Pathophysiology of right ventricular heart disease: the role of structure, apoptosis and inflammation
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CHAPTER I

The right ventricle myocardial: How right is seldom left or how dexter is not the same as sinister

“Science is organized knowledge. Wisdom is organized life.”

Immanuel Kant
Right Ventricle

Contrary to diseases of the left ventricle, right ventricular diseases remain rather underexposed and poorly understood. This discrepancy has been recognized, and in a recent decision-making summary, the National Heart, Lung, and Blood Institute concluded that right ventricular heart failure is distinctively different from left ventricular heart failure, and is recognized as an established mechanism of cardiovascular collapse [1].

1. Development, structure and function

From embryonic stage on, the right and left ventricles differ from each other. Numerous studies have suggested that the heart forms from two distinctly different cell populations that share a common progenitor cell population [2–4]. These progenitor cell populations are referred to as heart fields. The primary heart field contributes to the left ventricle (LV) and atria, while the secondary heart field contributes to the right ventricle (RV) and outflow tract. Specific cellular markers and transcription factors distinguish cells from these two distinct heart fields. Primary heart field cells are marked by the T-box transcription factor Tbx5 and the bHLH transcription factor Hand1 (which plays a role in cardiac morphogenesis). Some of the transcription factors required for the secondary heart field development and its derivates (outflow tract, right ventricle) are LIM-homeodomain protein Islet-1, Mef2c, Hand2, Foxh1, Foxc1/c2, Smyd (Bop). Other cardiac genes, however, such as the homeobox gene Nkx2-5, are expressed in both heart fields, but rely on distinctly different regulatory elements for expression [5]. Different cellular markers and potentially different intracellular signaling cascades may allow right and left cardiac myocytes to respond differently in terms of functional recovery after a noxious insult. It is also likely that the differences in origin of the cardiac
progenitor cells may contribute to the structural, functional, and geometric differences between the right and left heart.

Anatomically, the RV is crescent shaped in cross-section, as compared to the elliptically shaped concentric LV. Structurally, the RV is thin walled, and its mass is only about 1/6 that of the LV. It performs approximately 1/4 of the cardiac stroke work, and must overcome the pulmonary vascular resistance which is 1/10 of the systemic circulation [6]. The RV also has a lower volume to surface area ratio, thereby making it a highly compliant chamber. The primary function of the RV is to deliver deoxygenated blood to the lungs for gas exchange. The RV effectively serves as a reservoir for blood returning to the heart via the right atrium, thereby optimizing venous return and providing sustained low-pressure perfusion through the lungs. In contrast, the LV generates a high-pressure pulsatile flow through arterial vessels with low compliance. The RV is anatomically adapted for the generation of sustained low-pressure perfusion. It is comprised of two anatomically and functionally different cavities, namely the sinus and the conus. The sinus generates pressure during systole and the conus regulate this pressure [6-11]. The resulting effect of RV contraction is pressure generation in the sinus with a peristaltic motion that starts at the apex and moves toward the conus. The RV is very compliant; thus, the peak pressure is reduced but prolonged. This physiological effect sustains ejection into the pulmonary system until the RV is completely emptied.

Animal models for RV hypertrophy/failure

Developing animal models of RV failure is particularly useful in determining factors that initiate RV dysfunction. Models would also help to identify biomarkers and changes in gene expression and protein synthesis associated with RV failure. Furthermore, experimental studies help to
understand the differences, similarities and interplay of the left and right heart. This issue can be addressed with chamber specific studies in animal models, which examine changes in gene expression, protein synthesis, histology and geometry during development, injury and stress.

The monocrotaline (MCT) model is commonly used as a model of pulmonary hypertension that leads to RV hypertrophy and RV failure. The MCT model was introduced more than 40 years ago [12]. It is based upon a single injection of MCT (typically 60 mg/kg intraperitoneally or subcutaneously) which rapidly leads to severe pulmonary vascular disease in the absence of intrinsic heart and lung disease. The resulting pulmonary hypertension in turn induces RV hypertrophy and, eventually RV failure. The major advantages are the ease and rapidity of producing the model. The disadvantages of the MCT model are the reported effects not associate with RV failure (i.e. hepatic cirrhosis and megalocytosis, venoocclusive disease [13] disease and thrombocytopenia [14]). RV hypertrophy develops in humans living at high altitude and has been simulated in chronic hypoxia rats [15]. Both the MCT model and the hypoxia model appear to produce similar cardiac changes, especially selective RV hypertrophy, as in human pulmonary hypertension, although the pulmonary changes do not seem to be comparable in rats and human [16]. RV hypertrophy independent of pulmonary hypertension can be produced by increasing RV pressure following pulmonary artery banding [17]. This model would appear to mimic a minor cause of RV dysfunction in humans.

In conclusion, the use of relevant animal models for RV diseases may provide useful information allowing understanding of cause and progression of the disease as well as potential therapeutic interventions. The most common pathophysiological changes in the human cardiovascular system
(i.e. hypertension, cardiac hypertrophy and heart failure) have been successfully reproduced in animal models. No model mimics exactly all the symptoms of the human disease, partly because many of the changes in the human disease are not thoroughly understood. However, a thorough understanding of both advantages and disadvantages of each model is necessary if the results are to be extrapolated to humans.

2. Ventricular Remodeling

The pathophysiology of RV remodeling is a complex process. In addition to basic changes in geometry, wall thickness, and ventricular pressure-volume relationships, it includes structural and electrical alterations. Structural remodeling involves changes in myocyte dimensions and number as well as alterations in myocardial extracellular matrix. In addition, the biochemical milieu might be modified and infiltration of inflammatory cells might occur. Electrical remodeling involves alterations in gap junction and ion channel expression, distribution and/or their biophysical properties. Remodeling has been associated with pulmonary hypertension, RV failure, lung transplantation, LV pathology, Chagas' disease, arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVCD), and, recently Brugada syndrome. Disease progression may lead to further RV changes, including hypertrophy, dilatation, and subsequently to variable alterations in RV hemodynamic status.

2.1 Triggers for cardiac remodeling

*Pulmonary vascular-related right ventricular remodeling*

In a canine study of pulmonary hypertension, increased RV after-load was associated with increased RV weight, RV weight to body weight ratio, and ratio of RV weight to the combined LV plus septum weight [18]. However, none of these changes were accompanied by evidence of cardiac failure. In
addition to primary pulmonary hypertension, remodeling is often observed in pulmonary hypertension secondary to severe congestive heart failure. Eltzschig et al have described RV failure in the donor heart of heart transplant recipients, probably as a result of residual pulmonary hypertension secondary to heart failure [19]. Bhatia et al. monitored RV changes in donor hearts following transplant [20]. At 1-year post transplantation, there was an enlargement of the RV in donor hearts despite a decrease in pulmonary vascular pressure and resistance to high-normal levels by 2 weeks post transplant. These findings suggest that increased donor RV dimensions and wall thickening following transplantation persist despite normalization of RV after-load. Kowalewski et al. examined the effect of pulmonary resection on RV performance [21]. In addition to a significant RV dilatation following pneumonectomy, patients exhibited elevated RV end-diastolic pressure and end-systolic pressure as well as decreased RV ejection fraction. Nearly half of these patients developed arrhythmias, which were associated with even higher RV end-diastolic volume index and lower RV ejection fraction. These findings were attributed to postoperative increases in RV afterload [21].

*Right ventricular remodeling secondary to left ventricular dysfunction*

Changes induced by LV hypertrophy and/or dysfunction are also important in RV remodeling. Pathomorphological RV changes and RV remodeling in a post-infarction LV situation are most frequently associated with transmural scars located circularly or in septal/apical regions of the LV [22]. One mechanism by which LV myocardial infarction (MI) may lead to RV remodeling is via pulmonary hypertension following LV failure [22]. Hirose et al. suggested that post-MI remodeling is a biventricular process [23]. In response to an anterior LV infarction, RV remodeling may be primarily due
to septal remodeling resulting from changes in both LV loading and blood supply from the left anterior descending artery to the apical RV. Over a 5-year follow-up period, the same researchers concluded that increased RV chamber volumes mirror increases in LV chamber volume following anterior MI [24]. Another study noted initial RV changes and biventricular remodeling in patients with inferior wall MI in the absence of RV infarction [25]. This study also suggested that early RV distension has a favorable effect on LV remodeling, possibly because the expanded RV occupies a large portion of the rigid pericardial sac, preventing excess LV dilation immediately following MI [25]. Animal studies often use induced LV MI to study heart failure–associated changes, noting numerous RV changes secondary to subsequent LV volume overload [26-29].

**Neurohormonal factors**

Neurohormonal activation plays an important role in cardiac remodeling, especially when manifested through the renin-angiotensin-aldosterone system. Nahrendorf et al. suggested that pressure/volume overload is insufficient to induce RV hypertrophy, and that local and systemic activation of the renin-angiotensin-aldosterone system may underlie this change [26]. In addition to RV structural changes, angiotensin II type 1 (AT1) receptor mRNA expression was significantly increased in the RV following atrioventricular conduction block in dogs [30]. In addition to the role of the renin-angiotensin system in the development of RV hypertrophy there seems to be an interesting protective role of the ovarian function [31]. In this study both male and ovariectomized female rats with induced pulmonary hypertension exhibited increased renin mRNA expression in the RV, whereas male rats exhibited increased expression of angiotensinogen mRNA. Ovariectomized female rats exhibited significantly increased AT1a
and AT1b receptor expression in the RV, providing a basis for the use of angiotensin-converting enzyme inhibitors (ACE-I) and angiotensin receptor blockers (ARBs) to prevent maladaptive cardiac remodeling. This study also suggested that endogenous estrogen plays a protective role in remodeling, because ovariectomized females displayed more severe cardiac changes in response to pulmonary hypertension compared to rats with intact ovaries [31]. The role of estrogen in cardiac remodeling is likely linked to its ability to mediate inflammatory signaling and to increase nitric oxide synthase activities [32]. In a canine-based study of RV adaptive mechanisms, there was an increased α1- and β-adrenergic receptor density in the RV associated with increased preload-recruitable stroke work [18]. Stimulation of β-adrenergic receptor represents the major endogenous mechanism for increasing cardiac work, whereas α1-mediated signaling contributes to both cardiac development and the hypertrophic response in adult myocardium.

**Atypical causes of right ventricular remodeling**

ARVC/D is an autosomal dominant inherited disease with incomplete penetrance and variable expression. Causative mutations in genes encoding desmosomal proteins (for cell adhesion) and TMEM43 (transmembrane protein 43 that plays a role in maintaining nuclear envelope structure) are present in large proportion of the probands. ARVC/D leads to primary maladaptive RV changes, although LV may be involved as well. General pathologic features include gross RV volume increases, with diffuse, transmural fibro-fatty infiltration; decreased myocardial wall thickness; chronic pericardial inflammation; and myocyte elongation [33]. Occasionally a reduction in connexin43 expression has been observed [34]. These parameters display greater alterations in older patients, suggesting a progressive disease course. Moreover, many patients with ARVC/D display
increased RV end-diastolic volumes with subsequently impaired RV wall motion and decreased ejection fraction [35]. The pathophysiology behind the loss of RV myocardium in ARVC/D and the fibro-fatty replacement is unresolved. A consistent post-mortem finding in ARVC/D is patchy cell death with inflammatory infiltration [36]. This has spawned the proposal that ARVC/D has an infectious/inflammatory etiology, involving a primary chronic myocarditis that evokes an inflammatory injury, and culminates in fibro-fatty repair and progressive loss of RV myocardium.

Genetic mutations that result in loss-of-function of the cardiac voltage-gated sodium channel, Nav1.5, encoded by SCN5A gene are associated with various inherited arrhythmia syndromes that revolve around reduced cardiac excitability ("loss-of-function SCN5A channelopathy") [37]. The most prevalent is the Brugada syndrome (BrS) [38]. It has long been assumed that cardiac structural abnormalities are undetectable by clinical imaging methods in individuals with “loss-of-function SCN5A channelopathies”. This would be consistent with the conventional concept that Nav1.5 is only involved in maintaining electrical integrity of the heart. However, this paradigm has been challenged by the recent discovery that Nav1.5 may also be involved in maintaining structural integrity of the heart. Although unexpected, such an involvement was supported by experimental studies, which have indicated that Nav1.5 is part of a macromolecular complex that contains cytoskeleton proteins [39]. Moreover, loss-of-function SCN5A mutations were found in patients with dilated cardiomyopathy (DCM) [40]. While one study showed histopathological derangements in myocardial biopsies of BrS patients [41], the pathophysiologic role of these derangements was questioned in another study [42]. Cardiac magnetic resonance (CMR) imaging in BrS patients
showed subtle abnormalities, including mild dilation of the RV outflow tract (RVOT) and reduced contractile function [43-47]. As in ARVC/D, structural changes predominantly occur in RV (especially the RVOT in BrS), despite the fact that the genetic alterations affect cells from both RV and LV.

2.2 Hemodynamic and cellular changes of cardiac remodeling

Hemodynamic changes

The RV internal surface consists of rough trabeculae and a wide angle between inflow and outflow tracts, further complicating determinations of ventricular function. The complexity of the geometry of the RV often increases in relation to different diseases of the RV. Postmortem examination of patients with varying degrees of LV MI showed profound RV geometric changes, most often dilatation, followed by hypertrophic and aneurysmatic remodeling [48]. Paradoxically, dilatational remodeling led to decreased ventricular volume, probably because hypertrophy of the interventricular septum with subsequent lateral displacement of the RV and a subsequent change of the triangular shape. Secondary to remodeling both increased [25,49] and decreased RV ejection fraction have been described [21,50-52]. Furthermore, altered end-diastolic volume, [21,23,26,50,52,53] end-systolic volume, [51,50,53] end-diastolic pressure, [21,54] and end-systolic pressure [21,26,55] have been described. In addition to altered pressure and volume, RV hypertrophy is also common. Because baseline RV muscle mass is less than that of the LV, the RV is more sensitive to changes in after-load [21]. Accordingly, RV hypertrophy has been associated with increased RV after-load in numerous animal [27,28,54,56,57] and human investigations [48]. Though RV geometry, hypertrophy, and pressure-volume relationships are frequently interrelated,
studies have noted relatively stable RV hemodynamics despite RV hypertrophy [26,58], suggesting that RV hypertrophy is due to more than simple pressure/volume overload, and that hypertrophy alone may not be a harmful process.

**Cellular changes**

Several investigations have observed alterations in cardiac myocytes from increased RV myocytes diameter in congestive heart failure [59] to increased myocytes length with decreased cross-sectional area in dilated cardiomyopathy [60]. Olivetti et al. reported in long term pressure overload-induced RV hypertrophy increased mean myocytes cross-sectional area with a less elongated cellular shape in rats [54]. In a chronic high-altitude hypoxia rat model of RV remodeling, significantly wider myocytes were found in the youngest age groups, suggesting age-related changes in remodeling [61]. Furthermore, RV pressure overload in felines also increased mean myocardial cell volume, primarily through increased myocyte diameter [59]. In guinea pigs, myocyte volume increased following increased RV systolic pressure, secondary to LV failure [55]. Evidence suggests that remodeling may also manifest as increased extracellular space between myocytes [50]. Currently, it is accepted that apoptosis plays an important role in LV disease [62]. Moreover, accumulation of collagen was demonstrated in hypertrophied and failing hearts [63]. Apoptosis and fibrosis have been shown to act in isolation or in concert to reduce LV performance [64, 65]. In contrast, the association of apoptosis with RV disease progression is unclear [66, 67].

In addition, activation of immune-inflammatory response plays an important role in the pathogenesis and progression of LV heart failure [68,69]. Patients with LV heart failure have increased levels of circulating
inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), IL-1β, and various chemokines, e.g., monocyte chemo-attractant peptide (MCP)-1 and macrophage inflammatory protein (MIP)-1α [70-72]. Furthermore, levels of activated circulatory cytokines correlate directly with the severity of LV disease [71,73,74]. However, the knowledge of immune-inflammatory processes in RV remodeling and failure is limited.

3. Aims of the thesis

The fundamental involvement of the RV in both common and rare cardiovascular diseases and the lack of knowledge about its normal and pathophysiological function, were the main motivation for this study. Experimental model of RV heart failure secondary to chronic pulmonary hypertension, ARVC/D patients and Brugada patients were studied.

The first aim of this thesis was to determine the occurrence of apoptosis in RV disease progression. Also, we tested the feasibility of imaging apoptosis with the use of serial in vivo ⁹⁹mTc-annexin V scintigraphy.

The etiology of RV failure involves multiple triggers and conditions, but the progressive loss of cardiac myocytes is one of the most important pathogenic components. During the past few years, there has been accumulating evidence in both human and experimental models suggesting that apoptosis may be an important mode of cell death during heart failure. Despite the wealth of available data, there are still controversies on the role of apoptosis in heart failure. These controversies stem largely from the limitation of the techniques used to detect apoptosis and the difficulties in translating these to the clinical significance of apoptosis in heart failure. In recent years, in vivo imaging of cardiac apoptosis with the use of ⁹⁹mTc-
annexin V was proven feasible. $^{99m}$Tc-annexin V binds to exposed phosphatidylserine on the outer surface of apoptotic cells and allows for consecutive assessment of apoptosis in vivo. This may not only provide insight into the time course of apoptosis, but it may also aid in determining the optimal timing of anti-apoptotic therapy, and in assessing the therapy efficacy.

Thus far, the role of immune-inflammatory processes has been studied only in patients with left ventricular disease. The mechanism of inflammation includes structural and functional changes of the myocardium that are responsible for the development of symptoms, but also for the progression of disease. To study the association between immune-inflammatory activation and RV disease progression was therefore the second aim of this thesis.

Thirdly, we investigated the structural remodeling of the RV myocardium in rare diseases such as ARVC/D and Brugada syndrom. Using complementary findings from imaging assays and functional assay we aimed to obtain clinical evidence that SCN5A is involved in maintaining cardiac structural integrity. Visualization of the localization and extent of cell death and the inflammatory process within the myocardium in ARVC/D allows guided procedures, therapeutic decisions and initiation of appropriate treatment.
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