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CHEMOKINE PATTERNS AND RIGHT HEART FAILURE IN MECHANICAL CIRCULATORY SUPPORT

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Abstract

Background—Right Ventricular failure (RVF) complicates 9–44% of LVAD implants postoperatively. Current prediction scores perform only modestly in validation studies, and do not include immune markers. Chemokines are inflammatory signaling molecules with a fundamental role in cardiac physiology and stress adaptation. We aimed to study chemokine receptor regulation in LVAD recipients who develop RVF.

Methods—Expression of chemokine receptor genes 3–8 was examined in the peripheral blood of 111 LVAD patients, collected 24 hours prior to implant. RNA was isolated using a PAXgene protocol. Gene expression was assessed using a targeted microarray (RT2 Profiler PCR array, Qiagen). Results were expressed as PCR cycles to threshold and normalized to the average of 3 control genes- GAPDH, HPRT1 and B2M. Secondary outcomes studied were 1 year mortality and long-term RV failure (RVF-LT).

Results—Chemokine receptors CCR3, CCR4, CCR6, CCR7 and CCR8 were down regulated in LVAD recipients who had RVF. Within this cohort of patients, CCR4, CCR7 and CCR8 were further down regulated in those that required mechanical support of their RV. Additionally, under-

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expression of CCR3–8 was independently associated with an increased risk of mortality at 1 year, even after adjusting for RVF. Chemokine receptor expression did not predict RVF-LT in our patient cohort.

Conclusions—Pre-LVAD chemokine receptor down regulation is associated with RVF and increased mortality after implant. Inflammatory signatures may play an important role in prognostication in this patient population.

Keywords

Left Ventricular Assist Devices; Right Ventricular failure; Survival; Chemokine receptors; Inflammation; Prognostication

Introduction

Heart failure (HF) is an epidemic, with an estimated prevalence of more than 23 million worldwide (1). Left Ventricular Assist Devices (LVADs) are approved for use both as a bridge to transplant (BTT) and as destination therapy (DT) for New York Heart Association (NYHA) class IV heart failure based on significantly improved outcomes compared to medical therapy (2–5). LVAD utilization continues to increase, with more than 15,000 devices implanted thus far, and new implants approaching 2,500 per year (6).

Right ventricular failure (RVF) complicates 9–44% of LVAD implants post-operatively. The accompanying syndrome of multi-organ failure is associated with poor outcomes including reduced survival, higher resource utilization and poor long-term functional capacity (7,8). The complex underlying pathophysiology makes prediction difficult (9). Early, planned biventricular support is associated with better survival than delayed conversion, iterating the importance of identifying patients at high risk of RVF (10). While RVF is usually reported in the early post-operative period, it can occur as a late complication as well. Little is known about the incidence, determinants and outcomes of long-term RVF in LVAD recipients (11).

Current RVF prediction scores incorporate HF severity, laboratory evidence of end organ damage, right heart hemodynamics, and history of cardiac procedures, but perform only modestly with C statistics of 0.61–0.66 in external validation studies (8,12). Echocardiographic parameters have been found to predict RVF inconsistently, and are limited by inherent difficulties in technique, standardization and RV load dependency (12).

There is increasing evidence to implicate the immune response and resultant inflammation in the pathophysiology of HF (13). Chemokines are a family of chemotactic cytokines. They play an important role in modulating the cardiac stress response via autocrine and paracrine effects (14). Chemokines mediate their effects by binding to chemokine receptors, which are members of a class of seven-transmembrane G protein-coupled receptor proteins (15). While several studies have focused on abnormal chemokine activation in the immuno-pathogenesis of heart failure (16), few have studied chemokine receptor regulation. Early evidence suggests that down regulation of several chemokine receptors is associated with cardiomyopathy progression, and that up regulation may mitigate disease severity (17–23). However, these early findings have yet to be confirmed in clinical studies, and chemokine

receptor regulation has never been investigated in the setting of RVF. Therefore, we aimed to study chemokine receptor regulation in LVAD patients who develop RVF.

Methods

Patient population

We enrolled 111 LVAD patients from November 2007 to October 2014 at the University of Pittsburgh Medical Center in the study. Patients who signed informed consent were consecutively enrolled. This protocol is approved by the University of Pittsburgh Institutional Review Board. Additionally, 8 healthy controls and 6 “candidate” LVAD patients with advanced heart failure but who did not eventually receive LVADs were included in the study. Devices used are noted in Table 1.

Outcomes

Data was collected in a retrospective manner, and was blinded to chemokine receptor expression data. Outcomes were adjudicated by a multi-disciplinary team including cardiothoracic surgeons, cardiologists and bioengineers.

Primary outcomes

RVF: RVF was defined as symptoms or signs of persistent RV failure characterized by documentation and manifestations of elevated central venous pressures, and requiring >14 consecutive days of inotropes after implant and/or RV mechanical support (RV MCS).

RV MCS: RV MCS was defined as requiring right ventricular assist devices (RVAD), and included both temporary and permanent, and planned and unplanned RVADs. Planned RVAD recipients were those taken to the operating room with a pre-determined plan for biventricular support. Unplanned RVAD patients were initially thought to be candidates for isolated LVAD support, but developed acute RVF requiring unexpected RVAD placement after LVAD implantation.

Secondary outcomes

Mortality: Death during a follow-up period of 1 year after implant, with transplant events censored.

RVF-LT: Long-term RVF (RVF-LT) was defined as symptoms or signs of persistent RV failure characterized by documentation and manifestations of elevated central venous pressures, and hospital readmission for iv diuretics and/or inotropes and/or vasodilators and/or RV MCS, during a surveillance period of 1 year after implant or until transplant or death, whichever was earlier. Time to development of RVF-LT was defined as time from implant until date of first hospital admission for RVF-LT, as determined by the criteria above.

Gene expression

Expression of chemokine receptors was examined in patient’s peripheral blood cells collected 24 hours prior to LVAD; and in healthy controls and candidate LVAD patients.

RNA was isolated using PAXgene protocol. Gene expression was assessed using a targeted microarray (RT2 Profiler PCR array, Qiagen). Results were expressed as PCR cycles to threshold (Ct) and normalized to the average of 3 control genes- GAPDH, HPRT1 and B2M. More highly expressed genes have a lower Ct; ΔCt was calculated as the difference between the chemokine receptor gene Ct and the average of GAPDH, HPRT1 and B2M gene Ct. Log-fold differences in expression were reported using the $2^{-\Delta\Delta CT}$ method (24).

Statistical analysis

Descriptive statistics—For comparing baseline patient characteristics, continuous data was evaluated for normality. Accordingly, between-group comparisons with Student *t* or Mann-Whitney testing were performed. Categorical data was compared with Fisher exact test.

Primary outcomes—LVAD recipients were randomly divided into a derivation cohort (n=18) [Figure 1] and a validation cohort (n=93) [Figure 2] for the analysis of primary outcomes of RVF and RV MCS. Student *t* testing was performed to compare differences in gene expression between those with and without the primary outcome.

Secondary outcomes—Analyses for secondary outcomes of mortality and RVF-LT were performed on the complete cohort (n=111) of LVAD patients. Cox univariate analysis was employed to study the effect of baseline chemokine receptor expression on secondary outcomes. As no cut-off value of prognostic significance could be identified, normalized CCR expression values (ΔCt, calculated as noted previously) were used as a continuous variable in the analysis.

Multivariate modeling—Two multivariate models were constructed, one each for the outcomes of RVF and mortality. Univariate analysis was first performed on clinically relevant determinants of risk (age, gender, DT/BTT indication, etiology, pre-implant IABP requirement, pre-operative laboratory data: BUN, creatinine, total bilirubin, WBC count, INR, hematocrit, albumin and MELD score, pre-operative hemodynamics by right heart catheterization (RHC): right atrial pressure (RAP), mean pulmonary artery pressure, pulmonary capillary wedge pressure (PCWP), RA/PCWP ratio, trans-pulmonary gradient, cardiac index (CI), heart rate (HR), pulmonary vascular resistance and stroke volume index, pre-operative echocardiographic data: severity of TR, TR jet velocity) to determine which covariates to include in the multivariate model. Variables with $p < 0.05$ by univariate analysis were chosen for inclusion in the multivariate models, along with age and gender.

For RVF outcome, patients were dichotomized by median ΔCt value (ΔCt greater than median indicating gene under-expression) into two groups: > median or < to the median. Binomial logistic regression was performed to assess the relationship between CCR expression and RVF after adjusting for the correlates determined above. For survival outcome, Cox proportional hazards modeling using normalized CCR expression data (ΔCt) as a continuous predictor variable was employed, as no cut-off value of prognostic significance could be identified.

Data analysis was performed with SPSS (IBM, version 23.0). P values of less than 0.05 were considered statistically significant.

Results

Chemokine receptor expression compared to healthy controls

Compared to healthy controls (n=8), all chemokine receptors tested were down regulated in LVAD recipients (n=111): CCR3 (2.64 fold decrease, p=0.022), CCR4 (4.29 fold decrease, p< 0.001), CCR5 (1.62 fold decrease, p=0.016), CCR6 (5.66 fold decrease, p< 0.001), CCR7 (6.96 fold decrease, p< 0.001) and CCR8 (2 fold decrease, p=0.048). There was no significant difference in chemokine receptor expression between “candidate” LVAD patients (those who were evaluated for, but did not ultimately receive an LVAD) versus LVAD patients.

Chemokine receptor expression: Derivation cohort

Within the derivation cohort (n=18), 9 patients (50%) developed RVF and 6 (33.3%) required RV MCS. Compared to those patients that did not develop RVF, the following were under-expressed in RVF patients: CCR3 (3.25 fold decrease, p=0.042), CCR4 (2 fold decrease, p=0.037), CCR6 (2 fold decrease, p=0.024) and CCR8 (2.14 fold decrease, p=0.043). RV MCS patients had lower expression of CCR3 (4.92 fold decrease, p=0.011), CCR6 (2.46 fold decrease, p=0.004) and CCR8 (2.64 fold decrease, p=0.001) than their counterparts who did not require RV MCS. Baseline patient characteristics of the derivation cohort are noted in Table 1.

Chemokine receptor expression: Validation cohort

Within the validation cohort (n=93), 39 patients (41.9%) developed RVF and 29 (31.2%) required RV MCS. LVAD patients who met criteria for RVF had lower expression of CCR6 (1.41 fold decrease, p= 0.019) and CCR7 (1.41 fold decrease, p= 0.015), Figure 3a. Compared to patients that did not require RV MCS, those requiring RV MCS significantly under-expressed CCR4 (1.52 fold decrease, p= 0.007), CCR6 (1.52 fold decrease, p= 0.005), CCR7 (1.62 fold decrease, p= 0.003) and CCR8 (1.74 fold decrease, p= 0.005), Figure 3b. Baseline patient characteristics of the validation cohort are noted in Table 1.

Chemokine receptor expression within RV MCS cohort

Within the RV MCS cohort (n=35), there was no significant difference in chemokine receptor expression between those that required permanent (n=19) versus temporary (n=16) support of their RV. Additionally, there was no difference in chemokine receptor expression between patients who had planned (n=17) versus unplanned (n=18) RV MCS.

Chemokine receptor expression based on type of RV failure

Within the RVF cohort (n=48), those patients that required RV MCS (n=35) down-regulated CCR4 (1.62 fold decrease, p=0.039), CCR7 (1.62 fold decrease, p=0.044) and CCR8 (2.14 fold decrease, p=0.006) when compared to those patients that met RVF criteria due to inotrope requirement (n=13).

Chemokine receptor expression based on etiology of heart failure

There were no significant differences in chemokine receptor expression between ischemic (n=52) versus non-ischemic (n=53) cardiomyopathy patients.

Chemokine receptor expression and survival

There were 32 total deaths by 1 year post-implant within the entire LVAD cohort (n=111). Chemokine receptor down-regulation (increasing ΔCt values) was associated with an increased risk of 1 year mortality with each unit increase in ΔCt: CCR3 (HR: 1.12, 95% CI 1.06–1.19, p<0.001), CCR4 (HR: 1.15, 95% CI 1.07–1.25, p<0.001), CCR5 (HR: 1.09, 95% CI 1.01–1.17, p=0.019), CCR6 (HR: 1.14, 95% CI 1.06–1.23, p=0.001), CCR7 (HR: 1.09, 95% CI 1.03–1.15, p=0.003) and CCR8 (HR: 1.17, 95% CI 1.05–1.31, p=0.006). (Figure 4)

Chemokine receptor expression and Long-term RVF

Of 111 patients, 90 LVAD recipients who did not require permanent RV MCS (n=19) or chronic home inotropes for RV support (n=2) after LVAD implant were included in RVF-LT analysis. Mean follow-up period was 218.78 + 136.11 days. There were 9 patients (10%) that were adjudicated as having developed RVF-LT during a 1 year surveillance period after implant. Mean time to development of RVF-LT was 129 + 97 days. There was no association between chemokine receptor expression (CCR3–8) and development of RVF-LT. Baseline characteristics of these patients are noted in Table 2.

Multivariate modeling

RVF—Patients with a complete set of pre-operative demographic and laboratory data (n=111) were included in multivariate modeling. CCR expression was stratified by median values of ΔCt for the cohort (3.13, 5.48, 2.51, 6.05, 2.81, 9.04 for CCR3 though CCR8, respectively). After controlling for age, gender, pre-implant INR and hematocrit, patients who had decreased expression (higher ΔCt) of CCR5 (HR: 2.39, 95% CI 1.06–5.42, p=0.037), CCR6 (HR: 3.32, 95% CI 1.41–7.83, p=0.006) or CCR7 (HR: 2.77, 95% CI 1.15–6.65, p=0.023) each had increased risk of developing RVF. Patients who had decreased expression of CCR6 (HR: 5.04, 95% CI 1.85–13.72, p=0.002), CCR7 (HR: 4.15, 95% CI 1.5–11.43, p=0.006) or CCR8 (HR: 3.1, 95% CI 1.18–8.13, p=0.021) had an increased risk of requiring RV MCS.

Survival—Patients with a complete set of pre-operative demographic and laboratory data (n=106) were included in multivariate modeling. Normalized CCR expression data was used a continuous predictor variable in the model as no cut-off value of prognostic significance related to survival could be identified. After adjusting for age, gender, pre-implant WBC, hematocrit, albumin and MELD score, down-regulation of each chemokine receptor (CCR3–8) tested remained significantly associated with mortality: CCR3 (HR: 1.1, 95% CI 1.02–1.19, p=0.018), CCR4 (HR: 1.14, 95% CI 1.03–1.25, p=0.008), CCR5 (HR: 1.09, 95% CI 1.01–1.18, p=0.042), CCR6 (HR 1.13, 95% CI: 1.02–1.24, p=0.014), CCR7 (HR: 1.08, 95% CI 1.02–1.15, p=0.012) and CCR8 (HR: 1.17, 95% CI 1.03–1.32, p=0.015). When RVF was included as a covariate in the model, due to high risk of mortality from RVF, CCR 3–8 under-expression continued to be independently associated with increased hazard of death

with each unit increase in ΔCt (decreasing gene expression): CCR3 (HR: 1.12, 95% CI 1.01–1.23, p=0.025), CCR4 (HR: 1.18, 95% CI 1.06–1.31, p=0.003), CCR5 (HR: 1.12, 95% CI 1.03–1.22, p=0.006), CCR6 (HR: 1.15, 95% CI 1.03–1.29, p=0.011), CCR7 (HR: 1.11, 95% CI 1.03–1.19, p=0.004) and CCR8 (HR: 1.19, 95% CI 1.02–1.39, p=0.026).

Discussion

The primary findings of this study are that chemokine receptors CCR3–8 are down regulated in HF patients undergoing LVAD implantation, and CCR3, CCR4, CCR6, CCR7 and CCR8 are further down regulated in those patients that develop RVF. Within the cohort of patients that develop RVF, CCR4, CCR7 and CCR8 are further down regulated in those that require RV MCS. We found no significant difference in chemokine receptor expression between those requiring permanent versus temporary RV MCS; between those that had planned versus unplanned RV MCS; and between candidate LVAD versus LVAD patients. When stratified by median ΔCt, decreased expression of CCR6, CCR7 or CCR8 was able to predict the use of RV MCS, after controlling for covariates in a multivariate analysis. Additionally, under-expression of CCR3–8 was independently associated with an increased risk of mortality at 1 year, even after adjusting for RVF. Chemokine receptor expression did not predict RVF-LT that developed during 1 year surveillance in our patient cohort.

Chemokine receptor-ligand interactions influence cardiac homeostasis and response to injury. The human myocardium, under both diseased and normal conditions, expresses several chemokines and their corresponding receptors (25). There are several lines of evidence linking chemokine receptor down regulation to cardiac dysfunction. Bonaros et al. demonstrated that ischemic rat myocardium increases mRNA expression of the CCR3-binding chemokines eotaxin, RANTES, and MCP-3 (17). This study also showed that CCR3 mediates honing of CD34+ human bone marrow progenitor cells to areas of cardiac ischemia, potentially inducing neovascularization, inhibiting apoptosis, and promoting tissue repair. Migration of human angioblasts to ischemic rat myocardium was inhibited by administration of an anti-CCR3 monoclonal antibody. Neely and colleagues showed that cardiac specific RNAi-mediated silencing of the CCR4-Not components not3 and UBC4 in adult Drosophila results in myofibrillar disarray resembling dilated cardiomyopathy (18). Additionally, not3 haploinsufficient mice show reduced cardiac contractility and develop severe cardiomyopathy after aortic banding. Our study translates these early findings, linking CCR3 and CCR4 down regulation to a signal discriminating RVF in LVAD patients. Interestingly, while some of the preclinical data is variable between ischemic and non-ischemic etiologies of heart failure, we found no difference in baseline chemokine receptor regulation between these groups. A subset analysis by etiology, to study the relationship between these expression profiles and clinical outcomes, could constitute an important focus of future investigation.

Valaperti et al. reported that interleukin-1 receptor-associated kinase 4 (IRAK4), a major innate immunity signal transducer, worsens Coxsackievirus B3 induced viral myocarditis in mice by reducing early recruitment of protective CCR5+ monocytes/macrophages to the heart (19). Mortality and viral proliferation rates were increased in CCR5 knockout mice, highlighting the importance of CCR5 mediated effects on cardioprotection and antiviral

defense. In humans, Fernandez-Mestre and colleagues found the presence of a pCCR5-59029G/G genotype and putatively associated lower expression of CCR5 on the surface of peripheral CD8+ T cells occurred in 37% of Typanosoma cruzi-infected Venezuelan patients who developed chagasic cardiomyopathy (20). Talvani et al. found that patients with mild chronic chagasic cardiomyopathy had an increased expression of CCR5 on circulating peripheral blood mononuclear cells (PBMC) compared with noninfected individuals or those with severe disease, postulating a role for CCR5 in cardiac adaptation to this infection (21). In contrast, a study by Damas et al. showed increased CCR5 gene expression on PBMCs of HF patients compared to healthy controls (26). However, this study only included patients who had had stable chronic New York Heart Association functional class II–III heart failure for at least 6 months prior to participation. It is possible that, similar to findings by Talvani and colleagues (21), this increased expression is lost when patients develop worsening heart dysfunction. In our study, down regulation of CCR5 was observed in end-stage HF patients undergoing LVAD implantation, and associated with worse 1 year survival.

CCR7 deficient mice show LV dilatation and decreased wall thickness in response to experimental pressure overload by aortic banding, indicating a role for CCR7 in preserving LV geometry during cardiac stress (22). Additionally, while CCR7 knockout mice have improved survival in the first week following MI, they display an increase in markers of myocardial dysfunction including ANP, BNP, and β-MHC/α-MHC ratio, with accompanying LV dilatation 6 weeks post MI (23). Our findings are in agreement with Damas et al., who also reported significant reduction in CCR7 expression on PBMCs in HF patients (26). Taken together, the findings of these studies suggest a fundamental role for chemokine receptor mediated networks in cardiac physiology and the delicate balance between adaptation and maladaptation to injury.

Activation of the systemic inflammatory response pre-implant, represented by levels of circulating IL-6, has previously been associated with worse INTERMACS profiles, poor early outcomes, and longer hospital stays in LVAD recipients (27, 28). However, little is known about the effect of baseline inflammatory profiles on RVF and long-term survival in these patients. Furthermore, there is a paucity of data on chemokine receptor regulation and function in RV pathophysiology. Our study reports pre-implant chemokine receptor expression, and their correlation with RVF and 1 year mortality in LVAD patients.

There are several limitations to our study. The incidence of RVF requiring RV MCS may appear higher in this study than what has been previously reported. As our purpose was to determine if chemokine receptor expression as measured from peripheral blood cells prior to LVAD implant, is associated with a failing RV in need of additional support, we included both planned (n=17) and unplanned (n=18) biventricular support in our RV MCS outcome group. This doubled our reported rate of RVF requiring MCS and should be kept in mind when interpreting these results; the overall rate of post-operative RVF excluding planned biventricular support for this cohort was 27.9%, which is in keeping with recent reports (29, 30). Interestingly, there was no significant difference in chemokine receptor expression between planned and unplanned RV MCS, indicating a potential role for inflammatory signaling data in the comprehensive evaluation of the pre-LVAD patient. In our study,

baseline chemokine profiles were unable to discriminate development of late onset RVF, as well as which RVF patients would eventually come off RV MCS. Molecular mechanisms involved in chemokine receptor-ligand signaling are both complex and dynamic, and it is possible that surveillance analysis of chemokine receptor profiles in LVAD patients could yield valuable data for further prognostication. Unfortunately, this data was not available at the time of this publication. While our cohort is the largest reported to date that examines the incidence of late onset RVF, the number of RVF-LT events was limited (n=9), which may have contributed to the lack of signal seen with CCR expression.

In conclusion, chemokine receptor down regulation in LVAD patients is associated with right ventricular failure and 1 year mortality. Further studies are needed to elucidate the role of chemokine receptors within the intricate signaling nexus of the failing human heart in order to provide mechanistic insights into the underlying biology, and to develop novel predictive diagnostics and therapeutics.

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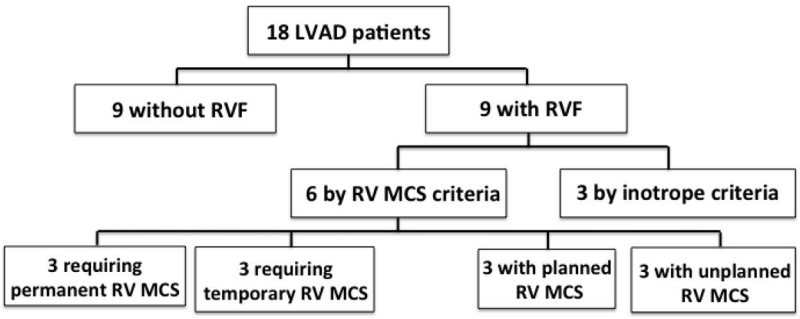

Figure 1.

Patient population: Derivation cohort. LVAD indicates left ventricular assist device; RVF, right ventricular failure; RV MCS, right ventricular mechanical circulatory support.

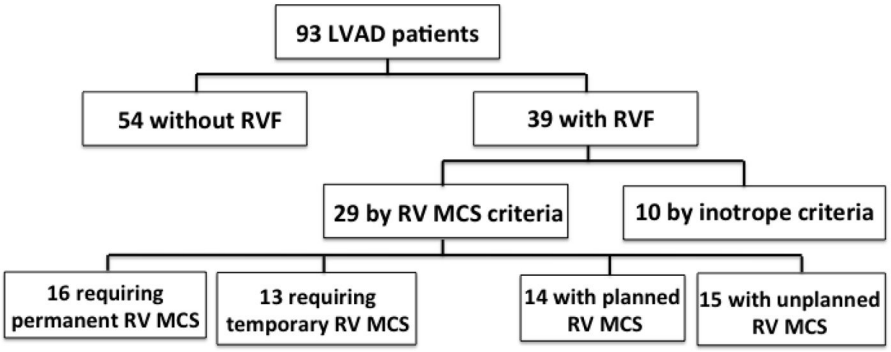

Figure 2.

Patient population: Validation cohort. LVAD indicates left ventricular assist device; RVF, right ventricular failure; RV MCS, right ventricular mechanical circulatory support.

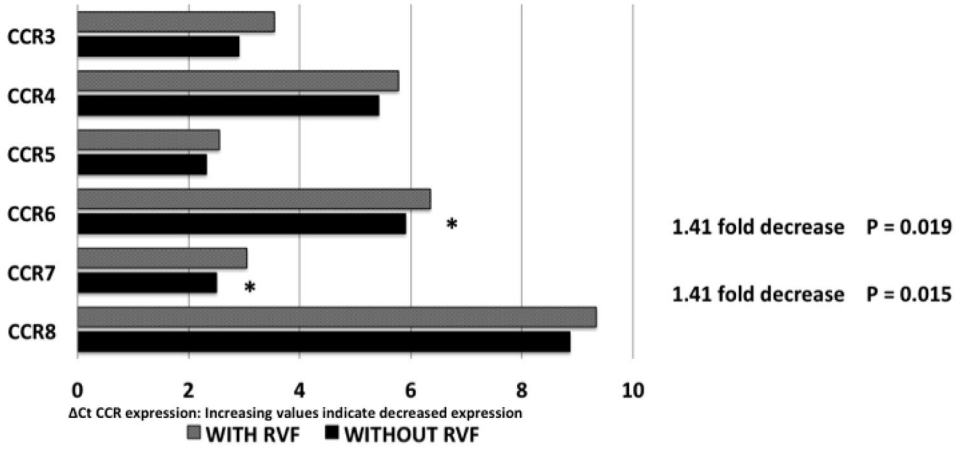

Figure 3a: ΔCt Chemokine receptor expression- with versus without RVF

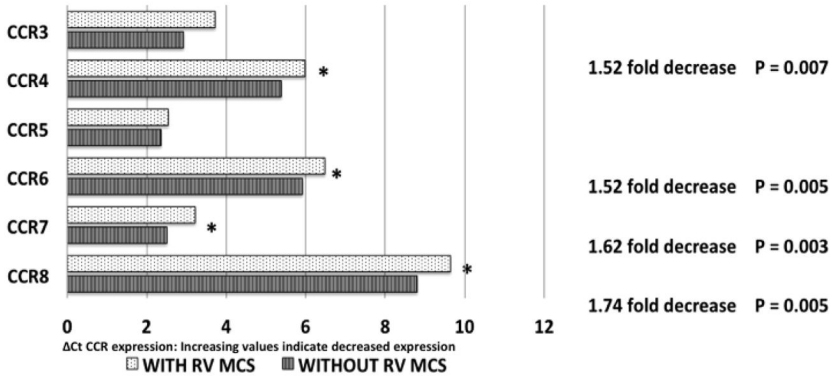

Figure 3b: ΔCt Chemokine receptor expression- with versus without RV MCS

Figure 3.

Chemokine receptor expression by RVF and RV MCS group. Chemokine receptor expression (ΔCt, PCR cycles to threshold normalized to the average of 3 control genes: GAPDH, HPRT1 and B2M) is shown for patients with versus without RVF (Panel A) and with versus without RV MCS requirement (Panel B). Higher ΔCt values correspond to lower gene expression. RVF indicates right ventricular failure; RV MCS, right ventricular mechanical circulatory support.

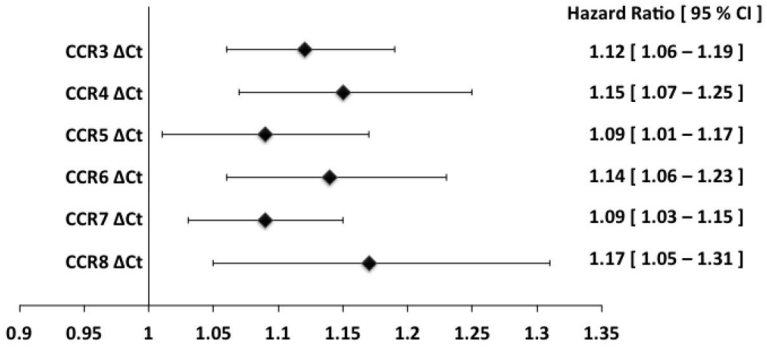

Figure 4.

Forest plot of survival according to normalized chemokine receptor expression (ΔCt) by Cox univariate analysis. Increasing ΔCt values correspond to lower gene expression. CI indicates confidence interval.

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Table 1

Patient characteristics by cohort

	Validation Cohort (n=93)			Derivation Cohort (n=l8)		
Patient Characteristics	**Without RVF**	**With RVK (n=3′J)**	**p value**	**Without RVt (n=9)**	**With RVK (n=9)**	**p value**
Demographics						
Age in years. mean. SD (n)	53.93 ±13.43(54)	53.28 ± 14.33(39)	0.82	53.89 ±7.72 (9)	52.33 ±9.43 (9)	0.71
Gender, Male, n (%)	47/54 (87,04%)	30/39 (76.92%)	0.20	8/9(88.89%)	6/9 (66.67%)	0,26
Etiology, n (%)			0.61			0.21
Ischemic	23/5–1 (4259%)	20/39 (5128%)		6/9 (66.67%)	3/9 (33.33%)	
Non-Ischeinic	28/54(5185%)	18/39(4615%)		3/9 (33.33%)	4/9 (44.44%)	
Other	3/54 (5.56%)	1/39(2.56 %)			2/9 (22.22%)	
Device Data						
Intention to treat, n (%)			0.02			063
Bridge to Transplant	34/54 (62 96%J	19/39 (48.72%)		5/9 (55.56%)	6/9 (66.67%)	
Destination Therapy	18/54 (33 33% J	11/39(28 21%)		4/9 (44.44%)	3/9(33.3 3%)	
DT - Modifiable	2/54 (370%)	9/39(23.08%)				
LVAD, n (%)						
HeartMato II	28/54 (51.85%)	14/39(35.90%)		5/9(55.56%)	2/9 (22.22%)	
Thoratec pVAD	4/54(7.41 %)	15/39(3846%)		2/9 (22.22%)	4/9 (44.44%)	
Thoratec iVAD	1/54(1.85%)	2/39(5.13%)				
HeartWare HVAD	18/54 33.33%)	4/39(10.26%)				
Vend Assist	3/54 (556%)	4/39(10.26%)		2/9(2222%)	3/9(3333%)	
RVAD, n (%)						
CcntriMae		14/29(48.28%)			3/6 (50.00%)	
Thotatcc pVAD		13/29 (44.83%)			3/6 (50.00%)	
Thoratec iVAD		1/29(3.45 %)				
Impella RVAD		1/29(3.45 %)				
RHC data						
RAP (mm Hg), mean, SD (n)	12.08 ± 6.5 (51)	1506 ± 875 (32)	0.08	1650 ± 475 (8)	1875 ± 888 (8)	054
mTAP (mm Hg), mean. SD (n)	38.60± 10.16(52)	38.27 ^6.87 (33)	0.87	42.50±5.45 (Si	41 13111 39(8)	0.76
PCWP (mm Hg), mean, SD (n)	2–1.79 ± 9.07 (52)	26.09 = 7.58 (33)	019	29.13 ±6.69 (8)	28 50 ± 867 (8)	0.87
HR (bpm). mean, SD (n)	88.08 ± 19.26(52!	93.53 ± 18.65(34)	0.20	91.38 ±24.63 (8)	104 00 ±24.13 (7)	0.34

	Validation Cohort (n=93)			Derivation Cohort (n=l8)		
Patient Characteristics	**Without RVF**	**With RVK (n=3′J)**	**p value**	**Without RVt (n=9)**	**With RVK (n=9)**	**p value**
CI (1/min/m 2), mean, SD (n)	2.16 ±0.66 (49)	2.29 ±0.79 (32)	0.44	2.13 ±0.36 (8)	1.93 ± 0.66 (7)	0.48
PVR (WU). mean. SD (n)	3 28 ± 1 48 (48)	2.96 ± 1.87 (32)	0.39	3.22 ± 1.92 (8)	3.89 ± 1. 57 (7)	0.48
Echocardi Doraphic data						
LVEF (%), mean, SI) (n)	16.82±6.21 1,52)	18 14 ± 9.12(39)	0.41	21.39 ± 11.12 (9)	17,50 ±8,37 (6)	0.48
TR jet velocity (m/s), mean, SD (n)	3.09 ± 0.42(44)	3.06±0.58(32)	0.75	2.71 ± 0.55 (7)	2.96 ±0.65 (6)	(147
RV global impression, n (%)			0.56			055
Normal	19/51(37.25%)	13/39(3333%)		3/9 (33.33%)	1/7 (14.29%)	
Mildly decreased	12/51 (23.53%)	10/39(211 51 i		1/9(11.11%)	2/7 (28.57%)	
Moderately decreased	13/51 (25.49%)	8/39(20.51 %)		4/9 (44.44%)	2/7 (28.57%)	
Severely decreased	7/51 (1313%)	10/39 (2564%)		1/9(11.11%)	2/7 (28.57%)	
TR grade, n (%)			0.24			037
Normal		1/35(2.86 %)			1/7(14.29%)	
Trace regurgitation	7/43(16.28 %)	7/35(20.00 %)		1/8 (12.50%)		
Mild regurgitation	15/43 (34.88%)	5/35(14.29 %)		5/8 (62.50%)	2/7 (28.57%)	
Moderate regurgitation	16/43(3721%)	18/35 (5143%)		1/8 (12.50%)	3/7 (42.86%)	
Severe regurgitation	5/43(11.63 %)	4/35(11.43 %)		1/8 (1250%)	1/7 (14.29%)	
TR grade, n(%)						
BUN.(mg/dl), mean. SD (n)	27.11 ± 1881 (54)	30.41 = 16.14(39)	038	39.00 ± 22.73 (9)	30.89 ± 15.27 (9)	039
Ct (mg/dl), mean, SD (n)	1.42 ±0.49 (54)	1.46 ± 0.54 (39)	0.73	1.77 ±0.78 (9)	1.72 ±0 53 (9)	0.89
TBild (mg/dl), mean. SD (n)	1.75 ±1.60 (51)	2.52 ± 5.27 (37)	0.33	1.80 ±2.37 (9)	3,83 ±5,79 (9)	0.34
WBC (10E9 cells/L), mean, SD (n)	8.68 ±3.28 (54)	9.48 ±3.59 (39)	026	10.22 ± 4.65 (9)	11.59 ± 4.86 (9)	0.55
Hct(%), mean, SD (n)	33.57 ±6.42 (54)	30.19 ± 4.78 (39)	0.01	30.79 ± 6.39 (9)	29.71 ±5.61 (9)	0.71
INK, (ratio), mean. SD (n)	1.29 ±0.18 (54)	1.42 ±0.36 (39)	0.02	1.49 ± 0.31 (9)	1 87 ±0.75 (9)	0.18
Albumin (g/dl) mean. SD (n)	3.22 ± 0.63 (53)	3.06± 0.71 (38)	0.26	2.82 ± 0.99 (9)	2.87 ±058 (9)	0.91
ALT (U/L), mean, SD (a)	39 54 ±44.41 (52)	75 74 ± 142.41 (39)	0.09	150.89 ± 277.34 (9)	449 22 ±513.11 (9)	014
AST (U/L), mean. SD In]	44 40 ± 78.69 (53)	77 28 ± 107.97 (39)	0.09	161.67 ± 287.29 (9)	473,11 ± 536 76 (9)	014
MELD (score), mean. SD, (n)	14 ±4.64 (51)	15 ±5.54 (37)	0.15	17 ± 5.61 (9)	20 ±8.56 (9)	031

RVb indicates right ventricular failure; SD, standard deviation; LVAD, left ventricular assist device; RVAD, right ventricular assist device; RAP, right atrial pressure; mPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; HR, heart rate; CI, cardiac index; PVR, pulmonary vascular resistance; LVEF, left ventricular ejection fraction; TR, tricuspid regurgitation; MELD, Model for hud-Stage liver Disease score, calculated as [3.7a*ln[TBili(mg/dl)])+(11.2*ln[INR])+(9.57+ln[Cr(mg/dl)]+6.43.

Table 2

Patient characteristics in relation to presence or absence of RVF-LT

Patient Characteristics Demographics	Without RVF-LT (n=81)	With RVF-LT (n=9)	p value
Age m years, mean. SD (n)	53.16 ± 13.74 (81)	54.89 ±9.45 (9)	0.71
Gender, Male, n (%)	67/81 (82.72%)	8/9 (88.89%)	0.64
Etiology, n (%)			0.48
Ischemic	39/81 (48.15%)	3/9 (33.33%)	
Non-Ischemic	38/81 (46.91%)	6/9 (66.67%)	
Other	4/81 (4.94%)		
Device Data			
Intention to treat, n (%)			0.17
Bridge to Transplant	47/81 (58.02%)	3/9 (33.33%)	
Destination Therapv	28/81 (34.57%)	6/9 (66.67%)	
DT - Modifiable LVAD, n (%)	6/81 (7.41%)		
HeartMate II	41/81 (50.62%)	5/9 (55.56%)	
Thoratec pVAD	11/81 (13.58%)		
Thoratec iVAD	1/81 (1.23%)		
HeartWare HVAD	19/81 (23.46%)	2/9 (22.22%)	
VentrAssist	9/81 (11.11%)	2/9 (22.22%)	
RHC data			
RAP (mm Hg), mean. SD (n)	13.41 ±6.69 (75)	15.33 ±5.92 (9)	0.41
mPAP (mm Hg), mean. SD (n)	39.28 ± 8.69 (76)	43.22 ±10.53 (9)	0.21
PCWP (mm Hg), mean, SD (n)	26.51 ±8.24 (76)	25.89 ±9.24 (9)	0.83
HR (bpm), mean SD (n)	90.91 ±20.24(77)	92.22 ±18.73 (9)	0.85
CI (l/mm/m2), mean. SD (n)	2.08 ± 0.58 (72)	2.27 ±0.63 (9)	0.38
PVR (WU), mean, SD (n)	3.22 ± 1.58 (71)	3.83 ±1.98 (9)	0.29
Echocardiography			
LVEF (%). mean. SD (n)	17.30 ±6.96 (77)	20.28 ±6.18 (9)	0.22
TR jet velocity (m/s), mean, SD (n)	3.05 ± 0.49 (66)	3.26 ±0.50 (7)	0.28
RV slobal impression, n (%)			0.66
Normal	24/77 (31.17%)	4/9 (44.44%)	
Milkly decreased	19/77 (24.68%)	2/9 (22.22%)	
Moderately decreased	22/77 (28.57%)	1/9(11.11%)	
Severely decreased	12/77 (15.58%)	2/9 (22.22%)	
TR grade. n(%)			0.36
Normal	1/68 (1.47%)		
Trace reauraitation	9/68 (13.24%)	3/8 (37.50%)	
Mild resuraitation	21/68 (30.88%)	3/8 (37.50%)	
Moderate resuraitation	30/68 (44.12%)	2/8 (25.00%)	
Severe regurgitation	7/68 (10.29%)		
Biochemical data			
BUN (mg/dl). mean.	27.41 ±	32.78 ±13.23	0.40

Patient Characteristics Demographics	Without RVF-LT (n=81)	With RVF-LT (n=9)	p value
SD (n)	18.29(81)	(9)	
Cr (mg/dl). mean. SD (n)	1.44 ±0.49 (81)	1.73 ±0.83 (9)	0.11
tBili (mg/dl). mean. SD (n)	1.73 ± 1.59 (78)	1.11 ±0.58 (9)	0.25
WBC (10E9 cells/L). mean. SD (n)	9.25 ±3.76 (81)	7.80 ±2.33 (9)	0.26
Hct (%), mean, SD (n)	32.47 ±6.19 (81)	32.27 ±4.87 (9)	0.92
INR (ratio), mean, SD (n)	1.39 ±0.36 (81)	1.21 ±0.14(9)	0.14
Albumin (g/dl). mean SD (n)	3.10 ±0.68 (80)	3.65 ±0.41 (9)	0.15
ALT (U/L). mean, SD (n)	97.59 ±219.33 (79)	37.67 ±40.67 (9)	0.42
AST (U/L), mean, SD (n)	95.59 ± 193.02 (80)	28.78 ±10.99 (9)	0.30
MELD (score), mean, SD (n)	15 ±4.65 (78)	14 ±5.26 (9)	0.56

RVF-LT indicates long-term right ventricular failure; SD, standard deviation; LVAD, left ventricular assist device; RAP, right atrial pressure; mPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; HR, heart rate; CI, cardiac index; PVR, pulmonary vascular resistance; LVEF, left ventricular ejection fraction; TR, tricuspid regurgitation; MELD, Model for End-Stage Liver Disease score, calculated as (3.78*ln[TBili(mg/dl)]X11.2*ln[INR]]+(9.57*ln[Cr(mg/dl]]]+6.43