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Adverse Remodeling of the Electrophysiological Response to Ischemia-Reperfusion in Human Heart Failure Is Associated with Remodeling of Metabolic Gene Expression

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Abstract

Background—Ventricular arrhythmias occur more frequently in heart failure during episodes of ischemia-reperfusion (I-R), although the mechanisms underlying this in humans are unclear. We assessed, in explanted human hearts, the remodeled electrophysiological response to acute I-R in heart failure, and its potential causes, including the remodeling of metabolic gene expression.

Methods and Results—We optically mapped coronary-perfused left ventricular wedge preparations from 6 human end-stage failing hearts (F) and 6 donor hearts rejected for transplantation (D). Preparations were subjected to 30 minutes of global ischemia, followed by 30 minutes of reperfusion. Failing hearts had exaggerated electrophysiological responses to I-R, with greater action potential duration (APD) shortening ($p < 0.001$ at 8 minutes ischemia; $p = 0.001$ at 12 minutes ischemia) and greater conduction slowing during ischemia, delayed recovery of electrical excitability following reperfusion (F 4.8 ± 1.8 vs. D 1.0 ± 0 mins, $p < 0.05$), and incomplete restoration of APD and conduction velocity early after reperfusion. Expression of 46 metabolic genes were probed using custom-designed TaqMan arrays, using extracted RNA from 15 failing and 9 donor hearts. Ten genes important in cardiac metabolism were downregulated in heart failure, with *SLC27A4* and *KCNJ11* significantly downregulated at a false discovery rate of 0%.

Conclusions—We demonstrate, for the first time in human hearts, that the electrophysiological response to I-R in heart failure is accelerated during ischemia with slower recovery following reperfusion. This can enhance spatial conduction and repolarization gradients across the ischemic border and increase arrhythmia susceptibility. This adverse response was associated with downregulation of expression of cardiac metabolic genes.

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Conflict of Interest Disclosures: None.

Keywords

heart failure; ischemia; reperfusion; electrophysiology; metabolism

Introduction

Acute ischemia and reperfusion (I-R) are profound arrhythmic triggers, with ischemia-induced ventricular tachycardia and fibrillation thought to be responsible for a significant proportion of sudden cardiac deaths,¹ and reperfusion-induced arrhythmias becoming more prevalent as a result of the modern practice of early coronary intervention for acute myocardial infarction.² Failing hearts are significantly more susceptible to ventricular arrhythmias during episodes of acute I-R,³ and although the pro-arrhythmic remodeling of the electrophysiological substrate in heart failure, encompassing the remodeling of conduction, repolarization and calcium homeostasis, has been extensively investigated,^{4,5} there have yet been no systematic studies into the remodeling of responses to external arrhythmic triggers such as acute I-R in human failing myocardium. The precise mechanisms for the enhanced arrhythmia susceptibility during I-R in failing human myocardium remain unclear, in part because of the absence of previous human studies, and a reliance on animal heart failure models, which have recently been shown to have significant electrophysiological differences to humans.⁶

From the cardiac energetics perspective, the failing myocardium is thought to be in a general state of energy-deprivation due to the remodeling of metabolic pathways.^{7,8} Derangements of energy metabolism in heart failure include impaired substrate transport and utilization, impaired oxidative phosphorylation, and impaired ATP transfer and utilization, all of which decrease the capacity for ATP supply.⁷ Because the failing myocardium is constantly on the verge of energy starvation, this can be expected to have a negative impact on the myocardial response to metabolic insults such as acute I-R, although experimental evidence for this is sparse, especially from humans.

We hypothesized that the electrophysiological response to acute I-R is adversely remodeled in human end-stage heart failure, which contributes to a more pro-arrhythmic substrate during I-R episodes. We also hypothesized that the adverse electrophysiological response is associated with the downregulation of transcription of genes critical in the multiple components of cardiac metabolism, including glucose metabolism, fatty acid metabolism and mitochondrial function. We systematically investigated, for the first time in intact human myocardium, the remodeled electrophysiological response to acute I-R in human heart failure by performing high-resolution optical mapping of transmembrane voltage on explanted human hearts during global I-R. We also probed the expression of selected genes involved in cardiac metabolic pathways using custom-designed low-density Taqman gene expression arrays to investigate the remodeling of metabolism in human heart failure.

Methods

The methods are briefly described here. Expanded methods can be found in the Supplemental Material.

Patient and donor characteristics

This study was approved by the Washington University in Saint Louis Institutional Review Board. Twelve explanted human hearts were used for optical mapping experiments (6 end-stage failing hearts (F) and 6 non-failing hearts from deceased donors that were rejected for transplantation (D) – details in Tables 1 & 2). Myocardial samples from 24 human hearts were used for metabolic gene expression studies (15 F, 9 D – Supplementary Table 1).

Optical mapping experimental protocol

The human isolated left ventricular wedge preparation, perfused via a marginal branch of the circumflex artery, was used for the optical mapping experiments, as previously described,^{6,9,10} (Supplementary Figure 1). Preparations were immobilized with 10 μ M blebbistatin (Tocris Bioscience, Ellisville, MO) and stained with 4 μ M di-4-ANEPPS (Molecular Probes, Eugene, OR), and the transmural surfaces of the preparations were optically mapped at a spatial resolution of 200-300 μ m/pixel and temporal resolution of 1000 samples/second. The experimental protocol is shown in Supplementary Figure 1C. An S1S1 restitution protocol was performed at baseline and the preparations were then subjected to 30 minutes of global no-flow ischemia, followed by 30 minutes of reperfusion. Following the reperfusion phase, the restitution protocol was repeated. The preparations were maintained at 37°C $\pm$ 0.5°C throughout the duration of the experiments.

Optical mapping data analysis and statistics

The optical mapping data were processed, filtered and analyzed using a custom MATLAB program as previously described, using MATLAB package R2013a.¹¹ For the analyses that involved three factors (e.g. disease group, cycle length, and myocardial region), the statistical methodology used was Linear Mixed Models for Repeated Measures, which takes into account correlated observations, heterogeneous variances and missing data. SAS 9.3 and SPSS 21 were used to obtain the estimated effects of interest and corresponding p-values. Details of statistical methods are presented in the Supplementary Methods. All data are presented as mean $\pm$ S.E.M, and p<0.05 was considered statistically significant.

Low-Density Taqman Gene Expression Arrays

Total RNA was extracted from left ventricular specimens from 24 human hearts (9 D, 6 failing – ischemic cardiomyopathy (F-ICM), and 9 failing non-ischemic cardiomyopathy (F-NICM)) using the RNEasy Fibrous Tissue Mini Kit (Qiagen, Valencia, CA). Custom-designed lowdensity Taqman gene arrays (Applied Biosystems, Foster City, CA) were then used to probe for the presence of 46 targets as previously described.¹² Genes selected for analysis included genes critical for glucose and fatty acid metabolism, mitochondrial function and regulation of energy metabolism, and are listed in Supplementary Table 2). Multiple comparisons statistical testing was conducted using the false discovery rate (FDR) method, as previously described.¹³

Results

Longer action potential durations and slower conduction velocities in failing hearts

We compared the electrophysiology of end-stage failing hearts (F) with non-failing donor hearts (D) by optical mapping of transmembrane voltage in intact human left ventricular preparations, and demonstrated significant differences at baseline. F hearts had longer action potential durations (APD) than D hearts across different stimulus cycle lengths (CLs) ($p=0.008$; Figures 1A & 1D). There was a gradient of APD across the transmural surface, going from longer APDs in the subendocardium to shorter APDs in the subepicardium (Figure 1B). Both groups exhibited APD restitution properties (Figure 1C). The transmural APD gradient ($APD_{\text{endo}}-APD_{\text{epi}}$) diminished significantly at shorter CLs ($p=0.02$; factors for repeated-measures ANOVA were disease (D vs. F) and CL). The reduction was statistically significant for D hearts (49.1 ± 7.3 ms at $CL=2000$ ms vs. 9.3 ± 4.6 ms at $CL=300$ ms; $p=0.01$) but not for F hearts. Mean transmural conduction velocities (CVs) were also slower in F vs. D hearts at baseline ($p=0.001$; Figure 2A & 2B). Both D and F hearts exhibited CV restitution, with greater restitution in F hearts (Figure 2C).

Accelerated APD shortening during ischemia and slower recovery after reperfusion in failing hearts

We then demonstrated that the APD response to I-R was exaggerated in heart failure. When the ventricular wedge preparations were subjected to global no-flow ischemia, an initial transient increase in APD was observed, reaching peak values at 2 minutes after ischemia onset, followed by progressive shortening of APD until there was a loss of electrical excitability (Figures 3A-B). Time to loss of electrical excitability during ischemia was not different between D and F groups: 11.7 ± 1.5 vs. 12.0 ± 1.9 mins (Figure 3D). Although the general trends were similar, the shortening of APD was significantly more rapid in F hearts during acute ischemia. Using Linear Mixed Models for Repeated Measures to fit the APD changes between 2 and 12 minutes of ischemia, slopes of the linear fits were steeper for F hearts compared to D hearts ($p=0.0004$; Supplementary Figure 2). As shown in Figure 3C, this resulted in greater absolute changes in APD for F hearts at 8 minutes ($p<0.001$) and 12 minutes ($p=0.001$) of ischemia.

Secondly, the recovery of APD was delayed in F hearts following reperfusion. All 6 D hearts regained electrical excitability at 1 minute following reperfusion, compared with only 2 out of 6 F hearts at this stage. The mean time to recovery of excitability was prolonged for F hearts compared to D hearts (4.8 ± 1.8 min vs. 1.0 ± 0 min, $p=0.04$; Figure 3D). Thirdly, there was only partial recovery of APD towards pre-ischemic values for the 2 F hearts that recovered electrical excitability in the first minute post-reperfusion (42% recovery of APD) compared with full recovery for all D hearts.

Greater CV slowing during ischemia and slower recovery during reperfusion in failing hearts

Similarly, the changes in CV during I-R were also more marked in heart failure. Acute ischemia caused slowing of conduction in both groups (Figure 4). After 10 minutes of ischemia, subendocardial CVs slowed by 43% in D hearts ($p=0.003$) and by 55% F hearts

($p=0.005$) (Figure 4B-D). As with APD, the slopes of the linear fits for CV changes between 0 and 12 minutes ischemia were again steeper for F hearts compared to D hearts ($p<0.0001$; Supplementary Figure 2). The restoration of CV towards pre-ischemic values was also delayed in F hearts, with CVs only being partially restored to baseline values early after reperfusion (61.8% recovery of CV at 2 minutes reperfusion vs. 85% recovery in D hearts).

Remodeled metabolic gene expression in end-stage heart failure

We also showed that the remodeled electrophysiological response in heart failure was associated with the remodeling of metabolic gene expression. The majority of metabolic genes we studied were downregulated in heart failure (Figure 5A), though not all achieved statistical significance. The fold-changes and the associated q-values for all genes probed are listed in rank order in Supplementary Table 3. At a false discovery rate (FDR) of 0%, 2 genes were significantly downregulated, *KCNJ11* (encoding for Kir6.2 subunit of K_{ATP} channels) and *SLC27A4* (encoding fatty acid transporter 4) with fold-changes of 0.55 and 0.55 respectively, and 2 genes upregulated, *NPPA* (atrial natriuretic peptide) and *PDK4* (pyruvate dehydrogenase kinase 4) with fold-changes of 9.13 and 2.32 respectively (Figure 5B). At a false discovery rate of 5.5%, a further 8 genes were significantly downregulated: *ATP2A2* (encoding SERCA2A), *SLC2A1* (glucose transporter 1), *KCNJ8* (K_{ATP} channel subunit Kir6.1), *TSPO* (translocator protein), *NT5C3* (5'-nucleotidase), *PPARA* (peroxisome proliferator-activated receptor- α), *SLC2A4* (glucose transporter 4), and *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase) (Figure 5C).

Discussion

In this study, we present evidence for the first time from human myocardium, demonstrating that heart failure adversely impacts upon the electrophysiological response to acute I-R, and that the remodeled electrophysiological response was associated with remodeling of metabolic gene expression. Specifically, our main findings were that, in human heart failure: (1) APD was prolonged and CV was slower at baseline, (2) the shortening of APD and the slowing of CV during ischemia was accelerated, (3) recovery of electrical excitability and the restoration of APD and CV towards baseline values was delayed following reperfusion, (4) the expression of genes critical in cardiac metabolism was downregulated.

Remodeling of the electrophysiological substrate in heart failure

At baseline, failing hearts had prolonged APDs, slower CVs and steeper CV restitution compared with non-failing hearts, in line with our previous studies into the remodeling of activation and repolarization in failing human myocardium.^{6,10} These well-characterized changes contribute to the pro-arrhythmic substrate in failing myocardium.^{4,5} We also observed relatively greater increases in APD at the subepicardial and midmyocardial layers compared with the subendocardial layer, resulting in a reduction in the calculated transmural APD gradient, again in agreement with our previous findings in human tissue.⁶ This was however in contrast to data from canine experiments showing an increase in transmural heterogeneity in heart failure models,¹⁴ highlighting the importance of validating findings from animal studies in human tissue.

Remodeling of the electrophysiological response to arrhythmic triggers in heart failure

(I) Accelerated APD shortening and CV slowing during acute ischemia—The ischemic myocardium is known to undergo a series of pro-arrhythmic electrophysiological changes that occur in response to the loss of ATP, acidosis and increased extracellular K^+ concentrations during ischemia.¹⁵ Acute ischemia causes APD shortening,¹⁶ and conduction slowing,¹⁷ which we observed both groups. However, a key finding was that these changes were significantly accelerated in failing hearts, with greater magnitudes and rates of APD shortening and CV slowing at any given duration of ischemia. These adverse electrophysiological responses to acute ischemia in heart failure were previously described in a rabbit model of combined pressure and volume overload.¹⁸ Our study, which is the first systematic study of activation and repolarization during I-R in human hearts, confirms these findings in humans for the first time. In both groups, we observed a transient increase in APD during ischemia before APD shortening, which may be due to a reduction in I_{Ks} function in response to ATP depletion before K_{ATP} channels open.¹⁹

The adverse response to acute ischemia in human heart failure has significant implications on arrhythmogenesis when extrapolated to regional ischemia, which occurs in the context of coronary artery disease. During regional ischemia, these accelerated changes in heart failure would be expected to enhance spatial gradients of APD and CV across the ischemic border zone, between the ischemic and non-ischemic myocardium (Supplementary Figure 5). The enhanced spatial dispersion and increased heterogeneity of APD and CV at the ischemic border zone would be expected to predispose failing hearts to ventricular arrhythmias by increasing the likelihood of unidirectional block and re-entry.

(II) Delayed recovery of electrical excitability, APD and CV following reperfusion—Following a period of ischemia, reperfusion restores the supply of ATP and returns the pH and K^+ concentration towards baseline values, thus triggering the rapid restoration of CV and APD towards that of pre-ischemic levels.²⁰ Electrical activity is often re-established in the previously ischemic myocardium within the first few seconds of reperfusion in experimental models.²⁰⁻²² During this immediate phase following restoration of flow, the myocardium is thought to be at greatest risk of re-entrant arrhythmias as inhomogeneities of APD and CV exist in and around the previously ischemic myocardium.^{23,24}

In our human studies, we observed that reperfusion can rapidly restore APD and CV to baseline values within 60 seconds of reperfusion in non-failing donor hearts. However, another key finding of this study is that the recovery of electrical excitability, APD and CV were all significantly delayed in the end-stage failing hearts, which to our knowledge, has not been previously reported in humans. In the context of regional ischemia, delayed recovery of APD and CV following reperfusion in heart failure would enhance spatial heterogeneities across the ischemic border zone in the first few minutes post-reperfusion, which would consequently increase the susceptibility to reperfusion arrhythmias.²⁴ Furthermore, the delay in recovery of electrical excitability can have the effect of prolonging the vulnerable time window postreperfusion, during which significant APD and CV inhomogeneities persist, thus also increasing the substrate for reperfusion arrhythmias.

Remodeling of metabolism in human heart failure

A possible factor responsible for the accelerated electrophysiological changes during acute ischemia and the delayed electrophysiological recovery following reperfusion in heart failure is the remodeling of metabolism.^{7,8} The reduced capacity for ATP supply and reduced baseline concentrations of ATP in heart failure would be expected to accelerate changes that occur during ischemia when ATP production is interrupted, and to delay recovery during reperfusion when the capacity for ATP production has just been restored.

The results from our metabolic gene expression studies confirm that multiple components of cardiac metabolism were remodeled in human heart failure. Transcription of genes responsible for fatty acid transport (*FATP4*), glucose transport (*GLUT1* and *GLUT4*), mitochondrial function (*TSPO*) and the purine metabolism pathway (*NT5C3*) were significantly downregulated, which can be expected to reduce the failing myocardium's capacity to maintain ATP production and adversely impact on the response to acute I-R. Furthermore, peroxisome proliferator-activated receptor- α (*PPARA*) was downregulated, which is thought to be responsible for the switch in substrate utilization from fatty acids to glucose,⁷ with glucose metabolism being less efficient in generating ATP than fatty acid metabolism. *PDK4* was significantly upregulated in heart failure, with recent reports suggesting that *PDK4* plays a critical role in angiotensin II-mediated insulin resistance and metabolic inefficiency.²⁵ Genes encoding two subunits of the K_{ATP} channel (*KCNJ8* and *KCNJ11*) were downregulated, although it remains unclear how this impacts overall channel function. We also observed the expected upregulation of *NPPA* and downregulation of *SERCA2A* in the failing hearts, both markers of heart failure, confirming the validity of our experiments.

Our results showing the remodeling of metabolic gene expression are in general agreement with previous studies in human heart failure.²⁶ The adverse remodeling of metabolism in our study was associated with the remodeled electrophysiological response to acute I-R, and although this link was associational in this study and further studies are required to prove causality, our data support a growing view that arrhythmia susceptibility may be intimately linked to cardiac metabolism and that altered metabolism may be arrhythmogenic under a number of circumstances.²⁷

Implications for anti-arrhythmic therapeutic strategies in heart failure

Our results underline the importance of considering the remodeling of responses to external arrhythmic triggers in any anti-arrhythmic therapeutic approach in heart failure. Current efforts to prevent arrhythmias in heart failure have focused on targeting the remodeled electrophysiological substrate.^{4,5} Our data suggest that the substrate should not be considered in isolation, and successful therapeutic strategies should also address the remodeled response to triggers. With respect to modifying the adverse response to acute I-R, one possible strategy may be to target the metabolic remodeling and correct the bioenergetics deficit associated with heart failure.^{7,27}

Study limitations

A limitation of the optical mapping studies is the need for excitation-contraction uncouplers, which can affect myocardial energetics by maintaining a state of ATP production reserve, which may then mask pathological changes that may otherwise have been evident during acute I-R. Our protocol did not include adrenergic stimulation, which is increased in heart failure, and the exclusion of catecholamines from our protocol is also likely to reduce the differences in metabolic states between F and D hearts. However, despite this, we were still able to detect differences between the failing and non-failing hearts, suggesting that even minor metabolic perturbations are sufficient to elicit differences in response between the two groups.

Another limitation is that left ventricular wedge preparation is of insufficient size to sustain re-entry (see Supplementary Results), and we therefore did not observe any spontaneous arrhythmias during the acute I-R experiments. We have had to extrapolate the observed exaggerated changes in failing hearts to increased arrhythmia susceptibility during regional I-R based on the knowledge that increased dispersion of repolarization and conduction is proarrhythmic (Supplementary Figure 5). Finally, we only measured APDs during acute ischemia, and did not specifically quantify effective refractory periods during ischemia, and therefore cannot exclude the possibility that there were differences in post-repolarization refractoriness during ischemia between groups that may further affect gradients of repolarization.²⁸

Conclusions

We provide novel mechanistic insights from human heart experiments showing that the electrophysiological response to acute I-R is adversely remodeled in end-stage heart failure, with accelerated changes during ischemia and delayed recovery following reperfusion, both of which can contribute towards a pro-arrhythmic substrate. We also demonstrate the downregulation of transcription of genes critical in cardiac metabolism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Optical action potentials and APDs at baseline. (A) Representative optical APs showing longer APs for a failing heart compared to a donor heart. (B) Transmural APD80 maps for a donor heart showing longer APDs in the subendocardium compared to the subepicardium. (C) APD80 restitution curves. (D) Longer mean APD80 in failing hearts (D vs. F: $p=0.008$; factors in the statistical analysis: disease (D vs. F), cycle length and transmural region).

Figure 2.

Transmural conduction velocities at baseline. (A) Activation maps for a D heart and an F heart during endocardial pacing at CL=1000 ms and CL=350ms. CV was slower for the F heart as evidenced by crowding of isochrones. (B) Mean CVs were slower in the F group compared to the D group (D vs. F: $p=0.001$; factors in the statistical analysis: disease (D vs. F), cycle length and transmural region). (C) CV restitution curves showing steeper CV restitution in failing hearts.

Figure 3.

Changes in APD during ischemia and reperfusion. (A & B) Representative optical APs and APD80 maps for a donor and a failing heart. Mean APD80 during I-R are also shown (n=6 each). APD80 prolonged in the first 2 minutes of ischemia followed by progressive shortening, until loss of electrical excitability. APDs returned to baseline following reperfusion, but this was delayed in the F hearts, with only partial recovery in the first few minutes following reperfusion. (C) The shortening of APD80 was more rapid in F hearts, with greater magnitudes of APD shortening (D vs. F: ** $p < 0.01$, *** $p < 0.001$; factors in the statistical analysis: disease (D vs. F), ischemia duration and transmural region). (D) Effective refractory periods were longer, and time to recovery of electrical excitability after reperfusion was prolonged, in the F group. There was no difference in time to loss of electrical excitability during acute ischemia (* $p < 0.05$).

Figure 4.

Changes in CVs during acute ischemia-reperfusion. (A & C) Activation maps at baseline and following 10 minutes of ischemia. Transmural CV was reduced following ischemia compared to baseline. (B & D) Mean CV during acute I-R. Conduction slowed progressively with acute ischemia and was slower in the F group at any given time-point during ischemia. CVs were restored to baseline following reperfusion, but this process was slower in F hearts.

Figure 5.

Downregulation of metabolic gene expression in heart failure. (A) Mean relative quantification (RQ) values for gene expression of probed genes for D and F hearts, with each point on the graph representing a gene. Genes below the dashed ($x=y$) line were downregulated in F hearts and genes above the dashed line were upregulated. Two genes (*NPPA*, *PDK4*) are not represented due to high RQ values. (B) At a false discovery rate (FDR) of 0%, two genes were significantly downregulated (*SLC27A4*, *KCNJ11*) and two genes upregulated (*NPPA*, *PDK4*). Gene names are shown in *italics* and the proteins shown in **bold**. (C) At an FDR of 5.5%, a further 8 genes were significantly downregulated in F hearts (*ATP2A2*, *SLC2A1*, *KCNJ8*, *TSPO*, *NT5C3*, *PPARA*, *SLC2A4* and *GAPDH*).

Table 1

Clinical information for the six donors of non-failing hearts (D), whose hearts were used for optical mapping studies

	Age	Gender	Cause of death	Reason unsuitable for transplantation
D #1	51	F	Stroke	Moderate LV hypertrophy
D #2	34	F	Stroke	Mild LV hypertrophy
D #3	65	M	Respiratory failure	Age
D #4	61	F	Stroke	Age
D #5	54	M	Stroke	Mildly impaired LV ejection fraction
D #6	61	F	Stroke	Age

LV: Left ventricular

Table 2

Clinical information for the six patients with end-stage heart failure (F), whose hearts were used for optical mapping studies.

	Age	Gender	Diagnosis	Other co-morbidities/relevant details
F #1	57	M	Non-ischemic CM	VT, CRT, ICD, LVAD, Type 2 Diabetes
F #2	35	M	Non-ischemic CM	AF, CRT, ICD, LVAD
F #3	65	M	Ischemic CM	AF, Type 2 Diabetes
F #4	48	M	Non-ischemic CM	VT, CRT, ICD, LVAD
F #5	68	M	Ischemic CM	ICD, LVAD
F #6	63	M	Non-ischemic CM	AF, CRT, ICD, LVAD, AVR

CM: Cardiomyopathy, VT: Ventricular tachycardia, CRT: Cardiac resynchronization therapy, ICD: Implantable Cardioverter Defibrillator, LVAD: Left ventricular assist device, AF: Atrial fibrillation, AVR: Aortic valve replacement