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Part 4

Summary and future perspectives
Chapter 4.1

Summary and future perspectives
The aim of this thesis is to review the current knowledge on atrioventricular septal defect (AVSD) development, early diagnosis and long-term follow-up (Part 1), to study the pathogenesis of AVSD (Part 2) and finally to analyze cardiac outcome long-term after AVSD correction (Part 3) with special emphasis on intra-cardiac flow patterns. Studies are performed with novel imaging techniques as fetal echocardiography and 4-dimensional velocity-encoded cardiac magnetic resonance imaging (4DFlow MRI).

Chapter 1.2 of this thesis contains a bench to bedside overview of AVSD. The spectrum of AVSD, comprises a variability in the extent of the defects in the septal tissue in proximity of the atrioventricular valves and of abnormalities of the atrioventricular valves themselves. As controversy exists in literature regarding terminology of AVSD, nomenclature and classification are addressed. The discovered developmental aspects (i.e. looping, mesenchymal cap, endocardial cushions and dorsal mesenchymal protrusion) and novel insights in genetic and maternal risk factors for AVSD are discussed. Additionally, an overview of innovations and challenges of diagnosis, which can already be made during the first trimester, is provided. The review displays that data on long-term follow-up of cardiac function after AVSD correction is limited and that literature mainly discourses the increasing survival rate and high incidence of surgical re-intervention because of left atrioventricular valve (LAVV) regurgitation. Finally, the review highlights differences between non-syndromic patients and patients with Down syndrome in the incidence of AVSD and in the associated cardiac anomalies. Early studies report poor outcome in AVSD patients with Down syndrome, whereas more recent studies report equal survival and even lower incidence of surgical re-intervention of valve regurgitation in patients with Down Syndrome as compared with non-syndromic patients. Recognition of anatomical factors related to outcome may aid identification of risk factors and predictors of outcome during follow-up of patients with AVSD.

PATHOGENESIS OF AVSD

Contributing developmental factors
In the first 10 weeks of gestation looping, remodeling and septation of the initially linear heart tube results in a heart with two atria, two atrioventricular valves, two ventricles and a septated outflow tract with a separated pulmonary valve and aortic valve. In Chapter 1.2 it is shown that development of an AVSD can be a result of incomplete development of different congenital structures (i.e. endocardial cushions, mesenchymal cap, dorsal mesenchymal protrusion). The detected genes associated with AVSD affect one or more of these structures. The combination of an inflow- and an outflow-tract defect, as seen in patients with AVSD with Tetralogy of Fallot, stresses the assumption that a defect in genes expressed in the progenitor cells dorsal of the heart, also referred to as the second heart field (e.g. genes like vascular endothelial growth factor described in Chapter 2.4), can be the culprit of both inflow- and outflow-tract defects. On
the other hand, the variety of involved genetic and epigenetic factor can explain the range in subtypes of AVSD.

Within AVSD, variation exists in the location and extent of the defect of the (atrioventricular) membranous septum and atrioventricular valves. Around a quarter of patients with Down syndrome develop an AVSD. There are, however, signs that patients with Down syndrome without AVSD also develop abnormalities, evidenced by the observation of prenatal tricuspid regurgitation and mitral valve prolapse. In Chapter 2.1 abnormalities of the membranous septum and atrioventricular valves are observed even in patients with Down syndrome without AVSD. Microscopy of fetal hearts shows differences, especially a larger membranous septum and dysplastic atrioventricular valves in fetuses with Down syndrome without AVSD as compared with normal hearts. Echocardiography reveals a shorter muscular ventricular septum in Down syndrome patients both with and without AVSD as compared with normal hearts. These findings confirm the hypothesis that the spectrum of AVSD forms a sliding scale. Therefore, an AVSD without shunting, i.e. an AVSD-valve morphology (common valve with linear insertion), where the common valve is attached to both the atrial and the ventricular septum, might also exist. We speculate that the extensiveness of AVSD is influenced by the degree of maldevelopment of the different structures involved in atrioventricular septation and may relate to differences in affected gene(s) involved.

Fetal echocardiography: the impact of flow

The primitive heart tube starts to beat in the 5th week of pregnancy (22 days after conception). Even though organs are not depending on active fetal circulation yet, blood flow is crucial for haematopoiesis and cardiogenesis. Studying blood flow during heart development might consequently give valuable information on normal and abnormal heart development. Current ultrasound techniques in human allow detection of an AVSD in the first trimester, between 10 and 14 weeks of gestation, by skilled staff with high-end ultrasound equipment, as is reviewed in Chapter 2.2. Visualization of the heart and cardiac blood flow is not (yet) possible during the stages of early cardiac development in human, due to limited ultrasound frequency. In mouse, high-frequency ultrasound as early as embryonic day 10.5 (6 weeks after gestation in human), allows analysis of cardiac function before complete septation. In Chapter 2.3 reference values are provided of functional parameters measured at three subsequent stages of development (embryonic day 12.5, 14.5 and 17.5) in wild type mouse embryos. Reliable assessment of left and right ventricular function is possible and diastolic and systolic function improves during normal heart development. Additionally, application of high frequency ultrasound in genetically mutated mouse models will potentially give further insight in hemodynamic during abnormal heart development. Vascular endothelial growth factor (VEGF) is in human related to development of AVSD and Tetralogy of Fallot. Murine Vegf120/1120 embryos, that overexpress the Vegf120 isoform, are known to develop a Tetralogy of Fallot phenotype. In Chapter 2.4, also abnormalities of the atrioventricular valves and an AVSD are described in Vegf120/1120 mouse embryos. Moreover, high
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frequency ultrasound reveals a reduced heart rate in Vegf120/120 embryos at three developmental stages, which is confirmed by ex-vivo optical mapping. Subsequently, different possible causes of the reduced heart rate are analyzed. Immunohistochemistry analyses reveals a smaller and less compact sinoatrial node (SAN), with hyper-vascularization, which might contribute to the sinus bradycardia. Furthermore, the observed increased expression of the high conductance gap junction Connexin43 in the SAN, which is normally expressed in the working myocardium, potentially increases loading time of SAN cells and reduces essential isolation of the SAN from the surrounding atrial myocardium, which may contribute to the observed bradycardia.

LONG-TERM FOLLOW-UP AFTER AVSD CORRECTION

During the surgical correction of AVSD, one or two patches are placed to close shunt(s) between the left and right heart. Most often the remaining ‘cleft’ in the LAVV is surgically closed to prevent regurgitation. Due to the different papillary muscle position, leaflet size and the surgical correction, the shape of the LAVV in corrected AVSD is different from the normal mitral valve. Survival after AVSD correction is as high as 100% in the current era. However, incidence of surgical re-intervention is reported between 2 and 29%, mainly because of regurgitation of the LAVV. Data on cardiac function long-term after AVSD correction is limited. Cardiac magnetic resonance imaging is the gold standard in the evaluation of cardiac function long-term after correction of a congenital heart defect. Furthermore, the recent introduction of 4DFlow allows comprehensive assessment of cardiac function and cardiovascular blood flow phenomena.

In Chapter 3.1 the use of 4DFlow MRI to assess cardiac function and the rapid development of applications to visualize and quantify cardiovascular blood flow are described. New visualization tools have enriched knowledge on blood flow hemodynamics and efficiency in the cardiovascular system. Most reports focus on normal and abnormal flow in the aorta, whereas data of intra-cardiac flow is limited. One of the available visualization tools is streamline visualization. Streamlines are lines instantaneously tangent to the local velocity vector in each position at a specific time point and connected to all points along the direction of this line. Therefore, streamlines can be used to visualize the flow direction only at a given time point. This limitation can be overcome with particle tracing. With particle tracing virtual particles are positioned at a predefined position and time and subsequently released inside the three dimensional (3D) velocity field and then followed backwards or forwards in time. The trajectory of the particles is calculated in 3D by either forward or backward tracing, by using the local velocity at each position and time point and calculating the next or previous position of this particle.

Disturbed left ventricular inflow

In Chapter 3.2 streamlines are used to analyze inflow direction in patients after AVSD correction. In these patients a dynamic and more lateral inflow direction is observed as compared
with healthy controls. In addition, more accurate measurement of the trans LAVV flow can be achieved by adjusting the measurement plane perpendicular to the peak velocities as visualized by streamlines. In **Chapter 3.3** the inflow is further investigated in 3D and in time with the use of particle tracing. In this study intra-cardiac particles are labelled as components of flow discriminating 1) *direct flow* entering and exiting the LV within the analyzed diastole and systole, 2) *retained inflow* entering during diastole but remaining in the LV during the following systole, 3) *delayed ejection flow* already present in LV prior to diastole but exiting the LV during the following systole and 4) *residual volume* present and remaining in the LV during the analyzed diastole and systole cardiac cycle and 5) *regurgitant flow*. Subsequently, quantification of flow is done with the use of the American Heart Association standardized 16-segment left ventricular cavity model. In patients after AVSD correction direct flow components are decreased and the delayed ejection components and residual volume components are increased. Inflow into the (infero- and antero-) lateral segments confirms the lateral inflow direction observed using streamline visualization in chapter 3.2. In addition, an increase of flow into the apical segments is found. Interestingly, the flow in the (inferior) lateral and apical segments is predominantly retained flow, suggesting flow in these directions to be less efficient.

An additional consequence of the more lateral inflow direction is that this may interfere with the assessment of diastolic function parameters with echocardiography as echocardiography relies on accurate alignment of the Doppler beam. One of these diastolic function parameters is velocity propagation. In **Chapter 3.4** a novel velocity encoded MRI method to assess velocity propagation is introduced and a comparison is made with the traditionally used Color M-mode echocardiography approach. In healthy subjects and patients with ischemic heart failure, the new method demonstrates good agreement with echocardiography to identify LV impaired relaxation. In contrast to echocardiography, this novel approach allows retrospective placement of the measurement line parallel to the inflow direction. This might be beneficial in patients with abnormal inflow direction, such as AVSD patients or patients with a poor echo window, such as patients with Duchenne muscular dystrophy.

**Disturbed vortex formation in the left ventricle**

As the inflow direction is disturbed in corrected AVSD patients, the subsequent study aims to investigate whether vortex formation in the left ventricle is also affected. When blood enters the left ventricle through the LAVV during diastole, shear layers separate and a vortex ring forms. A vortex ring is a compact region of swirling blood flow and is purposed to aid transport of blood flow, minimize kinetic energy loss, help LAVV closure and prevent thrombus formation. In a previous study (Elbaz et al. JCMR 2014) we have shown that a separate ring forms during early and late diastole, with the late filling having a position closer to the atroventricular valve and closer to the ventricular long axis. The shape of the late filling vortex ring is less circular compared with the early filling ring and this circularity is related to the circularity of the inflow jet. In **chapter 3.5** the vortex formation in corrected AVSD patients is compared with the vortex
formation in healthy controls. In contrast to controls, who all display a vortex ring during early filling, in 18% of patients after AVSD correction the vortex ring in the left ventricle is found to be absent. Absence of vortex ring formation is related to a high vortex formation time (due to high inflow velocity) and abnormalities of the LAVV (i.e. single papillary muscle and double orifices LAVV). Moreover, when a vortex ring is present, the center of the vortex core is positioned more towards the lateral wall, closer towards the apex and more anterior. Furthermore the vortex ring is more tilted with its septal side down and with a less circular shape. The abnormal orientation is related to the inflow direction on streamlines. Because patients included in the study are relatively asymptomatic, correlation between vortex formation and conventional function parameters is difficult. However, the results from this study prove the impact of the valve shape on vortex formation in vivo and in 3D, which was previously suggested in in vitro studies or in 2D analysis.

**Regurgitation of the left atrioventricular valve and atrial flow patterns**

Regurgitation of the LAVV is common after AVSD correction and surgical re-intervention incidence to correct LAVV regurgitation is high. Echocardiography is the technique most commonly used to evaluate regurgitation of the LAVV. However, poor inter-observer agreement is described for echocardiographic grading of regurgitation in corrected AVSD patients. In chapter 3.6 the regurgitation in corrected AVSD patients is characterized with streamlines. Multiple regurgitant jets are observed, with a dynamic behavior during systole, a non-circular shape and an overall eccentric (lateral) regurgitation direction. These findings explain why quantification of regurgitation with echocardiography is difficult in these patients, because Doppler quantification techniques (i.e. PISA and Vena Contracta measurements) are based on the assumption that the regurgitation jet is single and circular. Furthermore, echocardiography is known to underestimate eccentric regurgitation. Streamline visualization allows retrospective positioning of the measurement plane perpendicular to the jet during systole, which results in quantification of the amount of regurgitation with excellent internal flow validation. Correlation between grading of regurgitation with echocardiography and 4DFlow valve tracking is modest. The eccentric and dynamic regurgitation of the LAVV in corrected AVSD patients disturbs the normal left atrial flow pattern as is shown in Chapter 3.7. With the combined use of streamlines, vortex core extraction and particle tracing, a single recirculating flow pattern is displayed in the left atrium of healthy volunteers. The recirculating flow was predominantly formed from blood coming from the left pulmonary veins. In corrected AVSD patients with regurgitation, multiple recirculating flow patterns are seen around the regurgitant jet(s) and contribution of left pulmonary vein blood to the recirculating flow is diminished. Furthermore, the peak flow rate in the left pulmonary veins is delayed. The disturbed recirculating flow patterns in the left atrium may contribute to the poor clinical outcome of even asymptomatic patients with regurgitation of the LAVV.
CONCLUSIONS AND FUTURE PERSPECTIVES

AVSD development

This thesis shows diversity in subtypes of AVSD and highlights different developmental/genetic cascades that can result in an AVSD. The increasing amount of studies on genetic and epigenetic factors of heart development will step by step unravel the multiple factors involved in development of AVSD. Studies in other genetically mutated mouse models of Down syndrome related genes (e.g. DSCAM, CRELD1) or genes that are currently not related to Down syndrome (e.g. Pdgfr- α, Nkx2.5, Tbx5) will provide insight in the differences in AVSD between patients with Down syndrome and non-syndromic patients. Studies in mouse models will also provide better understanding of the effect that specific genes have on the different structures involved in AVSD pathogenesis and in which subtype of AVSD they result.

Furthermore, it is made plausible that AVSD is a sliding scale and that patients with Down syndrome without AVSD also have abnormalities of the membranous septum and atrioventricular valves. Future studies in these patients and in family members of AVSD patients are needed to further evaluate these cardiac abnormalities and determine if clinical outcome is also affected.

In the current thesis, high frequency ultrasound in mouse embryos shows to be a promising technique to study cardiovascular flow in early stages of heart development. In humans with congenital heart disease sick sinus syndrome is observed. Disturbed VEGF signaling has been associated with congenital heart defects (AVSD and Tetralogy of Fallot) in human and in mouse models. Whether disturbed VEGF signaling, that in the current thesis is shown to disturb SAN formation in mouse embryos, also plays a part in abnormal SAN development in humans with congenital heart disease remains to be investigated. Also, the relevance of VEGF signaling in other components of the cardiac conduction system or its innervation remains to be determined. In zebrafish, a reduced heart rate showed to disturb valve development via shear stress related genes as Kif2. From our studies it is impossible to distinguish whether the abnormal atrioventricular valves in Vegf^{120/120} embryos are solely an effect of the Vegf mutation or if a reduced heart rate also plays a part. Studies with pharmacological influenced heart rate combined with high frequency ultrasound in mouse embryos may gather more insight in the effect of heart rate on atrioventricular valve development and atrioventricular septation.

Intra-cardiac flow in patients with congenital heart disease

In part 3 of this thesis, 4DFlow MRI data reveals that patients with an abnormal LAVV after AVSD correction have aberrant intra-cardiac flow patterns. During diastole the inflow into the left ventricle is directed more towards the lateral wall, more towards the apex and vortex formation is abnormal. The disturbed inflow is most likely due to the congenitally different morphology and position of the LAVV, as well as the cleft closure during correction of an AVSD. This stresses the impact of valve surgery on intra-cardiac flow. Next to the aberrant inflow, during systole the dynamic and eccentric regurgitation of the LAVV disturbs the normal recirculating flow patterns
in the left atrium. We cannot preclude that the aberrant flow in the atrium and ventricle is a coping mechanism of the heart and has a favorable effect on the cardiac function. However, *in vitro* studies have shown that a lateral inflow direction and disturbed vortex flow, as found in our studies, cause an increase in energy dissipation. Furthermore, the disturbed flow in the left atrium and left ventricle may affect myocardial remodeling through changes in wall shear stress and in that way cause regional hypertrophy and stiffening. Newly available applications to quantify different forms of energy from 4DFlow MRI data, will potentially be able to tell whether the disturbed intra-cardiac flow is indeed related to more energy loss and less efficient flow. Future long-term follow-up studies have to show whether the observed changes in blood flow in corrected AVSD patients eventually result in poor clinical outcome.

In addition, in part 3 of this thesis it is shown that patients after AVSD correction have complex, multiple, dynamic and eccentric regurgitation jets. In patients after AVSD correction, streamline visualization from 4DFlow MRI can advance quantification of LAVV inflow and regurgitation. Retrospective valve tracking with the use of streamline visualization has the potency to be similarly beneficial in other patient groups with congenital and acquired heart disease. The adjustment of the measurement plane perpendicular to the flow during the cardiac cycle will especially be beneficial in patients with a disturbed inflow direction and eccentric and dynamic regurgitant jets, that are challenging to quantify with echocardiography. Development of computerized valve tracking will enable shorter post-processing time and potentially reduce observer variation.

Intra-cardiac flow analyses with 4DFlow MRI provided interesting new data in patients with a congenital heart disease, however the limitations of the different imaging techniques have to be kept in mind. In addition, validation of techniques with phantoms and echocardiography remain important. Evaluation of intra-cardiac flow in patients with a congenital heart disease is a relatively new field. The offered 4DFlow visualization techniques in the current thesis have the potency to provide valuable information of patients with other congenital heart defects (i.e. after single ventricular repair or in Tetralogy of Fallot). Additionally, knowledge on intra-cardiac flow may allow better understanding of diastolic (dys)function. Furthermore, quantification of flow component distribution and vortex cores in the left ventricle could potentially serve as an early predictor of cardiac dysfunction.

Continuing efforts to incorporate results of basic science studies into clinically oriented studies, will allow optimal comprehension of the mechanisms behind the phenomena observed in congenital heart disease patients, which will ultimately benefit clinical practice.