Chapter

Myocardial Remodeling with Ventricular Assist Devices

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Abstract

Most prominent functional abnormalities seen in the failing human heart are impaired contraction and slowed rates of relaxation of cardiac cells in the face of increased neurohormonal activation, sustained inflammation, mechanical and volume overload, and progressive maladaptive remodeling of the myocardium. Mechanical circulatory support devices (MCS) improve cardiac function and outcomes of patients with end-stage heart failure, allowing to bridge to heart transplantation and permitting the removal of MCS device as a bridge to recovery, in some patients with the sufficient recovery of heart function. Numerous reports have demonstrated favorable myocardial recovery and reverse remodeling after prolonged ventricular unloading by MCS. Ventricular unloading by MCS leads to a decreased concentration of peripheral natriuretic peptides in plasma, reduction in cardiac cytokines, kinases, collagens, and proteins involved in hypertrophy, fibrosis, programmed cell death, and necrosis in the heart. This chapter will summarize and review the effects and underlying mechanisms of myocardial remodeling during prolonged MCS in patients with end-stage heart failure. The mechanisms of myocardial recovery are multifactorial and remain to be further explored on cellular, organ, and systems levels.

Keywords: ventricular assist device, mechanical unloading, reverse remodeling, myocardial recovery

1. Introduction

Cardiovascular diseases (CVDs) were responsible for an estimated 17.8 million deaths globally in 2017 and half of all people diagnosed with heart failure (HF) die within 5 years of diagnosis [1]. The major cause for CVD morbidity and mortality is HF, a complex clinical syndrome caused by many CV and other diseases that impairs the ability of the ventricle to fill with or eject blood. The key pathophysiological features involved in the development of HF are hypertrophy, fibrosis, apoptosis/necrosis, microvasculature and extracellular matrix (ECM) abnormalities, and disturbances in electrophysiologic, adrenergic, and angiotensin signaling. Currently, heart transplantation is the gold standard treatment of patients with end-stage HF and the current 10-year survival rates of heart transplant recipients reach 53% [2].

During the last decades, mechanical circulatory support (MCS) devices with ventricular assist devices (VAD) have improved the outcomes of patients with advanced and end-stage HF, becoming a cornerstone therapy to bridge those patients to heart transplantation or recovery [3–7]. The synopsis of structural and molecular changes in the heart underlying the improved cardiac function after VAD implantation is called "reverse cardiac remodeling." Extensive investigations have been utilized to understand how the heart remodels to mechanical and volume unloading during MCS in a facet of stabilized neurohormonal and inflammatory responses [8–10]. MCS therapies lead to the improvement of HF symptoms with normalized cardiac size and shape with simultaneous biological remodeling on gene, molecular, cellular, and tissue levels [11–13]. Myocardial recovery is associated with improvements in structural, sarcomeric, sarcolemmal, and calcium handling-associated proteins expression and function [14–16]. Mechanical unloading has been shown to increase collagen cross-linking and myocardial stiffness [17], alter mitochondrial and metabolic processes [18], and promote repair and regeneration [19]. Moreover, studies focused on understanding the roles of biomarkers of neurohormonal activation, oxidative stress, and systemic inflammation pathways in patients with VAD support have identified a subset of vulnerable patients with risks of developing adverse events fostering the development of innovative applications of combined MSC and pharmacological agents [20, 21]. As a destination therapy MSC is critical in patients with the favorable restoration of cardiac function and this regenerative therapeutic strategy becomes a desirable alternative to heart transplantation [22]. Herein, we review and summarize research studies focused on understanding the roles of neurohormonal signaling, inflammation, signal transduction, cellular and subcellular remodeling, and transcriptional regulation in the failing human heart before and after MCS therapy.

2. Neurohormonal remodeling during LVAD therapy

HF is a highly complex clinical syndrome characterized by cardinal symptoms due to structural and/or functional abnormality of the heart, resulting in elevated intracardiac pressures and/or inadequate cardiac output [23]. The clinical symptoms of HF develop and progress through prolonged dyshomeostasis in the heart in response to various stressors, which include alterations of regulatory neurohormonal systems associated with the release of natriuretic peptides, proinflammatory cytokines as well as activations of the sympathetic nervous system (SNS), which in turn activates the renin-angiotensin-aldosterone system (RAAS) [8]. Some of these alterations appear to be reversible by VAD treatment in response to a decrease in cardiac pressure, volume overload, and ventricular wall tension and stretch [24]. These events lead to reduced cardiomyocyte hypertrophy, improved coronary perfusion, and decreased chronic ischemia in the heart [25]. Therefore, mechanical unloading of the failing heart by LVADs, coupled with neurohormonal and anti-inflammatory therapy, may further promote reverse remodeling and recovery of myocardial function.

2.1 Natriuretic peptides

The natriuretic peptide family consists of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) [26]. Under normal conditions, ANP is produced in the atrium and BNP is synthesized primarily by the

ventricles in response to cardiac mechanical stretch. Circulating in the plasma, ANP and BNP play compensatory diuretic roles by decreasing salt and water retention and inhibiting vasoconstrictor peptides. In contrast to ANP, levels of BNP are significantly elevated in plasma of HF patients in response to chronic volume overload and BNP concentration correlates with the status of ventricular dysfunction with high concentrations predicting poor long-term survival. Support with VAD in patients with end-stage HF reduces the myocardial wall stress and thereby may change BNP levels in the heart. Sodian *et al.* studied 21 patients with nonischemic cardiomyopathy on VAD support and demonstrated a significant decrease in BNP levels in plasma after initiation of MCS, reaching normal levels within the first week after VAD implantation [27]. Especially, an early decrease of BNP in plasma was indicative of cardiac function recovery during VAD support. The significant decrease in BNP serum concentration after VAD support coincides with a decrease in *BNP* messenger RNA (mRNA) and protein expression in the heart of patients with severe HF supported by VAD. They also showed a decrease in BNP production not only by cardiomyocytes, but also by endothelial cells, T cells, and macrophages infiltrating the heart [28].

ANP and BNP also exert local antihypertrophic, antifibrotic, and lusitropic effects in the heart *via* their interactions with guanylyl cyclase-A receptor (CG-AR) [29]. Comparative analysis of cardiac *ANP* and *BNP* mRNAs expression in patients with HF revealed normalization of *ANP, BNP,* and the NP-metabolizing *NPR-C* receptor after VAD support, while *GC-AR* mRNA expression levels remained intact, suggesting that reverse remodeling is associated with the local protective effects of ANP and BNP.

In chronic HF, expression of ANP and BNP serves as clinical markers of cardiac hypertrophy, decompensation, hypertension, and myocardial infarction. Acute coronary syndromes are linked with the expression of chromogranin A (CgA), CD56/NCAM (neural cell adhesion molecule), and endothelin-1 (ET-1) [30, 31]. Investigation of 33 paired myocardial and plasma samples demonstrated significantly increased ANP, BNP, and CgA in congestive HF (CHF) patients before LVAD support, and all of these indicators were significantly decreased by VAD support [32]. Concentrations of plasma ANP and BNP also depend on different types of devices and durations of MCS. The time courses of ANP and BNP concentration have been studied in patients supported by Thoratec (8 patients), TCI Heartmate (6 patients), Novacor (7 patients), and Lionheart (3 patients) by Milting *et. al* [33]. All patients supported with Novacor, and some patients supported by TCI Heartmate, showed a steady decrease in plasma BNP levels, reaching normal ranges at 30 to 50 days. In contrast, only few patients supported by Thoratec or Lionheart reached normal BNP plasma values during the entire duration of support, suggesting recognition of different time points in ANP and BNP decrease among various types of devices when weaning from MCS in patients without heart transplant is suggested.

In pediatric cohort, it has been demonstrated that BNP and N-terminal pro-BNP (NT-proBNP) were modified differently by MCS compared to adults, showing an increase up to 1 day after VAD implant with a subsequent decrease to the pre-VAD levels in one month. Another pediatric study found levels of BNP and NT-pro-BNP correlated with severity and unfavorable outcomes of acute decompensated HF and an incremental increase of those peptides within 48 hours of admission predicted the need for MCS [34]. Short-term VAD support in children with severe HF significantly decreased BNP levels in plasma from pre-VAD to post-VAD and reduced markers of apoptosis [35].

2.2 Renin angiotensin aldosterone system

Reduced blood supply causes renal hypoperfusion and stimulation of SNS and RAAS [36]. The key molecule that mediates RAAS activation is angiotensin II (Ang II), a potent vasoconstrictor. In early stages of HF, RAAS activation functions as a compensatory mechanism to increase cardiac output. However, with the HF progression, RAAS activation plays a detrimental role in myocardial ischemia, hypertrophy, and arrhythmia [37]. In end-stage HF, G-protein-coupled receptors (GPCRs) of RAAS, such as Ang receptors, AT1R and AT2R, are downregulated, while angiotensin-converting enzymes (ACE and ACE2), GPCR kinase (GRK), and β-arrestin are upregulated [38]. Following VAD support, a significant downregulation of Ang I, ACE2, GRK, and β-arrestin has been documented, while AT2R, JNK, and p38 were upregulated, indicating divergent and incomplete molecular reverse remodeling. Combined MCS with neurohormonal blockade drug therapy (NHBDT) improved survival and reduced adverse cardiac events in HF [39]. For example, ACE inhibition (ACEI) during VAD support was linked with decreased Ang II, cardiac collagen content, and myocardial stiffness [17, 40], demonstrating the pathophysiological benefits of combined therapy compared with VAD support alone. The ISHLT Mechanically Assisted Circulatory Support (IMACS) registry suggested positive effects for ACEI and angiotensin II receptor blockers (ARBs) therapy in adult HF patients with VAD implantation [41]. Among patients treated with an ACEI/ARB, significantly lower risk of cardiovascular death, gastrointestinal bleeding, and levels of creatinine has been demonstrated compared to those in patients treated with mineralocorticoid receptor antagonist (MRA).

3. Inflammation and cytokines

3.1 Tumor necrosis factor-alpha

Reports on inflammatory profiles in HF patients and LVAD recipients have recently been comprehensively summarized by Radley *et al.* [42]. Tumor necrosis factor-alpha (TNF-α) is a protein expressed in the myocardium that stimulates cardiac growth and cell death [43, 44]. High levels of TNF-α are found in patients with severe HF [45]. Expression of *TNF-α* mRNA and protein were both elevated in the heart and serum of VAD candidates with severe HF [46]. Moreover, interleukin 1 beta (IL-1β), IL-6, procaspase-9, and active caspase-9 were increased in the heart of those deteriorating patients who required VAD support. Torre-Amione *et al.* reported that prolonged MCS results in significant reductions in intracardiac TNF-α, with a greater reduction in myocardial TNF-α in VAD-treated patients with recovered cardiac function *versus* those who required cardiac transplantation [43].

3.2 Metallothionein

Metallothionein (MT) is a highly conserved cytokine-inducible protein whose role is the detoxification of heavy metals through the regulation of their metabolism [47]. High metal affinity to cadmium (Cd) of MT in renal tissue plays a major role in the kinetics and balance between CdMT and non-bound Cd, which is highly neurotoxic [48]. A study of heart transplant patients by Baba *et al.* demonstrated that MT expression correlated with IL-6 elevation in blood vessels and a decrease in plasma IL-2 [49].

Moreover, MT expression was associated with lower fractional shortening, increase in LV end-systolic diameter, and lower mean arterial pressure in the absence of rejection in transplant patients, implicating the role of MT in cellular stress response. Further immunohistochemical studies by the same group demonstrated a decrease of MT-positive cardiomyocytes and vessels in the subendocardial and subepicardial regions of the myocardium in 17 HF patients during prolonged VAD compared to pre-VAD state [50]. In addition, ventricular unloading leads to regression of cellular hypertrophy and a reversal of MT expression in the failing heart, suggesting the remodeling process with reduction of MT expression is due to diminished wall stress and improved blood supply. The authors also observed that MT reactivity was substantially lower in the hearts of patients supported longer than 88 days as compared to patients supported less than 88 days [50].

3.3 C-reactive protein and interleukins

C-reactive protein (CRP) is a protein that is produced in response to the release of pro-inflammatory cytokines when the body is in an inflammatory condition [51]. Patients with end-stage HF have almost 8-fold higher levels of circulating CRP (cCRP) in serum compared to normal references [52]. Batra *et al.* studied pre- and post-implant VAD patients and found that one-third of post-VAD patients have persistently high CRP levels. They concluded that high CRP levels are linked with high mortality risk and a higher possibility of having a stroke during VAD support. Longer VAD therapy (60 days after implantation) resulted in a 50% reduction of CRP levels compared to pre-VAD values, suggesting improved inflammatory status over time [53].

Interleukins (IL) are a group of small molecules and peptides secreted by a wide variety of body cells or cytokines that function in cellular signaling and communication. Serum levels of members of IL-1 family cytokines, IL-1β and IL-33, are highly elevated in HF and remained elevated after MCS [54]. Increased expression of IL-1β and correlated patterns of IL-1 receptors indicate enhanced IL-1β signaling in MCS patients, while expression of IL-33 correlates with CRP plasma levels in HF, but not in patients on MCS. Suppression of tumorigenicity 2 (ST2) is a receptor of IL-33 and coupling of IL-33 with its ST2 receptor (IL-33/ST2) triggers danger-associated cellular responses playing a pivotal role in tissue repair in many organs [55]. In the heart, IL-33 is expressed by activated cardiac fibroblasts and cardiomyocytes during cardiac stretch and then is released into the extracellular matrix (ECM), promoting cell survival by blocking pro-fibrotic intracellular signaling [56, 57]. A significant decrease in soluble ST2 (sST2) levels was observed in end-stage HF patients after VAD implantation, suggesting a lessening of fibrosis and inflammation [58]. Levels of other cytokines, including IL-6 and IL-8, were also linked to the severity of clinical course in end-stage HF patients and correlated with outcome after VAD implantation [59]. A significant correlation of those cytokines was also found with ET-1 and relaxin (RLX)-2, the vasoactive mediators involved in neurohormonal system responses in VAD-supported HF patients [60]. Elevated levels of galectin-3 (GAL-3) were associated with the severity of HF and dynamic changes in GAL-3 levels predicted post-VAD survival [61]. Although unloading with continuous-flow LVAD results in a decrease of GAL-3 levels early post-implant, GAL-3 levels become elevated after 6 months of VAD implantation [61, 62], suggesting that levels of GAL-3 may represent a higher risk of death in HF patients with long-term VAD support.

4. Myocardial remodeling during VAD support

The myocardium consists of cardiomyocytes, composing nearly 56% of the adult heart, fibroblasts (27%), endothelial cells (7%), smooth muscle cells (10%), and various immune cells that transiently reside in the ECM [63]. These cell types are important in preserving normal cardiac function and morphology. The cells interact with each other using reciprocally secreted auto and paracrine factors, secretion of which is regulated by numerous molecules-messengers involving integrins, ET-1, BMPs, PECAM-1, VE-cadherin, VEGF, and TGFβ [64, 65]. Engineered heart tissue (EHT), created *in vitro* by seeding decellularized porcine myocardial sections with primary cardiomyocytes and fibroblasts isolated from neonatal rat ventricular myocardium or with cardiomyocytes derived from human induced pluripotent stem cells (hiPSC), is a novel platform to study cardiac remodeling [66]. Characterization of EHTs demonstrated gradual normalization of stress-free tissue length after mechanical unloading and suggested that actomyosin contraction in cardiomyocytes and activity of fibroblasts may play crucial roles in reverse remodeling after mechanical unloading.

4.1 Cardiac fibroblasts and fibrosis

Cardiac fibrosis in the failing heart is a final product of a series of biomechanical, molecular, and cellular changes that causes an imbalanced increase in ECM production and decreased ECM degradation [67]. The resultant increase in ECM deposition is accompanied by inflammatory and fibrotic scar formation in the interstitial and perivascular areas of the myocardium, interfering with the normal array of cardiomyocytes along with the disturbing supply of oxygen and nutrients to the myocardium. Moreover, cardiac fibrosis triggers further pathological remodeling and functional decline of the heart [68]. According to Tseng *et al.*, an increase in inflammation and fibrosis in the failing heart was associated with an increase in sST2 levels [58]. Synthesis and degradation of collagens I and III are highly regulated processes in human cardiac ECM. Collagen I is a major collagen component establishing the myocyte-collagen matrix, while collagen III contributes to elasticity, and changes in content may influence LV stiffness and size [69]. In HF, predominantly increased accumulation of collagens I and III in ECM results in cardiomyocyte injury, cardiac fibrosis, and the release of collagen-derived peptides into circulation [70]. Bruckner *et al.* recorded a significant decrease in intracardiac TNF-α, collagen I (by 66%), and collagen III (62%) in post-VAD myocardial samples of 18 patients compared to their pre-VAD levels [71]. They also found a decrease in cardiomyocyte size by 26% at post-VAD, demonstrating favorable reverse remodeling in cardiac hypertrophy.

Insulin-like growth factor I (IGF-1), released preferentially from cardiac fibroblasts, functions to negatively regulate atrophy and apoptosis, and stimulate cardiac repair by interacting with stromal cell-derived factor (SDF) [19]. SDF induces IGF-1 expression in cardiac myocytes *in vitro*. Patients with VAD support combined with β2-AR agonist clenbuterol have shown elevated *IGF-1* mRNA at the time of VAD explantation relative to the time of LVAD implantation [72].

4.2 Extracellular matrix remodeling

Matrix metalloproteinases (MMPs) degrade the ECM, while tissue inhibitors of MMPs (TIMPs) prevent the ECM degradation during repair process of damaged

tissues and cells. There are four variants of TIMPs that selectively inhibit different types of MMPs [73]. Typically, TIMP1 and MMP1 are increased in patients with deteriorating HF [74]. The increased ratio of MMP-1 to TIMP-1 in DCM has been shown to be almost normalized after LVAD, favoring decreased collagen degradation [17]. Felkin *et al.* found that high myocardial MMP1 and MMP8 expression is associated with high collagen content and increased IL-6 and IL-1β expression in HF patients requiring VAD support [75]. After VAD support, expression of *MMP-2* mRNA and active MMP-2 protein has been shown to be significantly increased compared to pre-VAD ($P < 0.01$), which was associated with a reduction of collagen IV content in the cardiomyocyte basement membrane. Furthermore, this was associated with a decrease in the thickness of cardiomyocyte membrane as revealed by electron microscopy [76]. MCS support increases collagen cross-linking and the ratio of collagen I to III in the heart as a result of decreased tissue MMP-1-to-TIMP-1 ratio and increased myocardial Ang I and II levels that stimulate ECM synthesis [17]. Therapy with ACEI drugs decreased Ang II levels and myocardial collagen content, resulting in enhanced myocardial recovery during VAD support [40]. In elderly patients with end-stage HF, VAD therapy is associated with decreased collagen turnover and cross-linking and increased tissue Ang II, whereas combined VAD and ACEI therapy normalizes LV end-diastolic pressure-volume relationships [77].

4.3 Endothelial and vasculature remodeling

A gene ontology (GO) analysis implicated endothelial to mesenchymal transition (EndoMT) and *vice versa* (MEndoT) pathways in human end-stage HF based on dual expressed VE-Cadherin endothelial and FSP-1 mesenchymal markers [78]. Gene expression analysis of 19 paired pre-VAD and post-VAD human heart samples by Hall *et al.* revealed differential expression of neuropilin-1, *FGF9*, *Sprouty1*, SDF1, and endomucin, the genes involved in the regulation of vascular networks [79]. In addition, a significant downregulation of GATA-4 binding protein, a critical mediator of myocyte hypertrophy, was observed in these heart samples following mechanical unloading. Drakos *et al.* observed an increased density of endothelial cells by 33% and decreased microvascular lumen area (36%) in post-VAD *vs* pre-VAD myocardial samples of patients with chronic HF ($n = 15$). This was associated with the activation of endothelial cells evidenced by ultrastructural and immunohistochemical analysis [80]. In agreement with these findings, a significant increase in interstitial and total collagen content without structural changes in cardiomyocytes was suggestive of increased fibrosis accompanied by regression of cardiomyocyte hypertrophy.

4.4 Reversal of cardiac hypertrophy

The myocardium is typically subjected to three types of mechanical loading during every heartbeat, including cyclic stretch, static stretch, and shear stress, generated by blood flow and an increase in chamber volume and pressure. Cardiomyocytes are sensitive to mechanical stress, which is transduced to molecular transduction signaling by biomechanical sensors. Comparative analysis of cardiomyocyte size in pre- and post-VAD patients demonstrated a decrease of 26% (33.1 ± 1.32 to 24.4 ± 1.64 μm, $P < 0.001$) in all patients studied [71]. Long-term VAD support resulted in a 28% reduction in myocyte volume, 20% reduction in cell length, 20% reduction in cell width, and 32% reduction in cell length-to-thickness ratio [81]. Another study examined the effects of continuous-flow VAD on cardiomyocyte size and demonstrated

that cardiomyocyte cross-sectional area decreased after VAD, but not beyond that of normal donor hearts [82]. Electron microscopy, cardiac glycogen content, and echocardiographic assessment also did not suggest myocardial atrophy in post-VAD patients. Consistent with these findings, no upregulation of pro-atrophic genes and proteins of the ubiquitin-proteasome system (UPS) and no t-tubule pathologies have been demonstrated.

Myostatin (also called gdf-8) is a potent inhibitor of skeletal muscle growth from the TGF-β family and is secreted by cardiac muscle and adipocytes in response to pathological stress, such as myocardial infarction or obesity [83]. Myostatin has been shown to mediate the regression of cellular hypertrophy after unloading with LVAD support [84]. The nuclear factor (NF)-κB superfamily of transcription factors carries out broad functions by regulating immune cell maturation, cell survival, and inflammation in many cell types [85]. In the heart, NF-κB is shown to be cardioprotective during acute injury, however, prolonged activation of NF-κB enhances the release of TNF-α, IL-1, and IL-6 cytokines, triggering chronic inflammation, hypertrophy, and cell death [86, 87]. After VAD support, the NF-κB DNA-binding activity decreases in failing human hearts and this process has been associated with a decrease in cardiomyocyte diameter [88].

Several kinases such as mitogen-activated protein kinase (MAPK or MEK), ERK (extracellularly regulated kinase), AKT (protein kinase B, PKB), GSK-3b (glycogen synthase kinase-3 beta), JNK (c-Jun NH2-terminal kinase) and p38 are involved in the development of cardiac hypertrophy *via* kinase-mediated signal transduction pathways [89]. After VAD support, significantly decreased activities of ERKs and AKT were seen in failing hearts, while the activity of GSK-3β was increased [90]. These changes were associated with a decrease in TUNEL-positivity and myocyte diameter. The disparity in the regulation of MAPK activity with a concomitant decrease in ERK and JNK1/2 activities and an increase in p38 activity after VAD support has been also reported [91].

Osteopontin (OPN) is a pleiotropic extracellular signal-regulated bone sialoprotein. Expression and activity of OPN are increased in myocardial tissues in accordance with the severity of HF [92]. Levels of *OPN* mRNA in heart biopsy specimens decreased significantly after VAD support, while OPN protein remained intact [93]. Moreover, VAD support induced a decrease of OPN levels in the plasma of some patients with VAD support, whereas OPN plasma levels were reduced significantly in all patients after a heart transplant.

4.5 Cardiomyocyte apoptosis

While MCS improves the survival of end-stage HF patients by reversing many biological processes activated during progression of HF, the reports on modulation of apoptotic cell death in response to VAD remain controversial. Prescimone *et al.* found a significant increase of Bax (pro-apoptotic), Bcl-2 (pro-apoptotic), and Hsp72 (antiapoptotic) molecules and a mild increase in cardiac caspase (Casp)-3 activity in post-VAD hearts compared to pre-VAD, suggesting involvement of mitochondria in apoptotic signaling [94]. The authors also found an increase in Casp-1 after VAD implant in HF patients and lack of apoptotic nuclei [95]. Conversely, Francis *et al.* found Bcl-2 being downregulated after VAD implant [96]. Another study found no significant differences in Bcl-2, while autophagy markers such as beclin-1, autophagy-related gene 5 (Atg5), and microtubule-associated protein-1 light chain-3 (LC3) were all significantly decreased in response to unloading [97]. Moreover, Bedi *et al.*

observed a highly variable expression of Fas among patients who had undergone MSC therapy [98]. Fas, also called Apo-1 or CD95, is a membrane receptor recognizing Fas ligand (Fas-L) and Fas/Fas-L coupling initiates an apoptotic cell death through the activation of caspase cascade in the heart [99]. Although apoptotic DNA fragmentation was attenuated in the myocardium, expression of antiapoptotic *Bcl-XL* and *FasExo6Del/Fas* genes was dependent on the duration of MCS [100]. Overall, no significant differences in number of TUNEL-positive cells between pre- and post-VAD samples have been reported by several groups [96, 97, 101, 102].

Abnormal Ca^{2+} cycling in HF triggers activation of UPS with an increase of binding immunoglobulin protein (BiP), eukaryotic initiation factor (eIF2α), and X-box binding protein 1 (XBP1) [103]. MCS support significantly decreases the levels of BiP and XBP1 and phosphorylation of eIF2α [104]. Moreover, a decrease in apoptosis observed during short-term VAD support has been associated with a decrease in phosphorylation of SMAD2 (mothers against decapentaplegic homolog 2), however, a long-term VAD support increased apoptosis and fibrosis in the heart *via* enhanced SMAD2 signaling and increased phosphorylation of HDAC4 (histone deacetylase 4) [101].

4.6 Cardiomyocyte regeneration

Diploid cardiomyocytes that are abundant in animal heart have a substantial capacity for cardiac repair and regeneration [105]. In human failing heart, polyploidy of cardiomyocytes is often observed as a precondition of heart hypertrophy [106], suggesting that cardiomyocyte polyploidization in HF may be associated with regeneration [107]. A study by Wohlschlaeger *et al.* demonstrated a marked reduction in the size of cardiomyocyte nuclei and in ratios between number of nuclei and cardiac myocytes after implantation of VAD [108]. They also reported a significant decrease in DNA content and reduction of polyploid cardiomyocytes in 23 myocardial samples studied after VAD, suggesting a decline in protein synthesis. On the contrary, an increase in the number of diploid cardiomyocytes was seen by other groups in post-VAD samples [108]. The decrease in polyploidy and increase in diploidy in response to MCS suggested an abundance of diploid cardiomyocytes going through cell cycle progression with the completion of mitosis or increase in stem cells. Prolonged MCS unloading increased the number of cardiomyocytes positive for phosphorylated histone H3 and Aurora B and this was associated with a decrease in cardiomyocyte size and mitochondrial content [109].

4.7 Transcriptional changes during VAD therapy

Accumulating evidence shows that the changes in transcriptome and metabolome profiles associated with HF persist in the reverse-remodeled myocardium despite apparent normalization on organ and cellular levels [110]. To identify transcriptional adaptations in failing and VAD-supported hearts, a comprehensive transcription analysis was performed in 199 human myocardial samples from nonfailing, failing, and VAD-supported human hearts. Although over 3088 transcripts exhibited alterations in HF samples, the number of differentially expressed genes (DEGs) with greater than or equal to a 2-fold difference was insignificant between HF and post-VAD samples, suggesting that many HF-associated transcriptional changes may have a limited role in regulating cardiac structure and function [111]. Significant elevation in myocardial arginine/glycine amidinotransferase (AGAT) expression is observed in

HF patients and myocardial *AGAT* is one of the DEGs that had a significant decrease during recovery [112]. In HF patients recovering after combination therapy, levels of *AGAT* mRNA decreased by 4.3-fold [$P < 0.001$] and 2.7-fold [$P < 0.005$] in VAD combined and VAD alone groups compared to donors, respectively, and *AGAT* levels returned to normal after recovery. These data highlighted the involvement of elevated local creatine synthesis specific to HF and its reversal during recovery. The genetic response of pediatric myocardium to MCS is distinct with approximately 40% of DEGs compared to adult hearts with VAD support, highlighting the importance of understanding features of reverse remodeling specific to pediatric myocardium to improve clinical strategies and LVAD management in children [113].

In long-term analysis of gene expression, data of patients studied for an average of 3.8 years post-explant revealed a significant association of integrin signaling and its downstream EPAC2 (exchange protein activated by cyclic-AMP2) during recovery of ventricular function by combined LVAD and clenbuterol therapy [20]. Downregulation of EPAC2 that regulates calcium involving cAMP pathway was associated with improvements in cardiac contractility and metabolism [114].

4.8 miRNAs in response to LVAD therapy

MicroRNAs (miRNAs) are small, endogenous noncoding RNAs that regulate posttranscriptional processes by repressing the translation of targeted protein-coding genes *via* binding to the 3' UTRs of mRNAs [115]. Therefore, cardiac miRNAs and circulating miRNAs (c-miRNAs) are promising biomarkers for HF diagnosis and prognosis [116]. Comprehensive microarray profiling of miRNAs and mRNAs, comparing myocardial specimens from adults with end stage HF with VAD and nonfailing hearts, showed upregulation of 28 miRNAs with almost normalization of miRNA profiles by VAD treatment [117]. Cardiac miRNAs have also been compared in 13 HF children at pre-VAD and at the moment of heart transplant (post-VAD) by next-generation sequencing [118]. The investigators found hsa-miR-199b-5p, hsa-miR-19a-3p, and hsa-miR-1246 being differentially expressed at post-VAD compared to that at pre-VAD. The candidate targets of those differentially expressed miRNAs were sarcomeric troponins showing significantly higher post-VAD when compared with pre-VAD values, suggesting that miRNAs can be therapeutically targeted to improve heart function in pediatric HF. Levels of nine c-miRNAs were downregulated and four c-miRNAs were upregulated in the post-VAD samples *vs* pre-VAD levels [119]. In particular, the c-miR-409-3p has been shown to regulate coagulation factor 7 (F7) and F2, suggesting a role of c-miRNA-409-3p in thrombotic events during MCS.

4.9 Beta-adrenergic receptor remodeling

Myocardial beta-adrenergic receptor (β-AR) signaling is severely diminished in failing heart due to increase in phosphorylation of agonist-occupied β-ARs by GRK2 [120, 121]. In chronic HF, VAD support leads to the restoration of cardiac β-AR signaling *via* the reduction of myocardial GRK2 expression and activity [122]. Unloading with VAD normalizes the ability of cardiac muscle to respond to SNS stimulation, reversing the downregulation of β-ARs [123]. Both types of devices, continuous-flow and pulsatile, decreased the expression and activity of GRK2 and normalized neurohormonal homeostasis disturbed with HF [124]. In pediatric HF, VAD treatment also resulted in the recovery of total β-AR and β_1-AR expressions and reversal of several pathologic processes in the heart [125].

4.10 Cyclic guanosine monophosphate

Cyclic guanosine monophosphate (cGMP) is a cyclic nucleotide derived from GTP (guanosine triphosphate) that acts as a second messenger for activation of intracellular protein kinases in response to the binding of membrane-impermeable hormones to the cell membrane [126]. The important components of cGMP signaling include cGMP-dependent protein kinase G (PKG), ANP, BNP, natriuretic peptide receptor A and C (NPR-A and NPR-C, respectively), neprilysin, NOS3, soluble guanylyl cyclase (sGC), and PDE5 [127]. The cGMP-PKG cascade can decrease the level of calcium and alter the expression of glycoprotein IIb/IIIa. Both fluctuations impact the aggregation of platelets within the body [128]. cGMP levels were found to be higher in patients with implanted VAD compared to healthy individuals. According to Grosman-Rimon *et al.*, cGMP was associated with an elevated risk of gastrointestinal (GI) bleeding during LVAD support [128]. The researchers presume that the association between elevated GI bleeding and higher cGMP levels could be due to platelet abnormalities. The study also found significant alterations of the cGMP-PKG pathway (downregulation of ANP, NPR-C, and cGMP) in patients with dilated cardiomyopathy after VAD implant, while the duration of VAD support negatively correlated with expression differences of PKG I, PDE5, and sGC in patients with ischemic cardiomyopathy.

5. Cardiomyocyte intracellular remodeling

5.1 Remodeling of cytoskeletal and sarcomeric proteins

Cytoskeletal proteins are essential for the structure and function of the cardiac myocyte. Stetson *et al.* reported ventricular unloading in humans dynamically changes not only myocardial TNF-α, total collagen, and myocyte size, but also remodels the expression of structural proteins [129]. To understand if myocardial recovery was associated with changes in sarcomeric, nonsarcomeric, and membrane-associated proteins, microarray analysis has been performed on the paired HF samples before and after VAD [16]. Significant increase of lamin A/C, spectrin and integrins (α5 and β5), and decrease of integrins β1, β6, and α7 has been observed at VAD explantation compared to pre-LVAD. Expression of sarcomeric proteins such as β-actin, α-tropomyosin, actinin-α1, and filamin A increased, while troponin T3 and actinin-α2 decreased. Vinculin expression decreased 4.1-fold in the recovered group. Despite decreased cardiomyocyte size post-VAD, severe structural damage in cardiomyocytes persisted with partial improvement in the expression of actin, tropomyosin, troponin C, troponin T, and titin [130]. In pediatric HF, MCS increased the expression of structural proteins, including dystrophin and actin [35]. Furthermore, expression of genes involved in calcium homeostasis, cell differentiation, and growth, including *CNNA1, CDK2B, CSF2, E2F1, EGR1,* and *EGR2*, were normalized after VAD therapy, suggesting an active reverse remodeling process after MCS in pediatric HF.

5.2 Dystrophin remodeling

Dystrophin is a rod-shaped protein encoded by the *DMD* gene located on the X chromosome, the largest gene of 2.4 megabases (Mb) in the human genome [131]. Dystrophin connects the actin and cytoskeleton of muscle fibers to the myocyte membrane at its N-terminus. At the C-terminus, it connects the sarcolemmal complex known

as the dystrophin-associated protein complex (DAPC) to the ECM, providing structural support for myocytes. Mutations in *DMD* cause Duchenne and Becker muscular dystrophies [132, 133]. Mutations in genes encoding cytoskeletal and sarcolemmal proteins provide the genetic basis for dilation and contractile dysfunction *via* "final common pathway." Abnormalities in *DMD* such as mutations in the N-terminus of dystrophin or in the cardiac-specific promoter, preferentially affecting cardiac function are associated with X-linked cardiomyopathy [134]. Vatta *et al.* investigated the integrity and response of dystrophin in end-stage dilated or ischemic cardiomyopathy HF patients to VAD therapy and identified disruption of N-terminal dystrophin in 18 HF patients studied [135]. This disruption was shown to be reversible in four patients after VAD support.

5.3 Remodeling in calcium cycling

Regulation of Ca^{2+} cycling is a versatile signaling process that regulates cellular homeostasis in different cell types, including cardiac myocytes [136]. Reduced rates of relaxation and impaired contractile reserve are the major abnormalities seen in the failing heart as a result of disturbances in Ca^{2+} transients [137]. The proteins that regulate cardiomyocyte Ca^{2+} cycling include sarcoplasmic reticulum (SR) Ca^{2+} ATPase (SERCA), ryanodine receptor 2 (RyR2), phospholamban (PLB), and the sarcolemmal Na^{+}/Ca^{2+} exchanger (NCX) [138–140]. Chaudhary *et al.* demonstrated that improvement in cardiac function during LVAD support was associated with a favorable balance between SERCA and NCX, resulting from the isolated decrease in NCX without an increase in SERCA [141]. Reverse remodeling of SERCA2a expression has been shown to be completed by about 20 days of VAD support, while hearts supported by VAD for longer than 40 days have significantly increased relative collagen content [142]. Post-VAD recovery increased SR calcium content and shortened action potential duration due to rapid inactivation in L-type Ca2+ current [15]. Short-term VAD support recovered post-rest potentiation (PRP) response to a level close to that in nonfailing hearts, but recovery of impaired SR Ca^{2+} cycling was dependent on duration MCS [143]. Chronic unloading with recovery of contractile function demonstrated upregulation of *SERCA2a*, *RyR2,* and *NCX* genes after MCS [144]. Recovery of rate-dependent contractility in failing human hearts during early VAD support was associated with faster decay of Ca^{2+} transients, while long-term MCS triggered abnormal Ca^{2+} cycling [101, 143]. Moreover, long-term MCS resulted in significantly increased SMAD2 activity with downstream phosphorylation of Ca^{2+}/calmodulin-dependent protein kinase type-IIδ (CaMKIIδ), myocyte enhancer factor 2 (MEF2), and myostatin. Improvements in the Ca^{2+} handling also depended on the severity of myocardial fibrosis, and ECM pathologies and excessive fibrosis limited the ability to recover [13].

5.4 Mitochondria and metabolism remodeling

Unloading with VAD has been shown to contribute to reverse remodeling of mitochondria and recovery of energy metabolism of the failing heart. In healthy adult hearts, the generation of ATP as a source of energy relies on the oxidation of fatty acids, glucose and lactate in mitochondria, and fatty acid oxidation provides the majority (> 70%) of total ATP [145]. The balance between lactate production and consumption by lactate dehydrogenase (LDH) that converts it to pyruvate, which is then transported by mitochondrial pyruvate carrier (MPC) into the mitochondrial tricarboxylic acid (TCA) cycle is important in producing plentiful ATP. The MPC expression is lower in patients with HF compared to those of non-failing cohorts [146]. Thus, the failing heart runs

increased glycolysis and decreased fatty acid oxidation for ATP production and the proportion of glucose oxidation to fatty acid oxidation depends on the severity of HF [147]. The generation of ATP is disturbed in HF with an increased glycolytic pyruvate-derived lactate and a simultaneous decrease in lactate utilization [148]. In addition, the opening of mitochondrial permeability transition pore (mPTP) in HF disrupts the mitochondrial membrane potential and disturbs oxidative phosphorylation pathways for ATP production, causing mitochondrial swelling and inducing apoptotic and necrotic cell death.

MCS improves systemic and cardiac metabolism *via* improvements in fatty acid oxidation, insulin resistance, and reductions in myocardial lipotoxicity through improved activation of the insulin/PI3K/AKT signaling cascade [149]. Significant decrease in long-chain acylcarnitines levels was consistent with improved fatty acid oxidation and utilization during long-term VAD support [150]. Diakos *et al*. reported induction of glycolysis through TCA without a subsequent increase in pyruvate oxidation in post-VAD patients [148], which may be attributed to the poor post-VAD recovery of mitochondrial oxidative capacity. Recently, the same group reported the beneficial cardioprotective effects of induced glycolysis as a result of an increase in rate-limiting enzymes of the pentose-phosphate pathway and 1-carbon metabolism in post-VAD patients [151]. All these have been associated with significantly reduced reactive oxygen species (ROS) and improved mitochondrial density [151, 152]. These metabolic improvements enhanced the glycosylation of α-dystroglycan, which maintains integrity between cytoskeleton and ECM [18]. Moreover, using high-resolution respirometry, a reduction in mitochondrial ROS up to 40% [153] and increased MPC1 abundance and glucose and glucose-6-phosphate levels, particularly, in mechanically unloaded hearts of ischemic HF patients has been demonstrated [154].

Levels of Ca^{2+} in the mitochondrial matrix regulate the activity of kinases and phosphatases involved in ATP production and mitochondrial quality control [155, 156]. In HF, the opening of mPTP not only disrupts the mitochondrial membrane potential but also reduces Ca^{2+} uptake, alters pH, and induces inflammation, leading to necrosis and death of cardiac myocytes [157]. Impaired mitochondrial Ca^{2+} uptake is the result of reduced Ca^{2+} release from SR and stimulates Ca^{2+} –sensitive dehydrogenases of the Krebs cycle [158].

About 20% of the total lipid composition on the mitochondrial inner membrane is constituted by cardiolipin and loss of cardiolipin and tetralinoleoyl-cardiolipin in HF is linked to excessive ROS production and cardiomyocyte apoptosis [159]. During mechanical unloading with LVAD, cardiolipin arrangement normalizes, which in turn, improves mitochondrial coupling [160]. Cellular proteases, such as cathepsins, are involved in the progression of HF. Parallel activation of cathepsins and their inhibitors was observed after VAD support. The expression of cathepsins and their inhibitors was significantly higher in pre-VAD compared to the heart transplant group and VAD induced a further increase in the cathepsin system. Significant positive correlations were observed between cardiac expression of cathepsins and their inhibitors as well as inflammatory cytokines [59, 161].

5.5 Cardiomyocyte signal transduction pathways and signaling

5.5.1 Mitogen-activated protein kinases

There are several cell signal-transduction pathways regulated in the heart in direct response to changes in mechanical loading and stress. The family of MAPKs, such as ERKs, p38, and JNK1/2, are well-characterized signal-transduction pathways [162].

These kinases are involved in the regulation of cell growth, cardiac hypertrophy, and cell death [163, 164]. They are upregulated in patients with HF secondary to ischemic heart disease and cardiomyopathy [165, 166]. The ERKs activity regulates adaptive hypertrophy and prevention of cell death during the early phase of chronic pressure overload in response to stimulation of GPCRs and integrin activation [167]. Mechanical unloading with VAD support resulted in differential regulation of MAPK activity with a significant decrease in the activity of p44/42 ERK and JNK1/2 along with a subsequent increase in p38 activity after LVAD support [91]. The authors explained a decrease in ERK activity is likely due to its decreased phosphorylation at p44/42, while a combination of decreased phosphorylation and expression of JNK1/2 is responsible for decreased JNK1/2 activity in VAD-supported hearts. Activation of AKT regulates cardiac physiological hypertrophy, glucose metabolism, cell death, and angiogenesis [168]. In failing human hearts, a high grade of kinase phosphorylation in all 3 MAPKs and AKT have been observed [166]. After VAD support, ERKs and AKT activities were dramatically decreased in failing hearts, while GSK-3β activities were increased [89].

6. Conclusions

Neurohormonal imbalance, inflammation, apoptosis, and abnormal inter and intracellular signaling and remodeling on molecular and genetic levels are critical processes contributing to adverse events in HF patients. This chapter provides a comprehensive overview of reverse remodeling on neurohormonal, myocardial, and cardiomyocyte intracellular levels in response to MCS in patients with HF. Knowledge about molecular mechanisms of underlying effects of VAD support aid to understand the adverse effects of myocardial unloading and deterioration of patients undergoing VAD therapy. While cardiac reverse remodeling has been correlated with clinical recovery in most post-VAD patients, many of those patients have deteriorated back to the original HF phenotype after LVAD explantation, suggesting the importance of considering a higher degree of myocardial recovery that may persist after device removal [169]. We found a significantly lower number of pediatric reports on clinical and pathological features of reverse remodeling compared to adult HF patients undergoing VAD support. Hence, there is a need for research on pediatric patients with VAD support, so we can better understand features of reverse remodeling specific to pediatric myocardium.

Conflict of interest

None.

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