The stressed right ventricle and its impact on the left ventricle

A testament to the progress made in pediatric cardiology and cardiac surgery is the extraordinary improvement in survival in patients with congenital heart disease (CHD) with a nearly 30% reduction in mortality from the 1980s to the early 2000s. This is mostly attributed to improved outcomes in infants with severe forms of CHD such as right sided obstructive lesions and single ventricles and a resulting dramatic increase in adults living with repaired CHD, now representing 1.4 million individuals in the U.S. alone. These improved outcomes have led to an increasing number of infants, children and adults with heart failure. In particular, RV failure is an important determinant of clinical status and outcomes in children and adults with various types of CHD. As discussed earlier in this thesis, the RV is at risk for failure from a variety of causes including reduced contractile function (e.g. arrhythmogenic right ventricular cardiomyopathy and Ebstein anomaly), increased pressure-loading (e.g. RV-pulmonary artery (PA) conduit stenosis after repair of truncus arteriosus or pulmonary atresia and pulmonary hypertension), increased volume-loading (e.g. pulmonary regurgitation (PR) after repair of tetralogy of Fallot (rTOF)), electro-mechanical dyssynchrony (i.e. incoordinate contraction between different segments of the ventricle) induced by right bundle branch block (e.g. rTOF), increased myocardial fibrosis (e.g. rTOF), abnormal coronary perfusion (e.g. pulmonary atresia with intact ventricular septum, pulmonary hypertension), restricted filling capacity (e.g. Fontan circulation), inefficient energy transfer between the ventricle and the vasculature (e.g. pulmonary hypertension , Fontan circulation) and adverse interactions between the RV and LV (e.g pulmonary hypertension, hypoplastic left heart syndrome (HLHS)). In many instances the co-existence of multiple factors may lead to RV failure, such as in the systemic RV with tricuspid regurgitation.

In **Chapter 3**, I used the known differences in clinical course and outcomes between pediatric pulmonary hypertension versus RV pressure loading to understand differences in the RV response to these two pressure-loading conditions. Using echocardiography, we found that children with PAH demonstrate adverse global and regional RV remodeling and mechanics compared to those with PS. The mechanisms we observed of RV systolic dysfunction in PAH included decreased longitudinal strain (deformation), usually the dominant contraction vector of the normal RV, decreased or absent transverse shortening, which reflects a loss of the bellows action of the normal RV and post-systolic shortening, reflecting contractile inefficiency and increased segmental interactions. As detailed below, some of these markers, such as inefficient transverse shortening and post-systolic shortening, are not routinely used in current clinical practice, but may be useful to identify children at risk of RV failure and hence at risk of morbidity and mortality. Echocardiographic markers of RV dysfunction have been found by our group and others in other high-risk situations such as hypoplastic left heart syndrome (HLHS).
In a recent study we identified RV remodeling and decreased RV fractional

area of change (a surrogate of ejection fraction) to be powerful predictors of outcome.[1] Additionally, a more direct assessment of RV myocardial function via echocardiography strain imaging may be beneficial in assessment of HLHS. In a small study of 35 infants with HLHS during the first 6 months of life, RV strain analysis was found to identify at-risk HLHS infants with interstage strain values being worse in infants with HLHS who had a poor cardiac outcome as defined by cardiac death, heart transplantation, or persistent moderate or greater RV dysfunction.[2] Similarly, when assessed before the bidirectional cavopulmonary anastomosis, children with good RV function by echocardiographic measures such as RV fractional area change (a surrogate for ejection fraction) and RV strain had a low likelihood of death or heart transplantation.[3] In HLHS patients with normal RV fractional area change values, reduced strain may improve prediction of clinical outcomes.[3] Similarly, we found that longitudinal assessment of strain in children with PAH predicts clinical outcomes.[4]

The contractile dysfunction in PAH we observed by echocardiography may stem from the unique molecular responses of the stressed RV myocardium. As detailed in Chapter 2, investigators have highlighted differences in the mechanisms of right vs. left ventricular failure.[5] Indeed, data from animal models of RV stress mimicking residual lesions after repair of tetralogy of Fallot have shown extracellular matrix and cytoskeletal remodeling, upregulation of genes regulating reactive oxygen species production and downregulation of antioxidant protection, angiogenesis, energy production and mitochondrial function more so than that seen in the LV under stress.[6] Similarly, deranged mitochondrial function, energetics and microcirculation leading to myocardial dysfunction have been described in the pulmonary hypertensive RV.[7] As some of the molecular pathways, such as those regulating angiogenesis, metabolism, and mitochondrial dynamics, are similarly deranged in the RV and pulmonary vasculature, there is the possibility of therapies that treat the RV and pulmonary circulation.[8] Interestingly, RV volume and pressure overload may demonstrate similar changes; however, pressure overload shows a more severe phenotype at the molecular level.[6]

The differences in the molecular response of the RV and LV could impact the effectiveness of the drugs used to treat heart failure. In **Chapter 7,** we described in preclinical models of RV pressure-loading, that treatment with losartan led to an improvement in fibrosis and cardiac hypertrophy.[9] However, the REDEFINE trial in adults with repaired tetralogy of Fallot suggested that renin-angiotensin-aldosterone inhibition using losartan is not beneficial for patients with mild RV failure.[10] However, the findings from the REDEFINE study may suggest that once RV fibrosis has developed, as the molecular correlate of RV dysfunction, that it cannot be reversed with angiotensin receptor blockers, but that institution of angiotensin receptor blockers prior to the development of fibrosis might be beneficial. It is also possible that longer-term administration of angiotensin

receptor blockers might be required to limit the slow progression of RV fibrosis in these patients.

Despite the differences in the RV and LV response to stress, as described in **Chapter 2**, and in **Chapters 3-5** our studies demonstrate that RV remodeling leads not only to RV, but also LV dysfunction. We have further shown that RV dysfunction in association with volume or pressure loading is associated abnormal LV in other conditions such as Ebstein anomaly and repaired tetralogy of Fallot.[11,12] These results show that adverse RV-LV interactions are important in diverse conditions and impact clinical outcomes. RV dysfunction in LVF more than doubles mortality through direct interaction or secondary to PV remodeling[13]. In RVF, there is a linear relation between RV and LV dysfunction; and sudden death is higher in patients with co-existing LV dysfunction.[14] An abnormal LV also affects the stressed RV. We have recently shown that LV size and septal thickness are associated with worse outcomes in children with HLHS.[1] This data provides additional risk factors for adverse outcomes when counseling parents. From the data presented in these chapters, it is apparent that events in one ventricle profoundly affect the other; and that LV and RV function and failure cannot be regarded separately.

In **Chapters 5 and 6**, we showed that temporal RV-LV interactions combine with geometrical RV-LV interactions to affect biventricular function and that these can be imaged effectively with echocardiography-providing a clinically relevant bedside tool to image RV-LV interactions. Imaging of these geometrical interactions do not account for the disparate timing of RV and LV events that substantially impact ventricular-ventricular interactions in pulmonary hypertension. Given its high temporal resolution and the ability of M-mode to simultaneously image the RV, interventricular septum and LV, it is useful to study event timing using M-mode and Doppler echocardiography. From the PSAX view, at the level of the mitral leaflet tips or LV papillary muscles, an M-mode cursor can be placed through the RV free-wall, interventricular septum and LV posterior-wall. With raw DICOM images, some software allows post-processing placement of an anatomical M-mode on the 2-D image positioned to afford best visualization of RV, septal and LV thickening and excursion; but provides lower frame-rates than regular M-mode, an important disadvantage for event-timing. On the M-mode trace, the timing of semi-lunar and atrio-ventricular valve opening and closure can be superimposed directly or as a schematic representation as timed from Doppler images obtained at similar heart rates sampled in the RV and LV outflows and inflows.

In **Chapter 5 and 6** we showed how RV pressure-loading leads to RV dysfunction characterized by prolonged isovolumic contraction, contraction and isovolumic relaxation. This prolongs RV systole and shortens RV diastole. These components may be imaged by flow or tissue Doppler. Normally, RV isovolumic

contraction and relaxation are very short. In pulmonary hypertension, as the (failing) RV myocardium struggles to mount adequate force in early systole, isovolumic contraction prolongs. This can be measured from the onset of the ECG QRS complex to onset of pulmonary ejection from a Doppler sample in the RV outflow tract or pulmonary artery. Next, RV contraction is blunted and prolonged as the RV needs more time to maintain output through the high resistance pulmonary vasculature. This can be observed as reduced longitudinal strain, with myocardial shortening (contraction) that extends into early LV diastole or delayed RV free-wall peak excursion on M-mode. Despite prolonged RV contraction, paradoxically, pulmonary ejection time is shortened with short acceleration time, measured as onset to peak pulmonary ejection from Doppler flow, and a short ejection-time with low stroke-volume given the increased distal resistance. Thus, despite prolonged RV contraction, which pushes the septum leftward for a long portion of the cardiac cycle, ejection is short. The consequences are inefficient RV contraction and impaired LV filling. From an imaging standpoint, inefficient RV contraction and adverse RV-LV interaction, can be visualized as prolonged TR duration (corresponding to prolonged RV contraction), short pulmonary ejection and low pulmonary velocity time integral (corresponding to low stroke-volume) and impaired LV filling with short mitral filling and often reversed E/A ratio typical of delayed relaxation. As described in **Chapters 2, 4 and 5**, due to prolonged RV contraction, RV systole extends into early LV diastole. Peak RV shortening by 2-D speckle strain imaging occurs at time of mitral valve opening. These adverse interactions worsen LV filling. Because the RV continues to contract while the LV is relaxing, a sharp, early-diastolic leftward septal deflection can be seen on M-mode due to continuing high RV-pressures, while at the same time, LV pressures are falling in early diastole. Consequently, LV eccentricity index is maximal at this time. Thus, in pulmonary hypertension substantially impaired early LV filling can be imaged by conventional measures of LV diastolic filling and by LV diastolic strain-rate.

In **Chapter 7** I demonstrated how the adverse mechanics and RV-LV interactions RV pressure-loading described in **Chapters 2-5** translate into cardiomyocyte hypertrophy and myocardial fibrosis. As fibrosis is thought to worsen ventricular function in diverse cardiac conditions and constitutes a common final injury pathway in diverse organs (e.g. kidney, liver, skin, lung etc), there is an intense interest in fibrosis and anti-fibrotic therapy.

Tissue repair is fast because it mainly uses the extra-cellular matrix (ECM) to fix the damage as opposed to tissue or organ regeneration that restores the appropriate cell and ECM components.[15] Without controlled scarring, the myocardium would rupture after severe infarction or chronic overload. Similarly, in other organs, beneficial fibrotic tissue reduces pressure on chronically overloaded kidney glomeruli to maintain proper filter function and in the absence of stabilizing scar tissue, lung alveoli would tear under continuous breathing expansion. When

discussing myocardial injury, it is important to consider that "detrimental" fibrosis encompasses the same fundamental mechanisms and pathways that are involved in normal healing.[16] Both the normal healing and ultimately injurious fibrosis process is a highly regulated sequence of overlapping phases comprising hemostasis, inflammation, proliferation, and remodeling/maturation.[17] The ECM performs central functions at all stages, both as a reinforcing material and cell signaling instructor.[18] Thus, ECM remodeling and fibrosis are highly complex processes and therapeutically targeting a single component, of this complex system; which itself is just one component of the myocardium may be inadequate.

The main objective of losartan administration in my study (**Chapter 7**) was to demonstrate the importance of TGF-B1 signaling in mediating biventricular fibrosis in response to RV pressure loading. The results nonetheless demonstrate the potential to treat RV and LV myocardial fibrosis by targeting the TGFβ1 molecular axis. Likewise **Chapter 8** describes mechano-transduction pathways through β1integrin signaling that activate TGFβ1 signaling and may be a therapeutic target. In parallel, we also describe increased elastin deposition that may alleviate adverse RV-LV interactions through increased compliance and hence a new therapeutic avenue. Nonetheless, more specific targets are still needed.

In **Chapter 9**, 'The stressed right ventricle in repaired tetralogy of Fallot: Dyssynchronous activation induces regional disparities in stress and function' we explore additional factors that stress the RV, other than pressure or volume loading, specifically RV electro-mechanical dyssynchrony that stems from right bundle branch block. In adult left heart failure, a substantial portion of patients have la broad QRS that stems from left bundle branch block. This conduction delay to electrical activation of the LV free wall causes a complex pathophysiology of disparate contraction-relaxation between the late activated LV free wall and early activated septum which leads to disparate wall stress, regional activation of myocardial injury pathways and LV dysfunction. Consequently, a large number of such patients have greatly benefited from pacing therapy to 'overcome' the electrical conduction delay and mechanically resynchronize LV contraction with translation of these mechanical benefits to improved morbidity and mortality.

Although over 90% of rTOF patients have a broad QRS duration, mostly from RBBB, and while a wide QRS is thought to be a risk factor for increased morbidity and mortality in rTOF, electromechanical dyssynchrony has, to date, not been a substantial therapeutic target and overwhelming emphasis has been placed on the timing of pulmonary valve replacement to treat pulmonary regurgitation and RV volume loading. However, there are few conclusive data to show that PVR has reduced mortality or risk factors associated with mortality such as ventricular arrhythmia. Although, ventricular dilatation and remodeling, may be associated

with QRS widening, our study demonstrates that a broad QRS is as, or perhaps even more associated with RV dysfunction and exercise intolerance, than PR. This suggests that RBBB, as much as or more than PR should be a therapeutic target. Our results have strong implications for management of rTOF and at a minimum provide strong support for a clinical trial to evaluate the usefulness of RV cardiac resynchronization therapy in rTOF with RV dysfunction and heart failure symptoms.

Chapter 9, over and above the content findings of electromechanical dyssynchrony and its contribution to RV dysfunction in rTOF, presents a novel methodological approach that can be applied to a wide array of clinical problems. Consequently, this work is highly novel both in its implications for rTOF specifically and in its innovative approach using advanced statistical analysis on actual patient data combined with computer modeling to generate hypotheses, demonstrate pathophysiological mechanisms and potentially beneficial therapies across a spectrum of severity. The next natural step would be to apply this approach to specific patients contributing to a personalized medicine approach.

In summary, this thesis studies the pressure-loaded and dyssynchronous RV, both associated with regional inhomogeneities in RV wall stress and function that are associated with global RV dysfunction and adverse clinical status and outcomes. Moreover, the results of the studies presented in this thesis demonstrate that the pressure loaded RV impacts the LV, not only by septal shift and adverse hemodynamics, but also by temporal discordance of interventricular events. The biventricular geometrical aberrations, increased wall stress and dysfunction negatively impact the RV and LV, from regional tissue injury to global hemodynamics. Thus, our studies demonstrate a wide spectrum of associated abnormalities from adverse hemodynamics to myocardial pro-fibrotic signaling. These studies also translate the experimental and observational patient data to the bedside by providing non-invasive bedside tools, through echocardiography, to diagnose RV dysfunction and adverse RV-LV interactions, as well as suggesting novel therapeutic avenues to address them.