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Long-term perspectives in advanced heart failure therapies

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Long-term perspectives in advanced heart failure therapies

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Table of contents

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Editorial: Long-term perspectives in advanced heart failure therapies

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KEYWORDS

advanced heart failure (AHF), LVAD (left ventricular assist device), heart transplantation (HTx), short term mcs, medical therapy, heart failure

Editorial on the Research Topic
Long-term perspectives in advanced heart failure therapies

Advanced heart failure is increasingly prevalent and the most resource-consuming entity of the global heart failure pandemic.

In addition to established treatment options like cardiac transplantation and long-term mechanical circulatory support, other contemporary approaches, such as short-term mechanical circulatory support and medical therapies expand the therapeutic armamentarium.

This research collection addresses these aspects, as well as current developments in the diagnostics of advanced heart failure and treatment optimization after cardiac transplantation or LVAD implantation in terms of early postoperative, as well as long-term outcomes.

The article by Keyt et al. reviews the unique pathophysiology of thin filament mutations, an important recently-discovered etiology of cardiomyopathies leading to cardiomyopathy. They further emphasize the relevance of personalized treatment options and give an overview of current therapies for this specific scenario.

In regards to medical therapies in advanced heart failure, the selected articles focus particularly on the role of the phosphodiesterase-5 inhibiting drug sildenafil, and the calcium sensitizer and potassium channel opener levosimendan.

Abdelshafy et al. systemically review the current evidence of levosimendan in the treatment of advanced heart failure, especially in the perioperative LVAD setting to prevent or mitigate postoperative right heart failure. They found advantageous hemodynamic conditions, which so far did not translate into clinical benefits; the stage is set for a well-conducted prospective randomized clinical trial in this setting in the future.

Monzo et al. investigated clinical variables that could be associated with the hemodynamic response to sildenafil in a subset of patients with pulmonary hypertension fulfilling criteria for vasoreactivity testing. They identified serum potassium levels, serum aldosterone levels and atrioventricular valve regurgitation as potential cofounders of hemodynamic response to acute administration of PDE5i.

With temporary mechanical circulatory support becoming a more prevalent option as a bridge to recovery, or to durable advanced surgical therapies, potential complications and

problems inherent to intermediate-term support warrant further investigation. Sugimura et al. evaluated the outcome of patients supported by microaxial flow pump systems after re-implantation procedures. Re-implantation was required in approximately one in seven patients in their single center cohort, and could be performed safely. Importantly, half of all system exchanges were required due to pump thrombosis. These results merit further analysis of anticoagulation strategies.

Comprehensive and forward-thinking articles are collected exploring the latest advances in LVAD therapy.

Maw et al. were able to demonstrate the safety, short-term efficacy and physiological responsiveness of a sensorless automated speed control system for a centrifugal LVAD in a pilot study. They were able to increase support during physical exertion and decrease support during resting times. With this, an important first step towards "smart pumps" and therefore improved hemocompatibility (less suction events, as well as better quality of life (better exercise tolerance) has been made.

Kortekaas et al. evaluated the treatment success of systemic thrombolysis with a structured protocol as an alternative treatment strategy to pump exchange in patients with VAD thrombosis. They showed that a systemic thrombolysis is feasible and successful in the majority of cases and might be a reasonable treatment alternative, especially in patients at high surgical risk, and in those who would require a complete system exchange (e.g., HVAD to HeartMate 3).

Nozdrzykowski et al. compared the standard sternotomy surgical approach to LVAD implantation with less invasive surgery with respect to morbidity and mortality during the first postoperative year in a large contemporary LVAD cohort. They were able to demonstrate potential advantages of less invasive surgical procedures during the very early postoperative period.

Finally, four articles deal with relevant topics in cardiac transplantation medicine.

Starting with Rivinius et al., they carefully investigated whether preoperative type II diabetes mellitus in cardiac transplant recipients has an impact on postoperative outcome. They were not only able to present acute postoperative results, but also stratified outcomes up to five years post-transplant analyzing the impact of postoperative HbA1c control. Thus, they highlight the importance of optimal glycemic control after cardiac transplantation.

Kooij et al. summarize their institutional experience regarding the important topic of sinus node- and conduction-system disturbances during the early post-transplant period. In their analysis, theophylline remains a valuable alternative to permanent pacemaker implantation and might be able to facilitate weaning of chronotropic support.

Further insights into the early postoperative period after heart transplantation are gained with the findings from Zhao et al. They evaluated the impact of extracorporeal membrane oxygenation on right ventricular function after heart transplantation. Interestingly, recipients preoperative right ventricular characteristics most likely display a certain degree of pulmonary vasculature damage that has relevant influence on the postoperative need of ECMO and therefore a significant impact on short- and long-term survival after transplantation.

McDonald et al. studied the histopathological phenomenon of acute myocardial injury (AMI) after cardiac transplantation. AMI is a further finding in endomyocardial biopsies, scientifically underrepresented when compared with grading of humoral or cellular rejection. They were able to correlate these findings with primary graft dysfunction and reduced survival. The authors suggest ischemia and myocardial reperfusion injury as primary contributors to those structural changes.

Overall, this research collection strives to provide a comprehensive and forward-thinking perspective on some of the latest advances in advanced heart failure diagnosis and therapy, paving a path forward for improved patient outcomes and better quality of life. It emphasizes an urgent need for precision medicine and the expansion of our therapeutic options beyond the conventional approach.

Author contributions

FS writing of the editorial, FG reading, correcting editorial. CM reading, correcting editorial. IN reading, correcting editorial. All authors contributed to the article and approved the submitted version.

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.

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A Sensorless Modular Multiobjective Control Algorithm for Left Ventricular Assist Devices: A Clinical Pilot Study

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Background: Contemporary Left Ventricular Assist Devices (LVADs) mainly operate at a constant speed, only insufficiently adapting to changes in patient demand. Automatic physiological speed control promises tighter integration of the LVAD into patient physiology, increasing the level of support during activity and decreasing support when it is excessive.

Methods: A sensorless modular control algorithm was developed for a centrifugal LVAD (HVAD, Medtronic plc, MN, USA). It consists of a heart rate-, a pulsatility-, a suction reaction—and a supervisor module. These modules were embedded into a safe testing environment and investigated in a single-center, blinded, crossover, clinical pilot trial (clinicaltrials.gov, NCT04786236). Patients completed a protocol consisting of orthostatic changes, Valsalva maneuver and submaximal bicycle ergometry in constant speed and physiological control mode in randomized sequence. Endpoints for the study were reduction of suction burden, adequate pump speed and flowrate adaptations of the control algorithm for each protocol item and no necessity for intervention via the hardware safety systems.

Results: A total of six patients (median age 53.5, 100% male) completed 13 tests in the intermediate care unit or in an outpatient setting, without necessity for intervention during control mode operation. Physiological control reduced speed and flowrate during patient rest, in sitting by a median of −75 [Interquartile Range (IQR): −137, 65] rpm and in supine position by −130 [−150, 30] rpm, thereby reducing suction burden in scenarios prone to overpumping in most tests [0 [−10, 2] Suction events/minute] in orthostatic upwards transitions and by −2 [−6, 0] Suction events/min in Valsalva maneuver. During submaximal ergometry speed was increased by 86 [31, 193] rpm compared to constant speed for a median flow increase of 0.2 [0.1, 0.8] L/min. In 3 tests speed could not be increased above constant set speed due to recurring suction and in 3 tests speed could be increased by up to 500 rpm with a pump flowrate increase of up to 0.9 L/min.

Conclusion: In this pilot study, safety, short-term efficacy, and physiological responsiveness of a sensorless automated speed control system for a centrifugal LVAD was established. Long term studies are needed to show improved clinical outcomes.

Clinical Trial Registration: ClinicalTrials.gov, identifier: NCT04786236.

Keywords: left ventricular assist device (LVAD), mechanical circulatory support, physiological control, smart pumping, Valsalva maneuver, orthostatic transitions, submaximal bicycle ergometry

INTRODUCTION

Implantation of a Left Ventricular Assist Device (LVAD) is an established therapy for end-stage heart failure. Technological and patient management advances have resulted in continuously improving survival-rates. However, success of LVAD therapy is still limited by hemocompatibility associated adverse events and quality of life challenges (1).

Adaptation of the LVAD to the physiological demand of the patient, the final frontier of "smart pumping" has long been proposed as a potential contender in the race for better outcomes and quality of life. But this adaptation has arguably even regressed from the days of pulsatile fill-to-empty pumps (2).

Contemporary LVADs typically operate at a constant speed (CS), set by the VAD clinicians with only periodic check-ups (3). In CS, only the pump-specific pressure flowrate characteristic determines the function that maps pump flowrate to the difference between left ventricular and aortic pressure. The inherent conflation of afterload and preload is however quite different from the native control mechanism of cardiac output, which is much more preload sensitive, and much less afterload sensitive (4).

Short term fluctuations are not properly compensated by the pump-characteristic. Residual native adaptive mechanisms such as the Frank-Starling mechanism are often impaired but not completely absent in LVAD patients. Still, it has been shown that patients could benefit from additional pump support during activity (5, 6). On the other hand, patients were shown to exhibit high levels of ventricular suction, collapse of a ventricular structure onto the inflow cannula, often brought upon by unphysiologically low ventricular pressures, even when their CS set-speed has been optimally adjusted (7).

Thus, greater adaptation of pump support is warranted. This greater flexibility of pump support should ideally come within seconds, as quick hemodynamic changes are quite common in everyday life, such as while standing up or coughing. Other adaptations need to be made within minutes, or hours such as adaptation to prolonged activity or diurnal variations. Automatic physiologic control (PhC) promises to add this functionality to continuous flow LVAD.

Abbreviations: AM, Ambulatory ward; CS, Constant speed; DR, Demand response module; ER, Submaximal ergometry; HR, Heart Rate; IC, Intermediate care unit; IQR, Interquartile range; LVAD, Left Ventricular Assist Device; OR, Orthostatic changes; PhC, Physiological control; PS, Pulsatility-presuction module; RLI, Rate limited increase module; SU, Suction detection and reaction module; VA, Valsalva maneuver.

Clinical application of PhC algorithms is still limited. While the HeartMate 3 (Abbott Laboratories, Chicago, IL, USA) reacts to Pulsatility Index events with transient speed decreases, all other deviations from constant speed in currently clinically used continuous flow devices such as the Lavare^{TM} cycle (8) and the Artificial Pulse (9), are periodically triggered and thus not adaptive to patient demand.

Previous research efforts have identified numerous control strategies which have been validated *in silico*, *in vitro*, *ex vivo*, but only once in a clinical trial (10, 11). In these studies, it could be shown, that these controllers adapt rapidly to changes in patient state, such as changes in venous return, arterial resistance, and simulated exercise (12, 13).

In a previously conducted clinical trial, it could be shown that a PhC algorithm could safely increase flowrate while decreasing pulmonary capillary wedge pressure during ergometry. The used pump system was an axial pump system with included flowsensor (14).

In this paper, an adapted, modular sensor-less multi-objective controller for a centrifugal pump (Medtronic HVAD) is described and tested in a clinical pilot study.

METHODS

Data Availability

Additional data can be found in the **Supplementary Material**. Data in accordance with privacy and confidentiality restrictions is available from the corresponding author upon request.

Patients

After approval was obtained from the institutional review board of the Medical University of Vienna and from the Austrian National Authority for Medical Devices (BASG), six patients who received an HVAD at the Medical University of Vienna were enrolled and completed their measurements between December 2020 and June 2021, when the study was terminated, due to the global stop of sale of the Medtronic HVAD. The study was registered (Clinicaltrials.gov: NCT04786236) and informed consent was provided by the patients. Pre-test screenings included echocardiographic evaluation to assess functional status of the ventricles and to rule out intraventricular or aortic root thrombus. Home documentation and lab results were checked for proper anticoagulation. Patients with history of stroke or suspected pump thrombosis as well as patients with known coagulopathies were excluded.

Study Design

The single blinded, crossover study protocol consisted of a set of activities performed twice, once in CS mode, and once in PhC mode. The sequence of the speed modes was randomized via permuted block randomization. Measurements were performed either at the intermediate care (IC) unit, or during outpatient follow up in the ambulatory ward (AM).

Activity Protocol

Patients performed activities adapted to their capabilities. The standardized protocol consisted of three segments.

Orthostatic Changes

Patients performed postural transitions from a supine position to standing position and in the reverse direction. If this was not possible for them, a transition from supine to the sitting position was performed. For analysis, the transitions were subdivided into 3 phases: Steady state (SS = 5–10 s before transition initiation), initial phase (IP = 0–15 s after transition initiation) and late phase (LP = 15 – 60 s after initiation) (15).

Valsalva Maneuver

While seated, patients forcefully exhaled into a positive expiratory pressure device (BA-Tube, Flores Medical GmBH, Germany) for up to 15 s. For analysis, the maneuvers were again subdivided into a steady state (SS), straining phase (SP), and recovery phase (RP). SS was again defined as 5 s before initiation of strain. SP comprises phase I and phase II from conventional Valsalva classification: the period of forced exhalation. RP is defined as phases III and IV, or recovery back to baseline. This was defined as the first 30 s after strain release (16).

Submaximal Ergometry

Patients completed a ramped submaximal ergometry (ER) protocol (Daum Electronics Gmbh, Fürth, Germany). If possible, initial load was set to 20 Watts and increased each minute until 70% of the maximum power during ergometry in their latest maximal spiro-ergometry was achieved. If 20 Watts exceeded the capabilities of the patient a bed pedaling device was used instead. An additional 1-minute warm-up and cool down period was optional. For analysis, ER is subdivided into a warmup phase (WU: First 10% of total duration), an early phase (EP: 10–60%), a late phase (LP: 60–100%) and a cooldown phase (CD) of 1 min.

OR and VA were repeated three times for each control mode and outcome measures were averaged.

Control System

The developed PhC system consists of 3 hemodynamically functional modules and one supervision module. An overview is given in **Figure 1**. The demand response module (DR) defines a linear function of heartrate (HR) to desired maximal speed. HR is estimated from the pump current and gradual changes are considered for the module whereas sudden changes are seen as pathologic and ignored. A pulsatility presuction module (PS), aims at maintaining a constant flowrate pulsatility, if not limited by other modules. A suction reaction module (SU) detects suction and reacts by incrementally reducing speed. Finally, a supervision module arbitrates conflicting commands, and a rate limiter (RLI) module enforces relative and absolute speed limits. A comprehensive description can be found in **Supplementary Material S1**.

Hardware and Software Setup

PhC algorithms were developed for a centrifugal LVAD (HVAD, Medtronic, **Figure 2**, 1-5). These algorithms were implemented in Simulink/Matlab (MathWorks INC, Natick, MA, USA) and compiled onto a prototyping unit (**Figure 2**, #7) (MicroLabBox, dSPACE GmbH, Paderborn, Germany) which exchanges information with the laptop (**Figure 2**, #8). The laptop displays a custom graphical user interface. The processing unit sends and receives data from the controller (**Figure 2**, #4) via the serial data port. A switchbox is implemented as a safety measure (**Figure 2**, #6). It allows quick switching of the source of speed commands between standard manual operation via the clinical monitor (**Figure 2**, #1) and the processing unit.

Recorded Data

Pump power, current, speed and estimated flowrate were recorded at 50 Hz, which were then also used to calculate derived indices such as Aortic Valve opening (17), Suction Detection (18), and HR (19). For greater arrhythmia-detection accuracy, patients were additionally outfitted with a 5-lead Holter electrocardiographic (ECG) device (medilog® AR 12 plus, Schiller AG, Baar, Switzerland). RR Intervals were detected by the Pan-Tompkins algorithm (20) and arrhythmias were classified via the Medilog Adapt Algorithm (21). Finally, patient demographic data were extracted from the hospital database and inflow cannula angles were measured as described in (22).

Setpoint Design

CS speed was not modified from the pre-test setpoint established by the routine clinical team, which aims to maintain a neutral septum position and, if possible, intermittend aortic valve opening. Speeds are confined to the recommended range of 2,400–3,200 rpm in CS operation.

For PhC, HR range was determined by reviewing retrospective logfile recordings when available and selecting the 10th and 90th HR percentile. Otherwise, baseline HR was set to resting HR during pretest sitting and 30 bpm added for exercise HR. Speed range for DR was set to 200 rpm below and 200 above pre-test CS set speed for rest and exercise set speed, respectively.

Set pulsatility was set to 2 L/min below usual pulsatility, determined by logfile recordings and during premeasurement evaluation but at a value not smaller than 1 L/min.

The setpoints were set by the attending investigator, the values above served as guidelines, but could be overruled, thus in some patients, DR setpoints or the PS Setpoint were set deliberately high to achieve more PS contribution.

Statistical Analysis

All values are stated as: median and [interquartile range] or mean ± standard deviation.

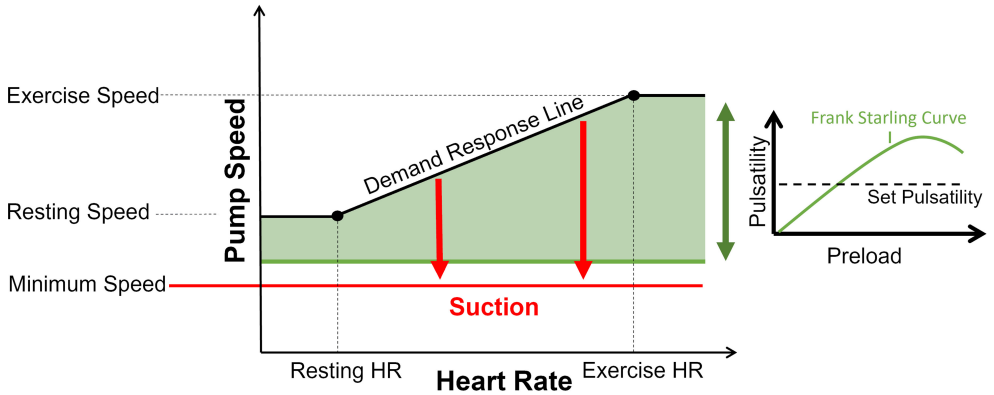

FIGURE 1 | Graphic representation of the control algorithm. The demand response submodule sets an upper speed limit based on heart rate. The flow pulsatility is partially governed by the Frank Starling curve of the ventricle (in the right panel). If flow pulsatility exceeds the set-point, sufficient filling is expected and speed is increased proportionally up to the demand response line (black). Conversely, if flow pulsatility is below set-value, speed is decreased, eventually down to the minimum speed limit. If suction is detected, speed is reduced by discrete steps until suction is cleared. If speed is decreased to minimum speed (red line) and suction is still present, speed is not further decreased. Speed is increased once suction is no longer present.

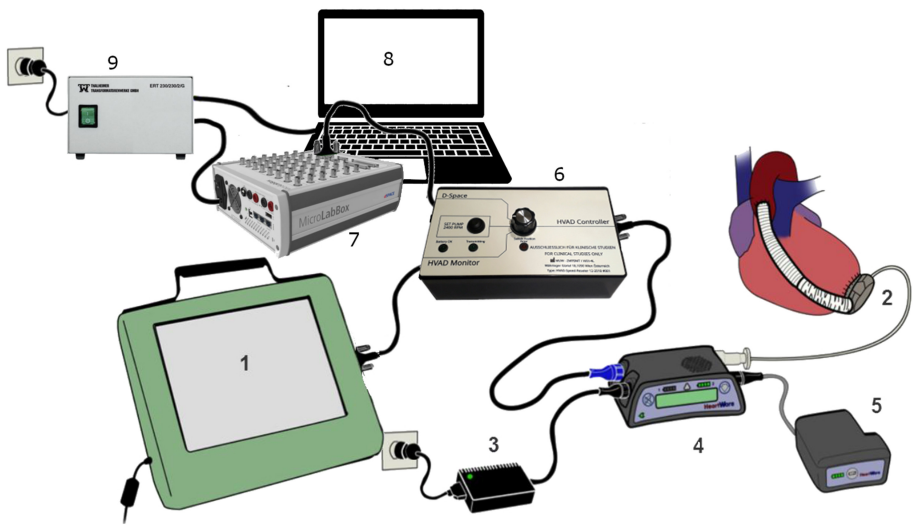

FIGURE 2 | The clinical routine hardware setup of the HVAD (1–5) was modified with the addition of a switchbox (6), the dSpace MicrolabBox (7), a laptop (8), and an isolation transformer (9). The switchbox routes serial transmission to the controller between the monitor, the internal microprocessor and the dSpace based system (1: Monitor; 2: HVAD pump; 3: Power supply; 4: Controller; 5: Battery; 6: Switchbox; 7: dSpace Microlabbox; 8: Laptop; 9: Isolation Transformer). Adapted from (8).

RESULTS

Demographics

A total of six patients partook in the investigation for a total of 13 measurements. A demographic overview can be found in **Supplementary Table S2-1**. All patients were male. All but one patient were on beta-adrenergic blocking agents and all but two received ACE Inhibitors, while three were on amiodarone. Patient 5 had grade II aortic insufficiency at baseline echocardiographic evaluation. Coronary cannula inflow angles ranged from −30° to 32°.

Overview of Tests

Three patients completed 3 tests, one patient 2 tests and two patients completed only one session for a total of 13 tests. Three tests took place at the IC and the remaining 10 at AM. In 10 measurements, the patients were able to complete the entire protocol. In 2 tests, patients were not able to perform ER and in 2 tests at the IC, ER was performed with the bed pedal exerciser. In 2 tests comparable data for VA could not be collected. For OR, in 8 tests patients could safely stand up, in 4 patients could sit up. In 1 test no comparable OR could be collected.

Pre-test sitting echocardiography confirmed partial assistance in 8/13 tests. CS pump settings averaged 2682 ± 79 rpm. Baseline

HR was 76 ± 16 bpm and mean arterial pressure was 79 ± 10 mmHg. Pump flow was 4.9 ± 0.4 L/min and pulsatility was 3.5 ± 0.6 L/min. **Supplementary Table S2-2** provides a detailed overview of the tests.

Setpoints

Six out of 13 tests were performed with setpoints as described in setpoint design. In 7/13, SU and PS modules were focused due to either increased DR setpoints (3/7) or pulsatility setpoints at or above baseline pulsatility (4/7) (see **Supplementary Table S2-2**).

Safety Outcomes

Throughout the entire study, there was no need for any safety switchover intervention. Neither CS nor PhC mode posed any risk to the patient as judged by the attending clinician. There were no study related adverse events.

Aggregate Comparative Results for Standardized Protocol

This section reports on the average differences between the PhC and CS. **Figures 3–6** provide an overview over the single standardized interventions. A per-test summary as well as a comprehensive collection of all the single snapshots can be found in the **Supplementary Materials S3**, **S4-6**, respectively.

Orthostatic Changes (OR)

Orthostatic Steady State

In CS pump-flowrate was highest in supine position at 5.3 (5.1, 5.5) L/min with reduced flowrate at sitting position at 5.0 (4.7, 5.3) L/min and lowest flowrate in standing posture [4.6 (4.2, 5.0) L/min]. Suction occurred in 5/10 sessions in standing, and in 2/13 in sitting.

PhC-mode resulted in a lower pump speed compared to CS and was lowest in supine [−130 (−150, 30) rpm] and similar for sitting [−75 (−137, 65) rpm] and standing [−79 (−150, −4) rpm]. Resulting in minor flowrate reductions in standing and supine posture but not during sitting.

Suction burden in standing posture was reduced in PhC compared to CS in 4 of the 5 tests, where suction occurred [0 SE/min (−18, 0)]. However, during most measurements patients did not experience suction in either mode.

Speed was mainly governed by DR in supine [81% (71, 84)] and sitting [73% (31, 92%)]. While standing, SU module was increasingly activated. See **Figure 3** for an overview and **Supplementary Figure S3-1** for an extended overview.

Orthostatic Transitions

In CS, flowrate increased from the baseline of 5.2 (5.0, 5.5) L/min at steady state to 5.5 (5.0, 5.7) in the initial phase due to increase in diastolic flow. In LP flowrate was decreased below baseline to 5.0 (4.4, 5.4) L/min. Suction occurred either in the initial phase (5/12) or in the late phase (6/12).

In PhC, speed was already reduced by −136 (−169, 30) rpm during steady state. From there, speed was slightly increased in the initial phase to −112 (−155, 51) rpm and further increased in late phase to −42 (−152, 47) rpm. This led to reduced flowrates in all phases compared to CS.

Suction in initial phase was decreased in 3/5 sessions and increased in 1/5 compared to CS. In the late phase it was reduced in 3/5 sessions and increased in 2/5. In the two tests with increased suction prevalence in late phase in PhC, speed was either set higher than speed set speed already at steady state (test 12) or repeated attempts at speed increase at low speeds (<2,500 rpm) retriggered suction events (test 9).

From predominant DR activation in steady state, increased activation of SU and PS (Contribution >20% in 4/12 tests) as well as the RLI module is recorded in the initial phase and late phase. In late phase, DR governed speed even less. High activation of PS in test 6 prevents suction without activation of the SU module. SU and RLI modules were also increasingly activated in the late phase. See **Figure 4** for an overview and **Supplementary Figure S3-2** for an extended overview. The mentioned single snapshots can be found in **Supplementary Material S4**.

Valsalva Maneuver

In the straining phase in CS, flowrate was reduced from a steady state of 5.0 (4.6, 5.1) L/min to 4.7 (4.0, 5.0) L/min due to suction events, which occurred in 7/11 sessions for a median suction burden of 14 (0, 24) SE/min, and persisted at least for some additional time after release in the recovery phase for a median suction burden of 5 (0, 21) SE/min. In 4/11 sessions there was almost no flowrate response during VA (example: test 3 in **Supplementary Material S-5**).

In PhC, upon straining, the decreased speed of steady state is mostly upheld or slightly reduced compared to CS [−90 (−153, 61)] rpm resulting in similar flowrates to CS. This led to reduced suction burden in the straining phase compared to CS for 5/7 Tests for a median reduction of −2 (−6, 0) SE/min (example: test 10), in the 2 tests with increased suction burden (test 9 and 13 in **Supplementary Material S-5**), suction occurred at low speeds (<2,600 rpm) or speed was already greatly increased at steady state due to pulsatility setpoint strategy and insufficient speed decrease before suction. Suction burden in recovery phase was reduced in PhC compared to CS in 5/11 Tests [0 (−9/2), SE/min].

DR-Module was again predominant during steady state. In straining phase PS gained relevance in 5/11 tests. Finally in recovery phase, SU was activated in 6/11 tests. See **Figure 5** for an overview and **Supplementary Figure S3-3** for an extended overview. The mentioned single Valsalva maneuver snapshots can be found in **Supplementary Material S5**.

Ergometry

Due to the activity involved in getting on the ergometer and starting movement, warmup flowrate in CS was already increased compared to sitting at rest by 0.4 L/min to 5.4 (5.2, 5.7) L/min. Only in 5/11 tests, flowrate further increased throughout the duration of ergometry by more than 0.3 L/min. The flowrate increase from warmup to late phase was 0.2 (0.0, 0.4) L/min. In 5/11 tests patients experienced suction events, especially in late phase and cooldown.

In PhC, speed at warmup was within 100 rpm of CS settings in 8/11 tests for a medium difference of −21 (−50, 78) rpm. It was increased by at least 100 rpm in early phase or late phase

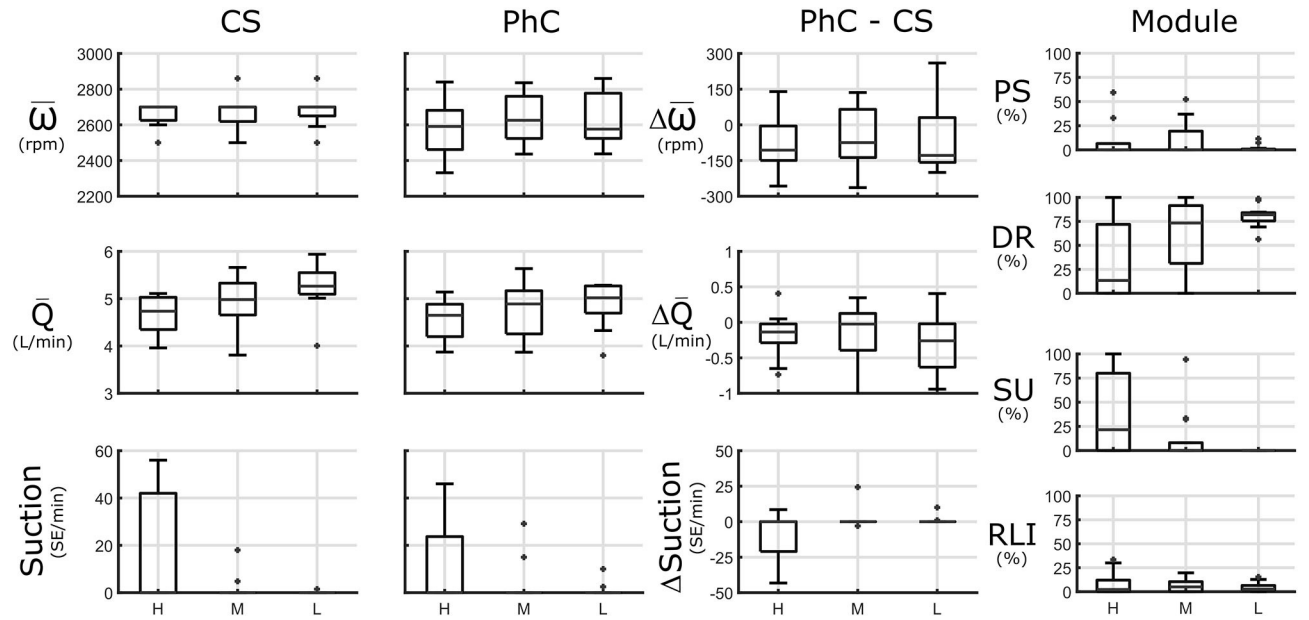

FIGURE 3 | Steady state posture differences between standing(H), sitting (M), supine (L). Left panels: Speed (ω), flowrate (Q), and suction [in Suction Events (SE)/minute] in constant speed (CS) and physiologic control (PhC) and their differences. Module panel: control module activation for pulsatility (PS), demand response (DR), suction response (SU), and rate limited increase (RLI) modules. Speed is reduced in all postures in PhC modes compared to CS resulting in reduced flowrates and reduced suction burden during standing. DR module mainly governs set speed in supine and sitting position with increasing contributions from the SU modules while standing.

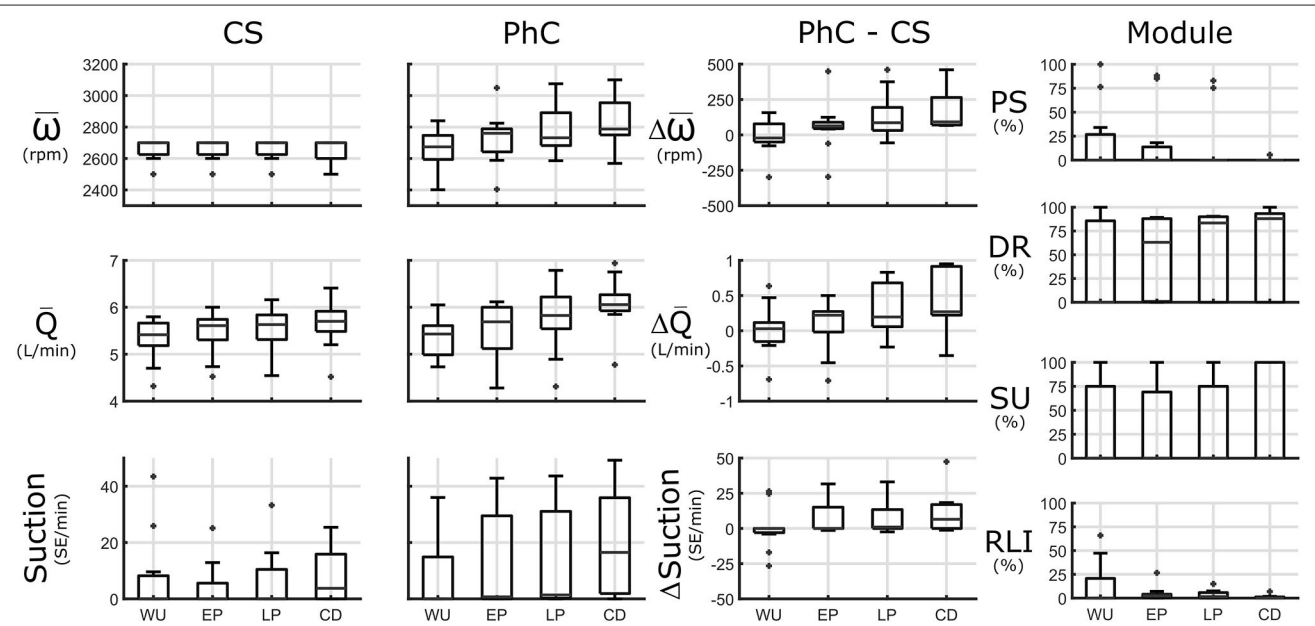

FIGURE 4 | Orthostatic transitions from the supine position to either sitting or standing. Left panels: Speed (ω), flowrate (Q), and suction [in Suction Events (SE)/minute] in constant speed (CS) and physiologic control (PhC) and their differences are presented in steady state (SS) initial transition phase (IP) and late phase (LP). Module panel: control module activation: activation for pulsatility (PS), demand response (DR), suction response (SU), and rate limited increase (RLI) modules. Speed is reduced in PhC compared to CS in all phases. Lower suction burden in IP and LP is observed. There is predominant DR activation at SS with increasing contributions of the PS and SU module at later stages of the transition.

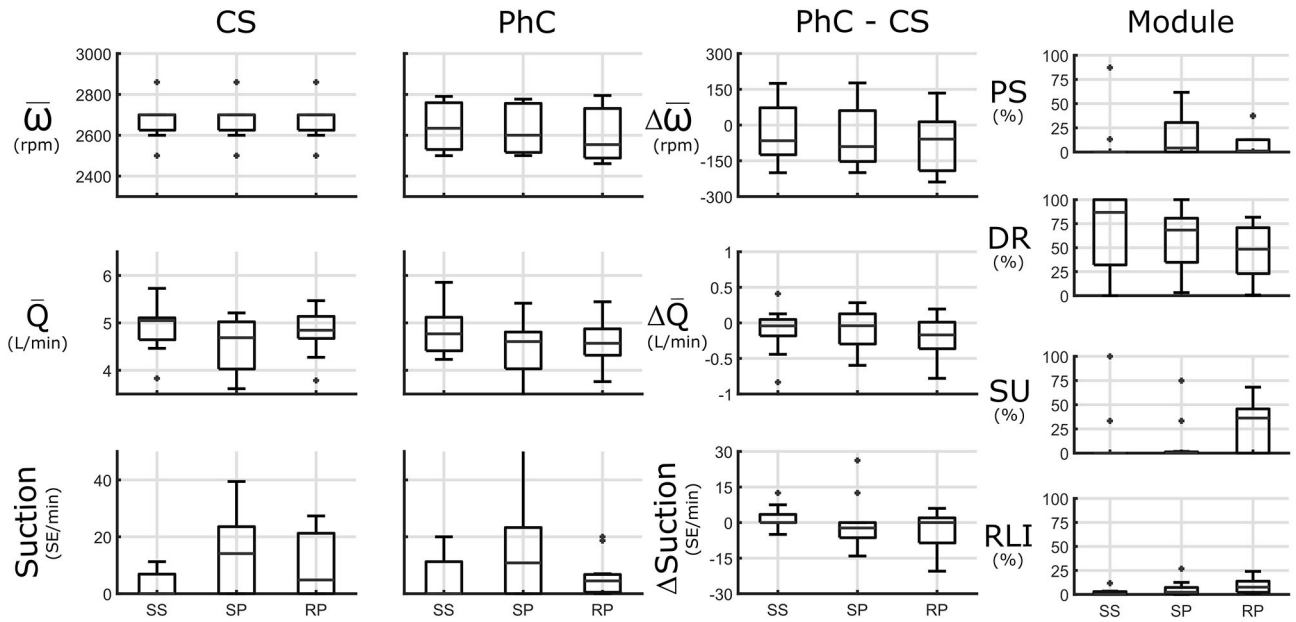

FIGURE 5 | Valsalva maneuver (VA) is subdivided into 3 phases: steady state (SS), strain phase (SP) and recovery phase (RP). Left panels: Speed (ω), flowrate (Q), and suction [in Suction Events (SE)/minute] in constant speed (CS) and physiologic control (PhC) and their differences are presented. Module panel: control module activation: activation for pulsatility (PS), demand response (DR), suction response (SU), and rate limited increase (RLI) modules. Speed is reduced in PhC compared to CS, resulting in lower suction burden.

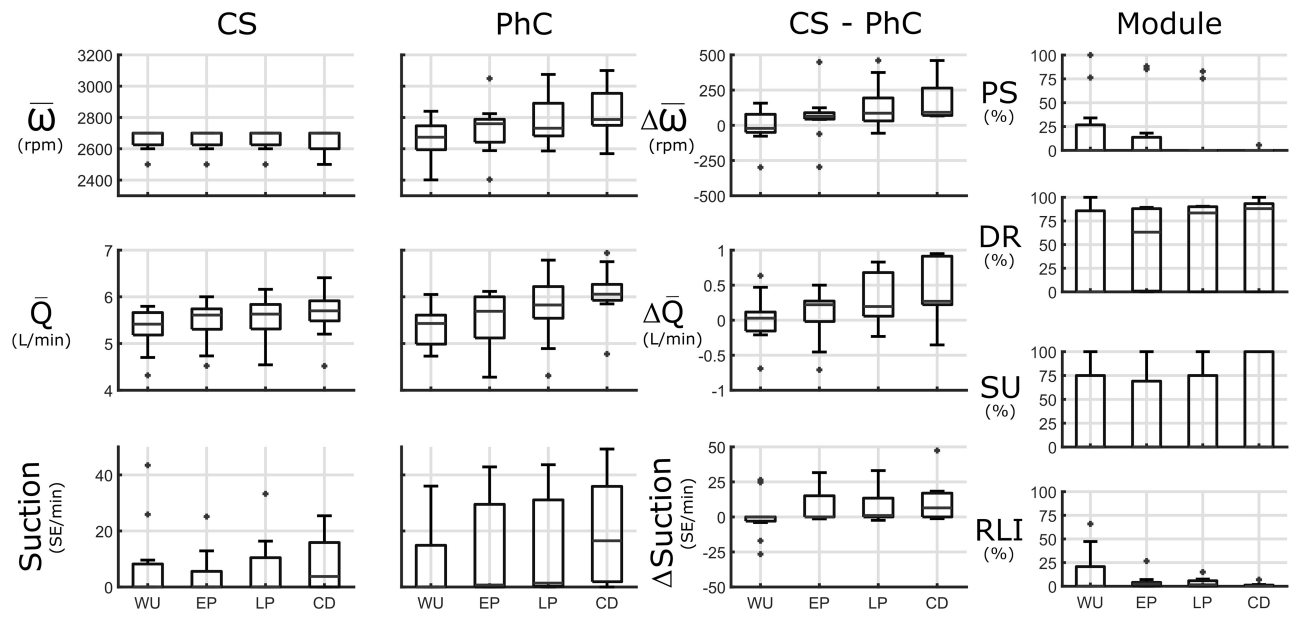

FIGURE 6 | Submaximal ergometry is subdivided into 4 phases: warm up (WU), early phase (EP), late phase (LP) and cool down (CD). Left panels: Speed (ω), flowrate (Q), and suction [in Suction Events (SE)/minute] in constant speed (CS) and physiologic control (PhC) and their differences are presented. Module panel: control module activation: activation for pulsatility (PS), demand response (DR), suction response (SU), and rate limited increase (RLI) modules. Speed during ergometry is increased in PhC compared to CS resulting in increased pump flowrates, especially at later stages of submaximal exercise.

for 8/11 tests for a median increase of 64 (44, 89) and 86 (31, 193) rpm, respectively. In patients, where upper limits of the DR Module were set higher, speed was increased by up to 500 rpm compared to CS (tests 11 and 13). Persistent suction upon speed increase (test 9) or a complete chronotropic incompetence and lack of pulsatility response shortly after implantation (test 1 and 4) restricted speed increase. In one test, speed was only increased in the late phase, as pulsatility remained low until then (test 8).

From warmup to late phase, flowrate was increased by more than 0.5 L/min in 5/11 tests for an overall median increase of 0.2 (0.1, 0.8) L/min. In 3/11 tests flowrate in late phase was increased by ∼0.9 L/min compared to CS.

Suction burden was increased in PhC compared to CS in 4/11 tests in early phase by 0 (0, 15) SE/min and late phase by 1 (0, 13) and 5/11 in the cooldown phase by 6 (0, 17) SE/min. There was no marked decrease in flowrate pulsatility before suction in these patients (minimum pulsatility > 3.5). The reasons for the suction increase were suction classifier discrepancy (test 2), or repeated unsuccessful attempts at speed increase past CS setpoint in test 9 and 12, which were by pat 4 and 2 with cannula inflow angles of 32° and −15°, respectively. In the 2 tests where speed was increased the most (>300 rpm) (tests 11 and 13) suction burden remained below 3 SE/min in both modes. In these patients (Pat. 1 and 5) cannula angles were more favorable at 20° and 9°, respectively.

During warm up the speed increase was rate limited by the RLI at least partially in 6/11 tests. In 3/11 tests pulsatility was not always sufficient to increase speed and in another 3/11 tests repeatedly encountered suction events triggered activity of the SU module. Speed was increasingly set based on the DR module with longer duration of exercise. See **Figure 6** for an overview and **Supplementary Figure S3-4** for an extended overview. the mentioned submaximal exercise snapshots can be found in **Supplementary Material S6**.

Rhythmological Summary

In 4/7 tests arrhythmias such as non-sustained ventricular tachycardia were recorded during OR (3 tests), VA (4 tests), and ER (2 tests). All 38 tachycardia episodes (30 of which occurred in test 12), were triggered by an immediately preceding suction event. However, not every suction event triggered arrhythmic episodes.

Arrhythmia duration was increased in PhC mode in ER in 2 Sessions (CS: 3.5 s, PhC: 13.2 s of arrhythmia). It was decreased in OR in 2 patients and increased in one. In VA unchanged in one test, reduced in two and increased in one session. In one session arrythmia only occurred in CS mode.

DISCUSSION

A multi-objective physiological control algorithm was developed and tested in a safe testing environment in a clinical pilot trial. The controller was able to modulate pump support without the use of additional sensors.

Controller Performance Overview

The controller set lower pump speeds, compared to CS in inactive patient states, such as supine and sitting posture. This led to a lower baseline speed during VA and OR, contributing to reduced suction burden in these suction-prone maneuvers. However, if speed was higher than baseline, speed decreases were generally too slow to avoid suction.

During ER speed was increased by up to 500 rpm compared to CS, generating higher pump flow. However, in other sessions, speed could not be increased above CS speed due to recurrent suction, even with arguably sufficient venous return.

Suction Reduction

Lower pump speed correlated with lower suction events, and suction burden could be reduced in some patients. In the presented dataset every single episode of arrhythmia was preceded by a suction event, similarly to previous experience (23). However, the exact impact of arrhythmia on patient outcomes is not fully understood. While transient episodes of arrhythmia seem to be well tolerated without greater risk for syncope or sudden cardiac death, a non-contractile or fibrillating RV might increase risk for thrombosis (24). Higher pump speeds have previously also been correlated with reduced aortic valve health (25), increased thrombogenicity (26), and increased bleeding (27), partially by increasing pulsatility. However, no definitive study so far has directly clinically linked suction to worse outcomes, possibly due to its low visibility (7).

Increased Support During Activity

On the other side of the spectrum, speed increase during activity has previously been shown to reduce PCWP (28) and improve submaximal capacity (6). It was shown that insufficient speed during exercise reduced peak cardiac output and peak oxygen consumption (29). In our collective we have shown that speed increases during ergometry can automatically be achieved for most patients. In some patients however, recurrent suction or lack of pulsatility does not permit speed increases.

Evaluation of Control Modules

The heart-rate driven DR module was the most activate module. Only in 3 tests HR range was <10 bpm. Two of these tests were within the first 20 post-operative days and one in a patient with multiple comorbidities that relied on a wheelchair. HR tended to be quite responsive during OR and early phases of ER.

The PS module was designed to become active at low ventricular preloads. Only in patient one pulsatility of <0.5 L/min could be achieved upon full unloading. In all other patients there remained considerable residual pulsatility before suction, sometimes without any decrease prior to onset, or a very rapid decrease, limiting the utility of the PS module. However, especially at the IC it was observed that pulsatility was quite responsive to posture changes, such that supine posture produced the highest pulsatility and standing posture the lowest, possibly due to venous pooling in the lower extremity. A pulsatility based controller will thus increase speed upon lying down, which is in line with preload-based paradigms.

In 2 of the 6 patients (33%) speed could not markedly be increased from the CS set speed even at later stages of ergometry, with arguably sufficient venous return. Here, geometrical challenges such as the cannula position, a small LV cavity or restricted filling might be prohibitive to speed increases by restricting pulsatility or triggering suction. Rapid onset of suction in the selected maneuvers often did not leave sufficient time for reaction when speed was already increased, however in some cases, speed reductions were able to break the "vicious cycle" of suction (7).

Absolute and relative speed restrictions were implemented for safety reasons but limited the control system, resulting in excessive speed if baseline speed was already higher at maneuver onset. RLI did not greatly delay PhC reaction to exercise.

Limits of Physiological Control

Non-optimal inflow cannula position, as it may happen if the patient gains weight after implantation, or small cavity size, can trigger suction even when venous return is otherwise sufficient. In our collective we observed suction even at low speeds of 2300 rpm.

Great interpatient variability needs consideration when adapting PhC algorithms. As shown here and also previously by Jain et al. (30) patient hemodynamic responses are highly variable during standardized maneuvers, for example due to baroreflex failures or different baseline preloads. Insufficient vasomotor tone resulting in excessive venous pooling could also explain some of the large responses especially at the IC to orthostatic transitions. Additionally, changes in medication such as omission of betablockers in patient 3 greatly changed hemodynamic response.

Differences to Previous Studies

Differences to previous implementations of the control algorithm in the axial Micromed Debakey LVAD, are the centrifugal pressure-flow characteristic of the HVAD (14, 31). Thus, pulsatility is more influenced by hyper- and hypotension and speed changes as the position on the HQ curve becomes more important due to the non-linearity. The pump-characteristic also causes higher pulsatility in lower flowrates. Additionally, differences in implantation techniques to previous devices might cause a higher rate of positional suction.

Implementation in Other Pump Systems

While the algorithms were only implemented in the HVAD in this study, feasibility of application to other pump systems ensures that progress is not lost with the discontinuation of this specific device. The control algorithms consist of modules that may be adapted for other systems. A suction detection module can be developed either by accessing motor-information of the pumps or with additional sensors within the ventricle or in the pump. Even without the high temporal resolution of 50 Hz provided by the HVAD, many suction detection features rely only on averages and extrema (18). Similarly, the pulsatility module requires only the signal extrema readily available in the HeartMate 3™ (Abbott Laboratories, Chicago, IL, USA) for example, allowing rapid translation. The cycle detection algorithm required for the DR module benefits from higher sample rates. However, all currently available devices are based on similar DC motor technology and thus have access to motor signals such as current and speed, however not all devices provide this information via a serial interface like the HVAD.

The sensorless control algorithms may even be used in systems with additional sensors, either as a redundant safety mechanism against sensor failure or to supplement additional information. Pressure sensor, placed either at the cannula tip, atrium or pulmonary artery could give important insight into patient hemodynamics, and especially about preload. However, current devices like the Cardio MEMS (Abbott Laboratories, Chicago, IL, USA) could not register all types of suction and could not detect ventricular contraction due to its placement in the pulmonary artery.

Limitations

Due to the nature of the pilot study, rigid setpoint protocols were not upheld, and setpoint options were explored, resulting in additional data heterogeneity. Furthermore, low patient numbers due to the discontinuation of the HVAD device, without the inclusion of female subjects, a short observation period, lack of invasive hemodynamic monitoring as well as a protocol not geared toward investigating physiological quantitative differences restricts conclusions drawn from the study.

Outlook

Due to the discontinuation of HVAD sales, follow-up studies with this implementation of the controller are not planned. Very similar algorithms have previously demonstrated efficacy in an entirely different pump system and due to the sensorless nature the presented control concepts are easily translated to additional other continuous flow pumps, such as the HeartMate 3™, Evaheart®2 (Sun Medical Technology Research Corp., Nagano, Japan) or even the family of Impella® pumps (Abiomed Inc., Danvers, Ma.), as well as other pump systems that rely on a DC motor.

CONCLUSION

A clinical pilot trial showed feasibility of a system for physiological control for a contemporary left ventricular assist device. It showed that support could be increased during physical exertion and decreased upon scenarios of overpumping, thereby decreasing suction burden and the correlated arrhythmias. Further study is needed to investigate how this closer adaptation to physiologic demand translates to long term patient outcomes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethikkommission Medizinische Universität Wien. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

The study was conceptualized, designed, and managed by HS and MM. Original manuscript draft was written by MM. Primary data analyses were done by MM and GW. The control algorithm was developed by MM, CG, D'AK, RS, and HS and preliminary tests were performed by MM, TS, and PA. TS and CM were responsible for patient screening and supervision. Investigations were performed by MM, TS, and HS with CM, A-KS, FW, DW, and DZ performing medical investigations. Regulatory responsibilities were shared by MM, DZ, and HS. Infrastructure was provided by HS and DZ. Additional supervision and manuscript editing was provided by FM. All authors reviewed and approved the final version of the submitted manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.888269/full#supplementary-material

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MINI REVIEW
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Perioperative Levosimendan Infusion in Patients With End-Stage Heart Failure Undergoing Left Ventricular Assist Device Implantation

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Left ventricular assist device (LVAD) therapy has been instrumental in saving lives of patients with end-stage heart failure (HF). Recent generation devices have short-to-mid-term survival rates close to heart transplantation. Unfortunately, up to 1 in 4 patients develop a life-threatening right-sided HF (RHF) early post LVAD implantation, with high morbidity and mortality rate, necessitating prolonged ICU stay, prolonged inotropic support, and implantation of a right-ventricular assist device. Pre-operative optimization of HF therapy could help in prevention, and/or mitigation of RHF. Levosimendan (LEVO) is a non-conventional inotropic agent that works by amplifying calcium sensitivity of troponin C in cardiac myocytes, without increasing the intra-cellular calcium or exacerbating ischemia. LEVO acts as an inodilator, which reduces the cardiac pre-, and after-load. LEVO administration is associated with hemodynamic improvements. Despite decades long of the use of LVAD and more than two decades of the use of LEVO for HF, the literature on LEVO use in LVAD is very limited. In this paper, we sought to conduct a systematic review to synthesize evidence related to the use of LEVO for the mitigation and/or prevention of RHF in patients undergoing LVAD implantation.

Keywords: levosimendan, LVAD, right-sided heart failure, inotropes, mechanical circularity support, heart failure

INTRODUCTION

Left ventricular assist devices (LVADs) have been proven to be effective in reducing morbidity and mortality in patients with end-stage heart failure (HF) (1). Furthermore, second and third-generation LVADs provide a significantly improved quality of life and lower complications compared to early generation devices, almost approaching mid-term heart transplant results.

Abbreviations: CI, cardiac index; HF, heart failure; LEVO, levosimendan; LVAD, left ventricular assist implantation device; MCS, mechanical circulatory support; PP, pulmonary pressure, PRISMA, preferred reporting items for systematic reviews and meta-analysis; RHF, right-sided heart failure; RVAD, right ventricular assist device; RVF, right ventricular failure, VP, venous pressure; WP, wedge pressure.

Unfortunately, early perioperative mortality remains high, mainly due to over 20% of LVAD patients developing right-sided heart failure (RHF), which is strongly associated with increased mortality, morbidity, prolonged ICU, and hospital stay (2). Overall, LVAD does not support the heart completely, so the ability of the right ventricle to provide sufficient output to fill the left heart remains essential. Therefore, optimization of patients in the pre-operative status, besides optimal decongestion, probably by pre-conditioning of the sick heart could mitigate and/or prevent RHF.

Levosimendan (LEVO) is a non-conventional inotropic agent that acts as a calcium sensitizer. It works by amplifying calcium sensitivity of troponin C, without increasing the intra-cellular calcium or exacerbating ischemia. LEVO acts as an inodilator, which reduces the cardiac pre-, and after-load. LEVO acts also as a potassium channel opener, which has an active metabolite (OR1896) that peaks approximately 80–90 h after administration and is associated with hemodynamic improvements that are sustained for a week (3). The advantages of LEVO include beneficial symptomatic, hemodynamic, and neurohormonal effects, and improved peripheral organ perfusion and renal function. Importantly, there is no effect attenuation in patients using beta-blockers (4), which is currently one of the main HF treatment agents. In early studies, LEVO has been shown to decrease mortality (5), improve hemodynamics and reduce symptoms. In two recent systemic reviews, our group clearly showed the incremental value of LEVO infusions in the setting of end-stage HF (6), and cardiogenic shock patients needing VA-ECMO support (7).

In this study, we sought to conduct a systematic review to synthesize evidence related to the use of LEVO for the prevention and/or mitigation of RHF in patients undergoing LVAD implantation.

This systematic review was performed and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (8). From inception to December 27, 2021, all relevant items were identified in collaboration with a Librarian at the Erasmus University Medical Centre. We searched Embase, Medline Ovid, Web of Science, Cochrane CENTRAL register of trials, and Google Scholar for articles published until the date of search. Adult ($\geq$18 years) patients with LVAD receiving intravenous LEVO infusions were included. We included all clinical studies containing $\geq$10 patients and published in the last 30 years. Case reports, editorials, reviews, studies included orally administered LEVO, and articles that are not in English language were excluded. Two researchers (HaE and MA) independently reviewed abstracts and full texts in an unblinded standardized manner. Disagreements between the researchers about whether to include a study were discussed and resolved before final approval. Furthermore, references in selected articles were independently cross-checked by the two researchers for other relevant studies.

The search strategy resulted in 506 studies. After removal of duplicates, 369 studies remained. After reviewing the title and abstract, another 359 studies were removed due to irrelevance. Of the remaining 10 studies, only two met the pre-defined inclusion criteria and were consequently included in this review. **Figure 1** displays the PRISMA flowchart. This systematic review included 106 patients from the two papers. A comparison between the two studies is shown in **Table 1**.

Sponga et al. (1) reported the pre-operative use of LEVO in 21 LVAD patients at a single center. The LVADs used in this study were the MicroMed DeBakey VAD (MicroMed Technology, Inc., Houston, TX, United States) and the Incor VAD (Berlin Heart AG, Berlin, Germany).

The inclusion criteria was LVAD patients with pre-operative borderline right ventricular function which was considered if one or more of the following echocardiographic criteria were unmet: right ventricular end-diastolic diameter <35 mm, right ventricular ejection fraction >30%, tricuspid regurgitation <grade II, short/long axis ratio of right ventricle <0.6, pulmonary pressure <35 mm Hg. Pre-operative use of centrifugal pump support was considered an exclusion criteria.

Intravenous infusion of LEVO was administered in the intensive care unit at a dose of 0.1 to 0.2 ug/kg/min for a maximum of 48 h without bolus. The survival rate was 86% at 30 days and 57% at both 1 and 2 years following LVAD support. Four patients died because of RHF associated with low cardiac output and multiorgan failure, three patients died of cerebral bleeding and two patients died of sepsis. Patients were divided in two groups: group 1 (N = 4, 19%) included patients who died due to RHF and group 2 (N = 17, 81%) included patients who survived or died from other reasons. RHF was defined by the occurrence of two of the following criteria: mean arterial pressure <55 mm Hg, central venous pressure >16 mm Hg, mixed venous saturation <55%, cardiac index <2 L/min/m^2, inotropic support >20 units.

Furthermore, hemodynamic data, using a pulmonary artery catheter, and NT-proBNP values were collected four times which are summarized in **Figure 2**. After 48 h, LEVO infusion improved hemodynamic: The cardiac index increased in a significant and progressive manner by 21% (p = 0.014); pulmonary pressure decreased by 12% (p = 0.003); wedge pressure and central venous pressure both decreased by 15% (p = 0.028 and p = 0.016). There was no clear trend in pulmonary or systemic vascular resistances. Heart rate, systolic arterial pressure, mean arterial pressure, and diastolic arterial pressure did not change significantly during the 48 h treatment period. The changes in mixed venous oxygen saturation were significant only after 24 h (p = 0.008). However, the hemodynamic assessment during the time in the two groups was not statistically significant.

Regarding the NT-proBNP, the Median value at 72 h increased by 3% compared to time 0 in group 1 and decreased by 39% in group 2 (p = 0.008). In every single patient, a reduction of <25% at 72 h is a predictor of mortality with a sensitivity of 100% and specificity of 75%. NT-proBNP value after 48 h of treatment was significantly (p = 0.019) higher (8797 vs. 6733 pg/ml) in group 1 than in group 2.

However, 24 h after the end of the treatment hemodynamic performance was worse than baseline in the group who died because of RHF. In contrast, the hemodynamic improvement in patients who survived or died because of other reasons persisted

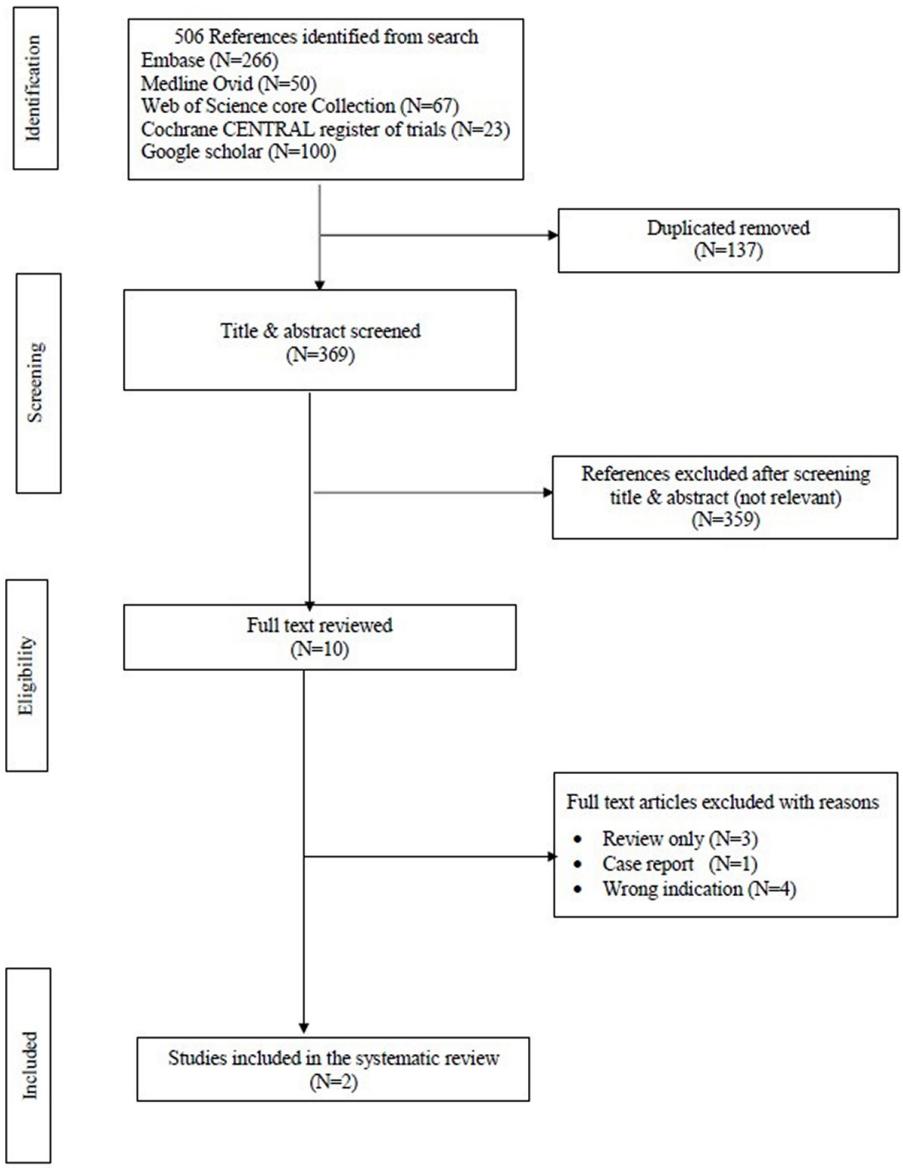

FIGURE 1 | Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow chart of the data.

longer. Therefore, the worsening of hemodynamic parameters despite the use of LEVO in RHF borderline patients is likely a marker of poor outcome in LVAD patients.

The second study by Kocabeyoglu et al. (9) was a retrospective single-center study that included 85 patients with end-stage HF who underwent isolated LVAD implantation. Patients with an Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) class-1 profile; pre-operative extracorporeal membrane oxygenation (ECMO) support; the need for a biventricular assist device and LVAD implantation using the off-pump technique; and without pre-operative optimization were excluded. The LVADs used in this study are shown in **Table 1**. The patients were divided into two groups: the LEVO group (N = 58) included patients who received LEVO infusion at a rate of 0.1 ug/kg/min for a maximum of 48 h without a bolus, 3–10 days before LVAD implantation in addition to other inotropes. The no-LEVO group (N = 27) included patients who received conventional inotropic support without LEVO. LEVO was administered in combination with dobutamine (0–10 ug/kg/min), dopamine (0–8 ug/kg/min), milrinone (0–0.5 ug/kg/min) and norepinephrine (0–0.5 ug/kg/min) in the LEVO group. The same inotropes were administered in the no-LEVO group.

Post optimization (pre-LAVD implantation) with inotropic therapy, hepatic and renal functions and serum albumin values improved in both groups. However, the improvement was better in the LEVO group than in the no-LEVO group, although 30-day and in-hospital mortality was similar in both groups. Likewise,

TABLE 1 | Comparsion between the two studies included in the mini-review.

	Sponga et al. (1)	**Kocabeyoglu et al. (9)**
Journal, Year	ASAIO Journal, 2012	European Journal of Cardio-Thoracic Surgery, 2020
Type of study	Single-center study. NR.	Single-center study. Retrospective study.
Recruitment period	NR	May 2013 and October 2018
Inclusion criteria	LVAD patients with pre-operative borderline right ventricular function which was considered if one or more of the following echocardiographic criteria were unmet: 1. RV end-diastolic diameter <35 mm. 2. RV ejection fraction >30%. 3. Tricuspid regurgitation <grade II. 4. Short/long axis ratio of RV <0.6. 5. Pulmonary pressure <35 mm Hg.	Patients (age >18 years) with end-stage heart failure who underwent isolated LVAD implantation.
Exclusion criteria	Pre-operative use of centrifugal pump support.	1. Patients with INTERMACS class-1 profile. 2. Pre-operative ECMO support. 3. The need for a BIVAD. 4. LVAD implantation using the off-pump technique; and without pre-operative optimization.
Aim of the study	1. Examine the hemodynamic effect of levosimendan infusion in patients with borderline right ventricular function before urgent LVAD implantation. 2. Evaluate the prognostic effect of the response to levosimendan infusion.	Examine the hemodynamic effects of pre-operative levosimendan infusion in patients who underwent LVAD implantation and evaluate their prognoses.
1ry endpoint	NR	Early RHF.
2ry endpoint	NR	30-day and in-hospital mortality, need for RVAD, late RHF, CPB duration, ICU stay, and recovery of end-organ function.
Patients numbers/ characteristics	21 patients, Myocarditis (1 patient), DCM (7 patients), and ICM (13 patients).	85 patients, DCM (44 patients), and ICM (41 patients).
LVAD types	MicroMed DeBakey VAD, and Incor VAD.	HVAD (n = 51), HM II (n = 5), HM III (n = 28), Reliant Heart (n = 1).
RHF definition	Occurrence of two of the following criteria: Mean arterial pressure <55 mm Hg. Central venous pressure >16 mm Hg. Mixed venous saturation <55%. Cardiac index <2 L/min/m^2. Inotropic support >20 units.	NR
Levosimendan protocol	0.1–0.2 ug/kg/min for a maximum of 48 h without bolus, 3 days before LVAD implantation.	0.1 ug/kg/min for a maximum of 48 h without a bolus, 3–10 days before LVAD implantation.
Patient cohorts	Group 1, patients who died due to RHF (n = 4, 19%). Group 2, included patients who survived or died from other reasons (n = 17, 81%).	Group A, levosimendan was administered in combination with other inotropes (n = 58, 86%). Group B, the same inotropes were administered without levosimendan (n = 27, 32%).
Results	The survival rate was 86% at 30 days and 57% at 1 and 2 years. Three patients underwent heart transplantation after a mean mechanical support time of 6 months. The main causes of death were RHF (4 patients), cerebral bleeding (3 patients), and sepsis (2 patients). Levosimendan improves pre-operative hemodynamic conditions in LVAD candidates with borderline RV function, and the response to levosimendan treatment helps to predict mortality and RHF.	The survival rates in groups A and B, respectively, were 77.2 and 73.1% at 30 days, 56.8 and 63.9% at 1 year and 46.4 and 53.2% at 3 years. 52 and 33 patients were bridged to transplant and destination therapy, respectively. The main causes of death were RHF (11 out of 20 patients, 55%), cerebrovascular accident (5 out of 20 patients, 25%; 3 patients with ischemic strokes, 2 patients with cerebral bleeding), and sepsis (4 out of 20 patients, 20%). The improvements in end-organ function were better in patients pre-conditioned with levosimendan; however, we found no difference between the 2 groups for the other outcomes.
RHF treatment	The four patients with RHF were treated with inhaled nitric acid, intravenous iloprost, and maximal inotropic support. No RVADs were implanted to treat RHF.	In group A, early RHF occurred in 15 out of 58 patients, 5 of these patients were treated with inhaled nitric oxide (with inhaled iloprost, if extubated), increased oral sildenafil (3 × 40 mg daily) and inotropic support—and RVAD implantation was needed in 10 patients unresponsive to medical treatment, 8 patients with ECMO and 2 patients with Levitronix (Abbott Inc., Chicago, IL, United States). In group B, early RHF was encountered in 5 patients (5 out of 27); only 2 patients responded to medical therapy and implantation of RVAD with ECMO was required in the remaining 3 patients.

BIVAD, biventricular assist device; CPB, cardiopulmonary bypass; DCM, dilated cardiomyopathy; ECMO, extracorporeal membrane oxygenation; HM II, heart mate II, HM III, heart mate III; HVAD, heartWare ventricular assist device; ICM, ischemic cardiomyopathy; ICU, intensive care unit; INTERMACS, interagency registry for mechanically assisted circulatory support; LVAD, left ventricular assist device; NR, not reported; RHF, right sided heart failure; RV, right ventricle; RVAD, right ventricular assist device.

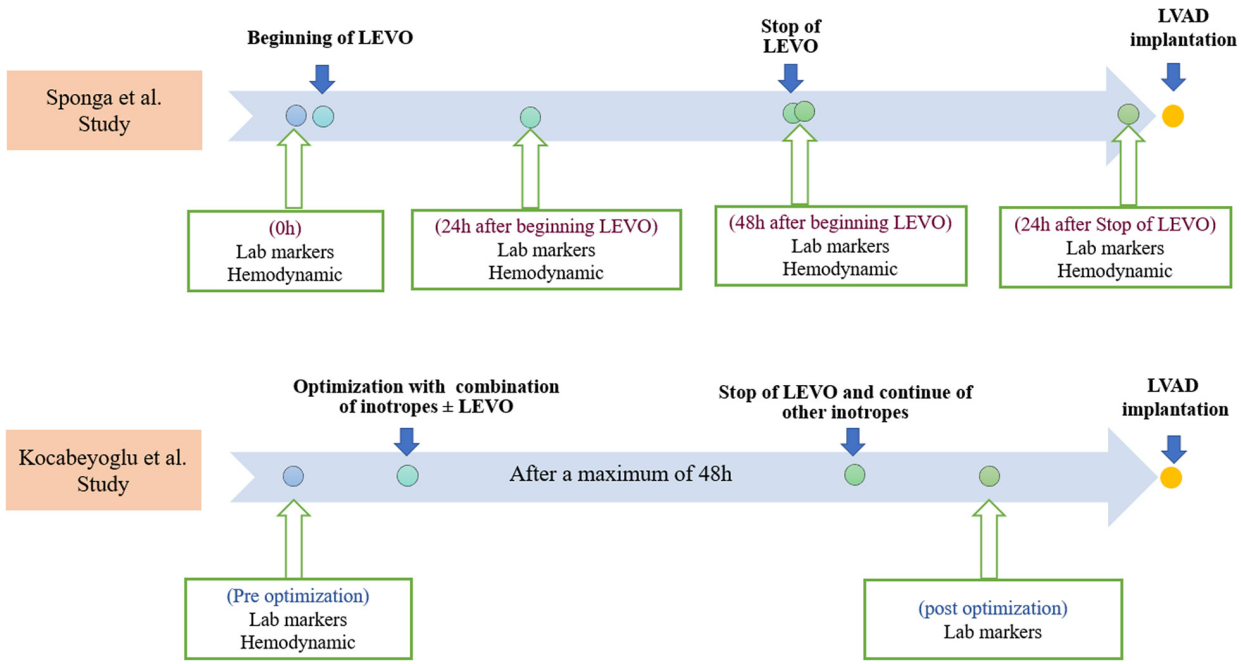

FIGURE 2 | Timelines of Levosimendan infusions, laboratory markers, and hemodynamic measurements in both studies. Lab, laboratory; LEVO, levosimendan; LVAD, left ventricular assist device.

no significant differences were seen between both groups in terms of early RHF, need for right ventricular assist device (RVAD) or late RHF.

In both studies (1, 9), the administration of LEVO was safe and well-tolerated without signs of arrhythmia, tachycardia or hypotension. There were also no cardiac arrest events recorded, and the administration of LEVO was not interrupted because of side effects. Both studies show that LEVO can be successfully administered before LVAD implantation.

In Sponga et al., LEVO improved pre-operative hemodynamic conditions in LVAD candidates. Furthermore, the hemodynamic changes after LEVO infusion could help in predicting the mortality and RHF along with the baseline hemodynamic and echocardiographic data.

In addition, Kocabeyoglu et al. showed that perioperative optimizations of LVAD candidate improved hemodynamic conditions and thus improved end organ functions. Furthermore, the improvements in end organ function were better in patients preconditioned with LEVO, particularly renal function. This emphasizes earlier reports that LEVO preserves renal perfusion and glomerular filtration rate (9).

In a recently published meta-analysis by our group, LEVO use in patients undergoing ECMO was associated with significant VA-ECMO weaning success and lower risk of mortality (7). In addition, another meta-analysis by our group demonstrated that LEVO use in ambulatory patients with refractory HF has been associated with wide range of improved hemodynamics, echocardiographic parameters, reverse LV remodeling, lower filling pressures, and lower biomarkers of LV failure (6). On the other hand, long-term treatment with conventional intravenous inotropes increases mortality (6). More recently, Yalcin et al. (10) reported a successful use of intermittent LEVO infusion for treatment of a late RHF patient post LVAD.

These data on LEVO use in LVAD, although limited, are encouraging and suggest that there is at least hemodynamic improvements alongside improved organ perfusion associated with the use of LEVO in patients undergoing LVAD. The lack of survival benefits in these two studies could be due to very small number of patients involved in these studies. This in turn emphasizes the need for initiation of a large-scale randomized clinical trial to ascertain the clinical benefits of using LEVO in LVAD patients.

CONCLUSION

In conclusion, current evidence of the use of Levosimendan in LVAD patients is very limited. So far, no survival benefits have been shown for the use of Levosimendan in LVAD patients, most probably due to underpowered studies. Therefore, further investigation, involving an adequately powered multicenter, randomized placebo-control study is warranted. In this proposed study, patients undergoing LVAD implantation and at risk for RHF should be randomized to receiving Levosimendan or placebo on top of the guideline-directed therapy. The primary safety endpoints should at least include the occurrence of arrhythmia, hypotension, tachycardia, termination of Levosimendan due to side effects. Efficacy endpoints should include at least, all-cause death, early and late RHF, hepatic

dysfunction, renal dysfunction, duration of ICU stay, duration of hospital stay and hemodynamic improvements.

AUTHOR CONTRIBUTIONS

OS and KC: conceptualization, methodology, and supervision. MA and HaE: studies screening, data extraction. MA, HaE, KC, and OS: writing—original draft preparation. MA, KC, AE, HaE, HeE, AS and OS: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Pulmonary Vasculature Responsiveness to Phosphodiesterase-5A Inhibition in Heart Failure With Reduced Ejection Fraction: Possible Role of Plasma Potassium

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Introduction: Phosphodiesterase-5a inhibition (PDE5i) leads to favorable changes in pulmonary hemodynamic and cardiac output (CO) in patients with advanced heart failure (HF) and reduced ejection fraction (HFrEF). The hemodynamic response to PDE5i could be heterogeneous and the clinical variables associated with these changes are scarcely investigated.

Materials and Methods: Of 260 patients with advanced HFrEF referred for advanced therapies [cardiac transplant/left ventricular assist device (LVAD)], 55 had pulmonary hypertension (PH) and fulfilled the criteria for the PDE5i vasoreactivity test. Right heart catheterization (RHC) was performed as a part of clinical evaluation before and after 20-mg intravenous sildenafil. Absolute and relative changes in pulmonary vascular resistance (PVR) were evaluated to assess hemodynamic response to PDE5i. Clinical, biochemical, and hemodynamic factors associated with PVR changes were identified.

Results: Sildenafil administration reduced PVR (− 45.3%) and transpulmonary gradient (TPG; − 34.8%) and increased CO (+ 13.6%). Relative change analysis showed a negative moderate association between baseline plasma potassium and changes in PVR (r = − 0.48; p = 0.001) and TPG (r = − 0.43; p = 0.005) after PDE5i. Aldosterone concentration shows a direct moderate association with PVR changes after PDE5i. A significant moderate association was also demonstrated between CO improvement and the severity of mitral (r = 0.42; p = 0.002) and tricuspid (r = 0.39; p = 0.004) regurgitation.

Conclusion: We identified plasma potassium, plasma aldosterone level, and atrioventricular valve regurgitations as potential cofounders of hemodynamic response to acute administration of PDE5i. Whether modulation of potassium levels could enhance pulmonary vasoreactivity in advanced HFrEF deserves further research.

Keywords: pulmonary hypertension, heart failure, phospodiesterase inhibitors, potassium, pulmonary vascular resistance

INTRODUCTION

Pulmonary hypertension (PH) is highly prevalent in patients with heart failure (HF) (1) and impacts detrimentally right ventricular (RV) function, exercise capacity, and survival (2, 3). PH in HF occurs due to the transmission of high left ventricular (LV) filling pressure into pulmonary vessels and due to an increase of pulmonary vascular resistance (PVR) (4). The resultant combined pre- and post-capillary PH can be particularly detrimental in patients with HF considered for left-sided mechanical support or heart transplant (5). Despite left ventricular assist device (LVAD), implantation significantly improves survival in end-stage HF (6), it is frequently complicated by right heart failure (RHF) (7) leading to a higher risk of adverse outcomes (8). Increased RV afterload due to high PVR is a critical determinant of acute graft failure immediately after transplantation (9), and it also contributes to RV dysfunction after LVAD (9, 10).

Phosphodiesterase-5a inhibitors (PDE5is) are selective pulmonary vasodilators that attenuate the degradation of cyclic guanosine monophosphate (cGMP) and are clinically used for the treatment of pulmonary arterial hypertension (11, 12). In some centers, PDE5is are used off-label to mitigate the detrimental impact of PH in advanced HF with reduced ejection fraction (HFrEF) patient prior heart transplantation or LVAD implantation (13–15). However, there are no randomized clinical trials to support this approach, which is still a subject of debate (4, 16, 17). In some transplant centers, such as ours, PDE5i may be used acutely for the testing of reversibility of increased PVR (18, 19).

In our practice, we noticed that acute hemodynamic response to PDE5i infusion could be heterogeneous, with some patients responding remarkably well in terms of PVR reduction (due to profound changes in its components, cardiac output (CO), and transpulmonary gradient [TPG]) (20), while others may have a small or even no response (21). To date, little research has been done about clinical variables that might influence hemodynamic response to PDE5i in patients with HF. If such variables are identified, then PDE5i therapy could be tailored to patients who benefit most, without exposing non-responders to potential side effects, such as hypotension or bleeding (22). Alternatively, factors associated with a poor response may be addressed to improve PDE5i responsiveness.

Given these premises, we explored clinical factors associated with acute hemodynamic response to PDE5i in an advanced HFrEF cohort.

MATERIALS AND METHODS

Study Population

The study retrospectively enrolled patients with chronic (> 6 months) advanced HFrEF (EF < 40%) electively hospitalized at the Institute for Clinical and Experimental Medicine (IKEM) in Prague between April 2017 and October 2019 for consideration of heart transplant or LVAD implantation. Patients with acute ischemia, uncontrolled cardiac arrhythmia, reversible cardiac dysfunction, active malignancy, endocrine disease, pre-existing PDE5i therapy, nitrate use, chronic or acute infection, or those unwilling to participate were excluded. Patients with hypervolemia on admission were enrolled in the study only if reached a normovolemic state (mean central vein pressure < 10 mmHg) after intravenous diuretics treatment. The testing of hemodynamic response to intravenous sildenafil was performed clinically as it is the current standard for our clinical evaluation protocol for pre-transplant assessment; as such no placebo control was administered. According to criteria used at our institution, indications for the PDE5i vasoreactivity test were increased PVR (> 4 Wood units) or TPG (> 15 mmHg) and systolic blood pressure > 90 mmHg. This study conforms to the declaration of Helsinki and was approved by the local ethics committee that did not considered it as a clinical trial. All patients signed informed consent with the procedure and with research data collection.

Study Protocol and Measures

Patients underwent, on the same day, morning fasting blood sampling for laboratory analysis, clinical history review, physical examination, echocardiography, ECG, and right heart catheterization (RHC). Echocardiographic exams were performed by an experienced physician, in the left lateral decubitus position with a commercially available standard ultrasound scanner (Vivid 7, General Electric Medical Systems, Wauwatosa, Wisconsin) using a 2.5 MHz phased-array transducer. LV function and dimensions were measured according to contemporary recommendations (23). Mitral and tricuspid regurgitation were assessed semiquantitatively and expressed in 4 grades according to current guidelines (24). RV function was evaluated by tricuspid annular plane systolic excursion (TAPSE) and systolic RV tissue velocity (S'-TDI). RV dysfunction was defined as a TAPSE < 17 mm or an S'-TDI < 9.5 cm/s (25). Body weight was measured by using an electronic scale (HBF-510W, Omron, Japan).

Right heart catheterization was performed after non-invasive examinations in the supine position using a 7F balloon-tipped triple-lumen Swan-Ganz catheter (Braun Melsungen AG, Germany) *via* right internal jugular vein and fluoroscopic guidance. Transducers were balanced by determining zero level at the mid-axillary line. Pressure waveforms were recorded as the average of at least 3 measurements and annotated by an invasive hemodynamic module (Mac-Lab, GE Healthcare, United States) and were measured in right atrium (RA), RV, and pulmonary artery (PA). The wedge position of the Swan-Ganz catheter was confirmed by X-ray. The value of PA wedge pressure (PAWP) was assessed at end-expiration with the balloon-tipped catheter at a steady state with the patient in a supine position (26). Cardiac output was measured by thermodilution as the average of at least 3 measurements with < 10% variance. TPG was computed by subtracting the PAWP from the mean PA pressure. PVR was calculated by dividing the TPG by the CO. PH was defined as a PA mean pressure > 20 mmHg (27). The effect of sildenafil on CO, PVR, and TPG was computed as absolute differences (post-PDE5i value − baseline) and as percent change from baseline (relative change: [(post-PDE5i

value − baseline)/baseline value] × 100. Systemic blood pressure was measured after 10 min of rest in the supine position using an automated oscillometric monitor. RHC with hemodynamic recording was repeated 10 min after the administration of 20 mg of sildenafil citrate (Pfizer, New York, NY, United States) into a central vein.

Data Analysis

Data are shown as mean ± standard deviation (SD) or median and [25th–75th interquartile range (IQR)] for continuous variables (according to distribution) and total count (n) with proportion (%) for categorical variables. Normality was assessed using the Shapiro-Wilks test. Significance of changes within subjects was tested using paired *t*-test or McNemar's test for paired comparisons as appropriate. For abnormally distributed data, Wilcoxon signed-rank test was used. The association between baseline prognostic clinically relevant covariates and hemodynamic changes induced by PDE5i administration was assessed by linear or logistic regression as appropriate. A two-tailed value of $p < 0.05$ was considered statistically significant. All analyses were performed using JMP pro 15.0 statistical software (SAS Institute, Inc., Cary, NC, United States).

RESULTS

A total of 260 consecutive patients referred for advanced HF therapies (cardiac transplant/LVAD) evaluation in our center were considered for the study. Of these, 55 patients fulfilled the criteria for hemodynamic testing of PVR reversibility (see section "Materials and Methods"). Baseline clinical characteristics of the population are summarized in **Table 1**. Our population predominantly consisted of severely symptomatic [71% New York Heart Association (NYHA) III class; 13% NYHA IV class] middle-aged male patients, on guideline-directed medical therapy at maximally tolerated doses and equally divided between ischemic and non-ischemic HF. In patients with non-ischemic HF, the etiology was mainly idiopathic dilated cardiomyopathy (80% of cases). Two-thirds of patients (65%) displayed RV dysfunction, and all of them had PH.

TABLE 1 | Baseline characteristics.

Clinical characteristics	
Age, years	57.2 ± 10.8
Male, *n* (%)	47 (85)
Body mass index, kg/m^2	27.3 ± 3.7
NYHA class, *n* (%): II; III; IV	9 (16); 39 (71); 7 (13)
Heart failure etiology, *n* (%): Ischemic; non-ischemic	28 (51); 27 (49)
Diabetes mellitus, *n* (%)	23 (43)
Atrial fibrillation, *n* (%)	5 (9)
Laboratory examinations	
Hemoglobin, g/L	141.7 ± 20.6
Sodium, mmol/L	137.8 ± 3.6
Potassium, mmol/L	4.3 ± 0.4
Creatinine, μmol/L	132.4 ± 40.7
BNP, ng/L	1,148 [507; 1,781]
Renin, ng/L	113 [54; 309]
Aldosterone, pmol/L	792 ± 567
Echocardiography	
LV end-diastolic diameter, mm	72.5 ± 9.1
LV end-systolic diameter, mm	64.7 ± 10.6
LV ejection fraction,%	21.5 ± 4.3
Left atrial volume index, mL/m^2	64.9 ± 20.1
Mitral regurgitation grade, *n* (%): 0–2; 3–4	22 (39); 33 (61)
Tricuspid regurgitation grade, *n* (%): 0–2; 3–4	39 (71); 16 (29)
Right ventricular dimensions, mm: RVD1; RVD2; RVD3	47.5 ± 6.5; 34.0 ± 4.9; 87.9 ± 6.4
TAPSE, mm	15.6 ± 3.8
Tricuspid annulus S'-TDI, cm/s	7.6 ± 2.3
Therapy	
Furosemide, *n* (%)	53 (96)
Furosemide daily dose, mg/24 h	126 ± 104
ACEi, ARB or ARNI, *n* (%)	32 (58)
MRA, *n* (%)	48 (87)
Betablocker, *n* (%)	48 (87)

ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARNI, angiotensin receptor neprilysin inhibitor; BNP, brain natriuretic peptide; MRA, mineralocorticoid receptor antagonist; LV, left ventricular; NYHA, New York Heart Association; RVD, right ventricular dimension; TAPSE, tricuspid annular plane systolic excursion; S'-TDI, peak systolic velocity of the tricuspid annulus by tissue Doppler imaging.

Hemodynamic Effects of PDE5i

At baseline, patients showed increased PA mean pressure, RA mean pressure, PVR, and PAWP. Intravenous administration of 20 mg of sildenafil was led to significant reduction in RA pressure (− 43.2 ± 9.8%) and PAWP (− 15.9 [2.5; 38.4]%). RA/PAWP pressure ratio, a global RV hemodynamic performance indicator, was significantly decreased (− 31.1 ± 30.2%), with a concurrent decrease in RV afterload (PA mean pressure − 25.4 ± 19.8%; all $p < 0.01$; **Table 2**). Sildenafil also led to a profound change in PVR (− 42.0 ± 25.7%, $p < 0.001$; **Figure 1A**) and in its components (TPG − 33.0 ± 26.5%; CO + 13.6 [1.9; 29.1]%, both $p < 0.01$). CO change was mainly driven by an increase in stroke volume (+ 6.5 [2.4; 14.2] ml; $p < 0.01$) rather than a modification in heart rate (0 [0; − 5] bpm; $p = 0.07$).

Interestingly, PDE5i-induced changes in TPG and CO were unrelated, i.e., some patients showed a large increase in CO with minimal change in TPG, and vice versa, indicating heterogeneity of response (**Figure 1B**). In the systemic circulation, PDE5i significantly reduced the mean blood pressure (− 7.4 [−2.2; − 15.1]%) and the systemic vascular resistance

TABLE 2 | Effect of sildenafil on hemodynamic.

	Baseline	After sildenafil	*p*-value
Right heart catheterization			
Cardiac output—L/min	3.7 ± 0.8	4.3 ± 0.8	<0.001
PVR—WU	5.1 ± 1.6	2.9 ± 1.4	<0.001
Transpulmonary pressure gradient—mmHg	17.9 ± 4.1	11.9 ± 4.5	<0.001
RA mean pressure—mmHg	8.2 ± 3.4	5.1 ± 3.4	<0.001
RV maximum pressure—mmHg	62.9 ± 13.6	45.4 ± 16.3	<0.001
RV minimum pressure—mmHg	3.5 ± 3.1	1.5 ± 3.0	<0.001
RV end-diastolic pressure—mmHg	11.6 ± 4.2	8.6 ± 6.2	0.002
PA systolic pressure—mmHg	64.2 ± 13.2	47.3 ± 15.5	<0.001
PA diastolic pressure—mmHg	29.5 ± 7.5	20.9 ± 6.7	<0.001
PA mean pressure—mmHg	42.4 ± 8.6	31.4 ± 9.9	<0.001
PA wedge pressure mean—mmHg	24.5 ± 7.1	19.4 ± 7.8	<0.001
Mean blood pressure—mmHg	88.6 ± 11.9	81.5 ± 13.9	<0.001
Heart rate—bpm	82.3 ± 14.0	80.4 ± 14.1	0.080
SVR—WU	22.8 ± 5.6	18.3 ± 4.9	<0.001
PVR/SVR ratio	0.22 ± 0.06	0.16 ± 0.07	<0.001

PA, pulmonary artery; PVR, pulmonary vascular resistance; RA, right atrial; RV, right ventricular; SVR, systemic vascular resistance; WU, Wood units.

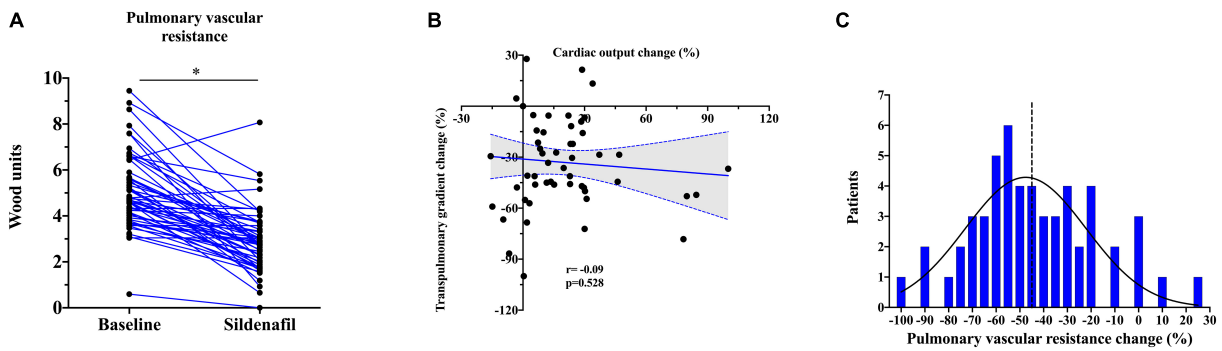

FIGURE 1 | (A) Changes in pulmonary vascular resistance (PVR) after acute PDE5i administration. The asterisk stands for $p < 0.001$. **(B)** Correlation between relative transpulmonary and cardiac output change after PDE5i infusion. **(C)** Distribution of sildenafil response in terms of PVR in our sample. The dashed line represents the median.

(SVR; − 19.1 [− 5.1; − 30.7]%). Vasodilation in the systemic circulation was less pronounced than in the pulmonary vascular bed as represented by a significant reduction in PVR/SVR ratio (− 31.9 [− 2.1; − 47.9]%, $p < 0.01$).

Clinical Variables and PDE5i Response

After PDE5i administration, PVR was reduced in average of − 45.3% (− 2.0 WU) when compared to baseline (**Figure 1C**). Concurrently, CO was increased on average of + 13.6% (+ 0.5 L/min) and TPG was reduced to − 34.8% (− 6.0 mmHg). PAWP showed generally a consistent reduction after PDE5i infusion, except in 10 patients (19%) who showed on the contrary a rise in PAWP. The median increase was usually mild (3 [2; 5] mmHg).

Baseline plasma potassium level showed a negative moderate association in both absolute (r = − 0.45; p = 0.002) and relative (r = − 0.48; p = 0.001) changes in PVR after PDE5i (**Table 3** and **Figure 2A**) and TPG change (r = − 0.43; p = 0.005; **Figure 2B**). Similarly, plasma potassium was inversely associated with relative changes in SVR (r = − 0.36; p = 0.018). A mild association was found between furosemide daily dose and pulmonary vasoreactivity (r = 0.35; p = 0.009). Aldosterone concentration showed a direct moderate association with PVR relative change after PDE5i (r = 0.42; p = 0.029; **Figure 2C**) but was inversely associated with baseline plasma potassium level (r = − 0.43; p = 0.025; **Supplementary Figure 1**). A significant moderate association was also demonstrated between CO improvement and the severity of mitral (r = 0.42; p = 0.002) and tricuspid (r = 0.39; p = 0.004) regurgitation (**Figure 3**).

Patients with increased PAWP after sildenafil showed a higher relative drop in PVR when compared to patients with a reduction in PAWP (− 54.0 ± 17.4% vs. − 39.3 ± 26.6%; p = 0.04). Baseline predictors of this kind of response had higher BNP, lower aldosterone, and a more severe degree of tricuspid regurgitation (**Supplementary Table 1**).

Left and right systolic functions, HF severity and duration, the use and the dose of beta-blockers, renal function, pulmonary, and

TABLE 3 | Correlation between baseline clinical variables and absolute and relative PVR change after sildenafil.

Parameters	PVR change (absolute)		PVR change% (relative)	
	Correlation coefficient [95% CI]	*p*-value	Correlation coefficient [95% CI]	*p*-value
Age, years	0.11 [−0.15; 0.37]	0.402	0.08 [−0.19; 0.34]	0.567
Body mass index, kg/m^2	0.26 [−0.01; 0.49]	0.056	0.08 [−0.18; 0.34]	0.549
NYHA class, I–IV	−0.13 [−0.39; 0.16]	0.372	0.02 [−0.26; 0.30]	0.884
HF duration, years	0.27 [0.01; 0.51]	0.046	0.19 [−0.08; 0.44]	0.157
Sodium, mmol/L	−0.09 [−0.38; 0.22]	0.567	−0.26 [−0.52; 0.04]	0.095
Potassium, mmol/L	−0.45 [−0.66; −0.17]	0.002	−0.48 [−0.68; −0.21]	0.001
BNP, ng/L	−0.27 [−0.50; −0.04]	0.047	−0.07 [−0.33; 0.19]	0.601
Aldosterone, pmol/L	0.36 [−0.01; 0.65]	0.063	0.42 [0.04; 0.69]	0.029
Renin, ng/L	0.33 [−0.12; 0.66]	0.145	0.31 [−0.14; 0.65]	0.171
PAWP, mmHg	−0.06 [−0.32; 0.20]	0.644	0.03 [−0.24; 0.29]	0.837
PA pressure (mean), mmHg	−0.29 [−0.52; −0.02]	0.031	−0.02 [−0.29; 0.24]	0.858
RV-EDP, mmHg	−0.07 [−0.33; 0.19]	0.598	0.03 [−0.23; 0.29]	0.811
RV maximum pressure, mmHg	−0.09 [−0.35; 0.18]	0.506	0.10 [−0.17; 0.36]	0.457
LVEF,%	−0.07 [−0.33; 0.19]	0.598	−0.11 [−0.36; 0.16]	0.420
TAPSE, mm	0.12 [−0.16; 0.39]	0.396	−0.13 [−0.39; 0.15]	0.364
Mitral regurgitation, grade 1–4	−0.25 [−0.48; 0.02]	0.066	−0.15 [−0.40; 0.12]	0.279
Tricuspid regurgitation, grade 1–4	−0.17 [−0.41; 0.10]	0.218	−0.18 [−0.43; 0.08]	0.172
Furosemide daily dose, mg/24h	0.26 [−0.01; 0.49]	0.051	0.35 [0.09; 0.56]	0.009

BNP, brain natriuretic peptide; HF, heart failure; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; PA, pulmonary artery; PAWP, pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; RV, right ventricle; RV-EDP, right ventricular end-diastolic pressure; TAPSE, tricuspid annular plane systolic excursion.

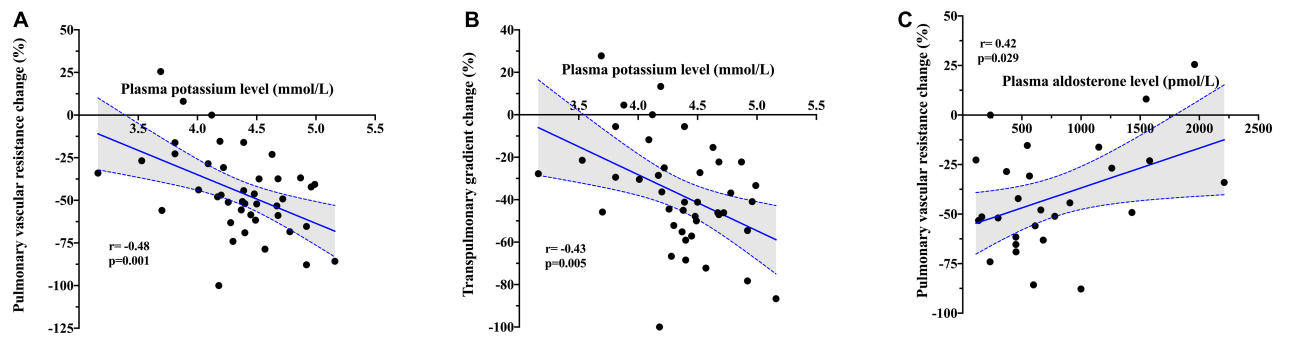

FIGURE 2 | Correlation between baseline plasma potassium level and **(A)** relative change in pulmonary vascular resistance and **(B)** relative change in transpulmonary gradient after PDE5i. **(C)** Correlation between baseline plasma aldosterone level and relative change in pulmonary vascular resistance after PDE5i.

RV hemodynamic were mostly unrelated to relative changes in pulmonary vasoreactivity.

DISCUSSION

In our study, we investigated clinical variables possibly associated with a better hemodynamic response to PDE5i in terms of PVR change and its components (TPG and CO). Specifically, we showed that a concentration of plasma potassium at the lower edge of normality has a moderate strength in predicting impaired pulmonary vasodilatory response to PDE5i, while the presence of severe mitral or tricuspid regurgitation was related to a more pronounced increase in CO. These findings might help to identify those patients with a possible favorable acute hemodynamic response to PDE5i in particular settings (i.e., LVAD or heart transplant aiming to reduce the risk of acute RHF) and may lead to actions to improve PDE5i responsiveness by increasing plasma potassium to the high range of normality.

Acute hemodynamic effects of PDE5i result from their ability to increase cGMP levels in vascular smooth muscle cells, thereby reducing vascular resistances and pressures (11, 28). Our study indicates that these effects may be modified by plasma potassium level and attenuated by a concentration of this cation in the lower range of normality. Potassium abnormalities are common in HF and are linked with excess morbidity, the risk for arrhythmia, and mortality (29, 30). Interestingly we showed, for the first time, that plasma potassium concentration was inversely associated with

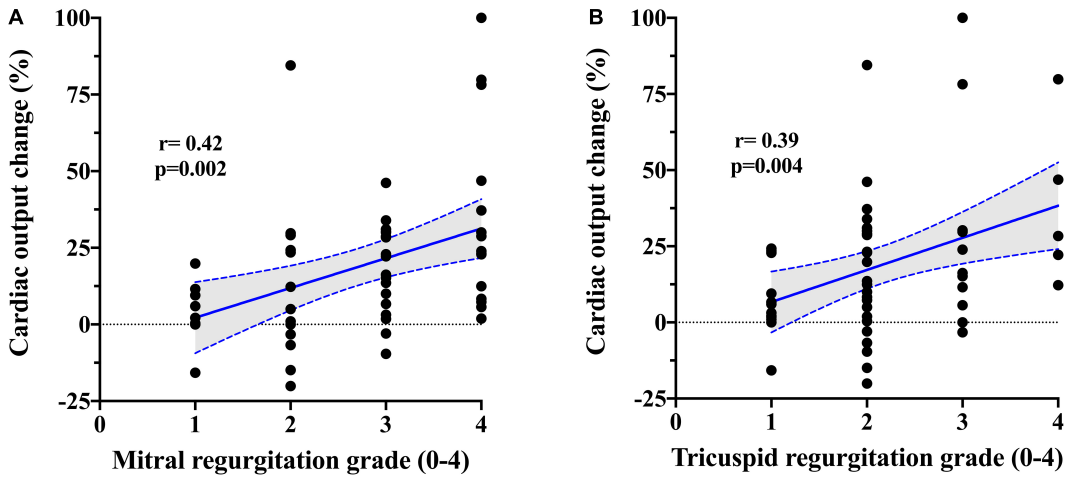

FIGURE 3 | Correlation between PDE5i-induced change in cardiac output and **(A)** mitral and **(B)** tricuspid regurgitation severity.

pulmonary vascular reactivity after acute PDE5i administration. Whether this is a mere coincidence or plasma potassium level is mechanistically involved in PVR reversibility cannot be sorted out from current data, although the latter is possible. As shown in our study, a lower potassium concentration could be also correlated with the use of high doses of loop diuretics, a typical feature of more advanced HF (i.e., increasing degree of backward RV failure) and, in turn, exhausted pulmonary vasoreactivity. A concentration of potassium in the lower range might increase transmembrane gradient (hyperpolarization) and reduce smooth muscular cell excitability, leading in turn to a blunted pulmonary vasodilatory response after PDE5i (31). Several studies identified voltage-gated potassium channels as key regulators of PA smooth muscular cell excitability (32). Two potassium channels largely represented in lungs, the potassium channel subfamily K member 3 (KCNK3) and the adenosine triphosphate (ATP)-sensitive potassium channels (KATP), are activated by protein kinase G-dependent phosphorylation, suggesting that their modulation might contribute to the vasorelaxant effects of PDE5i (33). This mechanism probably also applies to the systemic circulation, as demonstrated in our study by the attenuated SVR reduction after PDE5i among patients with a concentration of serum potassium in the lower range.

Another moderately good predictor of larger PVR reduction after PDE5i was plasma aldosterone level. Previous human and experimental studies showed that aldosterone concentration is increased in PH and correlated with cardio-pulmonary hemodynamic indexes and pulmonary vascular remodeling (34, 35). In PAH models, aldosterone reduces nitric oxide (NO) availability through increasing pulmonary endothelial oxidant stress (35), which promotes endothelial dysfunction. The administration of PDE5i may reverse this condition by reducing the GMP-mediated NO degradation and improving in turn pulmonary vasoreactivity. Accordingly, higher aldosterone concentrations might directly contribute to the blunted vasodilatory response after PDE5i showed in our study. Altogether, our findings provide new data to support the role of aldosterone signaling in mediating pulmonary vascular dysfunction among patients with advanced HF.

It was proposed that increased PVR may prevent fluid leak in the pulmonary capillary bed and consequent edema formation in patients with PH (36). Accordingly, it was suggested that pulmonary vasodilators may increase the risk of lung congestion in patients with HF (37). Our study challenges these concerns since sildenafil infusion mostly reduced both PVR and PAWP. As previously demonstrated by our group (15), while PVR reduction is a direct effect of PDE5i on pulmonary vasculature, the drop in PAWP is partly due to the diminished pericardial constraint as a consequence of the reduction of RV size. This mechanism is consistent with known contributions of external pericardial force to the LV filling pressure in patients with failing dilated hearts (38). Other mechanisms, such as reduction of venous return, a decrease of LV afterload, and improvement of LV/RV filling pressures with consequent improvement of paradoxical septal motion, could be involved in the observed drop in PAWP after PDE5i. About a fifth of our patients treated with sildenafil showed a rise rather than a reduction in PAWP. This condition was associated with higher BNP and more severe tricuspid regurgitation, possibly identifying a subset of sicker patients, with higher filling pressures and less prone to reduce RV size in response to sildenafil (and consequently with higher pericardial constraint). Lower aldosterone was also found to be a predictor of increased PAWP after sildenafil. This result is in line with responsiveness to PDE5i in terms of PVR and could be related to the same mechanism (i.e., higher PVR response due to lower aldosterone level could unveil an increase in PAWP after sildenafil).

The described profound PDE5i-induced afterload-reducing effects on both pulmonary and systemic circulation could probably also explain the showed moderate association between the severity of mitral regurgitation and the increase in CO. In chronic severe mitral regurgitation, a large portion of stroke volume regurgitates back into the atrium, and the effective

forward flow is curtailed and primarily determined by SVR (39). PDE5i administration reduced SVR, resulting in a significant increase in CO and in a consequent mitigation of mitral regurgitation severity (40). The same mechanism applies to the right side for hemodynamic relevant tricuspid regurgitation.

Different magnitude in response to PDE5i among subjects with HF may also come from a heterogeneity in PDE5 expression in the pulmonary vasculature. Indeed, it has been demonstrated that PDE5 isoform expressions were significantly increased in neomuscularized distal vessels and in smooth muscle cells of the medial layer of the diseased pulmonary vasculature (41). Accordingly, our patients with more severe PH seem to show a better hemodynamic response after PDE5 inhibition.

From a clinical perspective, our findings are hypothesis generating and might have relevant implications in the field of advanced HF. The most relevant insight is that PDE5i response seems to be associated with plasma potassium concentration. Indeed, a lower concentration of this electrolyte, even if in the normal range, could be a marker of poor response to PDE5i. In line, clinicians should be aware of a possible blunted pulmonary hemodynamic response to PDE5i when plasma potassium is close to the lower limit of normal. This finding assumes particular relevance in patients with HFrEF with severe PH candidates for LVAD or heart transplant, where the effect of pre-operative treatment with PDE5i could be improved by potassium level modulation (i.e., keeping the plasma potassium in the higher range of normality). Increased aldosterone concentration was also associated with attenuated response to PDE5i, suggesting a potential role of simultaneous use of mineralocorticoid antagonists and PDE5i to enhance PVR reduction in patients with HF and PH (42, 43).

Our analysis has some limitations. We do not have a placebo control group; however, it is unlikely that reported changes after PDE5i could be explained by the period or placebo effects. Plasma potassium and aldosterone levels are interconnected through a negative feedback mechanism; therefore, links between attenuated PDE5i response and low potassium or high aldosterone might be interdependent. Cardiac mechanics was assessed only at a steady state, without manipulation of pre-load. We did not study patients with mild HFrEF or patients with heart failure with mildly reduced ejection fraction (HFmrEF)/heart failure with preserved ejection fraction (HFpEF), and consequently, our findings cannot be extended to all spectra of HF. Due to the small number of patients, we cannot make any inference about the effect of PDE5i on specific subgroups. Individual variability in pharmacodynamics and pharmacokinetics or other unrevealed factors that might affect the response to sildenafil cannot be fully addressed in our study. In our population, virtually all patients showed a potassium level within the normal range (3.5–5.0 mmol/L); consequently, we cannot make any inference about the effect of hypo/hyperkaliemia on pulmonary vasodilatory response to PDE5i. Finally, differences in potassium or salt balance and/or circadian factors that could influence plasma levels of aldosterone and other hormones were not measured in this study.

CONCLUSION

We identified, for the first time, a possible role of plasma potassium level in the modulation of pulmonary vascular reactivity after acute administration of PDE5i. Our study is hypothesis generating; therefore, the causality and clinical relevance of these findings should be further investigated.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by IKEM Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LM and VM wrote the manuscript and analyzed the data. AR, HA-H, IJ, and ZH conducted the clinical studies, collected the data, and contributed to the manuscript. JK provided critical comments to the manuscript. All authors have participated in the work and have reviewed and agreed with the content of the article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.883911/full#supplementary-material

Supplementary Figure 1 | Correlation between baseline plasma aldosterone and potassium level.

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Pre-transplant Type 2 Diabetes Mellitus Is Associated With Higher Graft Failure and Increased 5-Year Mortality After Heart Transplantation

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Aims: Cardiac transplant recipients often suffer from type 2 diabetes mellitus (T2DM) but its influence on graft failure and post-transplant mortality remains unknown. The aim of this study was to investigate the long-term effects of pre-transplant T2DM in patients after heart transplantation (HTX).

Methods: This study included a total of 376 adult patients who received HTX at Heidelberg Heart Center between 01/01/2000 and 01/10/2016. HTX recipients were stratified by diagnosis of T2DM at the time of HTX. Patients with T2DM were further subdivided by hemoglobin A1c (HbA1c $\geq$ 7.0%). Analysis included donor and recipient data, immunosuppressive drugs, concomitant medications, post-transplant mortality, and causes of death. Five-year post-transplant mortality was further assessed by multivariate analysis (Cox regression) and Kaplan–Meier estimator.

Results: About one-third of all HTX recipients had T2DM (121 of 376 [32.2%]). Patients with T2DM showed an increased 5-year post-transplant mortality (41.3% versus 29.8%; P = 0.027) and had a higher percentage of death due to graft failure (14.9% versus 7.8%; P = 0.035). Multivariate analysis showed T2DM (HR: 1.563; 95% CI: 1.053–2.319; P = 0.027) as an independent risk factor for 5-year mortality after HTX. Kaplan–Meier analysis showed a significantly better 5-year post-transplant survival of patients with T2DM and a HbA1c $<$ 7.0% than patients with T2DM and a HbA1c $\geq$ 7.0% (68.7% versus 46.3%; P = 0.008) emphasizing the clinical relevance of a well-controlled T2DM in HTX recipients.

Conclusion: Pre-transplant T2DM is associated with higher graft failure and increased 5-year mortality after HTX.

Keywords: diabetes mellitus, graft failure, HbA1c, heart transplantation, mortality, survival

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a common comorbidity in patients with advanced heart failure and is often associated with a variety of extracardiac diseases such as obesity, impaired wound healing with increased risk of infection, thromboembolic complications, and renal dysfunction (1–8). Given these risk factors, T2DM is considered a relative contraindication for listing for heart transplantation (HTX), depending on the patient's diabetes status and severity of end-organ damage (4–8).

Encouraged by reasonable post-transplant outcomes of patients without evidence of end-organ damage from T2DM at the time of HTX (9–12), an increasing number of patients with T2DM were listed for HTX and subsequently transplanted. This development was supported by the growing number of patients with advanced heart failure and T2DM over the last decades (4–8). These early studies, however, included rather small numbers of carefully selected diabetic patients not necessarily reflecting clinical reality (9–12). It is therefore not surprising that recent literature is inconclusive as some studies found an elevated post-transplant mortality in patients with pre-transplant T2DM (13–16), whereas others could not observe such effect (17–21). Differences in study design, sample size, length of follow-up and analyzed post-transplant outcomes may have contributed to these inconsistencies (13–21). In addition, it should be noted that there was a distinct change in the composition of HTX study populations over time, since the reported rates of diabetic HTX recipients increased markedly from 13.7% to 18.3% in former studies (22, 23) up to 28.8–30.7% in recent studies (24, 25).

Another important aspect is the clinical management of patients with T2DM as a poorly controlled hemoglobin A1c (HbA1c) may be associated with increased post-transplant mortality. Furthermore, there might be an essential difference between T2DM patients with oral anti-diabetic medications and T2DM patients with insulin therapy. Yet, these questions have not been sufficiently answered in the literature. We therefore sought to investigate the effects of pre-transplant T2DM on survival and causes of death after HTX in a large contemporary population of HTX recipients.

PATIENTS AND METHODS

Patients

We performed this study in accordance with the ethical standards of the Declaration of Helsinki in its current form. Approval was granted by the institutional review board (IRB) of Heidelberg University (ethical approval number: S-286/2015, Version 1.2, 28-07-2020). Written informed consent was obtained from patients for inclusion in the Heidelberg HTX Registry allowing the clinical and scientific use of data. According to the ethical approval, no additional written informed consent was required for this observational study as merely routine clinical data were analyzed (26–35).

We screened all adult patients (≥18 years) for pre-transplant T2DM who received HTX at Heidelberg Heart Center, Heidelberg, Germany, between 01/01/2000 and 01/10/2016. Patients with type 1 diabetes mellitus or other forms of diabetes than T2DM were excluded. We also did not include patients who received a second HTX. The remaining patients were stratified by diagnosis of T2DM at the time of HTX: patients with T2DM at the time of HTX ("T2DM group") and patients without T2DM at the time of HTX ("no T2DM group"). Patients with T2DM at the time of HTX were further divided into patients with and without insulin therapy as well as into patients with a HbA1c $< 7.0\%$ at the time of HTX and patients with a HbA1c $\geq 7.0\%$ at the time of HTX.

Follow-Up

Post-transplant follow-up was performed according to Heidelberg Heart Center's routine clinical protocol. After hospital discharge, patients were monthly seen at the HTX outpatient-clinic during the first six post-transplant months, then bimonthly until the end of the first year after HTX, and thereafter three to four times annually (with additional visits when clinically required). Routine follow-up included medical history, physical examination, 12-lead electrocardiogram, echocardiography, endomyocardial biopsy, and blood tests including immunosuppressive drug monitoring (26–35).

Post-transplant Medications

Post-transplant medication including immunosuppressive drug therapy was administered according to Heidelberg Heart Center's usual standard of care. Patients perioperatively received an anti-thymocyte globulin-based immunosuppression induction therapy. Cyclosporine A and azathioprine were administered as the initial immunosuppressive regimen prior to 2001. From 2001 onward, mycophenolate mofetil subsequently replaced azathioprine, and tacrolimus consecutively replaced cyclosporine A from 2006 onward. Steroids were tapered incrementally during the initial post-transplant months and were discontinued 6 months after HTX (unless clinically needed) (26–35).

Statistical Analysis

Data were analyzed with SAS (Version 9.4, SAS Institute, Cary, NC, United States) and expressed as count (n) with percentage (%) or as mean ± standard deviation (SD). For measures of association, difference of mean or hazard ratio (HR) with 95% confidence interval (CI) were used. Depending on the variable type (categorical variables or continuous variables) and the underlying question, we applied chi-squared test, Fisher's exact test, Student's *t*-test, Mann–Whitney *U*-test, analysis of variance (ANOVA), or Kruskal–Wallis test, as appropriate. The Kaplan–Meier estimator was used to graphically show 5-year post-transplant survival. A *P*-value of < 0.050 was considered statistically significant (26–35).

The primary outcome of this study was 5-year mortality after HTX which was compared between patients with and without T2DM at the time of HTX. We could obtain 5-year follow-up data from all patients requiring no censoring. Five-year post-transplant mortality of patients with T2DM was further assessed by stratification into patients with and without insulin therapy as well as into patients with a HbA1c $< 7.0\%$ at HTX and patients with a HbA1c $\geq 7.0\%$ at HTX. Causes of death within

5 years after HTX were grouped into the following categories: graft failure, acute rejection, infection/sepsis, malignancy, and thromboembolic event/bleeding. We applied univariate analyses to search for intergroup differences including recipient data, previous open-heart surgery, principal diagnosis for HTX, donor data, transplant sex mismatch, perioperative data, immunosuppressive drug therapy, and concomitant medications. Analysis of 5-year mortality after HTX further included a multivariate analysis (Cox regression model) to investigate the impact of the eight variables which were statistically significant in the univariate analysis: recipient age (years), recipient body mass index (kg/m^2), recipient arterial hypertension (in total), recipient dyslipidemia (in total), recipient T2DM (in total), recipient previous coronary artery bypass graft (CABG) surgery (in total), ischemic cardiomyopathy (CMP) as principal diagnosis for HTX (in total), and cardiac amyloidosis as principal diagnosis for HTX (in total). We did not include additional parameters in this multivariate analysis for 5-year mortality after HTX in order to avoid biased regression coefficients and to ensure a stable number of events (deceased patients) per analyzed variable. Given the long study period (01/01/2000–01/10/2016), we additionally performed a sensitivity analysis to test the robustness of our findings and to examine a possible era effect using a subgroup of patients with tacrolimus and mycophenolate mofetil as the immunosuppressive drug regimen was changed from 2006 onward (26–35).

RESULTS

Baseline Characteristics of Patients With Type 2 Diabetes Mellitus at Heart Transplantation

This study included a total of 376 HTX recipients. About one-third of these patients (121 of 376 [32.2%]) had T2DM at the time of HTX. Patients with T2DM at the time of HTX were further divided into patients with a HbA1c $<$ 7.0% at the time of HTX (67 of 121 [55.4%]) and patients with a HbA1c $\geq$ 7.0% at the time of HTX (54 of 121 [44.6%]).

Comparison of recipient data showed a higher age ($P < 0.001$), a higher body mass index ($P < 0.001$), a higher percentage of arterial hypertension ($P < 0.001$), and a higher percentage of dyslipidemia ($P < 0.001$) in the T2DM group. We did not observe a statistically significant difference between both groups concerning recipient male sex, chronic obstructive pulmonary disease, or severe chronic kidney disease (all $P \geq 0.050$). Further evaluation of end-organ damage and clinical status of patients at the time of HTX showed no statistically significant difference between patients with or without T2DM concerning total bilirubin, hemoglobin, hospitalization before HTX, days on waiting list, high urgency status on waiting list, inotropic support, intra-aortic balloon pump, or initial hospital stay after HTX (all $P \geq 0.050$).

In terms of principal diagnoses for HTX, significantly more patients with ischemic CMP were found in the T2DM group ($P < 0.001$), whereas significantly more patients with cardiac amyloidosis ($P < 0.001$) were observed in the opposite group. In addition, patients with T2DM had a significantly higher percentage of CABG surgery before HTX ($P = 0.003$). Baseline characteristics stratified by T2DM at HTX are shown in **Table 1**.

Analysis of baseline characteristics stratified by HbA1c at the time of HTX indicated no statistically significant differences between T2DM patients with a HbA1c $<$ 7.0% and T2DM patients with a HbA1c $\geq$ 7.0% regarding recipient data, principal diagnosis for HTX, previous open-heart surgery, donor data, and perioperative data, except for donor (m) to recipient (f) sex mismatch which was significantly higher in T2DM patients with a HbA1c $<$ 7.0% ($P = 0.024$). Baseline characteristics stratified by HbA1c at HTX are presented in **Table 2**.

Medical Treatment of Patients With Type 2 Diabetes Mellitus at Heart Transplantation

Analysis of the immunosuppressive drug therapy showed no statistically significant differences between patients with or without T2DM at the time of HTX regarding the administration of cyclosporine A, tacrolimus, azathioprine, or mycophenolate mofetil (all $P \geq 0.050$). We also found no statistically significant differences between both groups concerning the administration of acetylsalicylic acid, beta blockers, ivabradine, calcium channel blockers, angiotensin-converting-enzyme inhibitors/angiotensin II receptor blockers, or statins (all $P \geq 0.050$). Medications after HTX stratified by T2DM at HTX are given in **Table 3**.

Likewise, we did not find any statistically significant differences between T2DM patients with a HbA1c $<$ 7.0% and T2DM patients with a HbA1c $\geq$ 7.0% concerning the immunosuppressive drug therapy or the concomitant medications (all $P \geq 0.050$). Medications after HTX stratified by HbA1c at HTX are shown in **Table 4**.

In terms of diabetes medications, metformin was the most common oral anti-diabetic drug in patients with T2DM at the time of HTX (49 of 121 [40.5%]). In addition, almost half of patients with T2DM at the time of HTX received insulin therapy (58 of 121 [47.9%]). Analysis of diabetes medications stratified by HbA1c at the time of HTX showed a significantly higher percentage of regular insulin ($P = 0.009$) and insulin glargine ($P = 0.028$) in T2DM patients with a HbA1c $\geq$ 7.0%. An overview of the diabetes medications of T2DM patients stratified by HbA1c at HTX is displayed in **Table 5**.

Survival of Patients With Type 2 Diabetes Mellitus at Heart Transplantation

Patients with T2DM at the time of HTX had a significantly higher 5-year all-cause mortality after HTX (41.3% versus 29.8%, difference: 11.5%, 95% CI: 1.1 – 21.9%, $P = 0.027$). Regarding the causes of death within 5 years after HTX, significantly more patients with T2DM died from graft failure (14.9% versus 7.8%, difference: 7.1%, 95% CI: 0.1 – 14.1%, $P = 0.035$). For further evaluation of the association between T2DM and graft failure, we performed a log rank test between patients with and without T2DM at HTX in regard to graft failure within 5 years after HTX analyzing the number of patients with graft failure and

TABLE 1 | Baseline characteristics – stratified by T2DM at HTX.

Parameter	All (*n* = 376)	T2DM (*n* = 121)	No T2DM (*n* = 255)	Difference	95% CI	*P*-value
Recipient data						
Age (years), mean ± SD	51.9 ± 10.4	56.0 ± 7.3	49.9 ± 11.0	6.1	4.2–8.0	<0.001*
Male sex, *n* (%)	287 (76.3%)	99 (81.8%)	188 (73.7%)	8.1%	–0.6 – 16.8%	0.085
Body mass index (kg/m^2), mean ± SD	25.2 ± 4.3	26.9 ± 4.4	24.4 ± 3.9	2.5	1.6 – 3.4	<0.001*
Arterial hypertension, *n* (%)	207 (55.1%)	91 (75.2%)	116 (45.5%)	29.7%	19.9 – 39.5%	<0.001*
Dyslipidemia, *n* (%)	242 (64.4%)	102 (84.3%)	140 (54.9%)	29.4%	20.5 – 38.3%	<0.001*
COPD, *n* (%)	94 (25.0%)	37 (30.6%)	57 (22.4%)	8.2%	–1.5 – 17.9%	0.085
Severe chronic kidney disease ^, *n* (%)	40 (10.6%)	17 (14.0%)	23 (9.0%)	5.0%	–2.1 – 12.1%	0.139
Principal diagnosis for HTX						
Ischemic CMP, *n* (%)	126 (33.5%)	60 (49.6%)	66 (25.9%)	23.7%	13.3 – 34.1%	<0.001*
Non-ischemic CMP, *n* (%)	187 (49.7%)	53 (43.8%)	134 (52.5%)	8.7%	–2.1 – 19.5%	0.113
Valvular heart disease, *n* (%)	16 (4.3%)	5 (4.1%)	11 (4.3%)	0.2%	–4.1 – 4.5%	0.935
Cardiac amyloidosis, *n* (%)	47 (12.5%)	3 (2.5%)	44 (17.3%)	14.8%	9.4 – 20.2%	<0.001*
Previous open-heart surgery						
CABG surgery, *n* (%)	47 (12.5%)	24 (19.8%)	23 (9.0%)	10.8%	2.9 – 18.7%	0.003 *
Other surgery°, *n* (%)	41 (10.9%)	17 (14.0%)	24 (9.4%)	4.6%	–2.5 – 11.7%	0.178
VAD surgery, *n* (%)	29 (7.7%)	11 (9.1%)	18 (7.1%)	2.0%	–4.0 – 8.0%	0.490
Donor data						
Age (years), mean ± SD	44.0 ± 12.8	45.0 ± 12.5	43.6 ± 12.9	1.4	–1.4 – 4.2	0.321
Male sex, *n* (%)	126 (33.5%)	43 (35.5%)	83 (32.5%)	3.0%	–7.3 – 13.3%	0.566
Body mass index (kg/m^2), mean ± SD	25.0 ± 4.5	25.5 ± 3.9	24.8 ± 4.7	0.7	–0.2 – 1.6	0.127
Transplant sex mismatch						
Mismatch, *n* (%)	186 (49.5%)	68 (56.2%)	118 (46.3%)	9.9%	–0.9 – 20.7%	0.072
Donor (m) to recipient (f), *n* (%)	12 (3.2%)	6 (5.0%)	6 (2.4%)	2.6%	–1.7 – 6.9%	0.179
Donor (f) to recipient (m), *n* (%)	174 (46.3%)	62 (51.2%)	112 (43.9%)	7.3%	–3.5 – 18.1%	0.184
Perioperative data						
Ischemic time (min), mean ± SD	248.1 ± 59.1	250.6 ± 60.7	247.0 ± 58.4	3.6	–9.4 – 16.6	0.588
Biatrial HTX, *n* (%)	5 (1.3%)	1 (0.8%)	4 (1.6%)	0.8%	–1.4 – 3.0%	0.557
Bicaval HTX, *n* (%)	146 (38.8%)	45 (37.2%)	101 (39.6%)	2.4%	–8.1 – 12.9%	0.653
Total orthotopic HTX, *n* (%)	225 (59.9%)	75 (62.0%)	150 (58.8%)	3.2%	–7.3 – 13.7%	0.559

*CABG, coronary artery bypass graft; CI, confidence interval; CMP, cardiomyopathy; COPD, chronic obstructive pulmonary disease; f, female; HTX, heart transplantation; m, male; n, number; SD, standard deviation; T2DM, type 2 diabetes mellitus; VAD, ventricular assist device; ^, estimated glomerular filtration rate < 30 ml/min/1.73 m^2; °, congenital, valvular or ventricular surgery; *, statistically significant (P < 0.050).*

the time from HTX until graft failure. Patients with T2DM at the time of HTX had a significantly higher rate of graft failure within 5 years after HTX (P = 0.019). In contrast, we did not observe statistically significant differences between T2DM groups concerning acute rejection, infection/sepsis, malignancy, or thromboembolic event/bleeding (all $P \geq 0.050$). Causes of death within 5 years after HTX stratified by T2DM at HTX are given in **Table 6**.

Analysis of causes of death within 5 years after HTX stratified by HbA1c at the time of HTX showed a significantly higher 5-year all-cause mortality after HTX in T2DM patients with a HbA1c ≥ 7.0% (53.7% versus 31.3%, difference: 22.4%, 95% CI: 5.1–39.7%, P = 0.013). Patients with T2DM and a HbA1c ≥ 7.0% at the time of HTX also had a higher percentage of death due to graft failure (18.5% versus 11.9%), infection/sepsis (24.1% versus 10.5%), and thromboembolic event/bleeding (9.3% versus 1.5%) within 5 years after HTX. Causes of death within 5 years after HTX stratified by HbA1c at HTX are provided in **Table 7**.

Multivariate analysis showed a more than 50% increased risk of death within 5 years after HTX in patients with T2DM at the time of HTX (HR: 1.563; 95% CI: 1.053–2.319; P = 0.027), while the other seven included variables (recipient age, recipient body mass index, recipient arterial hypertension, recipient dyslipidemia, previous CABG surgery, ischemic CMP as principal diagnosis for HTX, and cardiac amyloidosis as principal diagnosis for HTX) showed no statistically significant effect on 5-year post-transplant mortality. Multivariate analysis for 5-year mortality after HTX is given in **Table 8**.

Kaplan–Meier survival analysis displayed a significantly inferior 5-year post-transplant survival of patients with T2DM at the time of HTX (58.7%) in comparison to patients without T2DM at the time of HTX (70.2%, difference: 11.5%, 95% CI: 1.1 – 21.9%, P = 0.015). Patients with insulin therapy had in fact a lower 5-year post-transplant survival (53.4%) than patients without insulin therapy (63.5%) but this difference did not reach statistical significance (P = 0.243). Further stratification of T2DM patients at the time of HTX showed a significantly lower 5-year post-transplant survival of patients with a HbA1c ≥ 7.0% at HTX (46.3%) in comparison to patients with a HbA1c < 7.0%

TABLE 2 | Baseline characteristics – stratified by HbA1c at HTX.

Parameter	T2DM (*n* = 121)	HbA1c < 7.0% (*n* = 67)	HbA1c ≥ 7.0% (*n* = 54)	Difference	95% CI	*P*-value
Recipient data						
Age (years), mean ± SD	56.0 ± 7.3	57.0 ± 7.1	54.8 ± 7.5	2.2	–0.4 – 4.8	0.093
Male sex, *n* (%)	99 (81.8%)	51 (76.1%)	48 (88.9%)	12.8%	–0.4 – 26.0%	0.070
Body mass index (kg/m^2), mean ± SD	26.9 ± 4.4	26.2 ± 4.3	27.6 ± 4.5	1.4	–0.2 – 3.0	0.086
Arterial hypertension, *n* (%)	91 (75.2%)	51 (76.1%)	40 (74.1%)	2.0%	–13.5 – 17.5%	0.796
Dyslipidemia, *n* (%)	102 (84.3%)	57 (85.1%)	45 (83.3%)	1.8%	–11.3 – 14.9%	0.794
COPD, *n* (%)	37 (30.6%)	17 (25.4%)	20 (37.0%)	11.6%	–4.9 – 28.1%	0.166
Severe chronic kidney disease ^, *n* (%)	17 (14.0%)	9 (13.4%)	8 (14.8%)	1.4%	–11.1 – 13.9%	0.828
Principal diagnosis for HTX						
Ischemic CMP, *n* (%)	60 (49.6%)	35 (52.2%)	25 (46.3%)	5.9%	11.9 – 23.7%	0.516
Non-ischemic CMP, *n* (%)	53 (43.8%)	27 (40.3%)	26 (48.1%)	7.8%	–9.9 – 25.5%	0.387
Valvular heart disease, *n* (%)	5 (4.1%)	3 (4.5%)	2 (3.7%)	0.8%	–6.3 – 7.9%	0.832
Cardiac amyloidosis, *n* (%)	3 (2.5%)	2 (3.0%)	1 (1.9%)	1.1%	–4.3 – 6.5%	0.690
Previous open-heart surgery						
CABG surgery, *n* (%)	24 (19.8%)	15 (22.4%)	9 (16.7%)	5.7%	–8.4 – 19.8%	0.433
Other surgery°, *n* (%)	17 (14.0%)	9 (13.4%)	8 (14.8%)	1.4%	–11.1 – 13.9%	0.828
VAD surgery, *n* (%)	11 (9.1%)	6 (9.0%)	5 (9.3%)	0.3%	–10.0 – 10.6%	0.954
Donor data						
Age (years), mean ± SD	45.0 ± 12.5	46.1 ± 10.9	43.6 ± 14.3	2.5	–2.2 – 7.2	0.295
Male sex, *n* (%)	43 (35.5%)	25 (37.3%)	18 (33.3%)	4.0%	–13.1 – 21.1%	0.649
Body mass index (kg/m^2), mean ± SD	25.5 ± 3.9	25.6 ± 4.0	25.3 ± 3.7	0.3	–1.1 – 1.7	0.589
Transplant sex mismatch						
Mismatch, *n* (%)	68 (56.2%)	38 (56.7%)	30 (55.6%)	1.1%	–16.7 – 18.9%	0.898
Donor (m) to recipient (f), *n* (%)	6 (5.0%)	6 (9.0%)	0 (0.0%)	9.0%	2.1 – 15.9%	0.024*
Donor (f) to recipient (m), *n* (%)	62 (51.2%)	32 (47.7%)	30 (55.6%)	7.9%	–10.0 – 25.8%	0.394
Perioperative data						
Ischemic time (min), mean ± SD	250.6 ± 60.7	245.9 ± 64.3	256.4 ± 56.1	10.5	–11.2 – 32.2	0.338
Biatrial HTX, *n* (%)	1 (0.8%)	0 (0.0%)	1 (1.9%)	1.9%	–1.7 – 5.5%	0.263
Bicaval HTX, *n* (%)	45 (37.2%)	21 (31.3%)	24 (44.4%)	13.1%	–4.2 – 30.4%	0.138
Total orthotopic HTX, *n* (%)	75 (62.0%)	46 (68.7%)	29 (53.7%)	15.0%	–2.3 – 32.3%	0.092

*CABG, coronary artery bypass graft; CI, confidence interval; CMP, cardiomyopathy; COPD, chronic obstructive pulmonary disease; f, female; HbA1c, hemoglobin A1c; HTX, heart transplantation; m, male; n, number; SD, standard deviation; T2DM, type 2 diabetes mellitus; VAD, ventricular assist device; ^, estimated glomerular filtration rate < 30 ml/min/1.73 m^2; °, congenital, valvular or ventricular surgery; *, statistically significant (P < 0.050).*

TABLE 3 | Medications after HTX – stratified by T2DM at HTX.

Parameter	All (*n* = 376)	T2DM (*n* = 121)	No T2DM (*n* = 255)	Difference	95% CI	*P*-value
Immunosuppressive drug therapy						
Cyclosporine A, *n* (%)	124 (33.0%)	36 (29.8%)	88 (34.5%)	4.7%	–5.3 – 14.7%	0.359
Tacrolimus, *n* (%)	252 (67.0%)	85 (70.2%)	167 (65.5%)	4.7%	–5.3 – 14.7%	0.359
Azathioprine, n (%)	46 (12.2%)	16 (13.2%)	30 (11.8%)	1.4%	–5.8 – 8.6%	0.687
Mycophenolate mofetil, *n* (%)	330 (87.8%)	105 (86.8%)	225 (88.2%)	1.4%	–5.8 – 8.6%	0.687
Steroids, *n* (%)	376 (100.0%)	121 (100.0%)	255 (100.0%)	0.0%	n. a.	n. a.
Concomitant medications						
ASA, *n* (%)	47 (12.5%)	14 (11.6%)	33 (12.9%)	1.3%	–5.8 – 8.4%	0.707
Beta blocker, *n* (%)	85 (22.6%)	25 (20.7%)	60 (23.5%)	2.8%	–6.1 – 11.7%	0.534
Ivabradine, *n* (%)	44 (11.7%)	14 (11.6%)	30 (11.8%)	0.2%	–6.7 – 7.1%	0.956
Calcium channel blocker, *n* (%)	110 (29.3%)	42 (34.7%)	68 (26.7%)	8.0%	–2.1 – 18.1%	0.109
ACE inhibitor/ARB, *n* (%)	159 (42.3%)	52 (43.0%)	107 (42.0%)	1.0%	–9.7 – 11.7%	0.852
Diuretic, *n* (%)	376 (100.0%)	121 (100.0%)	255 (100.0%)	0.0%	n. a.	n. a.
Statin, *n* (%)	211 (56.1%)	73 (60.3%)	138 (54.1%)	6.2%	–4.5 – 16.9%	0.257
Gastric protection †, *n* (%)	376 (100.0%)	121 (100.0%)	255 (100.0%)	0.0%	n. a.	n. a.

ACE inhibitor, angiotensin-converting-enzyme inhibitor; ARB, angiotensin II receptor blocker; ASA, acetylsalicylic acid; CI, confidence interval; HTX, heart transplantation; n, number; n. a., not applicable; T2DM, type 2 diabetes mellitus; †, gastric protection defined as proton pump inhibitor (PPI) or histamine receptor (H_2) blocker.

TABLE 4 | Medications after HTX – stratified by HbA1c at HTX.

Parameter	T2DM (*n* = 121)	HbA1c < 7.0% (*n* = 67)	HbA1c ≥ 7.0% (*n* = 54)	Difference	95% CI	*P*-value
Immunosuppressive drug therapy						
Cyclosporine A, *n* (%)	36 (29.8%)	17 (25.4%)	19 (35.2%)	9.8%	–6.6 – 26.2%	0.241
Tacrolimus, *n* (%)	85 (70.2%)	50 (74.6%)	35 (64.8%)	9.8%	–6.6 – 26.2%	0.241
Azathioprine, *n* (%)	16 (13.2%)	9 (13.4%)	7 (13.0%)	0.4%	–11.7 – 12.5%	0.940
Mycophenolate mofetil, *n* (%)	105 (86.8%)	58 (86.6%)	47 (87.0%)	0.4%	–11.7 – 12.5%	0.940
Steroids, *n* (%)	121 (100.0%)	67 (100.0%)	54 (100.0%)	0.0%	n. a.	n. a.
Concomitant medications						
ASA, *n* (%)	14 (11.6%)	9 (13.4%)	5 (9.3%)	4.1%	–7.1 – 15.3%	0.476
Beta blocker, *n* (%)	25 (20.7%)	18 (26.9%)	7 (13.0%)	13.9%	–0.1 – 27.9%	0.060
Ivabradine, *n* (%)	14 (11.6%)	9 (13.4%)	5 (9.3%)	4.1%	–7.1 – 15.3%	0.476
Calcium channel blocker, *n* (%)	42 (34.7%)	21 (31.3%)	21 (38.9%)	7.6%	–9.5 – 24.7%	0.386
ACE inhibitor/ARB, *n* (%)	52 (43.0%)	27 (40.3%)	25 (46.3%)	6.0%	–11.7 – 23.7%	0.508
Diuretic, *n* (%)	121 (100.0%)	67 (100.0%)	54 (100.0%)	0.0%	n. a.	n. a.
Statin, *n* (%)	73 (60.3%)	44 (65.7%)	29 (53.7%)	12.0%	–5.5 – 29.5%	0.181
Gastric protection †, *n* (%)	121 (100.0%)	67 (100.0%)	54 (100.0%)	0.0%	n. a.	n. a.

ACE inhibitor, angiotensin-converting-enzyme inhibitor; ARB, angiotensin II receptor blocker; ASA, acetylsalicylic acid; CI, confidence interval; HbA1c, hemoglobin A1c; HTX, heart transplantation; n, number; n. a., not applicable; T2DM, type 2 diabetes mellitus; †, gastric protection defined as proton pump inhibitor (PPI) or histamine receptor (H_2) blocker.

at the time of HTX (68.7%, difference: 22.4%, 95% CI: 5.1–39.7%, $P = 0.008$). Kaplan–Meier estimators are shown in **Figures 1**, **2**.

Additional survival analysis revealed that patients without T2DM at the time of HTX had the best 1-year (210 of 255 [82.4%]), 2-year (196 of 255 [76.9%]), and 5-year post-transplant survival (179 of 255 [70.2%]), followed by patients with T2DM at HTX and a HbA1c < 7.0% at HTX who showed a broadly similar 1-year (51 of 67 [76.1%]), 2-year (49 of 67 [73.1%]), and 5-year post-transplant survival (46 of 67 [68.7%]). Of note, patients with T2DM at HTX and a HbA1c ≥ 7.0% at HTX had the worst 1-year (31 of 54 [57.4%]), 2-year (30 of 54 [55.6%]), and 5-year post-transplant survival (25 of 54 [46.3%]). An overview of 5-year post-transplant survival stratified by T2DM at HTX and HbA1c at HTX is provided in **Figure 3**.

Sensitivity Analysis

In order to investigate a possible era effect and to examine the robustness of our findings, we performed a sensitivity analysis with a subgroup of patients who were administered tacrolimus and mycophenolate mofetil as immunosuppressive drug therapy [252 of 376 patients (67.0%)]. This analysis provided similar results supporting the robustness of our findings and reducing the likelihood of a potential era effect.

DISCUSSION

Frequency and Clinical Relevance of Type 2 Diabetes Mellitus at Heart Transplantation

Given the unknown prognostic effect of T2DM at the time of HTX on post-transplant outcomes, we performed this large study with 376 HTX recipients to investigate the frequency and clinical relevance of pre-transplant T2DM. A total of 121 HTX recipients (32.2%) had pre-transplant T2DM. This is in line with recent studies describing a similar percentage of pre-transplant diabetic patients (24, 25). Chamarthi et al. (24) reported a pre-transplant diabetic rate of 28.8% (46 of 160) at Brigham and Women's Hospital, Harvard Medical School, between January 2000 and July 2012. Similarly, Feng et al. (25) published a pre-transplant diabetic rate of 30.7% (75 of 244) at Stanford University Medical Center between January 2008 and July 2018. In contrast, older studies covering earlier eras of HTX reported a considerably lower rate of pre-transplant diabetic patients ranging between 13.7 and 18.3% (22, 23). These data evidently highlight the rising percentage of diabetic HTX recipients (22–25).

From a clinician's perspective, this change is of high relevance as patients with T2DM face an increased risk of morbidity and mortality (1–8, 13–16, 36). Diabetic patients have a higher risk for post-transplant infections requiring hospitalization and often suffer from further deterioration of renal function, especially in combination with calcineurin inhibitors which are known nephrotoxic drugs (20, 21, 25, 37–39). In order to evaluate the degree of end-organ damage as well as the clinical status of patients at the time of HTX, we compared patients with and without T2DM at the time of HTX. We found no statistically significant differences between both groups in regard to severe chronic kidney disease, total bilirubin, hemoglobin, hospitalization before HTX, days on waiting list, high urgency status on waiting list, inotropic support, intra-aortic balloon pump, or initial hospital stay after HTX highlighting the importance of careful evaluation of T2DM patients before listing for HTX. Alternatively, patients with T2DM and severe end-organ damage may be considered for left ventricular assist device (LVAD) implantation (21). However, especially in younger patients, this may not be a valid long-term solution given the known LVAD complications (40, 41). Hence, patients with T2DM should be carefully evaluated before listing for HTX,

TABLE 5 | Overview of diabetes medications.

Parameter	T2DM (*n* = 121)	HbA1c < 7.0% (*n* = 67)	HbA1c ≥ 7.0% (*n* = 54)	Difference	95% CI	*P*-value
Oral anti-diabetic medications						
Alpha glucosidase inhibitors						
Acarbose, *n* (%)	3 (2.5%)	2 (3.0%)	1 (1.9%)	1.1%	–4.3 – 6.5%	0.690
Biguanides						
Metformin, *n* (%)	49 (40.5%)	27 (40.3%)	22 (40.7%)	0.4%	–17.2 – 18.0%	0.961
DPP-4 inhibitors						
Saxagliptin, *n* (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0%	n. a.	n. a.
Sitagliptin, *n* (%)	11 (9.1%)	9 (13.4%)	2 (3.7%)	9.7%	–0.1 – 19.5%	0.064
Vildagliptin, *n* (%)	3 (2.5%)	1 (1.5%)	2 (3.7%)	2.2%	–3.6 – 8.0%	0.437
GLP-1 receptor agonists						
Dulaglutide, *n* (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0%	n. a.	n. a.
Exenatide, *n* (%)	1 (0.8%)	0 (0.0%)	1 (1.9%)	1.9%	–1.7 – 5.5%	0.263
Liraglutide, *n* (%)	2 (1.7%)	1 (1.5%)	1 (1.9%)	0.4%	–4.2 – 5.0%	0.878
Meglitinides						
Nateglinide, *n* (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0%	n. a.	n. a.
Repaglinide, *n* (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0%	n. a.	n. a.
SGLT-2 inhibitors						
Dapagliflozin, *n* (%)	1 (0.8%)	0 (0.0%)	1 (1.9%)	1.9%	–1.7 – 5.5%	0.263
Empagliflozin, *n* (%)	1 (0.8%)	1 (1.5%)	0 (0.0%)	1.5%	–1.4 – 4.4%	0.367
Sulfonylureas						
Glibenclamide, *n* (%)	2 (1.7%)	1 (1.5%)	1 (1.9%)	0.4%	–4.2 – 5.0%	0.878
Glimepiride, *n* (%)	12 (9.9%)	8 (11.9%)	4 (7.4%)	4.5%	–6.0 – 15.0%	0.407
Gliquidone, *n* (%)	5 (4.1%)	3 (4.5%)	2 (3.7%)	0.8%	–6.3 – 7.9%	0.832
Thiazolidinediones						
Pioglitazone, *n* (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0%	n. a.	n. a.
Rosiglitazone, *n* (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0%	n. a.	n. a.
Insulin therapy						
Rapid-acting insulin						
Insulin aspart, *n* (%)	9 (7.4%)	5 (7.5%)	4 (7.4%)	0.1%	–9.3 – 9.5%	0.991
Insulin glulisine, *n* (%)	1 (0.8%)	0 (0.0%)	1 (1.9%)	1.9%	–1.7 – 5.5%	0.263
Insulin lispro, *n* (%)	3 (2.5%)	1 (1.5%)	2 (3.7%)	2.2%	–3.6 – 8.0%	0.437
Short-acting insulin						
Regular insulin, *n* (%)	45 (37.2%)	18 (26.9%)	27 (50.0%)	23.1%	6.1 – 40.1%	0.009*
Intermediate-acting insulin						
NPH insulin, *n* (%)	13 (10.7%)	6 (9.0%)	7 (13.0%)	4.0%	–7.3 – 15.3%	0.479
Long-acting insulin						
Insulin degludec, *n* (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0%	n. a.	n. a.
Insulin detemir, *n* (%)	4 (3.3%)	1 (1.5%)	3 (5.6%)	4.1%	–2.7 – 10.9%	0.214
Insulin glargine, *n* (%)	41 (33.9%)	17 (25.4%)	24 (44.4%)	19.0%	2.2 – 35.8%	0.028*

*CI, confidence interval; DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; HbA1c, hemoglobin A1c; n, number; n. a., not applicable; NPH, Neutral Protamine Hagedorn; SGLT-2, sodium-glucose transport protein 2; T2DM, type 2 diabetes mellitus; *, statistically significant (P < 0.050).*

TABLE 6 | Causes of death within 5 years after HTX – stratified by T2DM at HTX.

Parameter	All (*n* = 376)	T2DM (*n* = 121)	No T2DM (*n* = 255)	Difference	95% CI	*P*-value
Graft failure, *n* (%)	38 (10.1%)	18 (14.9%)	20 (7.8%)	7.1%	0.1 – 14.1%	0.035*
Acute rejection, *n* (%)	4 (1.1%)	1 (0.8%)	3 (1.2%)	0.4%	–1.7 – 2.5%	0.757
Infection/Sepsis, *n* (%)	66 (17.5%)	20 (16.5%)	46 (18.0%)	1.5%	–6.6 – 9.6%	0.719
Malignancy, *n* (%)	8 (2.1%)	5 (4.1%)	3 (1.2%)	2.9%	–0.9 – 6.7%	0.064
Thromboembolic event/bleeding, *n* (%)	10 (2.7%)	6 (5.0%)	4 (1.6%)	3.4%	–0.8 – 7.6%	0.056
All causes, *n* (%)	126 (33.5%)	50 (41.3%)	76 (29.8%)	11.5%	1.1 – 21.9%	0.027*

*CI, confidence interval; HTX, heart transplantation; n, number; T2DM, type 2 diabetes mellitus; *, statistically significant (P < 0.050).*

TABLE 7 | Causes of death within 5 years after HTX – stratified by HbA1c at HTX.

Parameter	T2DM (*n* = 121)	HbA1c < 7.0% (*n* = 67)	HbA1c ≥ 7.0% (*n* = 54)	Difference	95% CI	*P*-value
Graft failure, *n* (%)	18 (14.9%)	8 (11.9%)	10 (18.5%)	6.6%	–6.3 – 19.5%	0.312
Acute rejection, *n* (%)	1 (0.8%)	1 (1.5%)	0 (0.0%)	1.5%	–1.4 – 4.4%	0.367
Infection/Sepsis, *n* (%)	20 (16.5%)	7 (10.5%)	13 (24.1%)	13.6%	0.1 – 27.1%	0.045*
Malignancy, *n* (%)	5 (4.1%)	4 (6.0%)	1 (1.9%)	4.1%	–2.6 – 10.8%	0.258
Thromboembolic event/bleeding, *n* (%)	6 (5.0%)	1 (1.5%)	5 (9.3%)	7.8%	–0.4 – 16.0%	0.050
All causes, *n* (%)	50 (41.3%)	21 (31.3%)	29 (53.7%)	22.4%	5.1 – 39.7%	0.013*

*CI, confidence interval; HbA1c, hemoglobin A1c; HTX, heart transplantation; n, number; T2DM, type 2 diabetes mellitus; *, statistically significant (P < 0.050).*

TABLE 8 | Multivariate analysis for 5-year mortality after HTX.

Parameter	Hazard Ratio	95% CI	*P*-value
Recipient age (years)	1.018	0.996 – 1.041	0.103
Recipient body mass index (kg/m^2)	1.012	0.969 – 1.058	0.592
Recipient arterial hypertension (in total)	0.704	0.404 – 1.228	0.217
Recipient dyslipidemia (in total)	0.935	0.548 – 1.596	0.805
Recipient T2DM (in total)	1.563	1.053 – 2.319	0.027 *
Previous CABG surgery (in total)	0.783	0.434 – 1.412	0.415
Ischemic CMP (in total)	1.718	0.993 – 2.972	0.053
Cardiac amyloidosis (in total)	1.697	0.985 – 2.923	0.057

*CABG, coronary artery bypass graft; CI, confidence interval; CMP, cardiomyopathy; HTX, heart transplantation; T2DM, type 2 diabetes mellitus; *, statistically significant (P < 0.050).*

particularly in regard to severe end-organ damage, and should receive optimal individualized diabetes management before and after HTX (1–8).

Clinical Management of Patients With Type 2 Diabetes Mellitus at Heart Transplantation

In order to reduce microvascular and macrovascular complications in patients with T2DM, the 2019 European Society of Cardiology (ESC) guidelines on diabetes recommend a targeted HbA1c < 7.0% (1). We therefore compared in our study the diabetes medications of T2DM patients with a HbA1c < 7.0% and T2DM patients with a HbA1c ≥ 7.0%. We found no significant difference between both groups in regard to oral anti-diabetic medications of which metformin was the most common oral anti-diabetic drug in patients with T2DM at the time of HTX. In terms of insulin therapy which was administered to almost half of patients with T2DM at the time of HTX, T2DM patients with a HbA1c ≥ 7.0% had a significantly higher percentage of regular insulin (P = 0.009) and insulin glargine (P = 0.028).

With the introduction of new anti-diabetic medications such as dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists, and sodium-glucose transport protein 2 (SGLT-2) inhibitors, diabetes management has improved and a targeted HbA1c < 7.0% has become more achievable (1, 3, 25, 42–48). In addition to their excellent glucose-lowering profile, these novel agents exhibit multiple beneficial effects *via* reduction of body weight, blood pressure, major cardiovascular events and even mortality (1, 3, 25, 42–48).

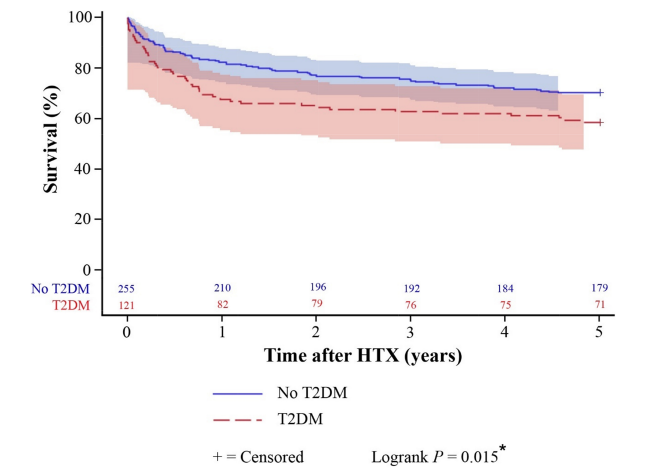

FIGURE 1 | Five-year post-transplant survival of patients with and without T2DM at HTX (Kaplan–Meier estimator). Patients with T2DM at HTX had a significantly worse 5-year post-transplant survival in the Kaplan–Meier survival analysis (58.7%) compared to patients without T2DM at HTX (70.2%, P = 0.015). HTX, heart transplantation; T2DM, type 2 diabetes mellitus; *, statistically significant (P < 0.050).

However, data regarding the safety and efficacy of these new drugs in HTX recipients with T2DM are limited to studies with small sample sizes (42, 45).

Cehic et al. (42) examined 22 HTX recipients with T2DM who were treated with empagliflozin. They observed no genitourinary infections and treatment with empagliflozin was associated with reductions in body mass index and HbA1c (42). Similar

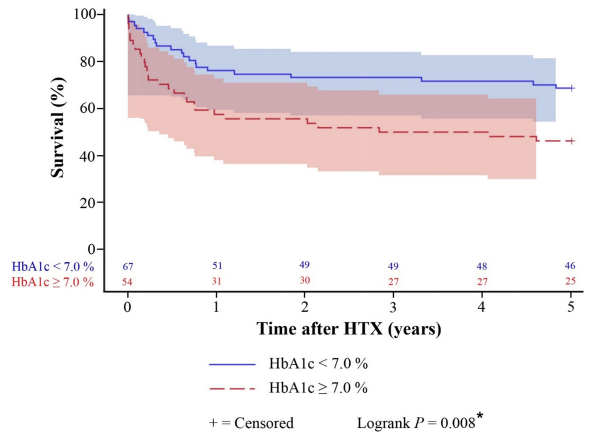

FIGURE 2 | Five-year post-transplant survival of patients with T2DM stratified by HbA1c at HTX (Kaplan–Meier estimator). Stratification of patients with T2DM at HTX showed a significantly lower 5-year post-transplant survival of patients with a HbA1c ≥ 7.0% at HTX (46.3%) in comparison to patients with a HbA1c < 7.0% at HTX (68.7%, $P = 0.008$). HbA1c, hemoglobin A1c; HTX, heart transplantation; T2DM, type 2 diabetes mellitus; *, statistically significant ($P < 0.050$).

findings were reported by Sammour et al. (45) who evaluated the safety and efficacy of GLP-1 receptor agonists and SGLT-2 inhibitors in HTX recipients with T2DM. Among 21 patients, they found a significant reduction of body weight, HbA1c, and low-density lipoprotein-cholesterol with no adverse events leading to discontinuation of either therapy (45).

In our study, only a minority of patients with pre-transplant T2DM received DPP-4 inhibitors, GLP-1 receptor agonists, or SGLT-2 inhibitors, as the majority of patients with oral anti-diabetic medications were still on metformin. This is in line with a recent study by Feng et al. (25) reporting likewise only a few HTX recipients on GLP-1 receptor agonists or SGLT-2 inhibitors. Further studies with large contemporary populations of HTX recipients with T2DM are therefore needed to determine the safety and efficacy of these medications but one should keep in mind that the use of anti-diabetic medications is just one part of diabetes management. A multimodal approach including nutrition counseling, increased physical activity, weight loss, smoking cessation in addition to anti-diabetic medications with new pharmacologic strategies is required to reduce the burden of morbidity and mortality in HTX recipients with T2DM.

Post-transplant Mortality of Patients With Type 2 Diabetes Mellitus at Heart Transplantation

Data regarding the impact of pre-transplant T2DM on mortality after HTX are inconclusive (9–21). Several studies reported an increased post-transplant mortality in patients with T2DM at the time of HTX (13–16), whereas others studies did not find a relevant difference (17–21).

In our study with a large contemporary population of HTX recipients, patients with pre-transplant T2DM had a significantly increased 5-year all-cause mortality after HTX (41.3% versus 29.8%, $P = 0.027$), along with a higher rate of death due to graft failure (14.9% versus 7.8%, $P = 0.035$). Multivariate analysis showed a more than 50% increased risk for 5-year mortality after HTX in these patients (HR: 1.563; 95% CI: 1.053–2.319; $P = 0.027$).

Discrepancies between former studies regarding post-transplant mortality in patients with T2DM at the time of HTX may result from differences in diabetes status (13–21).

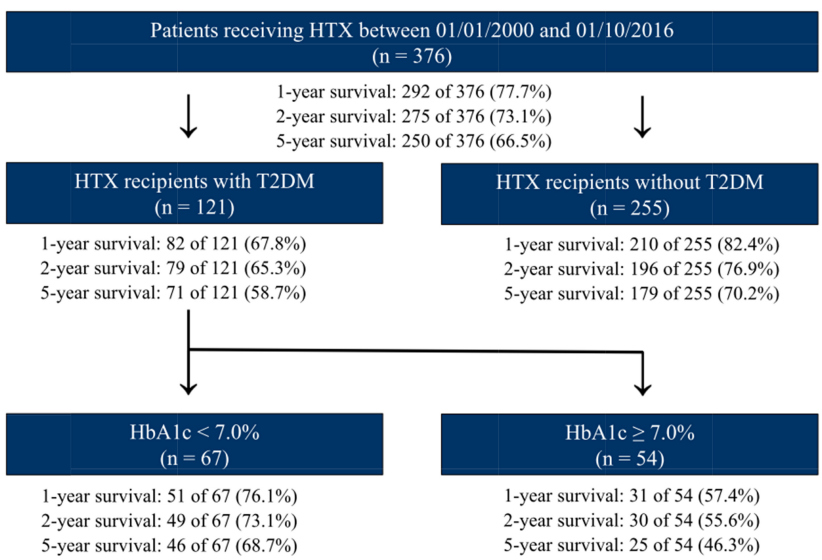

FIGURE 3 | Overview of 5-year post-transplant survival stratified by T2DM at HTX and HbA1c at HTX. Patients without T2DM at HTX showed the best 1-, 2-, and 5-year post-transplant survival, followed by patients with T2DM at HTX and a HbA1c < 7.0% at HTX, whereas patients with T2DM at HTX and a HbA1c ≥ 7.0% at HTX had the worst 1-, 2-, and 5-year post-transplant survival. HbA1c, hemoglobin A1c; HTX, heart transplantation; n, number; T2DM, type 2 diabetes mellitus.

A large study of the United Network of Organ Sharing (UNOS) database with 20,412 HTX recipients including 3,687 diabetic patients reported a significantly better post-transplant survival in non-diabetic HTX recipients than in diabetic HTX recipients in general ($P < 0.001$) but there was no statistically significant difference in post-transplant survival between non-diabetic HTX recipients and diabetic HTX recipients with uncomplicated diabetes status ($P = 0.080$) (21).

Stratification of patients with T2DM by HbA1c at HTX in our study showed a significantly higher 5-year all-cause mortality after HTX in T2DM patients with a HbA1c ≥ 7.0% (53.7% versus 31.3%, $P = 0.013$). Patients with T2DM and a HbA1c ≥ 7.0% also had a higher percentage of death due to graft failure (18.5% versus 11.9%), infection/sepsis (24.1% versus 10.5%), and thromboembolic event/bleeding (9.3% versus 1.5%) within 5 years after HTX highlighting the vulnerability of these patients. As insulin therapy is often needed in patients with advanced T2DM, we also compared patients with and without insulin therapy. HTX recipients with insulin therapy had in fact a lower 5-year post-transplant survival (53.4%) than patients without insulin therapy (63.5%) but this difference did not reach statistical significance ($P = 0.243$). This is in line with a report by Czerny et al. (13) who also found no significant influence of insulin therapy on survival after HTX.

Furthermore, a key message of our study is the finding that patients with T2DM and a HbA1c < 7.0% had a similar 5-year survival after HTX in comparison to patients without T2DM indicating that comparable long-term post-transplant survival rates of HTX recipients with T2DM are achievable if these patients receive optimal diabetes management and are followed-up closely after HTX.

Regarding the impact of diabetes on ventricular ejection fraction and cardiac allograft vasculopathy, results have been controversially discussed (4, 11, 13, 17, 49). Higgins et al. (17) reported that diabetic HTX recipients had an increased rate of transplant coronary artery disease (42% versus 13%; $P = 0.02$) as well as a lower left ventricular ejection fraction at 3 years after HTX (54% versus 61%; $P = 0.03$). In contrast, Munoz et al. (11) found no statistically significant difference in transplant coronary artery disease by the fourth year of follow-up (31% in diabetic HTX recipients versus 33% in non-diabetic HTX recipients). Similar results were reported by Czerny et al. (13) who also reported no statistically significant difference in transplant coronary artery disease (15% in diabetic HTX recipients versus 14% in non-diabetic HTX recipients) at 5 years after HTX.

In our study, survival of T2DM patients declined markedly within the first year after HTX, some patients with T2DM even died from graft failure within the first 3 months after HTX. As the development of cardiac allograft vasculopathy usually takes several months to years after HTX this may indicate that adverse graft survival in HTX recipients is rather related to generally impaired global organ function (13). However, given the importance of this aspect and the lack of contemporary knowledge, there is an urgent need for future studies focusing on the development of cardiac allograft vasculopathy by analyzing catheterization data of HTX recipients with T2DM.

Study Limitations

The findings of this study were derived from a large single-center registry (Heidelberg HTX Registry) including the highly elaborated data of 376 patients who received HTX at Heidelberg Heart Center. Given the known limitations of such a study design, our results should be interpreted carefully and within the context of the existing literature. However, we would like to point out that our study was comparable to multicenter studies in sample size and our patients received standardized treatment and follow-up, decreasing the likelihood of potential selection bias and confounders (26–35).

In order to detect long-term effects of T2DM in HTX recipients, we selected adult HTX recipients who received HTX at Heidelberg Heart Center between 01/01/2000 and 01/10/2016, enabling a minimum post-transplant follow-up of 5 years. This study included data of HTX recipients over a period of more than 20 years. A possible era effect as a result of changes in medical and surgical care may have therefore affected our results. As tacrolimus replaced cyclosporine A as the main immunosuppressive agent from 2006 onward, we investigated a possible era effect by comparing the immunosuppressive drug therapy of HTX recipients with or without T2DM. We could neither detect a statistically significant difference between HTX recipients with or without T2DM regarding the use of cyclosporine A or tacrolimus, nor concerning the use of azathioprine or mycophenolate mofetil supporting the robustness of our findings (26–35).

Our results should be interpreted as hypothesis-generating, especially in the context of post-transplant survival. We can therefore neither proof nor disproof a causal relationship between T2DM at the time of HTX and increased 5-year post-transplant mortality but merely indicate an association. In addition, the effects of the recently introduced SGLT-2 inhibitors on long-term post-transplant mortality in HTX recipients remain unknown and require further investigation, preferably in form of large multicenter trials.

CONCLUSION

The number of HTX recipients with pre-transplant T2DM has continuously been growing over the last decades. Many of these patients suffer from impaired wound healing, infections, renal dysfunction, thromboembolic complications, cardiac rejections, and cardiac allograft vasculopathy. Management of HTX recipients with T2DM is therefore very challenging but data about this topic are still very limited. In order to investigate the effects of pre-transplant T2DM on survival and causes of death after HTX, we performed a large study with a contemporary population of 376 HTX recipients including 121 patients with T2DM (32.2%). We observed a significantly higher 5-year all-cause mortality after HTX in patients with pre-transplant T2DM (41.3% versus 29.8%, $P = 0.027$) along with a higher percentage of death due to graft failure (14.9% versus 7.8%, $P = 0.035$). Multivariate analysis indicated pre-transplant T2DM as a significant risk factor for 5-year mortality after HTX (HR: 1.563; 95% CI: 1.053–2.319; $P = 0.027$). Stratification of HTX

recipients with pre-transplant T2DM showed no statistically significant difference in 5-year survival between patients with and without insulin therapy ($P = 0.243$) but patients with pre-transplant T2DM and a HbA1c $< 7.0\%$ had a significantly better 5-year survival than patients with a HbA1c $\geq 7.0\%$ ($P = 0.008$). Of note, patients with T2DM and a HbA1c $< 7.0\%$ had a similar 5-year survival after HTX compared to patients without T2DM. Therefore, patients with T2DM can successfully undergo HTX if they receive optimal diabetes management before and after HTX.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board (IRB) of Heidelberg University, Germany (ethical approval number: S-286/2015, Version 1.2, 28-07-2020). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RR, CG, TB, and LK: conceptualization, methodology, and investigation. RR, CG, MH, FD, TB, and LK: formal analysis, validation, and data curation. RR, CG, and LK: writing – original draft preparation, review and editing. RR, CG, FD, and LK: visualization. RR and FD: funding acquisition. RR, PE, WS, GW, SK, JS, NF, and LK: supervision and resources. All authors: contributed to the article and approved the submitted version.

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BRIEF RESEARCH REPORT
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Theophylline Use to Prevent Permanent Pacing in the Contemporary Era of Heart Transplantation: The Rotterdam Experience

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Introduction: Sinus node dysfunction and atrioventricular conduction disorders occur increasingly after orthotopic heart transplantation (HTX) due to aging donors and may require permanent pacemaker (PM) implantation. Theophylline has been used in the past in selected cases as an alternative to PM implantation.

Purpose: The aim of this study was to investigate the rate and success of oral theophylline administration after orthotopic heart transplantation preventing permanent PM implantation.

Methods: We included all patients treated with theophylline post HTX due to bradyarrhythmia's in our center from January 1985 to January 2020. Data was obtained retrospectively through electronic patient files. Re-transplants and patients who died within 1 month post HTX were excluded from the analysis.

Results: Of the total of 751 heart transplant recipients, 73 (9,7%) patients (mean age 46 ± 15.2 years; 73% male) were treated with theophylline for bradyarrhythmia's early post HTX. Of these patients, 14 (19%) patients needed a permanent PM during hospitalization and 10(14%) patients stopped using theophylline because of adequate heart rhythm. In the end, 49 (6.5% of the total) patients were discharged with a theophylline (mean maintenance doses of 354 ± 143 mg). At the outpatient clinics, additional 6 (12%) patients needed a PM within 7 months after discharge, with the rest stable sinus rhythm.

Conclusion: In this retrospective data analyses oral theophylline remained a viable alternative to permanent PM implantations in patients post HTX with increased heart rates, facilitating the withdrawal of chronotropic support and avoiding the need of permanent PM implantation.

Keywords: heart transplantation, pacemaker, bradyarrhythmia, theophylline, prevention, indication

In the last few decades, early permanent pacemaker (PPM) implantation has been increasingly utilized to treat persistent bradycardia following bradyarrhythmias occurring after transplantation, mainly due to the increasing age of suitable donors in Europe, especially in the Netherlands (1).

There are several disadvantages of PPM implantations such as complications, costs, and delayed hospitalizations related to the procedure within these vulnerable groups of patients. Furthermore, in many patients, the need for pacing vanishes in the months post-HTx (2).

Theophylline is one of the older drug medicines with several pharmacological actions, including bronchial dilatation, diuresis, and chronotropic and dromotropic effect. Therefore, theophylline has been used successfully in the past for the treatment of sick sinus syndrome to increase the heart frequency and avoid PPM implantation (3). The costs of theophylline therapy are minimal, and usually well tolerated. Despite its known use post-HTx, data on its efficacy are scarce.

Therefore, the aim of this study was to investigate the prevalence and efficacy of oral theophylline after orthotopic HTx to prevent PPM implantation.

In this study, data of all consecutive patients who underwent primary HTx at our center between January 1984 and January 2020 were retrospectively collected from the electronic patient records. The demographic and clinical data were collected along with the use and duration of theophylline, dosage, side effects, and early pacemaker implantations. Indications for PPM implantation were categorized into sinus node dysfunction and atrioventricular blockage. Clinically significant bradycardia was defined as a heart rate <60 beats/min. The primary endpoint of this study was the successful discharge of the patient with theophylline without the need of a PPM. Perioperatively, all patients received a temporary pacemaker and isoprenaline intravenously to maintain a heart rate > 100 beats per min in the first 3 days post-HTx. Thereafter, the target heart rate was decreased with 10 beats per min every day until the patient had an intrinsic heart rate of at least 60 bpm with stable hemodynamics. If the heart rate was not sufficient after 10–14 days, theophylline orally with extended release (daily dosage of 200–300 mg) was initiated to taper the isoprenaline intravenously. When theophylline therapy was successful with a stable sinus rhythm of ≥60 bpm and hemodynamics, the patient was discharged with oral theophylline without the need for a PPM. If this was unsuccessful, a PPM implantation was planned in weeks 4 to 6 post-HTX.

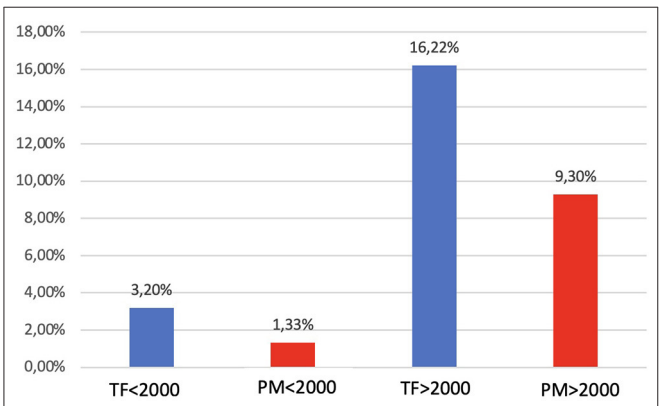

FIGURE 1 | Bar graph showing the percentage of theophylline use vs. permanent pacemaker implant at discharge before and after the year 2000 ($p = 0.56$). TF, theophylline; PM, pacemaker.

Of the total of 751 HTx recipients, 73 (9.7%) patients with a mean age 46 ± 15.2 years, 73% male, were treated with theophylline for bradyarrhythmia post-HTx. Of these patients, 10 (14%) patients stopped using theophylline because of stable sinus rhythm and in 14 (19%) treatment failed followed by a PPM during the hospitalization. Overall, 49 (6.5% of the total) patients were discharged with oral theophylline with a mean maintenance dose of 354 ± 143 mg. At the outpatient clinics, an additional six (12%) patients needed a PPM within the following months (longest: 7 months) post discharge. The prevalence of both theophylline uses (12 patients before and 61 patients after 2000) as well as need of a PPM increased significantly along with increasing donor ages in the past two decades. **Figure 1** shows the percentage of theophylline use vs. permanent pacemaker implant at discharge before and after the year 2000, a year in which the baseline immunosuppressive treatment and donor ages changed significantly in our center (1, 4). There were no significant correlations with successful vs. not successful treatment regarding the recipient age (mean 46.6 ± 14.0 vs. 43.8±14.9 years), donor age (mean 40.6 ± 14.7 vs. 42.5 ± 12.7 years), gender, preoperative use of amiodaron (53 vs. 67%), or underlying heart diseases (28 vs. 33 % ischemic heart diseases).

The major finding of the present study is that oral theophylline administration early after orthotopic HTx can prevent PPM implantation in a selected group of patients in the contemporary era of HTx. Our single-center experience confirms that the use of theophylline for post-HTx bradyarrhythmias successfully increased the baseline heart rate, facilitated the withdrawal of chronotropic support, and deferred the need for permanent pacing. We recently reported that the most common indication for early PPM implantation was sinus node dysfunction (SND) while atrioventricular block was more frequent in late PM implantation (1). Patients who had an older donor had an increased risk of having a PM implanted both early and late after HT. Unfortunately, the donor ages increased significantly in the last two decades to sustain the declining numbers of suitable heart donors (4). As bradyarrhythmias, especially sinus node dysfunction, usually improve over the weeks to months, the use of theophylline could prevent unnecessary delay of hospital stay post-HTx and save costly PPM implantation besides the low but always existent risk of complications of a PPM. However, randomized controlled trials are needed for a definitive answer in the choice of theophylline vs. early permanent pacemaker implantation.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of the Erasmus MC. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KC and TS-T contributed to conception and design of the study. CK and KC organized the database, performed the statistical analysis, and wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Outcome of Patients Supported by Large Impella Systems After Re-implantation Due to Continued or Recurrent Need of Temporary Mechanical Circulatory Support

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Despite the growing utilization of a large microaxial pump, i. e., Impella 5.0 or 5.5 (Abiomed Inc., Danvers, MA, USA) (Impella 5+) for patients with cardiogenic shock (CS), adverse events including the necessity of re-implantation have not been well discussed. In all 67 patients, in-hospital mortality was 52.2% (n = 35). Explantation of Impella 5+ was performed in 39 patients (58.2%), 22 of whom (32.8%) recovered under Impella 5+, and ten further patients (14.9%) survived after a successful transition to permanent mechanical circulatory support. Embolic events were considerable complications in each access. They occurred in the right arm after the removal of Impella 5+ *via* a subclavian artery (SA) (n = 3, 9.1%) or in the form of leg ischemia in patients with Impella 5+ *via* femoral artery (FA) (n = 2, 33.3%). Re-implantation was necessary for 10 patients (14.9%) due to 1) recurrent CS (n = 3), 2) pump thrombosis (n = 5), or 3) pump dislocation (n = 2), all of which were successfully performed *via* the same access route. In univariate analysis, FA access was a significant risk factor for Impella dysfunction compared to SA access (FA vs. SA, 42.9% vs. 9.8%, $p < 0.05$, odds ratio 6.88). No statistical difference of overall mortality was observed in patients with Impella 5+ re-implantation (n = 10) compared to patients with primary Impella 5+ support (n = 57) (80.0% (n = 8/10) vs. 47.4% (n = 27/57), p = 0.09). Our results suggested the acceptable clinical outcome of Impella 5+ despite a 15% re-implantation rate. Our observational data may merit further analysis of anticoagulation strategies, including risk stratification for embolic events.

Keywords: cardiogenic shock, Impella, complication, re-implantation, thrombosis

INTRODUCTION

In recent years, the percutaneous microaxial pump, i.e., Impella (Abiomed Inc., Danvers, MA, USA), has enabled antegrade flow support with unloading of the left ventricle (LV), which provides us with various therapy options to manage patients with cardiogenic shock (CS). Few previous studies have introduced some complications and adverse outcomes of Impella systems (1–6). In the

field of heart failure surgery, our attention is directed to large microaxial pump catheters, i.e., Impella 5.0 or 5.5 (Impella 5+), since patients with fulminant CS often need larger Impella systems to mimic the hemodynamic status under left ventricular assist device (LVAD) support and to bridge the permanent mechanical circulatory support (MCS) as well as orthotopic heart transplantation (oHTX).

Despite a comprehensive utilization of Impella 5+ for patients with CS, the reports regarding adverse events of Impella 5+ are scarce (7, 8). Moreover, reports that focused on re-implantation of Impella 5+ and its impact on patient outcomes are yet missing. In this study, we report our experience with respect to adverse events and the clinical outcomes after Impella 5+ support to elucidate the effective postoperative management of Impella 5+ as temporary MCS. In particular, we analyze those cases with re-implantation of Impella 5+.

MATERIALS AND METHODS

Ethics Committee Approval

The local ethics committee approved this retrospective study (Ref. 2020-1173).

Study Population

In consecutive 67 patients, a total of 78 Impella 5+ were implanted between November 2018 and February 2021 at our department, and all the cases were assigned to this study. Fifty-seven patients underwent Impella 5+ implantation as a single therapy action, whereas 10 patients (14.9%) received mechanical circulatory support *via* Impella 5+ more than one time (re-implantation). Among 10 patients with re-implantation of Impella 5+, 3 patients received the second Impella 5+ because of recurrent CS after an initial successful weaning of the first Impella 5+ and discharge from our department within the observation period. Besides, seven patients (10.4%) needed an exchange of Impella 5+ urgently due to Impella dysfunction, one of whom (1.5%) required an exchange of Impella 5+ two times (**Figure 1**).

Definition of Clinical Outcomes

Regarding clinical outcomes, we investigated whether the patient could be discharged from the specialized cardiac unit at the university hospital (cardiac surgery or cardiology department). For example, if the patient was transferred to other facilities only for rehabilitation purposes, such transfer was considered "discharge". According to this definition, overall survival considers patients after Impella 5+ support who could be discharged from the specialized cardiac unit. On the contrary, in-hospital mortality was defined as death without discharge from the cardiac unit. Besides, 30-day survival was defined as survival at 30 days after the first Impella 5+ implantation.

Further, the analysis of clinical outcomes was centered on the individual patients and not on each implanted Impella 5+ device. It means that only clinical outcomes since the last Impella 5+ support were analyzed in patients who required more than one Impella 5+ support to avoid artifacts in reported clinical outcomes. Furthermore, major bleeding was defined as prolonged or excessive bleeding, which was severe to control conservatively. It occurred spontaneously or following a medical maneuver, e.g., cardiopulmonary resuscitation (CPR) or surgical treatment. "Impella dysfunction" contains pump dislocation and pump thrombosis in this study. "Leg ischemia" means the leg hypoperfusion at the site where Impella 5+ was implanted.

Preoperative Assessment and Surgical Procedure

A CT angiography was performed only when feasible because of the constellation of patients with CS for preoperative assessment of the whole vascular systems, e.g., aorta, subclavian artery (SA), and femoral artery (FA). In principle, at our institution SA is the standard access route for implantation of Impella 5+, allowing early patient extubation and mobilization. As an aspect of hygiene control, the SA approach is considered to be superior to the FA approach. Thus, Impella 5+ was inserted through FA only when SA access was impractical or for some other apparent reason. No matter which approach of Impella 5+ implantation was selected, all patients received Impella 5+ pump over a 10-mm Dacron prosthesis (Gelweave, Vascutek, Terumo; Renfrewshire, Scotland) implanted in an end-to-side fashion onto the target artery (so-called chimney configuration). The prosthesis was led out through the skin *via* another additional incision. Insertion and final positioning were controlled using fluoroscopy combined with transesophageal echocardiography.

Concerning the surgical procedure of Impella 5+ explantation, we clamped the prosthesis after removing Impella 5+ catheter. After flushing thrombotic materials, we cut down the prosthesis leaving a 10–15 mm long stump, which was sewed over for closure. On the other hand, regarding the procedure of Impella re-implantation, in general, the proximal and distal side of the target site with the stump of the previous prosthesis was clamped after adequate heparin administration (activated clotting time; ACT $>$ 200 s). The remnant prosthesis was re-opened and then the included thrombotic materials were removed. A new 10-mm Dacron prosthesis was anastomosed on the stump of the previous prosthesis (end-to-end anastomosis). Of note, all prostheses implanted onto the target arteries were gelatin-coated and incubated with Rifampicin (600 mg) for minimum of 5 min before use.

Postoperative Management of Impella 5+

Postoperative management after Impella 5+ follows as we described before (9, 10). Briefly, all parameters displayed in Impella monitor, e.g., Impella setting, Impella flow, purge pressure, purge flow rate, and placement signal, were documented every 3 h. All patients underwent chest X-ray evaluation every day to examine the position of the Impella pump. Transthoracic echocardiographic (TTE) evaluation was performed if Impella position in chest X-ray was moved or at the timing of routine TTE check-up for cardiac functions or in case of abnormal sign of Impella parameters. The anticoagulation for Impella was administrated according to the current recommendation of the manufacturer. As far as anticoagulation for purge solution is concerned, 5% dextrose in water with heparin (50 U/ml) was used as the standard.

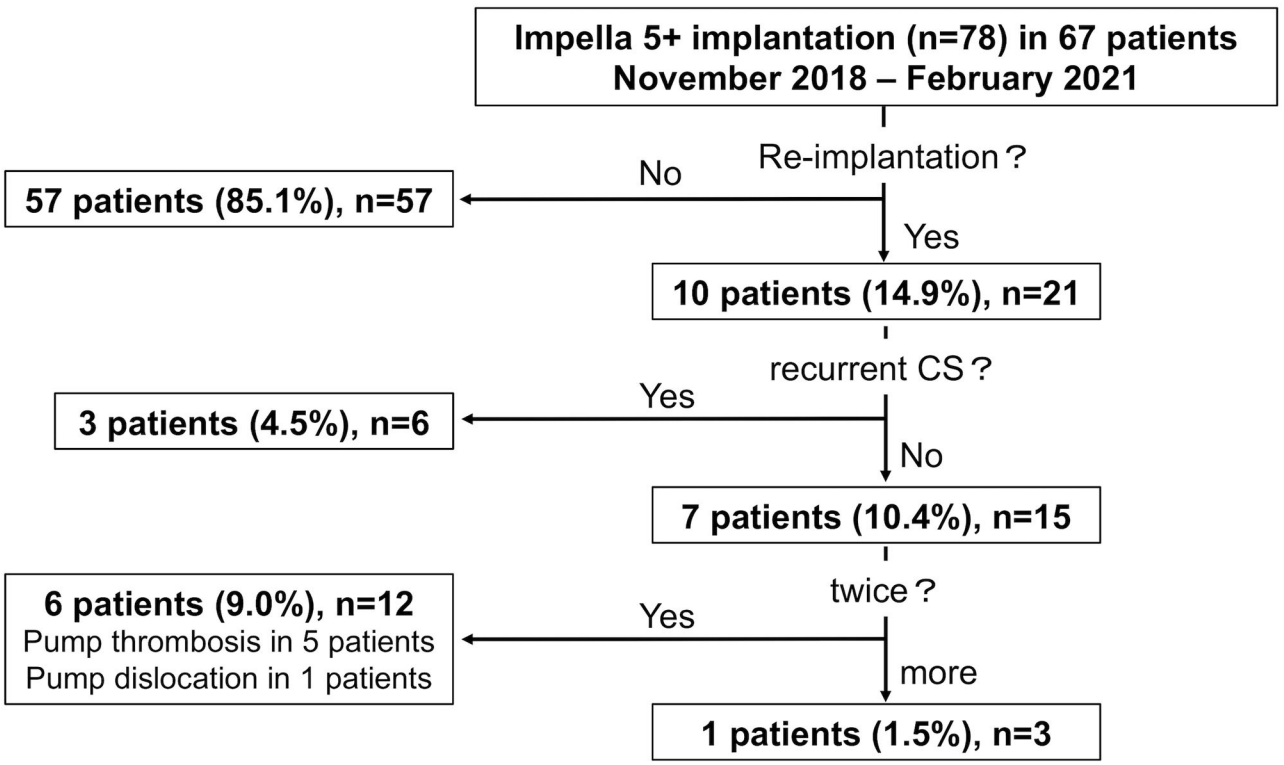

FIGURE 1 | The graphic flow chart of study population. Seventy-eight Impella 5+ (5.0 or 5.5) implantation in 67 patients. CS, cardiogenic shock.

Moreover, systemic administration of unfractionated heparin was also provided for optimal anticoagulation, with aPTT (activated partial thromboplastin time) monitored every 8 h until values became stable over 45 se. However, both anticoagulation agents were regulated appropriately in case of significant bleeding, i.e., major bleeding. In the case of heparin-induced thrombocytopeniaII, argatroban was administrated instead of heparin (10). For the evaluation of whole organs, blood gas analysis was routinely performed several times per day. The blood sampling test was also done every day.

Weaning Strategy of Temporary MCS

Weaning of temporary MCS was conducted in those patients who experienced a minimal level of cardiopulmonary recovery. The latter level of cardiopulmonary recovery was defined as cardiac index (CI) ≥2.2 l/min with a stable or less than moderate catecholamine support and an improved organ perfusion, declining core laboratory parameters (i.e., lactate, transaminase, and creatinine). In the setting of ECMELLA, generally the weaning of venous-arterial extracorporeal membrane oxygenation (va-ECMO) was prioritized over that of Impella. However, we decided an individual weaning strategy in a case-by-case manner, defining which device would be weaned first. As far as a transition to permanent MCS is concerned, we performed LVAD implantation or oHTX when 1) temporary MCS could not be weaned off, and 2) the patients were not too old (no limitation for LVAD implantation as destination therapy (our maximal age for LVAD recipients in this period was 76 years old), oHTX until 65 years), after exclusion of major neurological deficit using computed tomography (CT) and with prior informed consent given by the patients and/or their family.

The weaning of Impella 5+ was performed by gradually reducing the Impella flow setting until P2. Then, explantation of Impella 5+ was performed if patient remained hemodynamically stable. The decision of re-implantation vs. simple explantation in the setting of Impella dysfunction (i.e., exchange of Impella device) was made based on the whole clinical situation, such as persisting inappropriate hemodynamic status (CI < 2.2 l/min, more than moderate catecholamine support, missing LV ejection) following an interdisciplinary discussion.

Statistical Analysis

The statistical analyses were administrated with the Statistical Package for Social Sciences® (SPSS) 25.0 (IBM, Chicago, USA). Using this program, descriptive and comparative statistics were performed. Chi-Quadrat-Test and Odds Ratio (OR) were conducted for nominally scaled variables. However, Fisher's exact test was adapted instead of Chi-Quadrat-Test for expected values of <5. $P < 0.05$ were considered statistically significant.

RESULTS

Clinical Outcome of Impella 5+

Table 1 shows the baseline clinical characteristics of 67 consecutive patients enrolled in this study. The majority of patients were male (n = 56, 86.6%) with a mean age of 61.2 ± 11.4 years at Impella 5+ implantation. Acute coronary syndrome/ischemic cardiomyopathy (n = 55, 82.1%) is the most common underlying disease for acute CS, followed by decompensation due to dilated cardiomyopathy (DCM; n = 9, 13.4%). Adverse events associated with Impella 5+ support in all 67 patients are shown in **Table 2**. The 30-day survival was 55.2% (n = 37), of whom 5 patients were deceased in-hospital in the later course on postoperative day (POD) 32, 48 POD, 73 POD, 103 POD, and 210 POD, respectively. Thus, overall survival was 47.8% (n = 32), whereas in-hospital mortality was 52.2% (n = 35). We removed Impella 5+ from 39 patients (58.2%), of whom 22 patients (32.8%) recovered without permanent MCS, and 10 further patients (14.9%) survived after a successful transition to permanent MCS. On the other hand, 6 patients (9.0%) deceased after Impella 5+ removal, and 1 patient (1.5%) died from septic shock 102 days after combined oHTX and kidney transplantation (**Figure 2**). Interestingly, we found surgical site infection (SSI) only in this patient (1.5%), in whom revision surgery became necessary to remove the remnant Dacron prosthesis. Indeed, this patient underwent Impella 5.0 re-implantation before oHTX. Thus, SSI was found in 10% of patients who underwent re-Implantation of Impella 5+ (n = 1/10), whereas we found no SSI in patients with primary Impella support (0%; n = 0/57). Notably, an embolic event of the right arm after the removal of Impella 5+ *via* SA was observed in 3 patients (9.1%, among 33 patients who underwent the removal of Impella 5+ *via* SA), whereas 33.3% of patients with femoral Impella 5+ (n = 2, among 6 patients who underwent Impella 5+ implantation *via* FA) suffered from leg ischemia. Therapy withdrawal was performed due to cerebral vascular accidents (CVA) in 7 patients (19.4% of all mortality).

TABLE 1 | Baseline clinical characteristics.

	All patients (n = 67)		Re-Impella patients (n = 10)	
Age (y)	61.2 ± 11.4		58.3 ± 8.49	
Male, n (%)	58	(86.6)	7	(70.0)
INTERMACS profiles I, n (%)	32	(47.8)	4	(40.0)
Arterial hypertension, n (%)	40	(59.7)	2	(20.0)
Hyperlipidemia, n (%)	16	(23.9)	1	(10.0)
Diabetes, n (%)	22	(32.8)	3	(30.0)
Peripheral vascular disease, n (%)	6	(9.0)	0	(0.0)
Arrhythmia, n (%)	23	(34.3)	5	(50.0)
COPD, n (%)	5	(7.5)	1	(10.0)
Nicotine abuses, n (%)	22	(32.8)	3	(30.0)
Drug abuses, n (%)	2	(3.0)	0	(0.0)
Dialysis, n (%)	3	(4.5)	0	(0.0)
History of PCI, n (%)	20	(29.9)	5	(50.0)
Post CPR, n (%)	18	(26.9)	5	(50.0)
Biventricular failure, n (%)	38	(56.7)	4	(40.0)
ACS/ICM, n (%)	55	(82.1)	7	(70.0)
DCM, n (%)	9	(13.4)	3	(30.0)
Myocarditis, n (%)	2	(3.0)	0	(0.0)
CS after oHTX, n (%)	1	(1.5)	0	(0.0)
va-ECMO implantation, n (%)	47	(70.1)	6	(60.0)

Data documented as n (%) or mean ± standard deviation.
ACS, acute coronary syndrome; COPD, chronic obstructive pulmonary disease; CPR, cardiopulmonary resuscitation; CS, cardiogenic shock; DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; INTERMACS, interagency registry for mechanically assisted circulatory support; oHTX, orthotopic heart transplantation; PCI, percutaneous coronary intervention; va-ECMO, venous-arterial extracorporeal membrane oxygenation.

Femoral Access for Impella 5+

As described, Impella 5+ implantation was performed *via* FA only as a second choice if implantation *via* SA was deemed unfavorable, which is in line with current practice globally (11). In this sense, Impella 5+ was implanted *via* FA only in 6 patients

TABLE 2 | Clinical outcomes of Impella 5+ support focusing on adverse events.

Patients		All (n = 67)		ECMELLA (n = 47)		Solo Impella (n = 20)		p
30-day survival, n (%)		37	(55.2)	22	(46.8)	15	(75.0)	0.06
In-hospital mortality, n (%)		35	(52.2)	27	(57.4)	8	(40.0)	0.29
	MOF	25	(72.2*)	21	(77.8*)	5	(62.5*)	
	CVA	7	(19.4*)	4	(14.8*)	3	(37.5*)	
	SS/SIRS	3	(8.3*)	2	(7.4*)	1	(12.5*)	
HIT II, n (%)		6	(9.0)					
SSI, n (%)	Axillary access	1	(1.5)					
	Femoral access	0	(0.0)					
Arm embolism after removal, n (%)	Axillary access	3	(9.1**)	1	(3.0**)	2	(6.1**)	
Leg ischemia during support, n (%)	Femoral access	2	(33.3***)	2	(33.3***)	0	(0.0***)	
Re-implantation of Impella 5+, n (%)		10	(14.9)	7	(14.9)	3	(15.0)	

Data documented as n (%).
CVA, cerebral vascular accident; ECMELLA, venous-arterial extracorporeal membrane oxygenation + Impella; HIT, heparin-induced thrombocytopenia; MOF, multiple organ failure; SIRS, systemic inflammatory response syndrome; SS, septic shock; SSI, surgical site infection.
, % of all mortality in each group; **, % among 33 patients who underwent the removal of axillary Impella 5+; *, % among 6 patients who underwent Impella 5+ implantation via femoral.*

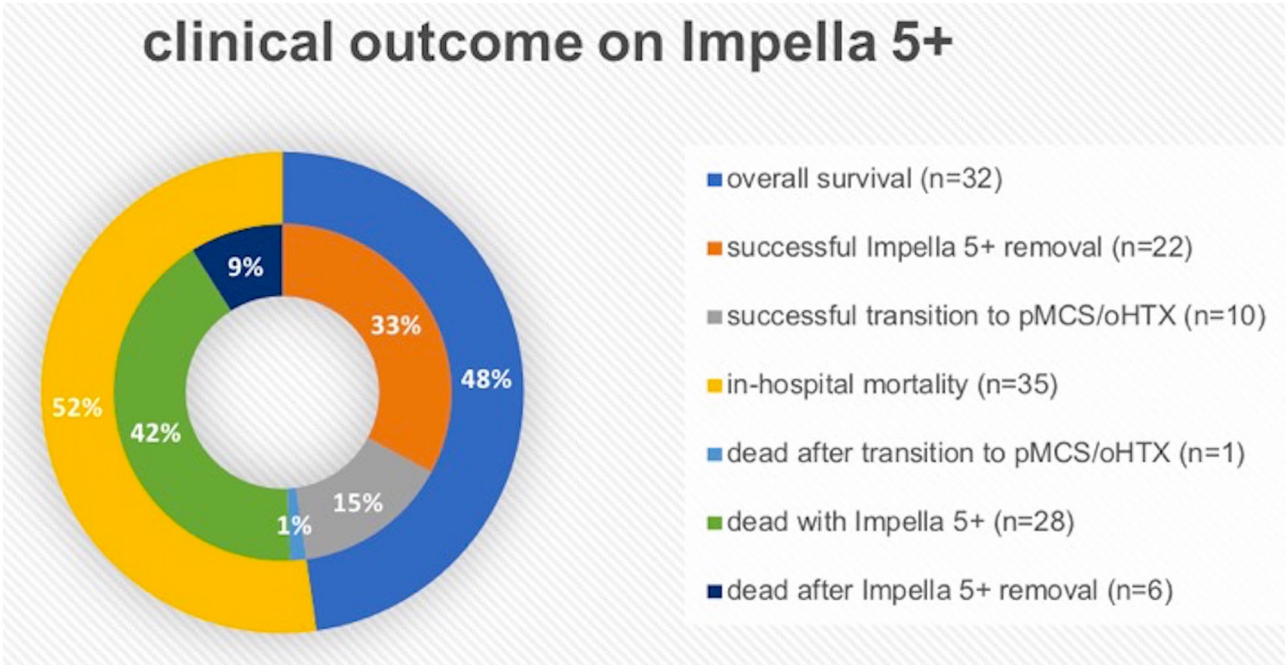

FIGURE 2 | The graphic explanation of clinical outcome on Impella 5+ (5.0 or 5.5). oHTX, orthotopic heart transplantation; pMCS, permanent mechanical circulatory support.

(9.0%) (**Table 3**). In four patients (66.7%), the implantation was converted to FA access intraoperatively. In one patient, the exchange from Impella CP to 5.0 was performed *via* the same FA. In contrast, in another patient, the implantation was performed *via* the same side of FA after open repair of FA due to massive bleeding after the attempt of punctual arterial cannulation of va-ECMO. In two patients with concomitant va-ECMO support simultaneous to FA Impella 5+, an antegrade reperfusion cannula directed to the distal leg was inserted on the side of Impella due to suspected leg ischemia (33.3%, $n = 2$). Interestingly, the mortality of the femoral Impella 5+ was tendentially higher as the group of Impella 5+ implantation *via* SA in all cohorts (FA vs. SA, 83.3% ($n = 5/6$) vs. 49.2% ($n = 30/61$), $p = 0.20$). Among only 47 ECMELLA patients, this tendency also remains (FA vs. SA, 83.3% ($n = 5/6$) vs. 41.5% ($n = 22/41$), $p = 0.22$) (**Table 4**).

Re-implantation of Impella 5+

Characteristics of patients undergoing re-implantation of Impella 5+ are presented in **Table 5**. A re-implantation of Impella 5+ was necessary in a total of 10 patients due to (1) recurrent CS ($n = 3$), (2) Impella thrombosis ($n = 5$), and (3) Impella dislocation ($n = 2$). Additionally, we observed 2 further patients with a change of left ventricular unloading therapy involving one Impella 5+, where in one patient LVAD implantation was performed as a direct transition after dislocation of Impella 5.0 (patient suppl. 1 in **Table 5**). In another patient (patient suppl. 2 in **Table 5**), Impella 5+ was upgraded from Impella CP due to the dislocation of Impella CP inserted *via* FA. Concerning Impella dysfunction including one Impella CP patient (patient suppl. 2 in **Table 5**), the FA access was a significant risk factor for Impella dysfunction compared to the SA access (FA vs. SA, 42.8% ($n = 3/7$) vs. 9.8% ($n = 6/61$), $p = 0.04$, OR 6.88, CI 1.23–38.3). This evidence was also found in the setting of ECMELLA (FA vs. SA, 42.8% ($n = 3/7$) vs. 7.3% ($n = 3/41$), $p = 0.03$, OR 9.5, CI 1.42-63.7). On the other hand, no statistical difference of overall mortality was observed in patients with Impella 5+ re-implantation ($n = 10$) compared to patients with primary Impella 5+ support ($n = 57$) (mortality 80.0% ($n = 8/10$) for ECMELLA setting vs. 47.4% ($n = 27/57$) for primary Impella setting, $p = 0.09$) (**Table 4**).

In all cases, we did not find complications associated with re-exploration of the artery or Impella re-implantation. As far as the operative technique of re-implantation of Impella 5+ is concerned, we used a new prosthesis as described above in nine patients. Only for one patient (patient 4 in **Table 5**), we used the index prosthesis for re-implantation *via* FA after removing thrombosis materials. Further, in one case (patient 10 in **Table 5**), the entire prosthesis used for previous Impella implantation was removed entirely before anastomosis of a new prosthesis.

Pump Thrombosis of Impella 5+

Details of patients with pump thrombosis are presented in **Table 6**. One patient (Case 6) did not receive an exchange of Impella due to adequate hemodynamics under ongoing va-ECMO support, and he recovered fully without permanent MCS in the further course. Thus, the prevalence of pump thrombosis was 9.0%, with 15.7 ± 8.89 days of mean support duration in this cohort. The patients of case 1 and case 4 had clinically severe

TABLE 3 | Patients profile of Impella 5+ implantation *via* the femoral artery.

Age (y)	Sex	Height (m)	Weight (kg)	Va-ECMO?	Impella size	Why not *via* SA?	Diameter of SA (mm)		Leg ischemia?	Re-implantation?	*Via* FA again?	Clinical outcome
							Pre. CT	Post. CT				
61.8	F	1.57	61	Yes	5.0	Inadequate diameter of SA	-	4.0	No	No	-	Living
60.1	M	1.74	70	Yes	5.0	Inadequate diameter of SA	-	4.9	Yes	No	-	Deceased
41.2	M	1.75	110	Yes	5.0	Inadequate diameter of SA	4.5	8.0	No	No	-	Deceased
40.7	M	1.85	100	Yes	5.0	Inadequate diameter of SA	5.8	-	Yes	No	-	Deceased
49.6	F	1.58	92	Yes	5.0	Impella change from CP to 5.0	-	-	No	Yes	Yes	Deceased
68.0	M	-	90	Yes	5.0	Opened groin; FA repair due to inguinal hemmorrage after emergent va-ECMO attempt	-	-	No	Yes	Yes	Deceased

CT, computed tomography; d, days; F, female; FA, femoral artery; M, male; post., postoperative; pre., preoperative; SA, subclavian artery; va-ECMO, venous-arterial extracorporeal membrane oxygenation; y, years; -, no available data or unnecessary information.

TABLE 4 | Representative outcomes in 47 ECMELLA patients depending on access site.

ECMELLA patients	All (*n* = 47)		Femoral access (*n* = 6)		Axillary access (*n* = 41)		p
Impella dysfunction, n (%)	5	(10.6)	3	(42.8*)	3	(7.3)	0.03
In-hospital mortality, n (%)	27	(57.4)	5	(83.3*)	22	(53.7)	0.22

*Data documented as n (%). ECMELLA, va-ECMO, venous-arterial extracorporeal membrane oxygenation + Impella; *, n (%) of 6 femoral Impella 5.0/5.5 + 1 femoral Impella CP.*

major bleeding (thoracic bleeding due to status post CPR and massive inguinal re-bleeding post-va-ECMO in case 1, bladder bleeding in case 4) so that the purge anticoagulation was changed to low dose heparin (heparin 20 U/ml) in the last few days, and systemic anticoagulation was ceased. In all cases, thrombus mass was observed as an obvious finding within the Impella pump at the time of explantation.

DISCUSSION

Although Impella has already been widely utilized for various CS situations, few reports focus on postoperative adverse events (1, 3–6). Further, clinical outcomes of Impella 5+ re-implantation have not been reported.

The key findings of this observational retrospective study are: (1) 15% re-implantation rate with 48% overall survival; (2) 9.0% incidence of pump thrombosis; (3) no complications of re-implantation procedure and a low incidence SSI when using rifampicin-incubated gelatin-coated Dacron prosthesis; (4) considerable morbidity (9.1 %) of arm embolism after the removal of Impella 5+ *via* SA and finally; (5) statistically significantly higher Impella dysfunction rate ($p = 0.04$, OR 6.88) with numerically higher mortality in the FA access sub-cohort.

In our study, pump thrombosis was the main indication for re-implantation of Impella 5+ (50%. $n = 5/10$). We do not know whether this result reflects a common range to be expected for Impella 5+ because the re-implantation rate and rate of pump thrombosis have not been well studied previously. However, according to our results, we can explain certain trends, which might affect the risk of pump thrombosis. As we know, the axial pump of the Impella system requires heparinized purge solution, which prevents blood from entering the motor as it flows through the Impella catheter. Besides, systemic anticoagulation to achieve therapeutic aPTT levels is recommended (7, 8).

Nevertheless, we often face the situation of restricting anticoagulation because of severe major bleeding, especially in patients with CS. In this sense, systemic anticoagulation, even anticoagulation administered *via* purge solution for Impella patients, sometimes becomes challenging. Depending on individual cases, we ought to decide on the ideal anticoagulation therapy. Previous studies underline the value of optimal anticoagulation therapy in a balance of prevention of thrombosis and the adverse result of major bleeding, respectively (1, 12–16).

TABLE 5 | Re-implantation of Impella 5+ in 10 patients.

Patient	Age (y)	Sex	Size of prev. Impella	Duration of prev. Impella (d)	Why re-implantation?	Window period (d)	Size of new Impella	Impella access site		*Via* prev. vasc. graft?	Remove prev. graft?	New graft on prev. graft?	Clinical outcome	Cause of death
								Prev.	New					
1	72.4	F	5.0	11	Recurrent CS	6	5.0	SA	SA	No	No	Yes	Deceased	CVA
2	54.9	M	5.0	8	Recurrent CS	13	5.0	SA	FA	-	-	-	Deceased	CVA
3	52.5	M	5.0	4	Recurrent CS	203	5.0	SA	SA	No	No	Yes	Living atter oHTX	-
4	49.6	F	5.0	13	Pump thrombosis	-	5.0	FA	FA	Yes	No	No	Deceased	SS
5	64.1	M	5.0	5	Pump thrombosis	-	5.0	SA	SA	No	No	Yes	Deceased	SS
6	60.7	M	5.0	27	Pump thrombosis	-	5.0	SA	SA	No	No	Yes	Living after LVAD	-
7	59.8	M	5.0	26	Pump thrombosis	-	5.5	SA	SA	No	No	Yes	Deceased After oHTX	SS
8	56.5	M	5.5	13	Pump thrombosis	-	5.0	SA	SA	No	No	Yes	Deceased	MOF
9	68.0	M	5.0	5	Pump dislocation	-	5.0	FA	FA	No	No	Yes	Deceased	MOF
10	44.5	F	5.0	39	Upgrade of Impella	-	5.5	SA	SA	No	Yes	No	Deceased	CVA
			5.5	28	Pump dislocation	-	5.0	SA	SA	No	No	Yes		
Suppl. 1	37.4	M	5.5	6	Pump dislocation	-	LVAD	SA	-	-	-	-	Living after LVAD	-
Suppl. 2	34.5	M	CP	1	Pump dislocation	-	5.0	FA	SA	-	-	-	Living afer oHTX	-

CP, Impella CP; CS, cardiogenic shock; CVA, cerebral vascular accident; d, days; F, female; FA, femoral artery; LVAD, left ventricular assist device; M, male; prev., previous; MOF, multiple organ failure; oHTX, orthotopic heart transplantation; SA, subclavian artery; SS, septic shock; suppl., supplemental; vasc., vascular; y, years; -, no available data or unnecessary information; 5+, 5.0 or 5.5.

TABLE 6 | Clinical characteristics of 6 patients at the timing of pump thrombosis.

Patient Nr. in Table 3	Case 1 Patient 4	Case 2 Patient 5	Case 3 Patient 6	Case 4 Patient 7	Case 5 Patient 8	Case 6
Basis diagnosis	ACS (4 year after oHTX)	DCM	ACS	ICM	DCM	Myocarditis
Impella size	5.0	5.0	5.0	5.0	5.5	5.0
Impella duration (d)	13	5	27	26	13	10
Va-ECMO ?	Termimated	No	Terminated	No	Yes	Yes
Other system ?	TandemHeart	No	No	No	No	No
Discharged ?	Yes	Yes	No	Yes	No	No
Antiplatelet medication ?	No	No	Aspirin + Clopidogrel	No	No	No
Purge anticoagulation	D5W+ heparin 20 U/ml	D5W+ heparin 50 U/ml	D5W+ heparin 50 U/ml	D5W+ heparin 20 U/ml	D5W+ heparin 50 U/ml	D5W+ argatroban 0.09 mg/ml
aPTT within 24h (sec)	27	48	70	30	79	30
INR within 24h	1.1	1.5	2.1	1.1	1.2	1.3
Hit II	Negative	Negative	Negative	Negative	Negative	Positive
Platelets ($\times$1000 μl)	85	119	216	98	130	75
Impella setting	P4	Pump stop	P7	P8	P2	P3
Impella flow (l/min)	Transient immeasurable		4.1	4.2	1.0	0.7
Purge flow rate (ml/h)	14.0		21.6	2.3	5.0	<1
Purge pressure (mmhg)	396		543	972	639	1065

ACS, acute coronary syndrome; aPTT, activated partial thromboplastin time; d, days; DCM, dilatative cardiomyopathy; D5W, dextrose 5% in water; h, hour(s); HIT, heparin-induced thrombocytopenia; ICM, ischemic cardiomyopathy; INR, international normalized ratio; min, minute; oHTX, orthotopic heart transplantation; sec, seconds; U, units; va-ECMO, venous-arterial extracorporeal membrane oxygenation; -, no available data or unnecessary information.

Despite the manufacturer's recommendation of anticoagulation management using ACT, systemic anticoagulation has been monitored by aPTT in the majority of past studies (aPTT > 45 s).

On the contrary, regarding anticoagulation of the purge solution, a reduction down to half of the heparin concentration (heparin 25 U/ml) has been described to result in a favorable outcome with no significant rise in thrombotic events (12). In our case series, we also administered less than half of the heparin concentration recommended for purge solution (20 U/ml) for one patient who unfortunately received pump thrombosis. Indeed, this patient had no systemic anticoagulation due to massive hematuria. Certainly, in such a case with purge solution's anticoagulation reduced to levels under the recommended therapeutic level. the Impella system parameters should be monitored closely.

It has been proposed that the longer the Impella support period, the higher the risk for thrombotic event rate due to the artificial profile. The presented data demonstrated a trend supporting this hypothesis. We performed an exchange of Impella 5+ in all patients with pump thrombosis. The utilization of tissue plasminogen activator (tPA) in the purge solution (0.04 mg/ml) has been recently introduced as an alternative to heparin (17, 18). In our opinion, tPA is a valid therapy option for patients with diagnosed or suspected pump thrombosis, although a decision for initiation of tPA should be made cautiously and in an individual case-by-case fashion due to the strong fibrinolytic effect of tPA.

Regarding the dislocation of Impella 5+, we encountered three cases (4.5%) in our study cohort. Only one patient also received va-ECMO support, whereas two patients had no other MCS. Thus, re-Impella or direct LVAD implantation had to be emergently performed. Bernhardt *et al.* had reported the first experience with Impella 5.5 in Germany. They reported the incidence of dislocation in 21.7% of patients in the first generation of the device with a shorter cannula (11). Since the device length has been modified, the incidence of dislocation would be improved. Although the dislocation of Impella may represent a critical situation, it is challenging to re-insert the Impella catheter through the aortic valve without a guidewire. One interesting method employing rapid ventricular pacing has been suggested to let the aortic valve "open" (19). This technique enables repositioning the Impella catheter at the "bedside," and it would be one of the most considerable merits for patients who are at risk for hemodynamic deterioration after Impella dislocation. However, further follow-up studies are warranted.

The surgical procedure for Impella 5 + re-implantation has not yet been thoroughly discussed. Excluding one case of femoral Impella 5+, we strictly utilized a new prosthesis, so that thrombus materials in the index prosthesis could be largely removed. Given our clinical results of no complications in the re-implantation, this concept seems to be favorable. Regarding the incidence of SSI and prosthesis-associated infection, an 1.5% incidence appears to be well tolerated with the consideration of complex settings in patients with CS. Thus, we conclude that the herein applied

surgical strategy for re-implantation of Impella 5+ may be used as a standardized strategy. However, it remains unclear whether rifampicin incubation is a causative contributor to the favorable outcome in terms of infection prevention.

With respect to arm embolism after removal of axillary Impella 5+, we regard this morbidity as an unacceptable one, although we observed no CVA that was directly associated with the explantation procedure. As a matter of fact, the Impella pump has been sometimes explanted without clamping of the distal artery in our previous series. Since we observed distal embolism after removing Impella in a few patients, we modified our standard procedure; recently, we clamped distal SA for embolic protection and sometimes performed the prophylactical thrombectomy using a Fogarty catheter. This technique was been already reported by Kawabori et al. (20). We regard this technique as a reasonable approach for the safe removal of Impella catheter.

In this cohort, the adverse outcomes of the femoral approach for Impella 5+ were significant. As we performed Impella 5+ implantation *via* FA as the second option, a direct comparison of the two access routes is not possible. However, our inferior outcomes of femoral Impella 5+, i.e., high incidence of leg ischemia, numerically higher mortality, and statistically higher morbidity of Impella dysfunction give us a word of caution and warrants high levels of awareness for early signs of complications when managing patients with femoral Impella 5+ support.

CONCLUSION

Our results suggest relevant rate of re-implantation (14.9%, $n = 10$) and considerable prevalence of pump thrombosis (9.0%, $n = 6$) in patients receiving Impella 5+. Re-implantation was safely feasible *via* the same access route. Particular attention is warranted regarding some complications, especially in FA access with potential impact on overall mortality.

LIMITATIONS

There are several limitations to this study. First, this manuscript deals with a retrospective observational study with a limited cohort size of non-randomized patients at a single center. Potential systematic measurement errors can affect the outcomes. Secondly, because of the limited cohort size in this study, our patients were analyzed as homogeneous patients with CS with no regard for baseline patients' characteristics and therapy variety, despite patients' heterogeneous backgrounds. Generally, the descriptive analysis of target patients is necessary to analyze more details of the study's main purpose. Further, long-term outcomes are missing in this study. We should evaluate patients with CS in the long observation period, which can provide us further insights into the clinical outcomes associated with Impella 5+ support.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The Ethic Committee in University Hospital Düsseldorf approved this retrospective study (Ref.2020-1173). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Conceptualization and validation: YS and PA. Methodology, software, formal analysis, investigation, resources, data curation, writing—original draft preparation, and visualization: YS. Writing—review and editing: SB, MI, AM, PR, RW, HA, UB, AL, and PA. Supervision and project administration: AL and PA. All authors have read and agreed to the published version of the manuscript.

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Impact of Extracorporeal Membrane Oxygenation on Right Ventricular Function After Heart Transplantation

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Aims: Acute right ventricular failure remains a common challenging clinical syndrome in heart transplant (HTx) recipients. While extracorporeal membrane oxygenation (ECMO) is a proven strategy for the treatment of this condition, the outcomes after weaning and during follow up remain understudied. We aimed to evaluate the right-sided heart function in ECMO survivors following HTx.

Methods: Between September 2005 and December 2019, 205 patients with end-stage heart failure who underwent standard orthotopic HTx were enrolled. In total, 68 (33.2%) patients were included in the ECMO group and 137 (66.8%) patients were included in the non-ECMO group.

Results: Of the 68 patients in the ECMO group, 42 (61.8%) were successfully weaned from ECMO. After a median follow-up period of 53 months, there were 25 (59.5%) and 27 (23.7%) deaths in the ECMO and non-ECMO groups ($P = 0.023$), respectively. Systolic pulmonary artery pressure (SPAP) before discharge ($P = 0.003$) was the unique predictor of all-cause mortality during follow up. Meanwhile, patients in the ECMO group with more than moderate SPAP increase before discharge had higher mortality than patients in the non-ECMO group without such increase ($P = 0.005$).

Conclusions: Recipient right-sided heart characteristics were strong predictors of ECMO need after HTx. ECMO patients had high mortality in the perioperative and follow-up periods, and the changes in right ventricular function in ECMO patients may be associated with pulmonary vessel injury before and after HTx.

Keywords: extracorporeal membrane oxygenation, cardiopulmonary bypass, heart transplantation, heart failure, right ventricular function

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INTRODUCTION

Heart transplantation (HTx) is the gold-standard treatment for end-stage heart failure (HF) (1). The most recent data from the registry of the International Society of Heart and Lung Transplantation (ISHLT) showed 1- and 5-year survival rates of 84.5 and 72.5%, respectively. ISHLT registry data show that acute right ventricular (RV) failure remains a difficult and common clinical syndrome in transplant recipients and is associated with 50% of all cardiac complications and 19% of all early deaths after HTx (2). Increased pulmonary vascular resistance and ischemia-reperfusion

injury of the myocardium are well-known risk factors for acute RV failure in HTx recipients (3). RV failure results in dilation, ischemia, and decreased contractility. Decreased pulmonary blood flow and leftward septal shift subsequently lead to lower left ventricular (LV) filling and low output syndrome (LOS). Despite multiple medical treatments, some patients cannot be weaned from cardiopulmonary bypass (CPB).

Extracorporeal membrane oxygenation (ECMO) is a proven strategy for the treatment of patients with LOS after HTx (4); it helps support organ system function in these patients during critical periods and allows the newly transplanted heart to work under less stress and eventually recover from the combined shock caused by ischemia-reperfusion injury and exposure to a previously unknown preload (5).

Considering short-term outcomes, studies have indicated that 45–80% of patients can be considered "ECMO survivors." However, the changes in pulmonary artery pressure (PAP) and RV function during follow up in ECMO survivors remain unknown. This study aimed to evaluate RV function in ECMO survivors after HTx during follow up.

METHODS

Study Population

Between September 2005 and December 2019, 242 patients with end-stage HF underwent standard orthotopic HTx (bi-atrial) at Anzhen Hospital. The indications followed the ISHLT guidelines for HTx. Patients with cardiac surgeries or re-transplantation, heart-lung transplantation or heart-kidney transplantation, and ECMO assistance before HTx were excluded. Sixty-eight of the remaining 205 patients that received ECMO after HTx for LOS were included in the ECMO group, while 137 were included in the non-ECMO group. All patients admitted to Anzhen Hospital provided written consent at hospital admission.

ECMO Procedures

According to the ISHLT guidelines, mechanical circulatory support should be initiated early if weaning from CPB after HTx fails. The decision to use VA-ECMO was made by the experienced heart transplantation team including a cardiac surgeon and intensivist. Indications for VA-ECMO therapy included difficulty weaning from CPB (44 patients, 64.7%) or postoperative refractory cardiogenic shock despite adequate volumes and high doses of inotropes (24 patients, 35.3%), such as norepinephrine, dobutamine, epinephrine, and milrinone. All procedures were performed by trained ECMO team members. Patients were evaluated daily for hemodynamic improvement and the possibility of weaning from circulatory support. Clinical and echocardiographic variables were serially assessed to determine if myocardial recovery occurred, and ECMO weaning was performed in patients who fulfilled our institutional weaning criteria and passed an ECMO weaning trial consisting of tolerance to decreasing and clamping ECMO flow. The protocol for ECMO used in our center has been previously described (6).

Echocardiography Assessment

Standard echocardiography was performed using commercially available equipment. Cardiac morphology was assessed using diameter and area measurements in standard 4- and 2-chamber views. The LV ejection fraction was calculated using the biplane Simpson method. Valve regurgitation was evaluated using color Doppler flow imaging and was graded as none, mild, mild to moderate, moderate, moderate to severe, and severe according to current guidelines (7, 8). Systolic PAP (SPAP) was calculated by adding the peak tricuspid regurgitation systolic gradient to the estimated central venous pressure. Further, RV function was quantified by fractional area change (FAC), tricuspid annular peak systolic excursion (TAPSE), Tei index of the RV, and tricuspid annulus systolic velocity (S'). FAC was measured via 2-dimensional planimetry in the 4-chamber view, while TAPSE and S' were evaluated using tissue Doppler imaging. The Tei index was evaluated using pulse Doppler imaging.

Follow Up

Postoperatively, all patients visited every 30 days in the first 6 months, every 60 days from 7 to 12 months, and every 6 months thereafter. The patients were invited for history-taking, physical examination, and echocardiographic evaluation during each visit. Major clinical events included all-cause deaths and major HTx complications such as rejection, infection, liver/kidney dysfunction, thrombus, hemorrhage.

Conventional triple-drug immunosuppressive therapy was administered to all recipients, including corticosteroids, calcineurin inhibitors, and antiproliferative agents, which were routinely used during follow up and approved by the guidelines.

Statistics

Baseline variables are represented as the median and interquartile range (IQR) for continuous variables and as percentages for categorical variables. The chi-squared and Kruskal–Wallis tests were used to analyze unadjusted associations between treatment variables and outcomes. Logistic regression was used to identify the risk factors for ECMO. Covariates were included in the multivariate logistic regression when their log-rank *P*-values were <0.2. Logistic regression with inverse probability for treatment weighting (IPTW), was used to identify the risk factors for perioperative all-cause death. Doubly robust estimates were used if imbalance still existed after adjusting for baseline variables, which was also defined as augmented inverse propensity weighting (AIPW). Similar covariate adjustments were also used in the Kaplan–Meier analysis and Cox regression to determine all-cause mortality during follow up. R version 3.5.2 was used for all statistical analyses, with the twang R package.

Ethics Approval

Ethics approval for this study was obtained from the Research Ethics Committee of Beijing Anzhen Hospital, Capital Medical University, Beijing, China (IRB approval no.: 2021096X, approval date: July 6, 2020).

TABLE 1 | Baseline characteristics.

	ECMO group ($n = 68$)	Non-ECMO Group ($n = 137$)	*P*
Recipients' characteristics at hospital admission			
Age, year (IQR)	49.0 (38.5–55.0)	49.0 (38.0–56.0)	0.661
BMI, (IQR)	23.5 (21.0–27.5)	23.4 (20.6–25.5)	0.477
Male sex, *n* (%)	58 (85.3)	104 (75.9)	0.120
Hypertension, *n* (%)	4 (5.9)	8 (5.8)	0.990
Diabetes mellitus, *n* (%)	9 (13.2)	26 (19.0)	0.304
Hypercholesterolemia, *n* (%)	5 (7.4)	15 (10.9)	0.414
Liver/kidney failure, *n* (%)	2 (2.9)	2 (2.9)	0.743
DCM, *n* (%)	51 (75.0)	96 (70.1)	0.461
Others*, *n* (%)	17 (25.0)	41 (29.9)	0.461
NYHA class, (IQR)	4 (3,4)	4 (3,4)	0.548
Recipients' laboratory and echocardiographic characteristics before HTx			
AST, U/L (IQR)	24.6 (22.4–26.6)	24.4 (21.3–27.4)	0.861
ALT, U/L (IQR)	23.4 (20.4–25.7)	23.1 (20.7–25.6)	0.929
TBIL, μmol/L (IQR)	17.4 (15.2–19.2)	17.2 (15.6–18.8)	0.623
DBIL, μmol/L (IQR)	6.16 ± 1.44	6.41 ± 1.27	0.223
CREA, μmol/L (IQR)	80.5 (66.3–96.8)	86.0 (71.0–94.0)	0.776
BNP, pg/ml (IQR)	1469.3 (523.5–2010.5)	1410.3 (511.5–2291.8)	0.654
EF, % (IQR)	27.0 (20.3–31.5)	27.0 (21.0–33.0)	0.496
SPAP, mmHg (IQR)	49.0 (44.3–58.8)	42.2 (33.0–51.0)	**<0.001**
PV, cm/s (IQR)	75.0 (63.0–87.5)	69.0 (55.0–81.2)	0.097
AV, cm/s (IQR)	102.5 (78.5–128.3)	98.0 (75.5–110.0)	0.186
LA diameter, mm, median (IQR)	50.0 (46.0–53.8)	47.0 (42.0–52.0)	**0.013**
RA diameter, mm (IQR)	54.0 (48.3–54.8)	47.0 (42.8–52.0)	**<0.001**
RV diameter, mm (IQR)	30.0 (25.2–34.6)	26.3 (22.0–30.0)	**<0.001**
RVOT, mm (IQR)	36.0 (32.3–38.8)	32.0 (28.0–35.1)	**<0.001**
TR (≥moderate), *n* (%)	57 (83.8)	99 (72.3)	0.068
Donors' information			
Donor's age, y, median (IQR)	31.5 (28.3–31.5)	31.5 (28.0–35.5)	0.503
Donor's weight, kg (IQR)	68.1 (61.5–74.5)	66.5 (60.0–75.0)	0.468
Donors male sex, *n* (%)	62 (91.2)	126 (92.0)	0.846
Surgical data			
Ischemic time, min (IQR)	365.3 (312.3–413.5)	328.0 (258.0–372.0)	**0.026**
CPB time, min (IQR)	173.9 (136.3–204.5)	148.0 (131.0–170.5)	**0.003**

Categorical data are reported as n (%) and continuous data are reported as mean ± SD or median (IQR). P -value: $\chi 2$ test with exact method for categorical variables and t -test or Mann-Whitney U test for continuous variables. P < 0.05 indicates significant statistical difference (bold values).

BMI, body mass index; DCM, dilated cardiomyopathy; CPB time, cardiopulmonary bypass; AST, aspartate transaminase; ALT, alanine aminotransferase; TBIL, serum total bilirubin; DBIL, serum direct bilirubin; CREA, serum creatinine; BNP, serum type B natriuretic peptide; EF, ejection fraction; SPAP, systolic pressure of pulmonary artery; PV, pulmonary valvular velocity; AV, aortic valvular velocity; LA, left atrial; RA, right atrial; RV, right ventricle; RVOT, right ventricular outflow tract; TR, tricuspid regurgitation.

Others including: VHD, 6 (8.8%) patients in ECMO group and 6 (4.4%) patients in non-ECMO group; CAD, 7 (10.3%) patients in ECMO group and 25 (18.2%) patients in non-ECMO group; CHD, 2 (2.9%) patients in ECMO group and 5 (3.6%) patients in non-ECMO group; RCM, 2 (2.9%) patients in ECMO group and 1 (0.7%) patient in non-ECMO group; Tumor, 4 (2.9%) in non-ECMO group.

RESULTS

Baseline Characteristics

Table 1 outlines the baseline characteristics of the recipients and donors, surgical data, and the recipients' echocardiographic data before HTx. The ischemic ($P = 0.026$) and CPB ($P = 0.003$) times were significantly higher in the ECMO group than in the non-ECMO group. The ECMO group had higher SPAP, left atrial diameter, right atrial diameter, RV diameter, and right ventricular outflow tract (RVOT) diameter in the preoperative echocardiographic evaluation ($P < 0.05$).

ECMO Outcomes

ECMO cannulation was femoral (arterial)-femoral (vein) for all patients. Of the 68 patients in the ECMO group, 42 (61.8%)

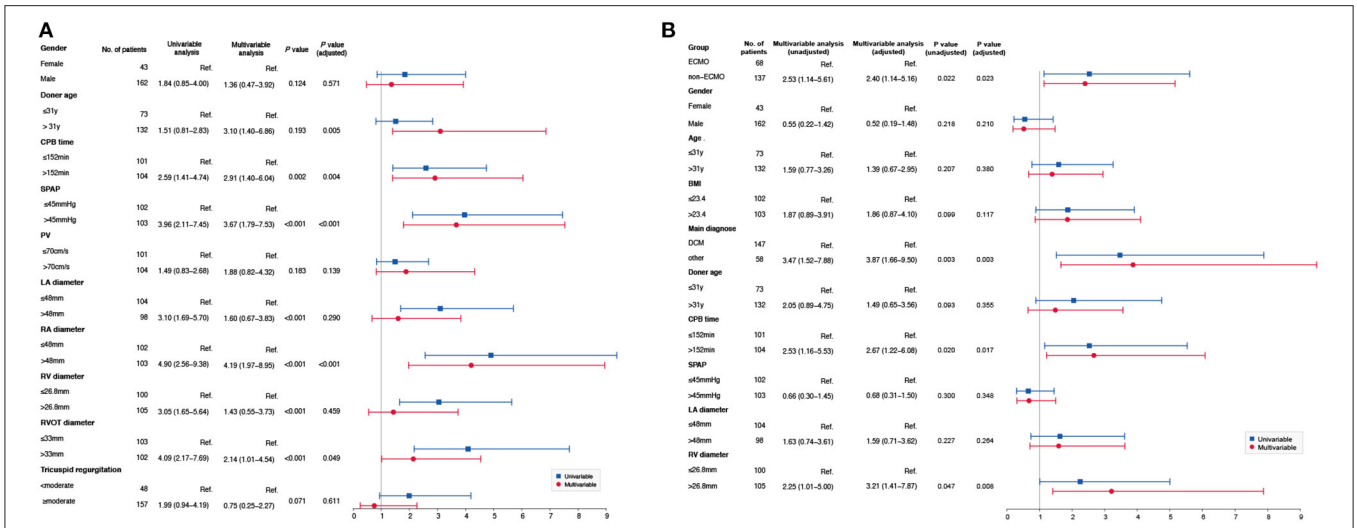

FIGURE 1 | (A) Risk factors for ECMO. Multivariate logistic regression analysis showed that donor age (OR 3.10, 95%CI 1.40–6.86, $P = 0.005$), CPB time (OR 2.91, 95%CI 1.40–6.04, $P = 0.004$), SPAP (OR 3.67, 95%CI 1.79–7.53, $P < 0.001$), RA diameter (OR 4.19, 95%CI 1.97–8.95, $P < 0.001$), and RVOT diameter (OR 2.13, 95%CI 1.01–4.54, $P = 0.049$) were risk factors for ECMO use. **(B)** Risk factors for perioperative death. Based on the baseline characteristics, multivariate logistic regression analysis showed that ECMO group (OR 2.53, 95%CI 1.14–5.61, $P = 0.022$), dilated cardiomyopathy (OR 3.47, 95%CI 1.52–7.88, $P = 0.003$), CPB time (OR 2.53, 95%CI 1.16–5.53, $P = 0.020$) and RV diameter (OR 2.25 95%CI 1.01–5.00, $P = 0.047$) were risk factors for perioperative death before AIPW. However, dilated cardiomyopathy (OR 3.87, 95%CI 1.66–9.50, $P = 0.003$), CPB time (OR 2.67, 95%CI 1.22–6.08, $P = 0.017$), and RV diameter (OR 3.21, 95%CI 1.41–7.87, $P = 0.008$) were risk factors for perioperative death after AIPW.

were successfully weaned from ECMO (median duration, 4.7 days [IQR 3–6]), and 26 (38.2%) died while on ECMO support. Forty (95.2%) patients in the ECMO group who were successfully weaned survived the perioperative period and were discharged from the hospital.

Multivariate logistic regression analysis showed that donor age (odds ratio [OR] 3.100, 95% confidence interval [CI] 1.40–6.86, $P = 0.005$), CPB time (OR 2.912, 95% CI 1.40–6.04, $P = 0.004$), SPAP (OR 3.672, 95% CI 1.79–7.53, $P < 0.001$), RA diameter (OR 4.196, 95% CI 1.97–8.95, $P < 0.001$), and RVOT diameter (OR 2.135, 95% CI 1.01–4.54, $P = 0.049$) were risk factors for ECMO use (**Figure 1A**).

Perioperative Death and Complications

Of the 205 patients, 51 (24.9%) died during the perioperative period, including 28 (41.2%) and 23 (16.8%) in the ECMO and non-ECMO groups, respectively. **Table 2** details the perioperative deaths and complications.

The ECMO group had a higher mortality rate ($P < 0.001$, 95% CI 0.18–0.45) and a higher proportion of deaths due to liver or kidney dysfunction ($P = 0.003$, 95% CI 0.12–0.56), while there was no significant difference in other causes of death between the groups.

For the overall population, multivariate logistic regression analysis showed that ECMO (OR 2.400, 95% CI 1.14–5.16, $P = 0.023$), CPB time (OR 2.670, 95% CI 1.22–6.08, $P = 0.017$), and RV diameter (OR 3.210, 95% CI 1.41–7.87, $P = 0.008$) were risk factors for perioperative death after adjustment (**Figure 1B**).

The incidence of rejection (13 patients, 50.0%) and liver/kidney dysfunction (10 patients, 38.5%) was higher in the perioperative period in the ECMO group; a higher incidence of rejection (13 patients, 56.5%) was also observed in the non-ECMO group. A higher incidence of postoperative hemorrhage ($P < 0.001$), embolism ($P = 0.014$), infection ($P = 0.004$), and liver/kidney dysfunction ($P < 0.001$) in the perioperative period was observed in the ECMO group (**Table 2**).

TABLE 2 | Perioperative deaths and complications.

	ECMO ($n = 68$)	Non-ECMO ($n = 137$)
Perioperative death, *n* (%)	**26 (38.2 of total)**	**23 (16.8 of total)**
Rejection, *n* (%)	13 (50.0 of death)	13 (56.5 of death)
Infection, *n* (%)	3 (11.5 of death)	2 (8.7 of death)
Liver/Kidney failure, *n* (%)	10 (38.5 of death)	1 (4.3 of death)
Other, *n* (%)	1 (3.8 of death)	7 (30.4 of death)
Perioperative complications		
Hemorrhage, *n* (%)	28 (41.2 of total)	5 (3.6 of total)
Embolism, *n* (%)	7 (10.3 of total)	1 (0.7 of total)
Infection, *n* (%)	10 (14.7 of total)	2 (1.5 of total)
Liver/Kidney failure, *n* (%)	22 (32.4 of total)	7 (5.1 of total)
Neurological, *n* (%)	5 (7.4 of total)	3 (2.2 of total)

Other perioperative death reasons contained gastrointestinal hemorrhage, cerebral hemorrhage, cerebral infarction, pulmonary embolism, and unexplained sudden death.

Follow Up

After a median follow up of 53 months [IQR 24–105], 25 (59.5%) and 27 (23.7%) deaths occurred in the ECMO and non-ECMO groups, respectively. One year after discharge, 142 (91.0%, 95% CI 0.86–0.95) patients survived, comprising 37 (88.1%, 95% CI

TABLE 3 | Complications during follow-up.

	Total ($n = 89$)	ECMO ($n = 15$)	non-ECMO ($n = 74$)	*P*
Infection, *n* (%)	25 (28.1)	5 (33.3)	20 (27.0)	0.625
Fungal, *n* (%)	6 (6.7)	2 (13.3)	4 (5.4)	0.265*
Bacterial, *n* (%)	8 (9.0)	3 (20.0)	5 (6.8)	0.254
Viral, *n* (%)	7 (7.9)	0 (0)	7 (9.5)	0.475
Cerebrovascular diseases, *n* (%)	6 (6.7)	0 (0)	6 (8.1)	**0.013**
Thrombus, *n* (%)	6 (6.7)	3 (20.0)	3 (4.1)	0.165
Hemorrhage, *n* (%)	2 (2.2)	0 (0)	2 (2.7)	0.525*
Hepatic failure, *n* (%)	2 (2.2)	1 (6.7)	1 (1.4)	0.447*
Renal failure, *n* (%)	5 (5.6)	1 (6.7)	4 (5.4)	0.849
CKD, *n* (%)	19 (21.3)	1 (6.7)	18 (24.3)	**0.042**
CAV, *n* (%)	1 (1.1)	1 (6.7)	0 (0)	0.334*
HBP after HTx, *n* (%)	33 (37.1)	4 (26.7)	29 (39.2)	0.351
DM after HTx, *n* (%)	21 (23.6)	4 (26.7)	17 (23.0)	0.762
Tumor, *n* (%)	1 (1.1)	1 (6.7)	0 (0)	0.334*

*Categorical data are reported as n (%). P-value: χ^2 test with exact method for categorical variables and t-test or Mann-Whitney U test for continuous variables. $P < 0.05$ indicates significant statistical difference (bold values). CKD, chronic renal dysfunction; CAV, cardiac allograft vasculopathy; HBP, hypertension; HTx, heart transplantation; DM, diabetes mellitus. *Fisher exact test.*

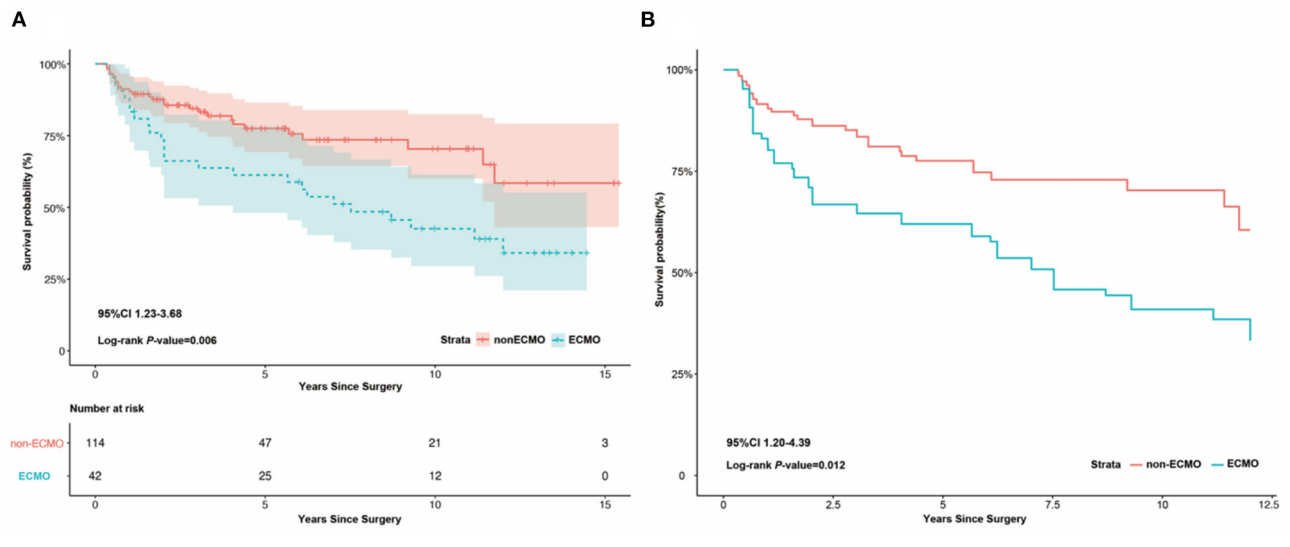

FIGURE 2 | Kaplan-Meier survival analysis between ECMO and non-ECMO group after discharge. **(A)** Kaplan-Meier survival analysis between ECMO and non-ECMO group after discharge, showing the ECMO group had a higher mortality than the non-ECMO group before adjust (log-rank $P = 0.006$, 95%CI 1.23–3.68). **(B)** Kaplan-Meier survival analysis between ECMO and non-ECMO group after discharge, showing the ECMO group had a higher mortality than the non-ECMO group after adjust (log-rank $P = 0.012$, 95%CI 1.20–4.39).

0.79–0.99) patients in the ECMO group and 105 (92.1%, 95% CI 0.82–0.97) patients in the non-ECMO group.

Five years after discharge, 119 (76.3%, 95% CI 0.66–0.81) patients survived, comprising 26 (65.0%, 95% CI 0.79–0.99) patients in the ECMO group and 93 (81.6%, 95% CI 0.82–0.97) in the non-ECMO group. The ECMO group had higher mortality before (OR 2.123, 95% CI 1.23–3.68, $P = 0.006$) and after (OR 2.295, 95% CI 1.20–4.39, $P = 0.012$) adjustment (**Figure 2**).

Rejection (38.5%), liver/kidney failure (25.0%), and infection (21.2%) were the main causes of death during follow up. Details of the complications during follow up were shown in **Table 3**.

Echocardiographic Evaluation

The results of echocardiographic evaluations, including the results of discharge and the latest follow-up results, were compared. Patients with the most recent echocardiographic

TABLE 4 | Latest echocardiography results.

	ECMO ($n = 37$)	Non-ECMO ($n = 97$)	*P*
EF, % (IQR)	66.0 (63.5–68.5)	63.0 (60.3–69.0)	0.693
LA diameter, mm (IQR)	38.1 (35.0–41.0)	40.0 (34.3–45.0)	0.388
RA diameter, mm (IQR)	40.0 (34.0–43.5)	35.8 (32.0–40.0)	0.094
RV diameter, mm (IQR)	23.0 (20.5–27.7)	20.8 (19.0–22.0)	**0.004**
RVOT diameter, mm (IQR)	29.0 (26.5–32.2)	26.2 (24.0–28.0)	**0.028**
FAC, % (IQR)	39.3 (31.6–49.1)	40.3 (35.6–45.4)	0.844
S', mm/s (IQR)	10.7 (9.2–11.0)	9.9 (9.1–11.3)	0.415
TAPSE, mm (IQR)	17.0 (16.0–20.9)	16.4 (15.0–17.9)	0.480
RV Tei index, (IQR)	0.46 (0.44–0.51)	0.43 (0.38–0.47)	**0.018**
SPAP, mmHg (IQR)	25.0 (25.0–30.0)	27.8 (25.0–30.0)	0.803
PAD, mm (IQR)	23.0 (22.0–24.5)	22.4 (21.0–24.0)	0.276
TR(≥moderate), *n* (%)	2 (5.4%)	7 (7.2%)	0.771

P-value: χ^2 test with exact method for categorical variables and t-test or Mann-Whitney U test for continuous variables.
P < 0.05 indicates significant statistical difference. (bold values)
EF, ejection fraction; LA, left atrial; RA, right atrial; RV, right ventricle; RVOT, right ventricular outflow tract; FAC, fractional area change; S', tricuspid annulus systolic velocity; TAPSE, tricuspid annular peak systolic excursion; PAD, pulmonary artery diameter; SPAP, systolic pressure of pulmonary artery; TR, tricuspid regurgitation.

evaluations accounted for 88.2 and 85.1% in the ECMO and non-ECMO groups, respectively. Some differences were observed at discharge between the groups, and these differences did not change over time during follow up; the mean SPAP of both groups notably declined in the latest echocardiographic evaluations compared to the discharge characteristics (ECMO group $P = 0.008$, non-ECMO group $P = 0.035$). The ECMO group also showed a significant improvement in tricuspid regurgitation ($P = 0.012$). However, this was not observed in the non-ECMO group. Horizontal comparisons were also performed between the groups during the same period. In terms of morphology, patients in the ECMO group had a larger RV diameter in both the discharge ($P = 0.002$) and latest ($P = 0.004$) echocardiographic evaluations.

SPAP and RV Function

Although there was no significant difference in other quantitative indicators used to evaluate RV function, the Tei index was significantly different ($P = 0.018$) in the latest echocardiographic evaluations between the groups. The ECMO group had a higher Tei index than the non-ECMO group. Details of the echocardiographic evaluations are shown in **Table 4**.

Patients were divided into subgroups according to the SPAP before discharge (whether it increased more than the moderate standard of ≥50 mmHg). According to the multivariate Cox regression analysis, graft ischemic time ($P = 0.025$, HR = 1.004, 95% CI 1.000–1.007) and a more than moderate standard increase in SPAP (≥50 mmHg) before discharge ($P = 0.002$, HR = 4.316, 95% CI 1.683–11.067) were strong predictors of all-cause mortality during follow up before AIPW. A more than moderate standard increase in SPAP (≥50 mmHg) before discharge ($P = 0.003$, HR = 3.347, 95% CI 1.517–7.381) was the unique predictor of all-cause mortality after AIPW. Kaplan–Meier survival analysis was also performed; patients in the ECMO group with a more than moderate SPAP increase had higher mortality than patients in the non-ECMO group without a more than moderate SPAP increase ($P = 0.005$, $X^2 = 8.010$) (**Figure 3**).

DISCUSSION

This study primarily showed that right-sided cardiac dysfunction in HTx recipients was a strong predictor of ECMO after HTx. Moreover, the ECMO group had significantly higher mortality than the non-ECMO group during the perioperative and follow-up periods, which may be associated with SPAP increase before discharge.

HTx is the gold standard treatment for end-stage HF (1). The most recent data from the ISHLT registry indicate that the median survival after HTx was 14.8 years (9). However, the patients in our study had a median survival of 12.0 years. The lower survival time in our study may be related to imperfect bridging before HTx in our country. As donor organs are limited, patients are often on ventricular assist device (VAD) support before receiving HTx in developed countries. Effective VAD bridging is associated with reduction in both perioperative complications and mortality in patients after HTx, especially in acute settings or under conditions of cardiac graft shortage (10, 11). Unfortunately, this alternative option has not been used in China during the last 20 years because VADs were not available until 2017 in the Chinese market. Simultaneously, owing to the unbalanced economic and educational development in our country, some patients had severe HF at hospital admission. The above reasons contributed to the lower survival time observed in our study.

LOS remains a common complication in patients early after HTx (12), and the incidence of LOS is reported to exceed 20% (13, 14). ECMO is a proven strategy for the treatment of patients with LOS (5). Our previous study showed that ECMO is acceptable for treating postcardiotomy cardiogenic shock in patients undergoing valvular surgery and was associated with good long-term outcomes in hospital survivors (6). According to the ISHLT guidelines for the management of HTx recipients, ECMO is the preferred mechanical circulatory support in patients with RV failure after HTx (15). Similarly, in this study, ECMO improved the hemodynamics of 68 of 205 (33.2%) patients. Despite growing evidence supporting ECMO use following cardiovascular surgery (4), the outcomes remain poor (16). In our study, 26 (38.2%) patients died while on ECMO support and 40 (58.8%) patients survived 30 days after ECMO implantation; this was similar to the findings of Bartko et al. where 14% of the patients died while on ECMO support and 46% died within 30 days after ECMO implantation (17).

Ischemia-reperfusion injury of the myocardium associated with graft preservation and pre-existing increased pulmonary vessel resistance are the two main causes of right-sided heart dysfunction in HTx patients postoperatively (18). In our study, CPB time, SPAP, and the pre-intervention anatomical diameters

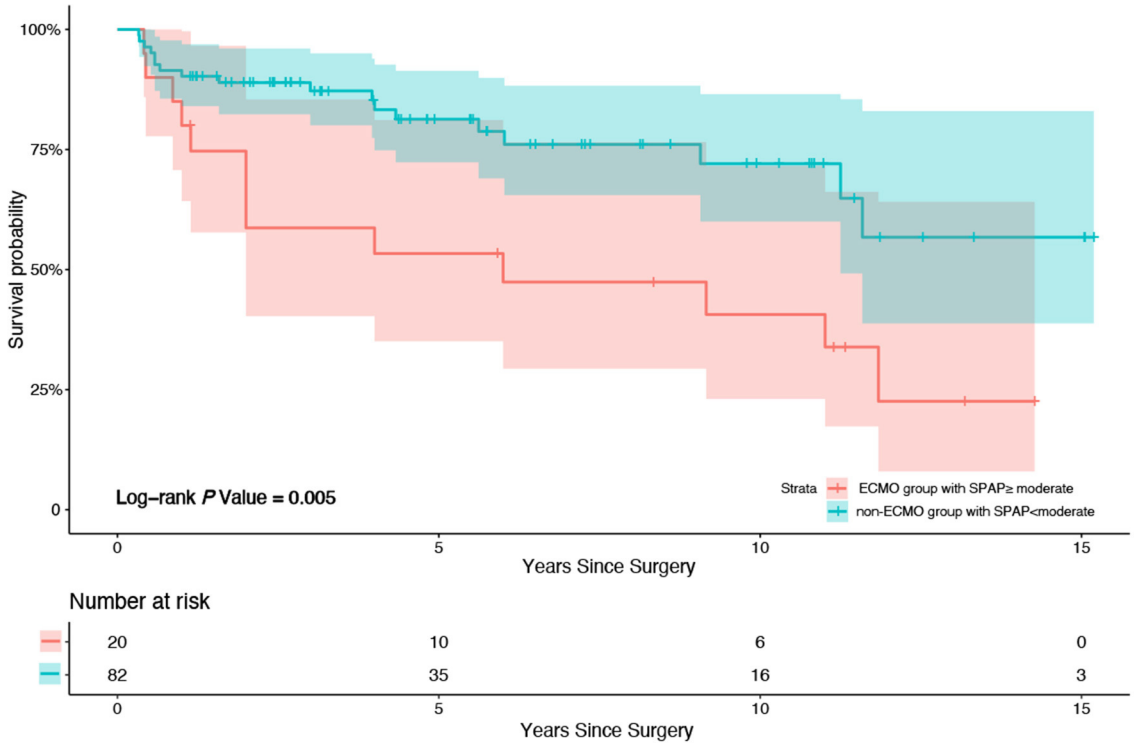

FIGURE 3 | Kaplan-Meier survival analysis between patients in ECMO group with SPAP increased more than moderate (50 mmHg) after HTx before discharge from hospital and patients in non-ECMO group without SPAP increased more than moderate (50 mmHg) after HTx before discharge from hospital. Patients in the ECMO group with a more than moderate SPAP increase after HTx before discharge from hospital had higher mortality compared to patients in the non-ECMO group without a more than moderate SPAP increase after HTx before discharge from hospital ($P = 0.005$, $X^2 = 8.010$).

of the right side of the recipient's heart were strong predictors of ECMO. Myocardial ischemia becomes more critical with prolonged CPB time, leading to worse cardiac rebounds after weaning. As reported by Banner et al. 30-day survival decreases linearly as ischemia time increases in HTx patients (19). Moreover, reperfusion worsens the extent of tissue damage and cardiac dysfunction (20). Similarly, in this study, prolonged CPB time was a strong predictor of ECMO after HTx. The average values of anatomical diameters related to the right side of the heart were greater in the ECMO group than in the non-ECMO group; this can serve as indirect evidence of increased right-sided heart afterload. The RV diameter was directly affected by SPAP and the RVOT diameter, being an intermediary variable between the RA and RVOT diameters, which showed no significant difference in multivariate logistic regression. However, this does not imply that there was no difference in the RV diameter between the groups. The morphological changes and increased SPAP suggested that the right-sided heart function of patients in the ECMO group was already impaired preoperatively. To ameliorate the right-sided heart dysfunction caused by myocardial ischemia and increased pulmonary vascular resistance, ECMO was performed for critical patients at our center.

The pathophysiological and prognostic significance of the right side of the heart was previously underestimated but has been recognized to have crucial prognostic significance (21). Bartko et al. suggested that the metrics of RV function were the strongest predictors of outcomes in patients who underwent cardiac surgery (17), which was in line with the results of our study. The ECMO group still showed significantly higher mortality than the non-ECMO group during follow up, even after adjustment. After a median follow-up period of 53 months, 35.0% (14 patients) and 25.0% (31 patients) of patients died in the ECMO and non-ECMO groups, respectively. This may suggest that patients requiring ECMO after HTx have a limited prognosis if right-sided heart function is impaired preoperatively.

In the latest echocardiographic evaluation in our study, the ECMO group had a larger mean RV diameter and RV outflow diameter than the non-ECMO group. Although there was no significant difference in other function parameters, such as FAC, S', and TAPSE, the Tei index was significantly associated with RV function. The Tei index is defined as the sum of the isovolumic contraction and relaxation times divided by the ejection time; it incorporates both systolic and diastolic time intervals to express global systolic and diastolic ventricular function (22, 23). Therefore, the Tei index avoids the irregular anatomical shape of the right side of the heart and the tracing error caused by the endocardial myocardium trabecula in echocardiography when measuring FAC, S', and TAPSE. Previous studies have shown that the RV Tei index is significantly related to SPAP (24) and is elevated owing to increased pulmonary vascular resistance and

decreased myocardial contraction (25). The ECMO group had a higher RV Tei index than the non-ECMO group, suggesting that these patients had increased pulmonary vascular resistance and decreased myocardial contraction during follow up. There was no significant difference in pulmonary pressure between the groups, which is probably related to the measurement method. In our center, we routinely use the tricuspid regurgitation method to non-invasively measure pulmonary pressure because right heart catheterization is an invasive procedure with associated risks that significantly limit its utility for routine monitoring of pulmonary hypertension. Indeed, SPAP is a standard assessment in contemporary echocardiography and can be measured in approximately two thirds of all echocardiograms. A good correlation has been found between right heart catheterization and echocardiography (26).

The progression of pulmonary vessel disease also plays an important role in the development of cardiovascular diseases. In a study by Crawford et al. (27), SPAP was associated with significant increase after HTx, which was in line with our findings. SPAP was a strong predictor of all-cause mortality during follow up in the multivariate Cox regression analysis in our study. In the subgroup analysis performed based on SPAP, the prognosis of patients in the ECMO group with more than moderate SPAP increase was much lower than that of the patients in the non-ECMO group without a more than moderate increase ($P = 0.005$, $X^2 = 8.010$). This suggests that patients requiring ECMO after HTx have a limited prognosis if the pulmonary vessels are impaired preoperatively. Han et al. (28) reported that cardiovascular involvement caused by pulmonary vessel disease is particularly relevant and is associated with impaired health status and worse mortality. Previous studies have found that in patients with rheumatic heart disease, even when rheumatism had been controlled and the implicated valves replaced, the damage to the pulmonary capillary vessels remained irreversible (29), showing the same trend as that observed in our study. Among patients with end-stage HF, LV systolic and diastolic function decreased, which led to increased filling pressures in the left heart. This initiates a series of adverse pathological and functional changes in the pulmonary vasculature and eventually in the right side of the heart. We consider that the most important and direct cause of right-sided heart dysfunction is the pre-HTx changes in the pulmonary vessels, which exist even after a healthy heart is replaced. Elevated pressure of the recipient's pulmonary artery leads to insufficiency of the right-sided heart, which leads to the application of ECMO early after HTx, also leading to higher mortality during long-term follow up. However, the changes in pulmonary vessels require further confirmation by pathology studies.

Study Strengths

To our knowledge, our study is the first retrospective study to investigate the impact of ECMO on right-sided heart performance in terms of both morphology and function after HTx. There were no significant differences in the baseline characteristics between the two groups except for some echocardiographic characteristics that cannot be ruled out. All survival analyses and right-sided heart evaluations were based on real-world data, which could be a reliable reference for clinicians to make treatment decisions for HTx patients, especially for those requiring ECMO.

Study Limitations

Our study was mainly limited by the small sample size, which made it difficult to determine reliable relationships among observed events through statistical analysis. For instance, the ECMO group had a high mortality rate, as such echocardiographic data were available for only a relatively small subset of patients. Additionally, a biopsy is difficult to perform regularly after HTx in China because of problems with cooperation. If biopsy was performed, the changes in the right side of the heart and pulmonary vessels would have been better evaluated. Although normal echocardiographic characteristics were measured at hospital admission during follow-up, there was no tracking of continuous changes in the assessment of right-sided heart function (RV tei index, S', and FAC). Especially there were some deficiencies in the echocardiographic data in agonal stage, we cannot determine whether the main cause of death was pulmonary hypertension or right heart failure. Finally, although differences in the measured covariables were minimized after propensity score weighting, unmeasured and unknown covariables were probably present. Therefore, further studies are required to confirm our findings.

CONCLUSIONS

Recipient right-sided heart characteristics were strong predictors of ECMO after HTx. ECMO patients had high mortality in the perioperative and follow-up periods, and the changes in RV function in ECMO patients may be associated with pulmonary vessel injury before and after HTx. Further studies are needed to assess the impact of ECMO on right-sided heart function.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethics approval for this study was obtained from the Research Ethics Committee of Beijing Anzhen Hospital, Capital Medical University, Beijing, China. IRB No. 2021096X Approve date: Jul. 6, 2020. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Thin filament cardiomyopathies: A review of genetics, disease mechanisms, and emerging therapeutics

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All muscle contraction occurs due to the cyclical interaction between sarcomeric thin and thick filament proteins within the myocyte. The thin filament consists of the proteins actin, tropomyosin, Troponin C, Troponin I, and Troponin T. Mutations in these proteins can result in various forms of cardiomyopathy, including hypertrophic, restrictive, and dilated phenotypes and account for as many as 30% of all cases of inherited cardiomyopathy. There is significant evidence that thin filament mutations contribute to dysregulation of Ca^{2+} within the sarcomere and may have a distinct pathomechanism of disease from cardiomyopathy associated with thick filament mutations. A number of distinct clinical findings appear to be correlated with thin-filament mutations: greater degrees of restrictive cardiomyopathy and relatively less left ventricular (LV) hypertrophy and LV outflow tract obstruction than that seen with thick filament mutations, increased morbidity associated with heart failure, increased arrhythmia burden and potentially higher mortality. Most therapies that improve outcomes in heart failure blunt the neurohormonal pathways involved in cardiac remodeling, while most therapies for hypertrophic cardiomyopathy involve use of negative inotropes to reduce LV hypertrophy or septal reduction therapies to reduce LV outflow tract obstruction. None of these therapies directly address the underlying sarcomeric dysfunction associated with thin-filament mutations. With mounting evidence that thin filament cardiomyopathies occur through a distinct mechanism, there is need for therapies targeting the unique, underlying mechanisms tailored for each patient depending on a given mutation.

KEYWORDS

thin filament, cardiomyopathy, TNNI3, TNNT2, TNNC1, TPM1, ACTC1

Introduction

Cardiomyopathies represent a group of inherited disorders that affect the myocardium with varying phenotypes including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), and arrhythmogenic right ventricular cardiomyopathy (1). Genetic mutations can affect any portion of the sarcomere including the thin and thick filaments, titin or calcium handling proteins, ultimately leading to cardiac muscle dysfunction (2, 3). In patients with cardiomyopathies due to sarcomeric mutations, thick filament mutations are the most common and best characterized, but thin filament mutations may account for a clinically significant portion of cases (4–7). Evolution in our understanding of cardiomyopathies and molecular structure of the cardiomyocyte has uncovered many different phenotypic presentations.

Although mutations of the thin filament proteins are a less common cause of inherited cardiomyopathies, it remains clinically relevant as they have distinct clinical presentations and some early studies suggest they are potentially associated with worse outcomes. Morbidity is significant in this subgroup as there are higher rates of restrictive physiology (8), earlier progression to advanced heart failure and higher rates of invasive intervention (9). Although controversial, some early studies have suggested that thin filament cardiomyopathies possibly have a higher rate of mortality, often presenting as sudden cardiac death (SCD) (10), although this remains to be confirmed in larger more contemporary trials.

Treatment has predominantly focused on symptom management related to outflow tract obstruction and heart failure, but it is less clear how to manage patients if these symptoms are not present. Novel targeted therapies currently under development, which address the underlying cause of disease (the dysfunctional sarcomere), may help treat the latter group. Improved understanding of genetype-phenotype relationships in thin filament cardiomyopathy are needed to develop new targeted therapies. The primary purpose of this review is to define the unique epidemiologic, genetic, pathophysiologic, and clinical features of thin filament cardiomyopathies with the intention of improving the recognition and overall management of this rare, but devastating disease subtype.

Role of the thin filament in the sarcomere

The thin filament is integral to the function of the sarcomere, serving primarily as a path for myosin function. The thin filament is formed through the combination of actin encoded by the gene *ACTC1*, tropomyosin (Tpm) encoded by the gene *TPM1*, and the troponin (Tn) complex, and requires precise coordination of all subunits to function properly. Cardiac actin, composed of two intertwined helical strands of actin requires leiomodin (Lmod), a strong filament nucleator, for nucleation and subsequent polymerization (11). Additionally, tropomodulin (Tmod) binds and stabilizes the minus terminus of actin, effectively end-capping and preventing dissocation of actin monomers (12). Cardiac actin is integrated with Tpm, a dimer of two α-helices wound into a coil that rests in the grooves of actin and binds every seventh successive actin protomer in a spiraling fashion (13, 14). The Tn complex is composed of cardiac troponin-T (cTnT), which associates with Tpm, cardiac troponin-C (cTnC), which contains a calcium binding site, and cardiac troponin-I (cTnI), a regulatory unit inhibiting the binding of actin to myosin. cTnT, cTnC, and cTnI are encoded by genes *TNNT2, TNNC1* and *TNNI3*, respectively.

Ionized calcium plays a key role within the myocyte by coupling electrical stimulation with sarcomeric contraction. Action potentials propagate along the sarcolemma to T-tubules, triggering the influx of calcium through L-type calcium channels into the cardiomyocyte cytoplasm which induces calcium-mediated calcium release from the sarcoplasmic reticulum. Muscle contraction is regulated by changes in intracellular calcium concentration, inducing myosin-dependent changes in the location of Tn and Tpm over the surface of the actin-based thin filament (15, 16). Intracellular calcium concentration is maintained exceptionally low at $1 \times 10^{-6.5}$M in resting cardiac muscle, during which the N-terminal EF-hand of cTnC is closed and without calcium bound, cTnI is tightly bound to actin and Tpm, and Tpm is stabilized in an inhibitory position, obstructing actin binding sites and preventing myosin from interacting (17, 18). With increased calcium levels in the cytoplasm, calcium bound to cTnC allows the regulatory switch of cTnI to interact with the opened EF-hand of cTnC, removing the inhibition of cTnC on Tpm and actin, exposing the myosin head binding sites on actin. With adequate phosphorylation potential (ΔG-ATP), myosin may bind to actin and initiate cross-bridge cycling, resulting in sarcomeric contraction (Figure 1).

Occurrence of thin filament cardiomyopathy

Large retrospective studies suggest mutations occurring in *TNNT2, TNNI3, TPM1* and *ACTC* likely account for ∼5–15% of all HCM cases, with some reports as high as 30% of HCM cases (4, 5, 19–22). Thin filament mutations can also present as DCM, with *TNNT2* mutations accounting for up to 3% of DCM cases (23–26). RCM has also been associated with thin filament sarcomeric mutations and can present with a severe phenotype with early need for cardiac transplant and premature death (26, 27). Furthermore, the same patient may progress from different phenotypes over the trajectory of their disease. For example, patients may present as a HCM phenotype initially

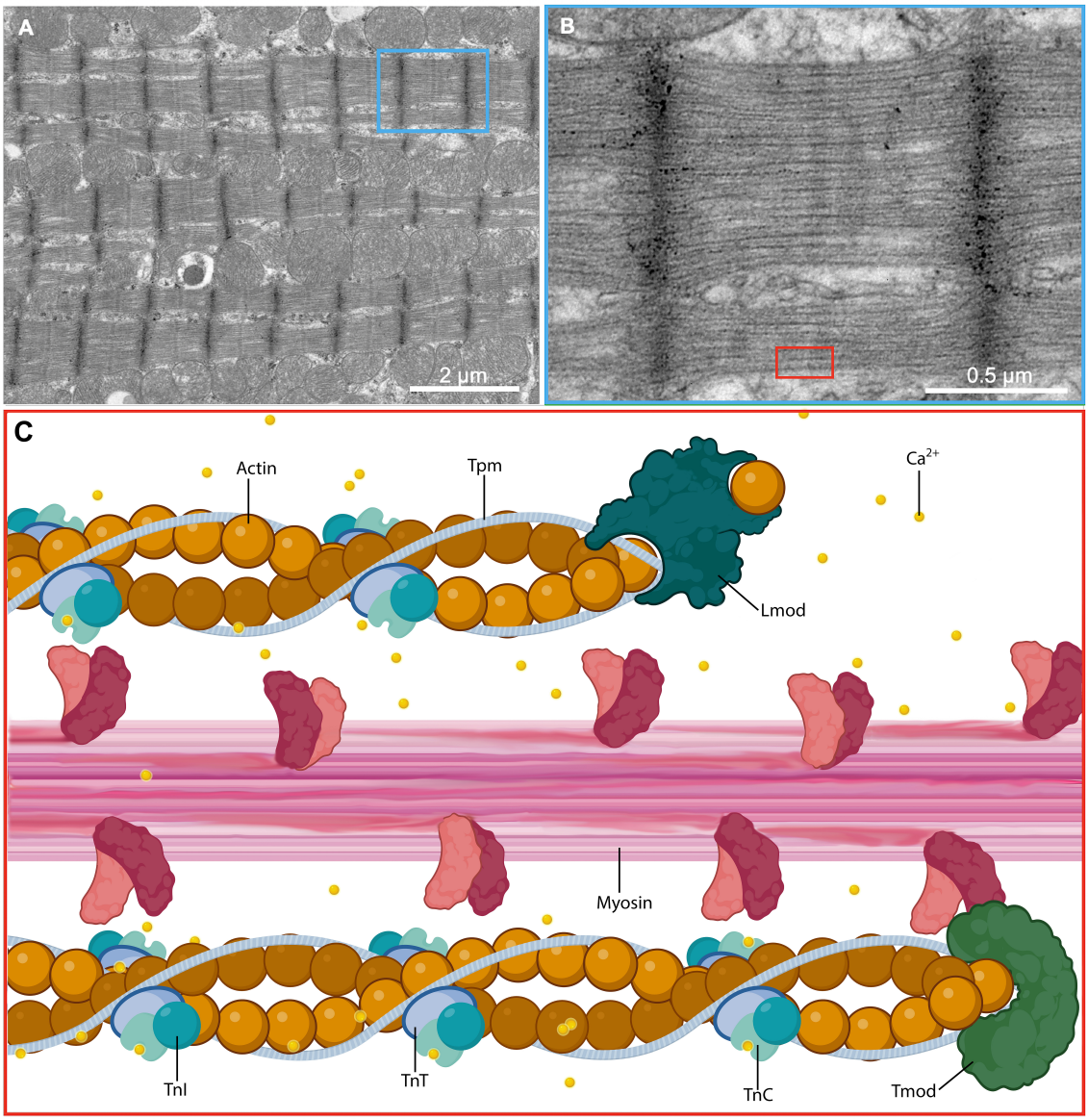

FIGURE 1
Molecular structure of the cardiac sarcomere. **(A)** Low and **(B)** high magnification electron micrographs showing the sarcomeres of a cardiac myocyte in a glutaraldehyde-fixed heart from an adult male C57BL/6 mouse. **(C)** A magnified schematic illustrating the molecular ultrastructure of the sarcomere and demonstrating the interaction between thick filaments that act as the motor apparatus of the cell and drive contraction and thin filaments that regulate the actions of the thick filaments in response to calcium flux. Ca^{2+}, calcium ion; TnC, troponin C; TnI, troponin I; TnT, troponin T.

and then develop more of a RCM/DCM phenotype as the disease progresses.

Because these inherited cardiomyopathies have variable penetrance, there is likely a significant proportion of individuals who are carriers, but are asymptomatic without any clinical manifestations (28). Therefore, the overall prevalence of thin filament associated cardiomyopathies may be higher than the estimates provided above.

Pathologic mutations and clinical presentation

Cardiomyopathies were once regarded as isolated, linear diseases of heart muscle with established clinical presentations and cardiac manifestations that progressed over time. This is consistent with the initial, gross portrayal of HCM, which was a disease of idiopathic subaortic stenosis caused by asymmetric septal hypertrophy (29). With the arrival of

molecular analysis and identification of sarcomeric mutations, cardiomyopathies are increasingly recognized as a multifaceted group with significant clinical overlap. Clinical diagnosis of cardiomyopathies can be challenging due to inconsistent presentations and apparent genotype-phenotype incongruity. For example, presentations may be phenotypically distinct (RCM vs. HCM) between family members despite carrying identical mutations (8, 30–32). However, despite the variability of cardiomyopathy presentation there appears to be a number of shared clinical findings observed in patients with thin filament mutations.

Depending highly on the specific amino-acid substitution and associated locus, mutations in each of the thin filament proteins have the potential to manifest as hypertrophic, dilated, and restrictive end phenotypes, with HCM occurring most frequently. Thin filament HCM is most associated with mutations in *TNNI3* and *TNNT2* and may present with "non-classic" findings, including apical hypertrophy, higher risk of SCD, restrictive phenotype, and lesser degrees of left ventricular hypertrophy and LV outflow tract obstruction than that observed with thick filament mutations (5, 9, 33–37).

Several studies have further described clinical manifestations of thin filament HCM through comparison against thick filament HCM. One such study by Coppini et al. suggested increased morbidity associated with thin filament HCM. Specifically, advanced heart failure was observed more frequently with thin-flament mutations than with thick filament mutations, as these subjects had more than twice the likelihood of progressing to NYHA functional class of III or IV, independent of LV outflow obstruction (9). Similarly, LV systolic dysfunction tended to be more severe and occurred at younger ages in patients with thin filament mutations (9), but there are conflicting reports (19).

Thin filament HCM patients may be associated with higher rates of catheter ablation procedures for atrial fibrillation but lower rates of invasive septal reduction therapies, compared to thick filament HCM. There were no differences in ICD implantation rates (9). Dynamic outflow tract obstruction was significantly less common in patients with thin filament HCM, presumably due to the less severe and atypical hypertrophy resulting in less anterior motion of the mitral valve leaflet (9). Diastolic dysfunction was more prominent in patients with thin filament HCM, as suggested by echocardiographic imaging studies (9).

Despite lower rates of hypertrophy and outflow tract obstruction, thin filament cardiomyopathy may convey a worse prognosis compared to its thick filament counterparts. Specifically, the degree of LV hypertrophy does not seem to directly correlate with risk of SCD (38). Patients affected by thin filament HCM have a high risk of SCD with positive family history, non-sustained ventricular arrythmias and abnormal blood pressure response with exercise (9, 33). In a retrospective pedigree analysis of families with HCM-associated *TNNT2* mutations, the overall mortality rate was 34%, but as high as 64% in young males (33). Additionally, the incidence of SCD was remarkably high, occurring in 7 of 22 (32%) subjects with *TNNT2* mutations, likely reflecting a particularly malignant mutation. Conversely, Coppini et al. did not find any significant difference between thin and thick filament HCM regarding SCD, cardiac mortality or all-cause mortality rates during a mean of 4.7 years of follow-up (9). Van Driest et al. similarly found no significant difference between subgroups with regards to SCD incidence (19), suggesting that the arrhythmogenicity seen in thin filament HCM may vary depending on the specific genotype. However, current guidelines do not distinguish between thin filament and thick filament HCM with regards to treatment, including implantable cardioverter-defibrillator (ICD) installation and dysfunction (39).

TNNT2

Located on chromosome 1q32.1, *TNNT2* encodes cTnT, a 35.9-kDa regulatory protein that combines with cardiac cTnI and cTnC to form the trimeric thin filament Tn complex (40–43). Cardiac TnT specifically functions as the Tpm binding subunit within the Tn complex and regulates striated muscle contraction in response to intracellular calcium levels (40, 41, 44).

The cTnT protein can be divided into two primary subsections: the N-terminal TnT1 (residues 1–181; also referred to as TnT_N), and TnT2 which is the C-terminal portion (residues 181–289) (45–47). The N-terminus is subject to alternative splicing, yielding multiple isoforms in cardiac tissue (48). TnT1 contains an α-helical tail, which tightly binds and restricts the translocation of Tpm dimers, while TnT2 associates with the Tn complex. The highly charged sequence between residues 112 and 136 is evolutionarily conserved among vertebrates and invertebrates, suggesting this segment is likely crucial to the overall function of cTnT and its anchoring to Tpm and actin (49). Moreover, the TnT1-Tpm region may be important for suppression of cross-bridge cycling during the low intracellular calcium phase of diastole (13). Genetic errors within the TnT1 coding region are typically missense or nonsense mutations, yielding a cTnT protein with altered function rather than a complete loss of function (49). TnT1 is of particular interest, as 65–75% of HCM-associated *TNNT2* mutations occur between residues 80 and 180, with hotspots surrounding residues 92–110 and 160–163 (49–53); the former hotspot appears to be the overlap binding region with Tpm and of clear importance. Mutations in these regions generally demonstrate reduced cooperativity of calcium activation, increased cTnT flexibility and ultimately, reduced interactions between cTnT and Tpm (50, 54). Therefore, it appears a likely central disease mechanism revolves around the altered calcium-dependent affinity of cTnT toward its associated sarcomeric subunits, namely Tpm.

Genetic mutations in *TNNT2* are responsible for up to 15% of all HCM cases (5, 19, 50) and as many as 50% of all thin filament cases (9) (Table 1). Cardiomyopathy caused by *TNNT2* mutations, particularly those altering the interaction with Tpm at residue 92, may portend a worse prognosis than other mutations, as one study of this mutation demonstrated a particularly high rate of sudden death with minimal evidence of hypertrophy (5), although this conclusion has not been validated in any other studies. While *TNNT2* is one of the most studied thin filament genes associated with HCM, the majority of its associated mutations remain inadequately understood. Of the nearly 400 *TNNT2* variations registered in ClinVar, more than one-third remain variants of unknown significance (VUS) and one-quarter demonstrate conflicting characterizations (73).

Derived primarily through *in silico* models, *in vitro* protein assays or *in vivo* animal models, the proposed disease mechanism resulting from pathogenic *TNNT2* variants follows a general theme of altered behavior of the carboxyl terminus toward Tpm, with varying degrees of abnormality observed depending on the particular nucleotide substitution. Specifically, HCM-associated mutations, particularly those within the TNT1 region, may alter folding of the Tn tail, potentially leading to abnormal calcium sensitivity and positioning of the Tn-Tpm complex on actin (74).

Interestingly, mutations within *TNNT2* also have the ability to manifest as dilated cardiomyopathy (DCM). Some DCM-causing mutations demonstrate a reduced affinity to calcium, thus increasing the threshold required for contraction and ultimately promoting systolic dysfunction (25). In contrast, using human induced pluripotent stem cells (hiPSCs) to characterize 51 *TNNT2* variants, Pettinato et al. demonstrated increased calcium affinity and increased concentrations of *NPPB*, a marker of cardiac hypertrophy, corresponding to increased thin filament activation (73). Thus, HCM and DCM can both result from altered calcium sensitivity of myosin ATPase regulation, highly dependent on the given nucleotide substitution, either promoting cardiac contraction causing hypertrophy or inhibiting cardiac contraction which may lead to DCM.

TNNI3

TNNI3 is the second most common genes associated with thin filament cardiomyopathies after *TNNT2* accounting for ∼5% of all cases of HCM (62). *TNNI3* mutations are potentially the most common cause of inherited RCM and are less commonly associated with DCM (75). The prevalence of *TNNI3* mutations vary geographically as *TNNI3* mutations occur more frequently than *TNNT2* in a number of subpopulations in Australia, Singapore, and the Czech Republic (76–80).

TNNI3 is located on chromosome 19q13.4, encoding cTnI, a ∼24-kDa regulatory protein found only in the cardiac sarcomere. Cardiac TnI is generally recognized as the "inhibitory" subunit of the thin filament, promoting cardiac relaxation by restricting actin-myosin cross-bridging during low intracellular calcium concentrations. As intracellular concentrations of calcium rise during systole, the C-terminus of calcium-bound cTnC interacts with cTnI, inducing a conformational change in cTnI. The conformational change of cTnI leads to a reduction in affinity toward actin and ultimately allows for actin-myosin cross-bridge formation (81). Thus, cTnI is an important regulatory protein, moderating cardiac contraction in response to intracellular calcium concentrations.

TNNI3 contains eight exons and the majority of disease-causing mutations occur in either exon seven or eight, which encode the regions interacting with actin and cTnC, respectively (36, 82). The cTnI peptide can be subdivided into five functional domains: (1) an N-terminal extension only found in cardiac TnI (residues 1–30), (2) the stiff α-helical IT arm which contains critical phosphorylation sites at serine 23/24 for protein kinase A, an N-terminal segment responsible for binding the C-terminal of cTnC during systole (residues 34–71) and a segment responsible for binding cTnT (residues 80–136), (3) the inhibitory domain that binds cTnC and actin-tropomyosin (residues 128–147), (4) the switch domain (also called the "triggering domain") possessing an flexible α-helix responsible that interacts with a calcium-binding pocket of cTnC (residues 147–163), and (5) the mobile domain at the C-terminus (residues 164–210) (46, 47). The majority of known pathogenic mutations to *TNNI3* affect the inhibitory or mobile domains.

The C-terminal third of cTnI containing the mobile region is the most conserved sequence of the peptide and is responsible for binding and stabilizing actin-Tpm (83, 84). This sequence is likely of significant importance, as deletion of only the final 3–5 residues leads to impaired function (85). Interestingly, it is this highly-conserved C-terminal sequence that houses a disproportionate number of HCM-associated mutations (77, 78, 86, 87). In fact, this nucleotide sequence may have the highest density of pathogenic mutations of any thin filament sequence (86). Mutations Asp190Gly, Arg192His, and Arg204 occurring within this conserved region demonstrate associations with both HCM and RCM phenotypes, suggesting single mutations are capable of multiple and/or overlapping phenotypes (75).

Similarly, the inhibitory region is also a hotspot for both HCM-associated and RCM-associated mutations, suggesting its functional importance (88). Specifically, residue 145 of the inhibitory region interacts with cTnC and Tpm-actin (89–91), and mutations in this location are associated with a highly variable cardiomyopathy penetrance even within the same family (82, 92). Arg145Trp is a particularly pathogenic mutation of the inhibitory region that contributes to a hypertrophic phenotype. It has been studied in detail *via* molecular dynamic analyses and demonstrates dissociated calcium-dependent phosphorylation of cTnI, leading to a reduction in interaction between residue 145 and cTnC (93–95). Similar to several

TABLE 1 Genes associated with thin filament cardiomyopathies and estimated incidences.

Gene	Protein	Percent of HCM cases	Percent of DCM cases	Percent of RCM cases	Associated cardiac diseases
TNNT2	Troponin T	5–15% (5, 9, 19, 50)	3–6% (55–59)	3–8% (60, 61)	HCM, DCM, RCM, LVNC
TNNI3	Troponin I	5% (62)	<1% (63)	3–17%(60, 61, 64)	HCM, DCM, RCM
TNNC1	Troponin C	1% (65, 66)	1% (57, 58, 63)	Unknown	HCM, DCM, RCM
TPM1	Tropomyosin	5% (67–70)	<1–2% (57, 59, 63)	3% (60)	HCM, DCM, RCM, LVNC
ACTC1	Actin	<5% (71)	<1% (57, 72)	8% (61)	HCM, DCM, RCM, LVNC, ASD

HCM, Hypertrophic cardiomyopathy; DCM, Dilated cardiomyopathy; RCM, Restrictive cardiomyopathy; LVNC, Left ventricular non-compaction cardiomyopathy; ASD, Atrial septal defect.

mutations in the C-terminal third, the Arg145Trp has also been found to contribute to RCM phenotypes, further indicating single mutations are capable of translational expression (31).

The function of the switch domain of *TNNI3* is less defined in the literature but is a site of important pathogenic mutations. Ala157Val occurring in the switch region is an interesting missense mutation that can manifest as HCM, RCM, and DCM, even in individuals within the same family (82, 96). A mouse model expressing this mutation recently developed by our lab recapitulates the key restrictive features of this disease in the absence of cardiac hypertrophy, which appear consistent with multiple large family descriptions (82, 97, 98). The mechanism remains largely unclear, but may involve altered binding of cTnC to the switch region of cTnI and subsequent malfunction of the inhibitory region of cTnI (99).

The mutation-hotspots of *TNNI3* suggest the site of mutation is highly important in cTnI-associated cardiomyopathies, as a disproportionate number of disease-causing mutations occur in the highly conserved regions responsible for interaction between sarcomeric subunits. Additionally, the repeated demonstration of identical *TNNI3* mutations manifesting as either HCM or RCM suggests the presence of unknown influencing factors, such as modifier genes or epigenetic influences, highlighting the challenge of predicting clinical manifestation and subsequent treatments based on mutation profile alone.

TNNC1

TNNC1 on chromosome 3p21.1 encodes the ∼18 kDa protein, cTnC, which serves as a sensor to changes in calcium concentration in the myoplasm. Composed of 161 residues, cTnC has two primary globular domains, a regulatory N-terminal domain (residues 1–86) connected *via* a flexible hydrophobic linker (residues 87–92) to the structural C-terminal domain (residues 93–161) (95). Each globular domain has unique EF-hand binding motifs capable of binding divalent cations (100). Specifically, the C-terminal possess two Calcium/Magnesium binding sites (sites III and IV) while the N-terminal has a single low-affinity calcium binding site (site II) (101). Site II of the N-terminus is chiefly responsible for the reversible binding of calcium throughout the cardiac cycle, hence its recognition as the regulatory domain (102). When bound to calcium cTnC conforms from the "closed" to "open" state and is strongly associated with cTnI, removing the inhibitive effect on Tpm and actin, and allowing for cross-bridge cycling (95).

Interestingly, many of the documented thin filament mutations resulting in HCM involve cTnC in some manner, affecting the calcium sensitivity of cTnC in various capacities. However, pathologic variants of *TNNC1* itself appear less frequently in population studies, with *TNNC1* only attributable in ∼1% of HCM cases, if at all (65, 66). In a recent meta-analysis, *TNNC1* conveyed the poorest prognosis of each of the cardiomyopathy-associated troponin genes, with the youngest age of diagnosis and highest rates of death, transplant and ventricular fibrillation (66). Furthermore, *TNNC1* demonstrated the highest rates of *de novo* variants, with ∼40% of patients lacking a family history of HCM (65, 66). There appear to be no well-defined variant hotspots within *TNNC1*, with one theory suggesting this is attributable to the poor prognosis of *TNNC1* mutations with fewer probands in the general population (66). Additionally, fewer cardiomyopathy-associated pathogenic mutations have been identified in *TNNC1* compared to other thin filament genes, such as *TNNT2* and *TNNI3*.

The disease mechanism of *TNNC1* pathogenic mutations seem to parallel other thin filament variations with increased sensitivity toward cytoplasmic calcium, leading to amplified contractility during systole and impaired relaxation during diastole (103). A number of mutations, such as Ala31Ser, demonstrated an increased calcium affinity at the N-terminal regulatory binding site II ($\Delta pCa_{50} = +0.17$), promoting contraction at lower calcium concentrations (102). However, other *TNNC1* variations, including as Ala8Val, Cys84Tyr, Leu48Gln, Leu29Gln, and Asp145Glu, result in significantly higher calcium sensitivity abnormalities through mechanisms not involving the N-terminal regulatory binding site, such as stabilizing the most active (M) state of the actin-Tpm-Tn complex and modifying the overall structural dynamics on cTnC

(65, 104–106). Interestingly, structural analysis of the Leu29Gln mutation revealed minimal structural alterations to cTnC, but rather abnormal calcium sensitivity and force-generating cross-bridging (107). Therefore, it appears *TNNC1* mutations impair cTnC function through a variety of mechanisms, ultimately increasing calcium sensitivity.

TPM1

Located on chromosome 15q22.2, *TPM1* contains 14 exons encoding the 32.7 kDa coiled-coil protein, tropomyosin 1.1 (Tpm1.1, also traditionally referred to as α-Tpm), a thin filament protein expressed in both cardiac and fast skeletal muscle fibers (108). Tpm1.1 was one of the first thin filament proteins to be associated with HCM more than 25 years ago (109), with approximately 30 culprit mutations identified since then (67, 110). Furthermore, Tpm1.1 mutations have also been implicated in DCM with associated reduced frequency of actin-myosin interaction. Assessments vary widely regarding the frequency of *TPM1*-associated cardiomyopathy cases, though it is likely low with most estimating *TPM1* is responsible for about 3–5% of all HCM cases (67–70). Interestingly, mutations in *TPM1* result only in cardiomyopathy, with no associated clinical myopathy despite also coding for skeletal muscle protein, possibly due to alternative splicing of the C-terminus (67).

Tpm1.1 can be organized into 7 sections or "periods," each with an N-terminal α band that interacts with actin and a C-terminal β band which interacts with myosin heads (111, 112). Tpm1.1 wraps longitudinally along the dual grooves of the actin filament, interacting with seven actin monomers and the Tn complex, and forming a regulatory constituent sterically blocking cross-bridge formation in low calcium concentrations. With calcium bound to cTnC, Tpm1.1 is allowed to shift its position, exposing strong cross-bridge binding sites on actin (113).

The α-helices of Tpm1.1 are determined by a strict heptad repeat $(a\text{-}b\text{-}c\text{-}d\text{-}e\text{-}f\text{-}g)_n$, with hydrophobic residues *a* and *d* internalized within the core and polar residues *e* and *g* facilitating electrostatic interactions with adjacent chains (114). Therefore, the primary structure and sequence of α-tropomyosin is of particular importance, making even single point mutations significant. Unlike some of the other thin filament genes, HCM-associated mutations are distributed throughout the majority of *TPM1* (67). However, a disproportionate number of HCM-associated mutations appear to occur at *g* and *e* positions of the heptapeptide repeat, including Arg21His (*g* position), Ala63Val (*g* position), Asp175Asn (*g* position), and Glu180Gly (*e* position), Glu180Val (*e* position), and Ser215Leu (*e* position) (109, 115–118). Often HCM-associated mutations following this pattern present with significant structural defects such as reduced alpha helix content (119). Furthermore, relatively few HCM-associated mutations have been demonstrated in the hydrophobic *a* and *d* positions, with notable exceptions including Ala22Ser, Ile172Thr, Ile284Val (*d* position) (19, 120, 121). Therefore, it appears changes in the highly-conserved structure of Tpm1.1 may be responsible for pathologic variations in function contributing to cardiomyopathies.

A number of models exist in the literature attempting to describe the mechanism by which *TPM1* mutations lead to HCM (67). One such "overlap" hypothesis is *TPM1* mutations alter Tpm1.1 interactions with the Tn complex, particularly cTnT, likely increasing calcium sensitivity within the myofilament (44, 112, 122). Residues 175–190 are located in exon 5 and are of particular significance, as they correspond to the region responsible for binding cTnT (123). Several mutations within this region exist, with Asp175Asn and Glu180Gly being the most heavily characterized, and may destabilize Tpm1.1-Tn interactions, ultimately causing abnormal cTn complex behavior and altered calcium sensitivity (124, 125). Interestingly, striated muscle tissue biopsies from the vastus lateralis of HCM patients possessing the Asp175Asn mutated α-tropomyosin also demonstrated a similar increase in calcium sensitivity, but no significant change in force conduction or shortening velocity (126). However, this is unlikely to be a unifying theory as dozens of HCM-associated mutations have been reported since the initial characterization of Asp175Asn and Glu180Gly that position outside of residues 175–190.

A second model describes *TPM1* missense mutations leading to weakened interactions between Tpm1.1 and actin, measured as increased free energy. Thus, it is plausible, that weakened Tpm-actin binding would lead to reduced steric inhibition of strong cross-bridge binding sites, loss of Tpm inhibitory function and increased transition into active Tpm states, eventually promoting contraction at lower calcium thresholds (127, 128). This model appears to hold true for mutations that occur at regions responsible for binding actin, for example Ala95Val (*d* position), which is thought to lead to increased binding of Tpm1.1 to actin (67, 123, 129), In summary, there are multiple models that may offer explanation for a particular subset of *TPM1* mutations contributing to the development of HCM.

Mutations in *TPM1* are also moderately associated with DCM, with about thirty identified mutations accounting for 30–35% of familial DCM cases (130, 131). Similar to HCM-associated mutations, those promoting DCM are distributed throughout Tpm1.1 likely are dependent upon positioning within heptad repeat. Mutations Glu40Lys and Glu54Lys (both *e* position), Glu114Gln and Glu62Gln are each reverse local charge from negative to positive and are associated with DCM (130, 132–135). Surface charge reversal (130, 135) appears to affect the stability of the coiled coil superstructure and may reduce affinity toward actin, theoretically leading to lower myofilament tension generation (136).

ACTC1

Actin is one of the most abundant proteins in human cells, with the most protein-protein interactions of any known protein (137). α-actin, the primary isoform of actin found in cardiac, skeletal, and smooth muscle tissue, is a 42.0 kDa protein composed of 377 residues encoded by *ACTC1* on chromosome 15q14 (40). Due to their respective positions within the overall actin filament, there are two primary domains of α-actin: the outer domain (also referred to as the smaller domain) containing subdomains 1 and 2, and the inner domain (also referred to as the larger domain) containing subdomains 3 and 4 (137).

Mutations in *ACTC1* have been linked with both HCM and DCM, as well as left ventricular non-compaction, with at least 12 mutations associated with HCM (138). Mutations in *ACTC1* account for approximately 1% or fewer of all HCM cases (71). HCM-associated mutations in *ACTC1* tend to primarily congregate in regions responsible for interacting with myosin, Tpm or both (138).

The well-characterized and notably arrhythmogenic mutation, Glu99Lys, was first described in 2000 as familial cardiac hypertrophy (139). Glu99Lys occurs in the myosin-binding region and has demonstrated increased calcium sensitivity with subsequent filament activation in human induced pluripotent stem cell-derived cardiomyocytes likely due to altered binding of myosin (140, 141). Additionally through *in vitro* motility assays, Glu99Lys demonstrated impaired relaxation and fewer motile filaments (142). Two similar mutations promoting HCM, His88Tyr, and Arg95Cys, also occur in the myosin-binding region and have been reported in pediatric populations (143).

Mutations Ala230Val and Arg312Cys, occur in actin regions important for binding Tpm and are associated with HCM (140, 144). Ala230Val produces a missense mutation occurring in the Tpm binding domain, which has demonstrated increased calcium sensitivity and subsequent hypercontraclitlity (140). No change in actin-myosin behavior was noted with Ala230Val, further suggesting it is a mutation affecting only the Tpm binding domain (145). Arg312Cys results in a hypertrophic phenotype, however calcium sensitivity data are lacking (145). HCM-associated mutation Tyr166Cys occurs in a region interacting with both Tpm and myosin, thus altering interactions with both subunits (146). Conflicting evidence exists regarding overall effect on ATPase rate and impact on calcium sensitivity is unknown.

To date, 4 documented mutations in *ACTC1* have been associated with DCM and may account for approximately 1% of DCM-associated mutations (147). Known DCM-causing mutations include Glu361Gly, Thr128Ile, Ile252Met, and Arg312His (72, 147). Interestingly, Arg312His, occurring in the same region as HCM-associated Arg312Cys mentioned above, promotes DCM (72). Despite the dilated phenotype, Arg312His has been shown to result in higher calcium sensitivity with lower calcium concentrations required for myosin activation, suggesting further characterization is required for this mutation along with Arg312Cys (72, 147, 148).

Interestingly, mutations associated with congenital heart defects, namely atrial septal defects, typically occur in the first half of *ACTC1*, while mutations resulting in cardiomyopathies are primarily found in the latter half of the sequence, suggesting *ACTC1* may play a unique role during embryonic development (139–141, 149).

Additional thin filament proteins

Lmods, are actin-binding filament nucleators that control the length of the thin filament by promoting polymerization of actin. Actin length is highly regulated within myocardiocytes and proper Lmod function is required for maturation of the cardiac sarcomere. Mutations in *LMOD2,* responsible for encoding Leiomodin subtype 2, have been associated with DCM. The prevalence of *LMOD*-associated DCM is unclear, though likely exceptionally rare in part due to the seemingly high mortality rate. This was demonstrated clearly in *LMOD2* knockout mice, that developed rapid-onset DCM with abnormally short thin-filaments and disorganized myofibrils (150). This was further exhibited through exome sequencing of a neonate with severe DCM (z score range 6.29–2.8), which revealed homozygous non-sense mutation Trp398*, interited from both asymptomatic heterozygous parents. The patient required prompt LVAD placement followed by heart transplant at 10 months (151). A similar case of neonatal DCM was demonstrated with biallelic *LMOD2* mutations: Leu415Val causing frameshift and Arg513* as non-sense. Interestingly, it appears heterozygous carriers of *LMOD2* mutations are often asymptomatic, suggesting only a low level of Leiomodin is required for normal function.

Tmod is an actin filament end-capping protein, responsible for stabilization of the pointed end of actin polymers from spontaneous disassociation. Though detailed structural information is somewhat lacking, *in vitro* models suggest it interacts with three actin subunits at the pointed end and also interacts with two Tpm subunits (12). Overexpression of *TMOD1*, encoding Tmod subtype 1 found in cardiac muscle, in mouse models demonstrated shorter thin filaments associated with a DCM phenotype and relatively high mortality rate, not unlike that seen with *LMOD2* mutations (150). Conversely, *in vitro* inhibition of Tmod *via* anti-Tmod antibody resulted in actin elongation from the pointed end (152). In both Lmod and Tmod loss of function studies, there does not appear to be any clear association with abnormal calcium sensitivity, unlike other thin filament mutations, which may indicate an alternative mechanism of cardiomyopathy that may be more dependent upon abnormal sarcomere structure.

The need for targeted therapies for thin filament cardiomyopathy

Thin filament cardiomyopathies are heterogeneous, with stark differences between seemingly similar mutations involving the same allele. Much remains unknown about the intermediary processes between the inciting mutation and subsequent cardiomyopathy, though there are several themes that remain consistent across all thin filament pathologic variants that ultimately distinguish this subtype of cardiomyopathy.

Common to each of the thin filament pathogenic variants previously discussed, a single nucleotide is typically replaced in a critical and/or highly conserved region of the genetic sequence, which encodes the substitution of a new, single amino acid and likely alters the higher global structure. Often, this occurs in a region responsible for the direct interface with another protein component of the sarcomere, distorting the normal cooperativity of these subunits, or in a key regulatory domain, changing the normal behavior of the protein itself. There is overwhelming evidence these mutations ultimately contribute to dysregulation of calcium within the sarcomere, as opposed to the leading hypothesis of increased ATPase activity observed with increased disordered relaxed state (DRX) of myosin in thick filament HCM (153–155). It is understandable how the theoretical increase in ATPase activity of thick filament HCM would directly promote increased contractility, oxygen consumption, and hypertrophy; however, the pathophysiologic mechanism is less clear in thin filament mutations. While there is increased oxygen and energy consumption with thin filament mutations, ATPase activity is conversely decreased, and myosin adopts the super relaxed state (SRX). This further suggests thin filament mutations promote cardiomyopathy through a separate mechanism compared to those of thick filament mutations (156, 157).

Though there is clinical overlap between subtypes and a high degree of variability at the individual patient level, a number of findings in thin filament HCM appear to be more correlated with thin filament mutations, including younger age of symptom onset, reduced LV hypertrophy with atypical distribution, reduced obstruction, increased diastolic dysfunction, increased progression to heart failure, elevated arrhythmogenicity, and potentially higher mortality (depending on the specific mutation) (9, 33). Additionally, mutations of the thin filament proteins seem to also correlate more with restrictive phenotypes and diastolic dysfunction more so than thick filament mutations (9). These distinctions are likely reflective of the different underlying pathophysiologic mechanisms driving each subtype of cardiomyopathy.

Current management of thin filament cardiomyopathies primarily consists of identification of disease through familial or genetic screening, control of symptoms through medication and lifestyle modification, and mitigation of adverse events such as sudden cardiac death. As recommended by current guidelines, early identification and characterization through genetic testing is paramount for at-risk individuals, such as those with family members diagnosed with HCM (39). However, with increasing use of high-sensitivity genetic testing there is a constantly expanding list of variants of unknown significance, often contributing more to patient confusion and undue distress rather than clinical intervention. As a potential solution, Mason et al. have demonstrated the use of a high-fidelity computational model of the cardiac thin filament to predict point mutation behavior at the atomic level, suggesting the future possibility of characterization of a given VUS without necessitating animal models or familial studies (158). But until mutation characterization matches available screening diagnostics, clinicians should be encouraged to limit any interpretation of genetic testing to only well-understood variants.

In patients with a primarily obstructive phenotype, though less common in thin filament cardiomyopathies, pharmaceutical agents with negative inotropic and chronotropic effects are recommended. Beta blockers are typically the first choice in such patients, as they are especially effective against exercise-induced obstruction and generally well-tolerated (39). However, if beta blockers are not tolerated or do not provide benefit, non-dihyropyridine calcium channel blockers may be trialed instead (39). The class Ia antiarrhythmic, disopyramide, has sarcomere-independent negative inotropic effects and is effective in reducing both obstruction and arrhythmic activity (159, 160). Furthermore, the combination of disopyramide with a beta blocker or non-dihyropyridine calcium channel blocker is likely one of the most effective symptom management strategies for patients with obstructive disease, with more than half of patients experiencing reduced resting outflow tract gradients and reduction in limiting symptoms (161, 162).

In patients with primarily obstructive disease with hypertrophic phenotype who fail medical management, septal reduction therapies, including alcohol septal ablation and septal myectomy, is an option with the intention of prolonging life and/or relieving symptoms. However, surgery is generally recommended only after failure of medical therapy and is infrequently performed in patients with thin filament cardiomyopathy, given the low prevalence of outflow tract obstruction in this population (161).

ICDs are life-saving devices that should be considered in individuals with high-risk features concerning for SCD. In general, ICD is recommended in individuals with HCM and previous cardiac arrest, sustained ventricular tachycardia, sudden death in a first-degree family member, LV hypertrophy >30 mm in any LV segment, a history of syncope due to arrhythmia, LV apical aneurysm or LV systolic dysfunction (39). A number of risk-stratifying tools have been developed to identify patients at risk of SCD, with the enhanced

ACC/AHA clinical risk factor strategy reporting a sensitivity for predicting SCD events of 85–95% (163). However, it should be noted that risk-stratifying tools heavily weigh outflow tract obstruction, which is less prevalent in thin filament cardiomyopathies despite an equal or greater rate of SCD. Therefore, these tools should be used with knowledge of potential underestimation of SCD risk in those with thin filament cardiomyopathies.

In HCM, genetic mutations facilitate interaction between myosin heads and actin, and increase duration of myosin in the attached state, ultimately leading to increased force generation, increased contractility and impaired relaxation. Mavacamten (MYK-461) and CK-274, novel reversible allosteric inhibitors of cardiac myosin ATPase, specifically target myosin and reduce actin-myosin cross-bridge formation, limiting contraction and cardiac thickening in a dose-dependent manner (164). In one particular myosin mutation (R403Q) mouse model, mavacamten demonstrated the ability to reduce ventricular hypertrophy if administered early in the disease process (165). A phase 2 clinical trial, PIONEER-HCM, demonstrated a reduction in the post-exercise LVOT gradient, increased exercise capacity and improved symptoms in patients with hypertrophic cardiomyopathy treated with mavacamten (166). Most recently, EXPLORER-HCM, a phase 3 trial involving 251 patients with obstructive HCM treated with either mavacamten or placebo for 30 days, demonstrated significant improvements in peak oxygen consumption, New York Heart Association (NYHA) class, post-exercise LVOT gradient and patient-reported symptom scores (167). Most interestingly, mavacamten appeared to rescue a number of effects associated with the thin filament mutations R92Q cTnT and R145G cTnI as demonstrated through *in vitro* cellular models. Specifically, mavacamten has been shown to reduce cytoplasmic calcium concentrations to below wild-type levels, suggesting mavacamten may also have the capacity to attenuate the heightened calcium sensitivity driving the majority of thin filament cardiomyopathies (168). Thus, it is possible the therapeutic efficacy of mavacamten will vary depending on the specific mutation and underlying disease mechanism. In theory mavacamten would be more likely to affect thick filament mutations with hypertrophy, however, genotypes were not clearly defined in EXPLORER-HCM and their future role or benefit in thin filament mutations, particularly those without hypertrophy or with a predominantly restrictive phenotype is unknown. Sarcomeric modulators, with cardiac myosin ATPase inhibitors being the novel example, certainly show early promise and may represent a landmark in the treatment of HCM, though long-term data involving diverse patient populations and potential drug-drug interactions are still needed (169).

Future therapies may target key alterations in proteins associated with calcium dysregulation observed extensively in thin filament cardiomyopathies. Using two cTnT mouse models with R92L and R92W mutations Lehman et al. demonstrated a significant increase in phosphorylation and subsequent auto-activation of calmodulin kinase II (CaMKII), a protein importantly involved in normal calcium homeostasis within the sarcomere, which was associated with a HCM phenotype (170). Most notably, inhibition of CaMKII lead to partial reversal of this phenotype in one of the models, with improved diastolic function and reduced atrial remodeling, suggesting a potential target for future therapeutics.

Altered calcium sensitivity has also been a focus for therapeutics in the treatment of acute systolic dysfunction. Levosimendan, a pyridazinone-dinitrile derivative and inodilator, was initially developed for acute treatment of decompensated heart failure and functions through multiple mechanisms, of which include increasing calcium sensitization of cTnC and ultimately decreasing the threshold for sarcomere contraction. The ALARM-HF trial demonstrated improved in-patient outcomes for patients with heart failure of various etiologies in acute decompensation (171). In both pediatric and adult patients with severe DCM, repetitive levosimendan infusions have demonstrated improvement in heart failure symptoms, hospilization rates and overall survival while in acute decompensation, however data are limited regarding longterm use with the longer published treatment duration measuring on the order of months (169, 172, 173). In theory, the heightening of calcium sensitization of the sarcomere would be an effective means to offset the lowered calcium sensitivity observed in DCM though extended use is likely limited to select patients with advanced heart failure rather than as a preventative measure in DCM.

With a greater understanding of the genetic mechanisms contributing to cardiomyopathy, there is increased effort to correct pathologic genetic variants prior to their clinical manifestation in patients. Unlike many genetic diseases, thin filament cardiomyopathies are caused by only a few errors in key nucleotide sequences, making them relatively approachable targets for genetic editing. While most gene therapeutic investigations focus on the treatment of thick filament cardiomyopathies, the pathologic mechanisms are similar enough in thin filament cardiomyopathies that much can be expected to translate.

Gene therapy, following the increased accessibility and utility of next-generation sequencing, allows for revision of erroneous genetic material and is often seen as one of the forefront therapies for inborn genetic diseases. One of the key challenges with gene therapy, however, is the transport of correctional material into the affected cells. Several delivery strategies have been used in cardiovascular diseases and offer potential to be used in thin filament cardiomyopathies, including the use of adeno-associated virus-9 (AAV-9), adenovirus, and lentivirus as vehicles for the introduction of genetic material (174–177).

In a proof-of-concept study focusing on loss-of-function hypertrophic mutation, *in vivo* gene transfer of *MYBPC3*

encoding cardiac myosin-binding protein-C (cMyBPC) was introduced *via* lentiviral vector-mediated transfer into cMyBPC-deficient mouse myocardium (178). Supplementation of functional *MYBPC3* resulted in near wild-type levels of cMyBPC with improved *in vivo* cardiac function, implying the possibility of delaying the clinical presentation of hypertrophy or circumventing the phenotype altogether. Alternatively, viral vectors have been used to deliver antisense oligoribonucleotides to deactivate gain-of-function mutations in *MYBPC3* through either exon skipping to mask enhancer sequences and subsequently remove a pathologic sequence without inducing a frame shift (179, 180), or trans-splicing, which splices engineered wildtype pre-trans-mRNA with mutant pre-mRNA and yields functional full-length mRNA (181–183). Limitations to these techniques include the potential for inadvertent mutagenesis and oncogenesis through inappropriate insertion, inconsistent or inefficient transduction throughout the myocardium (especially with trans-splicing) and possible host immune response toward viral vectors, such as adenovirus vectors (184).

Gene transfer *via* the cardiotropic adeno-associated virus 9 (AAV9) has demonstrated return toward baseline regulation of intracellular calcium, which may allow for therapeutic adjustment of calcium sensitivity altered in thin filament cardiomyopathies. The sarcoplasmic reticulum calcium-ATPase 2a (SERCA2a) is responsible for the rapid sequestration of cytosolic calcium into the sarcoplasmic reticulum after signal transduction in the myocardium. SERCA2a is inhibited by phospholamban in the unphosphorylated state, a micropeptide protein encoded by *PLN*. Gene transfer of *serca2a* using first-generation type 5 recombinant human adenovirus vector (AV-5) into neonatal mouse myocardium containing a hypertrophy-inducing mutation of thin filament protein tropomyosin demonstrated delayed hypertrophy and improved cardiac function at 6 weeks (185). SERCA2a delivery using a separate vehicle, adeno-associated viral vector, is determined to be a safe therapy in humans in a recent series of clinical trials focusing on chronic heart failure, the most recent of these being AGENT-HF (186–188). This trial failed to show any clinical improvement, though underpowered (186). Similar conclusions were drawn in the CUPID 2 trial, which demonstrated effective AAV1/SERCA2a gene transfer in heart failure patients, though ultimately no survival benefit (189). Interestingly, intracoronary gene transfer of adenylyl cyclase, a 130-kD membrane protein associated with increased SERCA2a calcium uptake, using adenovirus vector was shown to not only be safe but also demonstrated a dose-response improvement in cardiac function in patients with symptomatic heart failure and reduced ejection fraction (190). Although similar effects have been demonstrated through the deletion of phospholamban, leading to increased activity of SERCA2a, other deletions have yielded dilated cardiomyopathy in humans and likely prevent it from being used for treatment of HCM (191, 192).

The use of small interfering RNA (siRNA) cassettes is an alternative method to knock down the expression of pathologic missense mutations. A number of vehicles exist for the trafficking of siRNA, including lipid-based, cell-penetrating peptides, polymers and viral carriers (193). In one particular study, adeno-associated viral vectors were used to deliver siRNA into mouse models hemizygous for wildtype *MYH6* gene to silence the mutant allele, reducing pathologic transcripts by 25% and allowing for adequate production and function of alpha myosin heavy chain to prevent hypertrophy and fibrosis at 6 months (194).

Intracellular introduction of therapeutic material including low molecular weight drugs, proteins, peptides and genetic material including siRNA, and more recently mRNA, *via* lipid nanopartciles (LNP) represents a promising alternative delivery system absent of certain distinguishing drawbacks of viral vectors (195, 196). A number of theoretical advantages are offered by LNPs in the treatment of cardiomyopathies, including possible reduced immunogenicity and high carrying capacity compared to well-established viral vectors (197). However, specifically targeting myocardial tissues with LNPs will likely require further development in order for therapeutic use in cardiomyopathies, as constrated by the innate cardiac honing of AAV9 (198). Early findings from *in-vivo* animal studies demonstrate successful targeted delivery of mRNA to infarcted myocardium, suggesting a similar approach may be feasible in treatment of inherited cardiomyopathies (199).

CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeat—associated 9) technology has been used *ex vivo* to edit sperm cells containing a pathologic *MYBPC3* mutation associated with thick filament HCM. By introducing DNA nicks at regions adjacent to the targeted sequence and provided an engineered template, the pathologic sequence can be replaced through homologous recombination, resulting in correction of *MYBPC3* alleles in 72% of embryos (200). This therapy is purely experimental with the need to prevent adverse events, such as off-target insertions or deletions, and faces clear ethical concerns. Most recently NTLA-2001, a gene-editing therapy using CRISPR/Cas9, has been used for *in vivo* treatment of transthyretin amyloidosis associated with cardiomyopathy and heart failure. Among 6 patients undering going 28 days of NTLA-2001 infusion therapy, a dose-related reduction in misfolded transthyretin was demonstrated with minimal adverse effects. Though further evaluation is required, this is the first *in vivo* demonstration of the ability to eliminate mutations associated with cardiomyopathy (196, 197).

Conclusion

As screening methods improve and the progression of cardiomyopathy treatment advances from symptom

management to preventive and curative therapies, it is paramount to recognize thin filament cardiomyopathies as distinct diseases with a unique pathophysiologic mechanism and clinical presentation.

Author contributions

LK, JD, and EA contributed to the conception and design of the review article. All authors were involved in the drafting of the manuscript or revising it critically for important intellectual content and approved the final manuscript prior to submission.

Conflict of interest

JD reports personal fees from Lexeo during the conduct of the review. EA reports personal fees from Abiomed, Novartis, Abbott, non-financial support from Astra Zeneca, personal fees from Ionis Pharmaceuticals, Sana Biotechnology, Medtronic, other from Rocket Pharmaceuticals, Papillon Therapeutics, ResQ Pharmaceuticals, personal fees from Lexeo Pharmaceuticals, Cytokinetics, Endotronics, and during the conduct of the review. MM was employed by Providence Health.

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

Publisher's note

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Systemic thrombolysis in the management of pump thrombosis in patients with left ventricular assist devices

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Left ventricular assist device (LVAD) implantation as destination therapy (DT) is a valuable treatment option in patients with end-stage heart failure ineligible for heart transplant. However, this therapy can be complicated by life-threatening pump thrombosis (PT). This case series reports our single-center experience with a structured systemic thrombolysis protocol in case of PT. Consecutive patients undergoing DT LVAD (HVAD, Medtronic, Framingham, MA) implantation between 2010 and April 2021 at our institution were reviewed and those with PT identified. Clinical, laboratory and LVAD specific data were collected and analyzed retrospectively. All patients with PT were treated with systemic thrombolysis according to a structured bedside protocol. Treatment was defined successful if a patient was alive at 30 days follow-up and free of recurrent PT, stroke or device exchange. Fourteen out of 94 patients experienced a PT after LVAD implantation (11%). Systemic thrombolysis was successful in 10 of 14 patients (71%) at 30 days. Two patients died within 30 days due to a hemothorax and multi-organ failure. In three patients treatment was complicated by a major bleeding; twice a hemothorax (one fatal) and one right calf bleeding. No intracerebral hemorrhage was observed. Three patients experienced a thrombotic complication within 30 days; all recurrent PT. Eleven of the 14 DT patients were discharged home after a limited hospital stay after thrombolysis (average of 11 days). In conclusion, systemic thrombolysis may be a reasonable option for life-threatening PT in this vulnerable DT group in whom device exchange is often impossible due to comorbidity.

KEYWORDS

bleeding complication, left ventricular assist device (LVAD), pump thrombosis (PT), thrombolysis, thrombotic complication

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Introduction

Left ventricular assist device (LVAD) therapy is a viable option for patients with advanced heart failure (HF). Device innovation and a subsequent reduction in adverse events has resulted in a more widespread use of LVADs as destination therapy (DT). However, unintended device-related adverse events still exist, including pump thrombosis (PT) which may negatively affect clinical outcomes.

Patients require anticoagulant therapy because they face a significant risk of thromboembolic complications due to the presence of the artificial surfaces of the pump and the modified fluid dynamic pattern of the blood accompanied by shear forces (1). If PT occurs, the available treatment options are heart transplant, device exchange, intensification of anticoagulant treatment and/or systemic thrombolysis. Heart transplant is not an option in DT patients and (urgent) pump exchange implies major surgery in a vulnerable patient population with considerable surgical risk (2), leaving systemic thrombolysis as the main treatment option (3). This study aims to evaluate our experience in treating DT LVAD patients with PT by means of a structured systemic thrombolysis protocol.

Materials and methods

The study population consisted of all patients with advanced HF undergoing LVAD implantation (HVAD, Medtronic, Framingham, MA) as DT from November 2010 until April 2021. This single-center retrospective analysis of a prospectively collected cohort was reviewed by the local medical ethics committee (G20.182) who waived the need for official approval according to the Medical Research Involving Human Subjects Act.

Clinical, laboratory and LVAD specific data were collected and analyzed retrospectively from the patient information systems. Baseline characteristics, including laboratory values and medication use (antiplatelet regimen with clopidogrel preferred in our institution), were collected the day before PT (or most recent if not available). Pump thrombosis was defined as ≥2 signs or symptoms of PT in combination with an accompanying intervention such as intensified treatment with anti-coagulation, intravenous thrombolytics or pump replacement (4). The following signs and symptoms were considered suggestive of PT: (1) presence of biochemical signs of hemolysis, (2) worsening of HF and (3) abnormal pump parameters (4). All patients presenting with PT were initially treated with systemic thrombolysis as the first-line treatment, given the fact that all patients were no candidate for heart transplantation (our institution is a non-cardiac transplant center) and operative risk for pump exchange was deemed too high after a case by case discussion within our dedicated LVAD team including cardiologists, thrombosis and

TABLE 1 Baseline characteristics and laboratory values before pump thrombosis.

Patient no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Gender	Male	Male	Female	Male	Male	Male	Male	Female	Male	Male	Female	Male	Female	Male
Age (years)	67	65	39	60	64	67	56	69	62	68	59	70	59	71
BMI (kg/m^2)	27	25	29	23	24	26	27	26	35	22	29	25	23	26
Etiology of HF	Ischemic	Ischemic	Non-ischemic	Ischemic	Non-ischemic	Ischemic	Ischemic	Non-ischemic	Ischemic	Non-ischemic	Ischemic	Ischemic	Ischemic	Non-ischemic
Atrial fibrillation	No	Yes	No	No	No	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes
Creatinin (umol/L)	94	119	98	363	156	150	103	112	130	160	160	242	207	156
BUN (mmol/L)	5,3	4,1	6,5	13,9	9,8	39	10,7	10,3	6,8	18,9	10,7	27,5	12,4	16,0
Hemoglobin (mmol/L)	8,2	7,4	7,6	7,1	8,2	5,4	6,2	6,4	9,7	5,4	6,9	6,1	5,2	7,2
Hematocrit (%)	0,43	0,38	0,39	0,35	0,42	0,28	0,31	0,33	0,44	0,28	0,32	0,30	0,26	0,36
Phenprocoumon	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Clopidogrel	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes
Prasugrel	No	No	No	No	No	No	No	No	No	No	No	No	Yes	No

Patient data at baseline. All patients used phenprocoumon and antiplatelet therapy except patient 5 and patient 6. Patient 12 used phenprocoumon in combination with aspirin. Patient 3 was a 39 year-old female implanted as destination therapy because she was not eligible for transplant due to malignancy.
BMI, body mass index; BUN, blood urea nitrogen; HF, heart failure.

TABLE 2 Risk factors for pump thrombosis, laboratory values at the time of pump thrombosis and LVAD values peri-intervention.

Patient no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Recent bleeding	No	No	No	No	Yes	No	No	No	No	No	Yes	No	Yes	No
Recent inhibition OAC	No	Yes	No	No	Yes	Yes	No	No	No	No	Yes	No	No	Yes
MAP (mmHg)	87	68	62	78	79	70	65	50	81	75	100	108	71	77
Time between implantation and 1st PT (days)	335	66	1774	74	982	22	8	288	651	337	559	300	804	445
INR	2,1	2,5	4,3	2,9	1,1	1,2	3,9	2,7	2,2	2,2	2,3	2,5	3,1	2,6
LDH (U/L)	721	756	793	3424	2700	650	535	838	N/A	N/A	905	241	933	N/A
Total bilirubin (umol/L)	21	29	67	35	22	34	12	30	28	24	16	N/A	16	28
Conjugated bilirubin (umol/L)	7	15	22	<1	N/A	18	N/A	18	N/A	6	N/A	N/A	N/A	N/A
Haptoglobin (g/L)	N/A	<0,1	<0,1	N/A	<0,1	0,3	3,7	<0,1	<0,1	<0,1	<0,1	1,4	0,2	N/A
Free hemoglobin (umol/L)	N/A	9	283	N/A	N/A	2	1	7	83	61	50	N/A	42	344
Baseline														
LVAD flow (L/min)	4.7	5,7	2,5	4,2	3,1	5,1	4,6	4,4	5,0	3,3	3,2	3,3	4,1	3,9
LVAD power (watt)	3.3	2,8	2,7	2,7	3,0	3,5	3,1	3,3	4,2	3,2	3,3	3,1	3,5	3,1
LVAD speed (rpm)	2400	2300	2360	2340	2400	2560	2360	2480	2500	2400	2400	2400	2500	2400
Pre intervention														
LVAD flow (L/min)	7.1	9,5	7,0	9,1	8,0	6,9	7,7	2,6	9,3	6,9	5,5	0	9,2	9,0
LVAD power (Watt)	3.4	5,0	4,0	3,9	4,6	3,7	3,3	2,8	4,4	4,4	3,5	1,8	5,4	4,9
LVAD speed (rpm)	2400	2300	2360	2340	2400	2560	2360	2480	2500	2400	2400	2400	2500	2400
Post intervention														
LVAD speed (L/min)	4,6	3,7	2,8	4,0	3,0	5,2	5,4	9,5	4,1	3,0	3,6	3,2	4,7	3,4
LVAD power (Watt)	3,0	2,7	2,8	2,8	3,0	3,5	3,1	5	3,8	3,1	3,0	2,9	3,7	3,0
LVAD speed (rpm)	2400	2300	2360	2340	2400	2560	2360	2480	2500	2400	2400	2400	2500	2400

Risk factors for pump thrombosis and laboratory values at the time of pump thrombosis. Moreover, LVAD flow, power and speed values at baseline, during systemic thrombolysis and post intervention. The flow in patient 8 remained high post intervention because of persistent pump thrombosis. The flow pre-intervention in patient 12 was 0 L/min (for several short times).

INR, international normalized ratio; LDH, lactate dehydrogenase; LVAD, left ventricular assist device; MAP, mean arterial pressure; N/A, not applicable; OAC, oral anticoagulation; PT, pump thrombosis.

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haemostasis experts, and cardiothoracic surgeons. In principle, the systemic thrombolysis protocol for all patients comprised of 10 mg alteplase iv (bolus) followed by 90 mg in 2 hours (structured bedside protocol). In the absence of guidelines specifically providing guidance for thrombolysis in LVAD pump thrombosis, we choose to adhere to the high-risk pulmonary embolism protocol with deemed maximal fibrinolytic effect. All patients were admitted to the intensive care unit or cardiac care unit and vital signs were continuously monitored in addition to LVAD parameters, neurological signs and symptoms, bleeding related complications, and hemoglobin tests. In case of history of intracerebral hemorrhage or other bleeding related risk factors a tailored approach was applied after consultation of thrombosis/hemostasis experts. Bleeding complications were defined as major or minor according to the International Society of Thrombosis and Haemostasis (ISTH) criteria (5). After administration of alteplase, thrombolysis was deemed successful if the LVAD pump parameters normalized and markers for hemolysis improved. In accordance with previous studies, treatment success was defined successful if a patient was alive at 30 days follow-up and free of recurrent PT, stroke or device exchange (6, 7).

Results

Fourteen out of 94 patients experienced PT after LVAD implantation (11%). Table 1 depicts the baseline characteristics of these patients. All patients used a combination of phenprocoumon and antiplatelet therapy except for two patients. One patient (patient 5) was recently diagnosed with a intracerebral hemorrhage and received low molecular weight heparin (LMWH) in a therapeutic dosage. The second patient (patient 6) was still at the ICU due to a complicated clinical course after LVAD implantation, and received heparin guided by the aPTT (target 60–80 s). The heparin dosage was lowered for a tracheostomy and 2 days thereafter a PT was diagnosed (lowest aPTT value: 48 s). Of note, patient 12, with a history of atrial fibrillation and subdural hematoma after LVAD implantation, used phenprocoumon in combination with aspirin.

Table 2 shows the risk factors for PT and laboratory values used for the diagnosis of PT. Three patients experienced a recent bleeding (intracerebral hemorrhage in 2 patients, gastro-intestinal blood loss in 1 patient). In five patients oral anticoagulation was discontinued/lowered for invasive procedures or after intracerebral hemorrhage. After systemic thrombolysis, LVAD parameters normalized in all patients except one showing a persistent high flow (patient 8). This patient received three doses of systemic thrombolysis within 3 days. Extracorporeal membrane oxygenation as bridge to device exchange was used but the patient passed away due to multi organ failure. Also in patient 14 device exchange was considered, however, the patient refused this option and therefore received several doses of systemic thrombolysis.

Table 3 shows that systemic thrombolysis was successful in 10 of 14 patients (71%) at 30 days. Two of the 14 patients (14%) died within 30 days after an in hospital cardiac arrest due to a hemothorax (patient 7), and multi-organ failure (patient 8, as described above), respectively. In patient 7, systemic thrombolysis was considered the only suitable option despite the recent LVAD implantation. Unfortunately, this resulted in an early fatal bleeding. Three patients experienced a thrombotic complication within 30 days; all recurrent PT. Patient 8 died as a result of multi-organ failure (received 3 times systemic thrombolysis). Patient 13 and 14 received thrombolysis again, respectively 5 and 4 days after their first systemic thrombolysis. Patient 13 was free of PT thereafter and patient 14 experienced 3 additional events of pump thrombosis. Six patients had a 30-day bleeding complication of which 3 major bleedings according to the ISTH criteria (twice a hemothorax (one fatal ending) and one right calf bleeding, no intracerebral hemorrhage). No patient experienced an intracerebral hemorrhage after systemic thrombolysis. Eleven of the 14 DT patients were discharged home shortly after thrombolysis (average of 11 days).

After PT, no changes in regular medical regimen of clopidogrel/phenprocoumon were implemented in nine patients but often INR target range was revised. In three patients a switch to prasugrel was made because of inadequate inhibition of ADP-induced aggregation (i.e., clopidogrel resistance). In the remaining two patients nadroparin monotherapy was used awaiting the recovery of bleeding in the calf, and prasugrel/heparin was used awaiting the recovery of a hemothorax.

Discussion

The main finding of the current study is that systemic thrombolysis for PT is feasible and successful in the majority of the cases regarding resolution of PT in DT LVAD patients. Complications were often well manageable and must be interpreted in the light of no reasonable alternative treatment options for this life-threatening event in this vulnerable patient population.

Several studies reported on the feasibility and safety of medical therapy for PT in LVAD patients (6, 8, 9), and compared this with surgical device exchange (10). Medical treatment strategies for pump thrombosis vary among different centers. Direct thrombin inhibitors, tissue plasminogen activator, or glycoprotein IIb/IIIa antagonist have been reported as options (7).

TABLE 3 Treatment success and 30-day outcomes.

Patient no.	Treatment success	30-day mortality	30-day thrombosis complications	30-day bleeding complications	Time between thrombolysis and discharge (days)	Time between PT and mortality or latest follow-up (days)
1	Yes	No	No	No	5	1830
2	Yes	No	No	No	28	850
3	Yes	No	No	No	4	216
4	Yes	No	No	Hemothorax	41	862
5	Yes	No	No	Right calf bleeding	9	904
6	Yes	No	No	Psoas bleeding	N/A	135
7	No	Yes	No	Hemothorax	N/A	0
8	No	Yes	Recurrent PT	No	N/A	6
9	Yes	No	No	No	6	415
10	Yes	No	No	No	6	114
11	Yes	No	No	No	3	263
12	Yes	No	No	No	6	499
13	No	No	Recurrent PT	Epistaxis	11	129
14	No	No	Recurrent PT	Blood seeping from wound treated by VAC therapy	4	40
Total	71%	14%	21%	3 major bleedings (21%)	N/A	N/A

Treatment success, and 30-day mortality, thrombosis and bleeding complications after systemic thrombolysis are shown. Moreover, times between PT latest follow-up and -discharge and times between PT and mortality are depicted. Patient 14 has been discharged at day 4 following systemic thrombolysis. Flow and power returned to baseline levels. However, within 30 days he presented with a recurrent pump thrombosis and therefore there is no treatment success as defined by "patient alive at 30 days follow-up and free of recurrent PT, stroke or device exchange". He underwent recent reversal of his oral anticoagulation because of surgical drainage and VAC placement because of a driveline infection.
N/A, not applicable; PT, pump thrombosis; VAC, vacuum assisted closure.

In the literature, two studies reported the use of alteplase as initial and single medical treatment (not combined with other anticoagulant modalities) in the light of PT in patients with a HeartWare device. First, there is a case report from Kamouh et al. (8) in which they treated a patient successfully with a total dose of 30 mg alteplase (with a successful transplant 30 days after thrombolytic therapy). Second, there is another case report of Heim et al. (11) describing a case where they used 50 mg alteplase iv in total (20 mg over 60 minutes and the remaining 30 mg over the next 3 h thereafter). In conclusion, medical treatment options in general are heterogeneous and even within the group who receive tissue plasminogen activator it is mixed.

A recent meta-analysis of 43 studies comprising 28.728 LVAD patients suggests that surgical device exchange is superior to medical therapy for PT (7). This meta-analysis should, however, be regarded as indirect evidence as no randomized trial comparing medical therapy with pump exchange has been performed. Also, this meta-analysis included the whole LVAD population whereas we specifically focused on DT LVAD patients. Treatment success has previously been defined as patients alive and free from recurrent PT, stroke, device exchange or urgent transplantation at 30 days follow-up (6, 7). Although there is experience with systemic thrombolysis for the treatment of PT, the regimen used varies among different centers and success rate depends on early recognition of PT (12). In our study, medical therapy, using a structured thrombolysis protocol, was successful in 10 of 14 patients (71%) at 30 days, whereas the recent meta-analysis reported a much lower success rate of 45% for medical therapy (7). Moreover, the current 14% 30-day mortality rate compares favorably to the previously reported 17% mortality rate of surgical therapy (7).

The current findings are of particular relevance given the recent withdrawal of the HeartWare LVAD system of the market by Medtronic (13). A substantial number of the 4000 living patients have their HeartWare LVAD as *DT*, and exchange to a HeartMate 3 may be challenging in this vulnerable patient population (12). The exchange from HeartWare LVAD to a HeartMate 3 is surgically and technically feasible, but the surgery is more complex than just a regular exchange from HeartWare to HeartWare and the redo surgery *per se* is associated with increased post-operative risks and mortality. Therefore, medical treatment using systemic thrombolysis may be a viable option in this patient group with a PT as supported by our results. To

our knowledge, there has no study been performed comparing systemic thrombolysis with exchange from HeartWare LVAD to HeartMate 3.

Some limitations of this study need to be addressed. First, the small sample size with only DT patients and single center design might hamper the extrapolation of our data to *all* LVAD patients with a HeartWare LVAD. In the bridge-to-transplant or bridge-to-recovery cohort the occurrence of PT unresponsive to medical therapy can lead to urgent transplantation but in our DT group this is not an option. Second, the observational, retrospective study design does not allow for firm conclusions regarding the efficacy of thrombolysis to manage PT in DT LVAD patients. Still, adherence to a structured management protocol during the course of this study and complete data collection, strengthen the validity of our observations.

Conclusion

Systemic thrombolysis may be a reasonable therapeutic option in DT LVAD patients with a PT as final attempt to prevent death or life-threatening events, despite its risk of bleeding complications. The decision to give this therapy should be made in a multidisciplinary team with the patient and his/her family, thrombosis and hemostasis experts, cardiothoracic surgeons and cardiologists.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Leiden University Medical Center, Leiden, The Netherlands. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

KK was responsible for conceptualization, investigation, analysis, editing, and writing. PdE and LT were responsible for conceptualization, investigation, analysis, writing, and supervision. SB, MP, JJ, and MH were responsible for conceptualization and supervision. All authors contributed to the article and approved the submitted version.

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.

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Clinicopathological correlations in heart transplantation recipients complicated by death or re-transplantation

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Purpose: This study aimed to identify and correlate pathological findings with clinical outcomes in patients after orthotopic heart transplantation (OHT) who either died or underwent a re-transplantation.

Methodology and study design: Single-center retrospective analysis of primary OHT patients who died or were re-transplanted between October 2012 and July 2021. Clinical data were matched with corresponding pathological findings from endomyocardial biopsies on antibody-mediated rejection, cellular rejection, and cardiac allograft vasculopathy. Re-assessment of available tissue samples was performed to investigate acute myocardial injury (AMI) as a distinct phenomenon. These were correlated with clinical outcomes, which included severe primary graft dysfunction. Patients were grouped according to the presence of AMI and compared.

Results: We identified 47 patients with truncated outcomes after the first OHT. The median age was 59 years, 36 patients (76%) were male, 25 patients (53%) had a prior history of cardiac operation, and 21 patients (45%) were supported with a durable assist device before OHT. Of those, AMI was identified in 22 (47%) patients (AMI group), and 25 patients had no AMI (non-AMI group). Groups were comparable in baseline and perioperative data. Histopathological observations in AMI group included a non-significant higher incidence of antibody-mediated rejection Grade 1 or higher (pAMR $\geq$ 1) (32% vs. 12%, P = 0.154), and non-significant lower incidence of severe acute cellular rejection (ACR $\geq$ 2R) (32% vs. 40%, P = 0.762). Clinical observations in the AMI group found a significantly higher occurrence of severe primary graft dysfunction (68% vs. 20%, P = 0.001) and a highly significant shorter duration from transplantation to death or re-transplantation (42 days [IQR 26, 120] vs. 1,133 days [711–1,664], $P < 0.0001$). Those patients had a significantly higher

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occurrence of cardiac-related deaths (64% vs. 24%, P = 0.020). No difference was observed in other outcomes.

Conclusion: In heart transplant recipients with a truncated postoperative course leading to either death or re-transplantation, AMI in endomyocardial biopsies was a common pathological phenomenon, which correlated with the clinical occurrence of severe primary graft dysfunction. Those patients had significantly shorter survival times and higher cardiac-related deaths. The presence of AMI suggests a truncated course after OHT.

KEYWORDS

heart transplantation, endomyocardial biopsy, acute myocardial injury, primary graft dysfunction, rejection, cardiac allograft vasculopathy, C4d

Introduction

Advanced heart failure (HF) remains a detrimental condition associated with high mortality. While alternative strategies using mechanical circulatory support devices (MCS) may offer durable support for those patients, either before transplantation or as destination therapy, they are associated with a high comorbidity profile (1–4). Overall, orthotopic cardiac transplantation (OHT) remains the best therapeutic option to improve long-term survival and quality of life in patients with advanced HF (5–7). The current median survival time for adult patients after OHT is 11.5 years, with a contingent survival of 13.9 years for those who survive after the first year (5–7). Nevertheless, OHT recipients are exposed to the risk of several potential complications that may impair their outcomes (8, 9). These include graft-related complications, which include primary graft dysfunction (PGD), acute cellular rejection (ACR), antibody-mediated rejection (AMR), and cardiac allograft vasculopathy (CAV). Non-graft-related complications also impact transplant patients, including infections, renal dysfunction, and malignancy. While late mortality is commonly associated with the latter and with CAV, early mortality is often dominated by PGD and ACR (10–12). Specific donor-, recipient-, and surgery-related risk factors have been associated with PGD (9, 13), but the pathophysiological mechanisms remain largely unknown (9–12, 14–17). Standardized endomyocardial biopsies (EMB) are performed to detect rejection and adjust immunosuppressive therapy, but pathological changes associated with PGD are not routinely investigated in EMB samples. Further, no reliable marker exists to detect PGD to this date. Importantly, contraction band necrosis as a sign of acute myocardial injury (AMI) has been previously described in donor hearts supported with high inotropic support after brain death (9, 18–23). Still, a knowledge gap remains in whether these findings correlate with worse clinical outcomes. This study aimed to correlate the pathological findings in EMB with clinical outcomes of those OHT recipients who either died or underwent a re-transplantation.

Materials and methods

Study design

The observational and retrospective analysis included all adult patients who underwent a primary OHT at our tertiary care institution between October 2012 and July 2021. Only those OHT recipients were included who had a "hard outcome," defined as either postoperative death or re-OHT, whichever occurred first. Patients were followed up until death, re-OHT, or until July 31, 2021 (censor date). EMB biopsies were performed per our institutional protocol in every OHT recipient regardless of clinical status, at the end of weeks 1, 2, 3, 4, 6, 8, and 12, as well as at 6 months and optionally at 9 months after OHT, based on rejection history, level of immunosuppression and results of Allomap® and Allosure® assays for gene mapping and donor-derived cell-free DNA. For patients exhibiting adverse clinical features, the first EMB may be done sooner and repeat EMBs may be performed more frequently, which is considered in the time to event analysis. Upon de-identifying the included patients, their corresponding stored tissue samples were re-analyzed for rejection and stained additionally for the occurrence of cardiomyocyte (CMC) AMI necrosis (see below).

Further exploratory outcomes included histopathological signs of Grade $\geq$ 1 AMR, Grade $\geq$ 2 ACR, and CAV obtained from either EMB or an autopsy. All available EMB samples and tissue samples from the autopsy were analyzed. The clinical outcomes included peri-/postoperative extracorporeal membrane oxygenator (ECMO) support (differentiating between immediate ECMO support referring to intraoperative EMCO installment during index surgery, and delayed ECMO

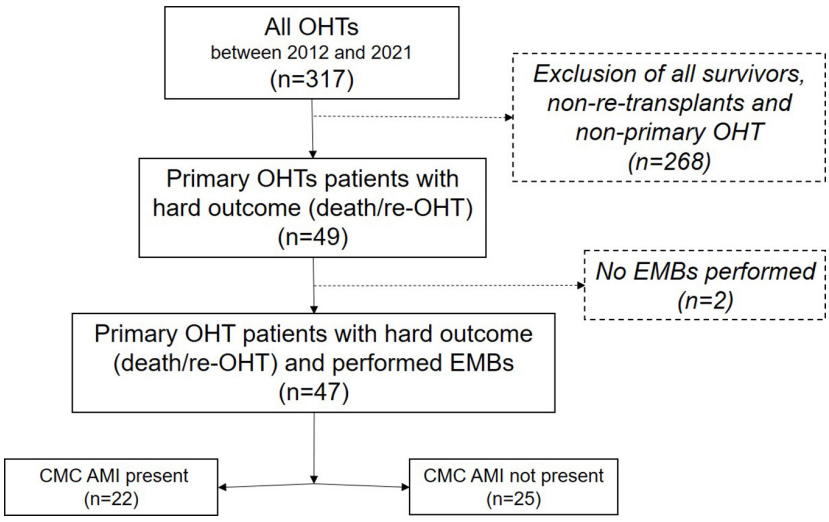

FIGURE 1
Study flow chart. The patient population is included in this retrospective review, and their interventions and outcomes are summarized in this flow chart. OHT, orthotopic heart transplantation; CMC, cardiomyocyte; AMI, acute myocardial injury; EMB, endomyocardial biopsy.

support, referring to postoperative ECMO installment), hospitalization duration after transplant, and the clinical occurrence of severe PGD, as defined by Kobashigawa et al. (9), until the hard outcomes of death or re-transplantation. Patient data included demographics, pre- and perioperative characteristics, and postoperative outcomes and was collected from internal electronic medical records by trained study personnel in an anonymized way. Patients were grouped according to the presence of CMC AMI in EMB ("AMI group" vs. "Non-AMI group"). This is summarized in the study flow chart (**Figure 1**). The study was conducted under the principles outlined in the Declaration of Helsinki and was approved by the institutional review board (HSC-MS-14-0139).

Pathology evaluation

All pathology specimens were identified from the electronic medical records, and two experienced cardiac pathologists (MM and LMB) performed a *de novo* pathological re-examination on all available tissue samples. The tissue samples were de-identified and anonymized, and the pathologist was blinded to the prior clinical and pathology reports during re-examination. The pathology specimens included tissue samples from all available post-OHT EMBs and donor transplant hearts at autopsy. EMB findings are presented regarding evidence of AMR and ACR in the standardized nomenclature (24, 25) and evidence of CMC AMI. The tissue samples were evaluated for the following histological types of AMI (26–28):

(i) hydropic/vacuolar change (moderate-sized, fluid-filled vacuoles);
(ii) fatty change (fine vacuoles containing triglycerides);
(iii) myocytolysis (colliquative myocytolysis) (swelling with loss of myofibrils);
(iv) contraction band injury/necrosis (coagulative myocytolysis);
(v) and coagulation necrosis (acontractile necrosis).

Only EMBs with the highest grades of injury and antibody-mediated and/or cellular rejection were reported. Evidence of AMI was also evaluated based on immunohistochemical detection with C4d (29). Therefore, a subgroup analysis was performed on a subset of those using C4d staining to confirm the histological findings and correlate C4d IHC with the H&E evidence of damage. It is important to note here that the pattern of C4d IHC staining in identifying acute cardiomyocyte damage is completely different from the pattern of staining associated with AMR. In C4d IHC, the marker is taken up by the CMC and does not highlight the capillaries as in C4d staining in AMR diagnostics.

Statistical analysis

The continuous or discrete data distribution was assessed with Shapiro–Wilk tests, with most variables showing a skewed distribution. Therefore, we consistently report medians and quartiles for continuous data and used non-parametric testing (Mann–Whitney *U* tests) for comparison between groups. Categorical data are summarized as counts and percentages and were compared between groups using Fisher's exact tests. Survival analysis techniques were used for time-to-event data; specifically, Kaplan–Meier curves and log-rank tests were

used to compare time-to-first event between the groups for comparison between groups for hard outcomes, as well as for the occurrence of worse documented rejection. The selected level of significance was $P < 0.05$, two-tailed. All statistical analyses were performed in Stata/IC 16.0 (StataCorp., College Station, TX, USA).

Results

Primary OHT was performed in 317 patients within the study observation period. Of these, 49 OHT recipients (15.5%) were identified who had either died or had to undergo a re-transplantation. Two were excluded due to missing EMB samples (4.1%).

The remaining 47 patients were evaluated for the distribution of pathological observations. The median age of these 47 patients was 59 years [interquartile range (IQR) 51, 67]. Thirty-six patients (76%) were male, with a median BMI of 28 kg/m^2 (23, 32). In summary, ischemic cardiomyopathy was present in 23 patients (49%) prior to OHT, and the most common comorbidities were arterial hypertension and dyslipidemia (65 and 51%, respectively). Twenty-five patients (53%) had a prior history of cardiac operation, and 21 patients (45%) were supported with a left ventricular assist device (LVAD) or total artificial heart (TAH) before OHT.

We found in EMB that CMC AMI was present in 22 (46.8%) patients and absent in 25 (53.2%). Patients were therefore grouped according to the presence or absence of CMC AMI. No significant differences were observed in any of the investigated demographic data between the groups (**Table 1**).

Perioperatively, no significant differences were observed between the groups except for the need for delayed ECMO support in the AMI necrosis group (55% vs. 24%; $P = 0.04$). The median allograft ischemic time was 203 min (170, 218) in the AMI group, and 174 min (105, 225) in the non-AMI group; although this difference was not significant ($P = 0.177$). The postoperative median duration of ECMO support was 10 days (6, 21) and was non-significant between groups.

Clinical outcomes

Altogether, six patients (13%) underwent a re-OHT; the cases were evenly divided between groups. The remaining 41 patients died (87%). Patients with CMC AMI had a significantly shorter time from OHT to death or re-OHT (42 days [26, 120] vs. 1,133 days [711, 1,664], $P < 0.0001$) (**Table 2** and **Figure 2**). The clinical course of patients with documented AMI in EMB was dominated by a significantly higher occurrence of severe PGD. This was observed in 15 patients (68%), compared to five patients (20%) where AMI was not observed ($P = 0.001$). The most common cause of death in the AMI group was cardiac-related death (14/22, 64% vs. 6/25, 24%), whereas non-cardiac-related death (causes due to multiorgan failure, sepsis, cerebrovascular event, cancer, or unknown causes) was significantly more common in patients without AMI (27% vs. 64%; $P = 0.020$).

Pathological findings

The CMC AMI was characterized by isolated coagulation necrosis in nine patients (41%), isolated vacuolar change in three patients (14%), isolated myocytolysis in two patients (9%), isolated contraction band injury in two patients (9%), coagulative necrosis with vacuolar change in two patients (9%), myocytolysis with vacuolar change in one patient (5%), contraction band injury with vacuolar change in one patient (5%), a combination of contraction band necrosis and fatty change in one patient (5%; **Figures 3A,B**), and myocytolysis with contraction band injury in one patient (5%).

Antibody-mediated rejection of grade pAMR 1 or 2 was observed in ten patients (21%), of which seven (32%) were found in the AMI group and three (12%) in the non-AMI group. No cases had a pAMR 3 grade. The median time to the first observation of the respective pAMR 1 or pAMR 2 in patients with documented AMI was 22 days (8, 28). In contrast, it was observed after a median of 1157 days (23, 1,392) in the three patients without AMI ($P = 0.087$). Severe ACR of grade 2R or 3R was observed in 17 patients (36%) but without evidence of a significant difference in the occurrence of severe ACR between groups ($P = 0.762$). The median time to severe ACR was 16 days (7, 31) for the AMI group and 56 days (15, 179) for the non-AMI group ($P = 0.064$). No significant difference was observed in the occurrence of CAV, which was observed in only one patient with documented AMI (5%), and in five patients (20%) in the non-AMI group ($P = 0.194$).

To evaluate the utility of C4d IHC in identifying AMI, 51 biopsies from 21 patients with hard outcomes in the first year after transplant were selected for C4d staining. The biopsies selected for staining were taken within the first two months after OHT. Of the 51 biopsies selected, twenty-eight (55%) had evidence of AMI by routine hematoxylin and eosin (H&E) staining. Focal C4d staining was found in 64.3% (18/28) of the biopsies with AMI damage, and only one biopsy (3.6%) without damage showed minimal focal C4d staining. In looking at the types of damage associated with C4d uptake, we noted six biopsies had only vacuolar change, five of which did not show C4d staining. The remaining 22 biopsies had more severe damage, and 17 of these biopsies showed C4d staining (**Figures 3C,D**). We also noted five biopsies with contraction bands, without necrosis—all of which showed C4d staining. In summary, of the 22 identified patients (45%) with AMI, C4d staining was performed in 21 patients, of which 15 (71%) had

TABLE 1 Baseline demographics and perioperative data.

Variable	All patients ($N = 47$)	Patients with AMI EMB ($N = 22$)	Patients without AMI ($N = 25$)	*P*-value
Demographics				
Age (years)	59 (51, 67)	56 (51, 63)	66 (50, 68)	0.290
BMI (kg/m^2)	28 (23, 32)	30.5 (28, 33)	26 (23, 31)	0.420
BSA (m^2)	1.98 (1.77, 2.25)	2.02 (1.82, 2.27)	1.98 (1.76-2.23)	0.655
Sex, male	36 (76%)	16 (73%)	20 (80%)	0.732
Race				0.264
White	19 (40%)	6 (27%)	13 (52%)	
African-American	16 (34%)	10 (45%)	6 (24%)	
Hispanic	8 (17%)	4 (18%)	4 (16%)	
Asian	3 (6%)	1 (4%)	2 (8%)	
Not disclosed	1 (2%)	1 (4%)	0	
Ischemic cardiomyopathy	23 (49%)	10 (45%)	13 (52%)	0.772
Arterial hypertension	30 (65%)	13 (61%)	17 (68%)	0.750
Diabetes mellitus	18 (39%)	10 (44%)	8 (36%)	0.753
Dyslipidemia	24 (51%)	10 (44%)	14 (56%)	0.543
Atrial fibrillation	12 (28%)	5 (28%)	7 (28%)	1.000
History of CVI	4 (9%)	3 (17%)	1 (4%)	0.293
Re-sternotomy	25 (53%)	14 (64%)	11 (44%)	0.244
MCS device before transplantation	21 (45%)	11 (50%)	10 (40%)	0.564
Perioperative data				
Total ischemic time (min)	202 (153, 222)	203 (170, 218)	174 (105, 225)	0.177
CPB time (min)	127 (110, 153)	145 (121, 182)	123 (109, 137)	0.115
Immediate ECMO post OHT	8 (17%)	6 (27%)	2 (8%)	0.123
Delayed ECMO post OHT	18 (38%)	12 (55%)	6 (24%)	0.040
Duration of ECMO support (days)	10 (6, 21)	10 (4, 21)	11 (7, 33)	0.413

All data as median (IQR) or counts (%) BMI, body mass index; BSA, body surface area; ICD, implantable cardioverter defibrillator; CVI, cerebrovascular insult; AMI, acute myocardial injury; EMB, endomyocardial biopsy; CPB, cardiopulmonary bypass; MCS, mechanical circulatory support; ECMO, extracorporeal membrane oxygenation.

TABLE 2 Clinicopathological outcomes.

Variable	All patients ($N = 47$)	Patients with AMI($N = 22$)	Patients without AMI ($N = 25$)	*P*-value
Clinical outcomes				
Severe PGD	20 (42%)	15 (68%)	5 (20%)	0.001
Re-OHT	6 (13%)	3 (14%)	3 (12%)	1.000
Cause of Death				0.020
Cardiac	21 (45%)	14 (64%)	6 (24%)	
Non-cardiac	20 (43%)	5 (23%)	16 (64%)	
Time from OHT to death/re-OHT (days)	446 (38, 1264)	42 (26, 120)	1133 (711, 1664)	$<$ 0.0001
Pathological outcomes				
EMB performed	47 (100%)	22 (100%)	25 (100%)	1
C4d Staining of CMC	21 (45%)	15/20 (75%)	0/1 (0%)	0.154
Worst Grade $\geq$ 1 AMR	10 (21%)	7 (32%)	3 (12%)	0.154
Median time to worst grade $\geq$ 1 AMR (days)	24 (17, 163)	22 (8, 28)	1157 (23, 1392)	0.087
Worst Grade $\geq$ 2 ACR	17 (36%)	7 (32%)	10 (40%)	0.762
Median time to worst grade $\geq$ 2 ACR (days)	20 (15, 61)	16 (7, 31)	56 (15, 179)	0.064
CAV	6 (13%)	1 (5%)	5 (20%)	0.194

All data as median (IQR) or counts (%) AMI, acute myocardial injury; PGD, primary graft dysfunction; OHT, orthotopic heart transplantation; EMB, endomyocardial biopsy; CMC, cardiomyocyte; AMR, antibody-mediated rejection; ACR, acute cellular rejection; CAV, cardiac allograft vasculopathy.

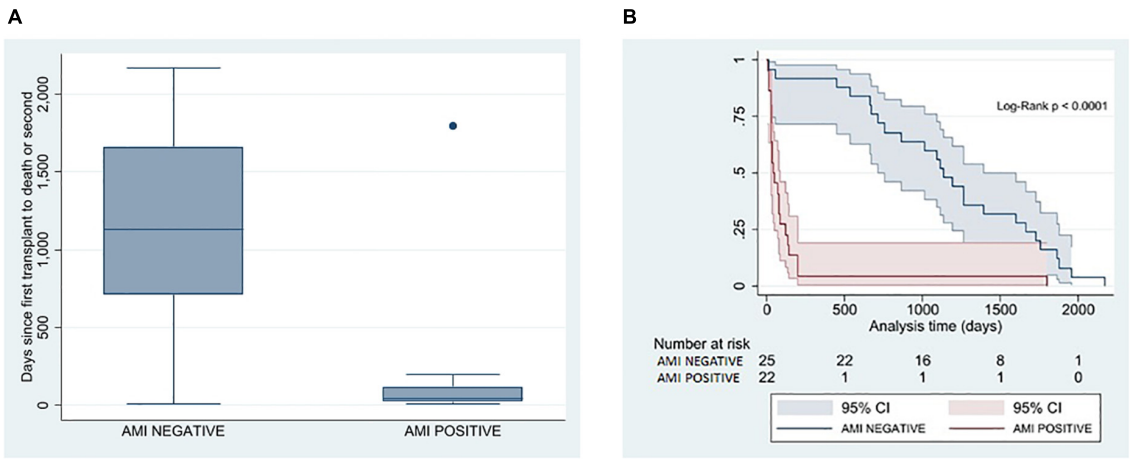

FIGURE 2
Correlation between pathological presence of AMI in EMB and time from the first transplant to death or re-transplant. **(A)** Box plot analysis between AMI and non-AMI patients on time from the first transplant to death or re-transplant in days. **(B)** Kaplan–Meier estimator on freedom-from hard outcome death or re-transplantation between the AMI (red) and non-AMI patients (blue). AMI, acute myocardial injury; EMB, endomyocardial biopsy.

one or more biopsies taken during the first two months post OHT, which stained positive for C4d.

CAV was observed in one patient with detected CMC AMI (5%), who died 73 days after OHT, and in five patients where no CMC AMI was detected (20%). These patents died or were re-transplanted after median 711 days (598, 1409).

Correlation between severe primary graft dysfunction and cardiomyocyte acute myocardial injury

Out of the 22 patients with detected CMC AMI in EMB, 15 patients (68.2%) had severe PGD and only seven (31.8%) had no evidence of severe PGD. Correspondingly, the remaining 25 patients did not have evidence for CMC AMI in EMB, and only five of those (20%) had evidence of severe PGD. The absence of CMC AMI in EMB had a high negative predictive value of 80% (95% CI 68–91), relatively high sensitivity of 75% (95% CI 62–87), and specificity of 74% (62–87) for severe PGD. A significantly higher occurrence of CMC AMI was observed in those who died within the first 90 days after OHT [88.9% (16/18) of patients vs. 20.7% (6/29) of patients; $P < 0.0001$]. Similarly, a significantly higher occurrence of severe PGD was observed in those who died within the first 90 days after OHT [77.8% (14/18) of patients vs. 20.7% (6/29) of patients; $P = 0.0002$].

Discussion

Our findings document that post-OHT EMB can provide evidence of CMC AMI, which can indicate primary graft dysfunction based on the strong correlation obtained from our data. We showed a spectrum of AMI injury types and severity seen in the biopsies. We also showed that the histopathological features correlated with diffuse C4d deposition in the injured CMC.

The C4d deposition is a consequence of sarcolemmal damage, a key component of myocardial ischemic injury (10, 11, 30). Manzoor and colleagues reported similar results regarding EMB findings in patients with PGD (23). Their study included 20 PGD EMBs, and 50% (10/20) showed myofiber injury/necrosis by either morphology and/or C4d/C3d IHC. One case had ACR (grade 1R, ISHLT 2004), and two had AMR 2 (ISHLT 2013). In a control group of 24 cases, 5 showed myofiber injury, 3 had ACR (grade 1R, ISHLT 2004), and 2 had AMR 2 (ISHLT 2013). Manzoor and colleagues concluded that myofiber injury, including coagulative necrosis, are the pathologic features of severe cardiac PGD. Their findings support AMI as a separate etiology and do not indicate ACR or AMR involvement. A similar observation was made in an older study by Baldwin et al., where they found C4d depositions in pericapillary regions in EMBs obtained within three weeks of transplantation in 15 (45%) of the 33 patients (31). Histopathological evidence of myocardial ischemic injury was detected in 11 (73%) of the 15 biopsies with C4d and/or C3d deposition, compared to 8 (44%) biopsies without C4d and/or C3d deposition (P = 0.005). This supports our findings of AMI as a separate entity, independent of ACR and AMR.

Regarding the clinical outcomes, the patients with detected CMC AMI had a significantly higher occurrence of severe PGD. Furthermore, patients with severe PGD had a significantly shorter survival time or time until re-transplantation. The

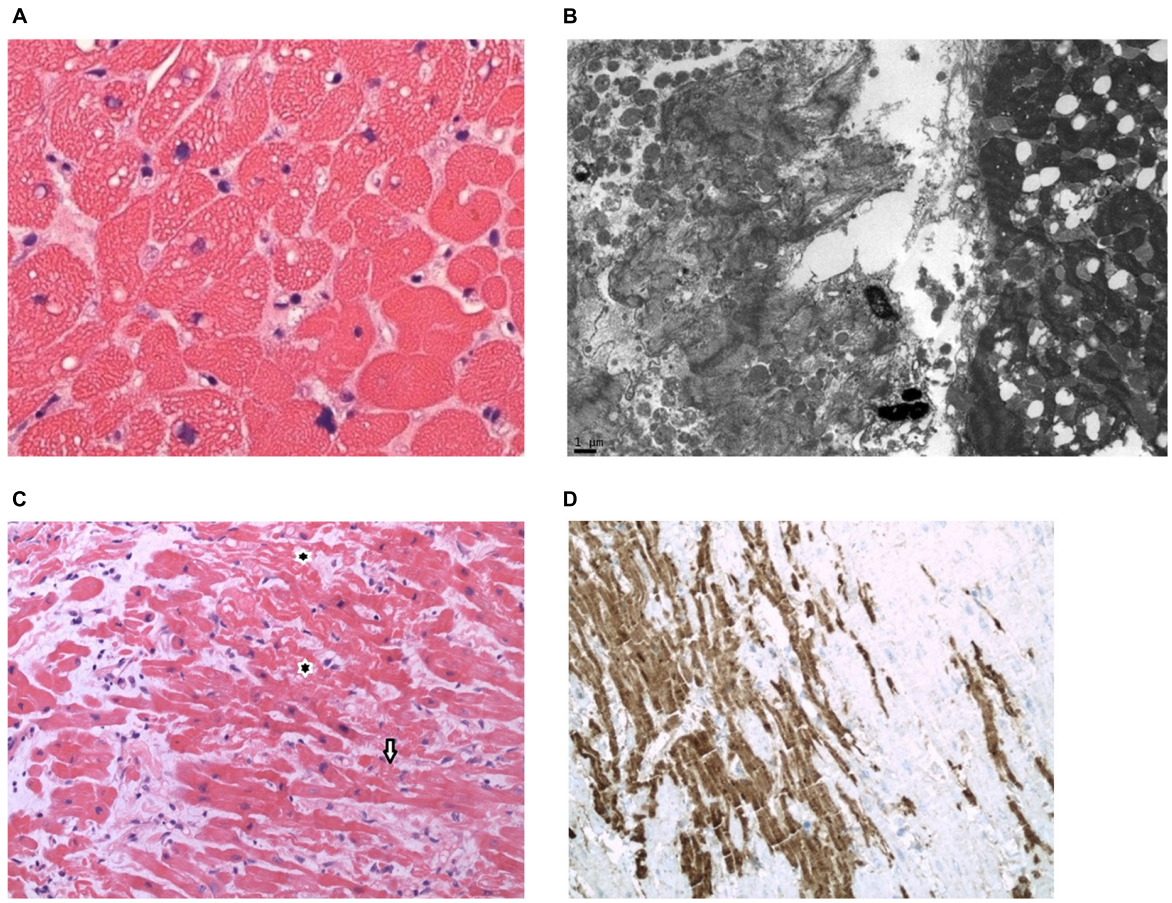

FIGURE 3
Pathological observations. **(A,B)**. Endomyocardial biopsy (EMB) on post-orthotopic heart transplant (OHT) Day 2 from a 55-year-old man with non-ischemic cardiomyopathy associated with cardiac sarcoidosis who manifested clinical features of early graft dysfunction. Triglyceride droplets in cardiomyocytes (CMC) are evidence of CMC injury. EMB confirmed the lipidosis and focal contraction band necrosis of CMC. Patient expired on post-OHT Day 28. **(C,D)**. EMB on post-OHT Day 5 from a 71-year-old man with non-ischemic cardiomyopathy who manifest clinical features of early graft dysfunction. EMB shows features of CMC injury with contraction bands (arrows) and marked C4d uptake into the damaged CMC. Patient expired on post-OHT day 35. [**(A)**, hematoxylin and eosin stain, high magnification; **(B)**, electron micrograph; **(C)**, hematoxylin and eosin stain, medium magnification; **(D)**, C4d immunostain, medium magnification].

findings of this study may suggest that CMC AMI may be a marker for pathological changes leading to PGD. Further, CMC AMI may be used as a predictor of early mortality in OHT recipients with a truncated outcome.

Cardiac transplantation is a clinical setting that involves risk for global myocardial ischemia during harvest, transport, and implantation of the donor heart (10–12). Techniques of cardioplegia derived from open heart surgery have been adapted to protect the donor heart. Nevertheless, myocardial ischemia and cardiac reperfusion injury can be major factors in the development of early graft dysfunction shortly after implantation of the donor heart (9, 32–37). It has been shown that the total myocardial ischemic time of four hours in the conventional static preservation methods is associated with significantly impaired overall survival (38, 39). However, this time may be significantly prolonged with novel procurement strategies with continuous *ex vivo* perfusion (36, 40–42). All of the "failed" OHT recipients in our study had an ischemic time kept under 240 min; however, non-significant longer ischemic times were observed in the AMI group (median 29 min longer ischemia time). While this difference is not significant, it is relevant since all baseline characteristics between groups were comparable. Nonetheless, patients in the AMI group had a higher incidence of ECMO support after their OHT, a higher incidence of PGD, and significantly shorter survival times. Therefore, the importance of graft ischemic time of <180 min in the non-AMI group on PGD and overall clinical outcome should be evaluated in larger studies and further examined.

This combination of ischemia and myocardial reperfusion injury is perhaps the main contributor to altered short-term outcomes after OHT, including PGD. The conventional

static preservation procurement strategies aim at reducing the preservation injury during cold ischemia. The main objectives of organ preservation are to establish hypothermia, prevent cell swelling, and minimize free radical-induced organ injury (43–48). During the harvest of the donor heart, two types of ischemia, cold and warm, follow each other. Cold ischemia occurs during the cold preservation time after hypothermic perfusion is instituted with the cessation of the donor's circulation. Then the heart is removed from the storage container. Warm ischemia occurs during the time interval between taking the organ out of cold storage and re-establishing warm reperfusion. Transplantation involves rewarming of the donor heart and re-establishment of its coronary circulation. This is accompanied by the release of cytotoxic products of metabolism and the formation of free oxygen radicals (FORs) which produce reperfusion injury and augment the immunogenic properties of the graft. (37, 43–46) While unclear, the observed CMC AMI injury might be due to these complex pathophysiological occurrences during graft ischemia and reperfusion; thus, it could be a reliable marker for severe PGD.

Primary graft dysfunction is a complex clinical phenomenon, often without an identifiable cause and with complex pathophysiological pathways still not fully understood. While the overall occurrence varies between 3.8 and 7.4%, the overall mortality in those with PGD after OHT remains alarmingly high at up to 31.8% (9, 35, 49–52). The identified risk factors for PGD occurrence are listed in the 2014 ISHLT consensus document (9), but a reduction of these risk factors is very limited in clinical reality. The management of severe PGD remains a challenge and, per definition, includes the necessity of MCS support other than an intra-aortic balloon pump. In our center, all patients with severe PGD and hard outcomes included in this study were supported with ECMO; most received delayed therapy after OHT. While postoperative ECMO support was observed in both groups, it was more frequent in those with AMI in their biopsies (82% of patients in the AMI group vs. 32% in the non-AMI group). However, our study does not assess the success of ECMO therapy.

While early mortality after OHT is commonly dominated by PGD and multi-organ failure (MOF), late mortality is often dominated by rejection, CAV, infection, and malignancy (39, 49), similarly presented in our study cohort. In addition, our data show a high correlation between AMI and PGD, with a negative predictive value of 80% for the absence of AMI in severe PGD patients. AMI had high sensitivity and specificity of 75% and 74% for severe PGD, respectively. This correlates with our findings; patients who died within the first 90 days after transplant had a significantly higher incidence of AMI (88.9% vs. 20.7%; $P < 0.0001$) and severe PGD (77.8% vs. 20.7%; $P = 0.0002$). This is relevant because it suggests a co-dependant correlation between AMI and PGD.

The duration of survival to death or re-transplantation in the AMI group amounted to just 1.5 months, whereas that time was well over three years in the non-AMI group. This significant difference is clinically relevant because the presence of AMI in EMB might help identify those patients with unfavorable outcomes or early death. The non-AMI group had a prolonged survival, where patients most frequently died of non-cardiac-related causes, concurring with the existing results (9, 39, 49).

When the clinical outcomes were compared to further pathological observations, we did not observe a significant difference between the AMI and non-AMI groups for pAMR 1/2 and 2R/3R ACR occurrence. However, a higher occurrence of pAMR 1/2 was noted in the AMI group, which also occurred much earlier after OHT than in the non-AMI group, but without significant evidence. Our patients are routinely checked for rejection through EMBs. In the event of rejection, the immunosuppressive regimen is adapted accordingly. While we investigated the worst documented rejection in each patient, we did not investigate the immunosuppressive treatment alterations each patient received. This is important because while an aggressive immunosuppressive treatment aims to prevent rejection, it inherently increases the risk for treatment-related side effects, including long-term effects such as cancer.

CAV is one of the common causes of late death and a major limiting factor for long-term graft survival (5–8). It was observed in 20% of those without AMI, with median time to either death or re-transplantation in those patients at median 23.4 months after OHT. Pathophysiologically, it is a progressive occlusion of arteries and veins of the transplanted heart with the involvement of both epicardial and intramyocardial vessels (53, 54). It commonly remains clinically silent because of the denervation of the transplanted heart and tends to be diagnosed at an advanced stage of the disease. Presentations of CAV include myocardial infarction, congestive heart failure, arrhythmia, and/or sudden cardiac death (53–59). Because of the serious sequelae of CAV, extensive investigation has focused on risk factors, prediction, and prevention. Nevertheless, the pathogenesis is not fully understood, and the management of CAV continues to pose a challenge. However, both immune and non-immune factors in the donor and recipient have been identified as related to the development of CAV (53–59). In addition, several biomarkers in blood and tissue are found to correlate with the presence of CAV, and that may be able to predict CAV (53, 54, 59). Recent evidence suggests that novel imaging techniques have high sensitivity and specificity for detection of CAV, such as intravascular ultrasound (IVUS), optical coherence tomography (OCT) and coronary computed tomography angiography (CCTA) (55–58), but these are not yet routinely used. Efforts are ongoing to identify changes in EMB that can be predictive of the development of CAV, but further studies are needed (54). Since CAV occurred in only five non-AMI patients, no conclusions can be made about the impact and

correlation with other investigated pathological observations due to the small event rate and sample size.

Limitations

Our study is subject to the inherent limitations of observational research, which include a small sample size and low event rates for some variables. We could not adjust for confounding factors due to the same reasons. Hence, as generally true in observational research, our results do not support causal inferences or conclusions but should be interpreted in terms of associations. The double-blinded principle of histopathological evaluations reduced informational and selection bias as well as the type-I error by two experienced pathologists (LMB and MMM) without prior knowledge of the clinical or prior pathological diagnosis. Furthermore, patients were included based on their clinical outcomes over a long observational period, reducing selection and time bias. As only the worse rejection was included for each patient, any prior or subsequent less-severe rejections were not considered in the analysis. Importantly, all OHT recipients undergo standardized EMB sampling, irrespective of whether a rejection is suspected or not. This adds to the representability and objectivity of the data collection. Furthermore, our institution specializes in treating advanced heart failure and has a specialized medical team, which has remained largely consistent throughout the years. The healthcare providers entering data into the study database were also trained in correctly using the database.

Conclusion

In those patients who underwent primary OHT and had a truncated postoperative course leading to either death or re-transplantation, acute CMC injury in EMB was a common pathological phenomenon, which correlated strongly with the clinical occurrence of severe PGD. The patients with observed AMI had significantly shorter survival times and a significantly higher occurrence of cardiac-related deaths. The presence of AMI necrosis in EMB biopsies may suggest a truncated course of disease in OHT recipients. Further studies are needed to investigate these findings.

Data availability statement

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

Ethics statement

This study was conducted under the principles outlined in the Declaration of Helsinki and was approved by the Institutional Review Board (HSC-MS-14-0139). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

MMM and MMi drafted and edited the manuscript and led the analysis. BZ, SN, SM, GO, MJ, RR, BK, and IG contributed to data collection, analysis, and edits of the manuscript. LB significantly edited the manuscript and supervised the work and submission. All authors agreed to be accountable for the content of the work.

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Conflict of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Stroke and pump thrombosis following left ventricular assist device implantation: The impact of the implantation technique

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Objectives: Several studies have shown the potential advantage of less-invasive surgery (LIS) for left ventricular assist device (LVAD) implantation. This study aims to determine the impact of LIS on stroke and pump thrombosis events after LVAD implantation.
Methods: Between January 2015 and March 2021, 335 consecutive patients underwent LVAD implantation using either conventional sternotomy (CS) or the LIS technique. Patient characteristics was prospectively collected. All patients were followed up until October 2021. Logistic multivariate regression and propensity-matched analyses were performed to account for confounding factors.
Results: A total of 242 patients ($F = 32$; 13.0%) underwent LVAD implantation with CS and 93 patients ($F = 8$; 8.6%) with the LIS approach. Propensity matching generated two groups, including 98 patients in the CS group and 67 in the LIS group. Intensive care unit stay for the LIS group patients was significantly shorter than that for the CS group patients [2 (IQR: 2–5) days vs. 4 (IQR: 2–12) days, $p < 0.01$]. There were no significant differences in the incidence of stroke events (14% in CS vs. 16% in the LIS group; $p = 0.6$) or in pump thrombosis (6.1% in CS vs. 7.5% in the LIS group; $p = 0.8$) between the groups. The hospital mortality rate in the matched cohort was significantly lower in the LIS group (7.5% vs. 19%; $p = 0.03$). However, the 1-year mortality rate showed no significant difference between both groups (24.5% in CS and 17.9% in LIS; $p = 0.35$).
Conclusions: The LIS approach for LVAD implantation is a safe procedure with potential advantage in the early postoperative period. However, the LIS approach remains comparable to the sternotomy approach in terms of postoperative stroke, pump thrombosis, and outcome.

KEYWORDS

left ventricular assist device, stroke, pump thrombosis, right ventricular assist device (RVAD), less-invasive surgery

Abbreviations

CPB, cardiopulmonary bypass; CS, conventional sternotomy; ECMO, extracorporeal membrane oxygenation; HR, hazard ratio; ICU, intensive care unit; INTERMACS, The Interagency Registry for Mechanically Assisted Circulatory Support; LIS, less-invasive surgery; LVAD, left ventricular assist device; MCS, mechanical circulatory support; RVAD, right ventricular assist device.

Introduction

The application of mechanical circulatory support (MCS) in patients with advanced heart failure (AHF) has increased in Europe recently because of a lack of donor organs (1). Further, MCS therapy is considered a valuable option as a destination therapy or bridge-to-transplant for patients who are currently not candidates for heart transplantation.

However, the major surgical challenge lies in the comorbidities of these patients and previous cardiac surgery operations. Conventional sternotomy (CS) using a cardiopulmonary bypass (CPB) machine is considered a standard approach for left ventricular assist device (LVAD) implantation. To reduce the invasiveness of the LVAD implantation, many centers started using less-invasive surgery (LIS) approaches. A series of studies have shown that the LIS approach for LVAD implantation results in fewer hemodialysis treatments, lower rates of right ventricular (RV) failure, fewer blood transfusions, and significantly shorter intensive care unit (ICU) and hospital stays (2–5).

Some clinicians opine that the minimally invasive approaches may be associated with a higher rate of pump thrombosis and stroke (6). This may be related to the difficulties encountered in the deairing procedure and the inability to adequately visualize the left ventricle in LIS patients. To our knowledge, there are no representative studies looking specifically at the differences in thromboembolic complications in patients undergoing LIS and CS. The main aim of this study is to investigate the impact of the VAD implantation technique on stroke and pump thrombosis events following LVAD implantation.

Materials and methods

Ethical statement

Written informed consent for data collection is available for all patients included in the study.

Study population

This was a retrospective review of prospectively collected data maintained in our institutional LVAD database. All subjects in this study had to meet INTERMACS (The Interagency Registry for Mechanically Assisted Circulatory Support) eligibility criteria. The conditions of the patients were discussed in detail with the heart failure team. Included patients were implanted during the period from January 2015 through March 2021. To prove the effectiveness of the surgical technique, the patients were grouped on the basis of the surgical approach, conventional full median sternotomy (CS cohort), or the less-invasive approach (LIS cohort). The decision to proceed with sternotomy or the LIS approach was based on the operating surgeon's discretion. Both surgical techniques were used for the duration of the study; however, the sternotomy technique was used more commonly in the early years. However, after the implementation of the new procedure (LIS) at our center, it is being exclusively used unless concomitant surgery is required. The use percentage of the LIS approach has been >70% of the cases starting from 2018. Indications and perioperative management of patients with AHF showed consistent results throughout the recruitment period. No changes were made regarding anticoagulation management of LVAD patients over the course of the study period. The ventricular assist devices implanted were HeartMate II®, HeartMate III (Thoratec Corporation, Pleasanton, CA, United States), and HeartWare® (HeartWare, Incorporated, Framingham, MA, United States).

Study variables, definitions, and outcome measures

Information related to patient demographics, comorbidities, interventions before LVAD implantation, laboratory parameters, and hemodynamic measurements was collected for all patients. Intraoperative data such as CPB time, total procedural time, and concomitant procedures were analyzed. The primary outcome was freedom from stroke and/or pump thrombosis. The secondary outcome was survival till discharge and during follow-up.

Stroke was defined according to the INTERMACS Protocol: any new, symptomatic, clinically documented neurologic dysfunction persisting beyond 24 h that is also associated with radiographic evidence of a cerebrovascular insult corresponding to the deficit. The treatment strategy for stroke was devised according to the directive of the guidelines and in consultation with a neurologist, neuroradiologist, and interventional radiologist. In patients who had undergone an LVAD implantation in the past 14 days, the indication for the intravenous application of alteplase (Actilyse® Boehringer Ingelheim Pharma GmbH&Co. KG, Ingelheim, Germany) was carefully considered and the potential increased risk of surgical-site hemorrhage was weighed against the anticipated benefits of reduced stroke-related neurological deficits. In select acute stroke patients who had a large vessel occlusion, mechanical thrombectomy was considered. Pump thrombosis was determined on the basis of clinical, biochemical, or hemodynamic findings or on the basis of device inspection or incontrovertible evidence of radiologic studies or in the absence of appropriate Doppler flow signals that confirm the presence of thrombus within the device or its conduits. The first case is referred to as "suspected" and the second one as "confirmed." All pump thrombosis events (suspected or confirmed) were judged and acted upon according to the definition outlined in **Supplementary Appendix**. The treatment of pump thrombosis depended on the diagnosis of the type of blood obstruction based on clinical status, hemodynamic values, echocardiographic evaluation, level of hemolysis, and end-organ function. Thrombolysis was performed with alteplase infusion consisting of a bolus of 10 mg, followed by a bolus of 20–40 mg over 20 min, and an infusion of 1 mg/h over 24 h. Hemolysis parameters were

monitored daily, such as lactate dehydrogenase, plasma-free hemoglobin, haptoglobin, total bilirubin, and hemoglobinuria. Surgical treatment option included surgical pump exchange. All explanted pumps were disassembled and visually inspected for thrombus formation. In the case of outflow graft thrombosis, the graft was stented. Stenting was performed under angiographic monitoring. In the case of recurrent pump thrombosis, the indication for urgent transplantation, according to Eurotransplant Heart Transplantation guidelines, was given. Postoperative complications were recorded according to INTERMACS definitions, including RV failure (RVF). Severe RVF was denoted by the use of a right ventricular assist device or postoperative inotropes for longer than 14 days.

Operative techniques

All LVAD implantations were performed at a single institution by two experienced surgeons, who performed both CS and LIS techniques. The LIS procedure was introduced at our institution in 2016, and since October 2018, it has been carried out in almost all patients, except for those requiring concomitant procedures (e.g., aortic valve replacement, atrial septum defect reconstruction, etc.). The switch from conventional sternotomy to a less-invasive approach was associated with hiring a new program director at our institution.

For the LIS approach, the patient was positioned with a slight elevation of the left chest. The location of the LV apex was identified through transthoracic echocardiography and marked on the patient's skin in order to perform a minimized incision for thoracotomy. Before starting the operation, a venous guide wire was placed in the femoral vein using ultrasound guidance. In the case of reoperation, an arterial guide wire was also placed in the femoral artery. At this stage, 2000IE of heparin was administered and the femoral vein was cannulated percutaneously using the Seldinger technique under transesophageal guidance. Subsequently, surgical access was made using a partial J-shaped sternotomy in the 3rd intercostal space (ICS). Then, the aorta was cannulated for CPB and a needle vent was inserted in the ascending aorta for deairing. Venous cannulation for CPB was done *via* the previously inserted percutaneous venous cannula. In the next step, anterolateral thoracotomy at the previously marked site was performed. The pericardium was opened and the insertion site of the LVAD was localized by echocardiographic assessment. The sewing ring was then secured with interrupted pledgetted sutures. Thereafter, full-dose heparin was administered *as per* standard protocol and the CPB procedure started. The majority of patients underwent operation with a CPB machine. The apex was incised within the sewing ring using a coring knife. The device was inserted into the ventricle and fixed. The driveline was tunneled using the C-Technique (7). The outflow graft was tunneled within the pericardium and anastomosed end-to-side to the ascending aorta after a partial clamping of the ascending aorta. Deairing was performed through the outflow graft and ascending aorta. The CS consisted of a pump and outflow graft insertion *via* a median sternotomy. In both approaches, a complete coverage of the pump using a polytetrafluoroethylene membrane and closure of the pericardium over the pump were achieved. Moreover, the pericardium over the ascending aorta was also closed. In this way, the dilatation of the right ventricle could be reduced, thus facilitating easier performance of later reoperations.

Notably, only a few patients underwent off-pump implantation in this series. The off-pump series was performed predominately with the HVAD pump. None of the HM 3 patients were implanted using the off-pump approach. Both cohorts were treated postoperatively in the cardiovascular ICU by a team of intensive care specialists by administering the same postoperative goal-directed therapy.

Statistical methods

Continuous variables are presented as a median with an interquartile range or as a mean with standard deviation, depending on the distribution. Categorical variables are presented as counts and percentages. Differences for continuous variables were determined by using the *T*-test or Mann–Whitney *U*-test and for categorical data with the Chi-square test or Fisher's exact test, and they were found to be appropriate. Patients operated using the CS approach were compared with those in whom the LIS approach was used. Because these two patient groups differed in terms of baseline parameters, a propensity score analysis was computed. The following variables were included in the propensity score match on the basis of the distribution in the groups and clinical expertise of the research team: patient age, sex, hypertension, intubation, previous cardiac surgery, whether the patient was on extracorporeal membrane oxygenation (ECMO) preoperatively, whether LVAD implantation was performed off-pump, VAD model (HeartWare, HM3), and INTERMACS profiles. First, the balance of the variables between both surgery groups was assessed and visualized using standard mean differences and absolute standard differences. Subsequently, propensity scores were generated using multivariable logistic regression analysis, and the matching of patients in the CS group with those in the LIS group was done in R with the MatchIt package using the nearest neighbor matching with a caliper distance of 0.2 standard deviation and ratio of 2, resulting in 98 CS patients matched with 67 LIS patients. For all analyses, two-tailed p-values <0.05 were considered statistically significant. Analyses were performed using R4.1.

Results

Study population and preoperative characteristics

Between January 2015 and March 2021, a total of 335 consecutive patients underwent LVAD implantation for advanced heart failure at our center; 242 implantations were performed through CS and 93 by LIS. The LIS approach was found feasible

in all patients, and none of the patients were switched from LIS to CS. The median age of the LIS group was 63 years (IQR 22–66) vs. 61 years (IQR 53–66) in the CS group ($p = 0.4$). There was also no significant differences in body mass index, sex, history of diabetes, peripheral arterial disease, chronic kidney disease, history of hypertension, and type of cardiomyopathy (Table 1). The CS group consisted of 167 HM3, 14 HM2, and 61 HVADs compared with 53 HM3, 1 HM2, and 39 HVADs in the LIS group ($p = 0.004$). A total of 67 patients (28%) in the CS group had a history of cardiac surgeries vs. 13 patients (14%) in the LIS group ($p < 0.01$). Patients in the CS group were more likely to be supported by veno-arterial ECMO compared with those in the LIS group [41 (17%) vs. 9 (9.7%); $p < 0.01$]. Furthermore, there was a significant difference in the INTERMACS profiles between the CS and the LIS groups, with patients in the CS group having worse INTERMACS profiles than the other group (Table 1). A

TABLE 1 Pre- and intraoperative patient characteristics in unmatched and matched cohorts.

Features	Overall cohort			Propensity-matched cohort		
	CS, $N = 242$	LIS, $N = 93$	*p*-Value	CS, $N = 98$	LIS, $N = 67$	*p*-Value
Age (median; IQR)	61 (53–66)	63 (55–66)	0.4	61 (55–66)	64 (58–67)	0.3
Female	32 (13%)	8 (8.6%)	0.2	7 (7.1%)	6 (9%)	0.7
BMI (median; IQR)	28.1 (24.9–31.6)	27.7 (24.0–30.9)	0.6	28.1 (25.2–31.6)	27.9 (24.0–31.1)	0.8
Cardiomyopathy etiology			0.2			0.8
Ischemic	118 (49%)	42 (45%)		46 (47%)	29 (43%)	
Dilatative	113 (47%)	50 (54%)		49 (50%)	38 (57%)	
Others	11 (4.5%)	1 (1.1%)		3 (3.1%)	0 (0%)	
Diabetes mellitus			0.9			0.8
Type I	4 (1.7%)	2 (2.2%)		1 (1.0%)	1 (1.5%)	
Type II	91 (38%)	34 (37%)		44 (45%)	26 (39%)	
AF preoperatively	120 (50%)	45 (48%)	0.8	52 (53%)	34 (51%)	0.8
PAD	33 (14%)	12 (13%)	0.9	17 (17%)	9 (13%)	0.5
Creatinin (µmol/L) (median; IQR)	118 (91–156)	122 (91–165)	0.7	120 (92–164)	127 (98–166)	0.4
eGFR (median, IQR)	55 (39–77)	59 (36–75)	0.7	54 (39–78)	51 (36–69)	0.4
CKD	131 (54%)	46 (49%)	0.4	63 (64%)	37 (55%)	0.2
Dialysis preoperatively	19 (7.9%)	9 (9.7%)	0.6	5 (5.1%)	6 (9.0%)	0.4
Bilirubin (µmol/L) (median, IQR)	15 (10–24)	14 (8–20)	0.2	15 (10–24) $n = 95$	13 (8–17) $n = 63$	0.2
Hemoglobin (mmol/L) (median, IQR)	6.4 (5.7–7.5)	6.8 (5.9–7.8)	0.09	6.7 (6.1–7.7)	6.9 (6.05–7.90)	0.5
COLD	23 (9.5%)	8 (8.6%)	0.8	9 (9.2%)	7 (10%)	0.8
Preoperatively tricuspid valve insufficiency ≥II	100 (43%)	40 (43%)	>0.9	42 (43%)	28 (42%)	0.9
TAPSE (median, IQR)	14.0 (12.0–17.0) $n = 206$	15.0 (12.0–17.0) $n = 83$	0.3	14.0 (12.0–16.0) $n = 91$	15.0 (12.0–16.0) $n = 66$	0.3
History of prior cardiac surgery	67 (28%)	13 (14%)	0.008	21 (21%)	10 (15%)	0.3
Preoperative v-a ECMO	41 (17%)	9 (9.7%)	0.095	12 (12%)	5 (7.5%)	0.3
LVAD type:			0.004			0.9
HVAD	61 (25%)	39 (42%)		26 (27%)	19 (28%)	
HM2	14 (5.8)	1 (1.1%)		–	–	
HM3	167 (69%)	53 (57%)		72 (73%)	48 (72%)	
Concomitant procedures at the time of VAD implantation	69 (29%)	0 (0%)	<0.001	0 (0%)	0 (0%)	>0.9
Hypertension	152 (63%)	55 (59%)	0.5	70 (71%)	42 (63%)	0.2
Intubation preoperatively	47 (19%)	5 (5.4%)	0.001	12 (12%)	5 (7.5%)	0.3
INTERMACS			<0.001			0.3
1/2	114 (47%)	23 (25%)		32 (32%)	16 (24%)	
3/4	67 (28%)	51 (53%)		67 (68%)	51 (76%)	
MAP (mmHg; median; IQR)	77 (70–88) $n = 177$	79 (70–86) $n = 67$	>0.9	80 (71–91) $n = 73$	79 (70–86) $n = 50$	0.3
CI (L/min/m^2; median; IQR)	1.80 (1.43–2.29) $n = 177$	1.7 (1.4–2.1) $n = 76$	0.3	1.70 (1.40–2.04) $n = 80$	1.73 (1.40–2.10) $n = 56$	0.8
PAP mean (mmHg; median; IQR)	37 (28–43) $n = 181$	35 (28–42) $n = 75$	0.3	37 (30–43) $n = 81$	35 (28–42) $n = 56$	0.3
RVSWI (g*m/m^2; median; IQR)	6.3 (4.7–8.6)	5.9 (4.6–7.5)	0.4	5.7 (4.5–8.7)	6.2 (4.6–8.0)	0.9
PCWP (median; IQR)	27 (21–32) $n = 168$	24 (18–31) $n = 68$	0.09	28 (21–33) $n = 75$	26 (18–31) $n = 50$	0.12
Off-Pump implantation	1 (0.4%)	11 (12%)	<0.001	1 (1.0%)	0 (0%)	>0.9
CPB time (min, median; IQR)	68 (51–99)	60 (43–80)	0.002	60 (48–77)	63 (49–80)	0.6
Total surgery time (min, median; IQR)	176 (142–236)	195 (169–220)	0.05	164 (135–196)	200 (182–231)	<0.001

AF, atrial fibrillation; BMI, body mass index; CI, cardiac index; CKD, chronic kidney disease; COLD, chronic obstructive lung disease; CPB, cardiopulmonary bypass; CVP, central venous pressure; ECMO, extracorporeal membrane oxygenation, eGFR, estimated glomerular filtration rate; HM2, HeartMate 2, HM3, HeartMate 3, HVAD, HeartWare ventricular assist device; INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support; IQR, interquartile range; LIS, less-invasive surgery; LVAD, left ventricle assist device; MAP, mean arterial pressure; PAD, peripheral artery disease; PAP, pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; RVSWI, right ventricular stroke work index [(meanPAP-CVP)*SI*0.0136]; TAPSE, tricuspid annular plane systolic excursion.

total of 47 patients (19%) in the CS group were on ventilator preoperatively vs. 5 patients (5.4%) in the LIS group ($p < 0.01$). Follow-up was complete in 100% of the patients. There was no big difference in the median follow-up time (until death, censoring for transplant/LVAD removal, or end of follow-up) between the groups [612 (IQR: 160–1,172) days in the CS group vs. 463 (IQR: 204–943) days in the LIS group, $p = 0.4$]. Sixteen LVADs (4.8%) were explanted during the follow-up after recovery of the left ventricular function (2.1% in the LIS and 5.8% in the CS groups).

Outcome

Overall population

The overall stroke rate, regardless of the implanted device, was 13.7% (46/335). When comparing the groups (CS and LIS) within the cohort, in the unmatched groups, stroke was more frequent in the LIS group (19% vs. 12%, $p = 0.06$) than in the other group (Table 2). Stroke was also more common in patients with the implanted HVAD than in those with HM3 (21% vs. 8.6%; $p < 0.001$). Among HVAD patients, stroke was more common in the LIS group (14.8% vs. 30.8%; $p = 0.09$). In HM3 patients, stroke was more frequent in the CS group (19.4% vs. 11.3%; $p = 0.6$). Table 3 shows the specific distribution of stroke type in the cohort. Stroke after LVAD implantation was associated with significantly higher mortality ($p > 0.001$). Figure 1A shows freedom from stroke for the unmatched cohort. Similarly, prior to matching, pump thrombosis was most common in the LIS group than in the CS group (9.7% vs. 5.8%, $p = 0.2$, Table 2). In the entire cohort pump, thrombosis occurred significantly more often in patients with the HVAD (16%) than in those with HM3 (1.8%; $p < 0.001$; Table 3). Figure 2A shows freedom from stroke for the unmatched cohort. A re-exploration for bleeding was necessary in 45 (19%) patients in the CS group compared with 7 (7.5%) in the LIS group ($p = 0.01$). Furthermore, postoperative dialysis and the number of patients with respiratory insufficiency who needed tracheotomy were significantly more often in the CS group (Table 2). Right heart failure (RHF) after LVAD implantation was more frequent in the CS group than in the LIS group. Postoperative right ventricular assist device (RVAD) use was also significantly higher in the CS group (23% in the CS group vs. 9.7% in the LIS group, $p < 0.01$). Moreover, support time with RVAD was significantly longer for patients in the CS group than in the LIS group [18 (IQR: 9–40) days vs. 10 (IQR: 9–11) days, respectively, $p = 0.03$]. Prior to matching, the concomitant cardiac procedures at the time of LVAD implantation, other than temporary right ventricle support, were performed only in the CS group ($n = 69$, 29%; $p < 0.001$). The most common procedure was aortic valve replacement ($n = 25$),

TABLE 2 Postoperative outcomes in conventional surgery and less-invasive surgery groups in unmatched and matched cohorts.

Outcome	Overall cohort			Propensity-matched cohort		
	CS, $n = 242$	LIS, $n = 93$	*p*-Value	CS, $n = 98$	LIS, $n = 67$	*p*-Value
Re-exploration for bleeding	45 (19%)	7 (7.5%)	0.01	18 (18%)	6 (9.0%)	0.09
Blood products (median; IQR)						
RBC	9 (3–19) *n*: 158	5 (3–8) *n*: 67	0.012	6 (3–13) *n*: 67	6 (3–8) *n*: 48	0.5
Plasma	5 (2–10) *n*: 152	3 (0–6) *n*: 63	0.027	4 (0–8) *n*: 65	4 (0–6) *n*: 45	0.6
Thrombocytes	2.0 (0–5) *n*: 157	2.0 (0–2) *n*: 66	0.016	2 (0–3) *n*: 66	2 (0–2) *n*: 68	0.3
RVAD implantation	56 (23%)	9 (9.7%)	<0.01	19 (19%)	7 (10%)	0.12
RVAD support duration days (median, IQR)	18 (9–40)	10 (9–11)	0.03	24 (12–56)	10 (10–12)	0.03
Stroke	28 (12%)	18 (19%)	0.06	13 (13%)	11 (16%)	0.6
Ischemic	17	12		10	9	
Hemorrhagic	11	6		3	2	
Pump thrombosis	14 (5.8%)	9 (9.7%)	0.2	6 (6.1%)	5 (7.5%)	0.8
Dialysis			0.01			0.06
Acute	63 (26%)	19 (20%)		21 (21%)	14 (21%)	
Chronic	34 (14%)	4 (4.3%)		16 (16%)	3 (4.5%)	
Tracheotomy	70 (29%)	14 (15%)	<0.01	29 (30%)	11 (16%)	0.04
Driveline infection	76 (31%)	25 (27%)	0.4	31 (32%)	20 (30%)	0.8
Wound infection	22 (9.1%)	3 (3.2%)	0.07	7 (7.1%)	2 (3.0%)	0.3
GIB	24 (9.9%)	9 (9.7%)	>0.9	8 (8.2%)	5 (7.5%)	0.9
Sepsis	26 (11%)	6 (6.5%)	0.2	14 (14%)	5 (7.5%)	0.2
Heart transplantation during follow-up	41 (17%)	19 (20%)	0.5	15 (15%)	14 (21%)	0.4
Follow-up days (median; IQR)	542 (133–1,118)	518 (236–995)	0.6	612 (160–1,172)	463 (204–943)	0.4
ICU stay days (median; IQR)	4 (2–11)	2 (2–4)	<0.001	4 (2–12)	2 (2–5)	<0.01
Hospital length of stay (median; IQR)	43 (27–66)	36 (25–50)	0.04	38 (26–55)	36 (25–55)	0.6
30-day mortality	21 (8.7%)	5 (5.4%)	0.3	5 (5.1%)	5 (7.5%)	0.5
Hospital mortality	42 (17%)	6 (6.5%)	0.01	19 (19%)	5 (7.5%)	0.03
1-year mortality	66 (27.3%)	15 (16.1%)	0.035	24 (24.5%)	12 (17.9%)	0.35

CS, conventional sternotomy; CPB, cardiopulmonary bypass; GIB, gastrointestinal bleeding; ICU, intensive care unit, IQR, interquartile range; LIS, less-invasive surgery; RBC, red blood cells; RHF, right heart failure; RVAD, right ventricular assist device.

TABLE 3 Distribution of stroke and pump thrombosis in the unmatched cohort regard to implanted devices.

	Unmatched cohort ($n = 335$)					
	HVAD ($n = 100$)			HM 3 ($n = 220$)		
	CS ($n = 61$)	LIS ($n = 39$)	p-Value	CS ($n = 167$)	LIS ($n = 53$)	p-Value
Stroke all	9 (14.8%)	12 (30.8%)	0.09569	13 (19.4%)	6 (11.3%)	0.6045
Stroke ischemic	6	7	0.3833	9	5	0.5793
Stroke hemorrhagic	3	5	0.297	3	1	1
Pump thrombosis	8 (13.1%)	8 (20.5%)	0.481	3 (1.8%)	1 (1.9%)	1

CS, conventional sternotomy; HM3, HeartMate 3; HVAD, HeartWare ventricular assist device; LIS, less-invasive surgery.

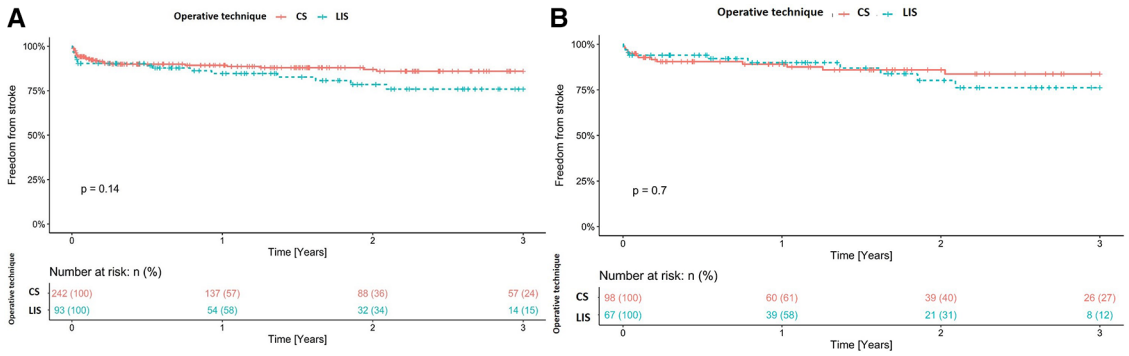

FIGURE 1
(A) Freedom from stroke for the unmatched cohort. (B) Freedom from stroke for the matched cohort. CS, conventional sternotomy; LIS, less-invasive surgery.

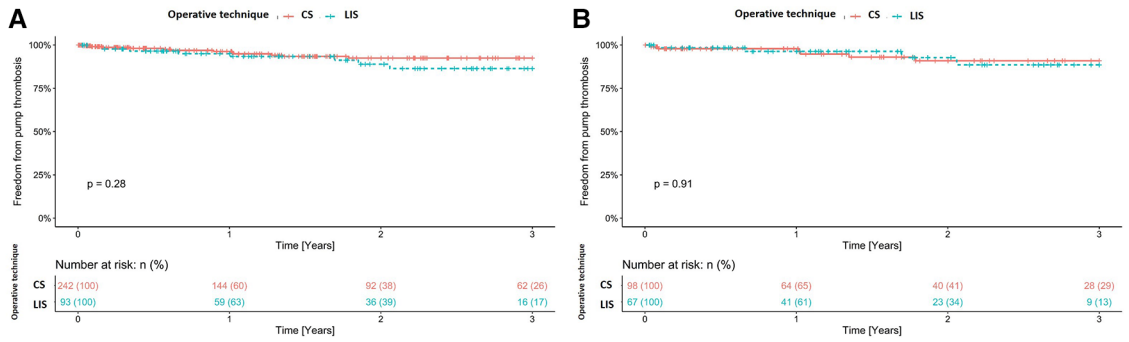

FIGURE 2
(A) Freedom from pump thrombosis for the unmatched cohort. (B) Freedom from pump thrombosis for the matched cohort. CS, conventional sternotomy; LIS, less-invasive surgery.

followed by a repair of intracardiac shunts ($n = 16$) and coronary artery bypass graft surgery ($n = 9$). Other valvular procedures (mitral or tricuspid valve repair, $n = 7$) and ascending aorta replacement ($n = 3$) were not commonly performed in our cohort. The duration of ICU stay for the LIS group patients was significantly lower than that for the CS group patients [2 (IQR: 2–4) days vs. 4 (IQR: 2–11) days, respectively, $p < 0.001$]. There was also a significant reduction in the hospital length of stay [36 (IQR: 25–50) days in the CS group vs. 43 (IQR: 27–66) days in the LIS group, $p = 0.04$]. All-cause hospital mortality was significantly higher in the CS group than in the LIS group (17% vs. 6.5%, $p = 0.01$). The most common causes of death were multiorgan failure (25/48; 52.1%), prolonged right heart failure (7/48; 14.6%), hemorrhagic stroke (4/48; 8.3%), ischemic stroke (4/48; 8.3%), and acute respiratory distress syndrome (4/48; 8.3%).

The cumulative mortality rate at 1 year was also significantly higher in the CS group (27.3%). In the univariable analysis, the type of the LVAD used and off-pump LVAD implantation were

identified as risk factors for stroke. Concomitant occlusion of the left atrial appendage has a preventive effect (**Supplementary Appendix**). Similarly, the type of LVAD has been identified as an influencing factor for the occurrence of pump thrombosis (**Supplementary Appendix**).

Propensity-matched cohort

In total, 67 patients in the LIS group were matched with 98 patients in the CS group. The variables used in the propensity score match were mentioned previously (statistical methods). After the groups were matched, no significant difference in postoperative severe acute RHF requiring RVAD implantation was observed (CS 19% vs. LIS 10%, $p = 0.12$). However, the RVAD support time was significantly shorter in the LIS group [10 (IQR: 10–12) days in the LIS group vs. 24 (IQR: 12–56) days in the CS group; $p = 0.03$]. Moreover, in the matched cohort, 53% patients with mild RVF, 15% with moderate RVF, 11% with severe RVF, and 20% with severe acute RVF were identified in the CS group. In contrast, in the LIS group, 69% patients with mild RVF, 7.5% with moderate RVF, 10% with severe RVF, and 12% with severe acute RVF were identified. In the statistical analysis, no significant different was observed.

The need to perform a re-exploration because of bleeding was less in the LIS group (9% vs. 18%, $p = 0.09$). LIS was also associated with a shorter ventilation time and a significantly lower rate of tracheotomy (16% vs. 30%, $p = 0.04$). The duration of ICU stay for the LIS group was significantly shorter than that for the CS group [2 (IQR: 2–5) days vs. 4 (IQR: 2–12) days, $p < 0.01$]. However, there was no significant reduction in the hospital length of stay [36 (IQR: 25–55) days vs. 38 (IQR: 26–55) days, $p = 0.6$]. The overall stroke rate in the matched cohort was 14.5% ($n = 24$). There was no significant difference in the incidence rate of stroke between both groups [14% (13/98) in the CS group vs. 16% (11/67) in the LIS group; $p = 0.6$; **Table 2**]. Perioperative stroke (within 30 days of surgery) occurred in 5 (5.1%) patients in the CS group and in 4 (6.0%) in the LIS group. In the first 6 months after LVAD implantation, we observed collectively 9 (9.2%) stroke events in the CS group and 4 (6.0%) in the LIS group. **Figure 1B** shows freedom from stroke for the matched cohort. The incidence rate of pump thrombosis during follow-up was also not significantly different between the groups [6.1% (6/98) in the CS group vs. 7.5% (5/67) in the LIS group; $p = 0.8$]. **Figure 2B** shows freedom from stroke for the matched cohort. In the matching cohort, no concomitant procedure was performed in both groups of patients. To better investigate the impact of the pump type on the outcome and after excluding HVAD and HM II patients, a total of 123 patients were identified in the matched cohort ($n = 74$ in the CS group and $n = 49$ in the LIS group). In this subanalysis, there was no statistical difference in the overall mortality rate during follow-up (41% in the CS group vs. 29% in the LIS group, $p = 0.2$); in the stroke rate (8.1% vs. 10%; $p = 0.8$), and in the pump thrombosis rate (2.7% vs. 2.0%, $p > 0.9$).

The hospital mortality rate was significantly lower in the LIS group (7.5% vs. 19%; $p = 0.03$). However, the 1-year mortality showed no significant difference 24.5% in the CS group vs. 17.9% in the LIS group ($p = 0.35$) (**Figures 3A,B**). Postoperative incidence of driveline infection, wound infection, gastrointestinal bleeding, and the need for hemodialysis were not significantly different between the groups during follow-up. Similarly, the rate of heart transplantation with the device did not differ between both groups.

Discussion

The outcomes of patients who received LVAD implantation have significantly improved over the last 10 years. The main reasons for this are advances in device design, better patient selection, and improved postoperative management (8). Nevertheless, stroke remains a significant complication after LVAD placement. It is a leading cause of death that affects not only outcomes but also the quality of life and transplantation candidacy (9–11). The overall stroke rate in our cohort,

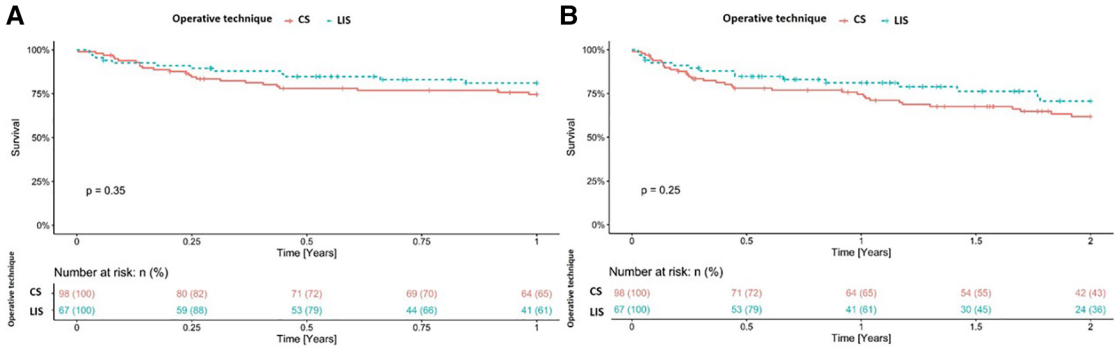

FIGURE 3
Kaplan–Meier curve for all-cause mortality at 1 year (A) and 2 years (B) in the propensity-matched cohort. CS, conventional sternotomy; LIS, less-invasive surgery.

regardless of the implanted device, was 13.7%, which is similar to that in previous published results (12–14). The analysis of the matched cohort showed no differences in the stroke incidence rate between the groups (CS 13% vs. LIS 16%, $p = 0.6$; **Table 2**). There are no large studies that have investigated the stroke rate and its correlation with the surgical approach. In the MOMENTUM 3 trial, the HM3 device was associated with almost half the risk of stroke compared with the HM2 device at 2 years of follow-up (10.1% vs. 19.2%; $p = 0.02$) (13). In contrast, data from the HeartWare HVAD pivotal trials showed an increased risk of stroke compared with the HM2 device. In the ENDURANCE DT trial, a significantly higher number of HVAD patients compared with HM2 patients experienced a stroke at 2 years (29.7% vs. 12.1%; $p < 0.001$) (14). Our results are similar to those of Chiang and colleagues (15). They conducted an unmatched single-center study of 247 total patients comparing HVAD ($n = 163$) vs. HM3 ($n = 84$) with regard to stroke during a median follow-up of 1.2 years in HVAD patients and 1.4 years in HM3 patients. In this context, it is important to mention that patients under ECMO support were excluded from this analysis. Their results showed an overall stroke rate of 12.2% (30/247). Stroke occurred in 24 (14.7%) HVAD patients (15 ischemic and 9 hemorrhagic) and 6 (7.1%) HM3 patients (4 ischemic and 2 hemorrhagic). In multivariate analysis, the HVAD was found to be associated with a significantly higher stroke risk (HR, 2.57; 95% confidence interval, 1.02–6.44; $p = 0.045$). In our unmatched cohort, we observed upon multivariate analysis, almost more than twice the number of stroke events in HVAD patients than in HM3 ones (hazard ratio, 3.31; $p = 0.017$). In the matched cohort, no statistical significance was seen because of the low number of events in HM3 patients (HR, 3.05; $p = 0.175$). There was no significant difference between the devices and surgical technique used in the unmatched cohort (**Table 3**). The LATERAL study was a multicenter, prospective, and nonrandomized trial that evaluated the lateral thoracotomy implantation of the HVAD and compared these results with previous historical data from the sternotomy approach (2). A total of 12 out of 144 (8.4%) subjects were reported to have had a stroke within 6 months postimplant, which was evaluated by using the modified Rankin Scale. These results are comparable to our observation. In our study, the stroke rate in the matched cohort within 6 months postimplant was 7.9% (13/165 for both the CS and LIS groups). The overall stroke rate for the LIS group was 21% in HVAD patients and 12.5% in HM3 patients. In our study, we included all postoperative stroke events (hemorrhagic and ischemic), which were validated by computed tomography; also incidental findings were reported without clinical correlation. This and the longer follow-up could explain the higher rate of stroke in HVAD patients. The LIS approach is technically more demanding. Consequent on such technical difficulties are usually longer operative times. However, in our matched cohort, we observed no difference in CPB time ($p = 0.6$). The detrimental effects of CPB are already well known. These include systemic immune inflammatory response with platelet damage and fibrinolysis, which cause renal dysfunction, acute lung injury, and stroke (16, 17). Moreover, platelet dysfunction and coagulopathy that occur after a CPB increase the risk of perioperative bleeding and the need for blood transfusion, which, in turn, contribute to volume overload and possible RHF. Alternatively, the less-invasive implantation can be performed without using the CPB machine. However, the off-pump approach is associated with a limited exposure of the left ventricular space and can potentially increase the risk of pump thrombosis because thrombi in the left ventricle may not be detected and removed. Hospital mortality was significantly higher in the CS group in the unmatched cohort. The overall mortality was still higher in the CS group but did not reach statistical significance. Following propensity score matching, the hospital mortality rate was significantly lower in the LIS group. This finding merits careful observation in future studies. Our results support the efforts of previous studies in investigating the use of the less-invasive LVAD implantation technique (2, 3, 18, 19). Another important observation in this study was the fact that even after the groups were matched, the LIS group was associated with a lower tracheotomy rate (16% vs. 30%, $p = 0.05$). This difference may be explained by the limited occurrence of surgical trauma and faster recovery for patients with LIS. This is very well mirrored by the shorter ICU stay in the LIS group (2 vs. 4 days, $p < 0.01$). Moreover, the full-sternotomy sparing operation is associated with a better postoperative stability of the thorax and thereby supports the respiratory function and faster weaning from mechanical ventilation. In this study, we observed a decreased incidence of severe RV failure when utilizing an LIS approach for LVAD implantation. This finding has now been well documented across several studies (4, 5, 20). There are many theories of possible protective effects of the LIS approach. Studies have indicated that pericardial opening promotes RV dilatation and changes in the pressure–volume relationship, resulting in impaired RV function (21). Therefore, the preservation of the pericardial restraint over the RV is crucial during the performance of the operation. Moreover, the minimal heart displacement during the LIS approach avoids potential coronary hypoperfusion and preserves the septal function. It has been shown that the septal function constitutes the highest share of the total RV function (22). In our cohort, in the LIS group, only limited pericardial opening was performed with additional closing of the pericardium directly or by the use of a membrane after LVAD implantation. In addition, we found that LIS was associated with a lesser need for postoperative re-exploration for bleeding. Moreover, the LIS patients demonstrated less blood product utilization including fewer packed red blood cells ($p = 0.012$), less plasma ($p = 0.027$), and fewer platelets ($p = 0.016$). Our results are similar to the previous findings (2–5). It is important to note that the avoidance of reoperation and less blood transfusion have a protective effect on the right ventricle function.

Limitations

Several limitations of this study merit consideration. The main limitation of this study is its retrospective nature and the fact that the patients were not randomized to a surgical approach.

Therefore, the patient groups were not identical. To achieve a high level of similarity in preoperative characteristics, we used both multivariable analysis regression and propensity matching. As a result, unlike randomized control trials, propensity score analyses have the limitation that some unmeasured confounding variables may still be present, thus leading to biased results. Because patients with a low INTERMACS of 1 or 2 (114/137 in CS vs. 23/137 in LIS, $p = 0.00019$) and a higher CRP (22 in the CS group vs. 8.05 in the LIS group, $p = 0.00036$) were more likely to receive total sternotomy, these patients were partially excluded by propensity matching as part of the preoperative comparability of patients. Therefore, our results in the matched cohorts have limited applicability to these patients. It should also be noted that in the years up to 2018, LVAD was performed more frequently by using CS rather than LIS, whereas in the last 3 years, the minimally invasive method was preferred. Also the observation periods of the individual patients postoperatively, depending on the time of implantation, can vary greatly and range from a few months to 6 years. However, we can exclude additional uncontrolled factors, which could influence survival and adverse events. Notably, two experienced surgeons performed the LVAD implantation, and this study was limited to a single institution. Our center usually performs a high volume of surgeries for heart failure. Nevertheless, while two different surgeons performed both procedures, this study remains limited to a single institution and may not cover other centers.

Conclusion

In summary, a less-invasive strategy for LVAD implantation is a good alternative procedure to conventional LVAD implantation by full median sternotomy with a potential advantage in the early postoperative period. Postoperative stroke and pump thrombosis remain comparable to the sternotomy approach. Further, no significant difference in all-cause mortality during follow-up was observed. A randomized controlled trial comparing the CS and LIS approaches may be necessary to confirm our findings.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Ethik-Kommission an der Medizinischen Fakultät der Universität Leipzig, Clinical Registration number: 069/15-ek. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MN did the conceptualization, data curation, investigation, and formal analysis, prepared the methodology, carried out supervision and visualization, and wrote the original draft. J-MB was involved in conceptualization, data curation, investigation, visualization, and writing of the original draft. US performed data collection. KJ was involved in data collection and investigation. MS, JJ-N, SF, and SE were involved in data collection. MB was involved in supervision. DS was involved in conceptualization, methodology preparation, project administration, supervision, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2023.974527/full#supplementary-material.

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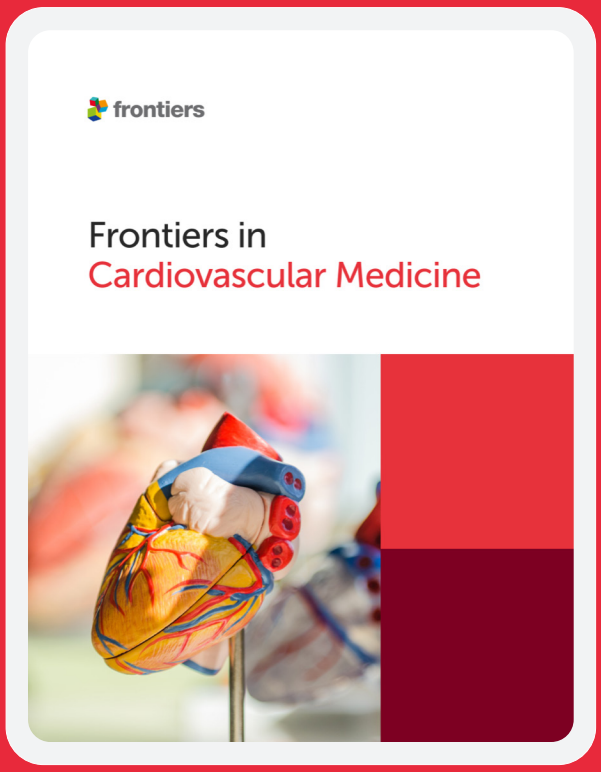

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