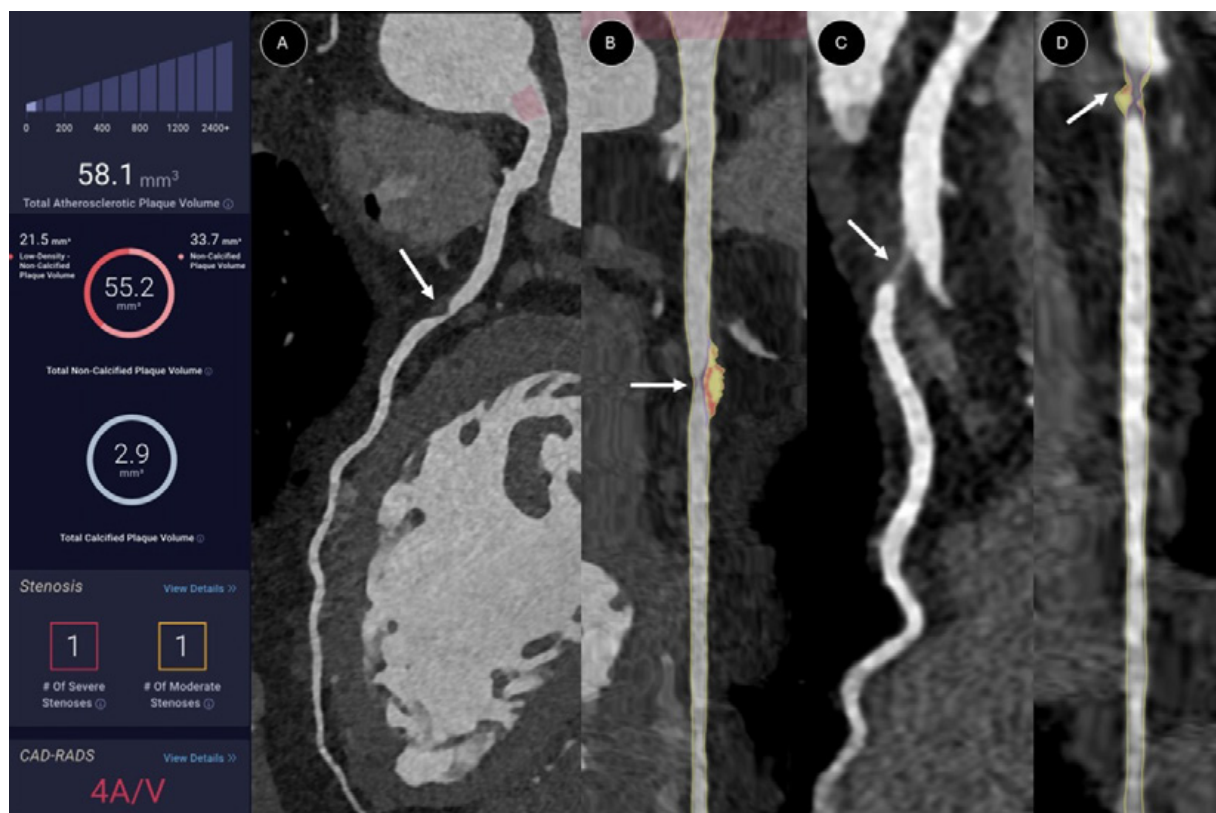


FIG. 1. Comparison of coronary computed tomographic angiography (CCTA) and AI-QCT analysis. A 56-year-old female patient with a T1D duration of 43 years and a coronary artery calcium score of 0. **(A)** presents CCTA of the left anterior descending (LAD) artery depicting focal moderate stenosis (a 66% stenosis) in the proximal LAD; **(B)** AI-QCT with color plaque overlay depicts that the stenotic plaque consists of low-density noncalcified (LD-NCP, red overlay) and noncalcified plaque (NCP, yellow overlay). **(C)** depicts the CCTA of the first diagonal artery with focal high-grade stenosis (81% stenosis) in the vessel origin (arrow) from the LAD. **(D)** AI-QCT with color plaque overlay depicts that the stenotic plaque (arrow) again consists of LD-NCP (red overlay) and NCP (yellow overlay). Both the LAD **(B)** and first diagonal plaques **(D)** are considered high-risk plaques as they have both LD-NCP and are positively remodeled (extend beyond the normal vessel wall boundary).

Given the limitations of traditional risk scores at the individual level, detecting subclinical atherosclerosis has emerged as a promising strategy to guide personalized prevention. While intensive control of cardiovascular risk factors can regress

noncalcified plaques, unlike calcified plaques, early detection becomes critical to intercept the disease before it progresses.²⁴

CCTA offers this capability by enabling the non-invasive detection and characterization of coronary plaques; however,

TABLE 3. CATEGORIES BY PERCENT ATHEROMA VOLUME AND HIGH-RISK PLAQUE PRESENCE BY STENO1 CATEGORIES (PANEL A) AND CORONARY ARTERY CALCIUM SCORE (PANEL B)

	PAV and HRP			
	PAV = 0	PAV >0% to 5% no HRP	PAV >0% to 5% and HRP	PAV >5% to 15% and HRP
Panel A				
STENO1				
5 to <10%	1	1	2	0
10 to <20%	6	6	14	1
>20%	3	5	13	0
Linear weighted kappa 0.02 (95% CI: -0.10 to 0.14).				
Panel B				
CAC				
0	10	3	6	0
>0 to 99	0	9	23	1
Linear weighted kappa 0.25 (95% CI: 0.13 to 0.36).				

Color denotes risk—green: low, orange: moderate, red: high to very high. PAV, percent atheroma volume; HRP, high-risk plaque presence.

TABLE 4. CLINICAL VARIABLES ASSOCIATION WITH AI-QCT VARIABLES

	<i>Percent atheroma volume</i>	<i>Noncalcified plaque volume</i>	<i>Low-density plaque volume</i>	<i>Calcified plaque volume</i>
Age, years	−0.264	−0.334*	−0.298*	−0.138
Diabetes duration, years	0.094	−0.005	0.041	0.089
HbA1c, mmol/mol	0.083	0.072	−0.128	0.094
Total cholesterol, mmol/L	−0.150	−0.169	−0.103	−0.240
HDL cholesterol, mmol/L	−0.343*	−0.393**	−0.271	−0.312*
LDL cholesterol, mmol/L	0.125	0.133	0.143	0.026
Triglycerides, mmol/L	0.040	0.109	0.032	−0.048
Lp(a), nmol/L	0.164	0.069	0.094	0.180
Apolipoprotein AI, g/L	−0.348*	−0.433**	−0.316*	−0.267
Apolipoprotein B, g/L	−0.130	−0.195	−0.206	−0.081
Apolipoprotein CIII, mg/L	−0.130	−0.195	−0.206	−0.081
NTproBNP, ng/L	−0.112	−0.182	−0.129	−0.103
Creatinine, μ mol/L	0.010	0.071	0.060	−0.019
Urea, mmol/L	−0.142	−0.156	−0.204	−0.135
ACR, g/mol	0.207	0.113	0.117	0.277
STENO1	−0.119	−0.192	−0.186	−0.043

Spearman correlation coefficients are shown.

* $P < 0.05$, ** $P < 0.01$.

ACR, albumin-to-creatinine ratio, HDL, high-density lipoprotein, LDL, low-density lipoprotein.

its utility has traditionally been limited to patients with suspected CAD. The FACTOR-64 trial,²⁵ which assessed CCTA screening in asymptomatic high-risk T2D patients, failed to demonstrate a reduction in major cardiovascular events. However, this may reflect a focus on revascularization over aggressive risk factor modification. Results of the FACTOR-64 trial align with the observation that coronary revascularization does not improve outcomes in stable CAD, even in patients with moderate to large ischemia.²⁶ In contrast, the SCOT-HEART study, which incorporated CCTA findings to guide preventive measures, demonstrated a significant reduction in coronary death and nonfatal myocardial infarction.²⁷ These contrasting outcomes underscore that the clinical utility of CCTA lies not in procedural escalation but in risk reclassification and improved risk factor control, especially LDL cholesterol.

Traditionally, patients with high or very high calculated ASCVD risk are referred for CCTA to detect obstructive disease. However, AI-QCT expands this paradigm by quantifying the total plaque burden, identifying HRP, and capturing nonobstructive disease—all of which carry prognostic value. Despite this, no prior studies have investigated the prevalence of clinically relevant coronary lesions in low-to-moderate-risk T1D patients. Our study fills this gap and reveals a surprisingly high prevalence of CAD in this subgroup. Notably, only 21% of those with CAC <100 were free of CAD, suggesting a significant underestimation of risk by CAC scoring alone, likely due to the predominance of non-calcified plaques in this relatively young cohort.

While clinical risk factors are commonly used to predict ASCVD risk in T1D, surprisingly, we did not find any association between PAV and parameters such as LDL cholesterol, age, DM duration, or STENO1 risk engine. This may be partially explained by the difference in vascular damage patterns in T1D and T2D.²⁸ The only parameters associated with PAV were HDL cholesterol and ApoA-1. This finding aligns with a recent systematic review and meta-analysis, which demonstrate that a decrease in LDL and an increase in

HDL are necessary for atherosclerotic plaque regression.²⁹ Furthermore, a central role of ApoA1 in inhibiting the progression of T1D and T2D has been suggested.³⁰

Although our study does not include longitudinal outcomes, we interpret our findings using validated thresholds from prior studies. In the study by Nurmohamed et al.,¹⁵ PAV 0 was associated with a 0% 10-year ASCVD risk, while PAV >0%–5% corresponded to a 10-year risk of 8.2%–10.0%, and PAV >5%–15% to 10.1%–15.3%. HRP presence increased the MACE risk over ninefold. Furthermore, the PREVENT trial demonstrated that preventive PCI of nonflow-limiting vulnerable plaques reduced MACE compared with optimal medical therapy alone.³¹ When applied to our cohort, AI-QCT reclassified 21% of patients compared with the STENO1 engine and 58% compared with CAC, underscoring its potential to improve risk stratification. This highlights the potential of AI-QCT analysis to reclassify a substantial proportion of T1D patients, offering a more precise approach to risk stratification and potentially guiding more targeted interventions.

Our findings have significant implications for clinical practice. CAC scoring has been recommended for ASCVD stratification in T2D patients.⁵ However, in the present study, only 17% of T1D patients had CAC >100. On the other hand, 79% of patients with CAC <100 had CAD detected by AI-QCT. Based on these findings, we propose that in T1D patients aged 45 years or older, AI-QCT should be performed to stratify risk. In individuals with an HRP or increased PAV, intensive risk factor control, including LDL cholesterol reduction of over 50% and low-dose aspirin consideration, should be considered.

Study limitations and strengths

While our findings are compelling, several limitations must be acknowledged. First, to identify individuals at low cardiovascular risk who may not benefit from AI-QCT, we used a combined criterion of CAC = 0 and absence of carotid atherosclerotic plaques. Therefore, our findings may not be

generalizable to all patients with a CAC score of 0. Future studies are needed to validate our assumption that this combination effectively identifies individuals with T1D who are at low risk. Second, the study's cross-sectional design precludes direct assessment of long-term outcomes associated with CCTA-detected features. However, numerous previous studies have confirmed the predictive value of the AI-QCT features evaluated in this study. Nonetheless, the ASCV risk associated with the analyzed features may be even higher in T1D patients than in the general population, as was previously shown for CAC.³² Third, no prior power analysis was conducted due to the absence of existing data on QCTA in the low-to-moderate-risk T1D population. Although the sample size in this study is limited, precluding definitive conclusions, our findings provide valuable preliminary insights to inform the design of a future, adequately powered study with hard clinical endpoints that could ultimately influence clinical practice. Although the study sample used in this study was adequate for an exploratory analysis, it limits the generalizability of the findings. Fourth, it is unclear whether interventions based on AI-QCT will decrease ASCVD risk. Future large-scale interventional studies should investigate the impact of AI-QCT-guided interventions on clinical outcomes in T1D.

A significant strength of this study is the use of validated AI software for quantitative plaque analysis. Manual quantitative plaque analysis is time-consuming, which precludes its clinical use in everyday practice. The Cleerly AI-QCT is superior to the consensus of level 3 expert readers in determining the severity of coronary artery stenosis, plaque volume, and composition.¹⁹ While different software may vary in plaque burden analysis based on the thresholds used, we have employed software that has demonstrated predictive power in previous studies.¹⁵

Conclusions

This study reveals a high prevalence of clinically significant coronary atherosclerosis in asymptomatic T1D patients with CAC <100, supporting the role of AI-guided QCT in enhancing risk stratification. These findings challenge the reliability of CAC alone in identifying low-risk individuals and underscore the value of advanced imaging in detecting subclinical disease and informing preventive strategies in T1D.

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Supplementary Material

Supplementary Table S1

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Cardiac Rhythm Conversions and the Outcome in Refractory Out-of-Hospital Cardiac Arrest: Extracorporeal Versus Conventional Resuscitation

Critical Care Medicine
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CLINICAL INVESTIGATION

OPEN

Cardiac Rhythm Conversions and the Outcome in Refractory Out-of-Hospital Cardiac Arrest: Extracorporeal Versus Conventional Resuscitation

OBJECTIVES: A Prague out-of-hospital cardiac arrest (OHCA) study has demonstrated that an invasive approach (early transport to the hospital, extracorporeal cardiopulmonary resuscitation [ECPR]) is a feasible and effective treatment strategy in refractory OHCA. This post hoc analysis of the Prague OHCA study aimed to stratify the prognosis of patients according to the detailed course of heart rhythm during prehospital and early hospital periods.

DESIGN, SETTING, AND PATIENTS: This analysis included all 256 patients (median age 58, 17% females) randomized to the Prague OHCA study. The sequence of heart rhythms during the prehospital period was analyzed in terms of neurologic outcomes after 180 days. The primary outcome was a composite of survival with Cerebral Performance Category 1 or 2 at 180 days.

INTERVENTIONS: None.

MEASUREMENTS AND MAIN RESULTS: Within the study cohort, 156 (61%) manifested ventricular fibrillation (VF), 45 pulseless electrical activity, and 55 asystole as the initial rhythm. Patients with an initial VF who reached a sustained recovery of spontaneous circulation (ROSC) had the highest proportion of reaching a primary outcome (32/44 [73%]). Patients who had one or more episodes of asystole during cardiopulmonary resuscitation had the lowest rate of primary endpoint (5/39 [13%]). Patients who experienced intermittent ROSC showed a higher success rate in achieving the primary outcome when treated with an invasive-based approach (including ECPR) compared with the conventional strategy (26/34 [76%] vs. 24/50 [48%]; $p < 0.05$).

CONCLUSIONS: Achieving ROSC is the best prognostic marker in OHCA patients with an initially refractory VF. Patients with intermittent ROSC after the initial VF and ongoing VF seem to be optimal candidates for an invasive approach. Asystole detection at any time during resuscitation is a strong negative prognostic marker, irrespective of the initial rhythm.

KEYWORDS: cardiopulmonary resuscitation; extracorporeal cardiopulmonary resuscitation; extracorporeal membrane oxygenation; heart rhythm; refractory cardiac arrest

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Out-of-hospital cardiac arrest (OHCA) places a considerable burden on society (1). For patients who fail to achieve a recovery of spontaneous circulation (ROSC), the likelihood of survival after transport to the hospital while undergoing ongoing cardiopulmonary resuscitation (CPR) is minimal, typically below 4% with the use of conventional CPR (CCPR) alone (2, 3).

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KEY POINTS

Question: The prognostic role of rhythm deterioration during refractory out-of-hospital cardiac arrest (OHCA) has not been fully described.

Findings: The post hoc analysis of the Prague OHCA trial identified the role of rhythm conversion on patient outcome. The most favorable predictor in patients with refractory OHCA was intermittent or sustained recovery of spontaneous circulation after initial ventricular fibrillation. Asystole at any point during resuscitation strongly indicates a poor prognosis, regardless of the initial rhythm or treatment strategy.

Meaning: Patients with intermittent recovery of spontaneous circulation are ideal candidates for invasive treatment, including extracorporeal cardiopulmonary resuscitation.

The temporary replacement of failing circulation by extracorporeal CPR (ECPR) has been recognized as a promising approach in refractory cardiac arrest (CA) (4–8). The Prague OHCA study offers encouraging evidence supporting the use of extracorporeal ECPR in select patients with refractory OHCA (9). While not universally applicable, ECPR has demonstrated the potential to improve survival outcomes in patients who meet specific criteria (10).

Initial cardiac rhythm, response time of bystander CPR, emergency medical services response time, duration, and quality of CPR critically affect the outcome in OHCA (11–14). We have shown previously that outcomes of refractory OHCA with initial rhythm being ventricular fibrillation (VF) are far more favorable than those with initial asystole or pulseless electrical activity (PEA) (15). However, many patients manifest rhythm changes during ongoing CPR (16, 17). Observational studies showed that clinical outcomes could differ in patients with a transient organized rhythm compared with uninterrupted VF or a transient asystole (16, 18–20). Furthermore, changing from a nonshockable to a shockable rhythm decreases the risk of unfavorable outcomes (21–23). Recently, we published data from a prospective registry showing that the rhythm at hospital admission affected ECPR outcomes (24). However, the relationship between the rhythm conversions in refractory OHCA and clinical outcomes has

not been fully elucidated. Further, the potential benefits of an ECPR-based approach compared with CCPR concerning different rhythm profiles remain unclear.

Therefore, we hereby present an analysis of the rhythm conversions during CPR and their impact on patient outcomes in refractory OHCA and aim to analyze the exploitability of the ECPR-based vs. conventional approach in a randomized population.

METHODS

The current study is a post hoc analysis of the Prague OHCA study, a randomized controlled trial comparing an ECPR-based/invasive approach (early transport to hospital under mechanical CPR, ECPR, and immediate invasive assessment and therapy) to standard/conventional treatment in the refractory OHCA population (9, 25). The study was performed according to good clinical practice and in accordance with the ethical standards of the responsible regional committee on human experimentation and with the Helsinki Declaration of 1975. The Prague OHCA study was approved by the Ethics committee of the General University Hospital in Prague (192/11 S-IV, date February 17, 2011).

Study Population

A detailed protocol of the main study and primary results have been described previously (9, 25). In brief, the study enrolled adults over 18 years old, with a witnessed OHCA of a presumed cardiac etiology, who were given a minimum of 5 minutes of advanced cardiac life support without ROSC and who remained unconscious. The patients were randomized in a 1:1 ratio into two study arms: invasive or standard. The termination of resuscitation efforts followed the valid European Resuscitation Council (ERC) guidelines (9, 25–27).

Intervention

Patients randomized into the standard/conventional arm were managed on-site by continued advanced cardiac life support. Drug use, further defibrillations, or other interventions followed the available ERC guidelines (26, 27). If ROSC was achieved (defined as an organized cardiac electrical activity with a palpable pulse), transportation to the hospital was initiated for further treatment as per current guidelines.

In the ECPR-based/invasive arm, the immediate intraarrest transfer directly to the cardiac center catheterization laboratory was initiated after randomization. Then, if ROSC was not achieved en route or at admission, ECPR (implantation of venoarterial extracorporeal membrane oxygenation [ECMO]) was performed. The use of drugs, further defibrillations, or other interventions during transportation followed the ERC guidelines (26, 27). Post-resuscitation care was standardized in both study arms.

Initial Rhythm and Rhythm Change

For the present study, the initial rhythm was defined as the first documented rhythm by the Emergency Medical System. Any prehospital rhythm conversions, either spontaneous or due to defibrillation, were evaluated. The rhythm at each assessment was placed into one of four groups: ROSC, VF, PEA, and asystole. The final rhythm was the last documented cardiac rhythm before withdrawal from the study or the rhythm at admission to the hospital.

Outcomes

The primary outcome was the composite of a 180-day survival with a favorable neurologic status, defined as no or minimal neurologic impairment (Cerebral Performance Category [CPC], 1 or 2).

Statistical Analysis

The numeric variables are expressed as medians and interquartile ranges. The two-sided Mann-Whitney, Kruskal-Wallis, and Fisher exact test (for a 2×2 table), chi-square test, and analysis of variance were used when appropriate. All the presented *p* values are two-tailed. The Cox regression analysis for prediction of an unfavorable clinical outcome was performed separately for data available during CPR and after the early hospital evaluation (within 1 hr). A *p* value of less than 0.05 was considered as statistically significant. Statistical analyses were performed with MedCalc Statistical Software, Version 19.7 (MedCalc Software, Ostend, Belgium; 2021), RStudio 2022.07.2+576 (RStudio Team [2020]. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA, URL: <http://www.rstudio.com/>).

RESULTS

Baseline Clinical Data, Prehospitalization Phase

During the study period, 256 patients (median age 58 yr, 17% females) were enrolled and analyzed. The baseline demographic and clinical data are described in **Table 1** and **Supplemental Table 1** (<https://links.lww.com/CCM/H762>). Out of the entire study cohort, 156 patients (61%) manifested VF as the initial rhythm. The rest of the patients had an initially nonshockable rhythm (45 [18%] PEA and 55 [21%] asystole).

Initial VF Group

Out of all patients with an initial VF, 28 (18%) had ongoing arrhythmia without any rhythm change, 84 (54%) manifested at least one episode of ROSC, 55 (35%) had at least one episode of PEA, and 39 (25%) converted to at least one episode of asystole. The final rhythm was as follows: VF in 41 (26%), ROSC in 69 (44%), PEA, and asystole in 22 (14%) and 24 (15%) patients, respectively. More details are in **Figure 1**.

Patients with stable ROSC after an initial VF (without any nonshockable rhythm) represented the highest proportion of those achieving 180-day survival at CPC 1 or 2 (32/44 [73%]). When any intermittent episode of a nonshockable rhythm was documented, the survival rates with favorable neurologic outcomes decreased (50/84 [59%]). Patients whose last rhythm was asystole or who had one or more episodes of asystole at any time during CPR had the lowest rate of 180-day survival with CPC 1 or 2 (0/24 [0%] or 5/39 [13%]). For more details, see **Table 2**.

In patients who had ROSC at any time during CPR (even though episodes of nonshockable rhythms were documented), the proportion of CPC 1 or 2 at the 180-day follow-up was higher in the ECPR-based/Invasive arm than in the standard arm. Similarly, a higher proportion of CPC 1 or 2 at the 180-day follow-up was documented in patients in the Invasive arm when the last rhythm was ROSC without exhibiting a nonshockable rhythm.

Ten of the 84 patients (12%) with an initial VF allocated to the standard arm crossed over to the invasive strategy. A cross-over from the invasive to the standard arm was done in two of 72 patients (3%) deemed ineligible for advanced invasive therapy. Differences in the primary endpoint rates between treatment strategies

TABLE 1.
Baseline Demographical and Clinical Data

Initial Rhythm	Ventricular Fibrillation (n = 156)	Pulseless Electrical Activity (n = 45)	Asystole (n = 55)	p
Age (yr)	56 (45–64)	62 (54–66)	58 (47–69)	0.07
Gender (male)	141 (90%)	31 (69%)	40 (73%)	0.0003
Bystander CPR	154 (99%)	44 (98%)	54 (98%)	0.89
Dispatcher assisted CPR	133 (85%)	24 (53%)	46 (84%)	< 0.0001
Randomized to				
Standard	84 (54%)	24 (53%)	24 (44%)	0.41
Invasive	72 (46%)	21 (47%)	31 (56%)	
Cross-over				
Standard → invasive	10/84	0/24	1/24	0.007
Invasive → standard	2/72	1/21	6/31	
Time of CPR (time to recovery of spontaneous circulation or ECLS) (min)	54 (33–69)	50 (42–68)	56 (37–67)	0.62
Admitted to hospital	136 (87%)	33 (73%)	41 (75%)	0.03
Declared death	33 (21%)	20 (44%)	22 (40%)	0.0015
ECLS implanted	57 (37%)	17 (38%)	18 (33%)	0.85
Cause of cardiac arrest (including autopsy findings)				
Acute coronary syndrome	89 (57%)	15 (33%)	23 (42%)	< 0.0001
Chronic coronary artery disease	29 (19%)	1 (2%)	2 (4%)	
Pulmonary embolism	1 (1%)	15 (33%)	8 (15%)	
Chronic heart failure	8 (5%)	2 (4%)	4 (7%)	
Cardiomyopathy	7 (5%)	1 (2%)	1 (2%)	
Myocarditis	5 (3%)	1 (2%)	2 (4%)	
Aortic stenosis	5 (3%)	1 (2%)	2 (4%)	
Aortic dissection type A	1 (1%)	1 (2%)	2 (4%)	
Intracranial hemorrhage	1 (1%)	1 (2%)	1 (2%)	
Other	4 (3%)	4 (9%)	4 (8%)	
Unknown	6 (4%)	3 (7%)	6 (11%)	
Primary endpoint	63 (40%)	3 (7%)	2 (4%)	< 0.0001

CPR = cardiopulmonary resuscitation, ECLS = extracorporeal life support.

Data are expressed as median (interquartile range) or *n* (%). Kruskal-Wallis test was used.

were not significantly affected by crossovers. Details are in Table 2.

Initial Nonshockable Rhythms

A rhythm conversion was documented in 68 patients (68%) with an initially nonshockable rhythm. Conversion of initially nonshockable rhythm to ROSC or VF during CPR was detected in 37 (37%) and 28 (28%) cases, respectively. Stable ROSC was present in

22 patients (22%) at hospital admission. The PEA subgroup had a higher prevalence of intermittent ROSC than the asystole subgroup. Differences in the last rhythm were recognized between patients with initial PEA and asystole. For more details, see Table 3 and Figure 1.

Only five patients with a nonshockable initial rhythm survived 180 days with CPC 1 or 2. **Supplemental Table 2** (<https://links.lww.com/CCM/H762>) shows the profiles of the surviving patients. In

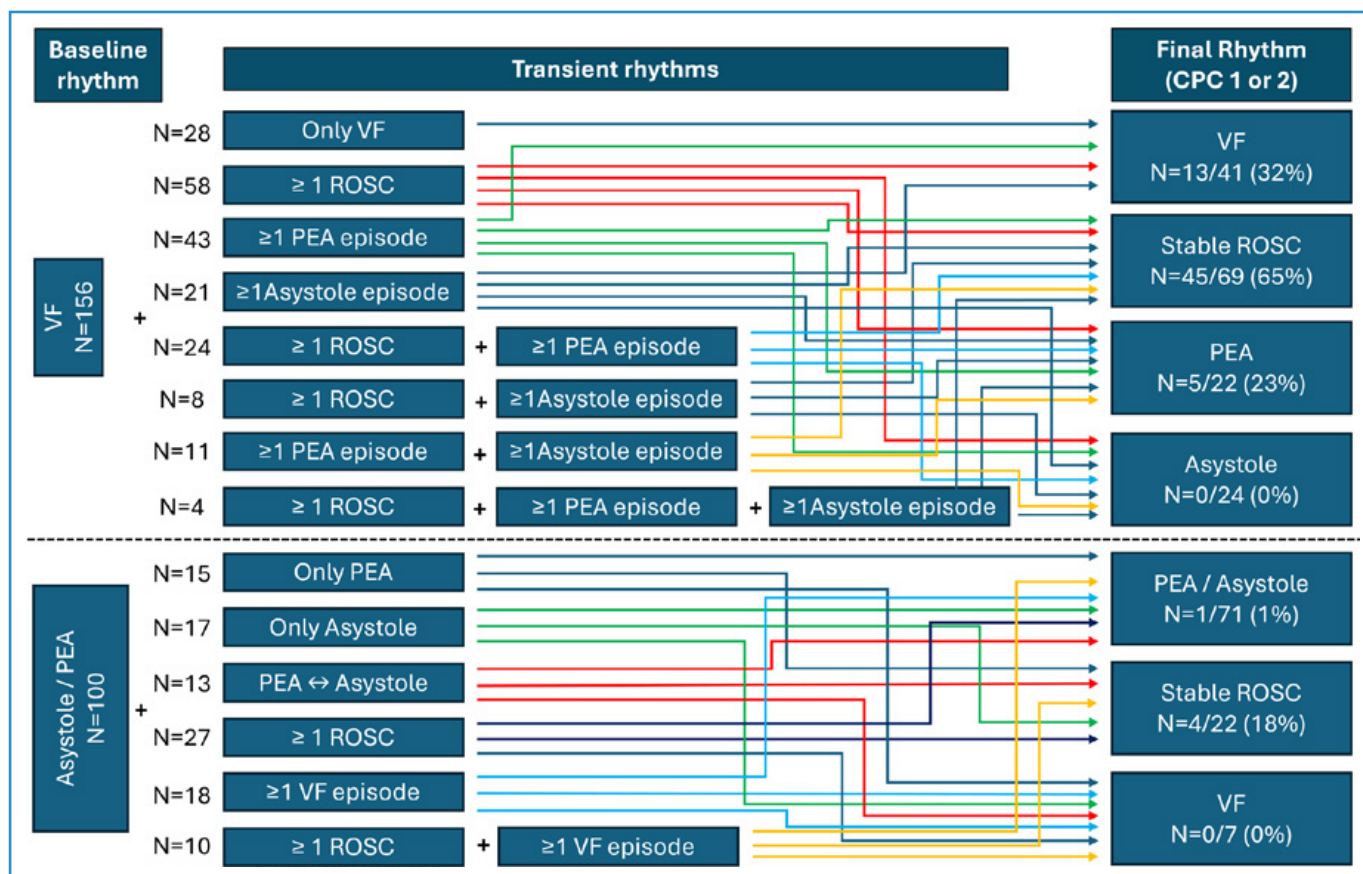


Figure 1. Rhythm profiles in patients with refractory out-of-hospital cardiac arrest during cardiopulmonary resuscitation. Many variants of heart rhythm scenarios with subsequent rhythm conversions could occur during prolonged cardiopulmonary resuscitation. CPC = Cerebral Performance Category, PEA = pulmonary endarterectomy, ROSC = recovery of spontaneous circulation, VF = ventricular fibrillation.

two patients, a rhythm change to a shockable rhythm during CPR was documented, constituting two of 28 (7%) of all patients with a conversion from a non-shockable rhythm to VF. Four patients had sustained ROSC at admission (4/22 [18%] of all patients with an initial nonshockable rhythm and sustained ROSC). The last patient, with an initial PEA, did not manifest any rhythm conversion and was treated with venoarterial ECMO (1/35 implanted ECLS in initially non-shockable patients).

Cox Regression Analysis in the Prediction of Unfavorable Clinical Outcome

In the first Cox regression model (including only variables known up to hospital admission), see **Table 4**, model A, an asystole documented at any time during CPR was independently associated with a higher probability of an unfavorable clinical outcome (the absence of both 180-d survival and a favorable

neurologic outcome). Documented ROSC during CPR reduced the probability of an unfavorable clinical outcome. The second Cox regression model, see **Table 4**, model B, included variables known after the initial in-hospital evaluation (within the first hour of admission). An unfavorable clinical outcome was associated with a CPR length of over 45 minutes, asystole at admission, and the absence of a sustained ROSC at admission when initially nonshockable patients were included.

DISCUSSION

This post hoc analysis of the Prague OHCA study contains several novel findings. An invasive strategy may be beneficial in patients with an initial shockable rhythm and intermittent or sustained ROSC. It is more likely in patients with ongoing VF or PEA as the last rhythm. On the contrary, patients with a deterioration of initial VF to asystole seemed to have an inauspicious

TABLE 2.
Primary Outcome According to Rhythm Profiles After an Initial Shockable Rhythm

Analysis Treatment Arm	Intention to Treat			As Treated		
	Invasive (n = 72)	Standard (n = 84)	p	Invasive (n = 80)	Standard (n = 76)	p
Ongoing VF (all other rhythms excluded)	7/20 (35%)	1/8 (13%)	0.48	8/22 (36%)	0/6 (0%)	0.2
Last rhythm VF (all intermittent rhythms included)	10/30 (33%)	3/11 (27%)	1.0	13/34 (38%)	0/7 (0%)	0.1
VF + ROSC anytime (nonshockable intermittent rhythms included)	26/34 (76%)	24/50 (48%)	0.02	27/36 (75%)	23/48 (48%)	0.02
VF + ROSC anytime (nonshockable intermittent rhythms excluded)	18/20 (86%)	17/29 (59%)	0.03	19/21 (91%)	16/28 (57%)	0.02
VF + last rhythm ROSC (nonshockable intermittent rhythms included)	21/26 (81%)	24/43 (56%)	0.06	22/28 (79%)	23/41 (56%)	0.09
VF → last rhythm ROSC (nonshockable intermittent rhythms excluded)	15/16 (94%)	17/28 (61%)	0.04	16/17 (94%)	16/27 (59%)	0.02
VF → intermittent ROSC → VF (nonshockable intermittent rhythms excluded)	3/4 (75%)	0/1 (0%)	x	3/4 (75%)	0/1 (0%)	x
VF + PEA anytime (asystole excluded)	7/16 (44%)	8/24 (33%)	0.74	9/19 (47%)	6/21 (29%)	0.37
VF + last rhythm PEA (asystole excluded)	3/6 (50%)	1/11 (9%)	0.2	4/8 (50%)	0/9 (0%)	0.06
VF → intermittent PEA → VF (asystole excluded)	0/5 (0%)	1/1 (100%)	x	1/6 (17%)	0/0 (0%)	x
VF → intermittent PEA → ROSC (asystole excluded)	4/5 (80%)	6/12 (50%)	0.56	4/5 (80%)	6/12 (50%)	0.56
VF → intermittent ROSC → PEA (asystole excluded)	2/3 (67%)	0/1 (0%)	x	2/3 (67%)	0/1 (0%)	x
VF + asystole anytime (PEA included)	3/16 (19%)	2/23 (9%)	0.65	4/18 (22%)	1/21 (5%)	0.25
VF + last rhythm asystole (PEA included)	0/7 (0%)	0/17 (0%)	x	0/7 (0%)	0/17 (0%)	x
VF → intermittent asystole → VF (PEA included)	0/1 (0%)	1/1 (100%)	x	1/2 (50%)	0/0 (0%)	x
VF → intermittent asystole → ROSC (PEA included)	2/5 (40%)	1/3 (33%)	x	2/6 (33%)	1/2 (50%)	x
VF → intermittent asystole → PEA (intermittent ROSC included)	1/3 (33%)	0/2 (0%)	x	1/3 (33%)	0/2 (0%)	x
VF → intermittent ROSC → asystole (PEA included)	0/1 (0%)	0/4 (0%)	x	0/1 (0%)	0/4 (0%)	x

PEA = pulseless electrical activity, ROSC = recovery of spontaneous circulation, VF = ventricular fibrillation.

x—the Fisher exact test cannot be calculated because there were not four values to construct a pivot table.

Data are expressed as n (%). The Fisher exact test was used.

prognosis despite an invasive approach. However, documentation of VF or sustained ROSC in patients with an initial nonshockable rhythm appears to be a favorable sign for a better outcome. Unfortunately, no evidence exists that an invasive strategy, including ECPR, would improve clinical outcomes in the initial asystole group. The presence of asystole is a significant adverse

prognostic marker regardless of the initial rhythm and the phase of initial resuscitation.

Rhythm Conversions of an Initial VF

Our findings on different outcomes in refractory OHCA for those who presented a transition from a

TABLE 3.
Rhythm Profiles in Patients With an Initial Nonshockable Rhythm

Initial Rhythm	PEA (n = 45)	Asystole (n = 55)	p
No rhythm change	15 (33%)	17 (31%)	Not significant
Rhythm change	30 (67%)	38 (69%)	
Intermittent rhythm			
ROSC	11 (24%)	4 (7%)	0.008
Asystole ^a	2 (4%)	38 (100%)	
VF	8 (18%)	13 (24%)	
PEA ^a	30 (100%)	2 (4%)	
Last rhythm			
ROSC	6 (13%)	16 (29%)	0.0002
Asystole	12 (27%)	29 (53%)	
VF	5 (11%)	2 (4%)	
PEA	22 (49%)	8 (15%)	

PEA = pulseless electrical activity, ROSC = recovery of spontaneous circulation, VF = ventricular fibrillation.

^aThe ongoing asystole and PEA were excluded from the calculation.

Data are expressed as n (%). The Fisher exact test was used.

VF to an intermittent or sustained organized rhythm, asystole, or with ongoing VF are in line with previously published data (16, 18), including refractory OHCA populations (19, 20, 24). Bhandari et al (16) presented that a transient organized rhythm had at least twice the survival rate to hospital discharge than patients with ongoing VF or detected asystole. In another study, Kandori et al (18) found in prospective multicenter observational data that the 1-month neurologic outcome of OHCA patients who manifested a conversion to a nonshockable rhythm at hospital admission was very poor compared with patients who had sustained VF. Furthermore, patients with a conversion to PEA had better prognosis than those with asystole (18). However, minimal data elucidates the impact of rhythm changes during CPR on the clinical outcome in refractory OHCA.

Before the Prague OHCA trial, only several studies, mainly retrospective, considered the impact of rhythm changes on patient prognosis in refractory OHCA. Maeda et al (19) showed in a single-center retrospective analysis that conversion to asystole at any time before ECPR in OHCA patients is

associated with in-hospital mortality and dismal in-hospital neurologic outcomes. In another study by Nakashima et al (20), patients with a sustained shockable rhythm before the establishment of ECMO support were more likely to have favorable neurologic outcomes at 6 months than those who showed a conversion from an initial shockable rhythm to nonshockable before ECMO initiation. Pozzi et al (28) reported that the occurrence of PEA or asystole at ECMO implantation was associated with a low survival rate and unfavorable neurologic outcomes in refractory OHCA patients. Recent analysis of the ECPR registry showed that heart rhythm at admission is a good predictor of 30-day mortality and neurologic outcomes (24).

The presented post hoc analysis of the Prague OHCA cohort was based on prospectively collected randomized data. Additionally, we enrolled truly refractory patients with prolonged resuscitations reaching 46 and 58 minutes in the standard and invasive arms. Furthermore, some studies refer to rhythm conversion only after initial defibrillation attempts (16, 18). Still, our data analyze the whole duration of the CPR period up to sustained ROSC, ECPR, or declaration of death. Furthermore, the Prague OHCA trial, including this post hoc analysis, compares conventional and invasive (ECPR-based) approaches. Finally, our study reports 180-day survival with a definitively established neurologic status, acknowledging that neurologic recovery can continue to evolve beyond 30 days following OHCA (9). Some previous studies were only focused on ECPR patients and did not evaluate subjects with ROSC (20, 24).

We also show the outcome of VF conversion to asystole separately from PEA. This has been separately analyzed only once in a study by Maeda et al (19). In agreement with their findings, the asystole was associated with higher in-hospital mortality and unfavorable neurologic outcomes in our study. Some previous studies have shown that PEA as the initial rhythm or when deciding to perform ECMO was associated with a trend toward improved survival rates and neurologic outcomes compared with those for asystole (29, 30).

Patients with a rhythm conversion from an initial shockable rhythm to a nonshockable rhythm, especially an asystole, before the initiation of ECPR, did not benefit from this advanced resuscitation strategy. Given that oxygen delivery and myocardial energy

TABLE 4.
Cox Regression Analysis in Predicting an Unfavorable Clinical Outcome

Model	Model A (Parameters Available During Ongoing CPR, <i>n</i> = 256)		Model B (Parameters After Initial In-Hospital Evaluation, <i>n</i> = 156)	
	HR (95% CI)	<i>p</i>	HR (95% CI)	<i>p</i>
Age ≥ 65 yr	1.09 (0.79–1.5)	0.61	0.94 (0.64–1.37)	0.76
Sex = woman	0.92 (0.64–1.34)	0.68	1.19 (0.77–1.86)	0.43
Telephone assisted bystander CPR = yes	0.91 (0.64–1.28)	0.58	0.84 (0.57–1.23)	0.37
Length CPR > 45 min = yes	–	–	1.85 (1.11–3.08)	0.02
Acute coronary syndrome = yes	–	–	0.92 (0.66–1.3)	0.65
ROSC anytime = yes	0.39 (0.28–0.53)	< 0.0001	–	–
Asystole anytime = yes	2.43 (1.79–3.3)	< 0.0001	–	–
Sustained ROSC at admission = yes	–	–	0.55 (0.32–0.93)	0.02
Asystole at admission = yes	–	–	2.37 (1.53–3.69)	0.0001

CPR = cardiopulmonary resuscitation, HR = hazard ratio, ROSC = recovery of spontaneous circulation.

Tested parameters available during ongoing CPR.

Dashes indicate the parameter was not included to the given model.

substrates might persist in patients with sustained VF, establishing sufficient perfusion of the injured myocardium with ECPR may lead to a higher chance of ROSC and organ recovery. There is also the effect of an invasive strategy on survival with a good neurologic status in patients with intermittent or sustained ROSC. The electrocardiogram rhythm at the presentation and during the CA reflects the etiology and the no-flow or low-flow time (28). If the time from collapse to ECPR onset is prolonged, rhythm conversion to a nonshockable rhythm will occur more frequently (31, 32). We speculate that the rhythm profile could be a better surrogate marker of many less addressable confounders. The invasive approach in the Prague OHCA trial did not only include the ECPR but also included intraarrest mechanical compressions, early transport to a dedicated center, and early invasive evaluation. We hypothesize that all those factors might remain invasive strategy beneficial for OHCA patients even though sustained ROSC is achieved, as suggested in our previous analyses (33), which is contrary to the previously reported deleterious effect of intraarrest transport (34).

Conversion of Initial Nonshockable Rhythm

The initial nonshockable rhythm appears to be a much better predictor of the clinical outcome than subsequent rhythms in patients suffering from an OHCA. In

a study by Cournoyer et al (21), the switch to a shockable rhythm was associated with an improvement in the odds of prehospital ROSC for patients presenting with an asystole. Rhythm conversion, however, did not significantly impact survival. Rajan et al (35) also observed that a conversion to a shockable rhythm was not a more robust prognostic marker than the initial rhythm itself. In a recent meta-analysis, patients who had an initial prehospital asystole experienced ROSC more often after a conversion to a shockable rhythm (22). Other studies confirmed the improved survival and clinical outcomes when subsequent shock delivery is applied (36–39). Goto et al (36) found that subsequent shock delivery in patients with an initial nonshockable rhythm was significantly associated with increased odds of prehospital ROSC, 1-month survival, and 1-month favorable neurologic outcomes when the shock delivery time was less than 20 minutes. It seems that outcomes after OHCA and subsequent shock delivery after an initial nonshockable rhythm may be substantially associated with shock delivery time from collapse (36, 37, 39).

We found that four of five patients with an initial nonshockable rhythm who survived 6 months with CPC 1 and 2 manifested a rhythm conversion to a shockable rhythm or ROSC before in-hospital admission. There was, however, one patient without rhythm conversion who survived while remaining in therapy

refractory PEA and applied ECPR as an equivalent to a subsequent shock delivery. Another explanation of rare survivors in a nonshockable group, as reported by Herlitz et al (38), might be misjudged fine VF interpreted as asystole, indicating the possibility of a successful later defibrillation.

Our study did not show the benefit of ECPR in patients with initial nonshockable rhythms. The fact that patients presenting with nonshockable rhythms do not respond to this advanced therapy might be more related to the course and etiology of CA than ECPR itself. We speculate that a shockable rhythm on presentation is a marker for earlier intervention. Indeed, even after conversion to a shockable rhythm, patients having an initial nonshockable rhythm might have more severe underlying total body ischemia concerning the duration of their arrest or the quality of their resuscitation. It is also possible that their underlying illness is less easily treatable than patients with an initial shockable rhythm, regardless of subsequent rhythm conversions.

Study Limitations

The main limitation of this study was the single-center design, which makes generalization difficult. The data are also limited to regions that have an ECMO center available in a reasonable time. The second limitation was a lack of timing of conversions in the prehospitalization phase. Data was based on medical emergency system staff reports. Third, we cannot rule out that intermittent ROSC was not captured in all cases. Fourth, we cannot rule out that many poorly assessable specifics of the individual OHCA event (i.e., bystander behavior, the effectiveness of CPR, other invasive interventions, etc.) may play a significant role in the outcomes. Finally, the numbers in the analyzed study subgroups are limited, and some crossovers were made. The multicenter study and data on other modalities are sorely needed.

CONCLUSIONS

The post-initial rhythm profile could more precisely identify an outcome in refractory OHCA patients. Deterioration of the initial shockable rhythm to asystole has a poor prognosis, even when ECPR is readily available. An invasive approach (including intraarrest compressions, early transport, early invasive evaluation, and

ECPR) seems beneficial in patients with intermittent or sustained ROSC. It is maybe beneficial in patients with ongoing VF. An initial nonshockable rhythm has an inauspicious prognosis, and a conversion to a shockable rhythm does not seem to improve outcomes.

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Significant hemolysis is present during irreversible electroporation of cardiomyocytes in vitro

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Significant hemolysis is present during irreversible electroporation of cardiomyocytes in vitro

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ABSTRACT

BACKGROUND Pulsed field ablation (PFA) of atrial fibrillation is a new method in clinical practice. Despite a favorable safety profile of PFA in atrial fibrillation ablation, rare cases of renal failure, probably due to hemolysis, have recently been reported.

OBJECTIVE The aim of this study was to determine the rate of hemolysis and cardiac cell death during in vitro PFA with different electric field intensities.

METHODS Blood samples from healthy volunteers and mouse HL-1 cardiomyocyte cell lines were subjected to in vitro irreversible electroporation using 216 bipolar pulses, each lasting 2 μ s with intervals of 5 μ s, repeated 20 times at a frequency of 1 Hz. These pulses varied from 500 V to 1500 V. Cell-free hemoglobin levels were assessed spectrophotometrically, and red blood cell microparticles were evaluated by flow cytometry. Cardiomyocyte death was quantified with propidium iodide.

RESULTS Pulsed field energy (1000 V/cm, 1250 V/cm, and 1500 V/cm) was associated with a significant increase in cell-free hemoglobin (0.32 ± 0.16 g/L, 2.2 ± 0.96 g/L, and 5.7 ± 0.39 g/L; $P < .01$) and similar increase in the concentration of red blood cell microparticles. Significant rates of cardiomyocyte death were observed at electric field strengths of 750 V/cm, 1000 V/cm, 1250 V/cm, and 1500 V/cm ($26.5\% \pm 5.9\%$, $44.3\% \pm 6.2\%$, $55.5\% \pm 6.9\%$, and $74.5\% \pm 17.8\%$ of cardiomyocytes; $P < .01$).

CONCLUSION The most effective induction of cell death in vitro was observed at 1500 V/cm. This intensity was also associated with a significant degree of hemolysis.

KEYWORDS Atrial fibrillation; Cardiomyocytes; Hemolysis; Irreversible electroporation; Pulsed field ablation

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Introduction

Pulsed field ablation (PFA), or irreversible electroporation (IRE), presents a new nonthermal energy that is increasingly used for catheter ablation of atrial fibrillation.¹ In preclinical studies, PFA was associated with high myocardial selectivity and preservation of surrounding nonmyocardial structures. Accordingly, no cases of pulmonary vein stenosis or atrial-esophageal fistula have been reported in clinical practice.¹ Targeted myocardial damage and retention of nonmyocardial atrial stroma also contribute to the preservation of nerve tissue and blood vessels in the atrial wall.

The principle of PFA is to produce irreversible pores in the cytoplasmic membrane by high-energy electric pulses of short duration and high frequency.² These pores allow the free passage of ions.³ Given that the extracellular concentration of Ca^{2+} is notably higher than the intracellular concentration, Ca^{2+} flows down its concentration gradient into the cell, causing an intracellular overload of Ca^{2+} , Ca^{2+} -related disruption of various metabolic pathways, and ultimately cell death.⁴

Although the method has been thoroughly studied, instances of acute renal failure have been attributed to

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procedural hemolysis, necessitating temporary hemodialysis in some cases.⁵ Numerous in vitro^{6–9} and in vivo^{10,11} studies have examined the impact of various electroporation protocols commonly used for IRE on cardiomyocytes, nonmyocardial cardiac tissue, and surrounding structures, such as pulmonary veins, the aorta, and the esophagus. Interestingly, the effect of PFA on erythrocytes was not described in these studies, although the effect of isolated high-voltage pulse on erythrocytes was described as early as the 1970s.

Our in vitro pulsed field (PF) system allows set up of electroporation parameters, including electric field strength, pulse length, pulse polarity, and pulse number of repetition. Our study aimed to evaluate the impact of PFA on hemolysis and cardiomyocyte cell death under in vitro conditions. We sought to determine how erythrocyte and cardiomyocyte injury varies with different electric field strengths.

Methods

Electric field modeling

Because it is not possible to directly measure the high electric fields achieved and the value in tissue depends not only on applied voltage but also on electrode shape, distance between electrodes, and electrical conductivity of particular tissue, numerical simulation had to be performed. Details of the simulation and parameters are shown in the [Supplemental Methods](#), and in [Supplemental Tables S1 and S2](#) and [Supplemental Figures S1–S3](#).

Ethics statement and obtaining blood samples

Blood samples were obtained from healthy volunteers according to the European Union regulation on collecting and using human body materials for research purposes. Both sexes were included in the study. All blood samples were obtained while patients were fasting. Samples were drawn from antecubital veins and without tourniquets; the first 5 mL of blood was discarded. Hemoglobin concentration was assessed in all volunteers, and all were within the normal range. The study was approved by the Ethics Committee of the University Hospital Kralovske Vinohrady (EK-VP/5810I02023) and was conducted according to the Declaration of Helsinki. All healthy volunteers signed an informed consent document before blood donation.

Cell culture

For cell experiments, the immortalized cell line of HL-1 cardiomyocytes (SCC065; Merck, St Louis, MO) was used. Cells were cultured in Claycomb medium (51800C; Merck) according to an optimized protocol provided by Dr Claycomb's laboratory.¹² For all experiments, passages 9–12 were used, all with at least 80% confluency (approximately 4 days after seeding). For electroporation in 96-well plates (Thermo

Fisher, Waltham, MA), cells were seeded at a density of 35,000 cells/well. All experiments were performed at 100% cell confluence.

In vitro IRE protocol

PFA was conducted with a commercial Tonapulse (Tonagen, Kladno, Czech Republic) electrical pulse generator with an electrode plate ([Figure 1A and 1B](#)). A detailed description of the electrodes is given in the [Supplemental Methods](#). A model of electrode placement in a single well is shown in [Figure 1C](#).

For the blood experiments, samples of whole blood were collected in the 10-mL sodium citrate vacutainers. Blood samples were injected with an Eppendorf pipette with a 200- μ L tip into 96-well plates (150 μ L/well). Each volunteer sample was exposed to all electroporation protocols in triplicate. The IRE protocol was as follows: 1 burst consisted of 216 bipolar pulses lasting 2 μ s, with 5- μ s pauses between the pulses ([Figure 1D and 1E](#)). Each burst was repeated 20 times with a 1-second pause in between. Electric fields ranged from 250 V/cm to 1500 V/cm. All blood samples were exposed to electric fields of 250 V/cm, 500 V/cm, 750 V/cm, 1000 V/cm, 1250 V/cm, and 1500 V/cm. One blood sample served as a control (no electric field was applied to the control well). Blood samples were collected and centrifuged for plasma separation (2500g, 15 minutes, 2 times). Plasma was collected and frozen at -80°C . A detailed description of the blood sampling and blood processing is given in the [Supplemental Methods](#).

The same electroporation protocol was used for the IRE of the HL-1 cell line of cardiomyocytes. Before electroporation of cardiomyocytes, the culture medium was replaced with 50 μ L of fresh medium. After electroporation, 200 μ L of medium was added to the cells.

Flow cytometry analysis

For red blood cell microparticle (RBC μ) analysis, we used 50 μ L of purified plasma. To detect fragments of disintegrated erythrocytes, each sample was incubated with 5 μ L of phycoerythrin-conjugated CD235a (glycophorin A; IgG1 clone 11E4B-7-6 KC16) and 1 μ L of fluorescein isothiocyanate-conjugated modified human recombinant annexin V for 20 minutes in the dark. After incubation, 400 μ L of ice-cold 1 $\times$ annexin V binding buffer and 50 μ L of flow-count fluorospheres were added to each specimen and mixed gently. All reagents were purchased from Beckman Coulter (Brea, CA). Further details are presented in the [Supplemental Methods](#) and [Supplemental Figure S4](#).

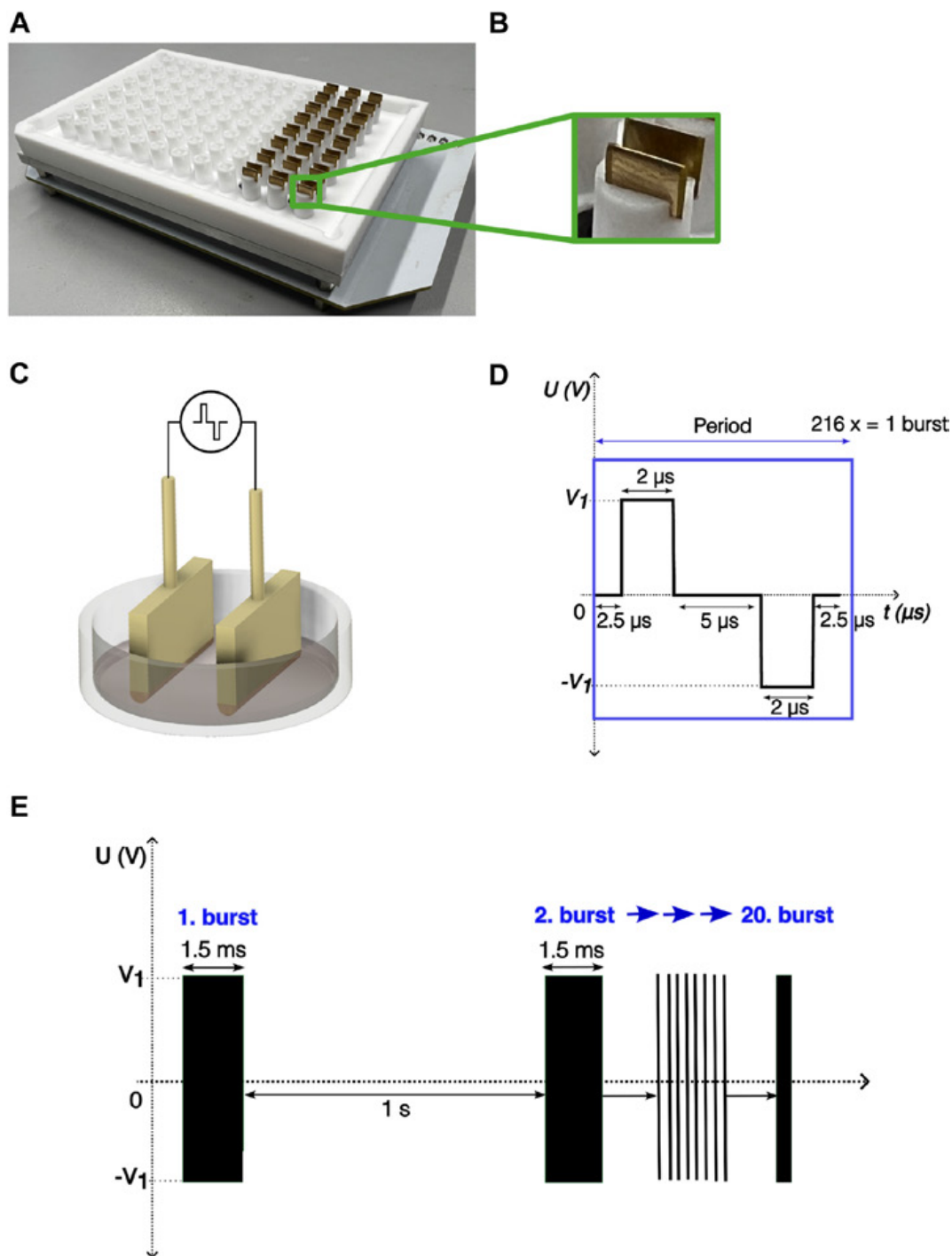
Analysis of cell-free hemoglobin

Because of the very high hemolytic range, we used the Harboe direct spectrophotometric method.¹³ This method is based on the absorbance of oxyhemoglobin at 415 nm with a background correction for impurities by interpolating the optical densities at 380 nm (nonspecific plasma interferents)

Abbreviations

IRE:	irreversible electroporation
PF:	pulsed field
PFA:	pulsed field ablation
RBC:	red blood cell
RBC μ :	red blood cell microparticle
RMSE:	root mean square error

omyocytes (SCC065; Merck, St Louis, MO) was used. Cells were cultured in Claycomb medium (51800C; Merck) according to an optimized protocol provided by Dr Claycomb's laboratory.¹² For all experiments, passages 9–12 were used, all with at least 80% confluency (approximately 4 days after seeding). For electroporation in 96-well plates (Thermo

**Figure 1**

Electroporation setup for in vitro adherent cultures. **A:** Planar array of semiflexible noble metal electrodes with a top-layer printed circuit board and polytetrafluoroethylene (Teflon) components compatible with a 96-well cell culture plate. **B:** Detail of the electrode for 1 well. **C:** Model of the electrode system inside the 1-cell culture well. **D:** Schematic of electroporation protocol for 1 burst. **E:** Schematic of burst setup.

and 450 nm (bilirubin/albumin complexes). The plasma samples were diluted 10 times in phosphate-buffered saline. Subsequently, the absorbance was measured as mentioned before. The concentration was calculated by Equation 1.

$$Hb \left(\frac{g/l}{l} \right) = \frac{k * (167.2 * A_{415} - 83.6 * A_{380} - 83.6 * A_{450})}{1000} \quad (1)$$

Cell death detection

The cells were incubated with 5 $\mu g/mL$ propidium iodide for 30 minutes at 37°C (Sigma-Aldrich, St Louis, MO) and 5 $\mu g/mL$ Hoechst (cat. no. 34580; Sigma-Aldrich) for 30 minutes after IRE. Cell death was studied for 1 hour after IRE with the fluorescence microscope Nikon ECLIPSE Ts2FL. A detailed

description of the cell death is given in the [Supplemental Methods](#).

Statistical analysis

All data were assessed for normal distribution by the Shapiro-Wilk parametric hypothesis test. Because the paired data of cell-free hemoglobin and RBC μ did not exhibit a normal distribution, Friedman 2-way analysis of variance by ranks with a Bonferroni correction was employed to assess the effect of field intensity. The Wilcoxon signed rank test was used to evaluate differences between the negative control (no IRE) and each IRE-exposed sample. Because the data for cell death had a normal distribution, the 1-way analysis of variance was employed to evaluate the influence of electric field strength on cell death. Accordingly, a paired t-test was used to compare the negative control and each electric field intensity. The level of statistical significance for all analyses was $P = .05$. In the Results section, the data without normal distribution are described by the median with interquartile range. The data with normal distribution are described by the mean with SD. The data in the figures are presented as the spots graph with

the median marked. All statistical analyses were carried out with MATLAB R2023a (MathWorks, Natick, MA).

Results

Exposure to higher intensity electric fields increases cell-free hemoglobin

The in vitro exposure of blood samples to stepwise increasing IRE induced a significant release of cell-free hemoglobin ($P < .0001$; [Figure 2](#)). A representative image of plasma after electroporation is depicted in [Figure 2A](#). Compared with controls, the concentration of cell-free hemoglobin did not increase after exposure to a lower intensity electric field (0–750 V/cm); however, starting with 1000 V/cm, a significant increase in cell-free hemoglobin was observed ([Figure 2](#)). Exposure to 1000 V/cm, 1250 V/cm, and 1500 V/cm fields led to increased concentrations of cell-free hemoglobin, 0.32 ± 0.16 g/L, 2.2 ± 0.96 g/L, and 5.7 ± 0.39 g/L, respectively (all $P < .01$). The observed concentrations were 4-fold, 27.5-fold, and 71.25-fold higher, respectively, than the negative control in which a concentration of 0.07 ± 0.04 g/L was seen ([Figure 2](#)). The concentration of free hemoglobin increases

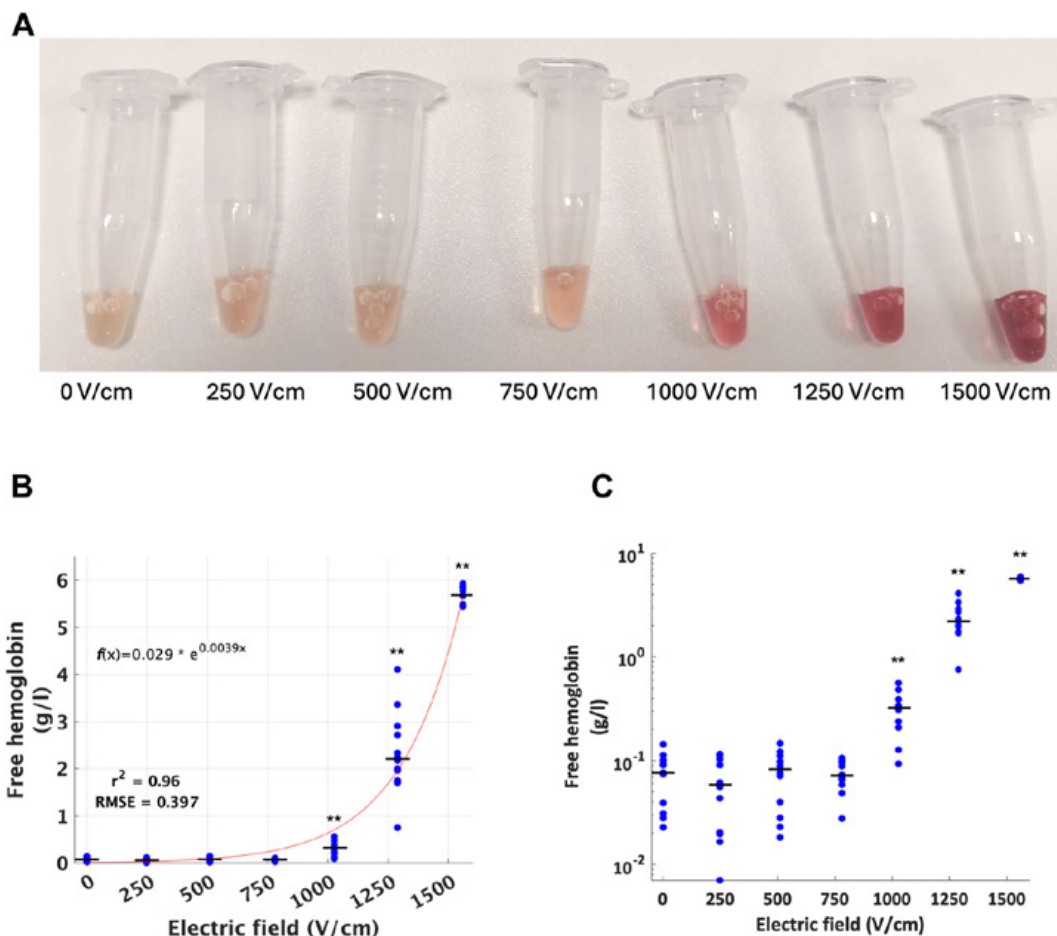


Figure 2

A: Plasma samples of blood exposed to irreversible electroporation with increased electric field. B, C: Concentration of cell-free hemoglobin in plasma. The data show a significant increase in free hemoglobin concentration starting with electric field strength of 1000 V/cm. Each point represents a measured value obtained from individual volunteers at the corresponding electric field strength. The black lines depict the median value. Free hemoglobin concentration is in linear (B) and logarithmic scale (C). ** $P < .01$ based on Friedman 2-way analysis of variance with post hoc pairwise Wilcoxon signed rank test, $n = 10$ biologic replicates. RMSE = root mean square error.

Table 1 Median values with interquartile range of cell-free hemoglobin and RBC μ and mean value $\pm$ SD of cardiomyocyte cell death from all measured parameters for each group

Pulse voltage amplitudes (V)	Simulated electric field (V/cm)	Cell-free hemoglobin (g/L)	RBC μ (abs/ μ L)	Cell death (%)
0	0	0.08 $\pm$ 0.06	68 $\pm$ 79	4.4 $\pm$ 1.4
75	$\sim$ 250	0.06 $\pm$ 0.08	331 $\pm$ 215	4.7 $\pm$ 2.1
160	$\sim$ 500	0.08 $\pm$ 0.07	539 $\pm$ 462	10.2 $\pm$ 2.9
241	$\sim$ 750	0.07 $\pm$ 0.03	1513 $\pm$ 1569	26.5 $\pm$ 5.9
320	$\sim$ 1000	0.32 $\pm$ 0.16	7586 $\pm$ 14,838	44.3 $\pm$ 6.2
400	$\sim$ 1250	2.2 $\pm$ 0.96	52,896 $\pm$ 35,992	55.5 $\pm$ 6.9
490	$\sim$ 1500	5.7 $\pm$ 0.39	155,358 $\pm$ 187,556	74.5 $\pm$ 17.8

RBC μ = red blood cell microparticles.

exponentially according to the equation $f(x) = 0.029 \cdot e^{0.0039x}$ with a correlation of 0.96 and root mean square error (RMSE) of 0.397 (Figure 2B). Figure 2C shows data with the logarithmic scale. Differences between sexes were not observed. All data are summarized in Tables 1 and 2.

Exposure of blood samples to all electric fields leads to RBC μ formation

RBC μ concentration increased at all electric field strengths during IRE ($P < .001$; Figure 3). The concentration of RBC μ was 4.9-fold, 7.9-fold, 22.3-fold, 111.6-fold, 777.9-fold, and 2284.7-fold higher in electric fields of $\sim$ 250 V/cm, $\sim$ 500 V/cm, $\sim$ 750 V/cm, $\sim$ 1000 V/cm, $\sim$ 1250 V/cm, and $\sim$ 1500 V/cm, respectively (all $P < .01$). Results are shown in Figure 3. Figure 3A shows all groups, and Figure 3B provides a more detailed representation of the electric field intensity up to 750 V/cm, enhancing the presentation of lower values within these groups. Figure 3C shows data with the logarithmic scale. The concentration of RBC μ increases exponentially according to the equation $f(x) = 86.2 \cdot e^{0.005x}$ with a correlation of 0.72 and RMSE of 43179 (Figure 3B). Differences between sexes were not observed. All data are also summarized in Tables 1 and 2. A representative example of the scatter graphs is presented in Supplemental Figure S4.

IRE-induced cardiomyocyte death in vitro

The same IRE protocol with increasing electric field strength was applied on a cell line of cardiomyocytes prepared in a 96-well plate, as described previously. Whereas electric fields $\sim$ 250 V/cm and $\sim$ 500 V/cm did not induce cell death, a statistically significant induction of cell death was present at $\sim$ 750 V/cm. The proportion of dead cardiomyocytes increased with applied voltages as follows: 27% $\pm$ 6%, 44%

$\pm$ 6%, 56% $\pm$ 7%, and 75% $\pm$ 18%, corresponding to electric field intensities of $\sim$ 750 V/cm, $\sim$ 1000 V/cm, $\sim$ 1250 V/cm, and $\sim$ 1500 V/cm, respectively (Figure 4). The ratio of dead cells increases exponentially according to the equation $f(x) = 7.7 \cdot e^{0.0015x}$ with a correlation of 0.88 and RMSE of 9.46 (Figure 4A). Figure 4B provides a view of the same data with logarithmic scale. Representative examples of fluorescence images are shown in Figure 4C. The red straight line in each picture represents dead cells due to contact with electrodes. All data are summarized in Tables 1 and 2.

Discussion

The key findings of the study can be summarized as follows:

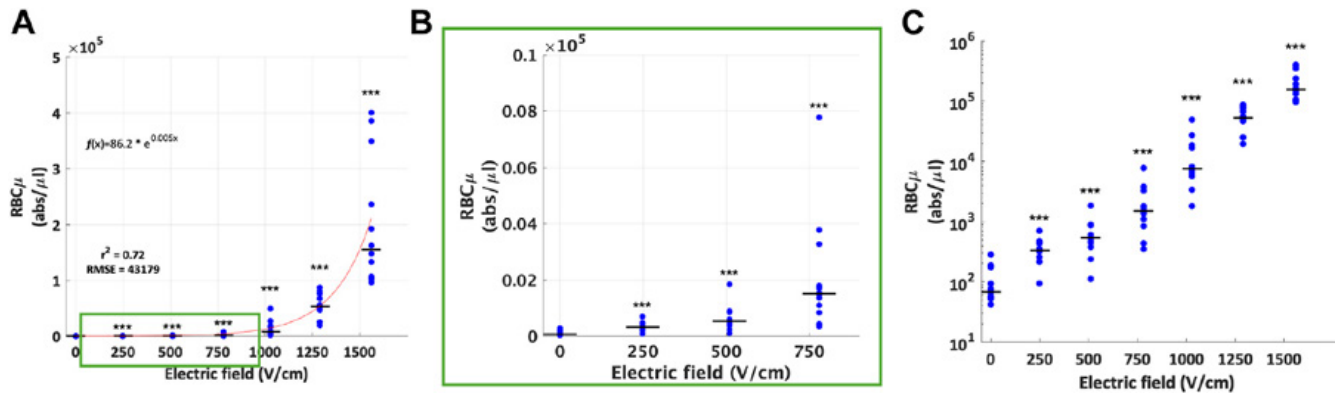
- During in vitro PFA, statistically significant hemolysis occurred around 1000 V/cm.
- A similar electric field intensity led to considerable cell death. Destruction of a cell line of cardiomyocytes started at 750 V/cm, but the highest percentage of cell death (about 75% of exposed cardiomyocytes) occurred at 1500 V/cm.

PFA has recently gained approval from regulatory authorities for clinical use. The effect of a single high-energy electric pulse on pore formation in erythrocytes in a physiologic solution was described in the 1970s.³ Surprisingly, although numerous preclinical animal and human studies have explored the effect of currently used PFA systems on cardiomyocytes and nonmyocardial cardiac structures, the effect on erythrocytes has not been studied. It was not until kidney failure due to hemolysis after PFA was reported that attention was drawn to the effect of PFA on erythrocytes. During catheter ablation using PF energy, the relatively broad (31 mm) catheter comes into contact not only with the endothelium of the pulmonary vein but also with the surrounding blood, leading to the release of PF energy into the surrounding blood elements. The cases of acute kidney injury due to hemolysis after PFA procedures have been reported after procedures with a large number of PF pulses.⁵ Our research group from the Department of Cardiology at University Hospital Kralovske Vinohrady observed increased red blood cell (RBC) breakdown in patients undergoing catheter ablation with PFA (determined by measuring the concentration of RBC μ by flow cytometry) immediately after the procedure, followed by a decrease in haptoglobin concentration 24 hours after the procedure.¹⁴ Furthermore, the amount of hemolysis

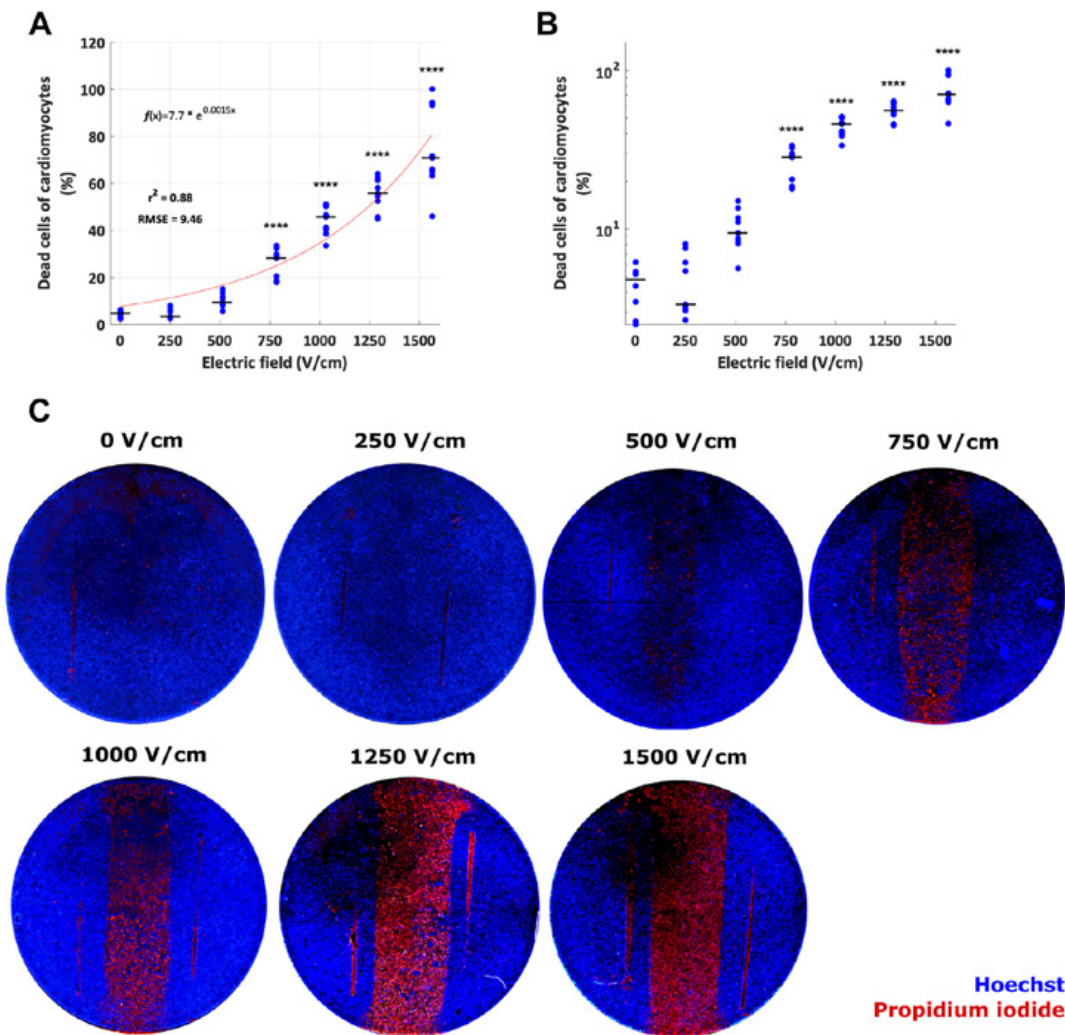
Table 2 Mathematical models of individual measured parameters

Measured parameter	Equation	Correlation	RMSE
Free hemoglobin	$f(x) = 0.029 \cdot e^{0.0039x}$	0.96	0.397
RBC μ	$f(x) = 86.2 \cdot e^{0.0039x}$	0.72	43179
Dead cells	$f(x) = 7.7 \cdot e^{0.0039x}$	0.88	9.46

RBC μ = red blood cell microparticles; RMSE = root mean square error.

**Figure 3**

Concentration of red blood cell microparticles (RBCμ). The data show significantly higher RBCμ concentrations in all groups exposed to irreversible electroporation. A: Concentrations of RBCμ at all electric field strengths. B: Detail representation of lower electric field strengths up to 750 V/cm. Each point represents a measured value from individual volunteers at the corresponding electric field strength. The black lines depict median values. C: Concentrations of RBCμ at all electric field strengths, Y axis in logarithmic scale. *** $P < .001$ based on Friedman 2-way analysis of variance with post hoc pairwise Wilcoxon signed rank test, $n = 12$ biologic replicates. RMSE = root mean square error.

**Figure 4**

Cell death of atrial HL-1 mouse cardiomyocytes. The data show significantly higher cell death with an electric field strength of ~ 750 V/cm. Each point represents a measured value from individual volunteers at the corresponding electric field strength. A: Percentage of dead cells in linear and in logarithmic (B) scale. C: Representative examples of fluorescence images of dead cells. Blue = living cells, red = dead cells. The black lines depict the median values. **** $P < .01$ based on a 1-way analysis of variance with post hoc Dunnett test, $n = 9$ biologic replicates. RMSE = root mean square error.

was related to the number of PF applications. Similarly, Nies et al¹⁵ described an increase in cell-free hemoglobin that was dependent on the number of PF applications, documenting the dose-dependent effect of PF on hemolysis.

Studying the effects of IRE *in vitro* let us assess and compare electric field strength thresholds that injure erythrocytes and cardiomyocytes. Moreover, in the future, the possibility of modifying the characteristics of the PF pulse in our PF generator to match those commonly used in clinical practice (as reported by the manufacturer) will enable us to test different electric field strengths and pulse durations on cardiomyocyte and erythrocyte death.

***In vitro* effect of PF energy on erythrocytes**

An electrode system compatible with 96-well culture microplates was used to expose blood samples and a cell line of cardiomyocytes to an electric field. The electrode system created a highly homogeneous distribution of the electric field; 50% of the area between the electrodes in the culture well produces a target electric field of $\pm 4\%$ (Supplemental Figure S3C and S3D). Blood samples were exposed to short (2- μ s) bipolar pulses structured as a set of microsecond-scale pulses of 20 bursts, each consisting of 216 pulses at a frequency of 1 Hz. The protocol follows the general principle of PFA, that is, an application of a fast sequence of high-intensity, short-duration bipolar electrical impulses.¹² Our protocol does not correspond to any specific PFA protocol currently approved for clinical practice. However, because it operates on a general principle of PFA (ie, a sequence of high-intensity electrical pulses of short duration), the results for cardiomyocyte death and hemolysis in our experiment are more comparable to *in vivo* conditions than results of experiments using a single electric pulse on erythrocytes in a physiologic solution.³ The electrical conductivity of isolated erythrocytes in a physiologic solution and that in whole blood differ significantly, and electrical conductivity is an essential parameter that affects the outcome of PFA. Moreover, studies from the 1970s examined the effect of only a single, isolated electrical pulse, which does not correspond to the currently used *in vivo* systems that employ a sequence of multiple high-energy pulses.

Owing to the different electrical conductivity of blood cell culture media and cardiomyocytes, slightly different electrical fields were used in the erythrocytes and cardiomyocytes in our experiments. Electric field strengths of 248 V/cm, 510 V/cm, 780 V/cm, 1027 V/cm, 1289 V/cm, and 1560 V/cm were used for blood samples, and strengths of 250 V/cm, 513 V/cm, 783 V/cm, 1031 V/cm, 1291 V/cm, and 1564 V/cm were used in the experiments with cardiomyocytes (for details, see the Supplemental Methods). Because of the minimal differences in electric field strength between blood and cardiomyocytes, these variations did not significantly affect either the rate of cell death or the rate of hemolysis.

A significant rate of hemolysis seen as increased concentrations of cell-free hemoglobin was observed starting with electric field strengths of 1000 V/cm; increased concentrations of RBC μ were observed with an electrical field of 250

V/cm. For example, an electric field of 1500 V/cm resulted in a concentration of cell-free hemoglobin of 5.7 g/L. With regard to RBC μ , which are fragments of damaged RBCs, concentrations were significantly increased with all applied electric field intensities, with the increase becoming more pronounced starting at an IRE of 750 V/cm. Under clinical conditions, high amounts of cell-free hemoglobin caused by rapid hemolysis can lead to tubular injury and even acute kidney injury.^{16,17} Our *in vitro* study cannot show the safe threshold for *in vivo* application of PF energy. However, our data clearly indicate that increasing the electric field intensity by short bipolar pulses contributes to significantly increased erythrocyte destruction. In addition, our data suggest that monitoring RBC μ by flow cytometry can detect IRE-associated RBC breakdown earlier than by measuring free hemoglobin in plasma. Thus, it may represent a more sensitive parameter of RBC damage than free hemoglobin.

A study from the 1970s demonstrated that applying a single 20- μ s pulse generating a transient electric field of 2000 V/cm or higher can induce rupture of isolated erythrocytes. The investigators attributed this effect to an increase in intracellular ion concentration leading to elevated osmotic pressure.³ Another study observed local elongation of attached isolated erythrocytes on a glass coverslip induced by high-frequency (MHz) electric pulses, where the length of 1 pulse is approximately 1 μ s with an electric field strength of 2000 V/cm without RBC rupture.¹⁸ RBC rupture was observed only when the electric field was increased to 5000 V/cm. Another study observed the induction of hemolysis in isolated mouse RBCs after exposure to a single 300- μ s pulse and an electric field strength of about 1100 V/cm.¹⁹ These studies confirmed that the length of the electrical pulse and the strength of the electric field can cause RBC rupture. Our findings corroborate the potential hemolytic effect of PFA under *in vitro* conditions and expand the threshold for erythrocyte and cardiomyocyte death by using not a single electrical impulse but a sequence of impulses similar to those used during PFA.¹²

***Cardiomyocyte and in vitro* PF energy**

When cardiomyocytes were exposed to the same electric field strengths, a statistically significant induction of cell death started at 783 V/cm, which caused death in 27% of all cardiomyocytes. Although this proportion of cell death was statistically significant in our *in vitro* conditions, ablation of only 27% of cardiomyocytes is unlikely to be clinically significant during catheter ablation. Effective ablation lesions should be close to 100% of targeted cardiomyocytes. The rate of cell death increased with increasing electric field strength, with cell death present in 75% of cardiomyocytes at 1500 V/cm. Although it is challenging to translate these *in vitro* results to clinical settings, 75% cardiomyocyte death is certainly closer to a clinically meaningful effect. Cardiomyocyte death after high-voltage pulses has been studied by others. Casciola and coworkers²⁰ demonstrated an increasing area of dead induced pluripotent stem cell-derived cardiomyocytes with

increasing electric field strength and number of electric pulses. The study observed cell death at 820 V/cm using 300 pulses of 5 μ s duration and 10 Hz frequency.²⁰ A study by Baena-Montes and coworkers⁷ observed effective induction of cardiomyocyte cell death at an electric field intensity of 750 V/cm (similar to our study) and a pulse length of 100 μ s. These results suggest that the electric field intensity required to destroy cardiomyocytes effectively is also sufficient to induce hemolysis. However, the mechanism of cardiomyocyte cell death may involve slightly different mechanisms, and further research is needed to show whether all IRE protocols associated with effective cardiomyocyte death are also associated with hemolysis. Unlike erythrocytes, cardiomyocytes form a syncytium in tissue culture, with no cells in suspension. The mechanism of cell death is likely due to the formation of pores in the cardiomyocyte cell membrane and subsequent triggering of cell death mechanisms. Further studies and a detailed examination of different electroporation protocols are needed to investigate ways to effectively induce cardiomyocyte cell death while minimizing hemolysis. These protocols will vary not only in electric field intensity but also in the length, number, and shape of pulses.

Study limitations

The effect of PF energy on RBCs and cardiomyocytes is influenced by the pulse length, number of pulses, pause duration between pulses, and intensity of the electric field. Because the characteristics of the pulse in our in vitro PF generator do not correspond to any particular approved device, our data cannot be easily extrapolated to any currently used or developed system. In addition, in vitro blood flow is absent, unlike in catheter ablation procedures in human patients. The design of the electrodes is different from those used in the clinical setting. The in vitro induction of cardiomyocyte cell death involved only a thin layer of cells, precluding the analysis of cardiomyocyte cell death in a 3-dimensional model.

Conclusion

In our study, electric field strength energy around 1500 V/cm is required to effectively induce cardiomyocyte cell death. However, this setting also leads to significant erythrocyte breakdown, which starts with electric field strength of around 1000 V/cm.

Clinical implications

Our proposed approach enables comprehensive testing of a diverse array of electroporation protocols to investigate the development of RBC hemolysis in vitro. The data demonstrate that hemolysis can be induced on the basis of the electric field strength value with use of short bipolar pulses, which are common in clinical practice. Therefore, it is crucial to assess a specific electroporation protocol in vitro for its potential hemolytic effects, thereby enhancing the safety of the procedure.

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Appendix

Supplementary data

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.hrthm.2024.08.019>.

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The role of ventricular remodeling in the early decompensation of cardiorenal syndrome: Insight from studies with Ren-2 transgenic hypertensive rats subjected to volume overload induced using aorto-caval fistula

Hypertension Research
Impact Factor: 4,6





The role of ventricular remodeling in the early decompensation of cardiorenal syndrome: Insight from studies with Ren-2 transgenic hypertensive rats subjected to volume overload induced using aorto-caval fistula

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Abstract

The aim of the present study was to evaluate the role of the left ventricle (LV) remodeling in the process of the transition from the compensation to the decompensation phase of cardiorenal syndrome. Ren-2 transgenic rats (TGR) with aorto-caval fistula (ACF) were used as the model of cardiorenal syndrome. Two weeks after ACF creation or sham operation, heart morphological parameters, cardiac structure and function were assessed by echocardiography and invasive pressure-volume analysis. This time point was chosen because two weeks after ACF the TGR still exhibit 100% survival rate and are in the transition phase from the compensation to the decompensation of cardiorenal syndrome. Our results at this stage show: (i) ACF TGR have already fully developed eccentric LV hypertrophy as compared with sham-operated TGR which exhibited signs of LV concentric hypertrophy; (ii) the increase in whole heart weight in ACF TGR was dominantly mediated by right ventricle (RV) hypertrophy, whereas the increase in the LV mass was minimal; (iii) ACF TGR displayed, besides bilateral ventricular dilatation, significant impairment of LV systolic functions whereas RV systolic functions were not impaired as compared with sham-operated TGR. Based on our present results, we propose that the inability of the LV to develop an appropriate hypertrophic response leads to maladaptive ventricular remodeling, which is likely a crucial factor in the process of the transition from the compensation to the decompensation phase of cardiorenal syndrome.

Keywords Cardiorenal syndrome · Ren-2 transgenic hypertensive rats · Volume overload · Cardiac remodeling · Cardiac functions

These authors contributed equally: Petr Kala, Matúš Miklovič

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Introduction

The profound bidirectional relationship between the kidney and the heart in the physiological regulation of cardiorenal function is now well-recognized [1–3]. The importance of this relationship in pathophysiological situations was described 190 years ago by Robert Bright [4], who

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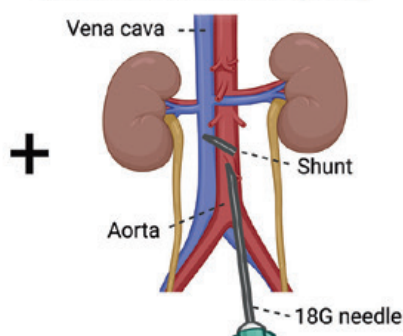
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Graphical Abstract

Ren-2 transgenic
hypertensive rats (TGR)

Aorto-caval fistula (ACF)


ACF TGR
 = Model of human cardiorenal
 syndrome

**Effects of 2 weeks ACF induction on heart parameters – comparison
 with sham-operated TGR**

Δ Whole Heart Weight	+32 ± 3%
Δ Left Ventricle Weight	+12 ± 3%
Δ Right Ventricle Weight	+101 ± 9%
Δ Cardiomyocyte Length/Width	+25 ± 2%

Conclusion: Based on our evaluation of cardiac morphology, structure and function in ACF TGR, we propose that the inability of the left ventricle to develop appropriate hypertrophic response (leading to maladaptive ventricular remodeling) is a crucial factor in the process of the transition from the compensation to the decompensation phase of cardiorenal syndrome.

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was the first to describe important cardiac structural changes in patients with advanced proteinuric kidney disease. Later, 70 years ago, Barger et al. [5] demonstrated for the first time the critical role of the kidney in the pathophysiology of heart failure (HF). At present, it is recognized that if HF is accompanied by impairment of renal function, the prognosis of patients is gloomy, even worse than in most types of common cancers [6–8]. The combination of HF with renal functional impairment is called “cardiorenal syndrome”; the term has entered medical terminology over the last 20 years and has been largely accepted - obviously because of the striking connection of the syndrome with the deterioration of morbidity and mortality of patients [9–12]. Cardiorenal syndrome was initially identified by Ronco et al. [13] as “disorders of the heart and kidneys whereby acute or chronic dysfunction in one organ may induce acute or chronic dysfunction of the other”. The syndrome was divided into 5 subcategories according to the organ of origin and depending on the nature of the initial insult: acute or chronic. This description was accepted for almost two decades [10–12, 14–18]. Still, recently, the

term has been conceptually modified to “chronic cardiovascular-kidney disorder” [19], which should better capture the disruption caused by disease states to organ function. Moreover, the modification was intended to achieve simplification and conceptual clarity. Regardless of the terminology used: “cardiorenal syndrome” or “chronic cardiovascular-kidney disorder”, awareness of the syndrome’s existence helped to propose adequate clinical trials and to introduce a new multidisciplinary approach in the treatment of the affected patients (“nephrocardiology”) [20–24]. Unfortunately, only scanty knowledge is available about the pathophysiological mechanisms underlying the development and particularly the progression of the syndrome. Undoubtedly, such knowledge is a prerequisite for introducing new therapeutic measures. These are urgently needed because currently available pharmacological strategies appear inadequate for effective management of the syndrome/disorder [11, 14–18, 25, 26]. Importantly, clinical investigations have demonstrated that patients with cardiorenal syndrome are known to display excessive renal sodium avidity, indicating that, at present, the research and

treatment have been focused on the renal aspects of the cardiorenal syndrome [11, 15–18, 27].

We employed here the rat model of volume overload induced by the creation of the aorto-caval fistula (ACF) in Ren-2 transgenic rat (TGR), a strain that combines endogenous activation of the renin-angiotensin system (RAS) and hypertension [28, 29] - the two factors critical for the development and progression of cardiorenal syndrome [9, 10, 14, 30–32]. Earlier, we demonstrated that ACF TGR represents a reasonable model of human cardiorenal syndrome [33–38]. However, our research, in parallel with the relevant clinical studies, focused on the “kidney side,” whereas the “heart side” was to some extent disregarded. This was so even though some data regarding cardiac ventricular remodeling have suggested that in this model of the cardiorenal syndrome, the inability of the left ventricle (LV) to develop hypertrophy comparable with that of the right ventricle (RV) might be one of the critical factors responsible for the development of decompensated phase of the disorder [36–38]. Admittedly, these data were obtained either in the very early phase of cardiorenal syndrome with enduring full compensation (i.e., one week after ACF creation) or in the phase of overt decompensation (i.e. three weeks after ACF creation). Therefore, it remains unknown if the inability of LV hypertrophy to increase further is already demonstrable during the transition phase from compensation to the decompensation phase of the cardiorenal syndrome. In this context, it is important to emphasize that in the present study we use the term of cardiorenal syndrome even though renal function was not evaluated, for methodical reasons. It is currently impossible to perform simultaneous evaluation of cardiac function and renal function in the same animals; a separate series of experiments would be required. We did not perform such studies to adhere to the Russell and Burch 3 R principle [39], which is now widely recommended in animal research [40] and highly recommended by the Animal Care and Use Committee of our institute (IKEM). Thus we made use here of our data obtained in our previous studies that were exclusively focused on the “kidney side” of the cardiorenal syndrome [33, 35–37].

Accordingly, the aim of the present study was to assess heart morphological parameters and cardiac structure and function using echocardiography and invasive pressure-volume analysis of the LV, and to examine gene expression of selected genes previously implicated in the development of LV failure [41, 42]. The studies were performed two weeks after ACF creation, the time point chosen because at that stage ACF TGR were in the transition phase of the cardiorenal syndrome and still displayed a 100% survival rate. We aimed to clarify if possible changes in cardiac

structure and/or function accompanied by gene expression alterations are a triggering cause of the onset of cardiorenal syndrome decompensation or are a consequence of already developed decompensation.

Methods

Ethical approval and animals

The study was performed in accordance with the guidelines and practices established by the Animal Care and Use Committee of the Institute for Clinical and Experimental Medicine (IKEM), Prague, which accords with the European Convention on Animal Protection and Guidelines on Research Animal Use and were approved by this committee and subsequently by the Ministry of Health of the Czech Republic (the decision number for this project is 18680/2020-4/OVZ). All animals used in the study were bred in IKEM, which is accredited by the Czech Association for Accreditation of Laboratory Animals. Experiments were performed in heterozygous TGR generated by breeding male homozygous TGR with female homozygous transgene-negative normotensive Hannover-Sprague Dawley (HanSD) rats. Homozygous breeder pairs of TGR and HanSD rats were also originally obtained from Max Delbrück Center, Berlin, Germany. The study complied with the ARRIVE (Animals in Research: Reporting In vivo experiments) guidelines [43].

HF model and exclusion criteria

Eight-weeks-old male TGR were anesthetized with intraperitoneal ketamine/midazolam mixture (Calypsol, Gedeon Richter, Hungary, 160 mg/kg and Dormicum, Roche, France, 160 mg/kg). The HF variant dependent on volume overload was induced by creating ACF using the needle technique. This procedure is routinely performed in our laboratory, and the details of the procedure have been reported previously [33–38, 41, 42]. Sham-operated rats underwent an identical procedure but without creating ACF. Animals were excluded from the study if a technical error occurred during the ACF creation procedure or pulsatile flow in the inferior vena cava could not be confirmed, suggesting a flawed ACF function. Two weeks after creation of ACF, TGR were considered to be in the transition phase from the compensation to the decompensation of HF (cardiorenal syndrome) and were employed for the studies described below.

Detailed experimental design

Experimental protocols for all series are outlined in Fig. 1.

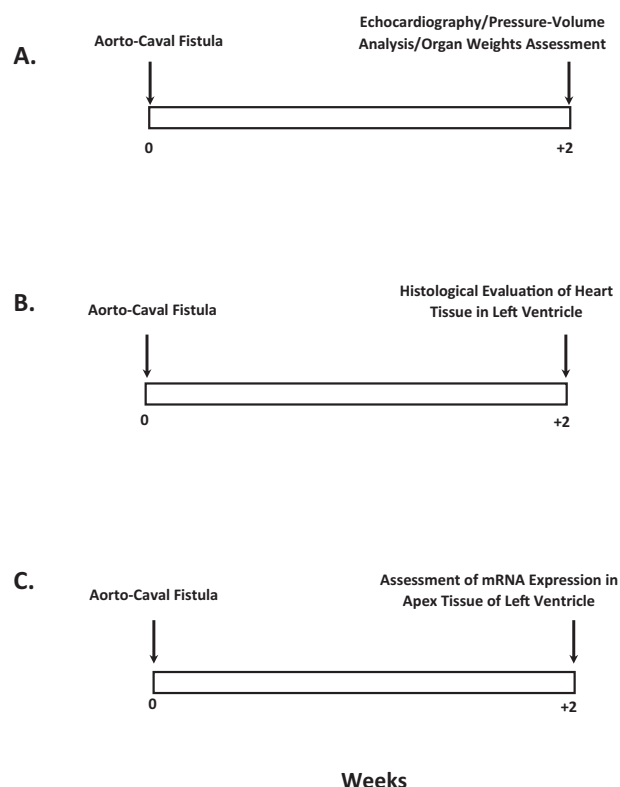


Fig. 1 The experimental design of the whole study, delineating individual experimental series and specific functional evaluation. Series 1 focused on quantification of cardiac function, hemodynamic and organ weight changes **A**, series 2 focused on histological changes of left ventricular tissue **B**, series 3 focused on gene expression changes in apex tissue of left ventricle **C**.

Series 1: Assessment of organ weights and cardiac structure and function by echocardiography and pressure-volume (PV) analysis in the phase of the transition from the compensation to the decompensation of cardiorenal syndrome

On the day of the experiment, rats were anesthetized with long-term anesthesia (intraperitoneal thiopental sodium, 50 mg/kg, VAUB Pharma a.s., Roztoky, Czech Republic), and echocardiography was performed as in previous studies from our laboratories [33, 41, 42, 44]. Briefly, the ventral thorax of animals was shaved, and for standard measurements of cardiac parameters, B-MODE and M-Mode images were recorded in parasternal long axis and parasternal short axis view at the papillary muscle level. Morphological parameters of the LV and right ventricle (RV), including dimension of LV and RV inner diameter, LV anterior and posterior walls, were measured in M-mode from long and short axis sections as previously described, and LV and RV cardiac functions were calculated by standard formulas [33, 41, 42, 44, 45]. All ultrasound studies were done by Vivid System (probe 10S, 5–11.5 MHz, Vivid 7 Dimension, GE Healthcare, Chicago, USA) and analyzed in EchoPAC

software (version 110.1.2 GE Healthcare, Chicago, USA). The mean of three optimally obtained measurements in each rat was used for each parameter. Subsequently, LV functions were invasively assessed by employing PV analysis using techniques and protocols developed and validated for mice and rats by Pacher et al. [46]. This method was introduced almost 15 years ago in our laboratory and is routinely used in our studies evaluating cardiac functions in rats. Detailed description can be found in our numerous previous studies [33, 38, 41, 42, 44, 47]. Briefly, rats were intubated with a plastic cannula to ensure chest relaxation during the whole operation. The left jugular vein was cannulated to secure central venous access for solutions administration as required. A balloon catheter (LeMaitre Single Lumen Embolectomy Catheter, 2 F, Burlington, MA, 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 PV measurement of the LV, the conductance and pressure signals of the Millar pressure-volume catheter (Millar, 2 F, Houston, TX, USA) were calibrated using MPVS software (V2.2, Millar, Houston, TX, USA) according to the manufacturer's instructions. Functions of the LV were invasively assessed by a PV catheter introduced into the LV via the right carotid artery as described in previous studies [38, 41, 42, 44, 47]. For basal measurements, intravenous pancuronium (1 mg/kg, 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. To effectively determine cardiac functions, the preload reductions were performed by slowly inflating the balloon catheter placed in the vena cava inferior with aqua pour injection. The volume signal was calibrated by end-diastolic and end-systolic volume, which were obtained shortly before invasive recordings. Data from PV loops were captured and analyzed using LabChart Pro software (ADInstruments, Bella Vista, NSW, Australia). At the end of the experiment, whole heart weight (HW) and then left ventricle weight (LVW) (including septum), right ventricle weight (RVW), and lung weight ("wet lung weight") were assessed and normalized to tibia length as described in our previous studies [34, 35, 37, 38, 48–50]. The parameters for evaluating cardiac function were calculated by employing formulas using the data obtained by echocardiography and PV analysis as originally described by Pacher et al. [46], now recognized as the "golden standard approach" in assessing cardiac systolic and diastolic function in small animals and also recently advocated by the American Physiological Society in the "guidelines paper" [51]. Therefore, this approach is standardly employed in our laboratories as documented by our previous studies [38, 47]. Here we remind some terms and formulas that are not commonly perceived by general medical community apart from professional cardiologists. Thus,

maximum rates of pressure rise $(+dP/dt)_{\max}$ represent the maximum rate of pressure change in the cardiac ventricle and is traditionally used as an index of ventricular contractility. Its limitation is that it is load-dependent and thought now to be inferior to parameters defined by the PV plane, such as preload recruitable stroke work (PRSW) and end-systolic pressure-volume relationship (ESPVR). PRSW is expressed as a linear relationship between stroke work (SW) and end-diastolic volume (EDV) and is considered relatively load-insensitive so it is not significantly influenced by changes in preload and/or afterload. In this context, SW is defined as the work performed by the ventricle to eject stroke volume into the aorta and is the product of stroke volume (SV) and mean arterial pressure, depicted as area enclosed by the PV loop. Mathematically, it is calculated as the integral ($\int$) of pressure with respect to volume, specifically the difference between $\int$ of systolic blood pressure with respect to volume and the $\int$ of diastolic blood pressure over volume. ESPVR describes the maximal pressure that can be developed by the LV at any given LV volume. The slope of ESPVR, known as end-systolic elastance (E_{es}) is an index of myocardial contractility and is, like PRSW, considered to be insensitive to changes in preload, afterload and also heart rate. The formula for the ESPVR is $P = E_{es} * (V - V_0)$, where P is the end-systolic pressure (ESP), V is the end-systolic volume and V_0 is the volume axis intercept. Likewise, the minimum rate of pressure rate $(-dP/dt)_{\min}$ represents the minimum value of the first derivative of pressure over time in the cardiac ventricle and is employed as a valuable tool for evaluation of isovolumic relaxation. Yet again, the limitation is that it may not be a valid measure of LV relaxation rate during acute alterations in contractility and/or afterload; therefore, this parameter is currently supplemented by evaluation of isovolumic relaxation constant [τ - (τ)]. τ represents the exponential decay of the ventricular pressure during isovolumic relaxation and the advantage is that it is believed to be a load-independent measure of isovolumic relaxation. The Weiss method (by which τ was computed) formula is: $P(t) = P_0 e^{-(t-t_0)/\tau}$, where $P(t)$ is the LV pressure at time t , P_0 is the pressure at the beginning of relaxation, and τ is the time constant of isovolumic relaxation [52]. End-diastolic pressure-volume relationship (EDPVR) describes relationship in the LV between the EDV and end-diastolic pressure (EDP) at the same point, and reflects passive filling properties of the myocardium. The slope of the EDPVR at any point along this curve is the reciprocal of LV compliance. Arterial elastance (E_a) is a measure of arterial load on the LV and the formula for its calculation is: ESP/SV . LV wall stress is a measure of the force exerted by the blood on the inner wall of the LV; it is a key indicator of the mechanical load of the myocardium and can be calculated using the law of Laplace; the formula used is: $\text{wall stress} = P \times r/2w$, where P is ESP (in our setup LV peak pressure), r is the internal radius of the LV and w is

the thickness of the LV wall. In this context another important parameter that is derived from echocardiography is relative wall thickness which is a measure of LV geometry and is calculated by the formula: $\text{relative wall thickness} = 2 * \text{LV posterior wall thickness} / \text{LV diastolic diameter}$. Ventriculo-Arterial Coupling (VAC) is a measure of efficient interaction between ventricular contractility and the resistance it faces from arteries. The formula is: $VAC = E_a/E_{es}$. VAC values >1.0 are considered as pathophysiological, indicating hemodynamic derangements and reduced cardiac efficiency. Potential Energy (PE) refers to the energy stored within the ventricular muscle at the end of systole, which is not immediately converted into mechanical work but rather dissipated as heat during isovolumic relaxation; it is depicted as the area enclosed by the PV loop. Mathematically, it is calculated as $\int$ area bound by both the ESPVR and EDPVR and the isovolumic relaxation limb of the same PV plot. The total pressure-volume area (PVA) represents the total mechanical energy produced by ventricle at each cardiac cycle and the formula is: $PVA = PE + SW$. It is known that PVA correlates linearly with myocardial oxygen consumption, which includes basal metabolism, intracellular calcium cycling and cross-bridge cycling, and is used for evaluating the efficiency of cardiac function. In this context, the efficiency of cardiac ventricle can be expressed as the ratio of the useful ventricular SW and the oxygen consumption as estimated by PVA. The formula is: $\text{Ventricular efficiency} = SW/PVA$. The following experimental groups were examined:

1. Sham-operated HanSD rats (no ACF) ($n = 10$)
2. Sham-operated TGR ($n = 10$)
3. ACF TGR in the transition from the compensation phase to the decompensation phase (i.e. two weeks after the creation of ACF) ($n = 13$)

Series 2: Histological evaluation of heart tissue in the phase of the transition from the compensation to the decompensation of cardiorenal syndrome

In separate appropriately matched (regarding the protocol as well as numbers) three experimental groups, i.e., sham-operated HanSD rats, sham-operated TGR (no ACF in both), and ACF TGR, the hearts were subjected to histological examination as described previously [36, 37, 45, 53]. The reason for the need for separate experimental groups for histological evaluation lies in the use of cardioplegia solution with subsequent immediate fixation in paraformaldehyde solution; this procedure precludes precise determination of HW, LVW, and RVW.

Briefly, two weeks after either sham operation or ACF creation, the rats were anesthetized with a combination of midazolam $50 \text{ mg} \cdot \text{kg}^{-1}$ (Dormicum, Roche Ltd., Prague,

Czech Republic) and ketamine 50 mg.kg⁻¹ (Calypsol, Gedeon Richter Ltd., Budapest, Hungary) i.p. The beating (pulsating) organ, i.e., the native heart, was perfused in situ with 20 ml of Thomas cardioplegia solution and subsequently fixed in 4% paraformaldehyde in phosphate-buffered saline and embedded into Tissue-Tek. The blocks were cut using a cryo-microtome, and cardiomyocyte width was measured in the subendocardium, midmyocardium, and subepicardium of the LV. Cardiomyocyte length was measured only in the midmyocardium; 30 cardiomyocytes were assessed in each layer. Only the cells in which the nucleus was visible were measured to avoid underestimation. Since there were no significant differences in the cardiomyocyte width between the layers, the data from the subendocardium, midmyocardium, and subepicardium were pooled, as was also practiced by other investigators [36, 37, 54]. LV fibrosis were analyzed in sections stained with Picrosirius red (Direct Red 80, Sigma Aldrich, MO, USA) as described in detail previously [55]. The interstitial collagen was analyzed in polarized light using 10 images of the LV and RV scanned from the midmyocardium, without perivascular areas (magnification 200x, microscope Nikon eclipse Ni-E, camera Nikon DS-L3, Tokyo, Japan). The percent area of myocardial fibrosis was calculated semiquantitatively using imaging software NIS-Elements Ar (LIM, Prague, Czech Republic). In this study we employed circularly polarized light for analysis which unlike the linearly polarized light used previously allows discrimination between mature and newly synthesized collagen fibers [56].

Series 3: Assessment of LV mRNA expression in the phase of the transition from the compensation to the decompensation of cardiorenal syndrome

In separate, appropriately matched three experimental groups ($n = 9$ in each), i.e., sham-operated HanSD rats, sham-operated TGR, and ACF TGR, the LV mRNA expression of selected genes (known as markers of myocardial stress, metabolism, inflammation, and fibrosis) was performed [41, 42]. The reason for the need for separate experimental groups for such analyses is the approach used to obtain tissue samples. Animals were killed by decapitation, and LV tissue samples that were taken from the apex of the LV were immediately harvested into liquid nitrogen and stored at -80°C until analysis. The tissue mRNA expression was determined using the standard technique described in previous studies, including ours [57–60]. The measurement of multiple mRNA expression was performed by the manufacturer's instructions (384-well microfluidics TaqMan array cards; custom setting of selected genes; Applied Biosystems, Foster City, CA,

USA). The investigated genes are listed in Supplemental Table 1, which shows the data, including abbreviations and the appropriate ID assay identification number given by the manufacturer. The relative gene expression was used to analyze changes in mRNA expression. This expression was calculated using the $2^{-\Delta\Delta\text{Ct}}$ method, the most frequently used method for such experiments [57, 58]. The method directly uses the Ct (threshold cycle) information generated from a qPCR system. Ct is the cycle number where the fluorescence generated by the PCR product is distinguishable from the background noise. To calculate relative gene expression in target and reference samples, a housekeeping gene was used as the normalizer. 18S rRNA was used as the normalizer because its expression level remains relatively stable in response to any treatment [57, 61], and it meets the "Minimum Information for Publication of qPCR Experiments" guidelines as introduced by Bustin et al. [62].

ΔCt of each sample was first calculated:

$$\Delta\text{Ct} = \text{Ct}(\text{gene of interest}) - \text{Ct}(\text{housekeeping gene})$$

The expression of mRNA of selected genes was related to a control group, i.e., HanSD rats treated with vehicle. The final results were expressed as the n -fold difference in gene expression of mRNA of target genes between the appropriate experimental group and control group calculated as follows:

$$n - \text{fold expression} = 2^{-(\Delta\text{Ct of experimental group} - \Delta\text{Ct of control group})}$$

Subsequently, the log transformation of the data was performed to make it more symmetrical, as recommended and generally accepted for evaluation of relative gene expression results [57, 62, 63]. Thus, the values in the graphs represent $\log_2 n$ -fold gene expression.

Statistical analysis

Statistical analyses were performed using Graph-Pad Prism software (Graph Pad Software, San Diego, California, USA). Statistical comparison was made by one-way ANOVA followed by Tukey's multi comparison test when appropriate, and all data were also analyzed by the D'Agostino-Pearson normality test, which is currently recommended as the best approach to quantify how far the distribution is from Gaussian distribution in terms of symmetry and shape, particularly for data obtained by qRT-PCR [63]. Pearson's correlation coefficient was used to assess the correlation between continuous variables. The difference of linear regression slope from the line of equality ($x = y$) or between two datasets was tested. Values are expressed as mean $\pm$ SEM. Values of p below 0.05 were considered statistically significant.

Results

Organ weights and cardiac structure and function assessed by echocardiography and PV analysis at the transition phase of cardiorenal syndrome

Organ weights

As shown in Fig. 2A–D, sham-operated TGR exhibited discernible LV hypertrophy as seen from the whole HW and LVW (with septum) values compared to sham-operated HanSD rats (Fig. 2A, B). There was no sign of RV hypertrophy as documented by the RVW and particularly by the decreases in RVW to LVW ratio (Fig. 2D). ACF TGR exhibited a further marked bilateral cardiac hypertrophy in this phase, as seen from the whole HW, LVW, and RVW – as compared to sham-operated TGR (Fig. 2A–C). Remarkably, the increases in RV hypertrophy were in all ACF TGR discernibly higher than that of the LV – as documented by increases in RVW to LVW ratio (Fig. 2D).

As shown in Fig. 2E there were no significant differences in the left atrium weight between sham-operated HanSD rats and sham-operated TGR, however, ACF TGR displayed significantly higher left atrium weight (Fig. 2E).

Sham-operated TGR (as compared with sham-operated HanSD rats) did not show any indication of lung congestion, as documented by wet lung weight (Fig. 2F). ACF TGR did not show significantly higher lung weight than sham-operated TGR, indicating that in this phase no important lung congestion was present in the former (Fig. 2F). However, it is important to emphasize that three out of thirteen ACF TGR showed markedly higher lung weight (almost twofold higher than the group average), indicating the development of important lung congestion (Fig. 2F). In addition, those three ACF TGR showed no increase in LVW compared to sham-operated TGR (the LVW was almost identical to the average in the group of sham-operated TGR – Fig. 2B), but exhibited the greatest increases in left atrium weight as compared with sham-operated TGR (Fig. 2E).

The RV hypertrophy in those rats was more pronounced than that seen from the increases in the ratio of RVW to LVW (the said three rats exhibited the highest RVW to LVW ratio in the ACF TGR group – Fig. 2D). There were no significant differences in kidney weight between experimental groups and therefore data are not shown.

Gross morphological changes in the heart are presented in Supplemental Fig. 1, which shows representative whole heart images in sham-operated HanSD rats, sham-operated TGR, and ACF TGR, as well as the images from the same groups in the transversely cut hearts. These images show that sham-operated TGR exhibited signs of concentric LV hypertrophy compared to sham-operated HanSD rats

(Figures Supplemental 1B and 1E versus Supplemental Fig. 1A and 1D). ACF creation in this phase already tended to produce some dilatation of both LV and RV cavities, with further increases in the wall mass and, notably, left atrial enlargement is clearly visible in the representative images (Supplemental Fig. 1C and 1F).

Cardiac structure and function analyzed by echocardiography

As summarized in Table 1, sham-operated TGR compared with sham-operated HanSD rats displayed increased LV anterior and posterior wall thickness without significantly changing LV diameter, leading to profound increase in relative wall thickness (by 56%), which indicated concentric LV hypertrophy and confirmed the data from gross morphological analysis (Supplemental Fig. 1). Otherwise sham-operated TGR did not show any signs of LV systolic or diastolic dysfunction as compared with sham-operated HanSD rats. Compared with sham-operated TGR, dilatation of the LV in ACF TGR echocardiography indicated eccentric remodeling with relative wall thinning (relative wall thickness was reduced by -52%) and also indicated LV systolic dysfunction (LV ejection fraction and LV fractional shortening were reduced by -10% and -20%, respectively). ACF TGR also displayed marked dilatation of the left atrium (by 109%) as compared with sham-operated TGR. ACF TGR also showed signs of impaired diastolic function as seen from the increased LV velocity of E-wave and the ratio of E-wave to A-wave as well as the ratio of E/E' waves – the features clearly different from those in sham-operated TGR. Moreover, echocardiography also showed that RV was markedly dilated in ACF TGR compared to sham-operated TGR. However, despite a clear tendency to lower RV fractional change in ACF TGR, the difference did not reach statistical significance, suggesting that RV systolic function was not yet significantly impaired in ACF TGR compared to sham-operated TGR. ACF TGR showed a marked increase in the vena cava inferior diameter compared to sham-operated TGR and sham-operated HanSD rats, confirming that substantial blood recirculation occurred via ACF.

Cardiac function assessed by invasive hemodynamics and PV analysis

Figures 3 and 4 and Supplemental Fig. 2 present the evaluation of cardiac function by the invasive hemodynamics method. Sham-operated TGR displayed higher LV peak pressure (Fig. 3A), maximum rates of pressure rise ($+dP/dt_{\max}$) (Fig. 3D), and arterial elastance (Fig. 4E) as compared with sham-operated HanSD rats; however, for all other parameters, there were no significant differences

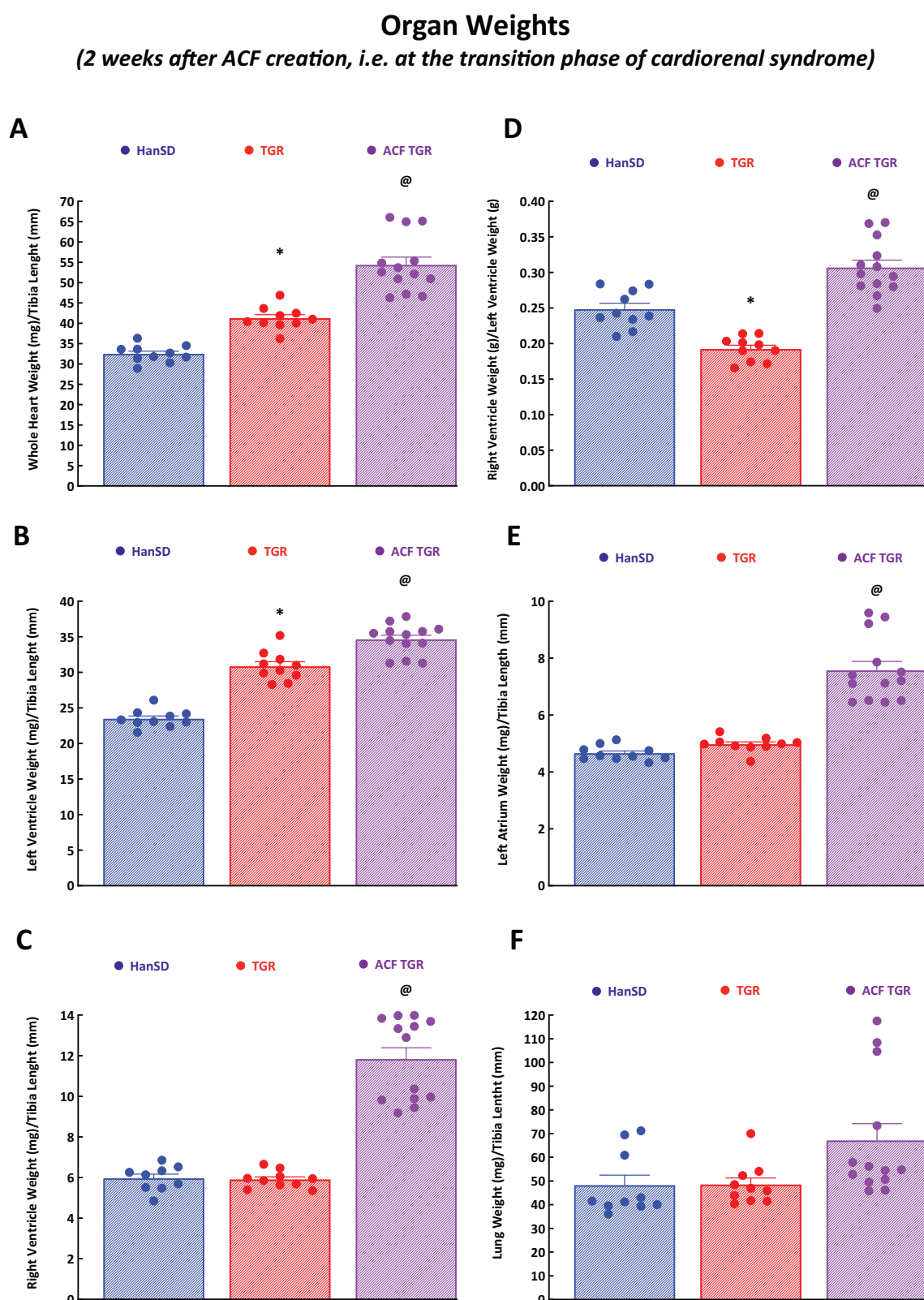


Fig. 2 Organ weight parameters. Whole heart weight **A**, left ventricle weight **B**, right ventricle weight **C**, the ratio of right ventricle weight to left ventricle weight **D**, left atrium weight **E**, and lung weight **F** in sham-operated normotensive transgene-negative Hannover Sprague-

Dawley rats (HanSD), hypertensive sham-operated Ren-2 transgenic (TGR) rats, and in the TGR two weeks after creation of the aorto-caval fistula (ACF). * $P < 0.05$ compared with sham-operated HanSD rats. @ $P < 0.05$ compared with all other groups. The values are means $\pm$ SEM

Table 1 Echocardiographic analysis of cardiac structure and function performed 2 weeks after creation of the aorto-caval fistula or sham-operation

	HanSD	TGR	ACF TGR
Heart rate (min ⁻¹)	357 ± 17	349 ± 12	332 ± 9
Left ventricle diastolic diameter (mm)	6.64 ± 0.20	6.69 ± 0.19	9.95 ± 0.19 [@]
Left ventricle systolic diameter (mm)	3.08 ± 0.24	3.15 ± 0.23	5.37 ± 0.27 [@]
Left ventricle anterior wall thickness in diastole (mm)	1.57 ± 0.06	2.52 ± 0.09 [*]	1.77 ± 0.07
Left ventricle posterior wall thickness in diastole (mm)	1.78 ± 0.04	2.41 ± 0.11 [*]	2.03 ± 0.05
Left ventricle relative wall thickness	0.539 ± 0.03	0.842 ± 0.06 [*]	0.406 ± 0.02 [@]
Left ventricle ejection fraction (%)	83.7 ± 2.2	83.1 ± 2.2	75.5 ± 2.7 [@]
Left ventricle fractional shortening (%)	57.4 ± 2.1	56.3 ± 2.5	45.1 ± 2.1 [@]
Left ventricle stroke volume (μl)	187.9 ± 9.3	192.9 ± 13.2	402.9 ± 17.1 [@]
Cardiac output (ml.min ⁻¹)	66.3 ± 2.5	70.8 ± 3.2	164.1 ± 6.3 [@]
Left atrium diameter (mm)	3.57 ± 0.14	3.48 ± 0.13	7.27 ± 0.26 [@]
Left ventricle E Velocity (m.s ⁻¹)	0.93 ± 0.05	0.89 ± 0.04	1.42 ± 0.06 [@]
Left ventricle E/A Ratio	11.9 ± 0.78	16.6 ± 0.76 [*]	19.9 ± 0.43 [@]
Left ventricle E/E' Ratio	1.21 ± 0.03	1.18 ± 0.04	1.61 ± 0.09 [@]
Right ventricle basal diameter in diastole (mm)	3.44 ± 0.11	3.36 ± 0.18	5.97 ± 0.26 [@]
Right ventricle midcavity diameter in diastole (mm)	3.08 ± 0.15	3.17 ± 0.13	5.39 ± 0.24 [@]
Right ventricle diastolic area (mm ²)	27.74 ± 2.75	27.53 ± 1.43	64.99 ± 4.51 [@]
Right ventricle systolic area (mm ²)	15.44 ± 1.55	15.01 ± 2.04	40.52 ± 2.93 [@]
Right ventricle fractional change (%)	44.16 ± 2.81	45.8 ± 2.38	37.41 ± 2.31
Diameter of Vena Cava Inferior (mm)	2.35 ± 0.19	2.31 ± 0.14	5.63 ± 0.34 [@]

The values are means ± SEM. TGR, Ren-2 renin transgenic rats; HanSD, Hannover-Sprague transgene-negative rats; ACF, aorto-caval fistula. ^{*}*p* < 0.05 TGR versus HanSD [@]*p* < 0.05 ACF TGR versus all other groups

E E-wave represents the velocity of blood flow during the rapid passive filling phase of the left ventricle; A A-wave represents the velocity of blood flow during the active filling phase of the left ventricle, which occurs after the contraction of the left atrium; E' the maximum velocity of mitral annular movement during the diastole

indicating that sham-operated TGR showed signs typical for hypertensive individuals in the compensation phase of hypertension. This notion is further supported by the finding that sham-operated TGR displayed similar VAC as that observed in sham-operated HanSD rats (Supplemental Fig. 2A). However, ventricular efficiency was already slightly impaired in sham-operated TGR as compared with sham-operated HanSD rats (Supplemental Fig. 2D).

ACF TGR displayed significantly lower LV peak pressure (Fig. 3A), (+ dP/dt)_{max} (Fig. 3D), ESPVR (Fig. 4A), PRSW (Fig. 4C) and arterial elastance (Fig. 4E) as compared with sham-operated TGR. In addition, ACF TGR showed higher LV end-diastolic pressure (Fig. 3B), LV end-diastolic volume (Fig. 3C), stroke work (Fig. 4D), and LV wall stress (Fig. 4F) as compared with sham-operated TGR. Moreover, ACF TGR displayed markedly elevated VAC, PE and PVA as compared with sham-operated TGR (Supplemental Fig. 2A–2C), resulting in profound decreases in ventricular efficiency (Supplemental Fig. 2D) in ACF TGR. There were no significant differences in (-dP/dt)_{min} (Fig. 3E), LV relaxation constant, Tau (Fig. 3F) and EDPVR (Fig. 4B) between experimental groups.

Histological evaluation of heart tissue at the transition phase of cardiorenal syndrome

Morphological changes at the cardiomyocyte level (Fig. 5A–C)

Figure 5 A–C shows that ACF TGR showed similar cardiomyocyte width as observed in sham-operated TGR; these values were significantly higher than in HanSD rats (Fig. 5A), whereas ACF TGR showed cardiomyocyte length higher than sham-operated TGR and sham-operated HanSD rats; this resulted in a higher ratio of cardiomyocyte length to cardiomyocyte width in ACF TGR as compared with sham-operated TGR (Fig. 5C). These findings corroborate the observation from the whole organ level (Fig. 2 and Supplemental Fig. 1) that ACF TGR showed signs of eccentric LV hypertrophy.

Myocardial fibrosis (Fig. 5D–F)

There were no significant differences in total LV myocardial fibrosis (expressed in %) between sham-operated HanSD rats and sham-operated TGR. However, ACF TGR

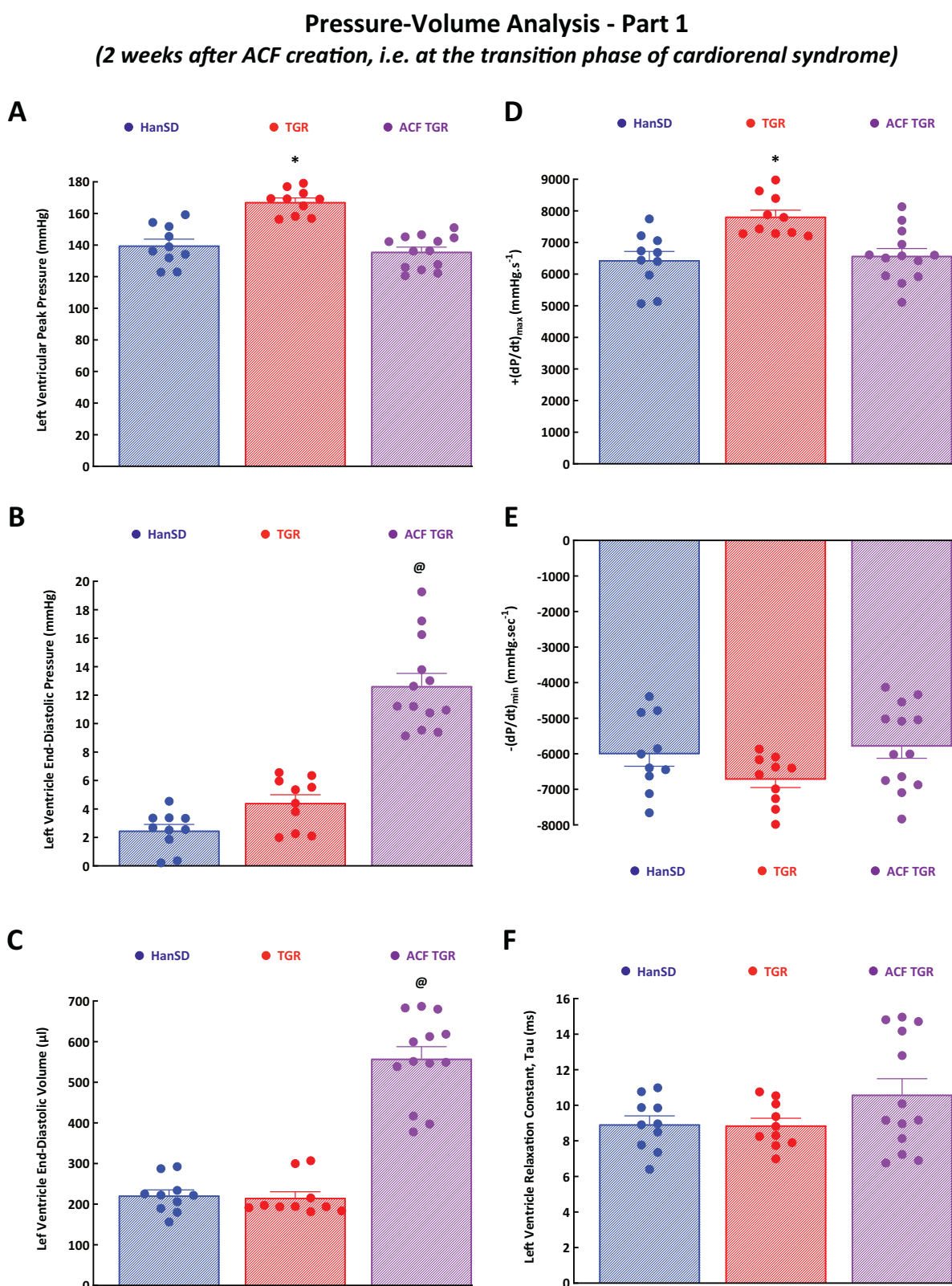


Fig. 3 Part 1 of the left ventricular cardiac function assessment by invasive hemodynamic analysis performed in sham-operated normotensive transgene-negative Hannover Sprague-Dawley rats (HanSD), hypertensive sham-operated Ren-2 transgenic (TGR) rats and in TGR two weeks after creation of the aorto-caval fistula (ACF). The data show 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 $-(dp/dt)_{min}$ **E** and relaxation constant tau of the left ventricle **F**. * $P < 0.05$ compared with sham-operated HanSD rats. @ $P < 0.05$ compared with all other groups. The values are means $\pm$ SEM

Pressure-Volume Analysis - Part 2

(2 weeks after ACF creation, i.e. at the transition phase of cardiorenal syndrome)

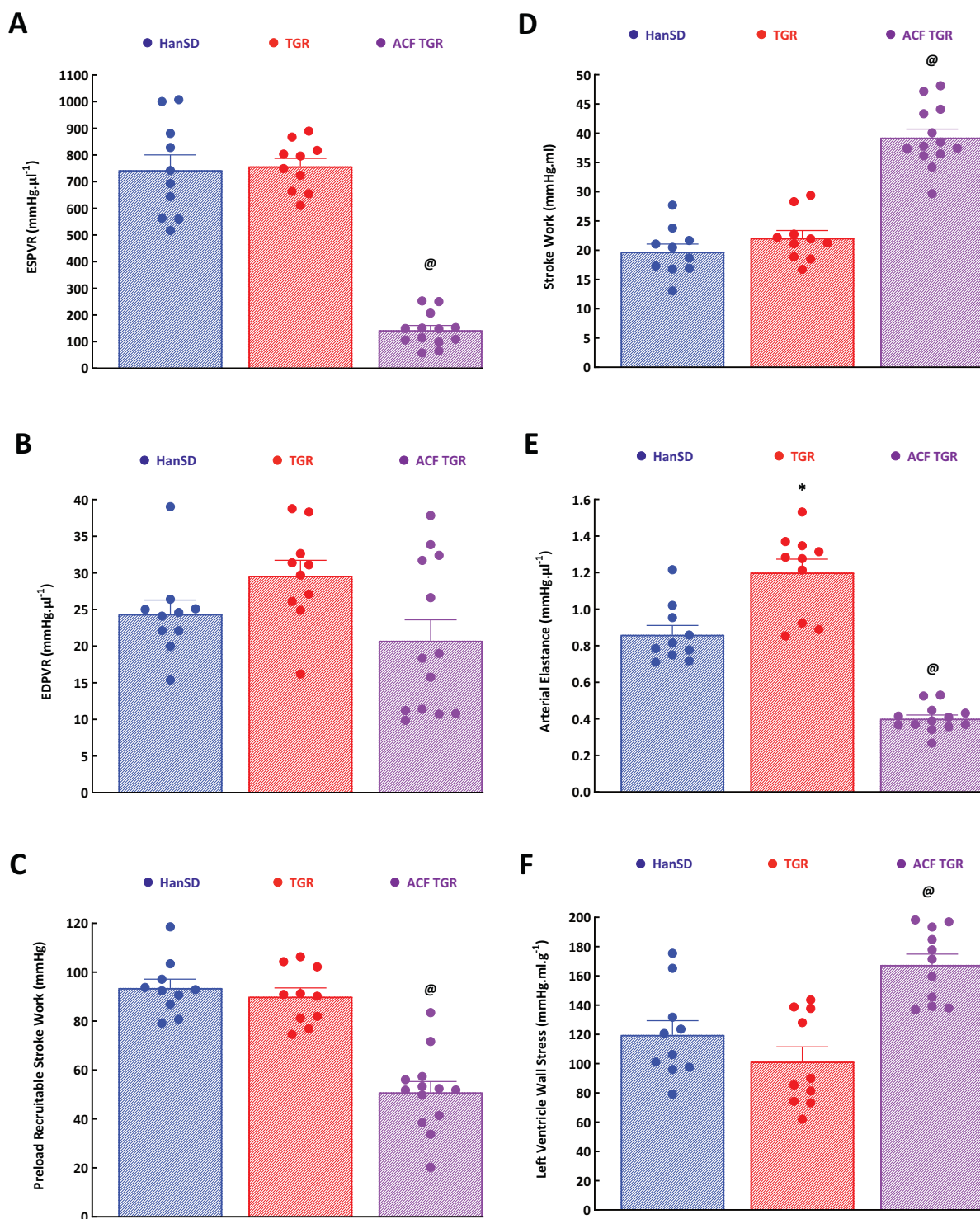


Fig. 4 Part 2 of the left ventricular cardiac function assessment by invasive hemodynamic analysis performed in sham-operated normotensive transgene-negative Hannover Sprague-Dawley rats (HanSD), hypertensive sham-operated Ren-2 transgenic (TGR) rats and in TGR two weeks after creation of the aorto-caval fistula (ACF). Shown are:

end-systolic pressure-volume relationship (ESPVR) **A**, end-diastolic pressure-volume relationship (EDPVR) **B**, preload recruitable stroke work **C**, stroke work **D**, arterial elastance **E** and left ventricle wall stress **F**. * $P < 0.05$ compared with sham-operated HanSD rats. @ $P < 0.05$ compared with all other groups. The values are means $\pm$ SEM

Histological Evaluation of the Heart Tissue (2 weeks after ACF creation, i.e. at the transition phase of cardiorenal syndrome)

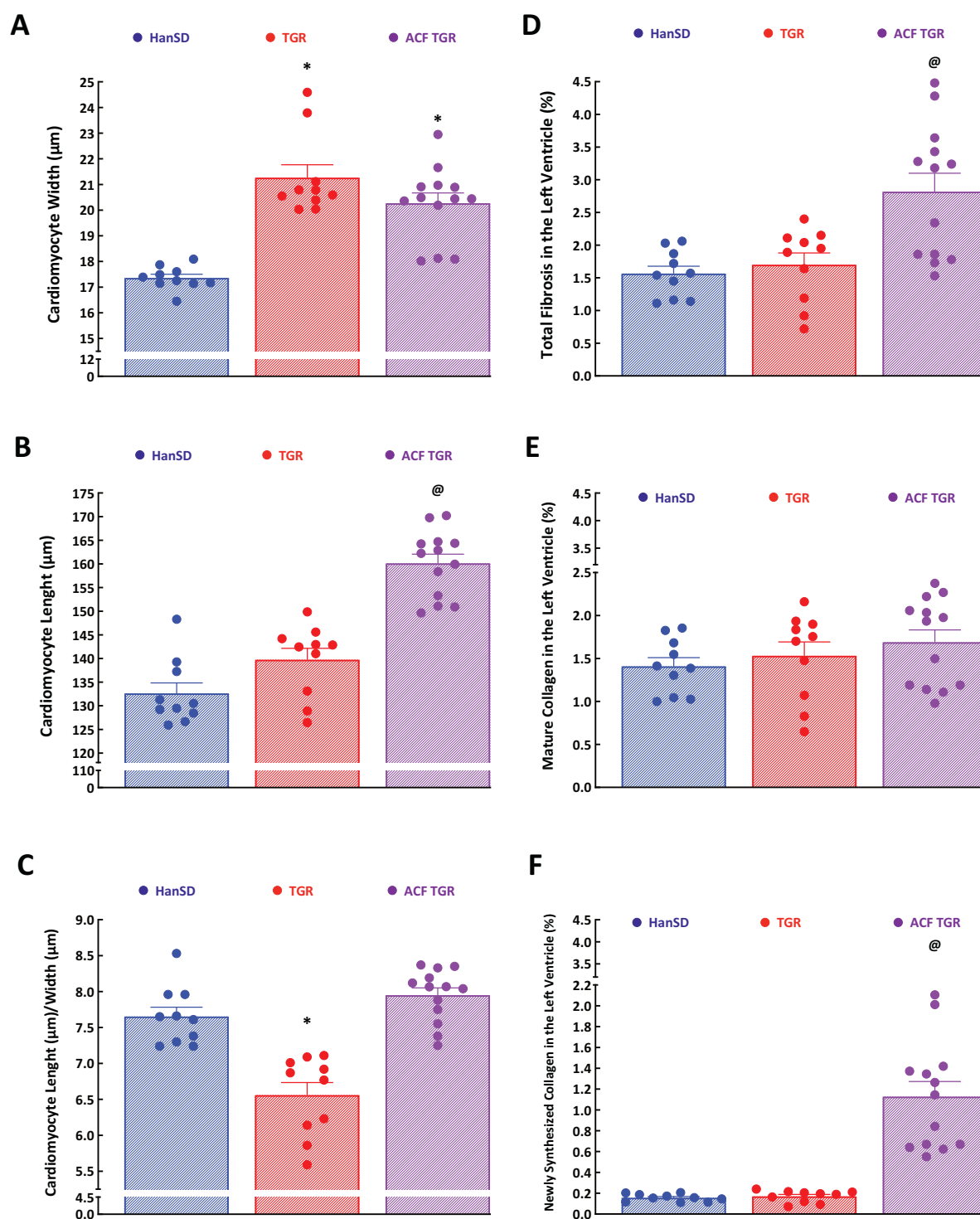


Fig. 5 Histological evaluation. Cardiomyocyte width **A**, cardiomyocyte length **B**, the ratio of cardiomyocyte length to cardiomyocyte width **C**, the total fibrosis in the left ventricle **D**, the amount of mature collagen in the left ventricle **E** and the amount of newly synthesized collagen in the left ventricle **F** - in sham-operated normotensive

transgene-negative Hannover Sprague-Dawley rats (HanSD), hypertensive sham-operated Ren-2 transgenic (TGR) rats and in TGR two weeks after creation of the aorto-caval fistula (ACF). * $P < 0.05$ compared with sham-operated HanSD rats. @ $P < 0.05$ compared with all other groups. The values are means $\pm$ SEM

displayed significantly higher total myocardial fibrosis than observed in both sham-operated groups of animals (Fig. 5D). Notably, there were no significant differences in the content of mature collagen between experimental groups (Fig. 5E), but ACF TGR showed markedly more of newly synthesized collagen as compared with sham-operated groups of animals (Fig. 5F). These data strongly indicate that the increase in the total LV myocardial fibrosis in ACF TGR is mainly mediated by increased collagen formation after ACF creation. Representative images of myocardial fibrosis in the LV are shown in Fig. 6.

Assessment of LV mRNA expression at the transition phase of cardiorenal syndrome

Gene mRNA expression analysis of cardiac markers of stress and contractility

As shown in Fig. 7, sham-operated TGR and ACF TGR displayed increased mRNA expression of myocardial stress genes as compared with sham-operated HanSD rats: natriuretic peptide type A (Nppa) (Fig. 7A), natriuretic peptide type B (Nppb) (it was more pronounced in ACF TGR than in sham-operated TGR) (Fig. 7B) and transglutaminase-2 (Tgm2) (Fig. 7F). In addition, sham-operated TGR and ACF TGR displayed increased mRNA expression of myocardial genes related to the process of contraction and relaxation: this was so with sarcoplasmic reticulum Ca^{++} -ATPase (SERCA) (Fig. 7D) and phospholamban (Pln) (Fig. 7E). ACF TGR showed an increased ratio of mRNA expression of myosin heavy chain beta (MYH7) to myosin heavy chain alpha (MYH6); this resulted from the combined upregulation of MYH7 and down-regulation of MYH6 (Fig. 7C).

Gene mRNA expression of cardiac markers of metabolism

As shown in Fig. 8, ACF TGR displayed increased mRNA expression of myocardial substrate metabolism genes as compared with sham-operated HanSD rats as well as sham-operated TGR: this was so in the case of glucose transporter type 1 (GLUT1) (Fig. 8A), Acyl CoA medium chain dehydrogenase (Acadm) (Fig. 8B), hexokinase 1 (Hk1) (Fig. 8C) and citrate synthase (Cs) (Fig. 8D). The mRNA expression of GLUT1 and Hk1 also significantly increased in sham-operated TGR compared with sham-operated HanSD rats (Fig. 8A and 8C).

Gene mRNA expression of cardiac markers of inflammation and fibrosis

As shown in Supplemental Fig. 3, sham-operated TGR and ACF TGR displayed increased mRNA expression of

myocardial inflammation and fibrosis genes as compared with sham-operated HanSD rats: this was so in the case of interleukin 6 (IL-6), transforming growth factor beta (Tgfb1), collagen type 1, alpha 1 (Col1a1) and collagen type 3, alpha 1 (Col3a1). However, there were no significant differences in mRNA expression of myocardial inflammation and fibrosis gene between sham-operated TGR and ACF TGR.

Overview of changes in lung and heart weights and cardiomyocyte parameters from the compensation phase (i.e. one week after ACF creation), via transition phase (i.e. two weeks after ACF creation) to the decompensation phase (i.e. three weeks after ACF creation) of cardiorenal syndrome

Below, we present percent changes in organ weights and cardiomyocyte parameters in ACF TGR compared to sham-operated TGR at the same time. The data for the compensation phase and the decompensation phase of cardiorenal syndrome are taken from our previous studies published in Hypertension Research [36, 37] and the data for the transition phase are taken from the current study.

Changes in organ weights

Changes are expressed as percent increases in ACF TGR compared to sham-operated TGR at the same time (sham-operated TGR represent 100% basal value).

As shown in Fig. 9A, there were similar increases in lung weight one and two weeks after ACF creation, but three weeks after ACF, the TGR displayed an almost twice higher increase in lung weight as compared with the previous phases.

As shown in Fig. 9B, there were progressive increases in whole HW across the three phases of cardiorenal syndrome. However, the simultaneous increases in LVW were relatively modest ($+2.5 \pm 0.3\%$, $+12 \pm 3\%$ and $+25 \pm 3\%$, respectively) (Fig. 9C), and the increases in whole HW were dominantly mediated (in all three phases) by increases in RVW (by $78 \pm 6\%$, $101 \pm 9\%$ and $232 \pm 12\%$, respectively) (Fig. 9D).

Changes in cardiomyocyte parameters

As shown in Fig. 10A, the cardiomyocyte width one week after ACF creation was not significantly different from the values observed in sham-operated TGR. On the other hand, the cardiomyocyte length was already at the beginning significantly higher and progressively increased across all three phases of the cardiorenal syndrome, whereas in sham-operated TGR, it remained stable (Fig. 10B); this resulted in a progressively increasing ratio of cardiomyocyte length to

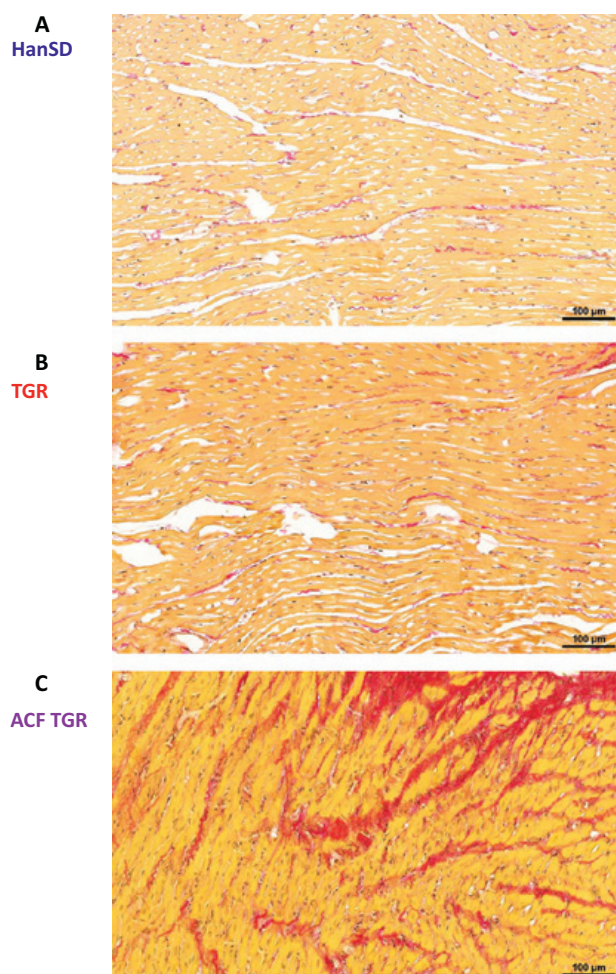


Fig. 6 Representative histological images of the left ventricle. Sections are stained with Picrosirius Red (200x), in these bright-field microscopy images, the collagen is red against a pale-yellow background. The scale bar in the figure is 100 µm. **A** sham-operated normotensive transgene-negative Hannover Sprague-Dawley rats (HanSD) (average fibrosis in this group is $1.57 \pm 0.11\%$), **B** hypertensive sham-operated Ren-2 transgenic (TGR) rats (average fibrosis in this group is $1.70 \pm 0.18\%$) and **C** in TGR two weeks after creation of the aorto-caval fistula (ACF) (average fibrosis in this group is $2.83 \pm 0.26\%$)

cardiomyocyte width in ACF TGR across all three phases of the cardiorenal syndrome (Fig. 10C).

Overview of changes in cardiac function assessed by PV analysis from the transition phase (two weeks after ACF creation) to the decompensation phase (three weeks after ACF creation) in cardiorenal syndrome

Below, we present percent changes in cardiac function evaluated by the invasive hemodynamics method in ACF TGR and compare with those seen in sham-operated TGR at the same time. The data for the decompensation phase of cardiorenal syndrome are taken from our previous study

published in Hypertension Research [38] and the data for the transition phase are taken from the current study. Percent changes in ACF TGR are shown and compared to sham-operated TGR at the same time (sham-operated TGR again serve as 100% basal value).

As shown in Fig. 11A, there was a similar decrease in LV peak pressure two and three weeks after ACF creation. Figure 11B–D show that three weeks after ACF creation the TGR displayed significantly higher decreases in $(+dP/dt)_{\max}$, ESPVR and PRSW as compared with the animals two weeks after ACF creation. As shown in Fig. 11E there were similar but especially well-marked increases in VAC two and three weeks after creation. As shown in Fig. 11F, the decreases in LV ventricular efficiency were not statistically different two and three weeks after ACF creation, even though there was a noticeable trend for more pronounced decreases in the latter group.

As summarized in Supplemental Fig. 4, there were no significant differences in the changes in $(-dP/dt)_{\min}$, LV relaxation constant, Tau and EDPVR between two and three weeks after ACF creation, anyway these changes were insignificant as compared with the values in sham-operated TGR (Supplemental Fig. 4A–4C). As shown in Supplemental Fig. 4D, the increase in LV wall stress was very pronounced two weeks after ACF creation (confirming the data expressed as absolute values) (Fig. 4F) but LV wall stress significantly decreased three weeks after ACF creation (compared with values observed two weeks after ACF creation, Supplemental Fig. 4D).

Discussion

The most important findings of our present study can be outlined follows: (i) Two weeks after creation of ACF, ACF TGR showed already fully developed *eccentric* LV hypertrophy, unlike the sham-operated TGR which exhibited signs of LV *concentric* hypertrophy; (ii) The increase in whole HW in ACF TGR was dominantly mediated by RV hypertrophy, whereas the increases in the LV mass were minimal; (iii) Two weeks after ACF creation, the group of ACF TGR did not show any consistent increases in lung weight as compared with sham-operated TGR; (iv) Two weeks after ACF creation, ACF TGR displayed (besides bilateral ventricular dilatation) a significant impairment of LV systolic as well as diastolic functions, whereas RV systolic functions were not impaired; (v) Two weeks after ACF creation, LV of the ACF TGR showed an upregulation of myocardial stress genes, upregulation of the genes reflecting enhanced glycolysis over fatty acid oxidation, and upregulation of the gene responsible for non-insulin dependent glucose uptake in LV.

It is seen that the first important set of findings relates to the organ morphological parameters assessed at the whole

mRNA Expression - Markers of Myocardial Stress and Contractility (2 weeks after ACF creation, i.e. at the transition phase of cardiorenal syndrome)

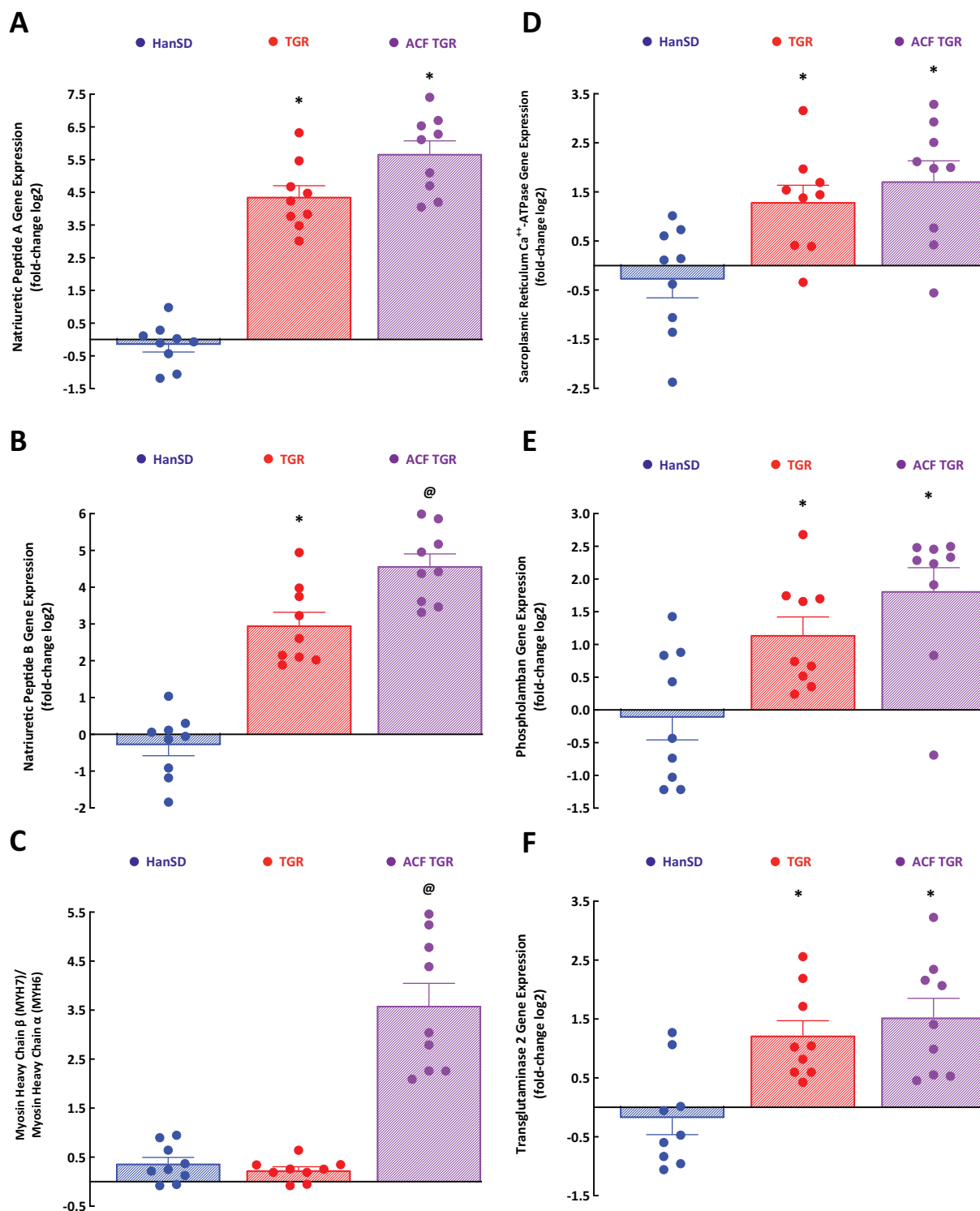


Fig. 7 The first part of the data for mRNA expression in the left ventricle – gene markers of myocardial stress and contractile function. Natriuretic peptide A **A**, natriuretic peptide B **B**, the ratio of the β -myosin heavy chain (Myh7) to α -myosin heavy chain (Myh6) **C**, sarcoplasmic reticulum Ca^{2+} -ATPase **D**, phospholamban **E** and transglutaminase 2 **F** in sham-operated

normotensive transgene-negative Hannover Sprague-Dawley rats (HanSD), hypertensive sham-operated Ren-2 transgenic (TGR) rats, and in TGR assessed two weeks after creation of the aorto-caval fistula (ACF). * $P < 0.05$ compared with sham-operated HanSD rats. @ $P < 0.05$ compared with all other groups. The values are means $\pm$ SEM

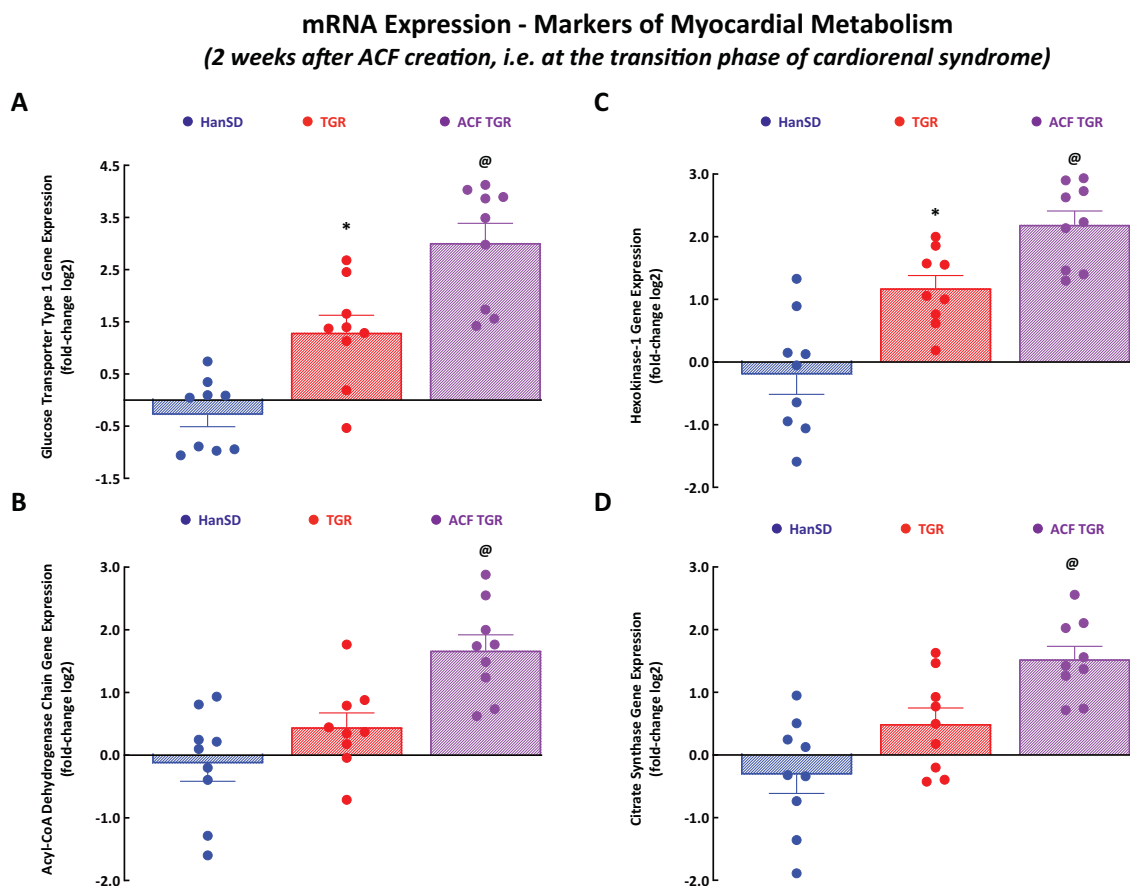


Fig. 8 The second part of the data for mRNA expression in the left ventricle – gene markers of myocardial metabolism. Glucose transport type 1 **A**, acyl-CoA dehydrogenase, C-4 to C-12 straight chain **B**, hexokinase-1 **C**, and citrate synthase **D** in sham-operated normotensive transgene-negative Hannover Sprague-Dawley rats (HanSD),

hypertensive sham-operated Ren-2 transgenic (TGR) rats, and in TGR two weeks after the creation of the aorto-caval fistula (ACF). * $P < 0.05$ compared with sham-operated HanSD rats. @ $P < 0.05$ compared with all other groups. The values are means $\pm$ SEM

organ level as well as at the cardiomyocyte level - in the phase of the transition from the compensation to the decompensation phase of cardiorenal syndrome. Previous studies (including ours) showed that organ weights (whole HW, LVW, RVW, and wet lung weight) are reliable indices of the sudden onset overt HF decompensation, at least in the ACF-induced high-output HF model [33–35, 64–71]. Based on our studies in ACF TGR [36, 37], the rat provides a realistic model of the cardiorenal syndrome we proposed, in accordance with the Linzbach theory [72, 73] (which states that once the myocardial hypertrophic response to the pathological stimuli is exhausted, a progressive ventricular dilatation occurs leading to increased wall stress with subsequent transition of HF to the phase of decompensation), that the inability of LV to develop hypertrophy comparable with the RV might be a critical factor in the transition to the overt decompensation phase of cardiorenal syndrome. For comparative analysis, we employed our present results - obtained exactly in the transition phase of cardiorenal syndrome - and our data obtained earlier in the phase of full

compensation and overt decompensation of cardiorenal syndrome [36–38]. As already mentioned in the Introduction, for ethical reasons we did not repeat experiments one week and three weeks after ACF creation and we use the data from our previous studies.

Our results clearly show that increases in LV hypertrophy across all three phases of the cardiorenal syndrome, i.e. from the compensation phase (one week after ACF creation via transition phase - two weeks after ACF creation) to the decompensation phase (three weeks after ACF creation) were modest as compared with increases in RV hypertrophy. Specifically, it is apparent that the RV hypertrophy was already one week after ACF induction 31-fold higher than LV hypertrophy, suggesting that the LV hypertrophic response is substantially delayed in the very early phase of cardiorenal syndrome. Furthermore, the LV hypertrophic response is still considerably hindered in the transition phase and decompensation phase of cardiorenal syndrome: the RV hypertrophy remained nine-fold higher than the LV hypertrophy (Fig. 2C, 2D). However, it is

Changes in Organ Weights (from the compensation phase via the transition phase to the decompensation phase of cardiorenal syndrome)

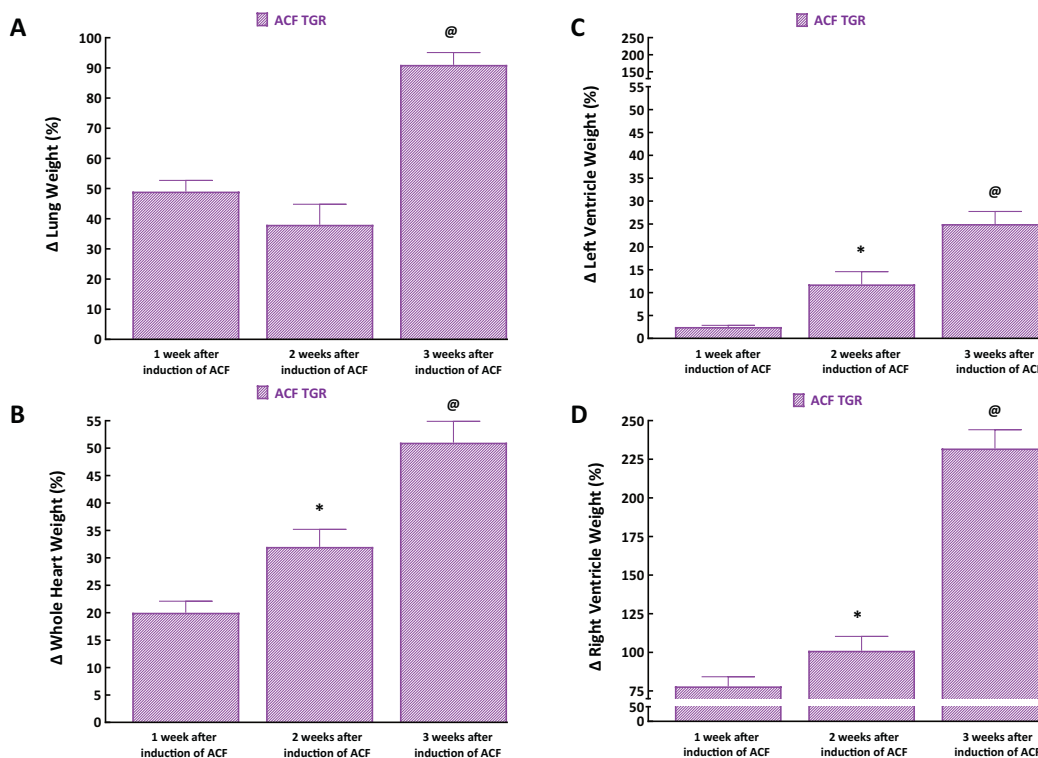


Fig. 9 Changes in organ weights from the compensation phase [one week after aorto-caval fistula (ACF) creation, data taken from reference #36], via transition phase (two weeks after ACF creation, data taken from the current study) to the decompensation phase (three weeks after ACF creation, data taken from reference #37). Changes are expressed as percent increases in hypertensive Ren-2 transgenic (TGR)

rats with ACF compared to sham-operated TGR at the same time. Changes in lung weight **A**, whole heart weight **B**, left ventricle weight **C**, and right ventricle weight **D**. * $P < 0.05$ compared with ACF TGR one week after ACF induction. @ $P < 0.05$ compared with ACF TGR one week as well as two weeks after ACF induction. The values are means $\pm$ SEM

noteworthy that the increase in LVW three weeks after ACF creation was almost twice higher than that observed in ACF TGR two weeks after ACF creation: this likely had a positive effect on the LV wall stress, as discussed below. Moreover, our findings at the cardiomyocyte level clearly showed that the development of eccentric remodeling of the LV dynamically progressed across all three phases of cardiorenal syndrome. Notably, the cardiomyocyte length to cardiomyocyte width ratio increased across all three phases of cardiorenal syndrome; thus, at the transition phase it was already about 25% higher than in sham-operated TGR (Fig. 5C). Furthermore, our present findings regarding the relationship between cardiac hypertrophy and lung congestion are of particular interest. Even though ACF TGR in the transition phase did not show higher lung weight as compared with sham-operated TGR, in three ACF TGR rats, the lung weight was about 70% higher than the average for the group and more than twice higher than observed in sham-operated TGR. Thus, marked lung congestion developed in the said three rats, and this occurred without any

significant LV hypertrophy (their LV weight was about 15% lower than the average of the group and was roughly the same as observed in sham-operated TGR) (Fig. 2B and Fig. 2F).

In this context, our findings regarding left atrium diameter (Table 1) and left atrium weight in ACF TGR two weeks after ACF creation are of special interest: they show that the relative absence of LV hypertrophy as compared with sham-operated TGR was accompanied not only by left atrium dilatation, but also by a marked increase in left atrium hypertrophy (particularly obvious in the said three rats that did not develop LV hypertrophy but developed marked lung congestion). Even though the pressure in the left atrium was not measured (enormous technical challenge!) the data suggest that discernable left atrium congestion is in ACF TGR present in the transition phase. Therefore, after a thorough consideration of our previous results and especially of the present morphological data, and taking the Linzbach concept into account [72], we propose that the inability of the LV to develop an appropriate

Changes in Cardiomyocytes Parameters

(from the compensation phase via the transition phase to the decompensation phase of cardiorenal syndrome)

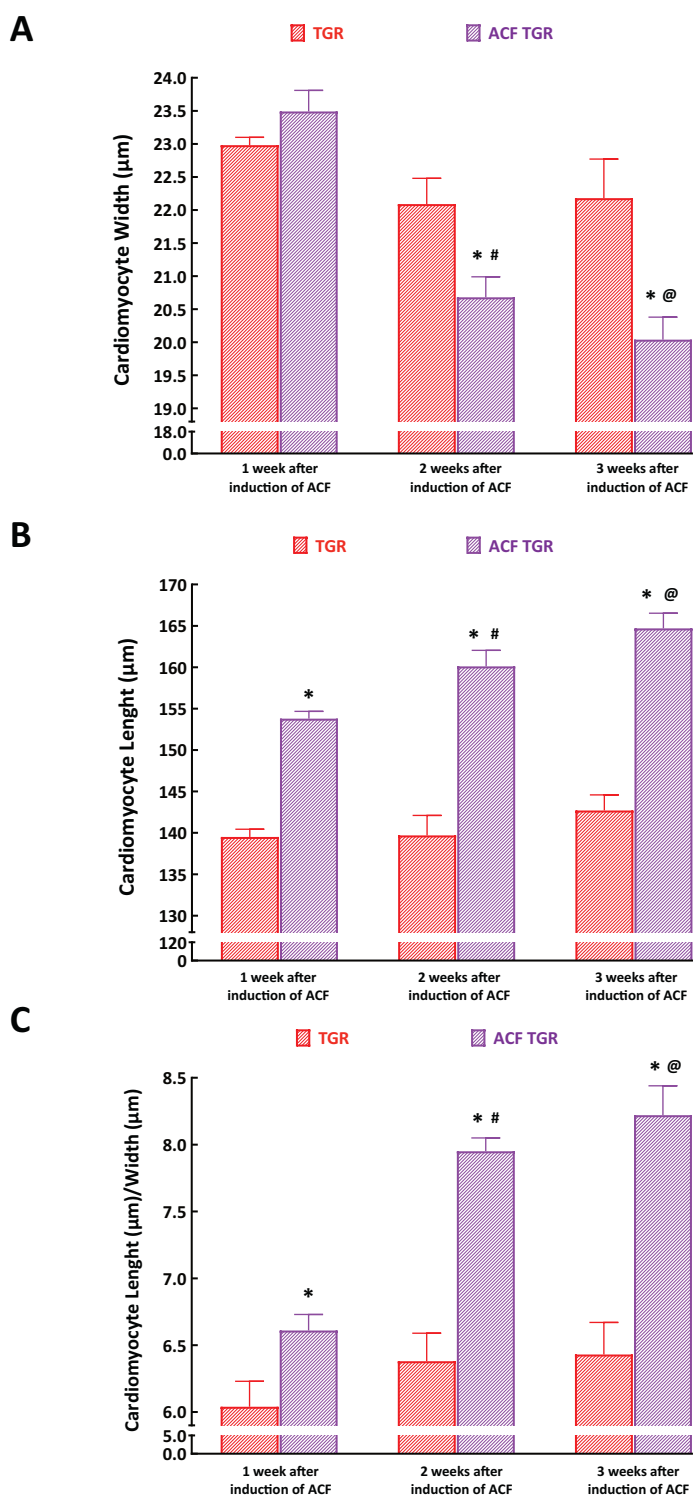
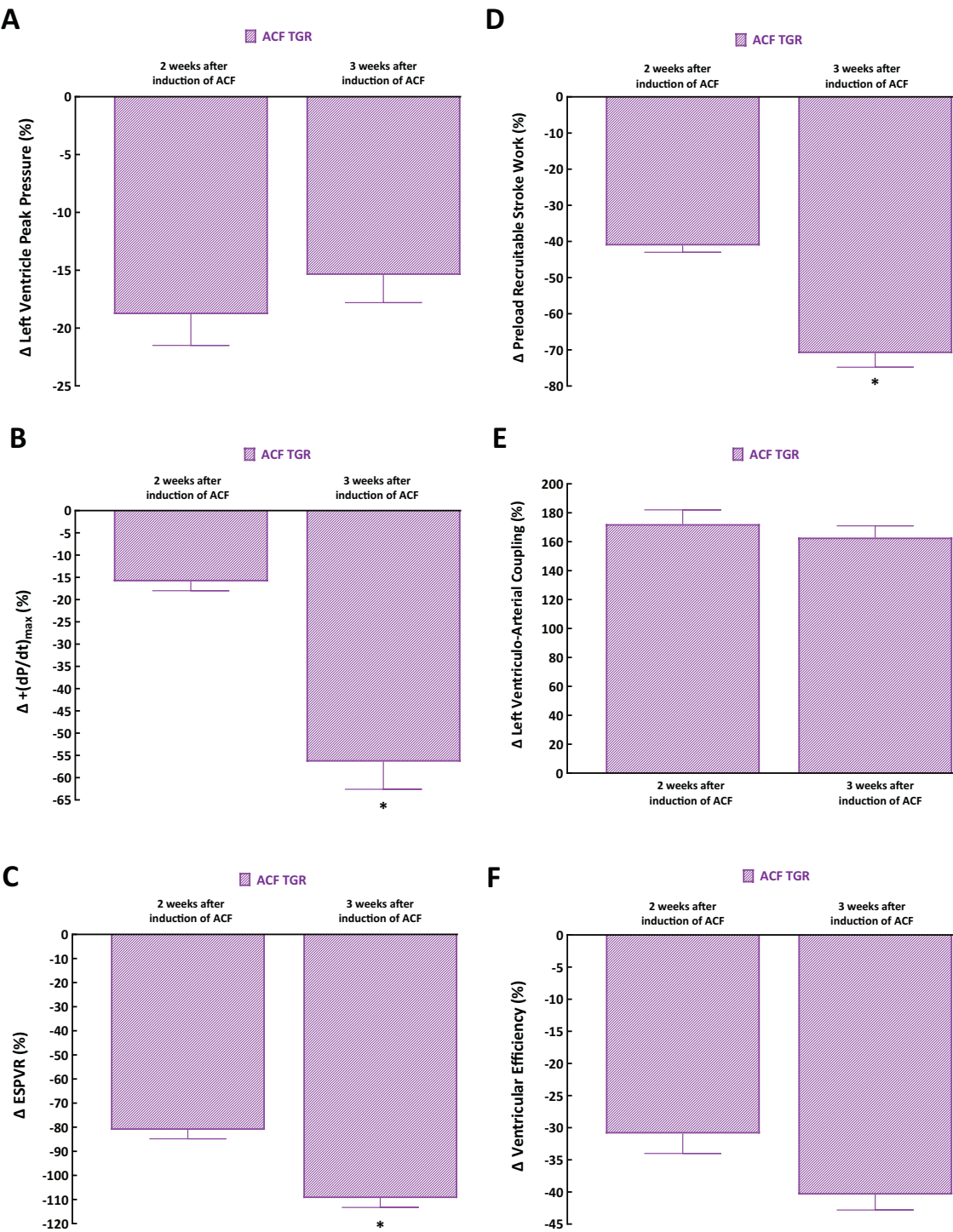


Fig. 10 Cardiomyocyte parameters from the compensation phase [one week after aorto-caval fistula (ACF) creation, data taken from reference #36], via transition phase (two weeks after ACF creation, data taken from the current study) to the decompensation phase (three weeks after ACF creation, data from reference #37). Cardiomyocyte width **A**, cardiomyocyte length **B**, and

the ratio of cardiomyocyte width to length **C**. ^{*} $P < 0.05$ compared with sham-operated TGR at the same time point. [@] $P < 0.05$: ACF TGR three weeks after ACF creation versus ACF TGR two weeks after ACF induction. [#] $P < 0.05$: ACF TGR two weeks after ACF creation versus ACF TGR one week after ACF creation. The values are means $\pm$ SEM

Changes in Left Ventricle Function - Pressure Volume Analysis - Part 1
(from the transition phase to the decompensation phase of cardiorenal synrome)



◀ **Fig. 11** Changes in left cardiac functions (assessed by invasive hemodynamic analysis) from the transition phase (two weeks after ACF creation, data taken from the current study) to the decompensation phase (three weeks after ACF creation, data taken from the reference #37). Changes are expressed as percent increases in hypertensive Ren-2 transgenic (TGR) rats with ACF compared to sham-operated TGR at the same time. Changes are shown in left ventricle peak pressure **A**, maximum rates of pressure rise ($+dp/dt_{\max}$) **B**, end-systolic pressure volume relationship (ESPVR) **C**, preload recruitable stroke work **D**, left ventriculo-arterial coupling **E** and left ventricular efficiency **F**. * $P < 0.05$ compared with ACF TGR two weeks after ACF induction

myocardial hypertrophic response to the volume overload in the ACF TGR is a critically important factor underlying the process of the transition from the compensation to the decompensation phase of cardiorenal syndrome. This first interim conclusion is further supported by our second important set of results of echocardiographic and invasive PV analysis of cardiac structure and function.

There were no significant differences in the LV and RV diameters between sham-operated HanSD rats and sham-operated TGR. Nor were there any significant differences between LV and RV function, based on the analysis of LV ejection fraction, the LV fractional shortening, the RV fractional change, the cardiac output, and the parameters of LV filling. All this was corroborated by PV analysis that demonstrated that load-dependent and load-independent parameters of LV contractility [$(+dp/dt)_{\max}$, ESPVR, PRSW] were similar in sham-operated HanSD rats and sham-operated TGR, indicating that the latter did not show systolic dysfunction despite significant hypertension and elevated arterial elastance. In addition, there were no significant differences in $(-dp/dt)_{\min}$, LV relaxation constant, and the EDPVR between sham-operated HanSD rats and sham-operated TGR, which indicated that sham-operated TGR did not exhibit any important impairment of diastolic function.

These conclusions are confirmed by the findings that the LV end-diastolic pressure and the LV wall stress were not elevated in sham-operated TGR compared with sham-operated HanSD rats. Thus, the concentric LV hypertrophy in sham-operated TGR (increased LV anterior and posterior wall thickness and particularly enhanced relative LV wall thickness) was able to offset the negative consequences of hypertension on the LV function.

In sharp contrast, two weeks after ACF creation, ACF TGR showed markedly increased LV diameter and decreased LV wall thickness (indices of the development of eccentric hypertrophy). Accordingly, there was enhanced LV wall stress, LV end-diastolic pressure, and subsequently impaired LV systolic function (documented by decreased load-dependent and load-independent parameters of the LV contractility). In this context, of particular interest is our

observation that ACF TGR in the transition phase did not show significant impairment of the RV systolic function (documented by RV fractional change that was not significantly diminished as compared with sham-operated HanSD rats as well as sham-operated TGR). Admittedly, the values tended to be lower in ACF TGR; however, unlike in the case of the LV, the increase in the RV mass two weeks after ACF creation was prominent. Other interesting findings relate to the diastolic function in ACF TGR observed two weeks after ACF creation: despite increased interstitial collagen content (mainly dependent on the newly synthesized component after ACF creation), and echocardiographic indices of diastolic function (increased E/A and E/E' ratios in ACF TGR in comparison to TGR and HanSD), ACF TGR did not show significantly impaired $(-dp/dt)_{\min}$, LV relaxation constant, and the EDPVR as compared with sham-operated TGR. This suggests that LV stiffness was not markedly enhanced and that ACF TGR in the transition phase may not yet display a pronounced impairment of diastolic function.

Our present data from the transition phase (two weeks after ACF creation) should be considered alongside with our recent data from the decompensation phase (i.e. three weeks after ACF creation) of the cardiorenal syndrome [38]. Comparison of those data shows that ACF TGR in both phases showed insignificant changes in $(-dp/dt)_{\min}$, LV relaxation constant, and the EDPVR as compared with sham-operated TGR (Supplemental Fig. 4A–4C). This suggests that impairment of diastolic function is not the critical component in the process of the transition to the decompensation phase of cardiorenal syndrome. In clear contrast is here the comparison of load-dependent and load-independent parameters of LV contractility. Evidently, $(+dp/dt)_{\max}$, ESPVR and PRSW further significantly decreased three weeks after ACF creation as compared with the values seen after two weeks. In fact, and there was a noticeable tendency for further worsening of LV ventricular efficiency (Fig. 11B–D and Fig. 11F). These data suggest that progressing impairment of LV systolic function is importantly involved in the transition to the decompensation phase of cardiorenal syndrome. Therefore, the issue of LV wall stress deserves special attention since it has been long regarded to be an essential determinant of myocardial oxygen consumption and the myocardial contractile status [72].

There is no doubt that the value of LV wall stress reflects in a modified way Laplace's law and according to the formula presented above in the Methods section it is directly proportional to the pressure and radius and inversely proportional to doubled wall thickness. Therefore, the marked increase in the LV wall stress in ACF TGR observed two weeks after ACF creation (dominantly mediated by the decrease in wall thickness) and the subsequent decrease in

the LV wall stress seen three weeks after ACF creation (Supplemental Fig. 4D) is explained by the degree of LV hypertrophy (and LV wall thickness) (Fig. 9C) that was much increased after three weeks.

Our present findings are also in agreement with observations made by Brower and Janicki [68], who, in the model of high-output HF, showed that in ACF HanSD rats, the observed LV hypertrophy exhibits an upper limit: above this, a marked LV dilatation and increased compliance occur. The authors called it “maladaptive ventricular remodeling,” which resulted in overt HF decompensation. Again, such findings are in accordance with the original Linzbach theory [72], which was further developed in detail by the Gerdes group [74, 75]. The above evidence further supports our current notion that the failure of the LV to develop an appropriate hypertrophic response to the volume overload in ACF TGR is likely a crucial factor underlying the process of the transition from the compensation to the decompensation phase of cardiorenal syndrome, and is predominantly due to progressive impairment of LV contractility.

The third important set of findings relates to the analysis of the LV gene expression of selected genes, those implicated in the development of HF [41, 42]. Our results show that ACF TGR displayed upregulation of myocardial stress genes, specifically of *NppB* and of the ratio of *Myh7/Myh6*, which suggests that it is linked to LV hemodynamic abnormalities induced by volume overload. In addition, ACF TGR consistently showed increased *GLUT1*, *Acadm*, *Hk1* and *Cs* mRNA expression in the LV, which suggested that volume overload induced enhanced insulin-independent glucose uptake and increased glycolysis (compared with fatty acid β -oxidation). The findings are compatible with the activation of the “fetal cardiac gene program” believed to be, in the long-term perspective, maladaptive. Admittedly, the issue remains controversial [76, 77]. However, our correlation analysis clearly showed no difference in the relationship between the critically important markers of the LV cardiac remodeling (LV mass and LV walls stress) and LV mRNA expression of the selected genes of myocardial stress and myocardial metabolism between ACF TGR and sham-operated TGR as well as sham-operated HanSD rats (Supplemental Figs. 5 to 7). This strongly indicates that alterations in gene expression of the selected genes (implicated in the development of HF [41, 42]) are not a causal factor in the transition from the compensation to the decompensation phase of cardiorenal syndrome. The observed gene upregulations in the LV would instead represent a consequence of the onset of cardiorenal syndrome decompensation. It is unknown whether these changes are, in the long-term perspective, adaptive or maladaptive – an issue that could be considered for future studies.

Limitations of the study

The first important limitation is that the study undertakes evaluation of cardiac structure and function only, even though we employ the term of cardiorenal syndrome, no assessment of renal function was done in this study. As already mentioned in the Introduction the major reason for this omission is that to perform simultaneous evaluation of cardiac function by PV analysis and of renal clearance and hemodynamic studies would be an extraordinary challenge. While our laboratories regularly employ these techniques in separate studies, we cannot so far perform simultaneous evaluation of cardiac and renal function in a way to achieve physiologically reliable results. There is no doubt that such combined studies would require long-term anesthesia and extensive interventional surgical procedures. Nevertheless, we are fully aware that such studies are of critical importance for thorough evaluation of the pathophysiology of cardiorenal syndrome; we believe that in near future we will be able to overcome the technical challenges involved and accomplish simultaneous evaluation of cardiac and renal function in our models of cardiorenal syndrome.

The second limitation of the present study is that it was conducted only in male and since there is increasing recognition that sex should be incorporated as a biological variable in preclinical research being prerequisite for greater reproducibility of research and for successful translation of results into a clinical research [78]. This is particularly valid if TGR are used as a model, because it is known that these rats exhibit important sex-linked differences in the course of hypertension [79, 80] and especially in view of our previous findings that ACF TGR showed important sex-related differences in the course of HF [81, 82]. We are aware of this important limitation and ongoing studies in our laboratories are employing male as well as female animals.

The third limitation of our present study is related to the selection of genes for the analysis of LV mRNA expression as markers of myocardial stress, metabolism, inflammation and fibrosis. These genes were selected based on previous studies in our laboratories [41, 42] that have shown their greatest correlations with changes during RV and LV remodeling. However, it should be noted that those earlier studies were performed under slightly different conditions, even though the same model of ACF-induced HF was employed (NB. only normotensive animals were used). Therefore, our gene selection is not unbiased and may not reflect the full spectrum of transcriptional adaptations in this phase of cardiorenal syndrome. Evidently, further studies are needed to comprehensively analyze transcriptional adaptation to the development of cardiorenal syndrome in this model.

Nonetheless, while admitting the limitations discussed above, we are still convinced that the present results

provide important information on the role of “heart side component” in the pathophysiology of experimental high-output HF, a reasonable model of human cardiorenal syndrome.

Conclusion

Based on our present comprehensive evaluation of cardiac morphology, structure, and function in ACF TGR – a model of high-output HF-induced cardiorenal syndrome - we propose that the inability of the LV to develop appropriate hypertrophic response (leading to maladaptive ventricular remodeling) is a crucial factor in the process of the transition from the compensation to the decompensation phase of cardiorenal syndrome.

Our present findings are important in the light of recent studies that demonstrated that decompensation in our model of the cardiorenal syndrome could not be simply ascribed to impairment of the autoregulatory capacity of renal hemodynamics and the kidney pressure-natriuresis relationship. In fact, we found previously that the pressure-natriuresis relationship was not impaired: it was even improved and facilitated sodium excretion under conditions of lower renal arterial pressure [36, 37]. Taken together, our present and recent findings [36, 37] strengthen the view that to understand the pathophysiology of cardiorenal syndrome (prerequisite for the development of new therapeutic approaches), integrated parallel evaluation of changes in the “kidney side” and in the “heart side” is needed and should be conducted at appropriate phases of this complex syndrome.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interest.

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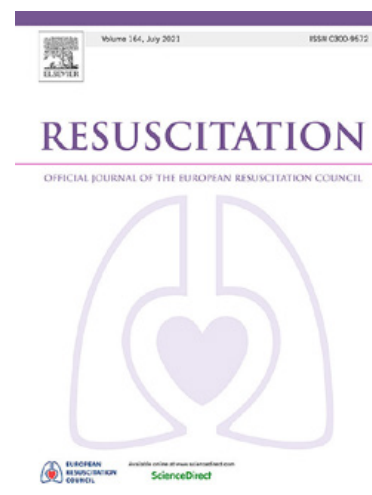
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Original paper

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Impact of rhythm changes during CPR on ECPR outcomes: an ELSO Registry Study

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Highlights:

1. Both initial and cannulation rhythms were associated with survival after ECPR.
2. Conversion to ventricular fibrillation improves, while asystole worsens outcomes.
3. Survival decline with longer CPR differs by rhythm, supporting rhythm-based ECPR triage.

Abstract

Background: Cardiac rhythm is a well-established prognostic factor in patients undergoing extracorporeal cardiopulmonary resuscitation (ECPR); however, existing evidence is primarily limited to small, single-center studies. This study aimed to evaluate the prognostic value of both initial rhythm and rhythm at the time of cannulation using data from the international Extracorporeal Life Support Organization (ELSO) Registry.

Methods: We analyzed adult ECPR cases (≥ 18 years) recorded in the ELSO Registry between 2020 and 2025. The primary outcome was survival to hospital discharge. Multivariable logistic regression was used to assess associations between survival and cardiac rhythm (initial and at cannulation), adjusting for age, sex, witnessed arrest, arrest location, and CPR duration.

Results: Among 5,489 adult ECPR cases, 3,382 were included in the complete case analysis. Median age was 57.1 years (IQR 45.7–65.5), 29.1% were female, and median CPR duration was 44 minutes (IQR 28–61). Survival proportions were highest in patients with persistent ventricular fibrillation (VF) or conversion from an initial non-shockable rhythm to VF, and lowest in those who either converted to or remained in non-shockable rhythm. In multivariable analysis, initial pulseless electrical activity (PEA) (OR 0.59, 95% CI 0.48–0.71) and asystole (OR 0.64, 95% CI 0.47–0.86) were associated with lower odds of survival compared to initial VF. Asystole at the time of cannulation was associated with worse outcomes (OR 0.61, 95% CI 0.38–0.99). The survival decline with prolonged CPR was steepest in those with asystole at cannulation (OR for 60 vs 40 minutes of CPR = 0.64; CI 0.54–0.76; $p < 0.001$) and most gradual in patients with VF at cannulation (OR for 60 vs 40 minutes of CPR = 0.84; CI 0.76–0.93; $p = 0.001$).

Conclusions: In this large, multicenter analysis, both initial and cannulation rhythms were independently associated with survival among ECPR recipients. Survival showed time-dependent variation across rhythm conversions. These specific survival patterns underscore the need for individualized, rhythm-informed ECPR decision-making algorithms to optimize patient outcomes.

Key words: Extracorporeal cardiopulmonary resuscitation, ECPR, Cardiac arrest rhythm, Extracorporeal membrane oxygenation, ECMO, ELSO Registry.

Main text

Background

Extracorporeal cardiopulmonary resuscitation (ECPR), defined as the rapid initiation of venoarterial extracorporeal membrane oxygenation (VA ECMO) in patients without sustained return of spontaneous circulation (ROSC) despite conventional cardiopulmonary resuscitation (CPR), offers a potentially life-saving option for selected individuals.¹⁻³ However, identifying suitable candidates during ongoing CPR remains challenging due to limited time and the absence of reliable real-time predictors.³⁻⁴

Patient selection for ECPR varies widely across institutions and guidelines, reflecting ongoing uncertainty and limited evidence to support uniform inclusion criteria.⁵⁻⁸ Among established prognostic indicators, initial cardiac rhythm, particularly the presence of a shockable rhythm, is consistently associated with improved survival in both conventional CPR and ECPR settings.^{4,9} Moreover, emerging observational data suggest that rhythm conversion during CPR, especially from shockable to non-shockable rhythms, is associated with worse outcomes.¹⁰⁻¹¹

Despite this, few studies have systematically evaluated cardiac rhythm at the time of ECPR cannulation, a clinically relevant and potentially modifiable factor during decision-making. Most available data are derived from small, single-center cohorts, which are limited by small to modest sample sizes and potential residual confounding.¹²⁻¹⁶ Furthermore, most existing studies focus on out-of-hospital cardiac arrest (OHCA), with limited evidence addressing in-hospital cardiac arrest (IHCA) populations, who may differ substantially in baseline characteristics, response time, and outcomes.

To address these gaps, we analyzed data from the Extracorporeal Life Support Organization (ELSO) Registry, the largest international, multicenter database of ECMO-treated patients, including those who received ECPR.¹⁷ This study aimed to assess the prognostic relevance of both initial rhythm and rhythm at the time of cannulation, drawing on the registry's global coverage, substantial statistical power, and inclusion of both OHCA and IHCA populations. By analyzing rhythm evolution, its prognostic implications, and its time-dependent relationship with survival, we sought to generate evidence that may support progress toward more standardized approaches in ECPR patient selection and management.

Methods

Study design

Data were extracted from the ELSO Registry, an international, multicenter database.¹⁷ Registry entries are submitted voluntarily by participating centers and include standardized data on patient demographics, clinical characteristics, ECMO management, and outcomes. All data are recorded using a uniform electronic case report form by trained and certified site personnel. This study was approved by the ELSO Scientific Oversight Committee following external peer review (ELSO request #2917). It was also approved by the local Ethics Committee of the General University Hospital in Prague (0078/25). Informed consent was waived, as the ELSO dataset contains only de-identified information.

Study population and data acquisition

This study included adult patients (≥ 18 years) who received ECPR between 2020 and 2025. The study period was restricted to 2020 onward to ensure inclusion of cases documented using the updated “ECPR 2020 Addendum” data form (publicly available at <https://www.else.org/registry/datadefinitions,forms,instructions.aspx>), which includes detailed information on cardiac rhythm at the time of ECMO cannulation. ECPR was defined as the rapid deployment of VA ECMO to provide circulatory support in patients for whom conventional cardiopulmonary resuscitation (CPR) failed to achieve sustained return of spontaneous circulation (ROSC). Sustained ROSC is deemed to have occurred when chest compressions are not required for 20 consecutive minutes and signs of circulation persist.¹⁸ Data were extracted for all eligible patients including baseline demographics, resuscitation characteristics, ECPR-specific clinical data, and outcomes. Patients with multiple ECMO runs and patients supported with VP, VV ECMO as their initial mode of support were excluded. Most included cases underwent femoral–femoral VA-ECMO cannulation but alternative arterial cannulation approaches (aortic, subclavian, or axillary access) were retained in the analysis. Additionally, patients with unknown total CPR duration, unknown initial rhythm, or unknown rhythm at cannulation were excluded from the complete case analysis because these variables were essential for the multivariable analysis. Finally, we excluded all patients with an organized rhythm at cannulation to avoid including cases with possible ROSC who may have been cannulated for hemodynamic instability rather than refractory cardiac arrest.

Outcomes

The primary outcome was survival to hospital discharge.

Statistical Analysis

Continuous variables were summarized using medians and interquartile ranges (IQR, 25th–75th percentiles), while categorical variables were reported as counts and percentages. Comparisons of continuous variables across groups and subgroups were performed using Welch’s t-test, or Mann–Whitney U-test, as appropriate. Categorical variables were compared using the chi-squared test. The primary analysis was conducted as a complete-case analysis, excluding patients with missing data on essential covariates. To address potential bias due to missing data, a secondary analysis with a multiple imputation approach was performed using Multivariate Imputation by Chained Equations (MICE) in R for key variables (initial rhythm, sex, and total CPR duration).¹⁹ We did not impute rhythm at cannulation despite its missingness because patients with organized rhythm at cannulation were excluded a priori from the analytic cohort. Imputing this variable would have required generating organized rhythms for patients who, by design, should not be included, thereby introducing misclassification. A multivariable logistic regression model was used to evaluate associations between variables (including age (per year), sex (male vs. female), witnessed status (yes vs. no), place of cardiac arrest (IHCA, OHCA-home, OHCA-public, OHCA-other), total CPR time (per minute), initial rhythm (VF/pVT, PEA, asystole), and rhythm at cannulation (VF/pVT, PEA, asystole) and the primary outcome, defined as survival to hospital discharge. Detailed definitions of variables of ELSO ECPR addendum are publicly available at <https://www.else.org/registry/datadefinitions,forms,instructions.aspx>. Primary complete case model robustness and temporal consistency were evaluated via cross-validation, treating each calendar year from 2020 to 2025 as a separate fold. The significance of individual coefficients was assessed using Wald tests. In addition, the overall contribution of total CPR time was evaluated using a likelihood ratio test. Moreover, to visualize time-dependent associations between total CPR duration and survival across different rhythm at the time of cannulation, we plotted mean survival probabilities with 95% pointwise confidence intervals (Figure 2A-C).

CPR duration was modelled using a quadratic term to account for non-linearity, and an interaction term between CPR duration and rhythm at cannulation was included to evaluate rhythm-specific time-dependent effects. This parametrisation was selected based on cross-validated model performance and the observed non-linear associations. Additional subgroup analyses were performed for out-of-hospital cardiac arrest (OHCA) and in-hospital cardiac arrest (IHCA) cohorts. All statistical analyses were performed using R statistical software (version 4.2.3).²⁰

Results

Baseline and resuscitation characteristics

Between 2020 and 2025, a total of 5,489 adult (≥ 18 years) ECPR cases with ECPR addendum were recorded in the ELSO Registry, of which 3,382 (61.6%) were included in the complete case analysis (Figure 1A). Although most cannulations were femoral–femoral, the final dataset also contained 232 patients (6.9%) who underwent alternative cannulation approaches, including aortic, subclavian, or axillary artery access. Details of exclusions are shown in Figure 1A.

Baseline characteristics stratified by initial rhythm are presented in Table 1. Patients with an initial rhythm of ventricular fibrillation/pulseless ventricular tachycardia (VF/pVT) or pulseless electrical activity (PEA) were older compared to those with asystole. Male sex was more common among patients with initial VF/pVT than among those with PEA or asystole. In 50.1% of VF/pVT cases cardiac arrest occurred in-hospital (IHCA), whereas higher proportions of IHCA were observed among patients with non-shockable rhythms PEA (79.9%) and asystole (69.7%) (Table 1). Total CPR duration differed substantially between IHCA and OHCA subgroups, with shorter durations in IHCA (median 35 minutes) compared with OHCA (60 minutes), consistent with findings from prior ECPR trials (Supplementary Table 2).¹⁻²

Conversion between initial rhythm and rhythm at cannulation

Rhythm conversion patterns for all patients are illustrated in Figure 1A. Among patients with initial VF/pVT, 56.4% remained in VF/pVT, 30.4% converted to PEA, and 13.2% to asystole. Among those with initial PEA, 73.1% remained in PEA, while 15.7% converted to VF/pVT and 11.2% to asystole. In the initial asystole group, 62.2% remained in asystole, 21.9% converted to PEA, and 15.8% to VF/pVT.

Survival to hospital discharge

Among patients with initial VF/pVT, overall survival to discharge was 32.9%, highest in those with sustained VF/pVT (40.1%), followed by those who converted to PEA (28.1%) and lowest in those who converted to asystole (13.6%) (Figure 1A). In the PEA group, overall survival was 23.8%, with highest rates in those who converted to VF/pVT (32.7%), followed by those with sustained PEA (23.7%) and conversion to asystole (12.1%). Among patients with initial asystole, the overall survival rate was 19.5%, with survival of 28.6% for those who converted to VF/pVT, 17.5% for conversion to PEA, and 17.8% for sustained asystole (Figure 1A).

Multivariable logistic regression of survival to discharge

Multivariable logistic regression results for all patients are summarized in Table 2. Compared to initial VF/pVT, both initial PEA (OR 0.59, 95% CI 0.48–0.71, $p < 0.001$) and initial asystole

(OR 0.64, 95% CI 0.47–0.86, $p=0.003$) were associated with significantly lower odds of survival. Asystole at the time of cannulation was similarly associated with significantly worse outcomes compared to VF/pVT (OR 0.61, 95% CI 0.38–0.99, $p=0.044$), while we did not detect an association between PEA at cannulation and survival (OR 0.76, 95% CI 0.54–1.07, $p=0.112$).

CPR duration showed a strong and independent association with survival ($p<0.001$), with a rhythm-specific, time-dependent effect (Figure 2A–C). The decline in survival with increasing CPR duration was steepest in patients converting to asystole (OR between 40min vs 20min CPR = 0.58; CI 0.48–0.70; $p<0.001$; OR between 60min vs 40min CPR = 0.64; CI 0.54–0.76; $p<0.001$), followed by those with PEA (OR 40 vs 20 min CPR = 0.72, CI 0.64–0.80; $p<0.001$, OR between 60min vs 40min CPR = 0.79; CI 0.71–0.86, $p<0.001$). The decline was most gradual in those with pVT/VF (OR between 40 vs 20 min CPR = 0.77; CI 0.68–0.86, $p<0.001$; OR between 60 vs 40 min CPR = 0.84; CI 0.76–0.93, $p=0.001$).

Other variables independently associated with lower survival were increasing age (OR per year = 0.99, 95% CI 0.98–0.99, $p<0.001$) and cardiac arrest occurring at home (OR 0.48, 95% CI 0.36–0.63, $p<0.001$), Table 2. Neither sex nor witnessed status was significantly associated with survival.

Multiple imputation model

The imputed model produced results that were consistent with the complete-case analysis (Supplementary Table 1), supporting the robustness of the identified variables. The negative association of initial and cannulation rhythms, prolonged CPR duration, and cardiac arrest occurring at home with survival remained significant after imputation. The only notable difference was a more pronounced effect of public location of OHCA in the imputed model (Supplementary Table 1).

OHCA and IHCA subgroup analyses

Baseline characteristics for OHCA and IHCA subgroups are provided in Supplementary Table 2. Compared with OHCA, the IHCA cohort was older, had a lower proportion of males, shorter CPR durations, more frequent witnessed arrests, and fewer defibrillation attempts.

Rhythm conversion patterns are shown in Figures 1B (IHCA) and 1C (OHCA). Initial PEA was more common in IHCA, while VF/pVT predominated in OHCA. Among patients with initial VF/pVT, IHCA cases were more likely to sustain VF/pVT (65% vs. 47.5%) and had lower rates of conversion to PEA (23.7% vs. 37.2%) or asystole (11.3% vs. 15.3%), ($p<0.001$). Across all rhythm transitions, survival rates were higher in IHCA than OHCA. Notably, OHCA patients with a non-shockable rhythm conversion had worse outcomes, especially in the subgroup PEA to asystole (Figure 1C).

Discussion

This study represents the largest multicenter analysis to date examining the association between cardiac rhythm, rhythm conversion, and survival in patients treated with ECPR. It incorporates both OHCA and IHCA populations and yields several clinically relevant insights.

Initial rhythm and rhythm at cannulation are strongly associated with outcomes

Our findings demonstrate that initial VF/pVT is associated with the highest survival, and that conversion from a non-shockable rhythm to VF/pVT is associated with higher survival compared to continued non-shockable rhythm. In contrast, persistence in or conversion to asystole is associated with the lowest survival, particularly among OHCA patients. These results highlight the prognostic importance of pre-cannulation rhythm evolution, which likely reflects the underlying pathophysiology and quality of resuscitation efforts. By including large cohorts of both non-shockable rhythms and IHCA patients, our study provides robust, multicenter validation of the prognostic relevance of rhythm conversion, confirming and extending prior observations from smaller studies.⁹⁻¹⁶

Our findings may offer several rhythm-specific implications for ECPR candidacy. First, patients presenting with initial VF/pVT demonstrated the highest survival across the cohort. Therefore, initial VF/pVT should remain a strong consideration for ECPR, even when subsequent rhythm deterioration occurs.

Second, patients with initial PEA who converted to VF/pVT had unexpectedly favorable survival (32.7% overall; 21.9% in OHCA). These results support considering ECPR for PEA → VF/pVT conversions, particularly when conversion occurs early and aligns with other favorable arrest characteristics.

Third, among patients with sustained non-shockable rhythms, survival varied markedly by pattern. PEA → sustained PEA showed 23.7% overall survival, indicating that selected patients with persistent PEA may still have reasonable outcomes, especially when cannulation occurs early. In contrast, the prognosis of PEA → asystole appeared strongly context-dependent: survival reached 15.8% in IHCA but was 0% in OHCA, suggesting that this conversion pattern may remain reasonable for consideration in controlled in-hospital settings but less so in the prehospital environment.

Finally, although asystole at presentation was generally associated with poor prognosis, a subset of patients (asystole → VF/pVT) demonstrated appreciable survival especially in IHCA.

Together, these findings underscore that dynamic rhythm trajectories offer more prognostic information than static rhythm assessment alone. Incorporating rhythm-conversion patterns may help refine ECPR decision-making beyond the current shockable/non-shockable paradigm, although these observations should be interpreted cautiously as hypothesis-generating given the observational design and absence of a non-cannulated comparator group.

Multivariable modeling demonstrated associations between initial PEA, asystole, cannulation asystole, and lower survival.

After adjusting for key confounders, both initial asystole and initial PEA were independently associated with significantly lower odds of survival compared to initial VF. Similarly, asystole at the time of ECPR cannulation was associated with worse outcomes, while we did not detect an association for PEA at cannulation, suggesting greater prognostic heterogeneity within this group. These findings validate previous studies and support incorporating real-time rhythm data into ECPR selection algorithms.^{12,13}

A time-dependent effect of CPR duration on survival varies substantially by rhythm at cannulation.

A time-dependent effect of CPR duration on survival varies substantially by rhythm at cannulation. Our data provide the first evidence in an ECPR cohort of a rhythm-specific association between increasing CPR duration and declining survival. Patients with VF at cannulation exhibited the most gradual decline in survival with prolonged CPR, whereas outcomes for conversions to PEA and especially to asystole deteriorated sharply over time. This pattern supports the hypothesis that each rhythm interacts differently with low-flow duration, likely reflecting variations in perfusion quality, metabolic status, and reversibility of the underlying cause.^{12,13} These observations parallel findings in conventional CPR, where time-sensitive and rhythm-dependent survival patterns have been described, although such interactions have not previously been evaluated in ECPR.²¹

The relevance of low-flow duration is further supported by prior OHCA ECPR studies and emerging IHCA ECPR data from Minnesota, all demonstrating steep survival declines with increasing time to cannulation.^{22–24} Our results suggest that the optimal time window for ECPR candidacy may differ by rhythm: patients with non-shockable rhythms may require particularly rapid cannulation to achieve meaningful survival, whereas those with persistent VF or conversion to VF may preserve viability over a longer period. If confirmed in prospective studies, this concept could inform real-time decision algorithms and rhythm-adapted ECPR inclusion criteria.

IHCA versus OHCA differences

This is the first study to directly compare IHCA and OHCA populations in terms of rhythm conversions and associated survival outcomes in ECPR. IHCA patients were more likely to present with PEA, sustain VF/pVT, and had generally higher survival across all rhythm categories. These differences likely reflect more controlled environments, faster response times, including shorter time to ECPR among IHCA. In contrast, OHCA patients with non-shockable rhythms and delayed cannulation exhibited worse outcomes, highlighting the need for rapid decision-making and rhythm-based triage in prehospital ECPR systems and confirming results of previous studies showing worse outcomes in OHCA patients with persistent non-shockable rhythms.^{12–13}

Limitations

This study is observational and registry-based, which inherently introduces potential limitations, including residual confounding, selection bias, and incomplete data capture. Although we applied robust multivariable modeling and multiple imputation to address missingness and confounding, the absence of variables (such as no-flow time and time to EMS arrival) may have influenced outcomes and limits external validity. Although time to cannulation was available, the registry does not capture no-flow duration as a separate variable, precluding assessment of these intervals independently. In addition, physiologic parameters (PaO₂, lactate etc.) were not included in the multivariable model as there is no pre-defined pre-ECPR measurement in ELSO registry. Further, the dataset did not include information on center case volume, which is a potentially important determinant of ECPR outcomes. Given the pragmatic, observational nature of the ELSO Registry, this study cannot establish causal relationships between rhythm patterns and survival; therefore, the findings should be interpreted as associations rather than evidence of causation. Prospective, standardized clinical studies including randomized and time-targeted ECPR trials are needed to validate these rhythm-specific differences and clarify their implications for clinical decision-making.

A major limitation is the lack of neurological outcome data, which prevents direct assessment of functional recovery as clinically the most important and meaningful endpoint. Survival with good neurological outcome will be lower than crude survival reported in this analysis. However, prior studies indicate a correlation between survival and favorable neurological outcomes in ECPR patients, and non-shockable rhythms have been associated with poorer neurological prognosis.^{12–13} Based on these data, we hypothesize that rhythm-based differences in outcomes may extend to neurological prognosis as well, but this requires confirmation in future studies. Another important limitation is that we assessed only survival to hospital discharge. We did not have access to long-term follow-up data, which may reveal a substantial decrease in survival, particularly among patients with non-shockable rhythms who are at higher risk for delayed mortality due to severe neurological injury or multiorgan dysfunction.

Finally, this study focused on initial and cannulation rhythms without information about the timing of rhythm conversions or the occurrence of multiple rhythm transitions during CPR. The lack of detailed rhythm transition dynamics may limit the granularity of patient stratification.

Conclusions

In this large, multicenter analysis, both initial and cannulation rhythms were independently associated with survival among ECPR recipients. Conversion to a shockable rhythm showed higher survival proportions than persistence in non-shockable rhythms, whereas persistence in or conversion to asystole showed the lowest survival proportions. Survival declined in a rhythm-specific, time-dependent manner with increasing CPR duration, underscoring the need for rhythm- and time-adapted ECPR decision pathways.

List of abbreviations

CI	Confidence Interval
CPC	Cerebral Performance Category
CPR	Cardiopulmonary Resuscitation
ECLS	Extracorporeal Life Support
ECPR	Extracorporeal Cardiopulmonary Resuscitation
ELSO	Extracorporeal Life Support Organization
IHCA	In-Hospital Cardiac Arrest
IQR	Interquartile Range
OHCA	Out-of-Hospital Cardiac Arrest
OR	Odds Ratio
PEA	Pulseless Electrical Activity
ROSC	Return of Spontaneous Circulation
VF	Ventricular Fibrillation

pVT Pulseless Ventricular Tachycardia

VA ECMO Veno-Arterial Extracorporeal Membrane Oxygenation

Declarations

Ethics approval and consent to participate

Informed consent was waived, as the ELSO dataset contains only de-identified information. The study was approved by the local Ethics Committee of the General University Hospital in Prague (0078/25).

Consent for publication

This study was approved by the ELSO Scientific Oversight Committee following external peer review (ELSO request #2917).

Availability of data and materials

The data that support the findings of this study are available from ELSO, upon request, to ELSO members.

Acknowledgements

We gratefully acknowledge all ELSO centers and the dedicated professionals in the ELSO community whose efforts in data collection and patient care were instrumental to this work. We also extend our sincere gratitude to all the patients and their families, whose courage in the face of critical illness continues to inspire our work.

Figure Legends

Figure 1 A, B, C. Patient flow chart and rhythm conversion patterns for all patients (A), the IHCA subgroup (B), and the OHCA subgroup (C) in the complete-case analysis. Unadjusted differences in survival proportions were evaluated across rhythm-conversion categories (initial VF conversions, initial PEA conversions, and initial asystole conversions), and the results (p-values) are presented at the bottom of the figure.

Abbreviations: CPR cardiopulmonary resuscitation, ECPR extracorporeal cardiopulmonary resuscitation, IHCA in-hospital cardiac arrest, OHCA out-of-hospital cardiac arrest, PEA pulseless electrical activity, VF ventricular fibrillation, pVT pulseless ventricular tachycardia.

Figure 2 A-C. Mean survival probabilities over the total CPR duration (for a patient with a mean age of 55 years) are shown with 95% pointwise confidence intervals for: A) initial VF/pVT, B) initial PEA, and C) initial asystole with subsequent rhythm conversion (to VF/pVT, PEA, or asystole). All panels (A–C) demonstrate a time-dependent effect that varied by rhythm. The steepest decline in survival was observed in patients who converted to asystole, followed by those with PEA, while the slowest decline was seen in patients with VF.

Abbreviations: CPR cardiopulmonary resuscitation, ECPR extracorporeal cardiopulmonary resuscitation, PEA pulseless electrical activity, VF ventricular fibrillation.

Tables Legends

Table 1. Baseline demographics and resuscitation characteristics by initial rhythm for complete case analysis.

Table 2. Multivariable logistic regression analysis of survival to hospital discharge for complete case analysis.

Supplementary Table 1. Multivariable logistic regression analysis of survival to hospital discharge for imputed analysis and mean survival probabilities across total CPR duration for imputed analysis.

Supplementary Table 2. Baseline demographics and resuscitation characteristics for IHCA and OHCA subgroups for complete case analysis.

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Table 1. Baseline demographics and resuscitation characteristics by initial rhythm for complete case analysis.

	Initial VF (n=1611)	Initial PEA (n=1329)	Initial Asystole (n=442)	P value
Age, median (IQR), years	57 (47-65)	58 (45-67)	54 (42-65)	0.001
Sex (men)	1261 (78.3%)	842 (63.4%)	295 (66.7%)	<0.001
Location of cardiac arrest				
IHCA	818 (50.8%)	1063 (79.9%)	308 (69.7%)	<0.001
OHCA - Home	359 (22.3%)	100 (7.5%)	60 (13.6%)	
OHCA - Public	345 (21.4%)	84 (6.3%)	52 (11.8%)	
OHCA - Other	89 (5.5%)	82 (6.2%)	22 (4.9%)	
Rhythm at time of cannulation				
VF	909 (56.4%)	208 (15.7%)	70 (15.8%)	<0.001
PEA	489 (30.4%)	972 (73.1%)	97 (21.9%)	

Asystole	213 (13.2%)	149 (11.2%)	275 (62.2%)	
Witnessed arrest	1458 (90.5%)	1269 (95.5%)	392 (88.7%)	<0.001
Total CPR time (median, IQR, min)	47 (30-65)	38 (25-55)	40 (26-59)	<0.001
Adrenaline (mg), median (IQR)	5 (3-9)	6 (4-10)	7 (4-10)	<0.001
Number of defibrillations prehospitally, median (IQR)	4 (3-8)	0 (0-2)	0 (0-2)	<0.001

Abbreviations: CPR: cardiopulmonary resuscitation, IHCA: in-hospital cardiac arrest, IQR: interquartile range, OHCA: out-of-hospital cardiac arrest, PEA: pulseless electrical activity, VF: ventricle fibrillation, VT: ventricle tachycardia.

Table 2. Multivariable logistic regression analysis of survival to hospital discharge for complete case analysis.

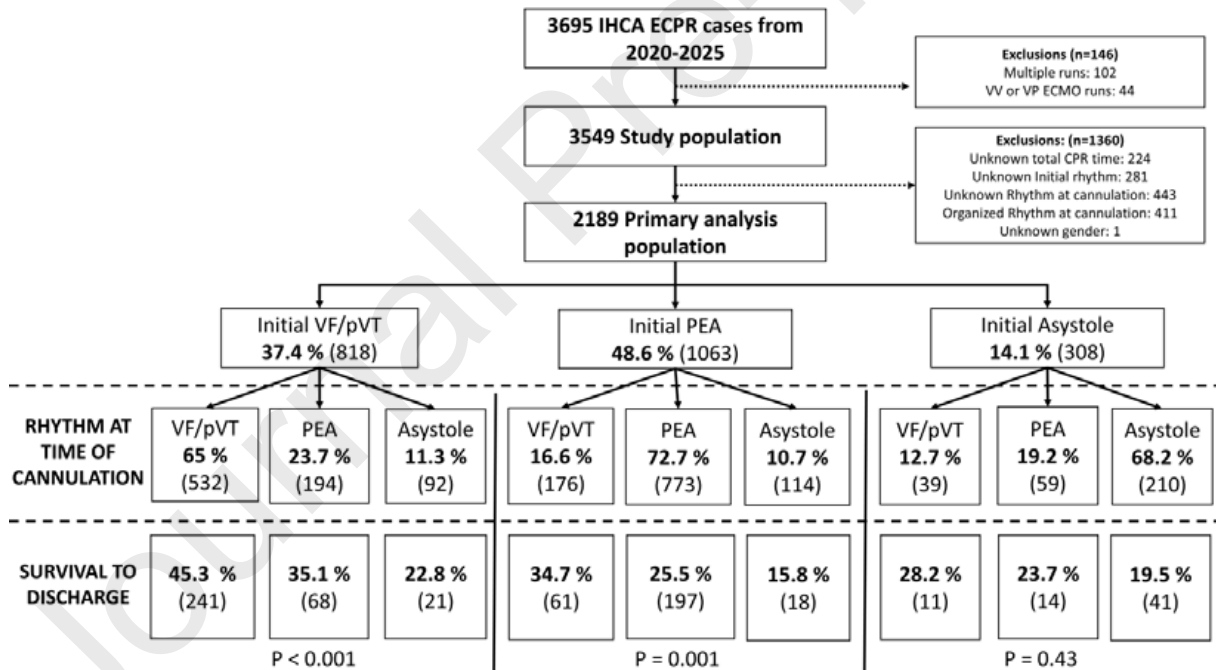
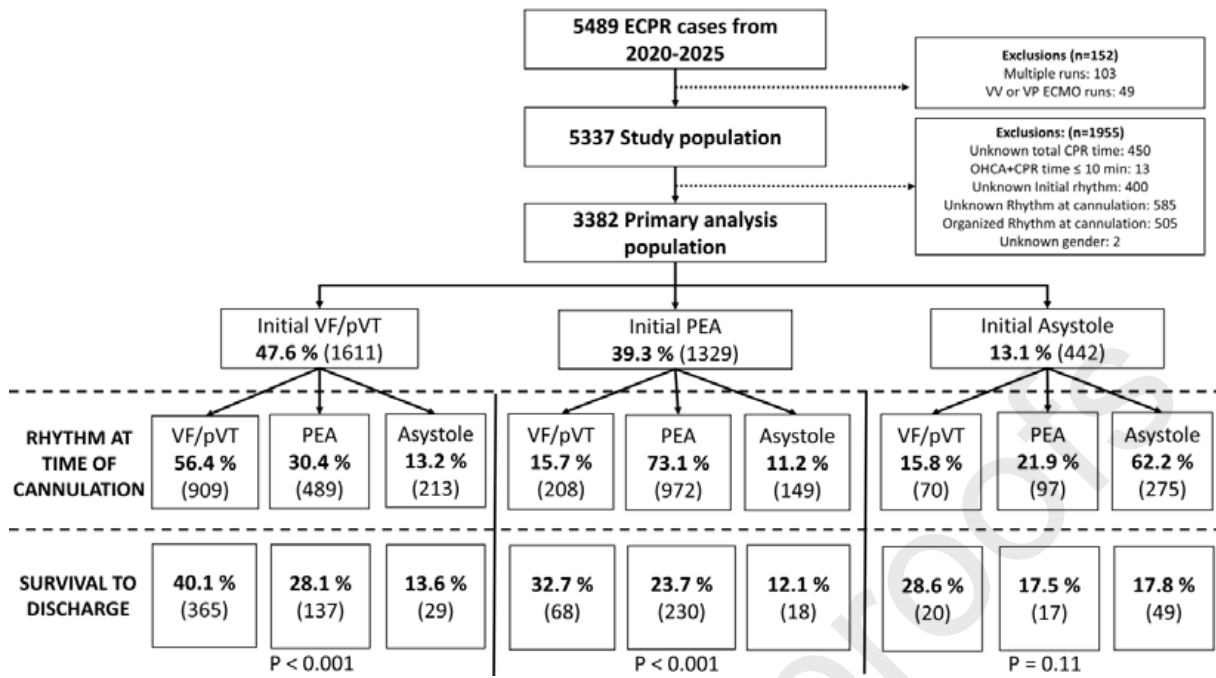
Factor	Odds Ratio	95 Confidence interval	P value
Age (+ 1 year)	0.99	(0.98; 0.99)	<0.001
Gender (male)	0.94	(0.79; 1.12)	0.479
Witnessed status (yes)	1.24	(0.89; 1.75)	0.221
Place of arrest (vs. IHCA)			
- OHCA-home	0.48	(0.36; 0.63)	<0.001

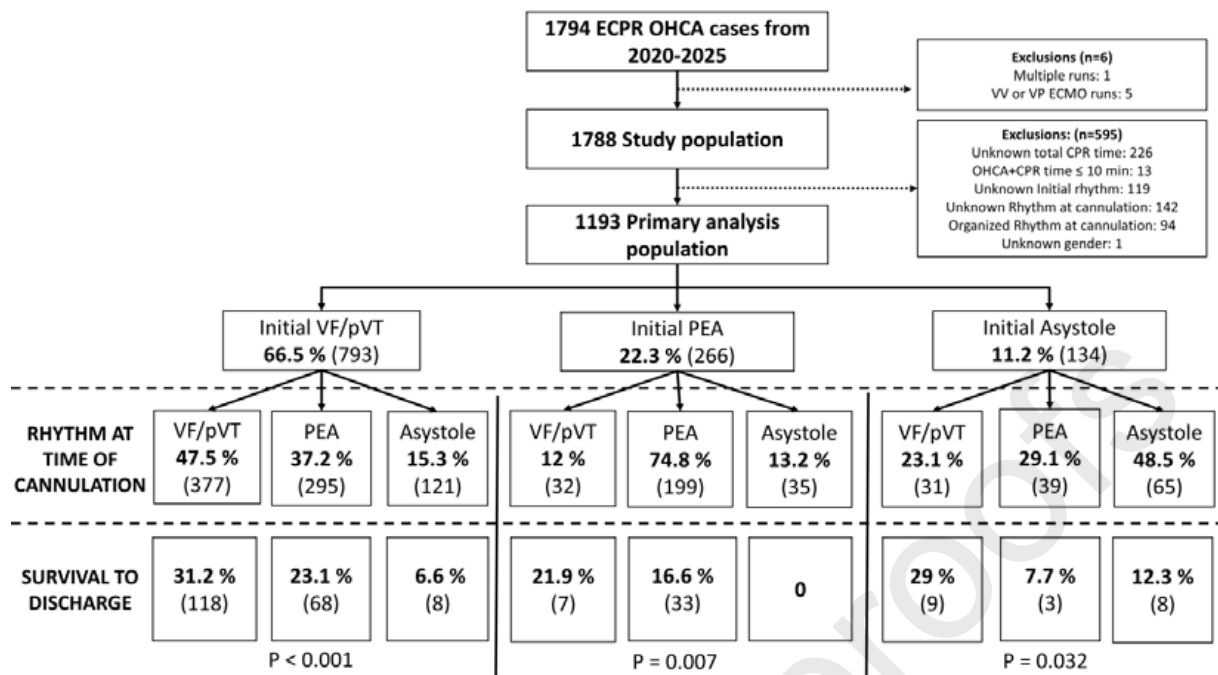
- OHCA-public	0.77	(0.59; 1.00)	0.052
- OHCA other *	1.07	(0.75; 1.51)	0.707
Initial Rhythm (vs. VF/VT)			
- PEA	0.59	(0.48; 0.71)	<0.001
- Asystole	0.64	(0.47; 0.86)	0.003
Rhythm at cannulation (vs. VF/VT)			
- PEA	0.76	(0.54; 1.07)	0.112
- Asystole	0.61	(0.38; 0.99)	0.044
Total CPR time	Figure 2**	Figure 2**	<0.001

* Ambulance transport/Ambulatory Medical Care/Other unspecified.

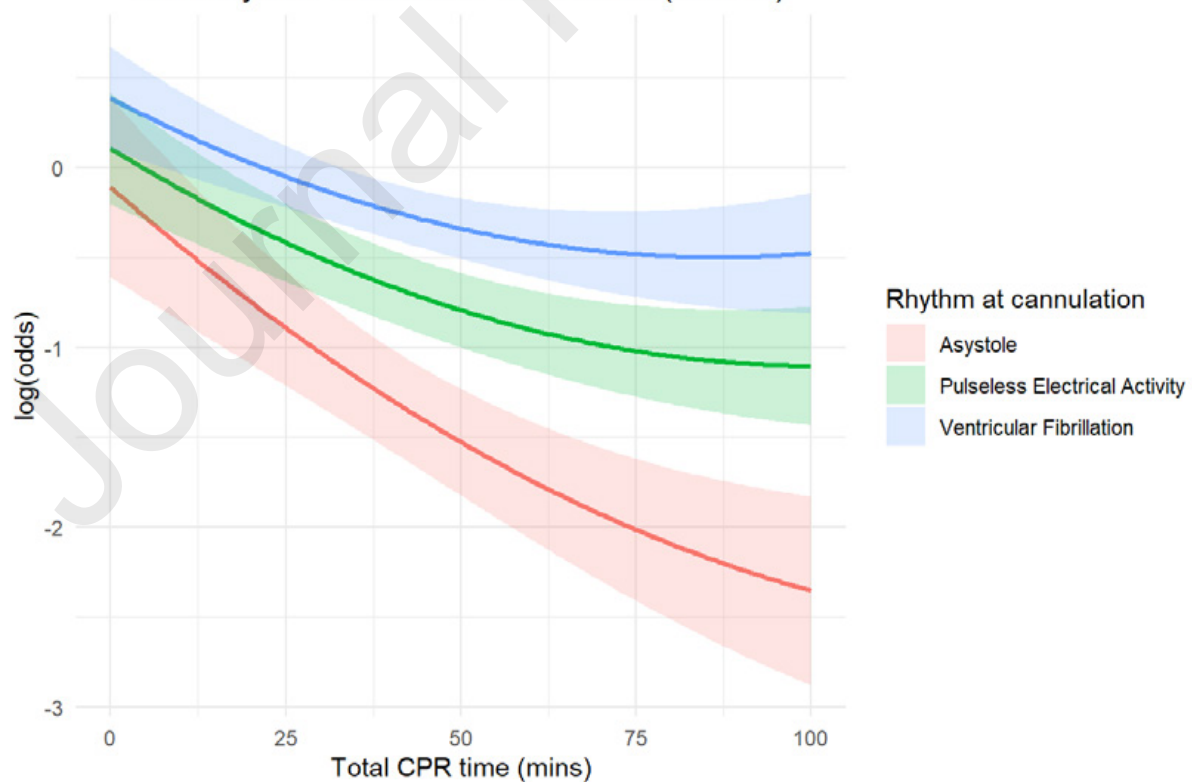
** The influence of CPR time on survival of discharge is presented in Figure 2 and is different for each time period and rhythm at time of cannulation.

Abbreviations: CPR: cardiopulmonary resuscitation, IHCA: in-hospital cardiac arrest, IQR: interquartile range, OHCA: out-of-hospital cardiac arrest, PEA: pulseless electrical activity, VF: ventricle fibrillation, VT: ventricle tachycardia.

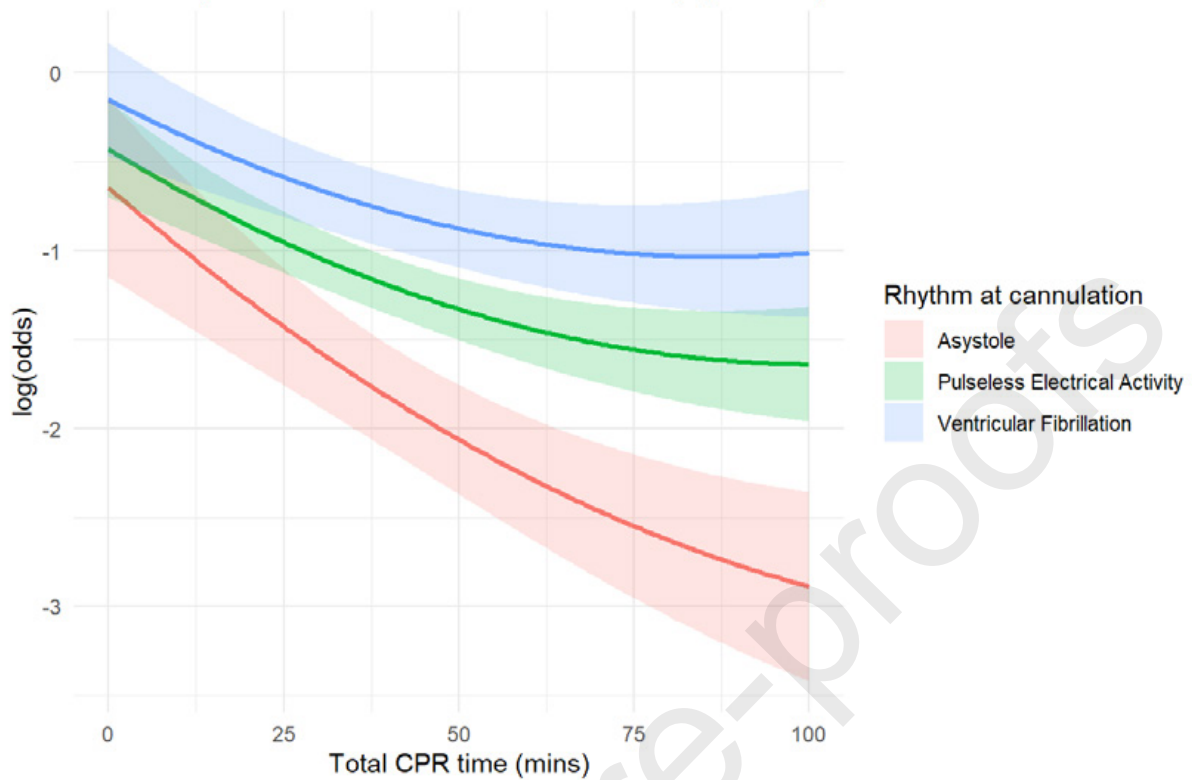




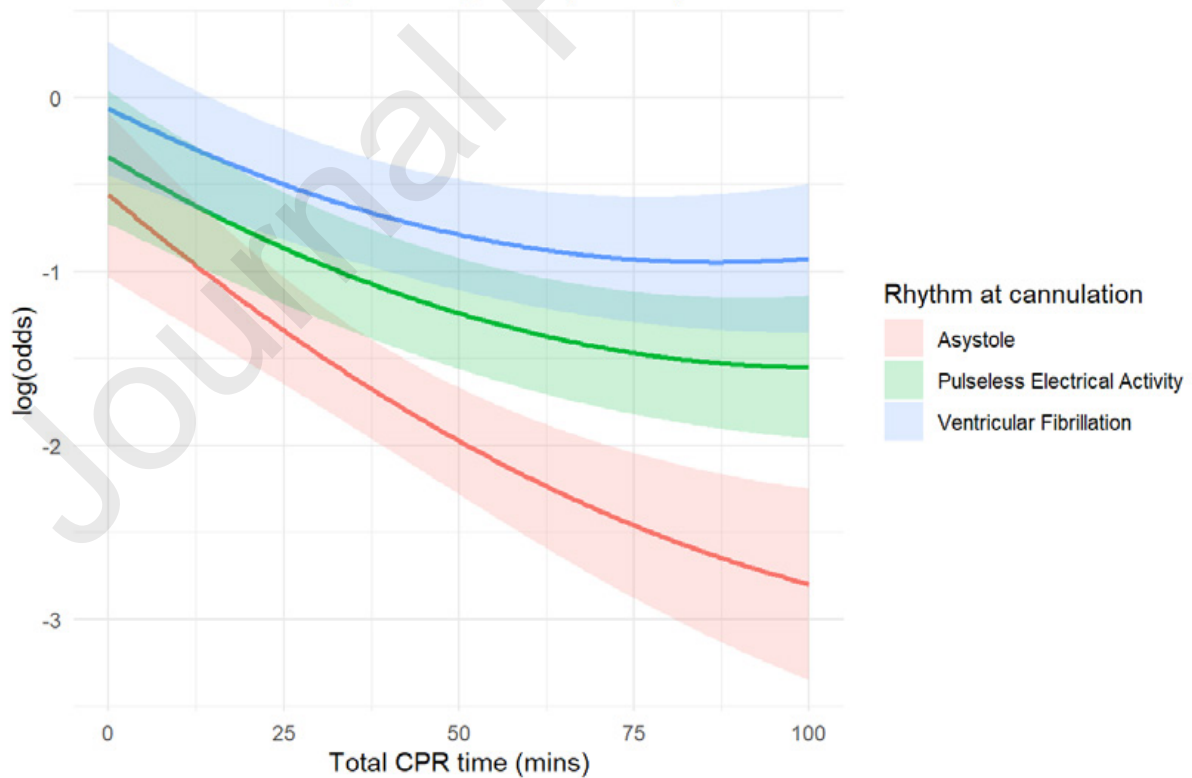
Effect of Total CPR time on survival at discharge
Initial rhythm: **Ventricular Fibrillation** (95% CI)



Effect of Total CPR time on survival at discharge
Initial rhythm: **Pulseless Electrical Activity** (95% CI)



Effect of Total CPR time on survival at discharge
Initial rhythm: **Asystole** (95% CI)



Competing interests

The first author (DR) has received lecture honoraria from Abiomed. The corresponding author (JB) has received lecture honoraria from the Abiomed, Getinge, Xenios, Resuscitec, Novartis, Astra-Zeneca, Boehringer-Ingelheim. The remaining authors report no conflict of interest.

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OPEN The impact of pre-existing aortic stenosis and mitral regurgitation on patients with acute myocardial infarction

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The prevalence of left-sided valvular heart disease (VHD) increases with age, but data on the impact of pre-existing VHD in patients with acute myocardial infarction (AMI) are limited. We aimed to define the clinical characteristics and outcomes of AMI patients with pre-existing left VHD. The analysis is based on data from three merged national registries. The dataset included 47,436 patients admitted with AMI over a 5-year period at all Cath Labs nationwide. Pre-existing VHD was diagnosed in 1,445 patients (3.0%), moderate-to-severe mitral regurgitation (MR) in 510 patients (35.3%), and moderate-to-severe aortic stenosis (AS) in 869 patients (60.1%). Patients with VHD had worse baseline characteristics, pre-existing coronary artery disease, more complicated in-hospital course with higher Killip class, lower left ventricular ejection fraction, and more comorbidities. Angiographically more frequent left main stenosis, TIMI flow 3 before PCI, less frequent stent implantation. Patients with pre-existing VHD had significantly higher 7-day (10.1% vs. 4.5%, $p < 0.001$), 30-day (16.0% vs. 7.0%, $p < 0.001$) and 1-year mortality (28.7 vs. 12.7%, $p < 0.001$) compared to patients without. Conclusions. Patients with pre-existing VHD and AMI are characterized by complicated in-hospital course with higher Killip class, lower ejection fraction, angiographically less severe stenosis, TIMI flow 3 prior to PCI, and less frequent stent implantation. This is a high-risk group with higher short – and long-term mortality and earlier intervention should be considered.

Abbreviations

AMI	Acute myocardial infarction
AS	Aortic stenosis
CAD	Coronary artery disease
LVEF	Left ventricular ejection fraction
MR	Mitral regurgitation
TIMI	Thrombolysis in Myocardial Infarction
VHD	Valvular heart disease

Acute myocardial infarction (AMI) remains the leading cause of mortality and morbidity worldwide, despite substantial improvements in management strategies over the past decades¹. Aortic stenosis (AS) and mitral

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regurgitation (MR) are the most common acquired valvular heart disease (VHD) in developed countries², and their prevalence is expected to nearly double by 2030³. The strong association between coronary artery disease (CAD) and VHD has been demonstrated in a number of large population-based studies^{4–7}. The left-sided VHD and CAD share common risk factors (smoking, dyslipidemia, diabetes and arterial hypertension) and underlying pathophysiological mechanisms (endothelial dysfunction, lipid deposition, inflammation)^{8,9}. Pre-existing VHD is more common in AMI patients than in the general population, with AS ranging from 2.7 to 16% and MR from 2.4 to 13.2% in AMI patients^{10,11}. VHD and AMI mutually promote a series of pathophysiological changes that lead to progression of VHD and worse outcome in AMI. Cardiac remodeling in AMI, left ventricular hypertrophy with increased myocardial oxygen demand, elevated interventricular pressure, microvascular compression, arteriolar remodeling and fibrosis, limit adequate coronary perfusion, resulting in an oxygen demand/supply mismatch and ischemia¹². In addition, AMI affects valvular changes with increased collagen production, thickening and remodeling of the aortic and mitral valves, leading to progression of AS and MR^{13,14}. With population growth, aging and increasing prevalence of cardiovascular risk factors, the global number of patients with AMI and VHD is expected to increase substantially^{2,3}. This analysis aims to investigate the prevalence and prognostic impact of pre-existing left VHD in patients with acute myocardial infarction.

Methods

Study population

Patients who underwent cardiac catheterization for AMI in the Czech Republic between 1 January 2017 and 31 December 2021 were included. Patients who underwent cardiac catheterization for diagnoses other than AMI were not included in the study. Demographic and clinical data at baseline were collected from the prospective national multicenter registry, which collects data on all cardiac catheterizations performed in the country. The medical reports contain demographic, clinical, electrocardiographic and angiographic characteristics, including complications. Enrolled patients with myocardial infarction were screened for the presence/absence of pre-existing significant left VHD, defined as moderate-to-severe and severe AS or MR. Data were collected from the National Registry of Reimbursed Health Services using ICD-10 CM codes - I35.0, I35.2 for AS and I34.0 for MR in one of the secondary diagnosis fields. The study endpoint was 30-day all-cause mortality. Mortality data were based on the National Death Registry.

Statistical analysis

Standard descriptive statistics were used in the analysis: absolute and relative frequencies for categorical variables and means with standard deviations for continuous variables. The statistical significance of differences between patient groups was tested using the maximum likelihood chi-squared test for categorical variables and the t-test for continuous variables. The Kaplan-Meier method was used to visualize the overall survival data; the Cox proportional hazards model was used to analyze the association between VHD and overall patient survival and to describe it using hazard ratios and their 95% confidence intervals. Overall survival was analyzed both since catheterization and after 30 days using the landmark analysis approach. The association between VHD, patient characteristics and the endpoint of 30-day all-cause mortality was analyzed using logistic regression and adjusted for potential confounders in the multivariate model. Analyses were performed using SPSS software (version 28.0.1.1.), and $p = 0.05$ was used as the level of statistical significance in all analyses.

Results

Prevalence and characteristics

A total of 48,881 patients with AMI were enrolled during the 5-year study period (2017–2021) in the Czech Republic. The 1,445 patients (3%) with significant AS or MR were assigned to the “VHD” group. The predominant valvular disease in the VHD group was AS, which was observed in 869 patients (60.1%). Within each group, the demographic, clinical and angiographic characteristics of the patients and their outcomes were compared with those of the non-VHD patients. Baseline characteristics are shown in **Table 1**. Based on information on electrocardiographic patterns, non-ST-elevation MI was a more frequent manifestation in the VHD group. Patients in the VHD group were older and more likely to be female. The prevalence of both AS and MR increased with age. Patients in the VHD group were also more likely to have diabetes, chronic renal failure, a history of previous ischemic heart disease or revascularization. On admission, VHD patients had a higher prevalence of mild to moderate heart failure and pulmonary edema (Killip > 2), and a higher incidence of left ventricular dysfunction with a higher proportion of left ventricular ejection fraction (LVEF) < 30%, compared to the non-VHD group. In terms of coronary intervention, the VHD group included a greater extent of coronary artery disease with multivessel and left main disease. The AMI patients with VHD had a higher proportion of Thrombolysis in Myocardial Infarction (TIMI) flow 3 prior to percutaneous coronary intervention and more frequent $\leq 80\%$ stenosis in the infarct-related coronary artery. Among the patients with VHD, the presence of AS was independently associated with more severe heart failure (Killip 3 and 4) and a greater use of mechanical ventilation. (**Table 2**)

Short- and long-term outcome

In-hospital and 30-day all-cause mortality was nearly double in patients with VHD compared to those without, 8.3% vs. 4.5% and 13.4% vs. 7%, respectively, $p < 0.01$ for all. In the VHD group, patients with significant AS had a higher in-hospital and 30-day mortality rates than did patients with significant MR, 10.1% vs. 5.7% and 16.0% vs. 9.4%, respectively, $p < 0.01$ for all. The adjusted risk for patients with pre-existing AS was high for both short- and long-term mortality (odds ratio (OR) 1.80; 95% confidence interval (CI) 1.51–2.13) and (OR 1.63; 95% CI 1.47–1.81), respectively $p < 0.01$ for all. (**Table 3**) In multivariate analysis, MR did not significantly increase the

		Non-VHD	VHD	p^1	MR	p^1	AS	p^1	p^2
		47,436	1,445		510		869		
Female gender, %		28.9	39.6	< 0.01	49.2	< 0.01	33.6	< 0.01	< 0.01
Age, mean ($\pm$ SD), yrs.		65.6 ($\pm$ 12.5)	73.9 ($\pm$ 10.6)	< 0.01	72.4 ($\pm$ 11.1)	< 0.01	74.7 ($\pm$ 10.1)	< 0.01	< 0.01
Age, yrs. %	< 40	1.8	0.6	< 0.01	0.6	0.04	0.7	< 0.01	1.00
	40–49	9.7	2.9	< 0.01	5.5	< 0.01	1.4	< 0.01	< 0.01
	50–59	19.4	5.6	< 0.01	5.5	< 0.01	5.8	< 0.01	0.91
	60–69	28.9	18.8	< 0.01	20.6	< 0.01	17.6	< 0.01	0.18
	70–79	26.2	40.6	< 0.01	40.6	< 0.01	40.6	< 0.01	1.00
	$\geq$ 80	14.0	31.5	< 0.01	27.3	< 0.01	33.9	< 0.01	0.01
AMI type, %	STEMI	57.7	41.2	< 0.01	44.1	< 0.01	39.1	< 0.01	0.12
	NSTEMI	41.3	58.9	< 0.01	55.9	< 0.01	60.9	< 0.01	0.07
Diabetes mellitus, %		20.0	27.6	< 0.01	22.7	0.13	30.1	< 0.01	< 0.01
Chronic kidney disease, %		5.0	11.9	< 0.01	13.5	< 0.01	11.2	< 0.01	0.20
Previous PCI, %		14.9	23.5	< 0.10	22.2	< 0.01	24.1	< 0.01	0.43
Previous CABG, %		5.3	23.4	< 0.01	16.9	< 0.01	26.4	< 0.01	< 0.01
Killip class,%	Killip 1	81.4	73.4	< 0.01	76.5	< 0.01	71	< 0.01	0.03
	Killip 2	7.7	11.6	< 0.01	10.8	0.01	12.4	< 0.01	0.40
	Killip 3	3.5	7.3	< 0.01	5.5	0.02	8.4	< 0.01	0.05
	Killip 4	5.7	5.9	0.69	4.3	0.21	6.9	0.12	0.05
	missing	1.7	1.8	-	2.9	-	1.3	-	-
OHCA, %		8.5	8.4	0.84	8.6	0.95	8.1	0.66	0.76
Mechanical ventilation, %		9.0	9.9	0.24	7.8	0.39	11.3	0.02	0.04
LVEF*, %	>50%	49.6	44.5	< 0.01	42.3	0.02	45.9	0.11	0.33
	30–50%	41.4	43.0	0.35	43.0	0.58	42.4	0.65	0.88
	<30%	9.0	12.5	< 0.01	14.7	< 0.01	11.7	0.05	0.26
Number of diseased vessels, %	1-VD	45.1	37.2	< 0.01	37.8	< 0.01	37.2	< 0.01	0.82
	2-VD	29.6	28.3	0.28	28.8	0.73	28.9	0.65	1.00
	3-VD	22.6	30.6	< 0.01	28.6	< 0.01	30.4	< 0.01	0.50
Infarct related coronary artery, %	LMCA	4.0	8.4	< 0.01	4.9	0.31	10.4	< 0.01	< 0.01
	RIA	44.8	44.2	0.67	44.1	0.79	44.3	0.78	0.96
	RCx	25.0	26.0	0.39	23.3	0.41	27.5	0.10	0.10
	ACD	35.2	30.9	< 0.01	34.3	0.71	29.1	< 0.01	0.05
Stenosis before PCI, %	$\leq$ 80%	13.1	18.8	< 0.01	16.3	0.04	20.0	< 0.01	0.09
	81–90%	21.5	25.9	< 0.01	25.5	0.03	26.7	< 0.01	0.66
	91–99%	26.7	27.0	0.79	27.5	0.69	26.0	0.70	0.57
	100,0%	38.8	28.4	< 0.01	30.8	< 0.01	27.3	< 0.01	0.17
TIMI flow before PCI, %	0	37.8	27.2	< 0.01	29.8	< 0.01	26.1	< 0.01	0.15
	1	7.6	8.9	0.09	8.6	0.41	8.9	0.18	0.92
	2	16.6	16.9	0.75	17.8	0.44	16.2	0.81	0.46
	3	38	47.1	< 0.01	43.7	< 0.01	48.8	< 0.01	0.07
Continued									
TIMI flow after PCI, %	< 3	8.2	9.3	0.11	10.4	0.07	8.4	0.81	0.25
	3	91.8	90.7	0.11	89.6	0.07	91.6	0.81	0.25
IABK		0.0	0.0	1.00	0.0	1.00	0.0	1.00	-

	Non-VHD	VHD	<i>p</i> ¹	MR	<i>p</i> ¹	AS	<i>p</i> ¹	<i>p</i> ²
	47,436	1,445		510		869		
ECMO	0.3	0.3	0.79	0.0	0.65	0.5	0.29	0.30
Middle term mech. support	0.1	0.1	1.00	0.0	1.00	0.1	0.55	1.00
Long term mech. support	0.1	0.1	0.57	0.0	1.00	0.1	0.40	1.00
MitraClip	0.1	0.1	1.00	0.2	0.39	0.0	1.00	0.37
TAVI	14.0	20.8	< 0.01	16.5	0.11	23.2	< 0.01	< 0.01

Table 1. Baseline and angiographic characteristics. ACD, arteria coronaria dextra; AS, aortic stenosis; CABG, coronary artery bypass grafting; CKD, chronic kidney disease; CPR, cardiopulmonary resuscitation; LMC, left main coronary artery; LVEF, left ventricular ejection fraction; MR, mitral regurgitation; NSTEMI, non-ST elevation myocardial infarction; OHCA, out of hospital cardiac arrest; PCI, percutaneous coronary intervention; RIA, ramus interventricularis anterior; RCx, ramus circumflexus; SD, standard deviation; STEMI, ST-elevation myocardial infarction; TIMI, thrombolysis in myocardial infarction; VD, vessel disease; VHD, valvular heart disease;. * Information on LVEF is available in 53–58% of patients, results are computed only for patients with available data. * *p*1 represents the comparison between non-VHD and each given VHD type, *p*2 represents the comparison between MR and AS.

		Mechanical ventilation		Killip > 2		Killip 3–4	
Predictor	N	OR (95%CI)	<i>p</i>	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>
NonVHD	47.436	reference					
MR	510	0.86 (0.62;1.19)	0.37	1.30 (1.04;1.61)	0.02	1.09 (0.81;1.46)	0.56
AS	869	1.29 (1.04;1.59)	0.02	1.88 (1.62;2.19)	< 0.01	1.78 (1.48;2.15)	< 0.01

Table 2. Valvular heart disease as risk factors of mechanical ventilation and heart failure in MI patients. AS, aortic stenosis; MR, mitral regurgitation; VHD, valvular heart disease.

risk of 30-day mortality after adjustment for covariates and potential confounders, but remained independently associated with long-term all-cause mortality (OR 1.21; 95% CI 1.04–1.40), *p* < 0.01. (Table 3) The Kaplan-Meier survival curves showed early divergence of the curves among the groups with AS, MR and non-VHD, with significantly higher 30-day mortality in AS, followed by MR and non-VHD (Fig. 1.). The difference between the AS and MR survival curves becomes smaller in a long-term period (Fig. 2) and the landmark analysis after 30 days demonstrates the later divergence of AS and MR curves (Fig. 3).

Discussion

The vast majority of studies report about valvular complications after AMI. We have analyzed patients with pre-existing significant VHD and have described that they are not uncommon in AMI patients, with a prevalence of about 3% and a tendency to increase with age. Published studies report VHD in 4.8% of ACS patients¹⁰, rising up to approximately 20% of ACS patients in the elderly population > 74 years of age¹¹ Unlike the aforementioned studies in selected population^{10,11}, our study analyzed a nationwide all-comers registry of AMI patients, which may explain the differences in prevalence. With the population growth, aging and increasing cardiovascular risk factors, pre-existing VHD in AMI is a rising problem.

The presence of VHD in AMI is associated with more severe course of the disease and outcome. According to our data, these patients are older, have a higher risk profile, have more comorbidities, they more often present with mild to moderate heart failure and pulmonary edema, with a lower LVEF, with a wider extent of coronary artery disease with multivessel and left main disease, they have more complicated in-hospital course with advanced stages of heart failure including cardiogenic shock and acute multiorgan failure, which is consistent with other published data^{10,11,15} Both AS and MR increase the workload on the left ventricle, lead to cardiac remodeling, and contribute to pulmonary congestion and dyspnea. In the inpatient setting, these conditions can progress rapidly to acute decompensated heart failure, requiring intensive management and possibly surgical intervention to prevent further deterioration. The presence of pre-existing significant VHD adversely affects the prognosis of AMI patients, leading to an almost twofold increase in in-hospital and 30-day mortality rate compared to AMI patients without VHD. The higher risk of cardiovascular mortality, three times higher risk of composite of all-cause death, AMI, stroke and rehospitalization for heart failure was reported for AMI patients with VHD in the study by Crimi et al.¹¹ According to our analysis the presence of AS is independently associated with worse prognosis and have higher short- and long-term mortality compared to the AMI patients with MR or AMI patients without VHD. As in Kaplan-Meier analysis, the mortality curve of AS separated early in the 30-day analysis and had a later divergence with MR curve in landmark analysis after 30 days, suggesting that the presence of AS in AMI patients has the greatest negative impact on the outcome of these patients during the first 30 days. This raises the question of whether earlier intervention in patients with significant AS leads to a better outcome of AMI patients. Our data provide a message that significant AS is not only independently associated

Factor		30 days		Long-term	
		OR (95%CI)	<i>p</i>	OR (95%CI)	<i>p</i>
MR		1.01 (0.82–1.46)	0.53	1.21 (1.04–1.40)	0.01
AS		1.80 (1.51–2.13)	< 0.01	1.63 (1.47–1.81)	< 0.01
Age		1.04 (1.04–1.05)	< 0.01	1.05 (1.05–1.05)	< 0.01
Woman		1.01 (1.02–1.18)	0.01	1.06 (1.02–1.11)	< 0.01
STEMI		1.44 (1.33–1.56)	< 0.01		
Diabetes mellitus		1.23 (1.13–1.32)	< 0.01	1.29 (1.23–1.35)	< 0.01
Chronic kidney disease		1.04 (0.93–1.17)	< 0.01	1.35 (1.27–1.44)	< 0.01
Previous PCI				1.05 (1.01–1.10)	0.05
Previous CABG		0.84 (0.73–0.96)	< 0.01	1.13 (1.05–1.21)	< 0.001
Killip class	Killip 1	Reference			
	Killip 2	0.55 (0.40–0.75)	< 0.01	1.72 (1.62–1.82)	< 0.01
	Kilip 3	1.41 (1.02–1.93)	0.04	2.05 (1.90–2.22)	< 0.01
	Kilip 4	1.71 (1.23–2.36)	0.01	3.74 (3.48–4.03)	< 0.01
OHCA		2.12 (1.85–2.45)	< 0.01	1.65 (1.53–1.78)	< 0.01
Mechanical ventilation		1.93 (1.76–2.12)	< 0.01	1.13 (1.04–1.22)	< 0.01
No. of diseased vessels	1 VD	Reference			
	2 VD	1.06 (0.97–1.16)	0.21	1.10 (1.05–1.16)	< 0.01
	3 VD	1.24 (1.14–1.36)	< 0.01	1.30 (1.23–1.36)	< 0.01
LVEF	> 50%	Reference			
	30–50%	1.81 (1.57–2.08)	< 0.01	1.48 (1.40–1.57)	< 0.01
	< 30%	3.22 (2.77–3.75)	< 0.01	2.40 (2.22–2.59)	< 0.01
LMC stenosis > 50%		1.35 (1.22–1.48)	< 0.01	1.20 (1.13–1.28)	< 0.01

Table 3. Multivariate model for short- and long-term all-cause mortality of MI patients. AS, aortic stenosis; CABG, coronary artery bypass grafting; CPR, cardiopulmonary resuscitation; LMC, left main coronary artery; MI, myocardial infarction; MR, mitral regurgitation; TIMI, thrombolysis in myocardial infarction; VD, vessel disease; VHD, valvular heart disease.

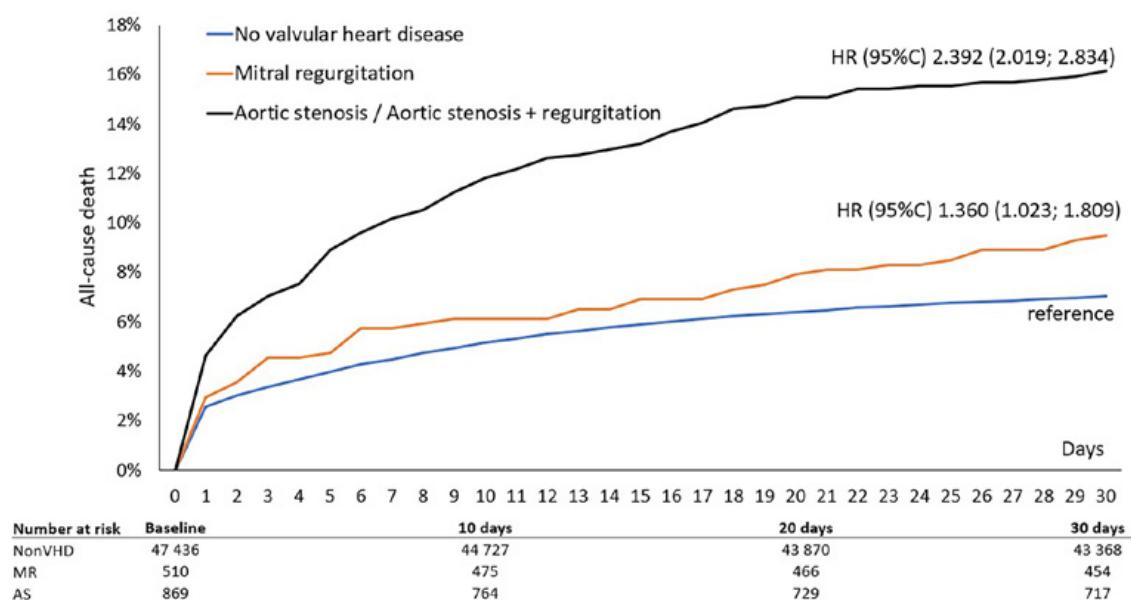


Fig. 1. Thirty days survival according to valvular heart disease.

with more severe heart failure and greater need for mechanical ventilation, but is also associated with mortality risk even after adjustment for possible covariates. Therefore, these patients must be treated with extreme caution.

The worse prognosis and survival of patients with AS is caused by the underlying pathophysiological mechanisms of AS, which result in oxygen demand-supply mismatch and ischemia, but also by the comorbidities and older age of these patients. Moreover, the acute ischemic event itself significantly enhances the progression

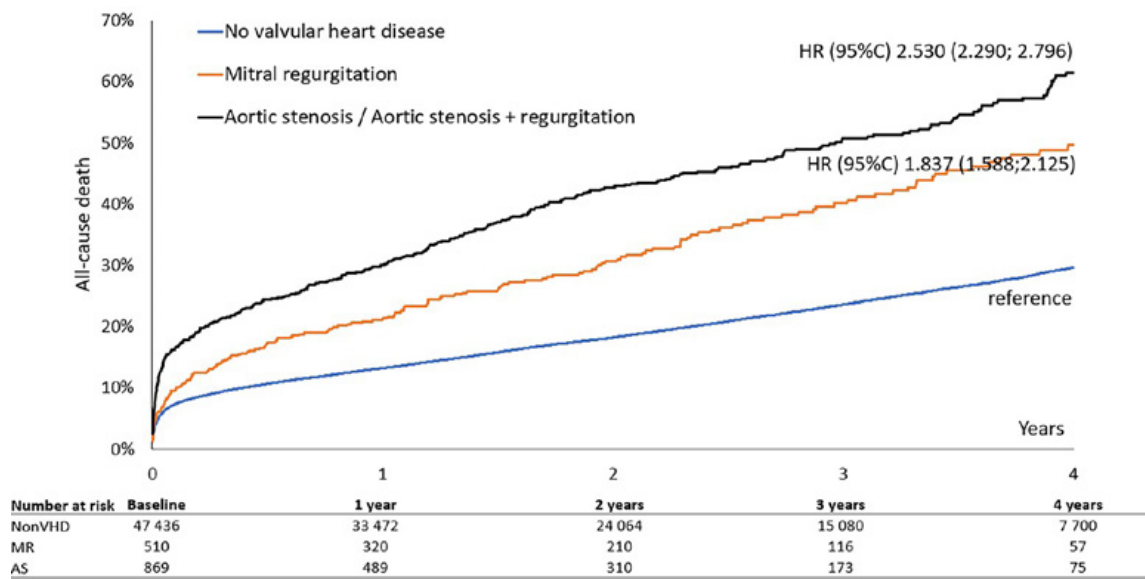


Fig. 2. Long-term survival according to valvular heart disease.

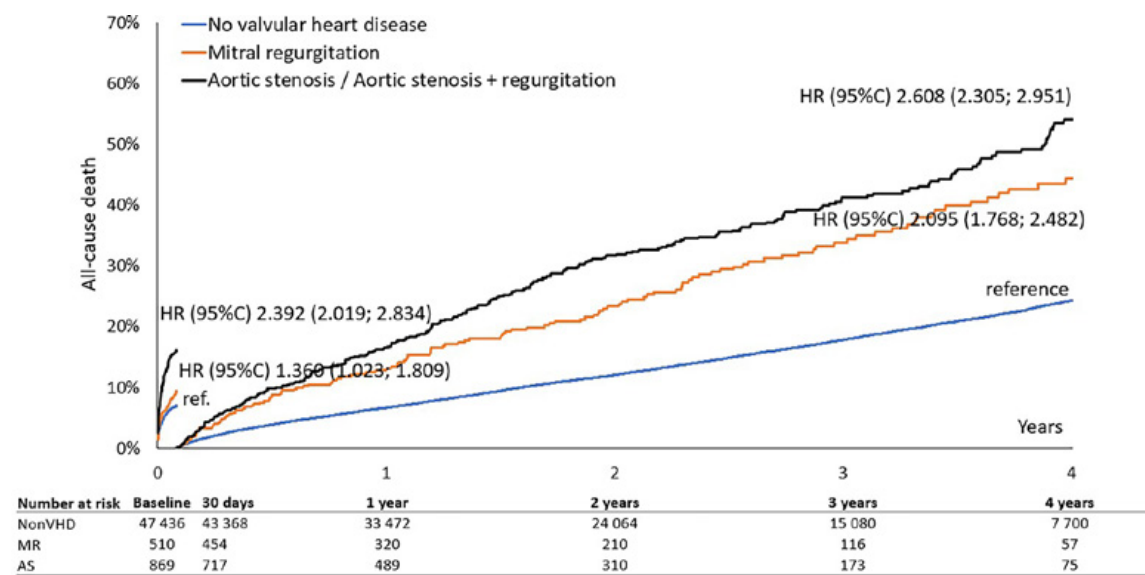


Fig. 3. Survival until 30 days and long-term survival since 30 days according to valvular heart disease – landmark analysis.

of AS and MR. The worsening of AS severity develops more rapidly shortly after AMI, and a decrease in the rate of AS progression has been observed in the later period of a few years after AMI¹³ While pre-existing VHD is a significant factor contributing to adverse outcomes in these patients, the co-occurrence of AS and CAD often increases with age. As patients age, the prevalence of other comorbidities such as CKD and DM also increase, further complicating prognosis and impacting patient outcomes. The presence of these additional risk factors, along with left-sided VHD, should be carefully considered when assessing prognosis and planning treatment strategies. Given the complexity of managing patients with both pre-existing left-sided VHD and multiple comorbidities, a multidisciplinary approach is critical. Coordination between cardiologists, nephrologists, endocrinologists, and other healthcare providers is essential to address the full spectrum of patient needs, optimize treatment, and improve clinical outcomes.

Interestingly, according to our data, MR did not significantly increase the short-term mortality in multivariate analysis, but remained independently associated with long-term mortality even after adjustment for covariates. The progression of VHD after AMI may be explained by the post-AMI valvular remodeling caused by the neurohumoral activation, including the renin-angiotensin-aldosterone system, activation of fibrotic pathways, and increased collagen production and valve thickness^{13,16,17} Therefore, it is important to assess the progression

of VHD severity and the need for valve intervention even shortly after MI and to continue in long-term follow-up. These patients need to be followed in hospitals with Heart teams and with facilities for surgical and transcatheter valve interventions.

Conclusion

Preexisting significant VHD modifies the risk profile, presentation, and prognosis of patients with AMI. Patients with AS have the worst prognosis, which may indicate the need for earlier planning of valvular intervention, especially in those with current coronary disease.

Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

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Author contributions

TM – analysis and interpretation of data, draft of the manuscript, ZM – the conception and design of the study, analysis and interpretation of data, revision of the manuscript, JJ – statistical analysis, methodology part, graphics and tables, OH, PK, MH, TV, JM, MS, MH, RP, PT, OI, PL, AC data acquisition and revision of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Conflict of interest statement

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.

Ethics approval and consent to participate

According to Czech legislation, all hospitals are mandated to report data on patients undergoing coronary interventions to the National Registry of Coronary Interventions (NRKI), which is part of the National Health Information System, as established by § 70 paragraph 1 of Act No. 372/2011 Coll. on health services and conditions of their provision (Health Services Act). Due to this legal obligation, the need to obtain informed consent was waived, and separate ethics committee approval was not required. All data were fully anonymized before analysis, and the study was conducted in accordance with relevant guidelines and regulations. The investigation conforms with the principles outlined in the *Declaration of Helsinki*.

Additional information

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P. Grenar et al.

Effect of point-of-care echocardiography by noncardiologists on patient management in acute chest pain

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Effect of point-of-care echocardiography by noncardiologists on patient management in acute chest pain

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Abstract

As point-of-care echocardiography (POCE) has expanded beyond cardiology into general emergency medical practice, there has been a lack of standardized, evidence-based imaging protocols and training, thus leading to challenges with respect to the quality of cardiac imaging for acute cardiovascular syndromes. The ENDEMIC study aims to assess the effectiveness of POCE based on structured training for noncardiologists on the management of patients with acute chest pain in emergency departments. A total of 150 patients presenting with acute chest pain possibly of cardiovascular aetiology were enrolled in this prospective randomized clinical trial. Patients were assigned to either the POCE-assisted management group or the standard management group. Physicians performing POCE examinations received focused training based on the British Society of Echocardiography level 1 standard. The primary outcome was the length of stay in the emergency department (ED), and the secondary outcomes included the time to decision, the time to coronary angiography and diagnostic accuracy of the initial examination. Quality of acquired images was also evaluated. The results revealed that the POCE group had a significantly shorter length of ED stay (209.0 vs. 271.0 min, median difference 64 (31–97) min., $p=0.0003$), time to decision (138.0 vs. 252.0 min, $p<0.0001$) and time to coronary angiography (29.6 vs. 120.3 h, $p=0.027$) than the control group. Furthermore, the error of initial diagnoses was lower in the POCE group (6.3% vs. 30.4%, $RR=0.21$ [0.047–0.90]). The implementation of POCE by trained noncardiologists significantly reduced the duration of ED stay and enhanced the management of patients with acute chest pain in the ED. Systematic training enables physicians without prior echocardiography experience to perform POCE accurately and efficiently. Preregistered number of clinical trial: NCT05306730 registered 2022-04-01.

Keywords Point-of-care echocardiography · Acute chest pain · Education · Emergency medicine

Petr Grenar and Martin Jakl are lead authors and contributed equally to the paper.

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Introduction

In the past, echocardiography was performed exclusively by dedicated specialists. Today, physicians from a variety of specialties, including emergency medicine, intensive care, anaesthesiology and even primary care, perform bedside echocardiography. This shift has been driven by the advent of compact, easy-to-use ultrasound devices that have made it easier for non-specialists to perform cardiac imaging. Although point-of-care ultrasound has emerged over the past three decades, its growth may have accelerated since 2020, likely due to the COVID-19 pandemic [1–3].

Point-of-care echocardiography (POCE) is defined as the acquisition, interpretation and immediate clinical integration of echocardiographic images performed by clinicians at the patient's bedside, rather than by a radiologist or cardiologist [4]. The different levels of cardiac ultrasound can be

categorised according to the extent of the examination [5, 6] (Fig. 1). The current study corresponds to a detailed FOCUS examination according to the scheme.

One of the key uses of POCE is to diagnose patients with acute chest pain [7, 8]. Acute chest pain is one of the most common yet challenging presentations in emergency clinical practice, requiring immediate and accurate diagnosis and management. In the context of emergency medicine, where time is of the essence, POCE has emerged as a pivotal diagnostic tool beyond the traditional confines of cardiology departments. The use of POCE by trained non-cardiologists has begun to revolutionise patient assessment, providing a real-time view of cardiac function at the bedside. However, there is little evidence on the optimal scope of the examination and the training of the examiner. A key question remains: Can structured training in POCE for non-cardiologists significantly improve the speed and quality of patient care? And at what cost?

Aims of the study

The study “Echocardiography by Non-cardiologist in Early Management of Patients With Chest Pain (ENDEMIC)” aims to assess the effect of controlled training of non-cardiologists on the use of POCE in the management of patients with acute chest pain. The primary objective was to assess the impact of POCE on the length of stay in the emergency department (ED). The primary safety endpoint was the occurrence of major adverse cardiovascular events (MACE) within 30 days of enrolment. Secondary objectives were to

evaluate the impact of POCE on the time to decision, the time to coronary angiography and to assess the diagnostic accuracy of the initial examination. Quality of acquired images was also evaluated.

Methods

We conducted a prospective, randomised cohort study at the Department of Emergency Medicine and First Department of Medicine—Cardioangiopathy, University Hospital Hradec Kralove, Czech Republic (ClinicalTrials.gov NCT05306730). The study protocol was approved by the Ethics Committee and was conducted in accordance with the Declaration of Helsinki. All patients provided informed written consent.

Training on point-of-care echocardiography

The training programme was described in detail previously [9]. The training program enrolled four physicians who had not undergone systematic training in echocardiography (including self-education) and had performed examinations up to the level of ultrasound-augmented physical examination. The British Society of Echocardiography (BSE) Level 1 protocol [10] was chosen as the basis for determining the scope of the training. The following modalities were added to this training: diameter of ascending aorta, colour flow mapping of aortic and mitral valve in short axis projection and measurement of tricuspid valve gradient. This additional training was added to the original protocol to cover common

Cardiac ultrasound categories					
Point-of-care ultrasound				Consultative ultrasound	
	UAPE	FOCUS	CCE	Limited ECHO	Comprehensive ECHO
Diagnostic expectations	„US stethoscope“ Used to augment bedside examination	Focused exams with specific imaging protocols based upon suspicion of specific disease	Focused on a collection of specific views/findings pertinent to the care of the critically ill (e.g. Cardiac output, fluid responsive)	Limited imaging protocol applied to answer a specific question	Comprehensive, all findings, use advanced techniques
Quantification	Usually absent	Optional	Typically	Typically	Mandatory
Documentation	Not recorded	Images archived, formal report	Images archived, formal report	Images archived on PACS, formal report	Images archived on PACS, formal report
Teaching required	Weeks	Weeks to months	Months	Months to years	Years
CCE, critical care echocardiography; POCUS, point of care ultrasound, UAPE, ultrasound assisted physical examination, FOCUS – Focused cardiac ultrasound Kimura BJ. Point-of-care cardiac ultrasound techniques in the physical examination: better at the bedside. Heart 2017; 103:987-994					

Fig. 1 Definition of the levels of echocardiographic examination

and serious conditions encountered in the emergency department (e.g. aortic dissection, severe valvular disease and significant pulmonary hypertension).

The training programme consisted of several steps: an introductory course in focused echocardiography, a supervised examination of patients in whom at least 29 predefined findings had to be identified (Supplementum 1) and a final examination of real patient assessed by a specialist with European Association of Cardiovascular Imaging accreditation in transthoracic echocardiography. The total training period was approximately 6 months.

Physicians began performing POCE on study participants immediately after successfully completing the course. To maintain the quality of the examinations, graduates were required to document at least fifty examinations per year. In addition, once a year, graduates spent four hours in the EchoLab under the supervision of a mentor to verify their continuing competence in performing POCE.

Selection of participants

Patients presenting to the ED with chest pain were eligible for the study. Inclusion criteria were as follows: 18 years of age or older; and pain that may have had a cardiovascular aetiology based on the patient's history, physical examination, and ECG findings. Exclusion criteria were as follows: possible ST-elevation myocardial infarction (STEMI), active pacemaker, pregnancy, performance status of 4 on the Zubrod scale, refusal or inability to give informed consent and no suspicion of a cardiovascular aetiology for the

patient's symptoms after initial clinical examination (for example, a young individual with vertebral pathology presenting with acute, precisely localised, palpation-sensitive, and position-dependent pain, accompanied by a normal ECG).

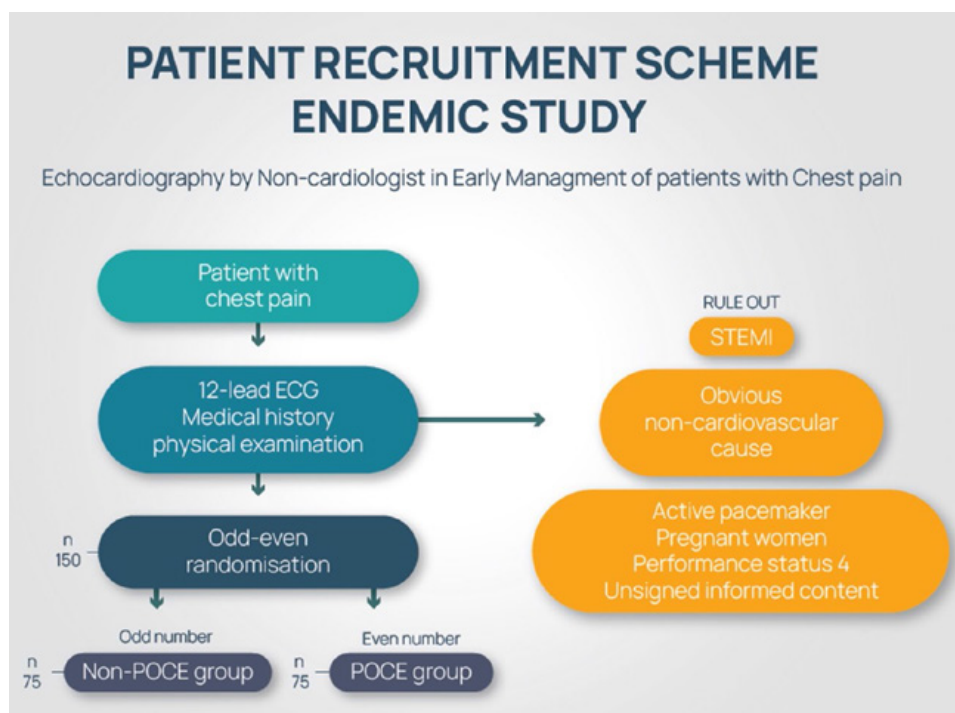
Possible STEMI was defined according to current guidelines as new ST segment elevation of more than 1 mm in two or more anatomically contiguous leads (measured at the J point) in all leads except V2–V3, where the threshold is over 2 mm in men older than 40 years, over 2.5 mm in men younger than 40 years, and over 1.5 mm in women of any age[11]. Such patients were referred immediately for coronary angiography that could not be delayed by the ultrasound examination.

Patients were not excluded on the basis of poor ultrasound visualisation. All patients were followed up for a minimum of 3 months. Patients were randomized in an even–odd manner to a POCE-assisted management group or a standard management group (Fig. 2).

Echocardiography examination

POCE was performed following ECG and standard clinical examination, but no later than 90 min after arrival at the emergency department. It was performed by an operator who had completed the aforementioned training programme. The recommended scope of POCE was based on the BSE Level 1 protocol. However, the examining physician was authorised to extend the scope of the examination if necessary.

Fig. 2 Patient recruitment scheme



All images of the examination were archived in the hospital information system and reviewed post hoc by an EACVI (European Association of Cardiovascular Imaging) accredited echocardiographer and the approved supervisor of the British Society of Echocardiography to assess the accuracy of the examinations performed.

In patients in control group, echocardiography was performed only if required by specific ESC guidelines (in case of 3-h troponine protocol etc.). In that case, the examination was performed by a cardiology fellow.

Outcomes

The primary endpoint of the study was the length of ED stay. The start of the ED stay was defined as the time of the patient's first contact with the ED, regardless of whether the patient was referred by the emergency medical service or self-referred. The end of the ED stay was defined as the moment the patient physically departed the ED, either through hospital admission or discharge home.

The time to decision was defined as the time at which the final decision regarding diagnosis or the initiation of a guideline-based therapeutic intervention was made in the ED, depending on which occurred later.

Time to revascularisation and occurrence of MACE were obtained from the patients' medical records and confirmed by evidence from the Czech National Population Registry.

MACE was defined as the composite endpoint of cardiovascular death, non-fatal myocardial infarction, or unplanned hospitalisation due to cardiovascular disease within 30 days of the clinical examination. Because of the absence of non-fatal myocardial infarction and very low incidence of death in discharged patients, discrepancy between working and final diagnosis was added as a secondary safety endpoint.

A working diagnosis was considered accurate if it shared both a pathophysiological mechanism and diagnostic criteria with the final diagnosis (e.g., a working diagnosis of acute coronary syndrome was considered accurate if the final diagnosis was myocardial infarction or unstable angina but not takotsubo cardiomyopathy). In case of discrepancy between the working and final diagnosis, the final diagnosis was determined by independent review.

Ethics approval

This study was approved by the local ethical committee (Ethics Committee of University Hospital in Hradec Kralove, accredited by the Office for Human Research Protections under the number IORG0008813). The study was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

Statistical analysis

Categorical variables are expressed as group counts and relative frequencies (%), and continuous variables are expressed as group means, standard deviations and totals (N). Effect size is expressed as median of difference (ΔM) with 95% confidence interval (95% CI).

As most of the continuous variables examined here did not follow a normal distribution (as indicated by exploratory analysis and the Shapiro–Wilk test), the non-parametric Wilcoxon rank-sum test was used to compare continuous outcomes between groups. Categorical variables were compared using Fisher's exact test. Statistical significance was set at $\alpha = 0.05$ for all tests. In cases of multiple testing of similar hypothesis (e.g. various ways of reducing management time), the Bonferroni–Holm correction for the nominal level of statistical significance was applied to keep the familywise Type I error rate α at 0.05. Statistical analyses were performed using R software (R version 3.5.3; R Foundation for Statistical Computing, Vienna, Austria).

In this study, we aimed to test the hypothesis that the implementation of POCE would lead to a reduction in the length of stay in the ED of at least 15% (≈ 30 min). We performed a power analysis to determine the appropriate sample size for our study. The desired power was set at $\beta = 0.8$. The effect size was estimated on the basis of preliminary data in which the length of stay in the ED was determined to be 176 ± 90 min. Taking these parameters into account, the sample size was determined to be 430 patients.

An interim analysis was performed after every 50 patients. If both the primary and secondary endpoints reached statistical significance (including the Bonferroni–Holm correction), the Data Safety Monitoring Board was asked to assess the ethics of continuing the study. Given the continued superiority of POCE in both reducing the length of stay in the ED and improving diagnostic accuracy, the Board recommended early termination of the study after enrolment of 150 patients.

Results

Characteristics of study subjects

Between September 2022 and October 2023, a total of 150 patients were enrolled in the study. The baseline demographic and clinical data of the patients are summarized in Table 1. The descriptive characteristics were well balanced across the groups.

In the POCE group, echocardiography was performed in all patients. All records were subsequently reviewed for quality of image acquired, and the real extent of the examination was recorded. Of the 16 projections based on the

Table 1 Comparison of baseline characteristics between the POCE and control groups

	POCE group (n = 75)	Control group (n = 75)	p
Age [years; mean $\pm$ SD]	61.1 $\pm$ 17.8	66.1 $\pm$ 15.6	0.072
Male sex [n (%)]	50 (66.7)	41 (54.7)	0.181
Caucasian ethnicity [n (%)]	75 (100)	75 (100)	1.0
Chronic coronary artery disease [n (%)]	19 (25.3)	20 (26.7)	1.0
Diabetes mellitus [n (%)]	19 (25.3)	12 (16.0)	0.226
Arterial hypertension [n (%)]	39 (52.0)	42 (56.0)	0.743
Severe valvular disease [n (%)]	4 (5.3)	4 (5.3)	1.0
Atrial fibrillation [n (%)]	7 (9.3)	10 (13.3)	0.608
Active smoker [n (%)]	14 (18.7)	14 (18.7)	1.0
Former smoker [n (%)]	15 (20.0)	17 (22.7)	0.690
Antiplatelet therapy [n (%)]	20 (26.6)	23 (30.7)	0.718
Anticoagulation therapy [n (%)]	8 (10.6)	13 (17.3)	0.239
GRACE score [points]	96.0 (69.0–125.5)	100.0 (64.5–134.0)	0.536
HEART score [points; median (IQR)]	5.0 (3.0–7.0)	5.0 (3.0–6.0)	0.648
Indication of further follow-up [n; %]	30 (40.0)	26 (24.7)	0.612
Patients requiring hospital admission [n; %]	25 (33.3)	14 (18.7)	0.062

POCE point-of-care echocardiography; GRACE Global Registry of Acute Coronary Events [1]; HEART History, ECG, Age, Risk factors and Troponin (18,665,203)[2]; ECG electrocardiography

BSE Level 1 protocol, 12.5 ± 2.5 ($78.4 \pm 15.9\%$) were actually performed. An additional 5.4 ± 1.6 projections were performed on average. A total of 91.0% of the projections were fully conclusive, 7.6% were partly inconclusive, and 1.4% were completely inconclusive (Table 2). Regional wall motion abnormality (RWA), including septal D-shape, was present in 38.5% of patients; however, it was present in only 21.4% of patients with confirmed acute coronary syndrome.

Main results

The use of POCE resulted in a significant shortening of ED stay, time to decision and time to coronary angiography (Table 3). The reduction in the time of ED stay depended on the ECG findings and final diagnosis, $p = 0.019$ and $p = 0.047$, respectively (Fig. 3 and Fig. 4; Supplementum 2 and 3). However, the small number of patients in some subgroups meant that meaningful subgroup analysis was not possible.

The incidence of adverse events was lower than expected; therefore, the study would be underpowered for a reliable analysis even if enrolment were not stopped. We found no evidence to support the hypothesis that the use of POCE increases the risk of an incorrect working diagnosis (6.3% in the POCE group vs. 30.4% in the control group, $p = 0.997$ for inferiority). Although this was not a prespecified endpoint, the risk ratio suggests that the use of POCE resulted in superior diagnostic accuracy: $RR = 0.21$ (0.047–0.90).

Discussion

The present study stands at the intersection of educational strategy and clinical effectiveness and provides insight into how systematic training in echocardiography can equip non-cardiologists with the skills needed to effectively manage patients with acute chest pain. We have shown that physicians with no previous experience can be effectively trained to perform POCE, and that their clinical performance resulted in shorter emergency department stays and more accurate diagnoses.

Despite the widespread use of echocardiography in patients with acute cardiovascular disease, recommendations for POCE in the emergency setting remain vague, particularly regarding the optimal extent and timing of examinations [7, 8, 12–14]. This lack of clarity is a cause for concern and is in line with the recognition of POCE as a major health technology risk [14]. In addition, evidence on the extent of necessary education is limited and primarily based on national standards, despite calls for high-quality, competency-based cardiac imaging service delivery [15].

The efficacy and accuracy of POCE has been evaluated in several previous studies. These studies have shown acceptable agreement between POCE and comprehensive echocardiography, as well as increased diagnostic confidence among physicians, although without significant clinical benefit [16–18]. Recent research has demonstrated reductions in patient management time but not mortality endpoints, but there is no detailed information on examiner training and scope of examination [19, 20]. However, we believe that it is not echocardiography itself that should be the focus of

Table 2 Extent of POCE examinations

Projection	Number patients with a projection performed [n (%)]
	fully conclusive [n (%)]
	partially inconclusive [n (%)]
Parasternal ascending aorta**	69 (92.0)
	67 (89.3)
	1 (1.3)
PLAX (2D)*	67 (89.3)
	61 (81.3)
	5 (6.7)
PLAX (LVIDd)*	66 (88.0)
	46 (61.3)
	14 (18.7)
PLAX with CFM aortic and mitral valve*	71 (94.7)
	58 (77.3)
	13 (17.3)
PSAX outflow (2D)*	46 (61.3)
	42 (54.7)
	4 (5.3)
PSAX outflow with CFM aortic valve**	54 (72.0)
	50 (66.7)
	3 (4.0)
PSAX outflow with CFM mitral valve**	34 (45.3)
	30 (40.0)
	2 (2.7)
PSAX base (2D)*	39 (52.0)
	38 (50.7)
	0 (0)
PSAX mid (2D)*	61 (81.3)
	61 (81.3)
	0 (0)
PSAX apex (2D)*	28 (37.3)
	28 (37.3)
	0 (0)
A4C (2D)*	71 (94.7)
	61 (81.3)
	10 (13.3)
A4C (M-Mode) TAPSE*	71 (94.7)
	71 (94.7)
	0 (0)
A4C with CFM tricuspid valve*	34 (45.3)
	34 (45.3)
	0 (0)
A4C Tricuspid gradient (CW)**	52 (69.3)
	52 (69.3)
	0 (0)
A4C with CFM mitral valve*	60 (80.0)
	56 (74.7)
	3 (4.0)

Table 2 (continued)

A5C (2D)*	18 (24.0)
	15 (20.0)
	3 (4.0)
A5C with CFM aortic valve*	28 (37.3)
	27 (36.0)
	1 (1.3)
A2C (2D)	68 (88.0)
	58 (77.3)
	8 (10.7)
A2C with FCM mitral valve	44 (58.7)
	40 (53.3)
	4 (5.3)
A3C	64 (85.3)
	58 (77.3)
	6 (8.0)
A3C with CFM aortic valve	38 (50.7)
	34 (45.3)
	4 (5.3)
Subcostal 4C (2D)*	63 (84.0)
	49 (65.3)
	12 (16.0)
Subcostal IVC (2D)*	67 (88.0)
	64 (85.3)
	3 (4.0)
Subcostal IVC (M-Mode)*	58 (74.7)
	56 (74.7)
	1 (1.3)
Suprasternal long axis	3 (4.0)
	3 (4.0)
	0 (0)
Left ventricular systolic function assessed by at least two independent projections	74 (98.6)
Mitral valve assessed by at least one projection	62 (82.6)
Aortic valve assessed by at least one projection	62 (82.6)
IVC assessed by at least one projection	56 (74.6)

* projections required by the BSE Level 1 protocol

** additional projections involved in pre-study education

POCE point-of-care echocardiography; *2D* 2D dynamic image without CFM acquisition; *CFM* colour flow mapping; *PLAX* parasternal long axis projection; *LVIDd* Left ventricular internal diameter in diastole; *PSAX* parasternal short axis projection; *A4C* apical 4-chamber projection; *TAPSE* tricuspid annular plane systolic excursion; *A3C* apical 3-chamber projection; *A2C* apical 2-chamber projection; *IVC* inferior vena cava

scientific interest, but rather the way in which it is taught, indicated and performed. In particular, it needs to be shown that POCE can be beneficial to ordinary emergency physicians, not just highly experienced specialists. This is where we see the greatest contribution of this study.

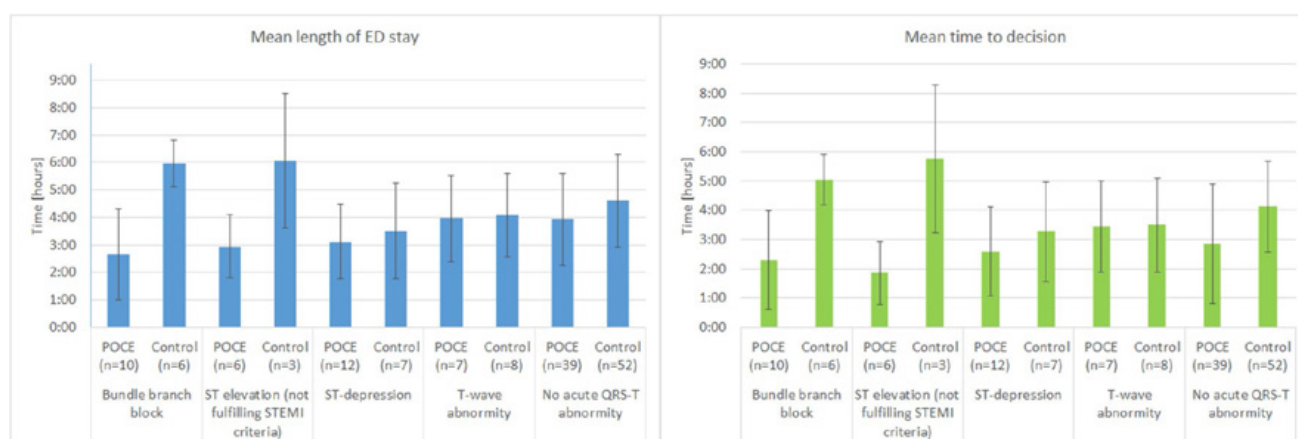
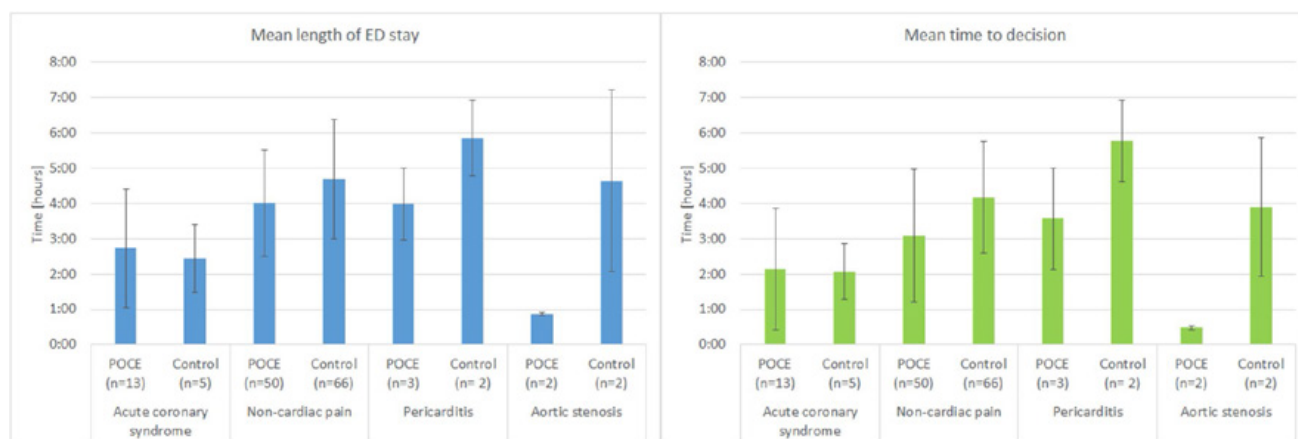
The rationale for performing early echocardiography in patients with chest pain is traditionally based on the pathophysiology of the ischaemic cascade [21] (Fig. 5). Interestingly, RWA was uncommon in patients with acute

coronary syndrome. However, when such impairment was detected, it was highly predictive of coronary artery occlusion. The long-standing classification of acute coronary syndromes into STEMI and non-STEMI, which was used in the study, is gradually being replaced by a new classification system that distinguishes between occlusive myocardial infarction (OMI) and non-occlusive myocardial infarction (NOMI) [22]. This paradigm shift is based on evidence that a significant proportion of non-STEMI

Table 3 Comparison of the POCE and control groups

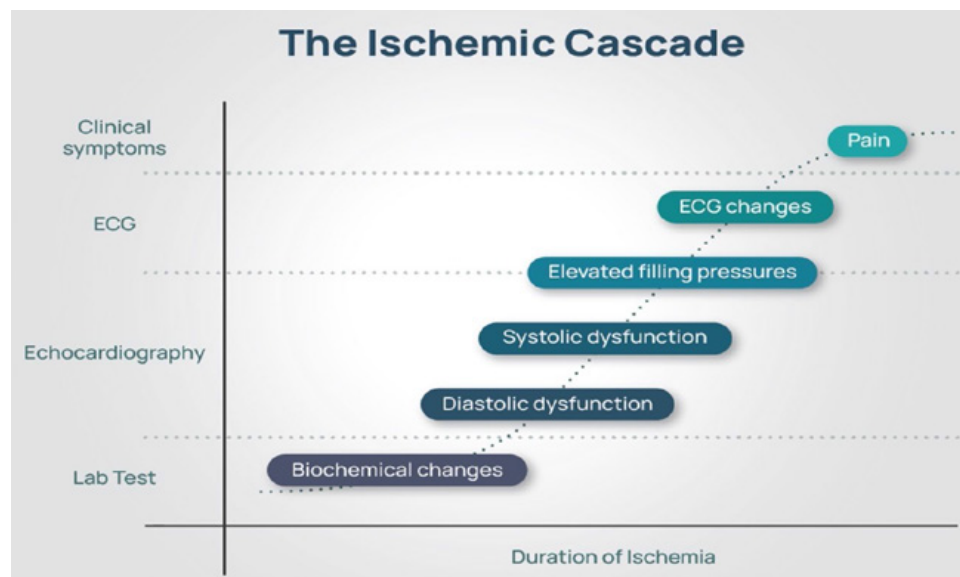
	POCE group (n = 75)	Control group (n = 75)	p
Time to decision in whole study group [min; mean (IQR)]	138.0 (68.5 – 230.5)	252.0 (165.5 – 304.0)	<0.0001
Duration of ED stay in whole study group [min; mean (IQR)]	209.0 (143.5 – 260.0)	271.0 (206.5 – 336.0)	0.0003
Time to decision among hospitalized patients [min; mean (IQR)]	112.0 (51.0 – 180.0)	183.5 (122.75 – 252.0)	0.024
Duration of ED stay among hospitalized patients [min; mean (IQR)]	178.0 (75.0 – 224.0)	219.5 (145.5 – 293.75)	0.058
Time to coronary angiography [hours]	29.6 (23.6–38.1)	120.3 (45.6–147.9)	0.027
MACE at day 30 [n; %]	0 (0)	0 (0)	1.0
All cause deaths at day 90 [n; %]	1 (1.33)	1 (1.33)	1.0

POCE point-of-care echocardiography; ED emergency department; IQR interquartile range; MACE Mayor cardiovascular adverse events

**Fig. 3** Reduction of management times depending on the initial ECG**Fig. 4** Reduction of management times depending on the final diagnosis

patients have occlusive coronary lesions and would therefore benefit from early coronary angiography [23]. It corresponds well with our finding that patients with difficult-to-interpret findings (e.g. bundle branch block, ST elevations not meeting STEMI criteria) benefited most from POCE. We hypothesise that the detection of RWA by

POCE of sufficient quality is superior to ECG for identifying high-risk patients and we advocate initiating research in this area. Nevertheless, it is important to emphasise that the standard troponin algorithm remains the primary method for ruling out acute coronary syndrome in most patients presenting with acute chest pain.

Fig. 5 The ischemic cascade

Archiving and sharing POCE records has been shown to be crucial in facilitating collaboration with consulting specialists, especially when RWA was present. We therefore strongly support the European Association of Cardiovascular Imaging's recommendation to archive POCE examinations [24].

Patients without ACS (such as those with severe valvular disease or pulmonary embolism) experienced a shorter Emergency Department stay. In these cases, POCE utilisation facilitated rapid diagnosis. Such patients also avoid time-consuming outpatient management, which may include coronary angiography. This is another main contributor to reducing the time to coronary angiography. Early recognition of less common causes of pain was also the main reason for the trend towards a higher number of hospital admissions in the POCE group.

Although the training in this study was based on the BSE Level 1 protocol, which is considered to be one of the most valuable focused protocols [25] and demonstrated very good clinical application in this study, physicians often expanded the scope of the examination to reliably detect regional wall motion abnormalities. This finding is consistent with previous findings that assessment of wall motion abnormalities is poorly predictive of acute coronary syndrome when insufficiently detailed POCE protocols are used [26]. We conclude that BSE Level 1 protocol is a good basis for routine use in clinical practice. However, we anticipate that a protocol specifically designed for efficient examining patients with acute chest pain will be introduced in the future.

We also recommend the development of evidence-based international recommendations for POCE training for basic clinical syndromes, which may broaden the impact of POCE and consolidate its role in emergency medicine [15]. The training programme described in this article is demanding

and may not be feasible in all centres. However, we believe that our data could help healthcare authorities and facility managers to make evidence-based decisions about the cost-effective training of non-cardiologists in POCE. In addition, we support the American Society of Echocardiography's recommendation that cardiologists should lead the training of non-cardiologists in echocardiography, with an emphasis on standardised, competency-based service delivery [6, 27–29].

Study limitations

The main limitation of this study is its monocentric design. We expect that the benefits of POCE may differ from those observed in tertiary cardiovascular centres. Acceleration of chest pain management may not be feasible in centres that are unable to respond immediately to the full range of POCE findings, either due to lack of resources or free capacity. The benefit may also be compromised by mistrust of other specialists in the reliability of POCE performed by emergency physicians.

The study was not designed to prove improved diagnostic accuracy by POCE. This is because, at the time the study was designed, there was more concern about the high error rate of POCE carried out by non-cardiologists. For this reason, the error rate assessment was only included as a safety endpoint.

The randomisation process cannot be blinded due to the nature of POCE, which requires direct interaction between the clinician and the process of performing the ultrasound examination. The chosen study design does not distinguish to what extent the disclosure of key findings or the increase in physician confidence in the diagnostic process is the cause of the acceleration of care.

Patients for whom POCE could not be performed within 90 min of arriving at the ED were excluded from the study. The number of such patients in the study population was very low. In chronically congested EDs, this condition may significantly limit the generalisability of the results. The number of patients included does not allow reliable subanalysis for rare diseases and ECG findings. Given the monocentric nature of the study, the impact of POCE on the management of less common conditions may also be biased.

Another limitation of the study is the small number of physicians performing POCE examinations. Without enthusiasm for the implementation of POCE in clinical practice and confidence in the method, the benefits may be reduced or absent.

Conclusion

The results of our study clearly demonstrate the significant benefits of POCE performed in the ED by non-cardiologists who have undergone structured training. In particular, the implementation of POCE led to a significant reduction in length of ED stay and decision-making times, which are critical measures of the efficiency of emergency care. The time to coronary angiography was also reduced. The accuracy of initial diagnosis improved significantly, highlighting the clinical value of POCE in the management of acute chest pain.

Where a standardised and comprehensive POCE education is required, the British Society of Echocardiography's Level 1 training framework represents an appropriate foundational protocol.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11739-025-04198-6>.

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Author contributions PG and MJ are lead authors and contributed equally to the paper. PG, MJ, KM and RP conceived the study and designed the trial. MJ, RP and JN supervised the conduct of the trial and data collection. PG and MC undertook recruitment of patients. PG and MJ managed the data, including quality control. JK administered the project. JV provided statistical advice on study design and analysed the data. JMH was responsible for funding the study. PG and MJ wrote the manuscript. MJ drafted the manuscript, and all authors contributed substantially to its revision. MJ takes responsibility for the paper as a whole.

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Data availability The data are available from the corresponding author upon reasonable request.

Declarations

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.

Ethical approval This study was approved by the local ethical committee (Ethics Committee of University Hospital in Hradec Kralove, accredited by the Office for Human Research Protections under the number IORG0008813). The study was conducted in accordance with the Declaration of Helsinki.

Consent to participate Written informed consent to participate in this study was provided by all patients.

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The impact of cardiogenic shock and out-of-hospital cardiac arrest on the outcome of acute myocardial infarction: a national-level analysis

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The impact of cardiogenic shock and out-of-hospital cardiac arrest on the outcome of acute myocardial infarction: a national-level analysis

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Abstract

Cardiogenic shock (CS) and out-of-hospital cardiac arrest (OHCA) are events with profound implications for patient outcomes. We aim to analyze the predictors of CS and OHCA in patients with acute myocardial infarction and their effects on mortality. The analysis is based on data from a national registry between 2016 and 2020. A total of 23,703 patients with ST-elevation myocardial infarction (STEMI) were analyzed: (A) patients without CS and OHCA (19,590), (B) after OHCA (2,262), (C) with CS (713), and (D) after OHCA with CS (1,138). Patients after OHCA without CS had the lowest mean age [62.0 ($\pm$ 12.6) years], while patients with CS without OHCA were the oldest [68.8 ($\pm$ 11.8) years] and had the highest proportions of comorbidities. CS was a predictor of 30-day and 1-year mortality, with odds ratios [OR; 95% confidence intervals (CI)] of 5.52 (4.51; 6.75) and 4.66 (3.87; 5.61) for patients after OHCA, and OR (95% CI) 9.28 (7.56; 11.38) and 7.33 (6.04; 8.89) for those without OHCA. For overall survival up to 30 days and in comparison to patients without CS and OHCA, the hazard ratios (95% CI) was 2.77 (2.40; 3.20) for patients with OHCA only, 14.36 (12.57; 16.40) for patients with CS only, and 16.96 (15.19; 18.92) for patients with both CS and OHCA. OHCA altered the 30-day mortality risk after STEMI for both patients with and without CS. CS is a predictor of both 30-day and 1-year mortality in patients with STEMI, irrespective of OHCA status.

Keywords Acute myocardial infarction · Out-of-hospital cardiac arrest · Cardiogenic shock · Outcome · Predictors

Introduction

Cardiogenic shock (CS) and out-of-hospital cardiac arrest (OHCA) are critical cardiovascular events with profound implications for patient outcomes. Acute myocardial infarction (AMI) remains a leading cause of both CS and OHCA [1]. There is limited evidence regarding treatment strategies in CS, which presents a challenge in contemporary cardiology and emergency medicine [2, 3]. A better understanding of epidemiology, risk factors, and prognostic implications of CS and OHCA could provide insights, eventually leading to improved patient care.

There has been an upward trend in the number of AMIs complicated by CS and OHCA [4–7], which both increase the mortality of patients with AMI [2, 8–10]. Cardiogenic

shock has been shown to complicate up to 10% of AMIs [3, 11]; moreover, despite some decline in mortality rates in registry data, randomized controlled trials (RCTs) reveal 30-day mortality rates as high as 40–60% [5, 9, 10, 12]. By comparison, the prevalence of OHCA in AMI patients treated with primary percutaneous coronary intervention (PCI) varies greatly, but can reach 30% in specific settings, with 30-day mortality rates exceeding 35% [13–16].

When OHCA occurs in AMI patients, it shifts the most frequent cause of death from primary cardiac reasons to neurological injury, outweighing the cardiac risks in the overall AMI population [6]. The population of AMI patients with OHCA admitted to hospital is heterogeneous and differs depending on the setting of prehospital care or the rules for the termination of cardiopulmonary resuscitation [13, 14, 16].

There is a paucity of data specifically addressing the interplay between CS and OHCA. Cardiac arrest occurring

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in patients with AMI-related CS confers increased short-term mortality risk with a diminishing effect over time [17]. However, it remains unclear what magnitude of risk for CS development OHCA confers due to the cardiac arrest itself (as opposed to the extent of AMI) and whether these patients have a different prognosis, potentially benefiting from modified treatment options. It is unknown how the interaction between CS and OHCA influences mortality in patients with ST-elevation myocardial infarction (STEMI), who have the largest ischemic myocardial mass.

Methods

This study aims to provide a national-level analysis of predictors of CS and OHCA in patients with STEMI and their effect on mortality based on an all-comers national registry.

The analysis is based on data from the National Registry of Cardiovascular Surgery and Interventions (NRCSI) in the Czech Republic combined with data from the National Registry of Deaths for mortality analysis.

National Health Information System (NHIS) data were collected by the Institute of Health Information and Statistics in the Czech Republic; NHIS provides data on the health status of the population, and on healthcare providers and personnel. The cardiovascular interventions module of the NRCSI started in 2004 and contains mandatory data collected on all coronary and noncoronary catheterizations and cardiovascular interventions performed in the Czech Republic. Data in the NHIS are collected in accordance with 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 from an ethics committee or informed consent from participants. The investigation conforms to the principles outlined in the *Declaration of Helsinki*. The analysis included all patients with STEMI in the NRCSI in the studied period with no exclusion criteria.

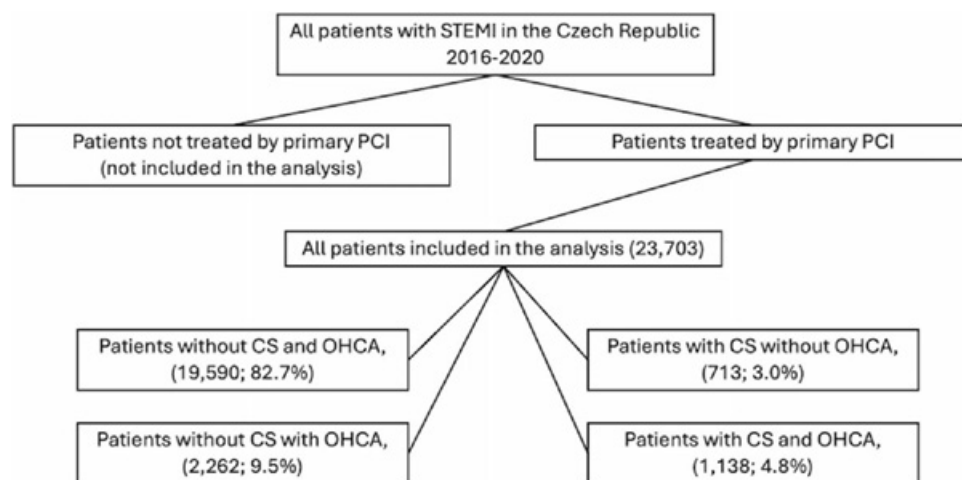
Therefore, all the patients included in this study were treated with primary PCI, as patients not undergoing PCI were not included in the NRCSI (Fig. 1). OHCA was defined as any cardiac arrest occurring in the out-of-hospital setting.

STEMI was defined as electrocardiogram changes of ST-segment elevation at the J point in at least two contiguous leads ≥ 0.25 mV in men < 40 years, ≥ 0.2 mV in men ≥ 40 years, or ≥ 0.15 mV in women in leads V2–V3, and/or ≥ 0.1 mV in all other leads.

CS was diagnosed if the patient had a systolic blood pressure < 90 mmHg, or catecholamine use was necessary to maintain a systolic blood pressure ≥ 90 mmHg, and presented clinical signs of impaired organ perfusion with at least one of the following: altered mental status, cold and clammy skin and limbs, oliguria with urine output < 30 ml per hour, or an arterial lactate level > 2.0 mmol per liter. This definition of CS was used in the design of NRCSI to allow all health care workers entering data into the registry a simple way to differentiate between patients with CS and without CS. Data regarding the medical history, clinical presentation, and PCI were entered into the NRCSI at the time of completion of primary PCI, and both CS and OHCA occurred between the onset of symptoms and the completion of primary PCI.

Standard descriptive statistics were applied in the analysis: absolute and relative frequencies for categorical variables and, for continuous variables, means supplemented with standard deviations and medians with interquartile ranges. The statistical significance of differences between groups of patients was computed using the maximum likelihood Chi-square test for categorical variables and t test for continuous variables. Logistic regression in univariate and multivariate settings with a forward stepwise algorithm applied for identifying the multivariate model was used to analyze factors influencing 30-day and 1-year mortality; its results were described using odds ratios (OR), and their 95% confidence intervals (CI) and statistical significance.

Fig. 1 Flowchart of the study



Time-to-event data were visualized using the Kaplan–Meier methodology with the landmark analysis approach. The Cox proportional hazards model was adopted for analyzing the combined influences of CS and OHCA in STEMI patients on overall survival described by hazard ratios (HR), and their 95% CI and statistical significance. The level of statistical significance was set at 0.05 for all analyses. Statistical computations were performed using SPSS 27.0.0.0. (IBM Corporation 2022).

Results

The period from January 1st, 2016, to December 31st, 2020 was chosen for standardized registry data, and 23,703 patients with STEMI were analyzed. The baseline characteristics of the patients are shown in Table 1.

Patient characteristics

We compared four subgroups of STEMI patients based on the presence of evaluated risk events—OHCA and initial CS: (1) without CS and OHCA (19,590; 82.7%), (2) after OHCA without CS (2,262; 9.5%), (3) with CS without OHCA (713; 3.0%), and (4) after OHCA with CS (1,138; 4.8%). Patients in group 2 had the lowest mean age, 62.0 ($\pm$ 12.6) years, while patients in group 3 were the oldest, at 68.8 ($\pm$ 11.8) years. Furthermore, patients in group 3 had the highest proportions of comorbidities, namely diabetes mellitus (DM) (25.0%), chronic kidney disease (CKD) (6.0%), prior PCI (18.8%), and prior coronary artery bypass graft (CABG) (5.3%), which were significantly more common than in all other subgroups with the exception of CKD (5.2%, $p=0.44$) and prior CABG (3.9%, $p=0.14$) in group 4. There were fewer comorbidities in groups 1 and 2, and no significant differences were observed between these groups in DM (16.6% vs 17.5%, $p=0.26$), prior PCI (13.9% vs 13.0%, $p=0.279$), prior CABG (2.7% vs 2.9%, $p=0.584$), and CKD (2.7% vs 2.2%, $p=0.44$). Patients in groups 2 and 4 had a shorter time from symptom onset to reperfusion, with median times (interquartile range, IQR) of 150 (115; 210) min and 153 (116; 228) min, than patients in group 4, who had a median time (IQR) of 210 (136; 370) min, $p<0.001$, and patients in group 1, with a median time (IQR) of 200 (138; 330) min, $p<0.001$.

Coronary angiography

An infarct-related artery (IRA) supplying a large myocardial mass was more common in groups 3 and 4 than in group 1, as was apparent for both the left main stem (LMS) [relative risk (RR) 9.82, 95% CI 7.95;12.13, $p<0.001$ in group 3; RR 9.23, 95% CI 7.67;11.10, $p<0.001$ in group 4] and the

left anterior descending artery (LAD) (RR 1.17, 95% CI 1.09;1.26, $p<0.001$ in group 3; RR 1.23, 95% CI 1.16;1.30, $p<0.001$ in group 4). The relationship with supplied myocardial territory was less pronounced, but still significant in group 2 (RR 1.61, 95% CI 1.21;2.14, $p<0.001$ for LMS; RR 1.11, 95% CI 1.06;1.16, $p<0.001$ for LAD). The distribution of IRAs among groups translated into ST-elevation localization, with anterior STEMI being less frequent in group 1 (42.8%) and with increasing risk in group 2 (RR 1.15, 95% CI 1.10;1.20, $p<0.001$), group 3 (RR 1.29, 95% CI 1.20;1.38, $p<0.001$), and group 4 (RR 1.31, 95% CI 1.24;1.38, $p<0.001$). The presence of triple-vessel disease was also less common in group 1 (18.1%), similar to group 2 (RR 1.01, 95% CI 0.92;1.11, $p=0.80$), and more common in group 3 (RR 1.92, 95% CI 1.76;2.09, $p<0.001$) and group 4 (RR 2.09, 95% CI 1.89;2.31, $p<0.001$). Groups 3 and 4 had lower initial as well as final thrombolysis in myocardial infarction (TIMI) flow in the IRA than groups 1 and 2 ($p<0.001$).

Mechanical ventilation and circulatory support

The use of mechanical ventilation was lowest in group 1 (2.2%), and the risk of ventilation support needs increased in group 3 (RR 12.34; 95% CI 10.60;14.36, $p<0.001$), group 2 (RR 32.70, 95% CI 29.68;36.02, $p<0.001$), and group 4 (RR 42.20; 95% CI 38.39;46.39, $p<0.001$). The CS status played a role in the use of mechanical circulatory support (MCS) devices. This was evident for intra-aortic balloon pump (IABP) use on the day of primary PCI, which was least frequent in group 1 (0.2%) and increased in group 2 (RR 3.15; 95% CI 1.62;6.09, $p=0.001$), group 3 (RR 37.47, 95% CI 24.06;58.35, $p<0.001$), and group 4 (RR 40.69, 95% CI 27.20;60.86, $p<0.001$), and even more in extracorporeal membrane oxygenation (ECMO) use on the day of primary PCI, which was again least common in group 1 (0.1%) and increased in group 2 (RR 6.38; 95% CI 3.20;12.71, $p<0.001$), group 3 (RR 30.38, 95% CI 16.41;56.26, $p<0.001$), and group 4 (RR 78.86, 95% CI 48.18;129.08, $p<0.001$).

Outcome

The presence of CS played a role in mortality. Additionally, the highest 30-day mortality rate was in the subgroup combining both studied risks, group 4 (48.2%), followed by group 3 (42.8%), group 2 (10.6%), and lowest in group 1 (4.0%). The predictors of 30-day mortality in the multivariate logistic model based on a forward stepwise algorithm are shown in Table 2 for patients after OHCA and in Table 3 for patients without OHCA. CS was a predictor of 30-day mortality with an OR (95% CI) of 5.53 (4.51; 6.75) for patients after OHCA and an OR (95% CI) of 9.28 (7.56;

Table 1 Baseline characteristics of patients with acute STEMI from the period 2016–2020

	1) CS: no. OHCA: no	2) CS: no OHCA: yes	1 vs. 2 <i>p</i> ^b	3) CS: yes OHCA: no	4) CS: yes OHCA: yes	3 vs 4 <i>p</i> ^b	1 vs 3 <i>p</i> ^b	2 vs 4 <i>p</i> ^b	2 vs 3 <i>p</i> ^b	1 vs 4 <i>p</i> ^b
Total ^a	19 590 (100.0%)	2 262 (100.0%)		713 (100.0%)	1 138 (100.0%)					
Gender										
Men	13 991 (71.4%)	1 676 (74.1%)	0.007	486 (68.2%)	870 (76.4%)	<0.001	0.062	0.134	0.002	<0.001
Women	5 599 (28.6%)	586 (25.9%)		227 (31.8%)	268 (23.6%)					
Age										
Mean ± SD	64.0 ± 12.6	62.0 ± 12.6	<0.001	68.8 ± 11.8	64.6 ± 12.3	<0.001	<0.001	<0.001	0.002	0.072
<40	431 (2.2%)	73 (3.2%)	<0.001	4 (0.6%)	24 (2.1%)	<0.001	<0.001	<0.001	<0.001	0.070
40–49	2 268 (11.6%)	317 (14.0%)		36 (5.0%)	114 (10.0%)					
50–59	4 313 (22.0%)	549 (24.3%)		113 (15.8%)	113 (15.8%)					
60–69	5 930 (30.3%)	677 (29.9%)		212 (29.7%)	212 (29.7%)					
70–79	4 384 (22.4%)	446 (19.7%)		201 (28.2%)	201 (28.2%)					
≥80	2 264 (11.6%)	200 (8.8%)		147 (20.6%)	147 (20.6%)					
Diabetes mellitus	3 246 (16.6%)	396 (17.5%)	0.260	178 (25.0%)	202 (17.8%)	<0.001	<0.001	0.86	<0.001	0.302
Previous PCI	2 716 (13.9%)	295 (13.0%)	0.279	134 (18.8%)	167 (14.7%)	0.020	<0.001	0.192	<0.001	0.446
Previous CABG	524 (2.7%)	65 (2.9%)	0.584	38 (5.3%)	44 (3.9%)	0.140	<0.001	0.126	0.003	0.024
Chronic kidney disease	536 (2.7%)	49 (2.2%)	0.102	43 (6.0%)	59 (5.2%)	0.440	<0.001	<0.001	<0.001	<0.001
Mechanical ventilation	432 (2.2%)	1 631 (72.1%)	<0.001	194 (27.2%)	1 059 (93.1%)	<0.001	<0.001	<0.001	<0.001	<0.001
Infarct-related artery										
LMS	291 (1.5%)	54 (2.4%)	0.002	104 (14.6%)	156 (13.7%)	0.597	<0.001	<0.001	<0.001	<0.001
LAD	8 571 (43.8%)	1 100 (48.6%)	<0.001	366 (51.3%)	611 (53.7%)	0.323	<0.001	0.005	0.208	<0.001
LCX	3 442 (17.6%)	486 (21.5%)	<0.001	163 (22.9%)	279 (24.5%)	0.415	<0.001	0.047	0.440	<0.001
RCA	8 178 (41.7%)	780 (34.5%)	<0.001	229 (32.1%)	309 (27.2%)	0.023	<0.001	<0.001	0.243	<0.001
Localization										
Anterior	8 380 (42.8%)	1 111 (49.1%)	<0.001	392 (55.0%)	636 (55.9%)	0.702	<0.001	<0.001	0.006	<0.001
Inferior/posterior	9 522 (48.6%)	911 (40.3%)	<0.001	253 (35.5%)	348 (30.6%)	0.029	<0.001	<0.001	0.022	<0.001
Lateral	1 494 (7.6%)	206 (9.1%)	0.015	47 (6.6%)	76 (6.7%)	0.942	0.296	0.015	0.031	0.232
Not known/LBBB	186 (0.9%)	34 (1.5%)	0.019	21 (2.9%)	77 (6.8%)	<0.001	<0.001	<0.001	0.018	<0.001
Unknown	8 (0.0%)	0 (0.0%)	0.186	0 (0.0%)	1 (0.1%)	0.324	0.324	0.335	-	0.512
Time from symptom onset to reperfusion ^c										
Median (IQR)	200 (105; 575)	150 (90; 320)	<0.001	210 (100; 760)	153 (89; 420)	<0.001	0.216	0.025	<0.001	<0.001
<2 h (<120 min)	2 980 (15.4%)	376 (28.4%)	<0.001	119 (17.9%)	290 (26.2%)	<0.001	0.085	0.111	<0.001	<0.001
2–4 h (120–239 min)	8 661 (44.7%)	695 (52.5%)	<0.001	264 (39.7%)	558 (50.4%)	<0.001	0.011	0.309	0.002	<0.001
4–8 h (240–479 min)	4 973 (25.7%)	199 (15.0%)	<0.001	161 (24.2%)	170 (15.4%)	<0.001	0.389	0.955	<0.001	<0.001
>8 h (≥480 min)	2 748 (14.2%)	54 (4.1%)	<0.001	121 (18.2%)	89 (8.0%)	<0.001	0.005	<0.001	<0.001	<0.001
Number of diseased vessels										
1 VD	9 437 (48.2%)	1 167 (51.6%)	<0.001	181 (25.4%)	374 (32.9%)	<0.001	<0.001	<0.001	<0.001	<0.001
2 VD	5 719 (29.2%)	648 (28.6%)		215 (30.2%)	332 (29.2%)					
3 VD	3 552 (18.1%)	415 (18.3%)		270 (37.9%)	396 (34.8%)					
Not available	882 (4.5%)	32 (1.4%)		47 (6.6%)	36 (3.2%)					
Left main stenosis ^d > 50%	587 (3.0%)	62 (2.7%)	<0.001	108 (15.1%)	163 (14.3%)	0.858	<0.001	<0.001	<0.001	<0.001

Table 1 (continued)

	1) CS: no. OHCA: no	2) CS: no OHCA: yes	1 vs. 2 <i>p</i> ^b	3) CS: yes OHCA: no	4) CS: yes OHCA: yes	3 vs 4 <i>p</i> ^b	1 vs 3 <i>p</i> ^b	2 vs 4 <i>p</i> ^b	2 vs 3 <i>p</i> ^b	1 vs 4 <i>p</i> ^b
TIMI flow before treatment (the lowest value of all treated lesions)										
0	10 279 (52.5%)	1 167 (51.6%)	0.015	486 (68.2%)	714 (62.7%)	0.096	<0.001	<0.001	<0.001	<0.001
1	1 663 (8.5%)	186 (8.2%)		71 (10.0%)	120 (10.5%)					
2	3 403 (17.4%)	453 (20.0%)		80 (11.2%)	159 (14.0%)					
3	4 245 (21.7%)	456 (20.2%)		76 (10.7%)	145 (12.7%)					
TIMI flow after treatment (the lowest value of all treated lesions)										
0	383 (2.0%)	84 (3.7%)	<0.001	61 (8.6%)	82 (7.2%)	0.021	<0.001	<0.001	<0.001	<0.001
1	158 (0.8%)	29 (1.3%)		41 (5.8%)	41 (3.6%)					
2	866 (4.4%)	120 (5.3%)		90 (12.6%)	116 (10.2%)					
3	18 183 (92.8%)	2 029 (89.7%)		521 (73.1%)	899 (79.0%)					
MCS										
IABP the same day as PCI	33 (0.2%)	12 (0.5%)	0.002	45 (6.3%)	78 (6.9%)	0.647	<0.001	<0.001	<0.001	<0.001
ECMO the same day as PCI	19 (0.1%)	14 (0.6%)	<0.001	21 (2.9%)	87 (7.6%)	<0.001	<0.001	<0.001	<0.001	<0.001
Other MCS the same day as PCI	7 (0.0%)	0 (0.0%)	0.216	5 (0.7%)	10 (0.9%)	0.676	<0.001	<0.001	<0.001	<0.001
IABP from 1 to 30 days after PCI	34 (0.2%)	8 (0.4%)	0.093	8 (1.1%)	7 (0.6%)	0.244	<0.001	0.290	0.024	0.008
ECMO from 1 to 30 days after PCI	22 (0.1%)	11 (0.5%)	<0.001	6 (0.8%)	21 (1.8%)	0.068	<0.001	<0.001	0.294	<0.001
Other MCS from 1 to 30 days after PCI	14 (0.1%)	3 (0.1%)	0.411	4 (0.6%)	7 (0.6%)	1.000	0.003	0.020	0.061	<0.001
Mortality at 30 days	776 (4.0%)	239 (10.6%)	<0.001	305 (42.8%)	549 (48.2%)	0.008	<0.001	<0.001	<0.001	<0.001
Mortality at 1 year	1 645 (8.4%)	387 (17.1%)	<0.001	387 (54.3%)	660 (58.0%)	0.116	<0.001	<0.001	<0.001	<0.001

CABG coronary artery bypass graft, ECMO extracorporeal membrane oxygenation, IABP intra-aortic balloon pump, LAD left anterior descending, LBBB left bundle branch block, LCX left circumflex, LMS left main stem, MCS mechanical circulatory support, OHCA out-of-hospital cardiac arrest, PCI percutaneous coronary intervention, RCA right coronary artery, SD standard deviation, STEMI ST-elevation myocardial infarction, TIMI thrombolysis in myocardial infarction, VD vessel disease

^aAbsolute and relative frequencies for categorical variables; mean supplemented with standard deviation (SD) for age

^bPearson Chi-square test for categorical variables; *t* test for age

^cComputed for patients with available data only

Table 2 Predictors of 30-day mortality in patients with acute STEMI after OHCA (multivariate model)

Characteristics ^a	OR (95% CI)	P
Cardiogenic shock	5.52 (4.51; 6.75)	<0.001
Age at intervention		
<40	Reference	
40–49	0.77 (0.36; 1.66)	0.510
50–59	0.83 (0.40; 1.72)	0.619
60–69	1.75 (0.86; 3.56)	0.122
70–79	3.13 (1.53; 6.43)	0.002
≥80	4.91 (2.35; 10.27)	<0.001
Diabetes mellitus	1.37 (1.08; 1.73)	0.010
Mechanical ventilation	1.89 (1.40; 2.55)	<0.001
LMS	2.14 (1.52; 3.02)	<0.001
Number of diseased vessels		
1 VD	Reference	
2 VD	1.12 (0.88; 1.42)	0.368
3 VD	1.46 (1.15; 1.86)	0.002
Not available	1.84 (1.01; 3.37)	0.047
TIMI flow after procedure 0–2	2.49 (1.96; 3.16)	<0.001
IABP the same day as PCI	1.95 (1.18; 3.21)	0.009
ECMO the same day as PCI	3.78 (2.36; 6.05)	<0.001
IABP from 1 to 30 days after PCI	4.28 (1.30; 14.12)	0.017
ECMO from 1 to 30 days after PCI	4.43 (1.83; 10.67)	<0.001
Other MCS from 1 to 30 days after PCI	0.12 (0.02; 0.78)	0.026

CI confidence interval, ECMO extracorporeal membrane oxygenation, IABP intra-aortic balloon pump, LMS left main stem, MCS mechanical circulatory support, OHCA out-of-hospital cardiac arrest, OR odds ratio, STEMI ST-elevation myocardial infarction, TIMI thrombolysis in myocardial infarction, VD vessel disease

^aModel is based on forward selection algorithm with cardiogenic shock forced into the model

11.38) for those without OHCA. Other independent predictors of 30-day mortality for all patients were age, DM, need for mechanical ventilation, IRA, multivessel disease, final TIMI flow, and the utilization of MCS. A further predictor for patients without OHCA was CKD. In addition, CS remained a predictor of 1-year mortality, with an OR (95% CI) of 4.66 (3.87; 5.61) for patients after OHCA and an OR (95% CI) of 7.33 (6.04; 8.89) for those without OHCA. The predictors of 1-year mortality in the multivariate logistic model based on a forward stepwise algorithm are shown in Table 4 for patients after OHCA and in Table 5 for patients without OHCA.

Figure 2 shows the Kaplan–Meier mortality curves of all compared patient groups. For overall survival up to 30 days in comparison to group 1, patients in group 2 had an HR (95% CI) of 2.77 (2.40; 3.20), patients in group 3 an HR (95% CI) of 14.36 (12.57; 16.40), and patients in group 4 an HR (95% CI) of 16.96 (15.19; 18.92); OHCA

Table 3 Predictors of 30-day mortality in patients with acute STEMI without OHCA (multivariate model)

Characteristics ^a	OR (95% CI)	P
Cardiogenic shock	9.28 (7.56; 11.38)	<0.001
Age at intervention		
<40	Reference	
40–49	1.03 (0.30; 3.53)	0.959
50–59	1.38 (0.43; 4.46)	0.589
60–69	3.73 (1.18; 11.78)	0.025
70–79	6.49 (2.05; 20.48)	0.001
≥80	17.03 (5.40; 53.75)	<0.001
Diabetes mellitus	1.27 (1.08; 1.49)	0.004
Chronic kidney disease	1.56 (1.18; 2.08)	0.002
Mechanical ventilation	2.38 (1.84; 3.08)	<0.001
LCX	0.75 (0.63; 0.91)	0.003
RCA	0.56 (0.48; 0.65)	<0.001
Number of diseased vessels		
1 VD	Reference	
2 VD	1.34 (1.12; 1.60)	0.001
3 VD	1.85 (1.55; 2.22)	<0.001
Not available	1.31 (0.93; 1.86)	0.125
LMS stenosis ≥ 50%	1.13 (1.07; 1.18)	<0.001
TIMI flow after procedure 0–2	3.29 (2.77; 3.89)	<0.001
IABP the same day as PCI	3.27 (1.84; 5.81)	<0.001
ECMO the same day as PCI	5.30 (2.43; 11.59)	<0.001
IABP from 1 to 30 days after PCI	16.70 (8.24; 33.86)	<0.001
ECMO from 1 to 30 days after PCI	17.95 (6.97; 46.22)	<0.001
Other MCS from 1 to 30 days after PCI	6.04 (0.98; 37.09)	0.052

CI confidence interval, ECMO extracorporeal membrane oxygenation, IABP intra-aortic balloon pump, LCX left circumflex, LMS left main stem, MCS mechanical circulatory support, OHCA out-of-hospital cardiac arrest, OR odds ratio, RCA right coronary artery, STEMI ST-elevation myocardial infarction, TIMI thrombolysis in myocardial infarction, VD vessel disease

^aModel is based on forward selection algorithm with cardiogenic shock forced into the model

significantly modified the 30-day survival in both patients with CS [HR (95% CI) of 1.17 (1.02; 1.35)] and patients without CS [HR (95% CI) of 2.77 (2.40; 3.20)]. This data shows a significant and combined effect of CS and OHCA on mortality, which lasted to a lesser extent even in patients who survived for the first 30 days. Compared to patients in group 1, patients in group 2 had an HR (95% CI) of 1.62 (1.35; 1.93), patients group 3 an HR (95% CI) of 4.33 (3.41; 5.51), and patients in group 4 an HR (95% CI) of 4.38 (3.57; 5.36) for overall survival up to 1 year. The modifying effect of OHCA remained significant only in patients without CS [HR (95% CI) of 1.62 (1.35; 1.93)] and diminished in those with CS [HR (95% CI) of 1.01 (0.75; 1.36)].

Table 4 Predictors of 1-year mortality in patients with acute STEMI after OHCA (multivariate model)

Characteristics ^a	OR (95% CI)	<i>p</i>
Cardiogenic shock	4.66 (3.87; 5.61)	<0.001
Age at intervention		
<40	Reference	
40–49	0.75 (0.37; 1.49)	0.406
50–59	1.11 (0.58; 2.14)	0.757
60–69	1.92 (1.01; 3.66)	0.048
70–79	3.53 (1.83; 6.80)	<0.001
≥80	5.82 (2.96; 11.46)	<0.001
Diabetes mellitus	1.44 (1.15; 1.80)	0.002
Chronic kidney disease	1.67 (1.04; 2.67)	0.033
Mechanical ventilation	2.03 (1.56; 2.63)	<0.001
LMS	2.05 (1.44; 2.91)	<0.001
Number of diseased vessels		
1 VD	Reference	
2 VD	1.32 (1.06; 1.63)	0.012
3 VD	1.78 (1.43; 2.22)	<0.001
Not available	2.18 (1.21; 3.92)	0.009
TIMI flow after procedure 0–2	1.88 (1.49; 2.38)	<0.001
IABP the same day as PCI	2.41 (1.40; 4.15)	0.002
ECMO the same day as PCI	4.15 (2.50; 6.89)	<0.001
IABP from 1 to 30 days after PCI	5.55 (1.59; 19.41)	0.007
ECMO from 1 to 30 days after PCI	3.96 (1.66; 9.45)	0.002

CI confidence interval, ECMO extracorporeal membrane oxygenation, IABP intra-aortic balloon pump, LMS left main stem, OHCA out-of-hospital cardiac arrest, OR odds ratio, STEMI ST-elevation myocardial infarction, TIMI thrombolysis in myocardial infarction, VD vessel disease

^aModel is based on forward selection algorithm with cardiogenic shock forced into the model

Discussion

Our analysis of patients with acute STEMI treated with primary PCI revealed differences between populations depending on their CS and OHCA status, as well as the impact of these risk conditions on patient prognosis.

Both CS and OHCA are complications of AMI that result in a markedly worse prognosis. The occurrence of CS among AMI patients is reported between 5 and 10% [3, 7, 11]. Our data showed a 7.8% prevalence of CS among patients with STEMI in an all-comers national registry, which is consistent with previous studies from other regions [3, 7, 11]. CS is a well-known predictor of mortality in AMI patients—RCTs commonly report 30-day mortality rates between 40 and 60% [5, 9, 10, 12]. CS also remains the major cause of in-hospital mortality in AMI patients [18]. The 30-day mortality rate for CS patients in our study was 42.8% in the subgroup without OHCA and 48.2% in cases complicated by OHCA. Chronic comorbidities, namely prior myocardial

Table 5 Predictors of 1-year mortality in patients with acute STEMI without OHCA (multivariate model)

Characteristics ^a	OR (95% CI)	<i>P</i>
Cardiogenic shock	7.33 (6.04; 8.89)	<0.001
Age at intervention		
<40	Reference	
40–49	0.99 (0.44; 2.25)	0.980
50–59	1.43 (0.66; 3.12)	0.366
60–69	3.45 (1.61; 7.42)	0.001
70–79	7.01 (3.27; 15.04)	<0.001
≥80	19.53 (9.11; 41.90)	<0.001
Diabetes mellitus	1.36 (1.20; 1.54)	<0.001
Previous PCI	1.38 (1.20; 1.58)	<0.001
Chronic kidney disease	2.10 (1.69; 2.60)	<0.001
Mechanical ventilation	2.17 (1.73; 2.72)	<0.001
LMS	1.57 (1.18; 2.08)	0.002
LAD	1.21 (1.05; 1.40)	0.007
RCA	0.85 (0.74; 0.99)	0.036
Number of diseased vessels		
1 VD	Reference	
2 VD	1.13 (1.00; 1.29)	0.056
3 VD	1.55 (1.36; 1.77)	<0.001
Not available	1.27 (0.98; 1.64)	0.071
LMS stenosis ^{>} 50%	1.07 (1.02; 1.11)	0.005
TIMI flow after procedure 0–2	2.34 (2.02; 2.70)	<0.001
IABP the same day as PCI	4.65 (2.61; 8.28)	<0.001
ECMO the same day as PCI	5.47 (2.45; 12.18)	<0.001
IABP from 1 to 30 days after PCI	19.45 (9.09; 41.64)	<0.001
ECMO from 1 to 30 days after PCI	20.20 (8.15; 50.07)	<0.001

^aModel is based on forward selection algorithm with cardiogenic shock forced into the model

CI confidence interval, ECMO extracorporeal membrane oxygenation, IABP intra-aortic balloon pump, LCX left circumflex, LMS left main stem, OHCA out-of-hospital cardiac arrest, OR odds ratio, RCA right coronary artery, STEMI ST-elevation myocardial infarction, TIMI thrombolysis in myocardial infarction, VD vessel disease

infarction, heart failure, DM, and CKD, in CS patients who present with AMI often lead to more severe coronary artery disease (CAD) and lower functional reserves and are associated with increased mortality [19, 20]. Our data confirmed the role of DM as an independent predictor of worse prognosis in STEMI patients, while the association with CKD was significant only in patients without OHCA.

The extent of CAD at the time of presentation is a logical predictor of severe myocardial ischemia. This relationship is most apparent in the presence of chronic total occlusion (CTO), which is supplied via collaterals from the IRA. Acute occlusion of the IRA then leads to double jeopardy of both myocardial territories, a greater risk of CS occurrence, and a worse prognosis [21]. While our registry did not include the presence of CTOs in our patients, the severity of CAD,

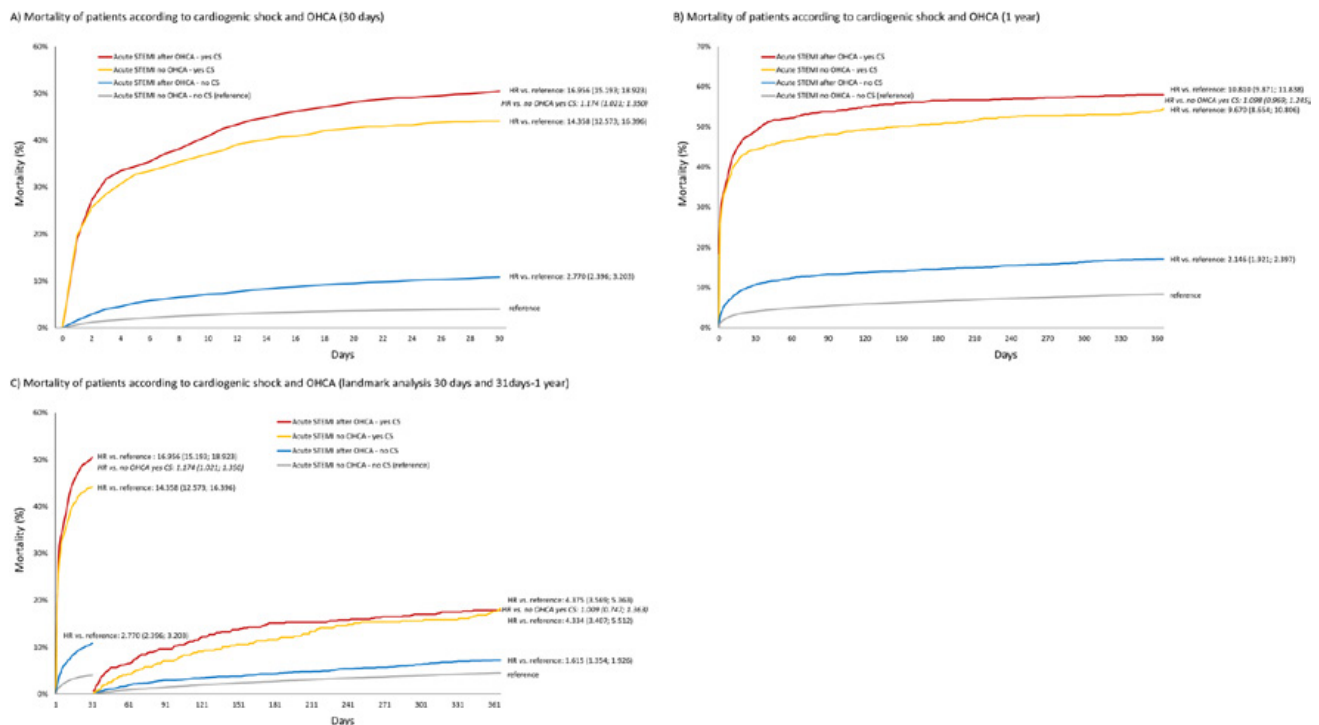


Fig. 2 **A** Mortality of patients according to cardiogenic shock and out-of-hospital cardiac arrest (30 days). **B** Mortality of patients according to cardiogenic shock and out-of-hospital cardiac arrest

(1 year). **C** Mortality of patients according to cardiogenic shock and out-of-hospital cardiac arrest (landmark analysis 30 days and 31 days–1 year)

as represented by multivessel disease and LMS disease, was associated with increased mortality. This association was again more pronounced in patients without OHCA.

OHCA complicating AMI in patients treated with primary PCI has been reported in 5–30% of patients [13–16]. There is variability in reporting outcome predictors in studies of CS complicating AMI [22]. As an example, for cardiac arrest—one of the most frequently reported prognostic factors—80% of RCTs report the condition in patients with CS after AMI; however, the prevalence ranging from 0 to 92% [22] highlights the heterogeneity of the studied populations. The all-comers national registry with the recorded resuscitation status of patients with STEMI treated with primary PCI depicts a reliable prevalence of OHCA in these patients, although it is modified by prehospital care for patients with OHCA and the rules for termination of resuscitation. In the Czech Republic, Emergency Medical Services have the authority to terminate resuscitation attempts outside the hospital, thereby conducting a selection process for patients with OHCA admitted to the catheterization laboratory. The prevalence of OHCA was 14.3% in our STEMI population and 61.5% in patients with CS. One of the factors that could influence the reported prevalence of OHCA in patients with AMI treated with primary PCI in various regions is the availability and use of extracorporeal cardiopulmonary resuscitation (ECPR) [13]. While ECPR use in the Czech

Republic is on the rise, including a local RCT on this topic [23], there are significant differences in the transport time to catheterization laboratories in urban and rural areas, and the effective use of ECPR remains limited to major cities. Thus, the national-level prevalence of OHCA in patients with STEMI may differ from that in specific settings of pre-hospital care for cardiac arrest in large cities [13].

The complex interplay between OHCA and CS within the STEMI population results in intriguing clinical and demographic distinctions among the studied subgroups. Our patients with CS and without OHCA combined the highest baseline risk represented by older age, extent of chronic CAD, and comorbidities with a high risk of the acute ischemic insult represented by an IRA supplying a large myocardial mass. On the other hand, patients with OHCA without CS were the youngest, with fewer comorbidities and smaller acute ischemic insult than patients with CS. Nevertheless, the myocardial territory at risk during AMI was still larger, and their comorbidities, despite younger age, were similar to those of patients without OHCA and CS. Therefore, these patients may represent a particularly vulnerable population among the STEMI population. We found the worst prognosis in patients with both CS and OHCA. This subgroup combined younger age, intermediate level of comorbidities, and high acute ischemic risk, suggesting that while OHCA occurs in younger and healthier patients, a

large ischemic myocardial mass is the most important factor in CS development.

OHCA increased the 30-day mortality of our patients to a similar absolute risk difference in both the CS and non-CS subgroups, but the relative risk difference was obviously much greater in the non-CS subgroup, where OHCA more than doubled the risk of 30-day mortality after STEMI. The effect of OHCA on the mortality of patients with CS was lower than previously reported, while the prevalence of OHCA in our patients with CS was slightly greater [17]. Combined with findings that patients with AMI after OHCA most commonly die due to neurological injury and not primary cardiac reasons [6], the different effect of OHCA on mortality in the CS population may reflect the heterogeneity of OHCA in various settings. We did not report the time to return of spontaneous circulation or specific cause of death in our registry, but the studied population included patients with low or no neurological impact of OHCA, as is perhaps best illustrated by the 72% prevalence of mechanical ventilation in patients with OHCA and without CS. Nevertheless, OHCA proved to be an important predictor of 30-day mortality in patients with STEMI, which was observed across the whole studied population with similar effect. Due to the clinical presentation, OHCA led to a significantly decreased time from symptom onset to reperfusion in patients with STEMI, but this reduction did not outweigh the deleterious effect of OHCA on survival.

The impact of OHCA on mortality in our analysis diminished over time and became statistically nonsignificant at 1 year among patients with CS. A similar effect has been reported previously and suggests that both baseline characteristics and the extent of ischemic insult during AMI in patients with OHCA influence the prognosis for a shorter duration than in patients with CS [17].

The limitations of our study are primarily inherent to its retrospective and observational design. In the national-level analysis, the number of collected variables was limited and we cannot rule out the possibility of an undetected confounding factor as we were unable to analyze all the granular patient-level data. The absence of time to return of spontaneous circulation in our dataset represents a possible bias, as it does not allow the assessment of OHCA duration on the prognosis of patients with STEMI. The shorter times from symptom onset to reperfusion in patients with OHCA may represent a survivor bias and attenuate the effects of OHCA on patient's mortality rates. The reduction of total ischemic time in these patients were probably due to the reduction in times from symptom onset to hospital admission, but these variables were not included in our study. The simplified definition of CS may lead to a bias, as patients with other types of shock fulfill this definition as well. Inclusion of patients with septic or hemorrhagic shock into the studied population may affect the results of our analysis. However, the

combination of shock, STEMI, and coronary artery lesions treated with primary PCI in all our patients with CS suggests that most of these patients were, in fact, suffering from CS. Due to the nature of NRCSI, only patients with STEMI treated with primary PCI could be included in our study. This represents a selection bias, as patients with STEMI not treated with PCI due to any reasons were not considered in our analysis, thus limiting the generalizability of the results to all patients with STEMI. The effect of COVID-19 pandemic on the results of our analysis was not studied and the pandemic may have had a specific effect on the prognosis of patients in the last year of the studied period.

In addition, the analysis was performed on a homogenous population, and its conclusions may not be generalizable to other populations. We specifically focused on patients with STEMI treated with primary PCI, and the results may differ from those of the broader population of patients with AMI. Further studies are needed to address these topics.

Conclusion

Our national-level analysis based on all-comers registry of patients with STEMI showed that OHCA significantly altered the 30-day mortality risk for both patients with and without CS. At 1 year, OHCA altered the mortality risk only for patients without CS. CS is a strong predictor of both 30-day and 1-year mortality in STEMI, irrespective of OHCA. Patients with STEMI after OHCA and CS at admission are at the highest risk of death. Patients with CS had the oldest age, the highest proportion of comorbidities, and the highest extent of CAD, while patients with OHCA had the youngest age, but an increased extent of CAD.

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Availability of data and material The datasets analyzed during the current study are not publicly available due to individual privacy, but are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethics approval and consent to participate Data in the NHIS are collected in accordance with 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 from an ethics committee or informed consent from participants. The investigation conforms to the principles outlined in the Declaration of Helsinki.

Consent for publication Not applicable.

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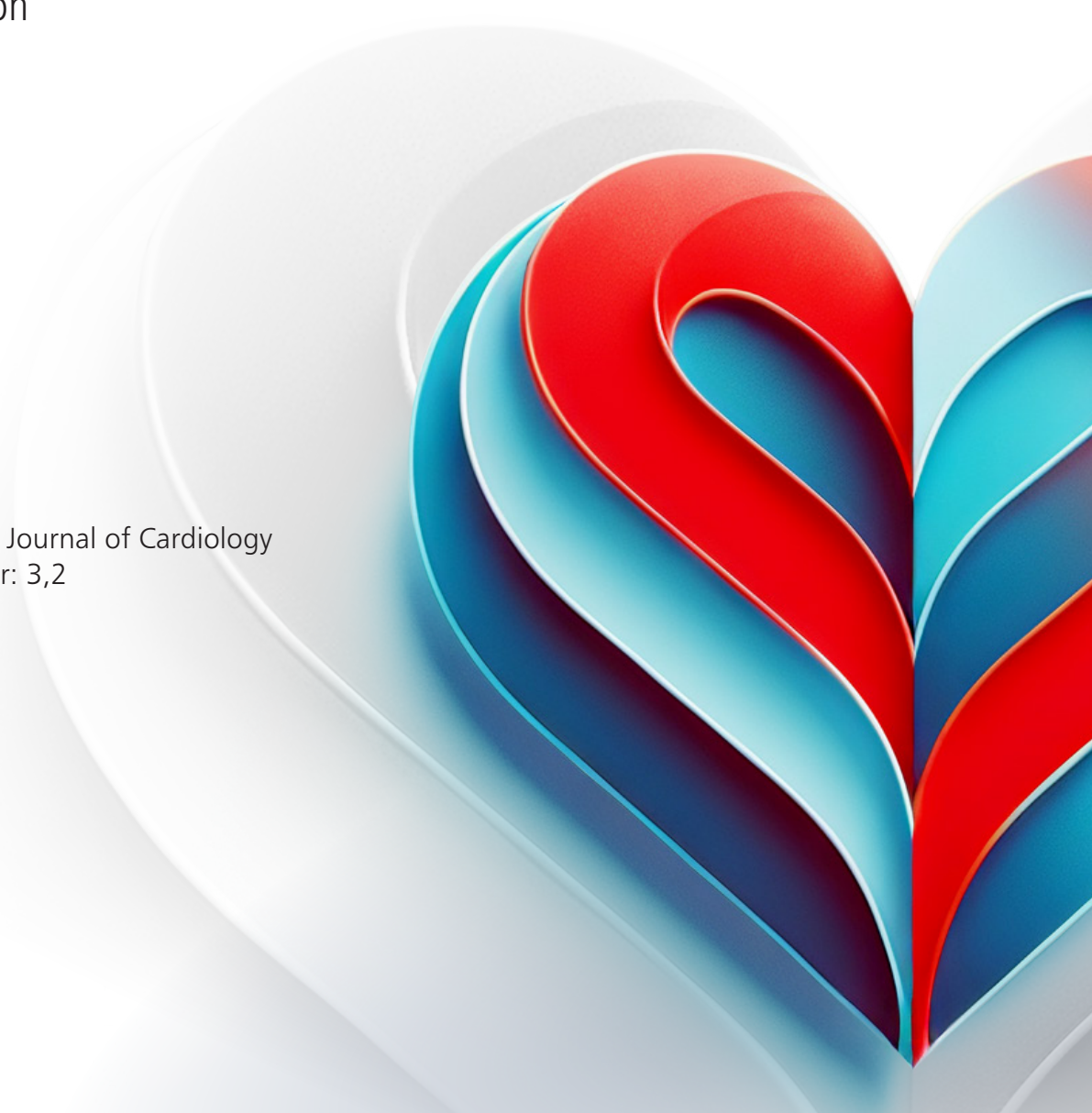
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Relative survival rate over twenty years of follow-up in younger patients with STEMI treated by primary percutaneous coronary intervention

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Relative survival rate over twenty years of follow-up in younger patients with STEMI treated by primary percutaneous coronary intervention

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ABSTRACT

Introduction: Primary percutaneous coronary intervention (pPCI) has been the gold standard treatment for patients with ST-elevation myocardial infarction (STEMI) in Europe for nearly two decades. While short- and medium-term outcomes are well described, data on very long-term mortality, especially in younger patients with longer life expectancy, remain limited.

Aim: To analyze very long-term mortality and 20 years relative survival rate compared to the general population in younger patients (≤ 65 years) with a first STEMI treated by pPCI.

Methods: We analyzed a high-volume, single-center registry of unselected STEMI patients treated with pPCI between January 1, 2000, and December 31, 2002. Patients aged ≤ 65 years with no history of myocardial infarction (MI) were selected. In cooperation with the Institute of Health Information and Statistics of the Czech Republic, we obtained mortality and cause-of-death data (classified by ICD-10) with a censoring date of December 31, 2023. Observed survival was assessed using the Kaplan–Meier method, and relative survival was estimated using the Pohar–Perme method, with expected survival derived from Czech national mortality tables.

Results: A total of 960 patients (71.4 % male, $n = 686$) with STEMI were treated with pPCI during the three-year study period. Among them, 385 patients (40.1 %) were ≤ 65 years with no previous MI. The mean age at STEMI was 55.03 ± 6.97 years for women ($n = 71$) and 54.16 ± 6.85 years for men ($n = 314$; $p = 0.168$). Over the entire very-long term follow-up period, 211 patients (54.8 %) died; cardiovascular causes were responsible in 111 cases (28.8 %). The 20-year overall survival was 50.6 %, while the 20-year relative survival was 79.5 %.

Conclusion: Despite high overall very-long term mortality in patients treated with primary PCI, the relative 20-year survival compared to the general population remained nearly 80 %. Furthermore, cardiovascular causes accounted for only half of all deaths.

1. Introduction

Primary percutaneous coronary intervention (pPCI) is the standard treatment for ST-elevation myocardial infarction (STEMI). Between 1998 and 2002, the Czech Republic established an extensive network of tertiary centers offering 24/7 pPCI access, achieving one of the highest PCI rates for STEMI in Europe. [1,2] Over the past two decades, a large number of patients have been treated using this strategy worldwide.

While short- and medium-term outcomes are well established, data on very long-term survival are scarce. [3–6]

The aim of this study was to analyze 20-years relative survival rate of younger patients with first STEMI treated with pPCI compared to general population. This study represents one of the longest follow-ups after implementation of this treatment strategy, marking a significant era in interventional cardiology.

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¹ This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation

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2. Methods

We analyzed a high-volume, single-center registry of unselected 960 patients treated for STEMI via pPCI between January 1, 2000, and December 31, 2002. Mortality and cause-of-death data (ICD-10 classification) were obtained from the Institute of Health Information and Statistics of the Czech Republic, with censoring on December 31, 2023. Patients not identifiable in the national registry during follow-up were excluded ($n = 39$). For patients with multiple STEMI hospitalizations ($n = 10$), only the earliest was considered.

For this analysis, we focused on patients aged <65 years without a history of MI, representing a lower-risk group with greater potential for very long-term survival. Mortality and cause-of-death data were analyzed for all patients, with a mean follow-up of 16,8 years and median 20,3 years. The patient was followed from the date of primary PCI until the date of death. If death was not recorded, the patient is censored on 12/31/2023. Cardiovascular deaths were defined as those with a primary ICD-10 code beginning with “I”. The expected survival of the general population (age- and sex-matched with STEMI cohort) was assessed based on mortality tables for the Czech population obtained from data provided by the Czech Statistical Office.

Descriptive statistics included frequencies for categorical variables and means $\pm$ standard deviations for continuous variables. Comparisons between groups were performed using the chi-square test for categorical variables and the Mann–Whitney U test for continuous variables. The Kaplan–Meier method was used for survival analysis, including confidence intervals. The log-rank test assessed survival differences. Relative survival was calculated via the Pohar–Perme method, comparing observed survival to that expected in the general population, adjusted for age and sex. Statistical significance was set at $\alpha = 0.05$. Analyses were performed using R (version 4.4.2; R Foundation for Statistical Computing, Vienna, Austria), employing the packages *dplyr*, *survival*, and *eha* for data manipulation and survival analysis.

3. Results

A total of 960 patients (71.4 % male, $n = 686$) with STEMI were treated with pPCI between 2000 and 2002. Mean age was 61.3 ± 11.4 years in women and 69.8 ± 11.2 years in men ($p < 0.001$). Of these, 385 patients (41.8 %) met criteria for the final analysis (age < 65 years, no prior MI). Mean age at STEMI in this group was 55.03 ± 6.97 years for women ($n = 71$) and 54.05 ± 6.82 years for men ($n = 331$; $p = 0.168$).

On admission, 85 % of patients were in Killip class I or II. Successful PCI with post-procedural TIMI 3 flow was achieved in 88 %. Clinical and

procedural characteristics of this cohort are presented in Table 1.

Thirty-day and one-year mortality were 3.2 % and 5.5 %, respectively. Over a median follow-up of 20,3 years, all-cause mortality was 54.8 %, and cardiovascular mortality was 28.8 %, accounting for 52.6 % of all deaths. Long-term survival did not differ significantly between men and women ($p = 0.19$). Relative survival rate since the primary PCI compared to general population at 5 years was 97.1 % (95 % CI 0.940; 1.002), at 10 years 93.4 % (95 % CI 0.888; 0.982), at 15 years 89.9 % (95 % CI 0.839; 0.965) and at 20 years 79.5 % (95 % CI 0.716; 0.882) as shown in Fig. 1.

4. Discussion

This study provides the longest follow-up of STEMI patients treated with primary percutaneous coronary intervention (PCI). In younger patients without a history of myocardial infarction (MI), the twenty-year survival rate was 20 % lower in comparison to that of the general population, demonstrating the long-term benefits of primary PCI in improving outcomes. No differences in survival were observed between men and women during the very long-term period.

Our results regarding relative survival are similar to those of other epidemiological studies assessing relative mortality or survival in the context of the general population. A recently published large-scale Danish study found that STEMI patients who survived the first 90 days had a 10-year mortality rate that was only 2 % higher than that of a matched general population. [7] Also, data from the UK Percutaneous Coronary Intervention Registry showed a crude five-year relative survival rate of 94.7 % for patients under 55 years old. Increasing age was associated with excess mortality up to four years following primary PCI. After four years, however, there was no excess mortality for patients aged 56–65 (excess mortality rate ratio: 1.27; 95 % CI: 0.95–1.70) [8]. Our study showed a similar survival rate to those of the two aforementioned studies over a ten-year period following primary PCI. Differences in relative survival compared to the general population only became apparent after 15 years. However, no other study has had such long-term follow-up, so we cannot compare it with other studies.

Our study did not find any sex differences in long-term survival. Similarly, a systematic review of this topic showed increased short-term, but not long-term, mortality in females with STEMI [9]. We also demonstrated that, during long-term follow-up after primary PCI, half of deaths were caused by non-cardiovascular factors. A sixteen-year follow-up of the DANAMI-2 trial found similar all-cause long-term mortality, with non-cardiovascular causes representing 64 % of deaths [10]. Thus, very long-term mortality should also be considered in this view, where at least half of deaths are not associated with long-term complications of percutaneous treatment of ST-segment elevation myocardial infarction (STEMI) in the patient's history.

5. Limitations

Our study is a single center study with limited number of patients, specially of women. Actual standard procedural technique of PCI has significantly evolved since the time when primary PCI was performed in patients of our study group (bare metal stents, femoral access, antithrombotic treatment). However, it has been shown that differences between drug-eluting and bare metal stents are mostly driven by target lesion revascularization during the first 5 years after primary PCI and do not affect further very long term follow-up. [11] Relative rate survival was calculated from all patients, not only from survivors as above mentioned studies did. However, hospital mortality in patients below 65 years undergoing primary PCI is very low and does not differ through years as we analyzed in our center. [12] Thus this analysis strategy should not have the significant impact on relative rate survival. Finally, as we did not have any information about risk factors from general population, we could not make any additional analysis in terms of comparison of very long term survival of STEMI patients and general

Table 1

Clinical and procedural characteristic of the study group ($N = 385$).

		N (%)
History of PCI, n (%)		3 (0.7 %)
History of CABG, n (%)		3 (0.7 %)
Diabetes mellitus, n (%)		
	Diet	62 (16.1 %)
	PAD	6 (1.5 %)
	Insulin	3 (0.7 %)
Hypertension, n (%)		168 (43.3 %)
Left main disease, n (%)		20 (5.2 %)
Vessels diseased	1	149 (38.7 %)
	2	123 (31.9 %)
	3	107 (27.8 %)
KILIP	1–2	334 (86.8 %)
	3–4	51 (13.2 %)
Culprit lesion		
	LM	3 (0.7 %)
	LAD territory	162 (42.1 %)
	LCx territory	63 (16.4 %)
	RCA territory	150 (38.9 %)
	Other	7 (1.9 %)
TIMI3 after pPCI		339 (88 %)

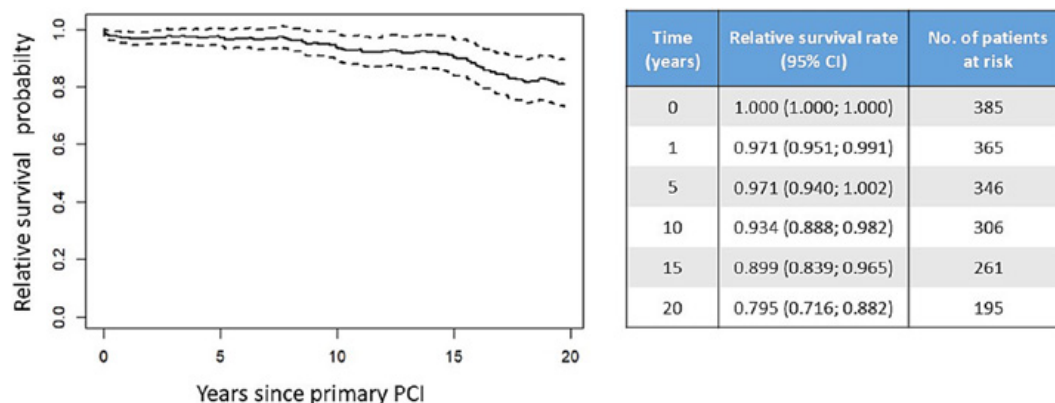


Fig. 1. Twenty years relative survival rate probability calculated for every 5 years period since primary PCI.

population including risk factors.

6. Conclusion

This study presents the longest reported follow-up of STEMI patients treated by pPCI. In younger patients without prior MI, very long-term survival is acceptable compared to the general population, underscoring the significant positive impact of primary PCI on long-term outcomes.

CRedit authorship contribution statement

Petr Toušek: Writing – original draft, Supervision, Project administration, Methodology, Investigation. **Dávid Bauer:** Formal analysis, Data curation. **Viktor Kočka:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Klára Benešová:** Writing – review & editing, Formal analysis, Data curation. **Andrea Kyselová:** Writing – review & editing, Formal analysis, Data curation. **Jiří Jarkovský:** Methodology, Formal analysis, Data curation. **Petr Widimský:** Writing – review & editing, Methodology.

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Declaration of competing interest

None declared.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

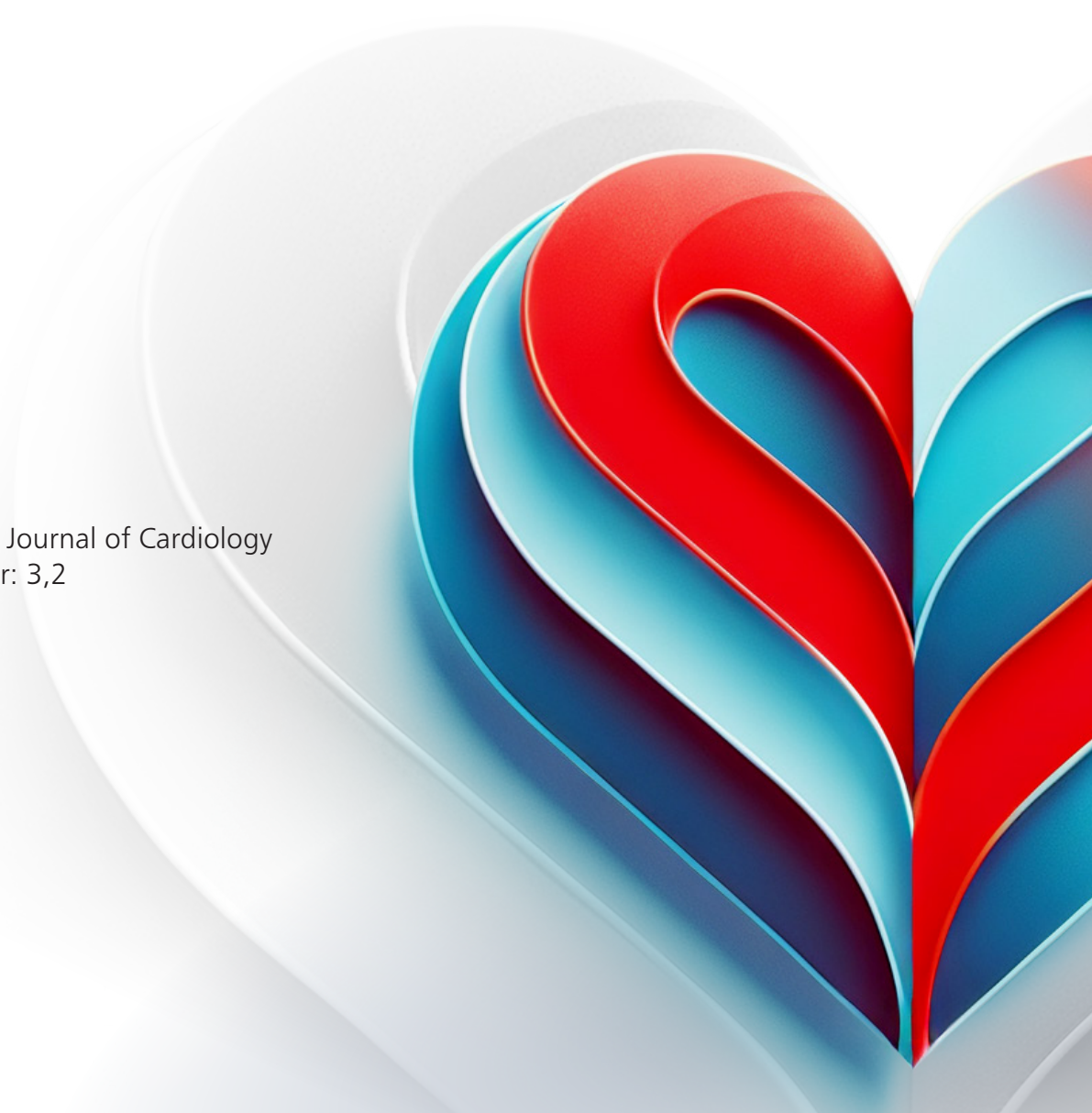
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Myocardial infarction in multivessel disease:
Does presence of chronic total occlusion make a difference?

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Myocardial infarction in multivessel disease: Does presence of chronic total occlusion make a difference?

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ABSTRACT

Background and aims: Myocardial infarction (MI) in multivessel disease (MVD) and chronic total occlusion (CTO) is associated with high mortality. However, all-cause mortality of matched cohort without a CTO is unclear. Our aim was to analyse clinical characteristics, presenting symptoms, and survival of patients with MI in MVD and the possible impact of CTO on 1-year mortality.

Methods: All MI patients with MVD (two or three vessel disease) hospitalized in our center from January 2020 to September 2022 (1309 patients) were selected. We conducted a propensity score matching (PSM) analysis based on age, gender, type of MI, and compared patients with CTO (CTO group, $n = 90$) and without CTO (Control group, $n = 90$).

Results: We observed no difference in presenting clinical symptoms and initial heart rhythm between the groups. 1-year follow-up shows all-cause mortality rate of 23.3 % ($n = 21$) in the CTO group (Mean survival [MS] = 292.1 days, 95 % CI = 263.8 to 320.4) and 18.9 % ($n = 17$) in the Control group (MS = 310.2 days, 95 % CI = 285.3 to 335.2), $p = 0.44$. PCI alone was performed in 64.4 % ($n = 58$) in both groups, CABG in 18.8 % ($n = 17$) and 24.4 % ($n = 22$) (CTO vs. Control group respectively). Combination of PCI and CABG occurred in 8.8 % ($n = 8$) in both groups. Conservative treatment was chosen for 7 CTO and 2 Control group patients.

Conclusion: We observed no 1-year mortality difference in patients with MI, MVD and a CTO compared to a matched cohort of patients with MI, MVD without CTO. Excellent 1-year survival was observed in patients treated by CABG, irrespective of CTO presence.

1. Introduction

Chronic total occlusion (CTO) of the coronary artery is a relatively common angiographic finding, occurring in 8–20 % of patients undergoing coronary angiography for various indications [1–6]. Although a universal definition of a CTO is lacking, typical characteristics include complete obstruction of coronary vessels known or presumed to be older than 3 months. Coronary flow is angiographically assessed by Thrombolysis in Myocardial Infarction (TIMI) flow. A true CTO is characterized by TIMI flow grade 0, while functional CTO is by TIMI flow grade 1 [7]. Other indirect signs of CTO include the presence of collaterals, inability to pass workhorse guidewire through the lesion or absence of contrast stain in proximal cap typical for acute thrombotic occlusion [8].

The most common symptom underlying the initial presentation of patients with chronic total occlusion is angina, followed by acute

coronary syndrome [3,9]. Although percutaneous coronary intervention on CTO lesions (CTO-PCI) is an established approach to mitigate symptoms, randomized controlled trials report conflicting results and have so far failed to show prognostic benefit [10,11].

A recent meta-analysis has shown that the presence of a CTO in patients with STEMI is a risk factor for short-term, as well as long-term all-cause mortality, cardiovascular mortality and major adverse cardiovascular events (MACE) rate [12]. An early revascularization of CTO lesions in patients with myocardial infarction (MI) has been associated with a possible improvement in their long-term prognosis [13].

Our investigation focuses on patients presenting with an acute MI, in the setting of MVD and a CTO. We aimed to provide a detailed account of the clinical and angiographic characteristics of these patients. Subsequently, we compared them with a matched cohort of patients with MI and MVD without CTO, including 1-year mortality data of both cohorts.

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2. Patients and methods

2.1. Study population and data selection

A prospective registry of patients with acute coronary syndrome (ACS) admitted to the University Hospital Královské Vinohrady Cardiocenter in Prague, Czech Republic, was created in 2018 and was previously described in detail [14]. ACS was defined based on, at that time current European guidelines for MI patients with ST-segment elevation (STEMI) and ACS without ST elevation (NSTEMI-ACS) [15,16]. There were no exclusion criteria for patients admitted with a final diagnosis of ACS. The registry was approved by the local ethics committee.

For the analysis, firstly, we selected all patients with MI (with ST segment elevation – STEMI; without ST segment elevation – NSTEMI) hospitalized from January 2020 to September 2022 and focused on patients with MVD (two or three vessel disease, characterized by a lesion of at least 50 % on the initial angiogram).

Secondly, we conducted a search in the hospital database of coronary angiograms for CTO presence. A CTO (vessel occlusion for more than 3 months) was defined based on an initial angiogram with TIMI flow 0/1, absence of contrast stain, culprit lesion in a different territory, or

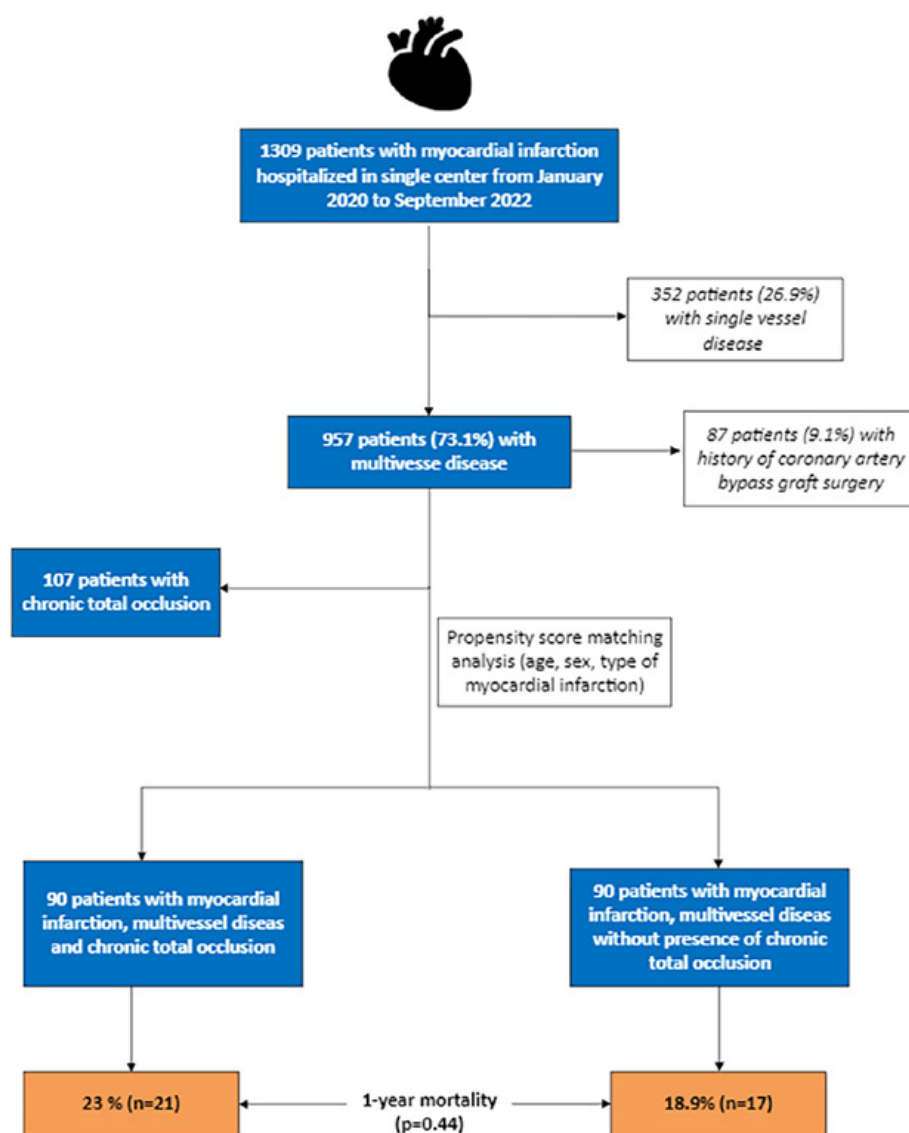
presence of collaterals. The final decision was left at the discretion of the interventional cardiologist performing the initial procedure. Angiograms were independently examined by a second experienced operator to minimize any potential error.

Subsequently, both datasets were unified to select patients with MI in the presence of MVD and CTO. We specifically focused on patients with native vessel disease, excluding patients with a history of coronary artery bypass graft (CABG), since these patients are prone to CTO formation in native vessels, as well as bypass grafts [17].

Finally, we conducted a propensity score matching analysis (PSM) based on age, gender, and type of MI. We compared patients with MI in the presence of MVD and CTO ($n = 90$) with patients with MI, and MVD without the presence of CTO ($n = 90$).

We retrospectively collected hemodynamic parameters (heart rate, systolic and diastolic pressure) and cardiac biomarkers at admission. The time from symptom onset to first laboratory results was considered with peak values for each patient.

All patients were treated by percutaneous coronary intervention (PCI), CABG, or conservatively. Importantly, no PCI of CTO was performed within 1-year of initial hospitalization for MI. Clinical characteristics, 30-day and 1-year mortality were analyzed. Mortality data were obtained in cooperation with the Institute of Health Information



Graph 1. Flowchart with patient selection.

and Statistics of the Czech Republic. [Graph 1](#) displays patients selection flowchart.

2.2. Statistical analysis

The Kolmogorov–Smirnov test for goodness-of-fit was used to determine the normality of the data distribution. Continuous variables are presented as means and standard deviations. Continuous variables were analyzed using Student's *t*-test or the Mann–Whitney *U* test. Categorical variables were analyzed using the χ^2 test and Fisher's exact test. Propensity score matching based on gender, age and type of MI was used to balance differences between the groups. Propensity scores were calculated using logistic regression analysis, and individuals were matched using the nearest neighbor matching algorithm with a caliper of 0.05. Kaplan–Meier survival analysis was used to calculate the cumulative survival at different time intervals; the log-rank test was used to assess differences in survival. Results were considered statistically significant at *P*-values <0.05. All statistical analyses were performed using SPSS Statistics software (IBM corp., Armonk, NY, USA). Graphical analysis was done using Sigma Plot, version 15.

3. Results

3.1. Myocardial infarction with multivessel disease with and without the presence of chronic total occlusion

Between January 2020 and September 2022, 1309 patients with MI were admitted to our center. Single vessel disease was present in 352 (26.9 %) and MVD in 957 (73.1 %) patients. CTO was present in 107 patients, which accounts for 8.2 % of all patients with MI (11.2 % in MVD). The basic clinical characteristics of both groups (MI in MVD with CTO vs. MI in MVD without CTO) are described in [Table 1](#). CTOs were

more prevalent in males, more likely with a history of heart failure and higher Killip class at admission, compared to patients without CTO. In-hospital mortality was significantly higher in patients with CTO.

3.2. Propensity score matching analysis

We conducted a PSM analysis based on age, gender, and type of MI (STEMI and NSTEMI). As can be seen in [Table 2](#), both the CTO group and the Control group (non-CTO) have similar clinical characteristics. There are apparent differences in lower ejection fraction, and higher Killip class at admission in the CTO group. Cardiopulmonary resuscitation and mechanical ventilation were also more often necessary in the CTO group. However, the difference was not statistically significant.

When comparing presenting clinical symptoms and cardiac rhythms, there was no statistically significant difference between the groups ([Supplemental Table 1](#)). Importantly, there was a difference in hemodynamic parameters at admission, for both systolic and diastolic pressure ([Supplemental Graph 1](#)), with no difference in heart rate. Cardiac biomarkers did not differ, with high sensitivity troponin I mean 31,275.1 ng/l ($\pm$ 40,274.5) in CTO and 29,659.2 ng/l ($\pm$ 39,711.7) in Control Group. The mean NTproBNP in the CTO group was 6176.9 ng/l ($\pm$ 8582.5) and in the control group 6076.1 ng/l ($\pm$ 10,159.3); ([Supplemental Graph 2](#)).

Culprit lesions were mostly located in LAD for both the CTO and Control groups. Most CTOs were in the right coronary artery. Basic angiographic characteristics of culprit lesions and CTOs are described in details ([Supplemental Table 2](#) and [Table 3](#), respectively). Significant left main stenosis ($\geq$ 50 %) was present in 11 patients in CTO and 9 patients in Control group.

3.3. Treatment

Most patients were treated by PCI, *n* = 58, for both groups. Seventeen patients in the CTO group and twenty-two patients in the Control group

Table 1
Myocardial infarction with multivessel disease with CTO and without CTO.

Clinical characteristics	MI, MVD and CTO (<i>n</i> = 107)	MI, MVD without CTO (<i>n</i> = 761)	p-value
Age (mean)	71.3 ($\pm$ 11.3)	68.9 ($\pm$ 11.8)	0.10
Men	85 (79.4 %)	523 (68.7 %)	0.02
STEMI	47 (43.7 %)	349 (45.9 %)	0.03
History of MI	26 (24.3 %)	163 (21.5 %)	0.53
History of PCI	23 (21.5 %)		
History of heart failure	11 (10.3 %)	41 (5.4 %)	0.05
History of stroke	19 (17.8 %)	92 (12.1 %)	0.12
Arterial hypertension	81 (75.7 %)	560 (73.6 %)	
Dyslipidemia	48 (44.9 %)	334 (43.9 %)	0.83
Diabetes mellitus			0.40
OAD	27 (25.2 %)	147 (19.3 %)	
Insulin	10 (9.3 %)	87 (11.4 %)	
Smoking			0.46
active	40 (37.4 %)	307 (41.2 %)	
former	23 (21.5 %)	201 (27.0 %)	
Killip class			0.003
I	66 (62.3 %)	565 (74.3 %)	
II	22 (20.8 %)	94 (12.4 %)	
III	3 (2.8 %)	46 (6.1 %)	
IV	15 (14.2 %)	55 (7.2 %)	
Ejection fraction (mean)	39.7 % ($\pm$ 12.0)	45.0 % ($\pm$ 11.7)	<0.001
CPR			
Prior to admission	16 (15.0 %)	60 (7.6 %)	0.03
In-hospital	12 (11.2 %)	58 (7.6 %)	0.20
Mechanical ventilation			
Prior to admission	11 (10.3 %)	60 (7.9 %)	0.45
In-hospital	15 (14.0 %)	51 (6.7 %)	0.02
In-hospital mortality	15 (14.2 %)	70 (9.2 %)	0.02

OAD – Oral antidiabetic drugs; CPR – cardiopulmonary resuscitation.

Table 2
Propensity score matching analysis, MI with MVD and CTO (CTO group) and without CTO (Control group).

	CTO group (<i>n</i> = 90)	Control group (<i>n</i> = 90)	p-value
Age (mean)	71.3 ($\pm$ 11.1)	70.7 ($\pm$ 11.0)	0.74
Men	72 (80.0 %)	72 (80.0 %)	>0.99
STEMI	36 (40 %)	36 (40 %)	>0.99
History of MI	19 (21.1 %)	23 (25.5 %)	0.60
History of PCI	17 (18.8 %)	20 (22.2 %)	0.58
History of heart failure	9 (10.0 %)	6 (6.7 %)	0.59
History of stroke	18 (20.0 %)	18 (20.0 %)	1.00
Arterial hypertension	69 (76.6 %)	72 (80.0 %)	0.72
Dyslipidemia	43 (47.7 %)	35 (38.8 %)	0.29
Diabetes mellitus			0.60
OAD	21 (23.3 %)	16 (21.1 %)	
Insulin	9 (10.0 %)	11 (12.2 %)	
Smoking			0.21
active	32 (35.6 %)	33 (36.7 %)	
former	20 (22.2 %)	31 (34.4 %)	
Killip class			0.11
I	57 (63.3 %)	63 (70.0 %)	
II	18 (20.0 %)	12 (13.3 %)	
III	3 (3.3 %)	9 (10.0 %)	
IV	11 (12.2 %)	6 (6.7 %)	
Ejection fraction (mean)	40.8 % ($\pm$ 11.4)	42.7 % ($\pm$ 11.6)	0.28
CPR			
Prior to admission	10 (11.1 %)	6 (6.7 %)	0.43
In-hospital	8 (8.9 %)	5 (5.6 %)	0.57
Mechanical ventilation			
Prior to admission	7 (7.8 %)	11 (12.2 %)	0.32
In-hospital	15 (16.7 %)	11 (12.2 %)	0.41
In-hospital mortality	6 (6.7 %)	6 (6.7 %)	>0.99

OAD – Oral antidiabetic drugs; CPR – cardiopulmonary resuscitation.

underwent CABG. A combination of both (CABG and PCI) was done in 8 patients in both groups. Seven patients from the CTO group and two in the Control group were treated conservatively.

3.4. Mortality

During the studied period, in-hospital mortality for all 107 patients with MI, MVD, and CTO was 14.0 % ($n = 15$). Mortality rate increased significantly within 30 days after admission to 19.6 % ($n = 21$). Interestingly, only a very small increase was observed in the mortality rate between 30 days and 1 year (20.6 %, $n = 22$).

After PSM, in-hospital mortality did not differ between the groups with 6.7 % ($n = 6$) for both CTO and the Control group respectively ($p \geq 0.99$). In the observational period up to 30 days, the CTO group mortality was 14.4 % ($n = 13$) and in the Control group was 10.0 %, ($n = 9$); $p = 0.36$, with a mean survival of 27.2 days (95 % confidence interval [CI] = 25.6 to 28.7) in the CTO group and 28.2 days (95 % CI = 26.9 to 29.5) in the Control group.

1-year follow-up shows all-cause mortality rate of 23.3 % ($n = 21$) in the CTO group ([MS] = 292.1 days, 95 % CI = 263.8 to 320.4) and 18.9 % ($n = 17$) in the Control group ([MS] = 310.2 days, 95 % CI = 285.3 to 335.2), $p = 0.44$. Landmark analysis emphasizes 1-year mortality distribution (Graph 2).

Patients treated by PCI had 27.6 % ($n = 16$) 1-year mortality in the CTO group, and 20.7 % ($n = 12$) in the Control group. Only 1 patient died within 1 year after CABG in both groups. Among the patients treated by the combination of CABG and PCI, one patient died in the CTO group, and two in the Control group. Conservatively treated patients had a high 1-year mortality rate of 42.9 % ($n = 3$) in the CTO group and 100.0 % ($n = 2$) in the control group.

4. Discussion

Our retrospective analysis describes in detail the high-risk subgroup of patients with MI, and MVD with and without the presence of a CTO. Subsequent PSM analysis provided several insights:

1. Presenting clinical symptoms and heart rhythms are similar between the groups.
2. Patients with myocardial infarction and multivessel disease are a vulnerable, high-risk subpopulation, with high in-hospital and 1-year mortality, irrespective of CTO presence.
3. When adjusted for age, sex, and type of myocardial infarction, there is no significant mortality difference within 1-year, regardless of CTO presence.

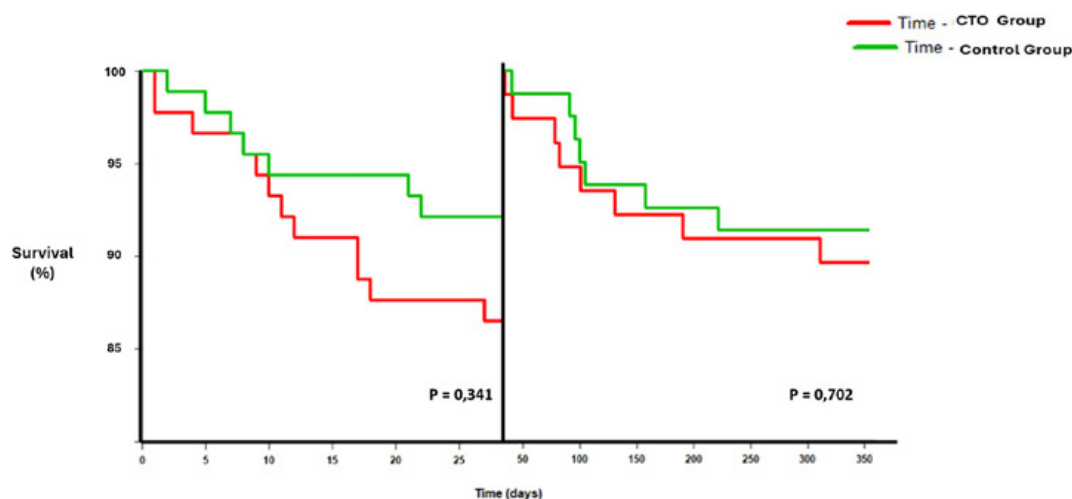
4. Patients treated by CABG had excellent outcomes.

First, presenting clinical symptoms and heart rhythms are similar between both groups (MI, MVD with and without CTO). In our cohort, more patients with CTO were oligosymptomatic. A possible explanation could be ischemic preconditioning, the process by which brief periods of decreased blood flow or hypoxemia prepare the myocardium for ischemic conditions, which allows the tissue to survive more prolonged periods of ischemia. Although the presence of CTO causes myocardial ischemia, even when collaterals are well-developed, preconditioning has been associated with reduced infarct size [18,19].

The presenting rhythms were also, somewhat surprisingly, similar among the groups – we did not find a difference between the groups in the prevalence of malignant arrhythmias prior to revascularization. The myocardial territory of a chronically occluded vessel is known to be arrhythmogenic. Scar tissue and scar border zone, hibernating myocardium, the persisting ischemic environment despite collaterals, areas of slow conduction, and heterogeneous repolarization all result in increased susceptibility to ventricular arrhythmias (VA). The VACTO Primary Study demonstrated that CTO presence is indeed an independent predictor of appropriate ICD therapy [20,21]. Importantly, patients with CTO who do not qualify for ICD (EF >35 %) have a substantial risk of VA [22]. Moreover, arrhythmic complications in MI, most frequently VA, occur between 10 and 20 % during hospitalization. More than half of VAs happen prior to revascularization [23,24]. Despite the fact, that a CTO alone poses an increased risk of ventricular arrhythmias (VA), in our cohort, the prevalence of VAs prior to revascularization for MI was similar between the groups (7.8 % in both groups).

Second, patients with MI and MVD with CTO represent a high-risk subpopulation with a high in-hospital and 1-year mortality. In accordance with literature findings, we documented that patients with CTO are older, more often men, with a history of heart failure, a higher Killip class at admission, and more commonly in need of a CPR and/or mechanical ventilation than patients without CTO. In our cohort, the in-hospital all-cause mortality was higher in patients with CTO ($n = 107$), compared with the non-CTO patients.

The presence of a CTO in STEMI patients is documented in 8–13 % and is associated with LVEF ≤ 40 % [1,2]. Investigators from the CREDO-Kyoto AMI registry group focused on the presence of CTO in STEMI patients with MVD, complicated by cardiogenic shock who underwent PCI within 24 h. In the 100 patients in the CTO group, there was significantly lower EF at admission, higher door-to-balloon time, and cardiac biomarkers (CK) were more elevated, compared to the non-CTO group. All-cause mortality was significantly higher in the CTO group, with outcomes worsening with multiple CTOs [25]. NSTEMI is more



Graph 2. Landmark analysis showing 30-day and 1-year mortality between the groups (CTO vs. Control group).

often associated with MVD, compared to STEMI, therefore CTOs are more commonly encountered. Although NSTEMI in the presence of a CTO is less studied, a recent multi-center observational registry of more than 17,000 patients documented a 10 % prevalence of CTO with significantly higher in-hospital and 1-year mortality [26]. In the CULPRIT-SHOCK trial, at least 1 CTO was present in 22.4 % in the culprit-lesion-only arm and in 24.0 % in the immediate multivessel PCI arm. The presence of a CTO did not influence the overall outcome. [27]

PSM based on age, gender, and type of MI, the presence of a CTO was not associated with a significant increase in the all-cause 1-year mortality in the studied cohort. Previous sub-study of the TOTAL trial had ascertained on a sample of 10,063 patients, comparing STEMI patients with a non-infarct-related artery (NIRA) CTO to those with SVD or MVD without a CTO, that the presence of a CTO was a reliable predictor of cardiovascular mortality up to 1 year [28]. In a study by Milasinovic and colleagues, studying the prognostic impact of NIRA CTOs on STEMI patients, the authors showed in a sample of 8679 patients that a NIRA CTO was associated with increased both 30-day and 5-year mortality, with a constant increase in the mortality rate after the 1st year, up to 5 years [29]. A CTO alone is considered a strong independent predictor for 30-day mortality (hazard ratio of 3.6), and its predictive value remains after 30 days [1,2]. The results from the HORIZON-AMI trial presented similar results [4]. Our results shows relatively high, but similar 1-year all-cause mortality in both groups. This may be due to the high-risk patient profile common to all patients with MVD, irrespective of CTO presence.

Finally, in our analysis, we compared two revascularization strategies: 1) PCI of culprit and chronic lesions with conservative treatment of the CTO, which makes in some perspective, this analysis unique, 2) a complete revascularization by CABG. Patients, who were treated by PCI had higher 1-year mortality of 27.6 % in the CTO group and 20.7 % in the control group. Contrary, our results have shown consistently positive results in patients treated by CABG, with only 1 patient dying within the 1 year in both groups. The non-random allocation of the treatment modality renders this observation difficult to generalize beyond the scope of the studied patient groups. Average age of CABG patients in both groups was 64.8 (SD 9.0) years with relatively low Euroscore II of $3.5 (\pm 5.1)$. Majority of patients (86 %) were in Killip class I at admission and only 1 patient required cardiopulmonary resuscitation and mechanical ventilation prior to hospital admission. 20 % of left main stenosis were treated by CABG and 44 % ($n = 11$) of CABG treated CTO patients had successful CTO revascularization. An important factor was patients frailty status assessed by biological age and mobility as one of surrogate indicators of successful postprocedural recovery and rehabilitation. This suggest that CABG, a complex and demanding procedure, underwent patients with better clinical status with higher probability of successful outcome. We therefore hypothesize that it is the meticulous patient selection process through the Heart Team that plays the pivotal role in achieving such a high survival rate. Nevertheless, contextualization of our results with the recent literature may contribute to the decision-making process, when selecting appropriate treatments for CTO patients.

There are multiple large-scale registries that reflect the recent trends in the treatment of CTOs globally. The Canadian Multicenter Chronic Total Occlusion Registry has shown that over 64 % of CTO patients were managed medically, 26 % underwent CABG, and 10 % PCI of CTO was performed with a 70 % success rate [3]. The recently published 10-year follow-up results compared patients who have been revascularized early (either by CTO-PCI or CABG) with patients who have not been revascularized, have shown a significantly lower mortality at 10 years for patients after early revascularization [30].

Choosing the appropriate modality of revascularization is a non-trivial task, and the published literature has been conflicting. There have so far been only a few randomized controlled trials (RCTs) focusing on the treatment of CTOs, most notably the EXPLORE trial, the REVASC trial, the EURO-CTO trial, the DECISION-CTO trial, the COMET-CTO

trial, and the awaited ISCHEMIA-CTO sub-study [31]. These have so far failed to demonstrate a definite mortality benefit [10,11] or improvement in cardiac function [32], but have indicated that symptomatic relief and a resulting improved quality of life may be provided by CTO-PCI [33]. CTO-PCI is, however, a complex intervention, not only due to the typical high-risk patient profile but also due to the technical difficulty involved and proficiency required. The creation of dedicated centers and training programs has in recent years provided a promising outlook for the future of the procedure. Recent data from the PROGRESS-CTO registry showed a success rate reaching 90 % with perioperative mortality at approximately 0.4 % [34].

Although mastering PCI techniques in recent years increased the success rate of CTO-PCI, CABG plays an important role in more complex anatomy. After adjustment for baseline variables, CTO-PCI has been associated with an increased risk of all-cause death, cardiac death, MI, and repeat revascularization compared to CABG at a 5-year follow-up. This highlights the need for a tailored approach, using anatomical complexity calculated by SYNTAX score II to find the optimal revascularization strategy. CABG of CTO in SYNTAX score II >8 has been associated with a lower risk of a 5-year composite endpoint compared to PCI [35]. In patients who have multiple CTOs, CABG provides a survival benefit compared to medical therapy alone, and an overall lower risk for repeat revascularization, and major adverse cardiac and cerebral events in comparison with CTO PCI [36].

The subpopulation of patients with MVD and a CTO in whom an acute MI occurs poses a subsequent question of whether further CTO intervention is feasible and provides benefit when culprit vessel PCI is already being performed. Even though the evidence is again conflicting, there could be a benefit for the patient in complete revascularization following an MI, including the CTO. Both CABG and CTO-PCI remain relevant alternatives, with little hard data to guide exact treatment allocation. The EXPLORE trial randomized over 300 STEMI patients with concomitant CTO into early CTO-PCI (within approximately 7 days) compared to conservative treatment. The recently published 10-year outcomes did not show a mortality benefit from early intervention of CTO. Moreover, cardiovascular death occurred significantly more frequently in the CTO-PCI subgroup. Nevertheless, relief of symptoms – dyspnea in particular – was significantly improved in the CTO-PCI subgroup [30,37]. In the aforementioned CULPRIT-SHOCK trial, a non-CTO RCT, recanalization of CTO was attempted in half of the patients with a 1/3 success rate. Importantly, CTO intervention did not improve the overall outcome [27].

There is yet to be an adequately powered, dedicated RCT investigating the various revascularization approaches and timing in CTO patients with MVD and acute MI. [38] Although CABG shows promising long term outcome compared to CTO-PCI in patients without MI, we emphasize that MI represent significant risk factor elevating peri- and postprocedural complications. Limited number of such high-risk patients highlights necessity of multicentre trial with large-volume expert CTO centers to achieve unbiased results. We believe long-term follow-up is required to provide objective results. Based on aforementioned, however, a key element which makes future RCT challenging is the proper patient selection for each treatment modality. We hypothesize that random allocation may not provide desired elucidation and presumably individually tailored approach may bring optimal results from both treatment modalities.

4.1. Limitations

Our study had several limitations that need to be addressed. First, it was a single-center retrospective analysis, and the statistical power of results is therefore limited. Second, we recognize matching samples of CTO patients in PSM analysis could be a source of bias. The attempt to properly match the control group based on sex, age, and type of MI may have resulted in very high-risk patients with CTO having been omitted from the analysis. This is documented by the stark contrast between the

in-hospital mortality rate among all of the 107 patients with CTO (14.0 %), and the subset of 90 patients selected for the matched analysis (6.7 %). However, the character of this analysis was to focus on particular differences between the groups with and without the presence of a CTO, irrespective of age and sex. Another disadvantage of PSM is neglecting many potential confounders. As most influential unmeasured variables we define functional (frailty) status, renal insufficiency and adherence to guidelines recommended treatment beyond hospital stay. Both pharmacotherapy and device-based therapy could have had prognostic impact. Third, there are missing data documenting cardiovascular complications (cardiovascular mortality, MI, stroke, etc.). The retrospective character of data collection in cooperation with the Institute of Health Information and Statistics of the Czech Republic allowed for a reliable assessment of general mortality only. Finally, the good 1-year outcome in CABG treated patients may have been a result of appropriate decisions by the local Heart Team, and its indication criteria in the revascularization selection process. Among others, younger age, lower Killip class at admission and lower number of left main stenosis presumably influenced the outcome.

A prospective validation of our results on a larger cohort is warranted.

4.2. Conclusion

Myocardial infarction in multivessel disease and the presence of chronic total occlusion is associated with high in-hospital and 1-year mortality. We observed no 1-year mortality difference in patients with MI, MVD and a CTO compared to a matched cohort of patients with MI, MVD without CTO. Excellent 1-year survival was observed in patients treated by CABG, irrespective of CTO presence.

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CRedit authorship contribution statement

Dávid Bauer: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Šimon Benjamin Špányi:** Writing – original draft, Visualization, Methodology, Data curation. **Marek Neuberger:** Visualization, Software, Formal analysis, Data curation. **Viktor Kočka:** Writing – review & editing, Supervision. **Petr Tousek:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijcard.2025.132984>.

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Atrial fibrillation's role in MitraClip patient outcomes:
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ORIGINAL ARTICLE

Atrial fibrillation's role in MitraClip patient outcomes: a retrospective analysis of mortality and heart failure hospitalization in a single-centre cohort

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ABSTRACT

OBJECTIVE Atrial fibrillation (AF) is common in patients with mitral regurgitation (MR) undergoing transcatheter edge-to-edge repair (TEER) with MitraClip; however, its impact on procedural hemodynamics and clinical outcomes remains inadequately characterized.

METHODS This retrospective single-center study analyzed 226 high-risk patients who underwent MitraClip implantation between 2010 and 2022. The primary endpoint was time to first heart failure hospitalization. Secondary endpoints included procedural hemodynamics and long-term mortality.

RESULTS AF was observed in 46.9% of the patients and was associated with distinct hemodynamic features, including significantly elevated right (11 vs. 9 mmHg, $P = 0.008$) and left atrial pressures (17 vs. 15 mmHg, $P = 0.023$). Despite similar procedural success rates, patients with AF experienced markedly accelerated time to first HF hospitalization (median 48 vs. 106 weeks, $P = 0.005$). Tricuspid regurgitation at discharge emerged as the strongest predictor of early heart failure hospitalization (HR 1.393, 95% CI: 1.009–1.924, $P = 0.044$). One-year mortality (16.0% vs. 16.7%, $P = 0.899$) and long-term survival remained comparable between groups.

CONCLUSION AF in TEER patients is characterized by elevated atrial filling pressures and substantially accelerated time to heart failure hospitalization, with tricuspid regurgitation at discharge predicting early events. Although these findings indicate the need for more intensive monitoring of patients with AF during the first post-procedural year, comparable survival rates suggest that AF alone should not preclude TEER in otherwise suitable candidates.

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ABBREVIATIONS

AF	= Atrial Fibrillation
ALT	= Alanine Amino transferase
AST	= Aspartate Amino transferase
CABG	= Coronary Artery Bypass Grafting
CI	= Confidence Interval
CRT	= Cardiac Resynchronization Therapy
EF	= Ejection Fraction
GGT	= Gamma-Glutamyl Transferase
HF	= Heart Failure
ICD	= Implantable Cardioverter-Defibrillator
IQR	= Interquartile Range
LA	= Left Atrium/Left Atrial
LBbB	= Left Bundle Branch Block
LV	= Left Ventricular
LVEDD	= Left Ventricular End-Diastolic Diameter
LVESD	= Left Ventricular End-Systolic Diameter
MI	= Myocardial Infarction
MR	= Mitral Regurgitation
NT-proBNP	= N-terminal pro-B-type Natriuretic Peptide
NYHA	= New York Heart Association
OR	= Odds Ratio
PCI	= Percutaneous Coronary Intervention
PG	= Pressure Gradient
RA	= Right Atrium/Right Atrial
RBbB	= Right Bundle Branch Block
RV	= Right Ventricle
TEER	= Transcatheter Edge-to-Edge Repair

1. INTRODUCTION

Mitral regurgitation (MR) is among the most common valvular heart pathologies, affecting an estimated 2% of the general population and significantly higher prevalence in older adults.¹⁻³ The global burden of MR continues to expand as populations age, creating mounting challenges for healthcare systems and necessitating evolving treatment strategies.⁴ Although surgical mitral valve repair or replacement has traditionally been the cornerstone of severe MR treatment, a substantial proportion of patients are deemed unsuitable for conventional open-heart surgery owing to prohibitive surgical risk, often resulting from advanced age, frailty, or significant comorbidities.⁵⁻⁸

The development of transcatheter mitral valve repair techniques, particularly transcatheter edge-to-edge repair (TEER) with the MitraClip system, has significantly advanced the therapeutic options for patients with severe MR who are at high surgical risk. This minimally invasive approach has emerged as a viable alternative for patients who were previously deemed inoperable, resulting in improvements in symptoms, quality of life, and clinical outcomes.⁹ As clinical experience with this technology grows, systematic evaluation of long-term outcomes, procedural complications, and factors influencing therapeutic success becomes increasingly important for optimizing patient selection and management strategies.¹⁰

Among the significant challenges in managing patients with MR is the high prevalence of atrial fibrillation (AF), which frequently coexists with or develops following mitral valve interventions. AF, the most common sustained cardiac arrhythmia, significantly impacts cardiovascular morbidity and mor-

tality through multiple pathophysiological mechanisms including myocardial strain, fibrosis, and inflammation.¹¹ AF represents more than a concurrent arrhythmia; it potentially influences procedural success and long-term clinical outcomes following MitraClip therapy.¹² Although the interaction between AF and MR is well-recognized, the impact of AF on MitraClip outcomes remains incompletely understood, with limited data available in current literature.¹³

By examining the relationship between AF and outcomes in patients undergoing TEER, this study

aimed to (1) evaluate the impact of pre-existing AF at the time to first heart failure (HF) hospitalization after MitraClip implantation, (2) assess the hemodynamic differences between patients with and without AF during the procedure, and (3) determine whether AF independently influences long-term mortality in this high-risk population.

2. METHODS

2.1. STUDY DESIGN AND OBJECTIVES. This single-center retrospective cohort study analyzed consecutive patients who underwent TEER with MitraClip between December 2010 and September 2022 at AGEL Trinec-Podlesí Hospital, Czech Republic. The primary objective was to evaluate the impact of pre-existing AF at the time to first HF hospitalization after MitraClip implantation. Secondary objectives included assessment of the relationship between AF and procedural hemodynamics, comparison of long-term clinical outcomes between AF and non-AF patients, and identification of predictors of early HF hospitalization. The primary endpoint was defined as the time to first HF hospitalization after MitraClip placement, with secondary endpoints encompassing procedural success, in-hospital complications, and all-cause mortality.

2.2. STUDY POPULATION. This analysis used data from our prospectively maintained “Patient Follow-up after MitraClip Registry” at AGEL Trinec-Podlesí Hospital, Czech Republic. The registry systematically collects comprehensive patient data through standardized electronic case report forms, including demographics, cardiovascular risk factors, comorbidities, cardiac status, echocardiographic parameters, procedural details, and clinical outcomes for all patients undergoing transcatheter mitral valve repair with the MitraClip system. The study cohort comprised consecutive patients who underwent index MitraClip implantation between December 2010 and September 2022, with data collection and follow-up extending through December 2023.

Patient were considered eligible for MitraClip intervention, if they had severe symptomatic MR with suitable anatomy, absence of surgical options, and no history of infective endocarditis or mitral stenosis. Pre-existing AF was defined as the presence of AF either in the medical history at admission or documented on admission electrocardiogram, regardless of AF type (paroxysmal, persistent, or permanent).

All patients provided written informed consent for the procedure and participation in the registry, in compliance with the General Data Protection

Regulation. The ethical use of these data for the registry and analyses was approved by the Ethics Committee of AGEL Hospital Trinec-Podlesi (EK 308/22). The study was conducted in accordance with the Declaration of Helsinki.

2.3. TRANSCATHETER EDGE-TO-EDGE REPAIR PROCEDURE. TEER Procedure with MitraClip implantation was performed according to the previously described standard techniques.^{5,14} The procedure was conducted under general anesthesia with anticoagulation and antibiotic prophylaxis. Following transfemoral venous access and transseptal puncture, the MitraClip delivery system was advanced into the left atrium. Under combined fluoroscopic and transesophageal echocardiographic guidance, the device was positioned and deployed to grasp and approximate the mitral leaflets, creating a double-orifice configuration. Additional clips were placed as needed to achieve optimal reduction in MR.

Hemodynamic assessment was performed via direct pressure measurements using calibrated fluid-filled catheters during transseptal puncture before clip deployment. These measurements included right and left atrial pressures as part of the standard procedural protocol.

At our center, the procedure was performed in a dedicated cardiac catheterization hybrid laboratory by a multidisciplinary heart team. Patients typically remained hospitalized for up to 3 days' post-procedure for monitoring. All procedures adhered to current guideline recommendations for TEER.⁹

2.4. FOLLOW UP. All patients underwent standardized follow-up evaluations at 1-, 3-, 6-, and 12-months post-procedure. Clinical assessment, including New York Heart Association functional class and adverse events documentation, was performed at each visit. Transthoracic echocardiography was conducted according to a standardized protocol, with the timing aligned with clinical follow-up visits except at the 3-month timepoint, where it was performed at the physician discretion.

Laboratory evaluations included NT-proBNP, complete blood count, renal and hepatic function tests, lipid panel, and inflammatory markers. Follow-up assessments were conducted either at our center or by referring cardiologists following a standardized protocol, with results systematically recorded in our registry database. Detailed follow-up examination schedules and specific laboratory parameters are provided in [Supplementary Table S1](#).

2.5. STATISTICAL ANALYSIS. All statistical analyses were performed using IBM SPSS Statistics version 29 (IBM Corp., Armonk, NY, USA). Owing to non-normal distribution of the data, as assessed using the Shapiro-Wilk test, all continuous variables were expressed as median (interquartile range [IQR]). Categorical variables were presented as frequencies and percentages.

Between-group comparisons for continuous variables were conducted using the Mann-Whitney *U* test. Categorical variables were compared using the Chi-squared test or Fisher's exact test when expected frequencies were <5. Time to first HF hospitalization was analyzed using the Kaplan-Meier survival analysis, with differences between AF and non-AF groups assessed using the log-rank test. To account for the potential confounders in the time-to-hospitalization analysis, a Cox proportional hazards model was constructed, adjusting for clinically relevant variables including age, sex, left ventricular ejection fraction, mitral and tricuspid regurgitation at discharge, and rhythm status.

Univariate analysis was performed to identify potential predictors of AF. Variables with a *P*-value < 0.1 in the univariate analysis were included in a multivariate logistic regression model to determine independent predictors of AF. The results of the logistic regression were presented as odds ratios (OR) with 95% confidence intervals (CI). A two-tailed *P*-value < 0.05 was considered statistically significant for all analyses.

3. RESULTS

3.1. STUDY POPULATION CHARACTERISTICS.

Among the 226 consecutive patients who underwent TEER, 106 (46.9%) had AF at baseline. The median age was 72 years (IQR: 67-77 years), with higher age in patients with AF (73 years vs. 70 years, *P* = 0.03). Males predominated overall (60.6%), particularly in the AF group (67.9% vs. 54.2%, *P* = 0.035). Major comorbidities included hypertension (84.1%) and coronary artery disease (81.9%) ([Table 1](#)).

Most patients (54.4%) presented in NYHA class III with impaired left ventricular function (median LVEF 32%, IQR: 25-50%). Secondary MR predominated (85.0%; 54.0% ischemic, and 31.0% non-ischemic). At baseline, 86.3% had severe MR (grade 4), with similar distribution between AF and non-AF groups. Complete baseline characteristics stratified by rhythm status are presented in [Table 1](#) and [Supplementary Table S2](#).

TABLE 1 Baseline characteristics of the study population according to the rhythm status (N = 226)

Characteristics	Total (N = 226)	Sinus rhythm (N = 120)	Atrial fibrillation (N = 106)	P-value
Demographics				
Age, years	72 (67-77)	70 (65-77)	73 (69-77)	0.03
Male sex	137 (60.6)	65 (54.2)	72 (67.9)	0.035
Body mass index, kg/m ²	28.9 (25.2-32.2)	28.7 (25.1-31.6)	29.0 (25.9-33.3)	0.251
EuroSCORE II	8.1 (4.1-14.0)	7.5 (4.1-12.1)	8.9 (4.3-17.5)	0.116
Cardiovascular comorbidities				
Hypertension	190 (84.1)	102 (85.0)	88 (83.0)	0.685
Coronary artery disease	185 (81.9)	103 (85.8)	82 (77.4)	0.099
Diabetes mellitus	101 (44.7)	51 (42.5)	50 (47.2)	0.481
Cardiac status				
NYHA functional class				0.8
II	27 (11.9)	16 (13.4)	11 (10.3)	
III	123 (54.4)	64 (53.3)	59 (55.7)	
IV	8 (3.5)	5 (4.2)	3 (2.8)	
Left ventricular ejection fraction, %	32 (25-50)	31 (25-49)	34 (25-50)	0.855
Left atrial diameter, mm	51 (47-57)	50 (46-53)	55 (48-60)	<0.001
Mitral valve characteristics				
Mitral regurgitation etiology				0.984
Primary	34 (15.0)	18 (15.0)	16 (15.1)	
Secondary	192 (85.0)	102 (85.0)	90 (84.9)	
Baseline MR severity, n (%)				0.511
Mild (Grade 1)	0 (0.0)	0 (0.0)	0 (0.0)	
Moderate (Grade 2)	4 (1.7)	1 (0.8)	3 (2.8)	
Moderate-to-severe (Grade 3)	61 (27.0)	37 (30.9)	24 (22.7)	
Severe (Grade 4)	161 (71.2)	82 (68.3)	79 (74.5)	
Tricuspid regurgitation grade	1.0 (1.0-2.0)	1.0 (1.0-2.0)	2.0 (1.0-2.5)	0.022
Right ventricular dysfunction	54 (23.9)	23 (19.2)	31 (29.2)	0.083

Note: Values are presented as median (interquartile range) for continuous variables and n (%) for categorical variables. P-values are derived from Mann-Whitney U test for continuous variables and Chi-squared or Fisher's exact test for categorical variables, as appropriate. NYHA = New York Heart Association; MR = mitral regurgitation.

3.2. PRIMARY ENDPOINT: TIME TO FIRST HEART FAILURE HOSPITALIZATION. Patients with AF experienced earlier HF hospitalization (median 48 weeks [IQR: 16-105 weeks] vs. 106 weeks [IQR: 50-183 weeks], $P = 0.005$) (Fig. 1). However, total hospitalization rates during follow-up were similar (AF: 31.1% vs. non-AF: 33.3%, $P = 0.724$) (Table 2).

3.3. SECONDARY ENDPOINTS: PROCEDURAL HEMODYNAMICS AND AF CHARACTERISTICS. The median procedure duration was 95 min (IQR: 75-130 min). First-generation MitraClip devices were the most common (54.9%), followed by third generation (23.5%). Single-clip implantation sufficed in 48.2% of the cases, whereas 42.9% required 2 clips, as shown in Supplementary Table S3.

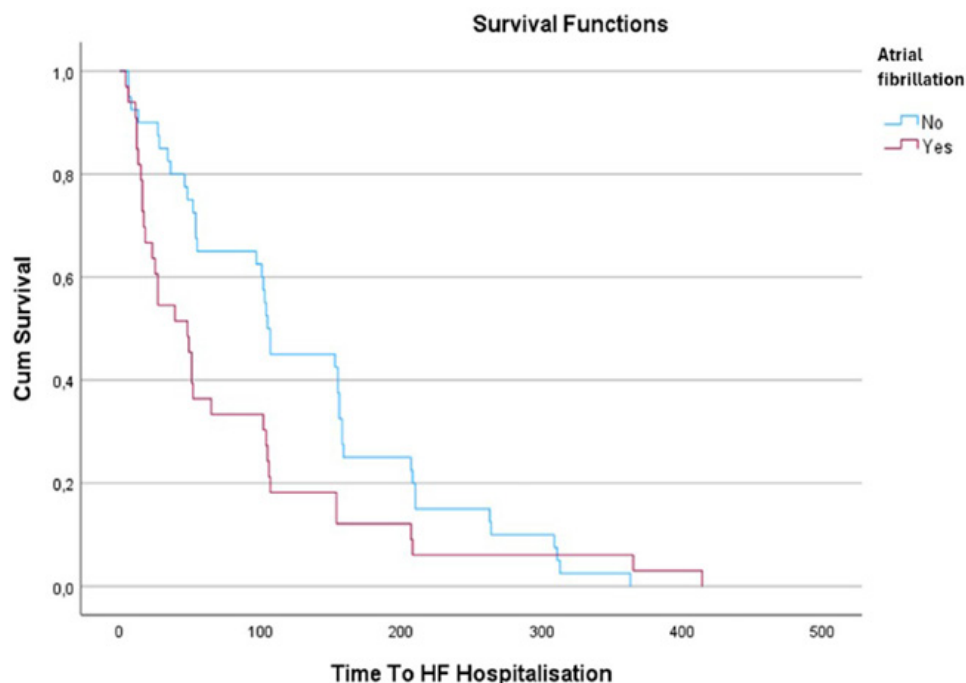
Procedural success, defined as a reduction in MR to moderate or less (grade ≤ 2), occurred in 95.8% of the cases, with comparable rates between AF and non-AF groups (94.3% vs. 97.1%, $P = 0.324$). At discharge, this improvement was maintained with

94.2% of the patients having mild or moderate MR (grade 1-2).

Early complications (≤ 72 h) were infrequent with bleeding (0.4%) and minor vascular complications (24.3%). Later complications (>72 h) included stroke (0.4%), bleeding (3.5%), and acute kidney injury (2.7%). No myocardial infarction or cardiac tamponade occurred during hospitalization. Detailed procedural characteristics and complications are presented in Supplementary Table S2.

Regarding hemodynamic differences, patients with AF showed distinct structural and functional features. Left atrial dimensions were larger (55 mm vs. 50 mm, $P < 0.001$), persisting at discharge (55 mm vs. 49 mm, $P < 0.001$). During the procedures, patients with AF demonstrated higher right atrial (11 mmHg vs. 9 mmHg, $P = 0.008$) and left atrial pressures (17 mmHg vs. 15 mmHg, $P = 0.023$).

Right ventricular dysfunction was more prevalent in patients with AF at baseline (29.2% vs. 19.2%, $P = 0.076$) and discharge (27.4% vs. 15.8%, $P = 0.035$).

FIGURE 1 Kaplan-Meier analysis of the time to first heart failure hospitalization using the atrial fibrillation status

Kaplan-Meier curves showing freedom from heart failure hospitalization after TEER in patients with and without atrial fibrillation. Kaplan-Meier curves demonstrating time to first heart failure hospitalization in patients with (red line, n = 106) and without (blue line, n = 120) atrial fibrillation following TEER. Log-rank test: $\chi^2 = 3.632$, $P = 0.057$

Tricuspid regurgitation was more severe in patients with AF (grade 2.0 vs. 1.0, $P = 0.021$). Complete hemodynamic and echocardiographic parameters are provided in [Supplementary Table S2](#).

3.4. LONG-TERM OUTCOMES AND PREDICTORS.

One-year mortality was comparable between the

groups (AF: 16.0% vs. non-AF: 16.7%, $P = 0.899$). Cardiovascular death predominated in both groups (AF: 56.3% vs. non-AF: 45.0%, $P = 0.152$), with similar non-cardiovascular mortality (29.7% vs. 30.0%, $P = 0.798$). Detailed clinical outcomes are presented in [Table 2](#).

TABLE 2 Clinical outcomes according to the atrial fibrillation status

Parameter	Total	Sinus rhythm	Atrial fibrillation	P-value
Heart failure hospitalization				
Time to first HF hospitalization, weeks	97 (27-156)	106 (50-183)	48 (16-105)	0.005
HF hospitalization during follow-up, n (%)	73 (32.3)	40 (33.3)	33 (31.1)	0.724
Follow-up and mortality				
Median follow-up, weeks	133 (52-260)	132 (44-261)	134 (53-234)	0.846
Total deaths, n (%)	124 (54.9)	60 (50.0)	64 (60.4)	0.121
Time to death, weeks	106 (50-209)	108 (34-209)	106 (52-209)	0.891
One-year mortality, n (%)	37 (16.4)	20 (16.7)	17 (16.0)	0.899
Cause of death				
Cardiovascular death, n (%)	62 (50.8)	27 (45.0)	36 (56.3)	0.152
Non-cardiovascular death, n (%)	37 (29.8)	18 (30.0)	19 (29.7)	0.798

Note: Data are presented as median (interquartile range) for continuous variables and n (%) for categorical variables. Time to first HF hospitalization was compared using the Mann-Whitney U test; categorical variables were compared using the Chi-squared test or Fisher's exact test when expected frequencies were <5 . HF = Heart Failure; FU = Follow-up.

Cox regression identified tricuspid regurgitation at discharge as the strongest predictor of early HF hospitalization (HR 1.393, 95% CI: 1.009-1.924, $P = 0.044$). Neither rhythm status (HR 1.026, 95% CI: 0.612-1.721, $P = 0.921$) nor residual MR (HR 1.019, 95% CI: 0.742-1.399, $P = 0.907$) independently predicted outcomes. Complete multivariate analysis results are shown in [Table 3](#).

4. DISCUSSION

Our study demonstrated that AF significantly influences the timing of HF hospitalization following TEER, and patients with AF experience this event approximately 58 weeks earlier than those in sinus rhythm. This acceleration of HF events appears to be mechanistically linked to the elevated atrial pressure observed in patients with AF, with both right (11 mmHg vs. 9 mmHg, $P = 0.008$) and left atrial pressures (17 mmHg vs. 15 mmHg, $P = 0.023$) being significantly higher than in those with sinus rhythm. Although AF was associated with earlier HF hospitalization, we found no significant impact on mortality at one year (16.0% vs. 16.7%) or during long-term follow-up.

These findings align with and diverge from the previous studies in important ways. Recent meta-analyses have demonstrated that AF presence is associated with higher rates of all-cause mortality (OR 1.55) and HF hospitalizations (OR 1.3) after TEER.¹⁵ The Transcatheter Mitral Valve Interventions (TRAMI) registry¹⁶ reported AF as a significant predictor of poor outcomes, with 53.1% four-year mortality, while the ACCESS-Europe A Two-Phase Observational Study of the MitraClip System in Europe (ACCESS-EU) registry¹⁷ showed higher early mortality in patients with AF.

The disparity between our mortality findings and previous studies may reflect the important

differences in patient populations and management strategies. Our cohort demonstrated more advanced structural heart disease compared to previous studies, with a higher proportion of secondary MR (85.0% vs. 77.1% in ACCESS-EU) and more severely impaired left ventricular function (median LVEF 32% vs. 60.0% $\pm$ 10.1% in EVEREST II).⁵ The MITRA-FR trial,¹⁷ which enrolled a similarly high-risk population, also found no significant difference in mortality based on the rhythm status, suggesting that in advanced disease states, the impact of AF may be overshadowed by the underlying structural heart disease.

The mechanistic link between AF and earlier HF hospitalization appears multifaceted. Recent findings from the COAPT trial demonstrated that patients with AF had significantly larger left atrial volumes and mitral valve orifice areas, suggesting an important atrial contribution to functional MR.¹⁸ This aligns with the emerging evidence that atrial functional MR represents a distinct entity that is frequently related to AF, characterized by isolated mitral annular dilatation and inadequate leaflet adaptation (Sanna et al., 2023).¹⁹ The elevated filling pressures observed in our patients with AF provide a potential mechanism for their accelerated pathway to HF hospitalization, supported by recent work indicating that intra-procedural hemodynamics provide important prognostic information.²⁰

Our finding that the severity of tricuspid regurgitation independently predicted early hospitalization adds to the growing evidence on the importance of right heart dysfunction in this population. Recent studies have demonstrated that residual tricuspid regurgitation after TEER carries important prognostic implications,²¹ suggesting the need for careful attention to right-sided heart function in these patients.

The absence of mortality differences in our cohort, despite earlier HF hospitalization in patients with AF, likely reflects several important factors. Recent advances in HF management and rhythm control strategies since the early registry studies have improved outcomes in patients with AF, with evidence showing that catheter ablation of concomitant AF can significantly improve the survival in TEER patients compared to medical therapy alone.²² Our center's protocol for intensive post-procedure monitoring and optimization of medical therapy may have further mitigated the impact of AF on survival. Additionally, successful TEER improves left atrial function and reduces arrhythmic burden,²³ suggesting benefits beyond pure hemodynamic improvement.

TABLE 3 Cox proportional hazards analysis for the time to first heart failure hospitalization

Variable	Hazard ratio	95% CI	P-value
Age	0.991	0.963-1.020	0.539
Sex (male)	0.682	0.399-1.165	0.161
Atrial fibrillation	1.026	0.612-1.721	0.921
LV ejection fraction (%)	0.982	0.964-1.001	0.059
LA diameter (mm)	1.027	0.995-1.060	0.1
MR etiology (primary)	0.89	0.745-1.404	0.723
MR discharge severity	1.019	0.742-1.399	0.907
Tricuspid regurgitation at discharge	1.393	1.009-1.924	0.044

Note: CI = confidence interval; LA = left atrial; LV = left ventricular; MR = mitral regurgitation.

Third, our center's protocol for intensive post-procedure monitoring and optimization of medical therapy may have mitigated the impact of AF on survival.

These findings align with previous systematic review evidence²⁴ and evidence demonstrating high success rates of catheter ablation in improving AF and functional MR in patients with HF with preserved ejection fraction.²⁵ As demonstrated in the EVEREST II 5-year study,²⁶ the durability of MR reduction appears to be consistent regardless of the rhythm status, suggesting that successful initial correction of MR may be more important than baseline rhythm in determining long-term survival. This aligns with the findings from the MITRA-FR trial,²⁷ where rhythm status did not significantly impact long-term outcomes in a similarly high-risk population with advanced structural heart disease.

Our procedural success rate (95.8%) and complication profile align favorably with those of major international registries, including ACCESS-EU (91.9%) and TRAMI (95.8%). This suggests that in experienced centers, the MitraClip procedure maintains its safety profile regardless of the rhythm status. However, the earlier occurrence of HF hospitalization in patients with AF underscores the need for tailored post-procedural management strategies.

These findings have important clinical implications. The significantly shorter time to HF hospitalization in patients with AF (48 weeks vs. 106 weeks) suggests the need for more intensive monitoring of this subgroup, particularly during the first year after the procedure. The combination of elevated filling pressures and significant tricuspid regurgitation may identify patients who are at the highest risk for early events. Furthermore, the similar mortality rates between AF and non-AF patients suggest that AF alone should not be considered to discourage the use of TEER in otherwise suitable candidates.

5. LIMITATIONS

Some limitations of our study should be acknowledged. First, this was a single-center retrospective analysis, which may limit the generalizability of our findings. Second, the relatively small sample size, particularly in subgroup analyses, may have limited our ability to detect smaller differences in outcomes. Third, our definition of AF included all types (paroxysmal, persistent, or permanent) at the baseline, and we did not systematically assess new-onset post-procedural AF as continuous rhythm monitoring was not part of our follow-up protocol. Future studies incorporating systematic rhythm monitoring

could provide valuable insights into the incidence and impact of new-onset AF after TEER. Fourth, although our follow-up was sufficient to assess the early outcomes and one-year mortality, longer follow-up might reveal additional patterns, particularly regarding the relationship between AF and later clinical events. Finally, as with all observational studies, unmeasured confounders might have influenced our results despite careful statistical adjustment.

6. CONCLUSIONS

In this retrospective study of 226 high-risk patients undergoing MitraClip implantation, we found that AF was associated with significantly elevated atrial filling pressures and accelerated time to first HF hospitalization (48 weeks vs. 106 weeks, $P = 0.005$). Tricuspid regurgitation at discharge emerged as the strongest predictor of early HF hospitalization, suggesting that the impact of AF is mediated through right-sided heart dysfunction and elevated filling pressures. Despite these differences, we observed no significant difference in one-year or long-term mortality between the groups.

These findings highlight the need for closer monitoring of patients with AF after MitraClip implantation, particularly those with significant tricuspid regurgitation, during the first year after the procedure. The similar long-term survival rates suggest that AF alone should not preclude TEER in otherwise suitable candidates. Further research is warranted to optimize the treatment approaches for this complex patient population.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work the authors used Claude 3.5 Sonnet (Anthropic) to improve language clarity. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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KEYWORDS Mitral regurgitation, MitraClip, Transcatheter mitral valve repair, Atrial fibrillation, Heart failure, Mortality

APPENDIX A. SUPPLEMENTARY

DATA Supplementary data related to this article can be found at <https://doi.org/10.1016/j.hjc.2025.03.004>.

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Comparison of the efficacy of intravascular lithotripsy and rotational atherectomy as adjunctive therapy before drug-eluting stent implantation in calcified coronary lesions (CCS- Coronary Calcification Study)

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RESEARCH

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Comparison of the efficacy of intravascular lithotripsy and rotational atherectomy as adjunctive therapy before drug-eluting stent implantation in calcified coronary lesions (CCS– Coronary Calcification Study)

Leos Pleva^{1,2*}, Pavel Kukla¹ and Adela Konde^{3,4}

Abstract

Background This prospective randomized study compares the efficacy of novel intravascular lithotripsy (IVL) to the standard preparation of calcified coronary lesions based on rotational atherectomy (RA).

Methods A total of 50 patients with 52 calcified lesions were enrolled in the study and randomized 1:1 to be treated with IVL or RA followed by drug-eluting stent (DES) implantation. The procedural success was chosen as a primary endpoint and the 12-month late lumen loss (LLL) as measured by quantitative coronary angiography, the incidence of binary in-stent restenosis (ISR), 12-month major adverse cardiac events (MACE) and target lesion failure (TLF) served as secondary angiographic and clinical endpoints.

Results Procedural success was achieved in 21 patients (84.0%) in the IVL group and in 24 patients (96%) in the RA group ($p = 0.349$). The secondary endpoints, including 12-month LLL (0.12 mm [IQR: – 0.06; 0.68] vs. 0.61 mm [IQR: 0.22; 0.72]; $p = 0.084$), the incidence of 12-month binary ISR (11.1% vs. 8.0%; $p > 0.999$), MACE (18.5% vs. 8.0%; $p = 0.422$), TLR (14.8% vs. 8.0%; $p = 0.670$) or TLF (18.5% vs. 8.0%; $p = 0.422$) did not show significant differences between the IVL and RA groups.

Conclusion Despite different approaches to the treatment of calcified coronary lesions, both therapeutic techniques achieved similar procedural, angiographic and clinical results. (ClinicalTrials.gov NCT04428177).

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Background

Calcified coronary lesions pose a significant challenge during percutaneous coronary intervention (PCI), often resulting in suboptimal procedural outcomes [1]. These lesions have been identified as independent predictors of PCI failure and adverse cardiovascular events. The extent of coronary artery calcification increases with age, the presence of cardiovascular risk factors, and associated comorbidities. Current plaque-modifying devices primarily utilize mechanisms such as tissue compression or debulking to fracture calcified plaques, aiming to enhance stent deployment and optimize procedural outcomes [1].

Coronary intravascular lithotripsy (IVL) represents an innovative approach that differs fundamentally from conventional methods. By delivering unfocused, circumferential, pulsatile mechanical energy, IVL effectively disrupts calcium deposits within the target lesion, ensuring safe and controlled plaque modification.

While several registries and small randomized trials have evaluated IVL or rotational atherectomy (RA) independently, few studies have provided randomized, long-term angiographic and clinical comparisons between IVL and RA.

Methods

Aim

This prospective, randomized pilot study was designed to compare the efficacy of intravascular lithotripsy with the standard approach of rotational atherectomy for the preparation of calcified coronary lesions prior to drug-eluting stent (DES) implantation.

Patients

The study included patients over 18 years of age with with $\geq 70\%$ diameter stenosis (%DS) and severe coronary calcification (defined as the presence of fixed radiopacities detected without cardiac motion before contrast injection, compromising both sides of the arterial lumen) [2] who were treated at the Department of Internal Medicine and Cardiology of the University Hospital Ostrava, between 2020 and 2024.

The exclusion criteria comprised comorbidities implying expected survival of < 12 months or limiting the feasibility of performing follow-up coronarography (e.g., advanced renal failure). Renal insufficiency was defined as chronic kidney disease stage 3b with estimated glomerular filtration of 30–44 ml/min/1.73m². Patients requiring bypass grafts or those with lesions in highly tortuous vessels where intervention was technically unfeasible were also excluded.

The procedural success was defined as the ability of IVL or RA to enable implantation and satisfactory stent expansion (residual diameter stenosis $< 50\%$ after

stenting) with no evidence of in-hospital MACE (major adverse cardiac events; cardiovascular death, recurrence of non-fatal myocardial infarction [MI], or need for repeat target lesion revascularisation [TLR]) and it served as the primary endpoint [2].

Secondary angiographic and clinical end-points included 12-month late lumen loss (LLL) as measured by quantitative coronary angiography (QCA) [3, 4], the incidence of binary in-stent restenosis ($\geq 50\%$ DS), the cumulative incidence of 12-month MACE and target lesion failure (TLF; cardiovascular death, target vessel-related MI or ischemia-driven TLR) [4].

The patients were randomized in a 1:1 ratio to treatment by intravascular lithotripsy or by rotational atherectomy, both of which were followed by the implantation of a drug-eluting stent (DES). The random permuted block method was utilised in order to effect randomisation.

Intervention

PCI was performed under standard conditions through radial or femoral access under X-ray control (Axiom X-ray imaging system *Siemens AG, Forchheim, Germany*). Adequate anticoagulation was achieved by administering unfractionated heparin with a target ACT of 250–300 s.

A Shockwave system (*Shockwave Medical, Inc, Santa Clara, USA*) was used for intravascular lithotripsy. An IVL balloon catheter with a diameter equal to the reference diameter of the target vessel was used for the procedure. After inflation of the balloon to 4 atmospheres, the required number of lithotripsy cycles (up to 8×10) was applied, followed by balloon inflation up to 6 atm to achieve its full expansion. The final number of pulses used was left to the operator's discretion.

Rotational atherectomy was performed using the Rotablator™ system (*Boston Scientific, Marlborough, MA, USA*). Once the guide wire (RotaWire™) was introduced, a relatively small burr (1.25–1.5 mm) was used to modify the plaque. The rotational speed ranged between 135 and 180,000 rpm with a rinse comprising an infusion of 500 ml of saline + 5 mg of nitrate + 5,000 IU of heparin.

Based on the results of IVL or RA, pre-dilation with non-compliant or scoring/cutting balloon catheters was performed if necessary and subsequently, an everolimus-eluting stent (Synergy™; by Boston Scientific, Marlborough, MA, USA or Xience™; by Abbott Laboratories, North Chicago, IL, USA) was implanted. Following the intervention, the patients received standard dual antiplatelet therapy for 6 months.

Patient follow-up

The patients were clinically examined on the Day of hospital discharge, at the Months 6 and 12 after the procedure and angiographic follow-up was performed at Month 12 (± 2 months) unless clinically indicated earlier.

All deaths were considered cardiac unless a non-cardiac cause was clearly demonstrated. Acute myocardial infarction was defined according to the ESC fourth universal definition of MI [5].

Angiographic examination

Angiographic imaging was performed in the appropriate orthogonal projections to avoid potential shortening or overlapping of the segment and lateral branches. Analogous projections were used in the 12-month coronary angiography. The angiographic parameters were evaluated offline by independent blinded investigators using the syngo Quantification software (Siemens AG, Forchheim, Germany).

The in-segment portions of the lesions were evaluated (± 5 mm from the proximal and distal edges), recording the following parameters: minimum lumen diameter (MLD), reference lumen diameter ($\text{RefD} = \frac{1}{2} [\text{proximal} + \text{distal diameter}]$), acute gain (AG), lesion length, percent diameter stenosis (%DS), and late lumen loss ($\text{LLL} = \text{MLD}_{\text{after intervention}} - \text{MLD}_{\text{control}}$); binary in-stent restenosis was defined as a recurrent $\geq 50\%$ DS narrowing in the stented segment [4].

Table 1 Baseline demographic, clinical and angiographic characteristics

	IVL	RA	<i>p</i>
Patients/lesion (n)	25/27	25/25	
Demographic and clinical parameters			
Male/female (n)	17/8 (68.0%/32.0%)	20/5 (80.0%/20.0%)	0.679 [§]
Age (years)	71 (64; 78) [†]	72 (68; 78) [†]	0.420 [*]
Ever smoked (n)	9 (36.0%)	8 (32.0%)	> 0.999 [§]
Diabetes mellitus (n)	10 (40.0%)	11 (44.0%)	> 0.999 [§]
Renal insufficiency (n)	2 (8.0%)	1 (4.0%)	> 0.999 [§]
LV EF (%)	55 (50; 60) [†]	55 (45; 60) [†]	0.409 [§]
CABG (n)	5 (20.0%)	3 (12.0%)	0.715 [§]
Previous MI (n)	8 (32.0%)	14 (56.0%)	0.227 [§]
2VD/3VD (n)	18 (72.0%)	21 (84.0%)	0.682 [§]
STEMI/NSTEMI (n)	4 (16.0%)	4 (16.0%)	> 0.999 [§]
Lesion and periprocedural parameters			
Ostial lesions (n)	4 (14.8%)	4 (16.0%)	> 0.999 [§]
Bifurcation (n)	3 (11.1%)	2 (8.0%)	> 0.999 [§]
Cutting predilatation (n)	16 (59.2%)	13 (52.0%)	0.664 [§]
NC postdilat (atm)	20 (18; 24) [†]	20 (18; 22) [†]	0.326 [*]
2nd stent (n)	15 (55.6%)	20 (80.0%)	0.298 [§]

The values represent absolute and relative frequencies (%) or medians and the interquartile ranges[†]; CABG means coronary artery bypass graft; ISR, in-stent restenosis; IVL, intravascular lithotripsy; LV EF, ejection fraction of left ventricle; MI, myocardial infarction; NC, non-compliant; NSTEMI, myocardial infarction without ST elevation; RA, rotational atherectomy; STEMI, myocardial infarction with ST elevation and 2VD/3VD- two or three vessel disease; The *p*-value was obtained with the Fisher's exact test[§] or the Mann-Whitney test^{*}

Statistical analysis

Numerical variables are represented with medians and interquartile ranges. Categorical variables are represented as absolute and relative frequencies (%). Between-group differences are analysed with the Mann-Whitney test, the Fisher's exact test, or the log-rank test as appropriate. The event-free survival is visualized with the Kaplan-Meier curves. The significance level was set to 0.05 and the statistical analysis was performed in R software (version 4.4.1).

Results

The course of the study is shown in the CONSORT study flow diagram (Fig. 1). A total of 50 patients with 52 calcified lesions were enrolled in this pilot study ($n=25$ in each group). Baseline demographic, clinical, and angiographic characteristics are provided in Table 1. No significant differences between the two treatment groups were detected in the basic demographic parameters, clinical risk factors, extent of the coronary disease, lesions, or periprocedural characteristics.

Procedural success was achieved in 21 patients (84.0%) in the IVL group and in 24 patients (96.0%) in the RA group ($p=0.349$). Two lesions in the IVL arm required adjunctive rotational atherectomy prior to lithotripsy due to the inability to cross the lesion with the IVL balloon, indicating a higher degree of calcific burden and lesion complexity. Conversely, in one case within the RA arm, intravascular lithotripsy was subsequently employed to address significant stent underexpansion, with a residual diameter stenosis of 58% following stent implantation.

The necessity for crossover between the groups was considered as a procedural failure. Two in-hospital MACE were reported in the IVL group: in one patient, a coronary artery rupture with tamponade and need for surgical revision occurred; the other patient developed a periprocedural MI due to side branch occlusion (ramus diagonals). Despite the absence of clinically significant complications in the RA group, the observed difference between the groups did not reach statistical significance ($p=0.491$) [Table 2].

Clinical data from the 12-month follow-up examination were obtained for all patients. The 12-month QCA was performed in 22 patients with 24 lesions in the IVL group (88.9%) and in 22 patients with 22 lesions in the RA group (88.0%).

The baseline, post-PCI and 12-month angiographic parameters QCA angiographic parameters are provided in Table 3. The distribution of LLL is displayed in Fig. 2. The secondary angiographic endpoints, including 12-month LLL (0.12 mm [IQR: -0.06; 0.68] vs. 0.61 mm [IQR: 0.22; 0.72]; $p=0.084$) and the incidence of 12-month binary ISR (11.1% vs. 8.0%; $p>0.999$), did not show significant differences between the IVL and RA

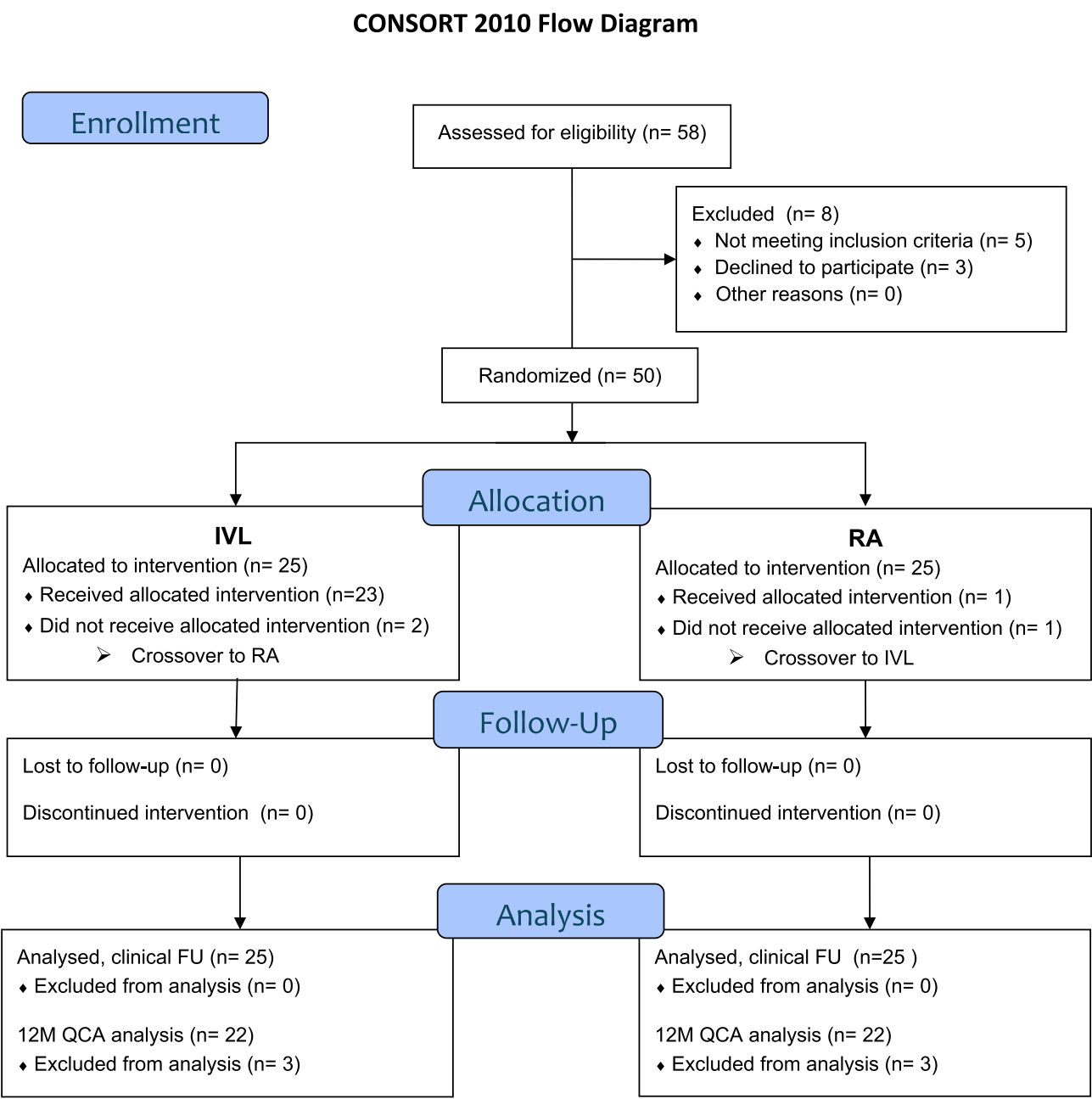


Fig. 1 CONSORT 2010 Flow Diagram

Table 2 Procedural success

	IVL	RA	<i>p</i>
Patients/lesions (n)	25/27	25/25	---
Procedural success	21 (84.0%)	24 (96.0%)	0.349
In-hospital MACE	2 (8.0%)	0 (0.0%)	0.491

The values represent absolute and relative frequencies (%); IVL, intravascular lithotripsy; MACE, major adverse cardiovascular events and RA, rotational atherectomy. The p-value was obtained with the Fisher's exact test

groups. The occurrence of 12-month MACE (18.5% vs. 8.0%; $p=0.422$), TLR (14.8% vs. 8.0%; $p=0.670$) or TLF (18.5% vs. 8.0%; $p=0.422$) suggested similar trends in both groups (Table 4), either. The estimates of event-free survival (EFS) are presented in Fig. 3. The log-rank test revealed comparable patterns in both groups with regard to EFS (time to MACE, $p=0.182$).

Table 3 Baseline, post-PCI and 12-month QCA angiographic parameters

	IVL	RA	p
Patients/lesions (n)	25/27	25/25	---
Baseline:			
Lesion length (mm)	10.68 (8.88; 24.91) [†]	16.22 (9.02; 26.43) [†]	0.517 [*]
RefD (mm)	2.67 (2.52; 3.42) [†]	2.66 (1.97; 2.80) [†]	0.129 [*]
MLD (mm)	0.65 (0.46;0.71) [†]	0.45 (0.33; 0.59) [†]	0.108 [*]
DS (%)	78 (73; 86) [†]	84 (77; 88) [†]	0.296 [*]
Post PCI:			
RefD (mm)	3.30 (2.92; 3.83) [†]	3.34 (3.06; 3.49) [†]	0.509 [*]
MLD (mm)	2.77 (2.27; 3.36) [†]	2.71 (2.32; 3.05) [†]	0.652 [*]
DS (%)	18 (7;25) [†]	19 (13;22) [†]	0.741 [*]
Acute Gain (mm)	2.00 (1.78; 2.41) [†]	2.33 (2.03; 2.75) [†]	0.063 [*]
12-month QCA:			
RefD (mm)	3.25 (2.66; 3.64) [†]	3.16 (2.73; 3.39) [†]	0.410 [*]
DS (%)	22 (12; 33) [†]	32 (20; 39) [†]	0.176 [*]
MLD (mm)	2.60 (1.90; 2.96) [†]	2.18 (1.71; 2.51) [†]	0.180 [*]
LLL (mm)	0.12 (−0.06; 0.68) [†]	0.61 (0.22; 0.72) [†]	0.084 [*]
Binary ISR, n (%)	3 (11.1%)	2 (8.0%)	> 0.999 [‡]

The values represent absolute and relative frequencies (%) or medians and interquartile ranges[†]

DS means diameter stenosis; IVL, intravascular lithotripsy; ISR, in-stent restenosis; LLL, late lumen loss; MLD, minimal lumen diameter; QCA, quantitative control angiography; RA, rotational atherectomy and RefD, reference lumen diameter. The p-value was obtained with the Fisher's exact test[‡] or the Mann-Whitney test^{*}

Discussion

Table 4 12-month clinical follow-up

	IVL	RA	p
Patients (n)	25	25	
MACE	5 (18.5%)	2 (8.0%)	0.422
CV death	0 (0.0%)	0 (0.0%)	---
MI	1 (4.0%)	0 (0.0%)	> 0.999
TLR	4 (14.8%)	2 (8.0%)	0.670
TLF	5 (18.5%)	2 (8.0%)	0.422
2nd MACE	1 (4.0%)	0 (0.0%)	> 0.999
Non-CV death	1 (4.0%)	0 (0.0%)	> 0.999

The values represent absolute and relative frequencies (%). CV means cardiovascular; IVL, intravascular lithotripsy; MACE, major adverse cardiovascular events; MI, myocardial infarction; RA, rotational atherectomy ant TLR, target vessel revascularisation. The p-value was obtained with the Fisher's exact test

Percutaneous coronary intervention (PCI) of calcified coronary lesions remains a clinical challenge, often resulting in suboptimal procedural outcomes and an increased incidence of MACE [1]. Effective treatment necessitates optimal lesion preparation prior to the implantation of the coronary stent, employing strategies such as predilation with high-pressure non-compliant balloon catheters, modifying (cutting or scoring) balloons, or ablation techniques like RA and orbital atherectomy. Unlike RA, which primarily targets superficial calcified plaques, intravascular lithotripsy (IVL) effectively addresses both superficial and deeply calcified plaques [1].

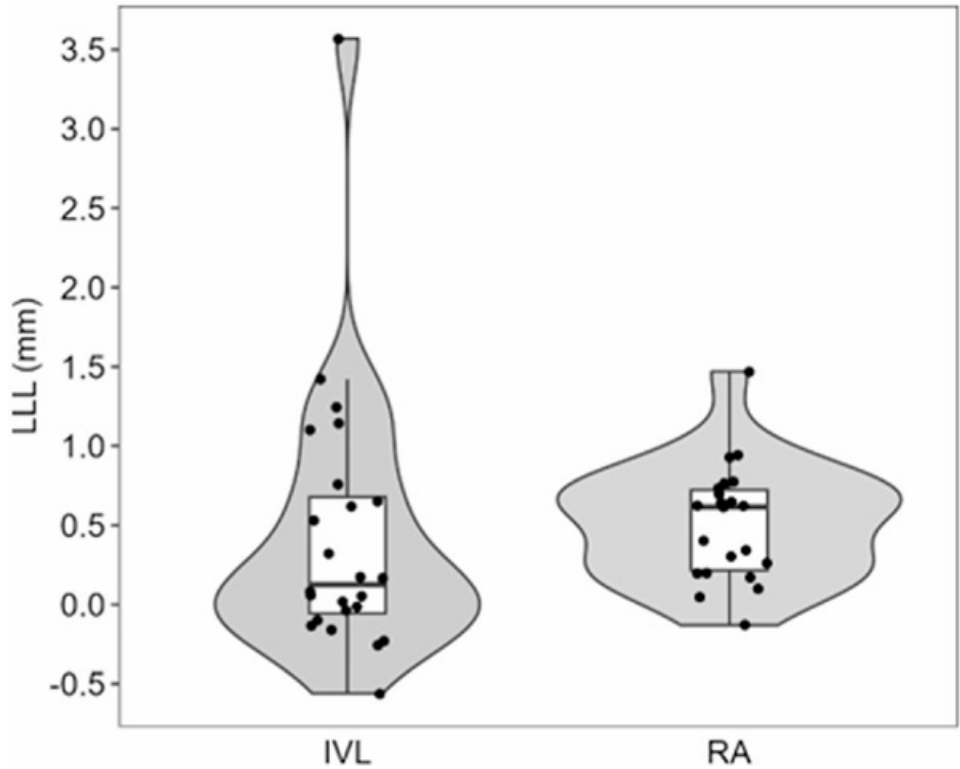


Fig. 2 Combined boxplot and violin plot of the LLL distribution

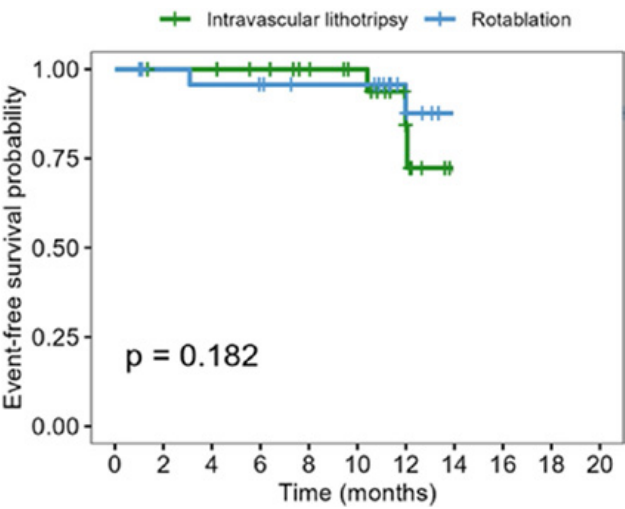


Fig. 3 Event-free survival

The efficacy of RA or IVL has been the focus of several clinical studies. The findings of the most significant studies are displayed in Table 5. The Rotaxus study, which compared RA to standard lesion pre-dilation before paclitaxel-eluting stent (PES) implantation, revealed a higher early acute gain in the RA group but demonstrated significantly worse LLL at 9 months ($p=0.04$). Importantly, differences in ISR, MACE, and TLR were not statistically significant. These results were consistent in the two-year follow-up, indicating no long-term clinical advantage of RA in this setting [6, 7].

The more recent Prepare-Calc study provided further insights into lesion preparation, comparing RA with cutting and scoring balloons (both followed by sirolimus-eluting stent). RA demonstrated a higher procedural

success rate ($p=0.0001$) and non-inferiority in 9-month LLL ($p=0.02$), while the clinical outcomes, including TLR ($p=0.17$) and target vessel failure (TVF; $p=0.78$), were comparable between the groups [8].

In our study, a trend toward lower 12-month late lumen loss (LLL) was observed in the IVL group, with a p-value of 0.084. However, it is indeed a non-significant result and should therefore be interpreted with a caution. A plausible underlying mechanism may involve the ability of intravascular lithotripsy to disrupt not only superficial but also deeper layers of calcified plaque, thereby reducing the constrictive forces acting on the implanted stent. Conversely, the observation of negative LLL in some cases in the IVL group may reflect positive remodeling of the fractured calcified plaques, which could predispose to late stent malapposition.

On the other hand, we hypothesize that the higher (though not statistically significant) need for a second stent in the RA group may be due to more extensive (longer) dissections following rotational atherectomy.

The safety and efficacy of IVL have been evaluated in the Disrupt CAD I-IV studies, which highlighted its ability to achieve minimal residual DS% after stent implantation ($7.8\pm7.1\%$) and a high procedural success rate (92.2%) [2, 9]. In the Disrupt CAD III and IV studies, freedom from 30-day MACE was 92.4%, while 12-month MACE rates were 13.8% and 9.4%, respectively, with comparable reductions in target lesion failure (TLF) [10, 11].

We used angiographic inclusion criteria consistent with the Disrupt CAD studies. Only lesions classified as having severe calcification were included in our study. We also adopted the $<50\%$ residual stenosis threshold based

Table 5 Summary of the most important studies

Study (year)	Type of study	Patients (n)	Treatment	Results
Rotaxus (2013,2016) ^{6,7}	randomised	240	RA vs. standard predilatation	AG: 1.5 ± 0.43 vs. 1.44 ± 0.49 mm; $p=0.01$ 9-month LLL: 0.44 ± 0.58 mm vs. 0.31 ± 0.52 mm; $p=0.04$ TLR 11.7% vs. 12.5% ; $p=0.84$ MACE: 24.2% vs. 28.3% ; $p=0.46$ 2-year MACE: 29.4% vs. 34.3% ; $p=0.47$
Prepare-Calc (2018) ⁸	randomised	200	RA vs. cutting or scoring balloon	Procedural success: 95% vs. 81% ; $p=0.0001$ 9-month LLL: 0.22 ± 0.40 mm vs. 0.16 ± 0.39 mm; $p=0.21$, $p=0.02_{\text{for NI}}$ TVF: 6% vs. 8% ; $p=0.78$
Disrupt CAD III (2022) ¹⁰	registry	431	IVL compared with Orbital II study	Procedural success: 92.4% (lower 1-sided 95%CI: 90.2%) vs. 83.4% ; $p<0.0001$ 30-day MACE free: 92.2% (lower 1-sided 95%CI: 89.9%) vs. 84.4% ; $p<0.0001$
ROTA.shock (2023) ¹²	randomised	70	RA vs. IVL	MSA: 6.60 mm ² (95%CI: $5.66\text{--}7.54$) vs. 6.10 mm ² (95%CI: $5.32\text{--}6.87$); $\Delta -0.50$ mm ² (95%CI: $-1.52\text{--}0.52$); for NI margin: -1.60 mm ² Stent expansion: $0.83\pm0.10\%$ vs. $0.82\pm0.11\%$; $p=0.79$
ROLLER COASTR-EPIC22 (2025) ¹³	randomised	171	RA vs. IVL vs. ELCA	Stent expansion: IVL vs. RA: $\Delta: -0.8$; 95%CI: -6.5 to 4.8 ; ELCA vs. RA: $\Delta: -6.1$; 95%CI: -11.9 to -0.4 ; for NI margin -7%

AG: means acute lumen gain; ELCA, excimer laser coronary angioplasty; IVL, intravascular lithotripsy; LLL, late lumen loss; MACE, major adverse cardiovascular events; MSA, minimal stent area; NI, non-inferiority; RA, rotational atherectomy; TLR, target vessel revascularisation and TVF, target vessel failure

on the Disrupt CAD studies, which define procedural success in this manner. Using similar criteria enables comparability with previously published data.

Contrary, in our study, the IVL arm exhibited a slightly higher 12-month MACE rate (18.5%), primarily driven by the need for TLR (14.8%), but this difference appeared to be statistically insignificant when compared to the RA arm ($p=0.422$). All TLRs in our study were ischemia-driven. Two in-hospital MACE were observed in the IVL group. The first involved a coronary artery rupture that did not occur during the lithotripsy itself, but rather during the subsequent post-dilatation with a non-compliant balloon, which was necessitated by a persistent significant waist. Emergency pericardiocentesis and surgical repair were required. The patient survived without long-term sequelae. It may be hypothesized that the use of a higher number of lithotripsy pulses could have mitigated this complication. The second event concerned the occlusion of a calcified side branch ostium, which could not be protected with a guidewire. This likely resulted from the rupture and displacement of a calcified plaque during the lithotripsy procedure. We believe that the slightly higher, though statistically insignificant, occurrence of MACE, despite a trend toward lower 12-month LLL, is primarily attributable to the two periprocedural complications mentioned above, both of which occurred in the IVL group.

Recently published the ROTA.shock trial had a similar setting as our study (i.e., the authors compared IVL with RA); however, this study with 70 patients randomized 1:1 assessed the immediately postprocedural outcomes. They found IVL to be non-inferior to RA in achieving minimal stent area (MSA) and stent expansion ($p=0.79$) [12]. These results support IVL as an effective alternative or adjunct to RA for calcified lesion treatment.

In the ROLLER COASTR-EPIC22 study, IVL was found to be non-inferior to RA in terms of stent expansion (Δ : -0.8; 95%CI: -6.5 to 4.8). In contrast, excimer laser coronary angioplasty (ELCA) did not demonstrate non-inferiority compared to RA (Δ : -6.1; 95%CI: -11.9 to -0.4) [13]. Both studies employed optical coherence tomography, which indeed represents a more advanced and accurate method for lesion assessment and stent optimization.

Rather than viewing RA and IVL as competing techniques, they may serve complementary roles, as exemplified by the “RotaTripsy” approach. This hybrid strategy employs smaller rotablation burrs (1.25–1.5 mm) to modify superficial calcifications and enable IVL balloon catheter delivery for addressing deeper calcium deposits. In our study, the “RotaTripsy” approach was used in two patients in the IVL arm due to uncrossable lesions. Additionally, IVL was employed in one patient in the RA arm due to stent underexpansion, a scenario increasingly

documented in case reports and registries. For example, the SMILE registry demonstrated an 87.1% success rate for IVL in underexpanded stents, bringing significant improvements in MSA ($p<0.00001$) [14].

In all three crossover cases in our study, both early postprocedural and long-term clinical outcomes were favorable, with no incidence of MACE during follow-up. Although the necessity for crossover between treatment modalities was classified as procedural failure, the overall study analysis adhered to the intention-to-treat principle, thereby preserving the methodological integrity and minimizing bias in the comparison of treatment strategies.

Further comparisons of IVL and RA from retrospective studies reinforce the advantages of IVL, including larger MSA in the STIFF study ($p=0.01$) [15], higher virtual FFR after IVL in Gallinoro et al. ($p=0.024$) [16], and fewer periprocedural complications after IVL in Zhao et al. ($p=0.027$) [17].

Despite the fact that intravascular imaging allows for a more precise evaluation of calcified coronary plaques, we utilized the 12-month angiographic late lumen loss (LLL) as the primary endpoint, similarly to the Rotaxus and Prepare-Calc studies. This methodological choice was predetermined by the pragmatic design of the trial and the availability of resources at the time. An angiography has inherent limitations, including its inability to provide detailed information on plaque composition and the precise extent of vessel wall calcification. Furthermore, it does not allow for the assessment of stent strut apposition, vessel remodeling, or other subtle morphological changes that may influence procedural outcomes. In contrast, intravascular imaging techniques offer more accurate and comprehensive characterization of coronary lesions, which is particularly advantageous in the evaluation of heavily calcified vessels. Although angiography is less accurate in the assessment of calcified lesions, it does allow comparison with the earlier studies mentioned above.

Further, multiple clinical studies have demonstrated a significant correlation, particularly between angiographic parameters, such as LLL or percentage diameter stenosis (%DS), and the incidence of target lesion revascularization (TLR) [3, 4].

Although our study represents a pilot investigation, it provides sufficient data to inform sample size and power calculations for future trials. Based on the observed results for the primary endpoint of procedural success, a post hoc power analysis was performed to estimate the minimum sample size required for a future, adequately powered study. Assuming a significance level of 0.05 and a statistical power of 0.80, and using the observed procedural success rates of 84.0% in the IVL group and 96.0% in the RA group, the analysis indicated that a minimum of 95 patients per group would be required.

Limitations:

Our study has some limitations that should be mentioned. One of them is that this study represents a pilot investigation characterized by a limited sample size, which inherently restricts the generalizability of the findings. To validate and expand upon these preliminary results, additional research involving larger cohorts and adequate statistical power will be required to ensure robust and reliable conclusions. The statistical power was not calculated prior to the patient recruitment, either; however, as this study was designed as pilot study and as at the time of its beginning, no data on which we could have based the study power calculations were available, this limitation could not be avoided. We acknowledge a potential selection bias due to exclusion of patients with extreme anatomical complexity or limited feasibility of imaging or follow-up, which may affect the generalizability of our results. Furthermore, the use of intravascular imaging could enhance understanding of the extent of calcified coronary lesions.

Conclusion

Despite the different technical principles, both of the compared methods, intravascular lithotripsy and rotational atherectomy, followed by DES implantation led to similar periprocedural, 12-month angiographic and clinical outcomes in the treatment of calcified coronary lesions.

Abbreviations

CABG	coronary artery bypass graft
CV	cardiovascular
DES	drug-eluting stent
FFR	fractional flow reserve
EFS	event-free survival
ELCA	excimer laser coronary angioplasty
ISR	in-stent restenosis
IVL	intravascular lithotripsy
LLL	late lumen loss
LV EF	ejection fraction of left ventricle
MACE	major adverse cardiac events
MI	myocardial infarction
MLD	minimal lumen diameter
MSA	minimal stent area
NC	non-compliant
NSTEM	myocardial infarction without ST elevation
PCI	percutaneous coronary intervention
QCA	quantitative coronary angiography
RA	rotational atherectomy
RefD	reference lumen diameter
STEMI	myocardial infarction with ST elevation
TLF/TVF	target lesion/vessel failure
TLR	target lesion revascularisation
%DS	diameter stenosis

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Author contributions

LP, PK: study design, literature search, data collection and data analysis; AK: statistical analysis; All authors read and approved the final manuscript.

Data availability

[illegible]

Declarations

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

Indexing words

indexing words
intravascular lithotripsy; rotational atherectomy; coronary calcification.

Ethics approval and consent.

The study protocol complied with the Declaration of Helsinki and was approved by the Ethics Committee of University Hospital Ostrava, Czech Republic. The study was registered at ClinicalTrials.gov (NCT04428177) on 09th June 2020. Written informed consent was obtained from each patient before enrolment in the study.

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CONSORT guidelines statement

We hereby declare that our study adheres to CONSORT guidelines and include a completed CONSORT checklist as an supplementary file.

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Z. Ramík et al.

Long-term Impact of Alpha-blockers on Renal Function - A Systematic Review and Meta-analysis

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A Systematic Review and Meta-Analysis: Long-Term Impact of Alpha-Blockers on Renal Function

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Keywords

Arterial hypertension · Glomerular filtration rate · Estimated glomerular filtration rate · Chronic kidney disease · Alpha-blocker · Doxazosin

Abstract

Introduction: Alpha-blockers are considered an additional option when the major antihypertensive drug classes are insufficient in reducing blood pressure. While the impact of alpha-blockers on blood pressure control seems comparable, data evaluating their effects on renal outcomes are lacking. This systematic review and meta-analysis assess the impact on renal function from a medium- to long-term perspective. **Methods:** A search and analysis according to the PRISMA statement across Medline, the Web of Science, and ScienceDirect was conducted, covering articles in English on adult populations without time restrictions to December 14, 2023, including all types of studies with a minimum follow-up of 12 weeks. **Results:**

Seventeen studies were included in the review, encompassing a total of 26,170 patients treated with alpha-blockers. Most studies were performed in the 20th century and often lacked an adequate number of participants and sufficient follow-up duration. Bayesian meta-analysis showed neutral effects of alpha-blockers on estimated glomerular filtration rate and serum creatinine, comparable with those of other antihypertensive agents. Compared with baseline, the data suggest an overall small but clinically unimportant increase in creatinine clearance in patients treated with alpha-blockers (95% credible interval: 1.61–9.97 mL/min/1.73 m²). **Conclusion:** A significant dearth of evidence concerning the long-term impact of alpha-blockers on renal function was revealed. The available evidence suggests that alpha-blockers have a neutral or non-inferior effect on renal function in comparison with other antihypertensive agents. Further research is needed to evaluate the role of alpha-blockers and their impact on preserving renal function.

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Plain Language Summary

High blood pressure is a major cause of kidney damage. Controlling blood pressure is essential for protecting kidney function. Alpha-blockers are a group of medicines sometimes used when standard treatments (like ACE inhibitors, calcium channel blockers, or diuretics) are not enough. While alpha-blockers are known to lower blood pressure, it is unclear whether they have a good or bad effect on kidney health in the long term. This study looked at all available research about how alpha-blockers affect kidney function. The authors searched several large medical databases for studies in adults that followed patients for at least 12 weeks. In total, they found 17 studies involving over 26,000 patients who were treated with alpha-blockers. Many of these studies were older and had limitations, such as small numbers of participants or short follow-up periods. The results showed that alpha-blockers do not significantly harm or improve kidney function. This was based on common measures of kidney health such as glomerular filtration rate (GFR), which tells how well the kidneys are filtering blood, and levels of creatinine, a waste product cleared by the kidneys. When compared to patients' values before starting treatment, there was a small improvement in one measure (creatinine clearance), but this change was not large enough to be considered important in practice. Overall, the findings suggest that alpha-blockers have a neutral effect on kidney function. However, more research is needed to understand their long-term impact, especially in patients at higher risk of kidney disease.

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Introduction

Adequate control of blood pressure is the primary approach to the prevention and treatment of chronic kidney disease (CKD), which is an independent cardiovascular risk factor [1, 2]. Although various antihypertensive agents are available for effectively achieving blood pressure control, the preservation of renal function has been well-established only in specific drug classes, and mostly when compared with placebo. Inhibitors of the renin-angiotensin-aldosterone system (RAAS), including angiotensin-converting-enzyme inhibitors, angiotensin receptor blockers, and mineralocorticoid receptor antagonists (MRAs), appear to have the best nephroprotective and antiproteinuric effects [3–13]. Nephroprotection is also observed with calcium channel blockers [4–6, 14], thiazide and thiazide-like diuretics [15–21], and an antiproteinuric effect is ob-

served even with β -blockers [22]. Conversely, some studies propose that the most important factor is achieving adequate blood pressure control, regardless of the chosen antihypertensive treatment [23–29].

The assessment of nephroprotection can involve both direct and indirect parameters. Direct parameters (hard parameters) usually include the long-term development of serum creatinine, often expressed as a slope [30–37], the development of directly measured glomerular filtration rate (GFR) or estimated GFR (eGFR), and composite renal endpoints, such as a defined decrease in eGFR (e.g., 25%, 50%, or 75%), increased serum creatinine, or the need to initiate renal replacement therapy (RRT) [38, 39]. Indirect parameters do not directly evaluate the renal function, but there is evidence of association between these parameters and their effects on renal function. For example, this involves the reduction of albuminuria and proteinuria [40–42], optimal control of CKD risk factors such as blood pressure and glycaemic control [43], as well as emerging parameters currently under intensive investigation, such as urinary post-translationally modified fetuin-A and urinary Dickkopf-related protein 3 [44–46].

Alpha-blockers, along with centrally acting agents and vasodilators, are considered backup treatments when normal blood pressure cannot be achieved despite the use of the previously mentioned agents. Data on the long-term effects of alpha-blockers on renal function appear to be insufficient. Some sources suggest alpha-blockers have antiproteinuric properties due to a favourable intrarenal haemodynamic effect, while others do not [47–53]. We conducted a systematic review and subsequent meta-analysis of current data to evaluate the long-term nephroprotection of alpha-blockers using direct renal parameters.

Materials and Methods

The protocol for this systematic review and meta-analysis was registered on the Open Science Framework (OSF). The review was conducted in accordance with PRISMA 2020 guidelines.

Search Strategy and Selection Criteria

Database searches and the selection of eligible studies were independently conducted by two authors (Z.R. and T.K.). The selection process is summarised in Figure 1. A search was conducted from December 1 to December 14, 2023, in the Medline and Web of Science databases, and ScienceDirect platform,

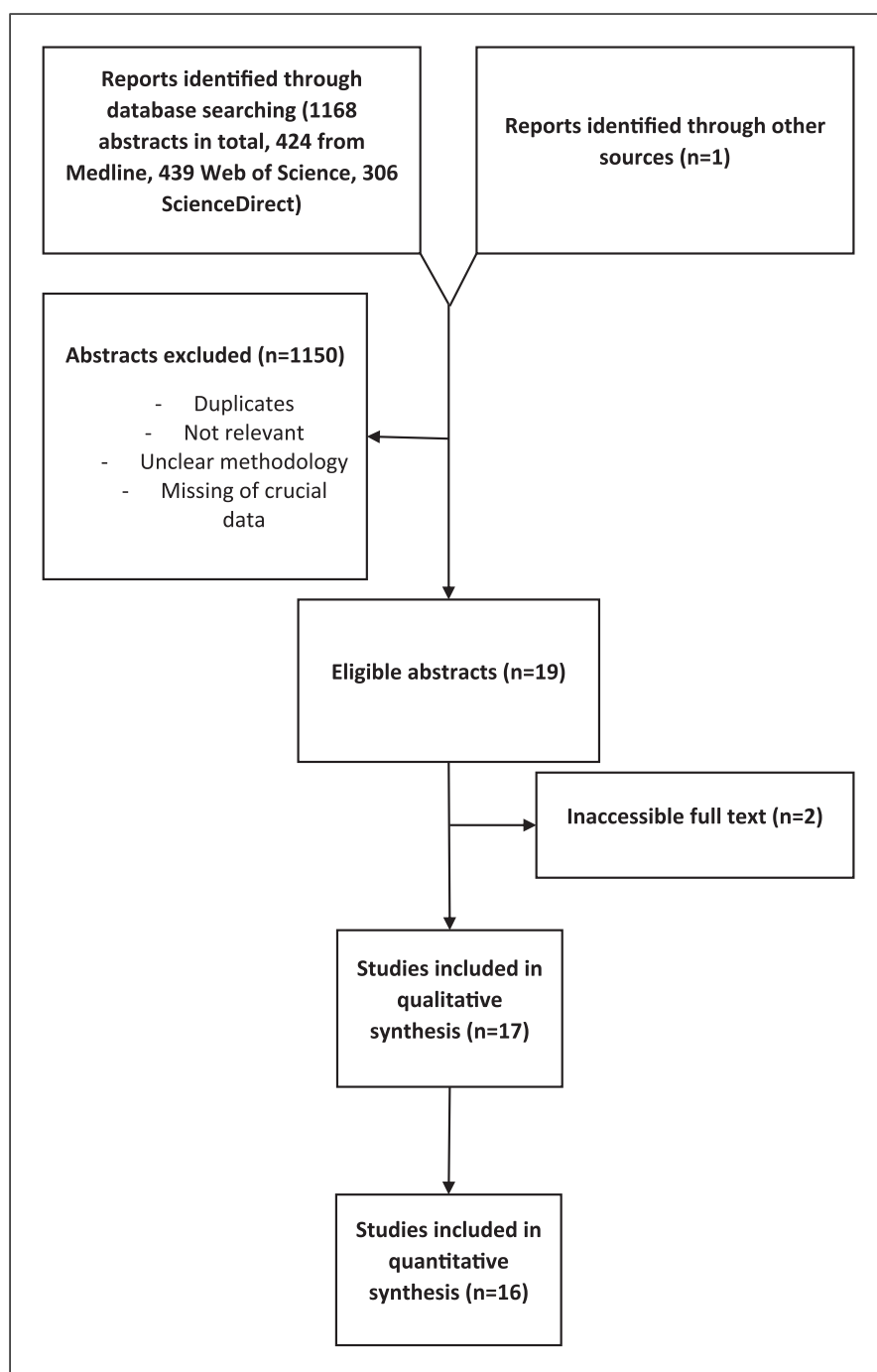


Fig. 1. Flowchart of trial selection.

encompassing all types of articles in the English language. No time restrictions were applied. Trials from 1982 to 2023 in Medline, 1945–2023 in the Web of Science, and 1988 until 2023 in ScienceDirect were included. Key terms used were eGFR*, GFR*, filtration rate*, creatinine*, and alpha-blocker*, alpha blocker*, bunazosin*, doxazosin*, terazosin*, pra-

zosin*, and urapidil*. These terms were searched in abstracts and titles. The search covered both open-access and subscription literature. One study, PATHWAY-2, was not identified through the database search but was included in the analysis because it involved treatment with doxazosin and provided sufficient data on renal function in the supplementary

material. The trials were grouped according to the comorbidities of the included patients in the systematic review.

Inclusion criteria were human studies with a baseline diagnosis of hypertension, a minimum follow-up of 3 months (12 weeks) to ensure at least a moderate or long-term treatment effect, and the evaluation of direct renal endpoints in the trial (development of serum creatinine, GFR or eGFR, or composite renal endpoint) in both adults and children, with no age restriction applied. All study designs were considered for the quantitative analysis, including randomised controlled trials, quasi-randomised trials, and non-randomised trials (with or without a control group). Retrospective studies were included in the review and discussion to ensure a comprehensive interpretation of the available evidence. The included trials adhered to the principles outlined in the Declaration of Helsinki and its subsequent amendments.

Exclusion criteria comprised a follow-up shorter than 12 weeks, the absence of hard renal endpoints (e.g., only proteinuria without the development of serum creatinine, eGFR, or composite renal endpoint), studies including patients at baseline with acute kidney injury, decompensated congestive heart failure (CHF), or cirrhosis, and patients on dialysis and pregnant women. Studies not in English and trials with the inaccessible full text were excluded. Studies lacking crucial data in their methods and results – for example, numerical data for the monitored renal parameters or duration of follow-up – were also excluded. Additionally, studies evaluating alpha-blockers exclusively for the treatment of benign prostatic hyperplasia were not considered eligible.

Data Extraction

Data extraction from the selected studies was performed collaboratively by Z.R. and M.M. The following parameters were extracted from the selected articles: author and year of publication, study design, basic trial population characteristics including age and sex, number of participants, follow-up duration, control group details (if applicable), drug dosage, the measured parameter of renal function along with its results, identification of potential sources of bias, and information on treatment tolerance and side effects. For the review, the effect of medication was categorized as negative when a significant decline in renal function was observed, neutral, if there was no effect during follow-up, or positive when a significant increase of renal function was observed.

The risk of bias in the included trials was assessed collectively by all authors except M.M. The RoB 2 tool was used for randomised and cross-over trials [54]. Bias

in non-randomised studies was evaluated using the ROBINS-I tool [55]. These tools assess 5–7 domains through signalling questions. Final plots were visualised using the Robvis tool [56].

Bayesian Meta-Analysis

A subsequent aggregate data meta-analysis of the studies from the review was performed by M.M. For the meta-analysis, we separately collected and evaluated changes in renal parameters compared with pre-treatment baseline values and compared with an active treatment. All of the comparisons to active treatments included in the meta-analysis are derived from controlled intervention studies, and with the exception of Ibsen et al. [57] (who allocated patients consecutively) all employ random treatment allocation. For the comparison to baseline, we combined both randomised and non-randomised studies as randomisation does not prevent biases in within-arm comparisons and such comparisons are thus very similar to observational evidence (see, e.g., the discussion in) [58].

For each study and outcome, we aimed to determine a point estimate and 95% confidence interval (CI, for visualisation) of the effect of interest. We note that confidence intervals are widely recommended for reporting of clinical outcomes [59, 60]. Unfortunately, direct reports of uncertainty were seldom available. We employed several strategies to reconstruct those values:

1. When direct comparison was not reported but individual patient data at baseline and after alpha-blocker treatment were presented in a table or a figure, we extracted the data and performed a paired *t* test to obtain the CI. We used Automeris Web-PlotDigitizer version 4.7 to reconstruct individual data from Figure [61].
2. When a paired comparison was reported with a *p* value, we reconstructed the CI using standard formulas [62, 63]. We applied the same formulas to approximately reconstruct the CI from reported *p* values from other tests that respect the paired structure of the data (e.g., from the multi-level model reported in PATHWAY-2).
3. Some studies reported only the mean and standard error or standard deviation of the marker value at baseline and after treatment, making it impossible to reconstruct the paired comparison. In these cases, we approximated the CI with an unpaired Welch's *t* test. This method is conservative, as it will not provide narrower intervals than a paired *t* test on the original data would.

4. When comparisons of laboratory markers between treatment arms were not reported, we used the reported means and standard deviations or standard errors of posttreatment values to reconstruct a Welch's *t* test.
5. When the variability in laboratory markers between treatments was not directly reported, but comparisons between all treatments and baseline were available, we derived the between-treatment comparisons from the two baseline comparisons, assuming independence (this was only the case for PATHWAY-2).
6. We converted all data to mL/min/1.73 m² (GFR, eGFR, and creatinine clearance) and μmol/L (serum creatinine, assuming 1 mg/dL = 88.4 μmol/L). Unfortunately, in some studies GFR, eGFR, and creatinine clearance were not standardised for body surface area, and the body surface area of participants was not reported. In those cases, we assumed that all patients had exactly the typical 1.73 m² body area, that is, that the standardised and non-standardised numbers were directly comparable. This assumption should increase between-study variability but not result in a substantial bias.

Finally, the obtained CIs were transformed into standard errors assuming normality as standard errors were never directly reported in any of the studies. The full code for the reconstruction of the CI and the extracted data in machine-readable format are available in the online supplementary material (for all online suppl. material, see <https://doi.org/10.1159/000547273>).

We conducted a Bayesian random-effects meta-analysis on the study-level estimates and standard errors [64] using the brms package with very weakly informative Student-*t* priors on all parameters [65]. When multiple comparators per study were available for analysis, a random intercept for comparators was added. Results are reported as posterior mean and 95% posterior credible interval (CrI). For individual studies, point estimates + 95% CI are shown in the online supplementary material. For the pooled estimate, we show the 95% CrI. Absolute heterogeneity between trials is expressed as the standard deviation of the random effect, and relative heterogeneity is reported as *I*². The full code to reproduce the meta-analysis is available in the online supplementary material.

Results and Discussion

A total of 1,169 studies were initially identified and screened for the eligibility of the review: 424 from Medline, 439 from the Web of Science, and 306 from

ScienceDirect. The decision to include all relevant study types was made to provide a comprehensive overview of the available evidence on alpha-blockers and their long-term effects on renal function, considering the overall limited number of eligible studies. Ultimately, 17 studies were included in the review after re-evaluation of the inclusion and exclusion criteria (Fig. 1). The included studies encompassed a total of 57,578 patients, of whom 26,170 were treated with an alpha-blocker. Only studies with adult patients were found (refer to Tables 1–2, online suppl. Tables 1 and 2, and online suppl. Figure 1 for the characteristics of the studies). Only two prospective trials included a substantial number of patients (24,335 and 335) [66, 67], and two retrospective trials involved 32,176 and 301 patients [68, 69]. The remaining trials included fewer than 100 patients each. Only one review addressed the impact of alpha-blockers on kidney function, discussing only two trials, and no cardiovascular or renal benefits were identified [70]. Of the studies included in this review, 5 claimed a positive effect on renal function, 10 claimed no effect or inconclusive evidence, and 2 reported a negative impact. Sixteen studies were included in the meta-analysis. The trial reported by Murakami et al. [69] did not provide matching data that could be compared with other trials.

Population with Hypertension and/or Increased CV Risk

The ALLHAT trial, a randomised, double-blind active-controlled study, assessed chlorthalidone, amlodipine, lisinopril, and doxazosin's impact on the incidence of composite fatal coronary heart disease and myocardial infarction in a hypertensive population with at least 1 risk factor for coronary heart disease. A total of 9,067 subjects were randomised to the doxazosin group, and 15,268 received chlorthalidone. Patients with baseline serum creatinine levels above >177 μmol/L were excluded. The doxazosin group was halted early, after a median follow-up of 3.3 years, due to higher incidence of CHF. The drugs had comparable effect on the primary endpoint in reducing incident or fatal coronary artery disease. Doxazosin was slightly less effective in controlling blood pressure compared with chlorthalidone. It was suggested that doxazosin might not be suitable for patients at high risk of heart failure and that another antihypertensive drug such as RAAS or diuretic could be more suitable in this population, due to a risk of fluid retention and increased levels of norepinephrine, both of which might be unfavourable in patients with high risk of or manifest heart failure [80]. In contrast, the ASCOT trial evaluated adding doxazosin as a third-line agent to

Table 1. General characteristics of randomised controlled trials

Author/ trial, year of publication	Type of study	Patients and characteristics, N	Duration of follow-up	Sex	Age, years	Medication and patients, N	Dose/day	Comparison treatment and patients, N	Dose/day	Monitored hard renal parameters	Effect on kidney function (comparison with baseline)
ALLHAT [29, 60, 68] (2000)	RCT, double blind	24,335, hypertonics with high CV risk	3.3 years (median)	53% men, 47% women	67	Doxazosin (N = 9,067)	2, 4, or 8 mg	Chlorthalidone (N = 15,268)	12.5–25 mg	Risk ratio of new-onset ESRD	Neutral RR of ESRD 1.04 (0.76–1.42)
PATHWAY- 2 [67] (2015)	RCT, cross- over	335, with resistant hypertension	12 weeks 4x rotation	69% M, 31% W	61.4±9.6	Doxazosin (N = 282)	4–8 mg	Spirolonactone (N = 285)	25–50 mg	Serum creatinine (μmol/L), eGFR (mL/min)	Neutral (similar with other antihypertensive agent) Creatinine increased from 83.75 to 91.04, $p =$ 0.003; eGFR 92.7–85.38 mL/ min, $p = 0.023$
Schmieder et al. [71] (1997)	RCT, double- blind	43, new hypertonics, without severe eHMOD	6 months	29 M, 14 W	52±9	Bunazosin (N = 23)	6 mg or 12 mg, or + HCTZ	Metoprolol (N = 20)	100 mg or 200 mg, or + HCTZ	Serum creatinine (mg/dL), GFR inulin (mL/min/ 1.73 m ²)	Neutral AB (serum creatinine 1.00±0.14 vs. 0.93±0.12 mg/dL; $p < 0.001$; GFR 112±20 to 115±18, NS) BB: NS for both
Erley et al. [72] (1993)	RCT, cross- over	17, hypertonics with microalbuminuria	12 weeks 4x rotation	7 men, 10 women	39±14	Doxazosin (N = 17)	2 mg (mean)	Felodipine Metoprolol Ramipril; (N = 17 each)	8 mg 85.5 mg 5 mg (mean)	GFR inulin clearance (mL/min/ 1.73 m ²)	Neutral GFR 112±17 to 111±19 ($p =$ NS) after doxazosin treatment, NS in every antihypertensive
Giordano et al. [49] (1996)	RCT, doxazosin open, other double-blind	30, hypertonics with DM	12 weeks	24 men, 6 women	50±3	Doxazosin (N = 10)	2–8 mg	Captopril (N = 9), nifedipine (N = 11)	25–50 mg, 30–60 mg	Creatine clearance (mL/min/ 1.73 m ²)	Positive Rise in CrCl in doxazosin from 99±8 to 122±8 mL/1.73/ m ² ($p < 0.01$) A rise was also observed in the captopril group ($p < 0.05$), but not in the nifedipine group

Table 1 (continued)

Author/ trial, year of publication	Type of study	Patients and characteristics, <i>N</i>	Duration of follow-up	Sex	Age, years	Medication and patients, <i>N</i>	Dose/day	Comparison treatment and patients, <i>N</i>	Dose/day	Monitored hard renal parameters	Effect on kidney function (comparison with baseline)
Rachmani et al. [47] (1998)	RCT, cross- over, open label	76, hypertonics with T2DM and albuminuria	12 months	37 men, 19 women	55±6	Doxazosin (<i>N</i> = 25)	2–8 mg	Cilazapril alone (<i>N</i> = 25)	2.5–10 mg	Creatinine clearance (mL/min/ 1.73 m ²)	Positive doxazosin and doxazosin + cilazapril led to increased eGFR in first phase: 87±7 to 91±6 mL/min (<i>p</i> = 0.046) 86±6 to 90±7 mL/ min (<i>p</i> = 0.05), crossover led to a decrease in eGFR in the cilazapril group
						Doxazosin + Cilazapril (<i>N</i> = 26)	2–8 mg + 2.5–10 mg	Addition of HCTZ after 8 months to all			
Martínez- Castelao et al. [73] (1998)	RCT, open label	88, after kidney transplant on cyclosporine with hypertension	3 years	54 men, 34 women	43±8.6; 41±9.7; 44.5±11	Doxazosin (<i>N</i> = 40)	1–16 mg	Verapamil (<i>N</i> = 24)	240–480 mg	Serum creatinine (μmol/L), % of Graft survival	Positive Doxazosin 183±101 to 194±74 μmol/L and 90% survival Enalapril 178±59 to 193±108 μmol/ L and 67% g. Survival Verapamil 196±86 to 265±221 μmol/ L and 79% g. Survival
								Enalapril (<i>N</i> = 24)	5–40 mg		
Svarstad et al. [74] (2001)	RCT, open label	28, T1DM on insulin with hypertension	7.6 men	21 men, 7 W	45±2	Doxazosin (<i>N</i> = 15)	8±1 mg (mean)	Captopril (<i>N</i> = 13)	112±7 mg (mean)	GFR 125I- iothalamate (mL/min/ 1.73 m ²)	Neutral Doxazosin: 122.8±6.8 (58.5–169.9) vs. 121.7±8.8 (59.3–191.0) mL/ min/1.73 m ² , <i>p</i> ≥ 0.10 captopril 110.0±8.1 (53.1–144.0) 110.8±9.9 (42.3–149.0) mL/ min/1.73 m ² , <i>p</i> ≥ 0.10

Table 1 (continued)

Author/ trial, year of publication	Type of study	Patients and characteristics, N	Duration of follow-up	Sex	Age, years	Medication and patients, N	Dose/day	Comparison treatment and patients, N	Dose/day	Monitored hard renal parameters	Effect on kidney function (comparison with baseline)
Ibsen et al. [57] (1979)	Comparative open-label	30, hypertonics on propranolol (120–640 mg/ day)	At least 3 months	10 women, 20 men	46.5 and 46.2	Added prazosin (N = 15)	2–20 mg (mean 9)	Added hydralazine (N = 15)	100–200 mg (mean 150)	GFR Single injection of ⁵¹ Cr-EDTA (mL/min)	Negative Propranolol + prazosin 79±16.1 (53–111) to 76±16.2 (47–108) mL/min, <i>p</i> < 0.02 Propranolol + hydralazine 72±16.5 (40–107) to 73±16.5 (42–108), mL/min, <i>p</i> = NS

AB, alpha blockers; BB β -blockers; CrCl, creatinine clearance; CV, cardiovascular; DM, diabetes mellitus; ⁵¹Cr-EDTA, chromium-51 labelled ethylenediaminetetraacetic acid; eGFR, estimated glomerular filtration rate; GFR, glomerular filtration rate; ESRD, end-stage kidney disease; g, graft; HCTZ, hydrochlorothiazide; HMOD, hypertension-mediated organ damage; HR, hazard ratio; PAH, para-aminohippurate; RCT, randomised controlled trial; RR, risk ratio; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

amlodipine + perindopril or to atenolol + bendroflumethiazide in a population of 19,257 hypertensive patients and did not find increased risk of heart failure. In the ALLHAT trial, the increased evidence of new-onset CHF might be also caused by heart failure misdiagnosis and discontinuation of diuretic treatment before the start of the trial in order to randomise the patients to doxazosin, and the decision to stop the doxazosin arm is nowadays considered controversial in European hypertension guidelines for the aforementioned reasons [81]. The risk of fluid retention might be attenuated by combining the alpha-blocker with an antihypertensive agent with a diuretic effect [82]. Concerning renal function, the incidence of new-onset end-stage kidney disease (ESRD) was comparable between the doxazosin and chlorthalidone groups [66, 70, 83, 84].

The PATHWAY-2 study, a placebo-controlled randomised controlled trial (RCT), compared the effects of spironolactone, bisoprolol, and doxazosin to placebo in terms of a 4th line antihypertensive agent added in 335 patients with resistant hypertension in 12-week courses. The study showed the superior effect of spironolactone on blood pressure control compared with bisoprolol and doxazosin. During the treatment, a significant slight drop of eGFR and an increase in serum creatinine were observed in all treatment groups. This was attributed to renal perfusion pressure and an initial eGFR drop after treatment initiation typically seen in RAAS blockade and others [85]; compared with other agents, doxazosin showed at least a similar effect on eGFR [67].

A double-blind randomised controlled trial by Schmieder et al. [71] compared the effect of the alpha-blocker bunazosin in 23 patients with newly diagnosed hypertension with metoprolol in 20 patients for 6 months of follow-up. Treatment with the alpha-blocker led to a slight decrease in serum creatinine levels and a corresponding increase in eGFR compared with the β -blocker, but the differences were not statistically significant. Additionally, urinary protein excretion was significantly lower in the β -blocker group [71].

Ibsen et al. [57] conducted a comparative open-label trial of adding hydralazine and prazosin to patients treated with propranolol without inadequate blood pressure control, with 15 patients in each group and followed for at least 3 months. GFR was evaluated by clearance of ⁵¹Cr-EDTA. After the addition of hydralazine, there was no significant change in GFR and a small significant drop in serum creatinine from 106.1 to 100.8 μ mol/L. Hydralazine did not reduce blood pressure

Table 2. General characteristics of unblinded, uncontrolled, and retrospective studies

Author/ trial, year of publication	Type of study	Patients and characteristics, N	Duration of follow-up	Gender	Age, years	Medication and N of patients	Dose/day	Monitored hard renal parameters and units	Effect on kidney function (comparison with baseline)
Bailey et al. [75] (1977)	Uncontrolled, interventional	38 with hypertension and moderate to severe CKD	2–26 months	20 women, 18 men	47.6	Prazosin (N = 33 and 16)	Mean 7 mg	Serum creatinine (mmol/L, N = 33) GFR Single injection of ⁵¹ Cr- EDTA (mL/s, N = 16)	Neutral Serum creatinine: 0.33 mmol/L to 0.38 mmol/L GFR 0.41 mL/s to 0.43 mL/s, <i>p</i> = NA
Bauer et al. [76] (1984)	Uncontrolled, interventional	14 white hypertensive males, without DM, manifested CVD or advanced CKD	5–8 months	0 women, 14 men	49±13	Prazosin (N = 14)	Mean 5.4 mg (1–10 mg)	Serum creatinine (mg/dL) Creatinine clearance (mL/ min/1.73 m ²) GFR (mL/min/ 1.73 m ²)	Neutral Creatinine 1.0±0.1 to 1.0±0.1 mg/dL, creatinine clearance 106±5 to 105±8 mL/ min/1.73 m ² , inulin clearance (GFR) 85±5 to 100±6 mL/min/ 1.73 m ² , <i>p</i> = NS for all
Krusell et al. [77] (1992)	Uncontrolled, interventional, blinded	15 with mild to moderate hypertension 4 weeks of placebo	4 months	7 women, 8 men	45.7±10.2	Doxazosin (N = 15)	8.9±8.5 mg (mean)	Creatinine clearance (mL/ min), GFR 125I- iothalamate (mL/min)	Neutral CrCl: 112±21 to 111±31 mL/min eGFR: 98±14 to 94±17 mL/min, <i>p</i> = NS.
Martínez- Castelao et al. [73] (2003)	Uncontrolled, interventional	20 kidney transplant recipients with hypertension, divided as responders and non-responders according to T/P ratio	12 weeks	10 women, 10 men	52.5±10.9	Doxazosin GITS (N = 10)	5.7±3 mg (mean)	Serum creatinine (μmol/L)	Neutral Responders: 161±64 to 141±66 μmol/L, <i>p</i> = NS Non-responders: 129±34 to 133±34 μmol/L, <i>p</i> = NS

Table 2 (continued)

Author/ trial, year of publication	Type of study	Patients and characteristics, N	Duration of follow-up	Gender	Age, years	Medication and N of patients	Dose/day	Monitored hard renal parameters and units	Effect on kidney function (comparison with baseline)
Mori et al. [78] (2001)	Uncontrolled, interventional	15 with hypertension and advanced CKD treated with furosemide (mean CrCl 24±2 mL/min)	6 months	7 women, 8 men	58±11	Doxazosin (N = 15)	5.6±0.4 mg (mean)	Serum creatinine (mg/dL) Creatinine clearance (mL/ min), eGFR (mL/min)	Positive Serum creatinine 3±0.3 to 2.5±0.3 mg/ dL, <i>p</i> < 0.05; CrCl increased from 24±2 to 30±3 mL/min, <i>p</i> = 0.0003; eGFR 24.5–29.7 mL/min, <i>p</i> < 0.05
Bartels et al. [79] (1988)	Uncontrolled, interventional	17 hypertonics with moderate to advanced CKD	14 weeks	10 women, 13 men	51.4±4.1	Doxazosin (N = 17)	9.8±1.4 mg (mean)	Serum creatinine (μmol/L)	Neutral 319±17 μmol/L to 333±22 μmol/L, <i>p</i> = NS
Hundemer et al. [68] (2021)	Retrospective	32,176 hypertonics with CKD older than 66 years	Minimum 90 days to maximum 3 years	37% women, 62% men	75±7	Alpha- blocker (N = 16,088), non-AB N = 16,088	NA	HR endpoint: 30% eGFR decline, dialysis, or kidney transplantation	Negative eGFR decline: HR 1.14; 95% CI: 1.08–1.21 Dialysis or kidney Tx: HR 1.28; 95% CI: 1.13–1.44 But lower risk of CV events and mortality in the alpha-blocker group
Murakami et al. [69] (2016)	Retrospective	301 Asian patients with hypertension and end-stage CKD	From 1990 to 2015	188 men, 113 women	68.0±13.7 (18–91)	NA	NA	Slope of reciprocal serum creatinine in multivariate analysis	Positive alpha- blockers were nephroprotective in patients followed from 2010 to 2015, standardised β coefficient 0.207, <i>p</i> = 0.04
AB, alpha-blocker; CKD, chronic kidney disease; CV, cardiovascular; CVD, cardiovascular disease; CrCl, creatine clearance; DM, diabetes mellitus; ⁵¹ Cr-EDTA, chromium-51 labelled ethylenediamine tetra-acetic acid; eGFR, estimated glomerular filtration rate; ESRD, end-stage kidney disease; GFR, glomerular filtration rate; GITS, gastrointestinal therapeutic system; HCTZ, hydrochlorothiazide; HMOD, hypertension-mediated organ damage; HR, hazard ratio; NA, not applicable/available; non-AB, non-alpha-blocker; PAH, para-aminohippurate; RCT, randomised controlled trial; RR, risk ratio; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; T/P, through-to-peak ratio; Tx, transplantation.									

AB, alpha-blocker; CKD, chronic kidney disease; CV, cardiovascular; CVD, cardiovascular disease; CrCl, creatinine clearance; DM, diabetes mellitus; ⁵¹Cr-EDTA, chromium-51 labelled ethylenediamine tetra-acetic acid; eGFR, estimated glomerular filtration rate; ESRD, end-stage kidney disease; GFR, glomerular filtration rate; GITS, gastrointestinal therapeutic system; HCTZ, hydrochlorothiazide; HMOD, hypertension-mediated organ damage; HR, hazard ratio; NA, not applicable/available; non-AB, non-alpha-blocker; PAH, para-aminohippurate; RCT, randomised controlled trial; RR, risk ratio; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; T/P, through-to-peak ratio; Tx, transplantation.

significantly. Prazosin added to propranolol led to a small significant reduction in GFR, by 4% ($p < 0.02$), and a significant drop in both supine and standing blood pressure was observed. However, plasma creatinine did not change significantly after the addition of any of the drugs.

The effect of prazosin was evaluated in 38 patients with moderate to severe hypertension who were followed for up to 25 months in an uncontrolled trial by Bailey. Fourteen patients had concomitant treatment with furosemide, and 11 patients had one or more antihypertensive agents. Renal function was evaluated during course of 6 months in 16 patients with CKD (serum creatinine was $>170 \mu\text{mol/L}$), and to estimate GFR, a ^{51}Cr -EDTA clearance single injection method was used. At the beginning, the mean serum creatinine was $330 \mu\text{mol/L}$ and mean GFR 24.6 mL/min . At the end of follow-up, serum creatinine had risen to $380 \mu\text{mol/L}$ and GFR was 28.5 mL/min . Among all patients, 5 progressed to end-stage CKD, 9 had significant worsening of renal function, 8 patients had stable GFR, and 11 had a significant improvement of renal function. Five patients died of co-morbidities during follow-up, which probably corresponds to the severity of hypertension in the group [75].

Bauer et al. [76] studied the effect of prazosin in 14 hypertensive white males followed for 6 months in an uncontrolled study. The exclusion of females introduced a potential source of bias. No difference was observed in the development of creatinine clearance: 106 ± 5 to $105 \pm 8 \text{ mL/min/1.73 m}^2$.

A population of 15 patients with mild to moderate hypertension was treated for 4 months with doxazosin in a trial by Krusell et al. [77] Only a significant drop in diastolic blood pressure was observed. GFR and creatinine clearance remained unaffected by the treatment at the end of follow-up (GFR changed from 98 ± 14 to $94 \pm 17 \text{ mL/min}$, and creatinine clearance from 112 ± 21 to $111 \pm 31 \text{ mL/min}$, $p = \text{NS}$).

Population with Diabetes Mellitus

Rachmani et al. [47] focused on the effects of various antihypertensive agents on blood pressure control and renal function in a randomised cross-over open-label trial involving 76 type 2 diabetic patients with hypertension and albuminuria. Patients underwent 4-month courses of doxazosin or cilazapril, or reduced doses of both. Patients on single-drug treatment received in addition 25 mg of hydrochlorothiazide per day after 9 months in the study. During the follow-up, a modest increase in serum creatinine was observed in all three

groups. The replacement of cilazapril with doxazosin led to further increase in creatinine clearance (86 ± 5 to $88 \pm 6 \text{ mL/min}$), and the substitution of doxazosin with cilazapril led to a slight decline in creatinine clearance (91 ± 6 to $88 \pm 5 \text{ mL/min}$). Both results were not statistically significant, likely corresponding to the well-known initial drop in GFR after treatment initiation with RAAS blockade [85]. The anti-albuminuric effect was most prominent in the combination treatment of cilazapril and doxazosin ($p = 0.001$). Doxazosin alone showed only a modest decline in albuminuria compared with cilazapril (relative reduction of 41% vs. 14%, $p = 0.001$ vs. 0.05) [47].

The effects of doxazosin, captopril, and nifedipine were compared in randomised controlled trial by Giordano et al. [49] involving 30 non-insulin-dependent diabetic patients with hypertension. Patients were followed for 12 weeks. Only doxazosin and captopril led to significant increases eGFR; however, exact numerical data are missing, so only creatinine clearance, not eGFR, could be included in the meta-analysis. Although the study maintained a double-blind design for the captopril and nifedipine groups, every third patient was assigned to the doxazosin group, introducing potential bias from the randomisation process. Every drug caused a significant decrease in proteinuria [49].

The effects of the alpha-blockers compared with captopril were also studied in the open-label RCT of Svarstad et al. [74] involving 28 patients with type 1 diabetes on insulin and with hypertension, with a mean treatment duration of 7.6 ± 4 months. eGFR and effective renal plasma flow remained unchanged in both groups.

Population with CKD

Eighty-eight hypertensive patients after kidney transplantation were followed for at least 3 years in randomised controlled open-label trial of Martínez-Castelao et al. [73] Plasma creatinine increased by $\sim 3.6 \mu\text{mol/L/year}$ during follow-up, similar to the enalapril group ($\sim 5 \mu\text{mol/L/year}$) and significantly less than in the verapamil group ($\sim 21 \mu\text{mol/L/year}$). Approximately 30%–40% of all patients required the addition of another antihypertensive treatment. Most interestingly, graft survival after 3 years was highest in patients treated with doxazosin (90%), compared with those receiving verapamil (79%) or enalapril (67%). The trial had potentially significant selection bias, as patients with baseline proteinuria $>1.5 \text{ g/day}$ were automatically assigned to the enalapril group. This likely resulted in a worse prognosis for the enalapril group and prevented

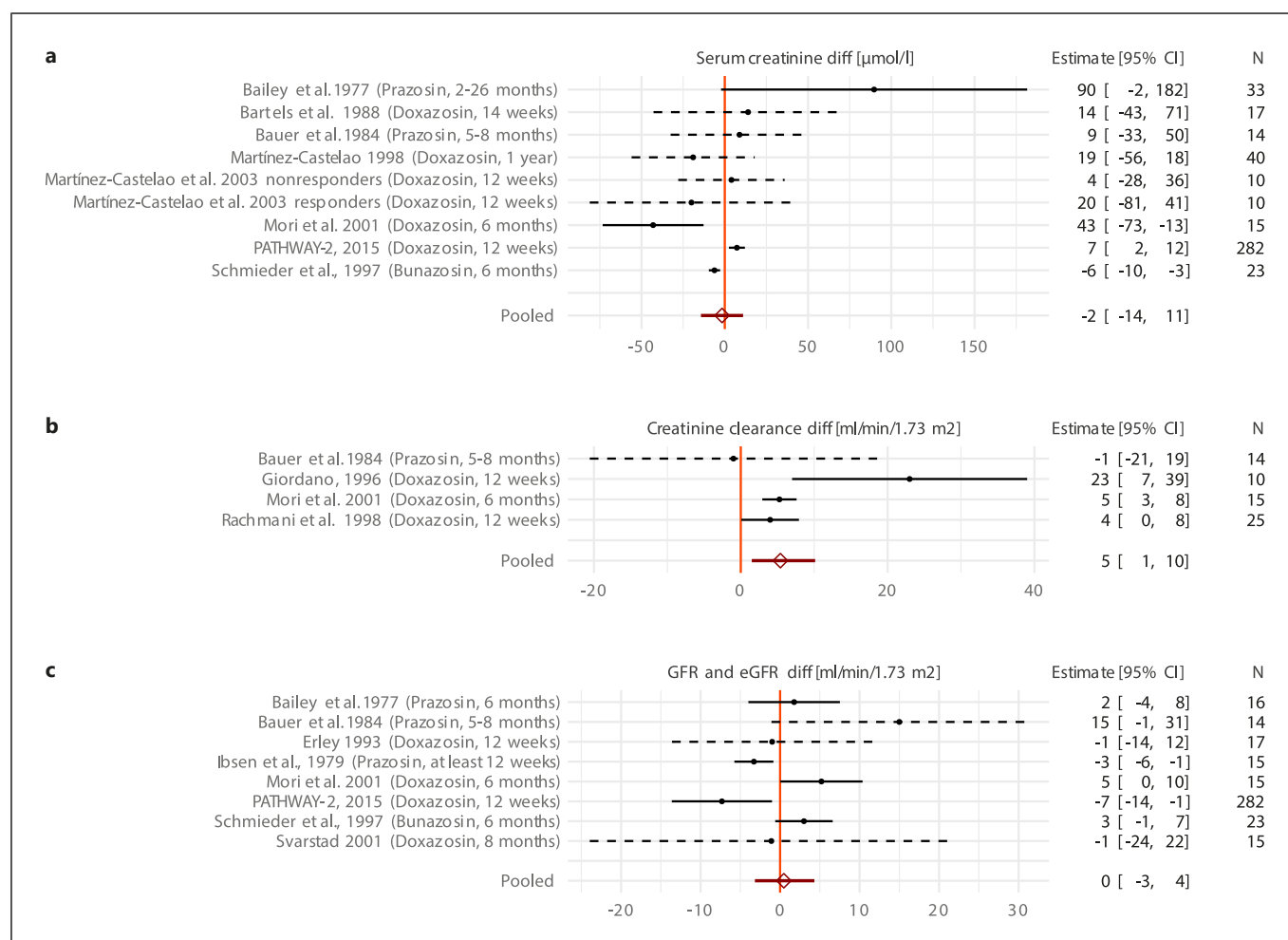


Fig. 2. Changes in renal parameters compared with baseline in patients receiving alpha-blockers (including both randomised and non-randomised studies). **a** Change in serum creatinine from baseline. **b** Change in creatinine clearance from baseline. **c** Change in GFR and eGFR from baseline. Dashed lines represent confidence intervals that could not be exactly reconstructed (they are likely overly wide).

the maintenance of the double-blind design for these patients [73].

The same author conducted a smaller uncontrolled study in 20 kidney transplant patients using doxazosin (gastrointestinal therapeutic system [GITS], a long-acting alternative) to evaluate its effect on blood pressure control over 12 weeks. No change in serum creatinine was observed during follow-up [86].

Erley et al. [72] conducted a double-blind cross-over trial in 17 patients with mild to moderate hypertension with albuminuria. The effects of doxazosin, felodipine, metoprolol, and ramipril on renal haemodynamic and microalbuminuria were evaluated for 12 weeks. Serum creatinine levels did not show any significant change during follow-up in any group. All drugs led to a

significant reduction in microalbuminuria ($p < 0.05$) [72].

An uncontrolled trial by Mori et al. [78] focused on 15 patients with advanced CKD (mean creatinine clearance 24 ± 2 mL/min, proteinuria 1.8 ± 0.3 g/day), who were followed for 6 months. After 2 weeks of furosemide treatment, doxazosin was administered with gradual up-titration according to blood pressure. An increase in creatinine clearance was noted, from 24.5 ± 2 to 29.7 ± 3 mL/min (serum creatinine was reduced from 265 to 221 $\mu\text{mol/L}$). Proteinuria during follow-up decreased from 1.8 g/day to 1.3 g/day ($p < 0.05$) [78].

Doxazosin was used in the uncontrolled trial of Bartels et al. [79] in a population of 23 hypertensive patients with CKD (baseline serum creatinine

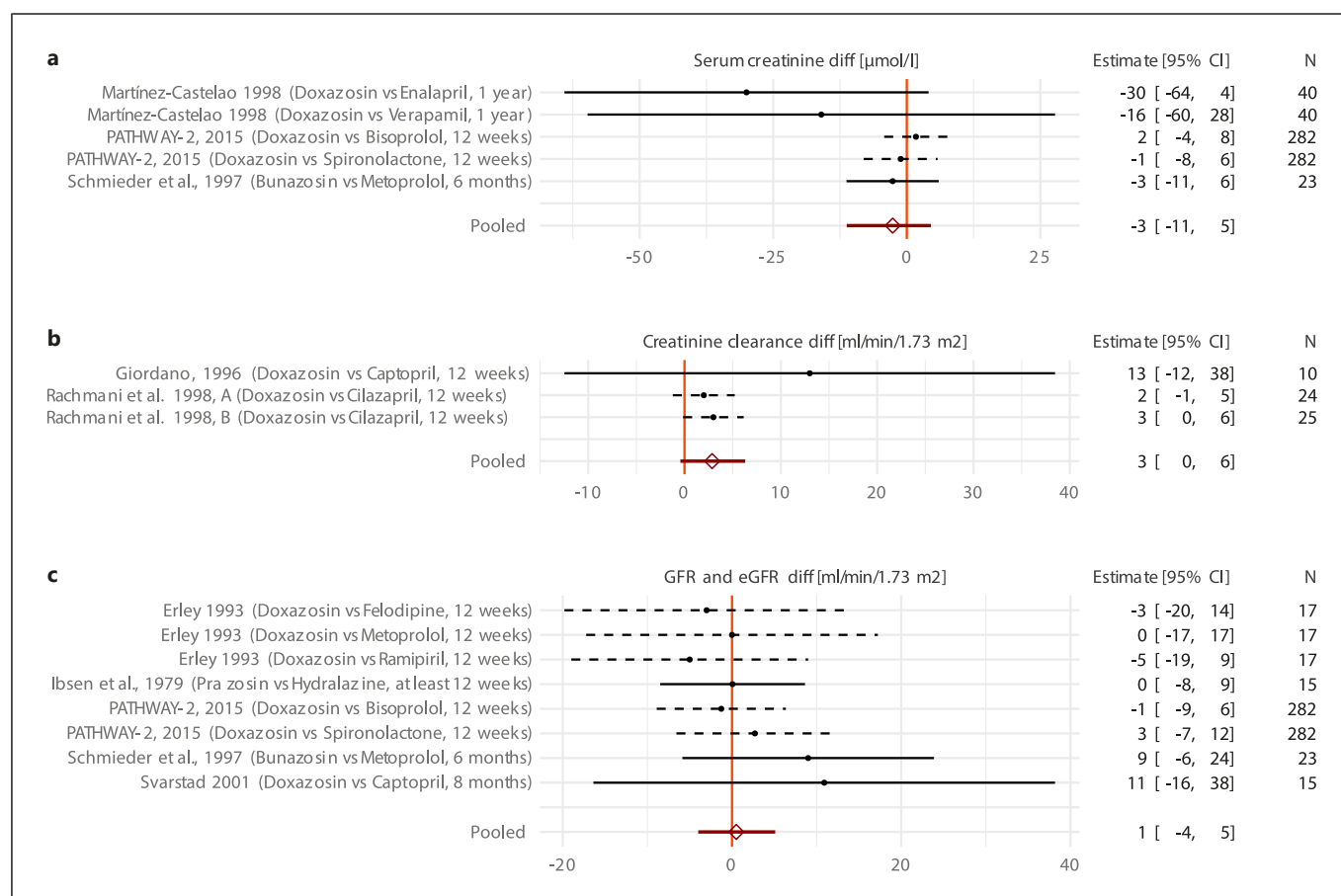


Fig. 3. Changes in renal parameters in patients receiving alpha-blockers compared with an active treatment in controlled trials. **a** Change in serum creatinine compared with active treatment. **b** Change in creatinine clearance compared with active treatment. **c** Change in GFR and eGFR compared with active treatment. Dashed lines represent confidence intervals that could not be exactly reconstructed (they are likely overly wide).

221–442 $\mu\text{mol/L}$) for a total of 14 weeks. Seventeen patients had concomitant antihypertensive medication, most frequently β -blockers ($N = 15$). Serum creatinine did not show any significant change during follow-up: the baseline level was $319 \pm 17 \mu\text{mol/L}$, and at the end of follow-up it was $333 \pm 22 \mu\text{mol/L}$. The trial exhibited a significant bias in patient selection and missing data. Of the 23 recruited patients, 18 did not meet all inclusion criteria but were included at the investigator's discretion. Additionally, data from 6 patients were considered valueless for efficacy [79].

Two retrospective studies evaluated the effect of alpha-blockers on kidney function. Hundemer et al. [68] evaluated data of 16,088 patients with CKD who were newly prescribed alpha-blockers (doxazosin, terazosin, or prazosin) compared with 16,088 matched CKD patients with new non-alpha-blockers, all aged 66 years or older. Patients were followed for

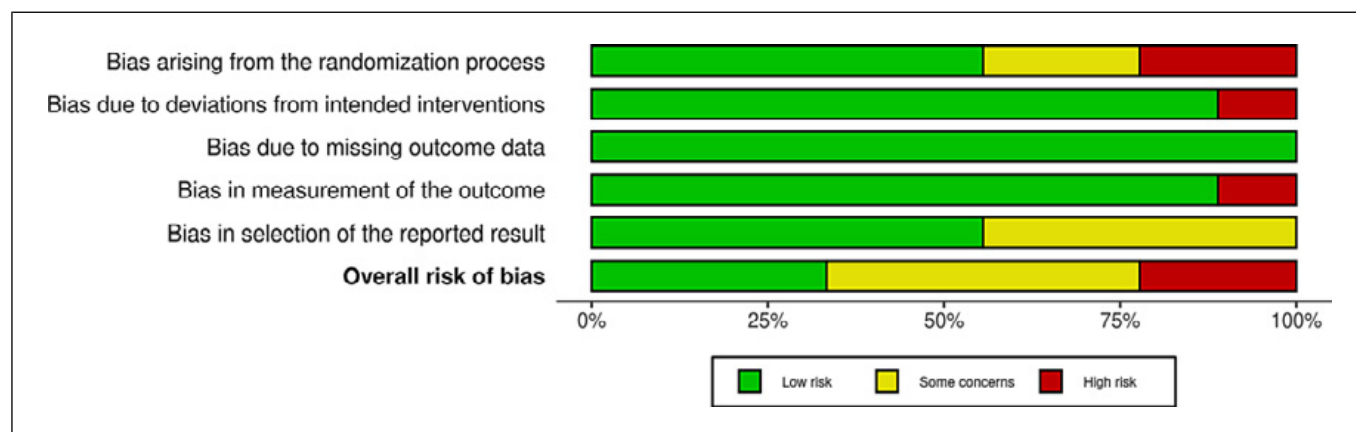
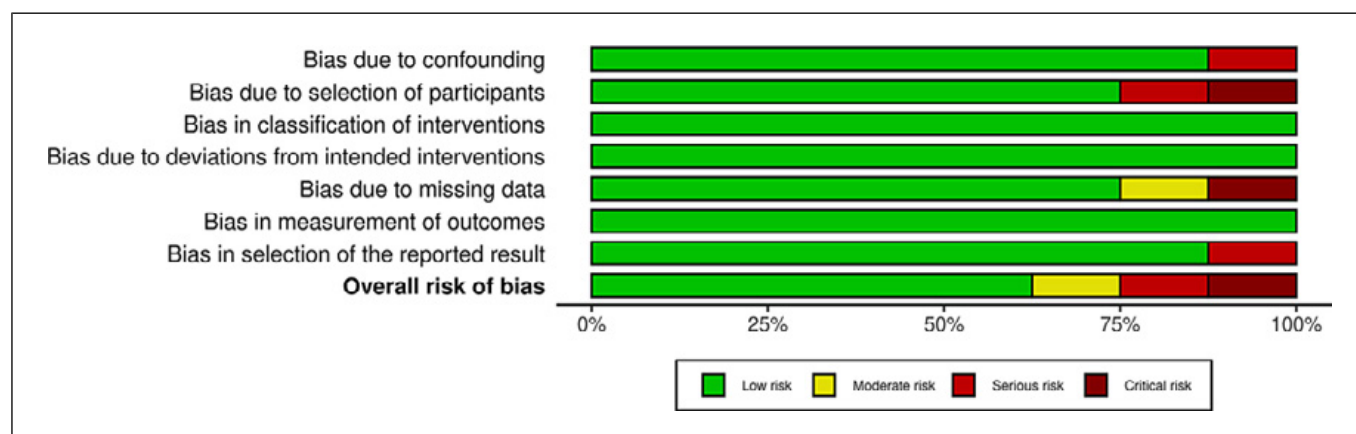
a minimum of 90 days and a maximum of 3 years. The use of alpha-blockers compared with non-alpha-blockers was associated with a higher risk of $\geq 30\%$ eGFR decline (HR: 1.14; 95% CI: 1.08–1.21), dialysis, or kidney transplantation (HR: 1.28; 95% CI: 1.13–1.44). There was a small incidence of RRT in patients with eGFR $>60 \text{ mL/min/1.73 m}^2$ (alpha-blocker 45 vs. 47 events), but an increase was observed in patients with eGFR $<60 \text{ mL/min/1.73 m}^2$ (alpha-blocker 597 vs. 428 events). On the other hand, the use of an alpha-blocker was associated with a significantly lower risk of cardiac events and mortality, which is contradictory to the result of the ALL-HAT trial [68].

Murakami et al. [69] evaluated data from 301 patients with advanced CKD 60 months before the initiation of dialysis, from 1995 to 2015. The development of renal function was evaluated by using the slope of reciprocal serum creatinine. The data were evaluated for every

Table 3. Numerical results of meta-analysis

Outcome	Unit	Pooled difference	95% CrI	tau (95% CI)	I^2 (95% CI)
Serum creatinine difference vs. baseline	[$\mu\text{mol/L}$]	-1.75	(-14.21; 10.69)	13.22 (4.15; 29.59)	0.28 (0.11; 0.48)
Serum creatinine difference vs. active treatment	[$\mu\text{mol/L}$]	-2.61	(-0.86; 4.27)	4.59 (0.17; 15.25)	0.14 (0.01; 0.38)
Creatinine clearance diff vs. baseline	[mL/min/ 1.73 m ²]	5.39	(1.61; 9.97)	2.76 (0.09; 9.77)	0.23 (0.01; 0.57)
Creatinine clearance diff vs. active treatment	[mL/min/ 1.73 m ²]	2.88	(-0.41; 6.54)	1.98 (0.07; 6.81)	0.24 (0.01; 0.57)
GFR or eGFR difference vs. baseline	[mL/min/ 1.73 m ²]	0.46	(-3.2; 4.34)	4.27 (1.39; 8.87)	0.35 (0.16; 0.54)
GFR or eGFR difference vs. active treatment	[mL/min/ 1.73 m ²]	0.49	(-4.03; 5.18)	2.41 (0.09; 7.84)	0.06 (0; 0.17)

CI, confidence interval; CrI, credible interval; tau, standard deviation of the random effect.

**Fig. 4.** Risk of bias in randomised controlled and cross-over studies.**Fig. 5.** Risk of bias in unblinded and retrospective studies.

decade. In summary, the spectrum of antihypertensive treatment changed significantly over the years, with increased use of RAAS inhibitors in patients with advanced CKD in the last decade. From 2010 to 2015, the use of alpha-blockers, like the use of alpha-methyldopa and RAAS inhibitors, was identified as an independent predictor of slower renal function decline [69]. Data from this trial could not be implemented in the meta-analysis.

Effect on Blood Pressure, Side Effects, and Tolerability of the Treatment and Effect on Metabolism

Studies have consistently demonstrated that alpha-blockers significantly reduce on blood pressure. The most substantial data are available for doxazosin, with slightly less pronounced effects observed in the ALLHAT trial, showing a 2–3 mm Hg smaller reduction compared with the effect of chlorthalidone, and in the PATHWAY-2 trial, showing a 4-mm Hg smaller reduction in comparison with that observed with spironolactone.

Most studies have shown good tolerability of treatment with alpha-blockers, comparable to that of other antihypertensive regimes. The most common side effect was orthostatic hypotension, particularly at the first dose, accompanied by reflex tachycardia, weakness, vertigo, fatigue, or agitation, and potentially leading to collapse. In most cases, these symptoms resolved spontaneously or after dose reduction or treatment termination. Other reported side effects include dry-mouth, headache, nausea, diarrhoea, sweating, rash, leg oedema, weight increase, and fluid retention. Alpha-blockers have a neutral effect on lipid profile, glucose in diabetic patients, and serum ions.

Meta-Analysis

The changes in serum creatinine, creatinine clearance, and GFR from baseline were analysed, as presented in Figures 2 and 3 and Table 3. Due to the limited number of available trials, data on GFR and eGFR from both randomised and observational studies were pooled for comparisons with baseline values. This approach is justified because within-arm comparisons in randomised trials carry a similar risk of bias to observational studies, making their combination methodologically acceptable. All between-group comparisons with other antihypertensive treatments (Fig. 3) were derived exclusively from randomised trials, except for eGFR estimates reported by Ibsen et al. [57]. Although the Ibsen study was consecutive rather than randomised, its methodological design approximates that of a randomised trial more closely than a typical observational study. Nevertheless, sensitivity analyses indicated that inclusion of the Ibsen study did not

significantly influence the overall results for eGFR. The available data for other parameters, for example, HR and RR, were insufficient for comparison (online suppl. Fig. 2). The full-data of the meta-analysis models are available in online supplementary Figures 3–7.

There was relatively low overall heterogeneity between studies in the measured outcomes, and heterogeneity appeared to be lower in comparisons with active comparators than with baseline comparators (Table 3). It should be also noted that in the study by Rachmani et al. [47], one group crossed over from doxazosin to cilazapril, and one from cilazapril to doxazosin, resulting in two independent estimates from the same population. Martínez-Castelao et al. [86] reported results separately for “responders” and “non-responders” and did not provide sufficient details to reconstruct a combined estimate. In both cases, we have treated each subgroup as a separate study. The effect of combining antihypertensive treatment with an alpha-blocker, such as in the trial by Ibsen et al. [57], was not evaluated, because the number of trials was insufficient to perform a meaningful analysis.

Compared with baseline, the data suggest an overall small but clinically unimportant increase in creatinine clearance in patients treated with alpha-blockers (95% CrI: 1.61–9.97 mL/min/1.73 m²). For other comparisons, such as plasma creatinine and eGFR and GFR, when compared with baseline and with active treatment, the data are consistent with no change in renal parameters and rule out clinically important differences. This suggests a neutral effect of alpha-blockers on renal function, comparable with other antihypertensive agents included in analysis (see Table 3).

Risk of Bias

Figures 4 and 5 illustrate the prevalence of bias across the included trials, expressed as percentages. Approximately 20% of the RCTs and 25% of unblinded and retrospective studies exhibited a high risk of potential bias. The most frequently reported biases were selection bias and missing data, as mentioned previously in individual trials.

Discussion

Our study is, to our knowledge, the only meta-analysis focusing on alpha-blockers and nephroprotection. Although the included studies and meta-analysis generally indicate a neutral or non-inferior effect of alpha-blockers on renal function comparable to other antihypertensive agents, the scarcity of long-term evidence limits the strength and generalizability of our conclusions.

Most studies have focused on doxazosin, with less frequent inclusion of other drugs. Notably, no trials were identified that investigated urapidil, despite its widespread use. Among the limited studies available, only 3 RCTs were published in the 21st century, with one being open-label [74]. Merely four RCTs had a follow-up duration of at least 3 months, which is insufficient to capture significant CKD progression, as meaningful renal outcomes such as a $\geq 50\%$ decline in eGFR, ESRD, or the need for RRT typically take years to develop [87]. Most RCTs from the 20th century lacked a control group and included insufficient number of participants, typically fewer than 100 participants. The renal effect of alpha-blockers was previously summarised in a comprehensive review by Ptinopoulou et al. [70] who similarly reported limited evidence on the association between alpha-blocker therapy and renal outcomes. Additionally, Ahmad et al. [88] reviewed current evidence regarding alpha-blockers, highlighting their renal and cardiovascular effects in the context of the trial by Hundemer et al. [68]. The authors emphasized the necessity for group-specific, adequately powered RCTs designed explicitly to investigate the long-term renal effects of alpha-blocker therapy [88].

Alpha-blockers remain clinically important as later-line therapy for hypertension inadequately controlled despite first-line antihypertensive agents, including RAAS inhibitors, calcium channel blockers, and diuretics – therapies previously demonstrated to provide significant cardiovascular protection. Moreover, alpha-blockers continue to play a crucial role in the medical management and preoperative preparation of patients with pheochromocytoma and paraganglioma, conditions for which alpha-blockade remains the treatment of choice [89]. Additional clinical indications include management of benign prostatic hyperplasia [90]. Alpha-blockers are commonly prescribed to patients with impaired renal function, including those with end-stage kidney disease, due to their neutral impact on serum potassium levels and favourable pharmacokinetic profile, typically eliminating the need for dose adjustments in renal impairment [91]. The modest positive effect of alpha-blockers on creatinine clearance observed in our analysis may reflect their unique renal haemodynamic effects. Specifically, blockade of alpha-1 adrenergic receptors induces vasodilation, which may improve renal blood flow and enhance glomerular perfusion, thus increasing GFR or facilitating waste product clearance. This hypothesis is supported by small clinical studies, including the study by Mori et al. [78], in which alpha-blocker therapy was associated with re-

ductions in blood urea nitrogen and serum creatinine. Beneficial renal effects were suggested in two studies including patients following kidney transplantation. However, the available evidence is strongly limited: both studies were small, from the same author, and further complicated by issues with drug group selection [73, 86].

In patients with diabetes mellitus, preserving renal function is paramount, as diabetes represents the leading cause of CKD worldwide [87]. Current clinical practice strongly favours the use of RAAS blockade for blood pressure management, due to its established renoprotective effect in diabetic nephropathy. Notably, alpha-blockers may offer a favourable metabolic profile, having been associated with improved insulin sensitivity and beneficial effects on lipid parameters. Therefore, alpha-blockers may represent a potentially advantageous treatment option in diabetic populations, in contrast to certain other antihypertensive agents, such as thiazide diuretics or β -blockers, which may adversely affect glucose metabolism [92].

Regarding heart failure, initial concerns were raised about potentially worsening outcomes with alpha-blocker treatment, as suggested by the ALLHAT trial. However, these findings were not confirmed in the robust ASCOT trial, and current evidence has cast doubt on the increased incidence of heart failure previously reported in ALLHAT, as discussed earlier. Additionally, the COMET trial directly compared the effects of metoprolol with carvedilol – a combined alpha – and β -blocker – and demonstrated superiority of carvedilol in reducing all-cause mortality in patients with chronic heart failure. A proposed mechanism underlying this observation is that alpha-blockade reduces afterload and neurohumoral activation, potentially leading to improved clinical outcomes. Therefore, combined alpha- and beta-adrenergic blockade, aimed at comprehensive adrenergic inhibition, represents an important direction for future research [93].

The frequency of side effects associated with alpha-blocker use was comparable to that of other antihypertensive agents. The most reported side-effect associated with alpha-blocker treatment was orthostatic hypotension. However, this side-effect typically occurs after the first dose and can generally be mitigated by careful dose titration, employing extended-release formulations, or administering the medication at bedtime to minimize the risk of daytime hypotension [88, 94].

Given their favourable or neutral metabolic effects, good tolerability, and limited side-effect profile, alpha-blockers warrant further investigation, particularly in combination

with other antihypertensive agents. They may potentially be useful in fixed-dose combinations for patients with resistant hypertension, in whom adherence to treatment remains a significant clinical challenge [95]. Combining alpha-blockers with diuretics in heart failure could mitigate fluid retention while preserving beneficial effects on afterload reduction, thus enhancing cardioprotective outcomes. Additionally, in patients with CKD, diabetes mellitus, and heart failure, combinations of alpha-blockers with RAAS inhibitors or newer therapeutic classes such as sodium-glucose cotransporter-2 inhibitors (SGLT2i) and non-steroid MRA (e.g., finerenone) merit exploration, as these drug classes have demonstrated significant reductions in mortality and morbidity in these conditions [10, 31].

The primary limitation of this systematic review is the relatively small number of available studies assessing the effect of alpha-blockers on renal function. To comprehensively summarise the existing evidence regarding the long-term renal impact of alpha-blocker therapy, retrospective studies were also identified and discussed; however, these studies were not eligible for inclusion in the quantitative synthesis.

The present meta-analysis has several limitations, primarily due to the relatively small number and limited size of the included randomised trials. Notably, a significant risk of bias was identified in approximately 25% of included trials, predominantly attributable to selection bias. Two additional technical factors lessened the precision of our analysis: firstly, renal function assessment in the earlier studies frequently used outdated biochemical methods without adjustment for body surface area, and secondly, most trial results were incompletely reported.

Conclusion

Although our findings suggest that alpha-blockers generally exert a neutral or non-inferior effect on renal function compared with other antihypertensive agents, robust conclusions are limited by the scarcity of high-quality, long-term evidence. Most available trials predominantly investigated doxazosin, with only limited evaluation of other alpha-blockers.

Alpha-blockers remain clinically important as the second-line therapy for hypertension resistant to first-line antihypertensive agents and are frequently prescribed to patients with impaired renal function due to their neutral metabolic profile and pharmacokinetic advantages. Clinically, alpha-blockers may

be particularly beneficial for diabetic patients given their favourable effects on insulin sensitivity and lipid profiles. Future research should systematically evaluate alpha-blockers in combination with other antihypertensive agents such as RAAS inhibitors, SGLT2i, non-steroidal MRAs (e.g., finerenone), diuretics, or β -blockers. Furthermore, the renal impact of less frequently studied alpha-blockers, such as urapidil, warrants specific investigation. Additionally, fixed-dose combination therapy with alpha-blockers may improve treatment adherence and clinical outcomes in patients with resistant hypertension. Preliminary evidence also suggests a potential nephroprotective benefit of alpha-blockers in renal transplant recipients, which merits further exploration.

Overall, current evidence highlights the critical need for rigorous, adequately powered RCTs designed explicitly to evaluate long-term renal and cardiovascular outcomes associated with alpha-blocker therapy. Future research should prioritise diverse patient populations, extended follow-up durations, exploration of fixed-dose combinations, and comprehensive outcome reporting to clearly delineate the therapeutic role and potential nephroprotective and cardiovascular benefits of alpha-blockers.

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Following the creation of the original manuscript, we employed ChatGPT version 4.5, an artificial intelligence model developed by OpenAI, to evaluate and correct the language in the initial draft, which was subsequently edited by a native speaker. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Statement of Ethics

Ethical approval for conducting the systematic review and meta-analysis was not required due to the nature of the collected data. Participants in the interventional trials provided written informed consent prior to enrolment in the studies, and ethical approval for data evaluation was granted by the Local Ethics Committee.

Conflict of Interest Statement

There are no relevant relationships with the industry and no other competing interests. The authors have no conflicts of interest to declare.

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Author Contributions

Z.R. corresponding author has full access, and was responsible for prepared the first draft of the manuscript, conceptualization, methodology, data integrity, the data analysis, and project administration. Z.R. and T.K. collaboratively selected the eligible

studies. Data curation was performed by Z.R. and M.M. Statistical analysis was conducted by M.M. L.J., M.D., Z.L., B.Č., D.M., and T.V., and all remaining authors contributed to manuscript review, critical revision, validation, visualisation, and editing. Any discrepancies were resolved through consultation with J.V., who supervised the process. The final version of the manuscript was approved by all authors.

Data Availability Statement

All available data related to the original analysis are included in the article or in online supplementary Tables 1–4 and online supplementary Figures 1–7. The protocol for this systematic review and meta-analysis is available at <https://doi.org/10.17605/OSF.IO/A4SME>.

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