Right ventricular adaptation to chronic abnormal loading

Hagdorn, Quint

DOI:
10.33612/diss.135804654

10

Discussion

The incredible improvement in survival and clinical status of patients with tetralogy of Fallot is a success of modern medicine.[1] However, as discussed in the introduction, great challenges remain in the long-term follow-up of these patients.[2] Considering that patients with repaired tetralogy of Fallot, or other types of congenital heart diseases, have a life expectancy that is far higher than only a few decades ago[3], the amount of adult and elderly patients with congenital heart disease increases. The problems and complications that these patients increasingly face in long-term follow-up, such as arrhythmia and heart failure, will form a growing burden on both individual patients, but also on health care systems.[4,5] It has even been stated that the increase in hospitalizations for congenital heart disease has been "dramatic"[4] in recent years, and we are about to face a "tsunami of arrhythmia".[6] The overarching focus of this thesis was aimed at improving the long-term functional status of the growing group of patients with repaired tetralogy of Fallot. To do so, by means of both experimental studies and imaging-based studies, the aims of this thesis were:

- To characterize RV adaptation to chronic abnormal loading. By studying functional and molecular characteristics of RV adaptation in animal models, we aimed to identify molecular pathways involved in the decline towards RV failure, to aid the development of future targets of treatment.
- To identify prognostic factors on cardiac magnetic resonance imaging. Prognostic factors are essential for the development of risk stratification schemes, to allow a tailored and preventive approach for patients with repaired tetralogy of Fallow in long-term follow-up. Therefore, we aimed to assess the predictive value of cardiac magnetic resonance, a cornerstone of long-term follow up, in patients with repaired tetralogy of Fallot.
- To study sex differences in RV adaptation to chronic abnormal loading. Currently, it remains unknown whether differences between sexes emerge not before puberty or already prior to pubertal development, and whether such differences merit a tailored approach with regards to cardiac imaging. Therefore, we aimed to describe sex differences both an imaging-based study in patients with repaired tetralogy of Fallot and an animal model of childhood RV pressure load.

Chronic experimental RV volume load

The systematic review and meta-analysis that **chapter 2** describes, provide an overview of studies that experimental studies on right ventricular (RV) volume load. It became apparent that studies that focus on chronic, RV volume load are scarce, exposing the existence of a relatively blind spot in our understanding of long-term RV failure. Despite that the low number of studies on this topic, in combination with high heterogeneity and lack of consistent study designs and outcome variables hampered meta-analyses of cellular and molecular responses, one of the conclusions that were drawn was that long-term experimental volume load seemed

to be associated with RV fibrosis. This could imply that fibrosis in the volume-loaded RV might be a future target of treatment. However, as the causal relation of RV fibrosis in the pressure loaded RV is increasingly disputed[7,8], further investigation of the relation between volume-loaded RV failure and fibrosis was warranted.

Right ventricular fibrosis

Therefore, in **chapter 4**, we aimed to assess whether myocardial fibrosis is truly associated with long-term volume load. We utilized an animal model of chronic volume overload leading and established clinical long-term RV failure, and described the temporal pattern of ventricular adaptation, fibrosis, and pro-fibrotic signaling. In this chapter, the main finding was that, despite the development of end-stage RV failure, there was no overt myocardial fibrosis. This finding challenges the dogma that myocardial fibrosis, either as cause or consequence, is associated with volume load-induced end-stage RV failure. Also, this also highlights the importance of this study: while suggestions in **chapter 2**, based on little and heterogeneous data, first seemed to suggest that long-term experimental volume load seemed to be associated with RV fibrosis, the comprehensive assessment on this topic in **chapter 4** demonstrated otherwise. Potential factors that may have contributed to the initial suggestion raised in our review, include the use of various animal species, different methods of volume load induction and different methods to quantify fibrosis in the studies included. For example, one of the studies that report fibrosis induces volume loading by creating pulmonary valve insufficiency in mice and describes subendocardial fibrosis.[9] Potentially, the myocardial stitches that have been used to induce valvular insufficiency may have triggered subendocardial hypo-perfusion and subsequent formation of fibrotic areas. Another study uses pigs in which aortocaval shunt was created, but these pigs demonstrated increased RV pressures, inducing a mixed load, instead of isolated volume load.[10] At last, publication bias might have caused that studies that assessed fibrosis, but found no fibrosis, did not report these measurements. Such confounding factors are not present in **chapter 4**, also underscoring its relevance and importance.

Although the results described in **chapter 2** and **chapter 4** provide new insights on cardiac adaptation to chronic abnormal loading conditions of the right ventricle, these have not resulted in an easily applicable new treatment strategy. However, by discouraging targeting fibrosis in RV volume loading, it may prevent useless pharmacological therapies and clinical studies, which can potentially be harmful. Additionally, besides fibrosis and pro-fibrotic signaling, other pathways have been studied for involvement with RV failure. For example, gene expression of the cardioprotective four and a half LIM domains protein 2 (FHL2) was decreased, which might form a treatment target.[11–13] Pirfenidone, a drug mostly known for its anti-fibrotic effect[14], also independently stimulates FHL2 activity[15] and could therewith be a candidate drug for the volume loaded RV.

Furthermore, the organic compound juglone was used to treat rats with pulmonary hypertension and isolated RV pressure load in **chapter 5**. Juglone has been previously shown to decrease myocardial fibrosis of the LV in various models.[16–18] However, in the rats that were subjected to RV pressure, no reduction in myocardial fibrosis could be demonstrated. This is yet another example of an anti-fibrotic treatment strategy that has proven to be successful in LV fibrosis, but not in RV fibrosis; many have preceded juglone in the context of the RV under pressure.[7,19–21] It increasingly seems that RV fibrosis, and RV molecular adaptation in general, have a very different mechanistic role in myocardial dysfunction, when compared to the LV, with different driving factors and response to therapy.[22] Perhaps it is therefore that no pharmacological treatment is available that directly and effectively targets RV dysfunction in congenital heart disease[23], as most therapies are developed for the LV, and then later tested in the RV. Therefore, basic studies that specifically aim for understanding and targeting RV adaptation, as performed in this thesis, are essential in order to be able to directly and successfully support this once forgotten chamber in the near future.[24]

Prognostic value of CMR

Besides heart failure, the other leading cause of cardiovascular death in adults with congenital heart disease is sudden death and arrhythmia.[25,26] Identifying patients who are at high risk is therefore of utmost importance, to be able to timely identify patients at risk to prevent or reduce further deterioration, and eventually select patients who are eligible for implanting an ICD if risk reduction appears to be insufficient. For secondary prevention, in every patient who has experienced cardiac arrest, VT, or unexplained syncope, without reversible causes, ICD implantation is indicated.[27,28] RV and LV dysfunction, non-sustained VT, QRS duration > 180ms, RV fibrosis on CMR and inducible VT during electrophysiological (EP) testing have been described as risk factors for SCD.[29–35] However, despite the existence of predictors and as previously discussed, there is no clear treatment algorithm for primary prevention.

This thesis describes the prognostic capacities of CMR variables for ventricular tachycardia in patients with repaired tetralogy of Fallot in **chapter 7** and **chapter 8**. RV end-diastolic volume (EDV), RV end-systolic volume (ESV) RV mass, BMI and QRS duration were independent prognosticators of VT. These results are partly confirmatory of previous studies, in which RV dimensions and hypertrophy have been demonstrated to predict VT and/or SCD. However, despite that RV mass has been described to be associated with VT and/or SCD, it is currently not mentioned as a risk factor in leading guidelines.[27,28,36] Perhaps, this is due to a lack of measuring RV mass in CMR measurements in older CMR studies. In **chapter 7**, a value of 50g/m^2 is proposed as the optimal cut-off point for prediction of VT, with 83% specificity and 63% sensitivity. Confirmation of this cut-off point in other populations is necessary, but we do believe

that our data confirm that RV mass, as an independent predictor of arrhythmia, deserves a solid place in guidelines for risk stratification for VT in the repaired tetralogy of Fallot population.

Also, an often-described predictor of VT, namely RV EDV, was again confirmed to be an independent predictor. Additionally, in **chapter 8**, RV EDV is described as a predictor of deterioration of ventricular function. Although it may not seem of particular importance to confirm this nowadays almost undisputed predictor, this does remind us of its importance, in the same year that its prognostic value is disputed by results from the INDICATOR cohort.[36] This study demonstrated no predictive value of pre-operative RV EDV for death and VT, in a cohort of patients who underwent pulmonary valve replacement (PVR). In an editorial that accompanied this study, it was even questioned whether RV volumes should be thrown out of decision making in repaired tetralogy of Fallot.[37] However, it seemed that the authors of these citations ignored the potential bias due to the widely spread use of RV EDV as an the indication for PVR itself. PVR is in most centers indicated whenever multiple thresholds in a list of relative indications are met. Patients who are in a relatively good physical and functional state, but who only have a very high RV EDV, are thus generally not operated on, since high RV EDV would be the only relative indication in the list of indications. However, patients who are in symptomatic state may already reach indication for PVR, despite a potentially low RV EDV. Therefore, patients that are enrolled in the study with lower RV EDV may actually be in a 'worse' condition, compared to patients with higher RV EDV. When a cohort of patients who are operated on is studied, one cannot ignore the fact that variables which are clinically used indications for operation induce selection bias. Only prospective randomized controlled trials that test various indication thresholds would be able to fully eliminate such biases, but such studies would need to include many patients with a long follow-up, severely limiting feasibility. **Chapter 7** and **chapter 8** describe cohorts of patients without this operation indication selection bias and still demonstrate that RV EDV is a strong predictor of outcome. Therefore, RV volumes remain important determinants of outcome in the follow-up of these patients, and should thus not be thrown out of decision making in repaired tetralogy of Fallot.

Another finding with relevance for counseling patients with tetralogy of Fallot, is the strong association of increased BMI with both VT and atrial tachyarrhythmia (ATA). The described hazard ratio of 1.09 and 1.11 for VT and ATA, respectively, mean that a patient with a BMI of 30kg/m^2 has a two- to threefold higher risk of VT and/or ATA, compared to a patient with a BMI of 20kg/m^2. This may not seem surprising, as this association is also similarly present in the general population.[38,39] However, it does suggest that BMI could potentially be a future target for both patients and their caretakers, to beneficially influence patients' health status and long-term outcome. Since the current data only demonstrate that BMI is a predictor at baseline, studies that attempt to intervene, for example by means of dietary counseling or

weight reduction programs, are needed to establish whether obesity could truly be a target of treatment. Since the increased risk associated with obesity adds to the already increased risk of arrhythmia, this is of particular importance for patients with repaired tetralogy of Fallot.

Additionally, we used the newer and more advanced post-processing technique of 'standard' cine-CMR images: feature tracking deformation analysis and assessed its prognostic capacities for VT in **chapter 8**. Deformation analysis enables analyses of cardiac motion in different, e.g. longitudinal shortening or circumferential shortening, while conventional CMR measures only measure ventricular volumes and masses, and subsequently EF. Deformation analyses would only have incremental value to conventional CMR variables if they provide any information, that conventional functional variables cannot already provide. Therefore, it is important to recognize the theoretical relations of deformation and EF. Deformation, or strain, is shortening of the myocardium in a certain direction. When shortening of all myocardial area's is 'added up', one theoretically comes to EF.[40] Therefore, when two populations differ in EF, it is to be expected that strain variables also differ. In this situation, there is little additive information that can be derived from deformation analyses. However, in the situation that two populations do not differ in EF, there may still be differences in contraction patterns. For example, longitudinal strain can be decreased but compensated for by circumferential strain.[40] This phenomenon of altered contraction patterns to compensate for abnormal loading conditions has long been recognized.[41] In the repaired tetralogy of Fallot population, two studies have examined the prognostic capacities of deformation analyses in the prediction of VT or SCD, described by Moon and colleagues, and Orwat and colleagues.[42,43] These authors describe deformation variables of both ventricles to be prognostic of VT, albeit with EF also being a predictor. Moon and colleagues have performed multivariable analysis with all CMR variables included, showing no additive value of CMR deformation variables to conventional CMR variables. In contrast, Orwat and colleagues showed prognostic capacities of deformation variables, independent from conventional CMR measurements. We describe a cohort of tetralogy of Fallot patients, in which there was no difference in EF between patients with VT, and patients without VT. Using CMR deformation analysis, however, LV circumferential strain appeared to be prognostic of VT, and superior compared to conventional measures. Thus, this study demonstrates that deformation analyses can provide information that, in this population, conventional analyses could not.

Sex differences in RV adaptation to chronic abnormal loading conditions

There is an increasing body of evidence that substantial differences between sexes exist in cardiac adaptation to chronic abnormal RV loading conditions. However, such differences are currently not accounted for in clinical guidelines, and it remained unclear whether differences between sexes are already present prior to pubertal development. **Chapter 9** demonstrates that in patients with repaired tetralogy of Fallot, male patients have substantially higher RV

volumes and masses, even when indexed for body surface area. However, when related to reference values of healthy subjects by means of Z-scores, the extent of RV dilatation was similar between sexes. This can be explained by the fact that in healthy subjects, indexed RV volumes and mass are also higher in males, compared to females. It is thus normal for males to have bigger and heavier hearts, also in patients with repaired tetralogy of Fallot.[44–46] Keeping this in mind, the commonly used unisex thresholds for RV volume as indication for pulmonary valve replacement may erroneously cause that PVR in female patients is considered in a later, more advanced stage of dilatation, compared to male patients. Furthermore, in **chapter 7**, male sex was identified as a predictor of VT. Therefore, sex-specific treatment recommendations, for instance sex-specific cutoff values or the use of Z-scores, are needed.

Also, it was demonstrated that female patients with repaired tetralogy of Fallot have higher biventricular ejection fraction, compared to male patients. Since sex hormones carry cardioprotective properties on RV function[47–50], one can speculate that the favorable profile of female patients with repaired tetralogy of Fallot results from their beneficial hormonal environment. However, these differences between males and females were ubiquitously present in various ages, thus also in patients between 8-12 years of age. Most patients in this age group are pre-pubertal, and although some patients may have reached puberty prior to the age of 12, the amount of time exposed to post-pubertal levels of sex hormones is little. These observations are exactly in line with our experimental findings, which demonstrate that already prior to pubertal development, female rats show less clinical symptoms and a beneficial systolic adaptation pattern, compared to male rats. The results of both these studies indicate that sex differences in RV adaptation are not solely a phenomenon of adulthood, but already exist in pre-pubertal RV failure. Also, the two chapters provide a clear picture that sex and sex hormones merit attention in future clinical and experimental studies, as well as in clinical practice. Sex-tailored recommendations in relevant guidelines are warranted in the strive for precision medicine for this group of patients.

Blind trust in numbers: the Achilles' heel of research?

Ejection fraction has recently been named the *Achilles' heel of Cardiology* by the European Society of Cardiology on social media when referring to an elegant review about heart failure classification.[51] In this review, the authors argue that the use of EF, and the use of arbitrary cut-off points that have gradually created separate disease entities, have led to oversimplification of the scientific view of complex syndromes. This is a telling example of how an initially practical and clever method of categorizing subjects, with increasing knowledge, eventually becomes an oversimplification. The same phenomenon can be observed in indexing cardiac measures in small animal models, as described in **chapter 3**. The common practice of decades appeared to be a simplification of mathematical relations, inducing substantial error. Another example of

oversimplification is the meta-analysis of extremely heterogeneous data from a limited number of studies, as recently described in a study regarding patients with Fontan circulation.[52] In a letter to the editor[53], we criticize this study for overrating the available evidence, which automatically becomes a higher level of evidence when labeled "meta-analysis". Caution and modesty are therefore appropriate when writing meta-analyses.[54] These statements may seem anecdotal, but share the denominator of oversimplified or misused quantification. The use of quantification methods can of course not at all be regarded as the Achilles' heel of research. However, they merit continuous validation and skepticism, and should never be regarded as objective truth, but rather as a subjectively chosen method to strive for objectivity. When something 'has always been done like this', presumably even more caution is warranted.

Future prospects

Data from various chapters in this thesis identify variables that may be used to select specific patients at risk of arrhythmia who potentially qualify for treatments, such as PVR or ICD implantation. As time progresses, more and more knowledge is being obtained, and subsequently, more and more variables are incidentally or repeatedly reported as a predictor. In 2013 it was already stated that 'numerous' predictors of sudden cardiac death have been identified, yet without adequate risk stratification schemes.[2] Without clear treatment algorithms or cutoff values, the clinical relevance of these findings remains little. Therefore, to aid clinical utility, we proposed specific thresholds to be used when RV mass or LV strain-rate are used as predictors of VT. Nevertheless, opportunities lie in translating the abundance of data into practical guidelines or treatment strategies. An interesting and promising modality in all fields of medical research, but also in (congenital) cardiology and cardiovascular imaging, is machine learning: an overarching term for computer-aided methods to learn how to make predictions.[57–61] Using machine learning, one can create practical algorithms or even decision-making tools from a multitude of variables more efficiently than could have been created by conventional statistics. For example, machine learning improved the prediction of deterioration of ventricular function in patients with repaired tetralogy of Fallot where conventional statistics could not.[62] Furthermore, machine learning proved to be useful in enhancing imaging quality, estimating prognosis, guiding therapy, and predicting critical events in congenital heart disease.[63–65] However, in comparison to industry and other fields of medicine, such as dermatology and ophthalmology, the advance of machine learning into cardiology came relatively late[60], which applies perhaps even more to congenital cardiology. However, machine learning could especially be helpful in complex and heterogeneous diseases as repaired tetralogy of Fallot, as these techniques can help unravel the relative value of each predictor in the near future. Machine learning-derived risk stratification models or treatment indication models could therewith soon have a major impact on daily clinical practice, especially in third world countries.[66]

Concluding remarks

The clinical state and long-term outcome of patients with repaired tetralogy of Fallot have dramatically improved over the last decades. However, therewith, long-term complications become increasingly prevalent. This thesis has characterized RV adaptation to chronic abnormal loading, has identified prognostic factors for adverse events, and has identified sex differences, both in animal models and in studies with repaired tetralogy of Fallot patients. Herewith, this thesis eventually contributes to improving outcome and clinical state of this growing group of patients.

REFERENCES

1. Apitz C, Webb GD, Redington AN. Tetralogy of Fallot. Lancet (London, England). 2009 Oct;374(9699):1462–71.
2. Villafañe J, Feinstein JA, Jenkins KJ, Vincent RN, Walsh EP, Dubin AM, et al. Hot topics in tetralogy of Fallot. J Am Coll Cardiol [Internet]. 2013;62(23):2155–66. Available from: http://dx.doi.org/10.1016/j.jacc.2013.07.100
3. Yu C, Moore BM, Kotchetkova I, Cordina RL, Celermajer DS. Causes of death in a contemporary adult congenital heart disease cohort. Heart. 2018;104(20):1678–82.
4. Burchill LJ, Gao L, Kovacs AH, Opotowsky AR, Maxwell BG, Minnier J, et al. Hospitalization trends and health resource use for adult congenital heart disease-related heart failure. J Am Heart Assoc. 2018;7(15).
5. Mazor Dray E, Marelli AJ. Adult Congenital Heart Disease: Scope of the Problem. Cardiol Clin [Internet]. 2015;33(4):503–12. Available from: http://dx.doi.org/10.1016/j.ccl.2015.07.001
6. Ceresnak SR. The coming Afib Tsunami in GUCH patients. What to expect. How to deal with it. In Annual Meeting of the Association for European Paediatric and Congenital Cardiology, Sevilla; 2019.
7. Crnkovic S, Egemnazarov B, Damico R, Marsh LM, Nagy BM, Douschan P, et al. Disconnect between Fibrotic Response and Right Ventricular Dysfunction. Am J Respir Crit Care Med. 2018;1–48.
8. Borgdorff MAJ, Dickinson MG, Berger RMF, Bartelds B. Right ventricular failure due to chronic pressure load: What have we learned in animal models since the NIH working group statement? Heart Fail Rev [Internet]. 2015;20(4):475–91. Available from: http://dx.doi.org/10.1007/s10741-015-9479-6
9. Reddy S, Zhao M, Hu D-Q, Fajardo G, Katznelson E, Punn R, et al. Physiologic and molecular characterization of a murine model of right ventricular volume overload. Am J Physiol Heart Circ Physiol [Internet]. 2013;304(10):H1314-27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23504182
10. Modesti PA, Vanni S, Bertolozzi I, Cecioni I, Lumachi C, Perna AM, et al. Different Growth Factor Activation in the Right and Left Ventricles in Experimental Volume Overload. Hypertension. 2004;43(1):101–8.
11. Johannessen M, Møler S, Hansen T, Moens U, Van Ghelue M. The multifunctional roles of the four-and-a-half-LIM only protein FHL2. Cell Mol Life Sci. 2006;63(3):268–84.
12. Arimura T, Hayashi T, Matsumoto Y, Shibata H, Hiroi S, Nakamura T, et al. Structural analysis of four and half LIM protein-2 in dilated cardiomyopathy. Biochem Biophys Res Commun. 2007;357(1):162–7.
13. Friedrich FW, Reischmann S, Schwalm A, Unger A, Ramanujam D, Münch J, et al. FHL2 expression and variants in hypertrophic cardiomyopathy. Basic Res Cardiol. 2014;109(6).
14. Schaefer CJ, Ruhrmund DW, Pan L, Seiwert SD, Kossen K. Antifibrotic activities of pirfenidone in animal models. Eur Respir Rev. 2011;20(120):85–97.
15. Jin J, Kadoya K, Tulafu M, Namba Y, Iwai M, Watanabe J, et al. Pirfenidone attenuates lung fibrotic fibroblast responses to transforming growth factor- β 1. 2019;1–14.
16. Liu X, Liang E, Song X, Du Z, Zhang Y, Zhao Y. Inhibition of Pin1 alleviates myocardial fibrosis and dysfunction in STZ-induced diabetic mice. Biochem Biophys Res Commun [Internet]. 2016;479(1):109–15. Available from: http://dx.doi.org/10.1016/j.bbrc.2016.09.050
17. Wu X, Li M, Chen SQ, Li S, Guo F. Pin1 facilitates isoproterenol-induced cardiac fibrosis and collagen deposition by promoting oxidative stress and activating the MEK1/2-ERK1/2 signal transduction pathway in rats. Int J Mol Med. 2018;41(3):1573–83.
18. Wu D, Huang D, Li LL, Ni P, Li XX, Wang B, et al. TGF-β1-PML SUMOylation-peptidyl-prolyl cis–trans isomerase NIMA-interacting 1 (Pin1) form a positive feedback loop to regulate cardiac fibrosis. J Cell Physiol. 2019;234(5):6263–73.
19. Borgdorff MA, Bartelds B, Dickinson MG, Steendijk P, Berger RMF. A cornerstone of heart failure treatment is not effective in experimental right ventricular failure. Int J Cardiol [Internet]. 2013;169(3):183–9. Available from: http://dx.doi.org/10.1016/j.ijcard.2013.08.102

20. Andersen S, Axelsen JB, Ringgaard S, Nyengaard JR, Nielsen SH, Genovese F, et al. Pressure overload induced right ventricular remodeling is not attenuated by the anti-fibrotic agent Pirfenidone. Pulm Circ. 2019;204589401984865.
21. Borgdorff MAJ, Bartelds B, Dickinson MG, Boersma B, Weij M, Zandvoort A, et al. Sildenafil enhances systolic adaptation, but does not prevent diastolic dysfunction, in the pressure-loaded right ventricle. Eur J Heart Fail. 2012;14(9):1067–74.
22. Friedberg MK, Redington AN. Right versus left ventricular failure: Differences, similarities, and interactions. Circulation. 2014;129(9):1033–44.
23. Brida M, Diller GP, Nashat H, Strozzi M, Milicic D, Baumgartner H, et al. Pharmacological therapy in adult congenital heart disease: Growing need, yet limited evidence. Eur Heart J. 2019;40(13):1049–56.
24. Rigolin VH, Robiolio PA, Wilson JS, Harrison JK, Bashore TM. The forgotten chamber: the importance of the right ventricle. Cathet Cardiovasc Diagn. 1995 May;35(1):18–28.
25. Oliver JM, Gallego P, Gonzalez AE, Garcia-Hamilton D, Avila P, Alonso A, et al. Impact of age and sex on survival and causes of death in adults with congenital heart disease. Int J Cardiol [Internet]. 2017;245(June 2017):119–24. Available from: http://dx.doi.org/10.1016/j.ijcard.2017.06.060
26. Verheugt CL, Uiterwaal CSPM, Van Der Velde ET, Meijboom FJ, Pieper PG, Van Dijk APJ, et al. Mortality in adult congenital heart disease. Eur Heart J. 2010;31(10):1220–9.
27. Baumgartner H, Bonhoeffer P, De Groot NMS, De Haan F, Deanfield JE, Galie N, et al. ESC Guidelines for the management of grown-up congenital heart disease (new version 2010). Eur Heart J. 2010;31(23):2915–57.
28. Stout KK, Daniels CJ, Aboulhosn JA, Bozkurt B, Broberg CS, Colman JM, et al. 2018 AHA/ACC Guideline for the Management of Adults With Congenital Heart Disease: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Vol. 139, Circulation. 2019. 637–697 p.
29. Ghai A, Silversides C, Harris L, Webb GD, Siu SC, Therrien J. Left ventricular dysfunction is a risk factor for sudden cardiac death in adults late after repair of tetralogy of Fallot. J Am Coll Cardiol [Internet]. 2002;40(9):1675–80. Available from: http://dx.doi.org/10.1016/S0735-1097(02)02344-6
30. Gatzoulis MA, Balaji S, Webber SA, Siu SC, Hokanson JS, Poile C, et al. Risk factors for arrhythmia and sudden cardiac death late after repair of tetralogy of Fallot: A multicentre study. Lancet. 2000;356(9234):975–81.
31. Cochet H, Iriart X, Allain-Nicolaï A, Camaioni C, Sridi S, Nivet H, et al. Focal scar and diffuse myocardial fibrosis are independent imaging markers in repaired tetralogy of Fallot. Eur Hear J - Cardiovasc Imaging. 2019;33:1–14.
32. Khairy P, Landzberg MJ, Gatzoulis MA, Lucron H, Lambert J, Marçon F, et al. Value of Programmed Ventricular Stimulation after Tetralogy of Fallot Repair: A Multicenter Study. Circulation. 2004;109(16):1994–2000.
33. Bokma JP, De Wilde KC, Vliegen HW, Van Dijk AP, Van Melle JP, Meijboom FJ, et al. Value of cardiovascular magnetic resonance imaging in noninvasive risk stratification in tetralogy of Fallot. JAMA Cardiol. 2017;2(6):678–83.
34. Khairy P, Harris L, Landzberg MJ, Viswanathan S, Barlow A, Gatzoulis MA, et al. Implantable Cardioverter-Defibrillators in Tetralogy of Fallot. Circulation. 2008;117:363–70.
35. Diller GP, Kempny A, Liodakis E, Alonso-Gonzalez R, Inuzuka R, Uebing A, et al. Left ventricular longitudinal function predicts life-threatening ventricular arrhythmia and death in adults with repaired tetralogy of fallot. Circulation. 2012;125(20):2440–6.
36. Geva T, Mulder B, Gauvreau K, Babu-Narayan SV, Wald RM, Hickey K, et al. Preoperative Predictors of Death and Sustained Ventricular Tachycardia After Pulmonary Valve Replacement in Patients With Repaired Tetralogy of Fallot Enrolled in the INDICATOR Cohort. Circulation. 2018;138:2106–15.
37. Tretter JT, Redington AN. Risk Factors and Biomarkers of Poor Outcomes Time to Throw Out Right Ventricular Volumes in Repaired Tetralogy of Fallot? Lessons From the INDICATOR Cohort. Circulation. 2018;138:2116–8.

38. Jouven X, Desnos M, Guerot C, Ducimetière P. Predicting Sudden Death in the Population. Circulation. 1999;1978–83.
39. Stritzke J, Markus MRP, Duderstadt S, Lieb W, Luchner A, Döring A, et al. The Aging Process of the Heart: Obesity Is the Main Risk Factor for Left Atrial Enlargement During Aging. The MONICA/KORA (Monitoring of Trends and Determinations in Cardiovascular Disease/Cooperative Research in the Region of Augsburg) Study. J Am Coll Cardiol [Internet]. 2009;54(21):1982–9. Available from: http://dx.doi.org/10.1016/j.jacc.2009.07.034
40. Pedrizzetti G, Lapinskas T, Tonti G, Stoiber L, Zaliunas R, Gebker R, et al. The Relationship Between EF and Strain Permits a More Accurate Assessment of LV Systolic Function. JACC Cardiovasc Imaging [Internet]. 2019; Available from: https://doi.org/10.1016/j.jcmg.2019.03.019
41. Tezuka F, Hort W, Lange PE, Nurnberg JH. Muscle fiber orientation in the development and regression of right ventricular hypertrophy in pigs. Acta Pathol Jpn. 1990 Jun;40(6):402–7.
42. Moon TJ, Choueiter N, Geva T, Valente AM, Gauvreau K, Harrild DM. Relation of biventricular strain and dyssynchrony in repaired tetralogy of fallot measured by cardiac magnetic resonance to death and sustained ventricular tachycardia. Am J Cardiol [Internet]. 2015;115(5):676–80. Available from: http://dx.doi.org/10.1016/j.amjcard.2014.12.024
43. Orwat S, Diller G-P, Kempny A, Radke R, Peters B, Kühne T, et al. Myocardial deformation parameters predict outcome in patients with repaired tetralogy of Fallot. Heart [Internet]. 2016;102(3):209–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26715570
44. Maceira AM, Prasad SK, Khan M, Pennell DJ. Reference right ventricular systolic and diastolic function normalized to age, gender and body surface area from steady-state free precession cardiovascular magnetic resonance. Eur Heart J. 2006;27(23):2879–88.
45. Maceira AM, Prasad SK, Khan M, Pennell DJ. Normalized left ventricular systolic and diastolic function by steady state free precession cardiovascular magnetic resonance. J Cardiovasc Magn Reson. 2006;8(3):417–26.
46. Sarikouch S, Peters B, Gutberlet M, Leismann B, Kelter-Kloepping A, Koerperich H, et al. Sex-specific pediatric Percentiles for ventricular size and mass as reference values for Cardiac MRI assessment by steady-state free-precession and phase-contrast MRI flow. Circ Cardiovasc Imaging. 2010;3(1):65–76.
47. Ventetuolo CE, Ouyang P, Bluemke DA, Tandri H, Barr RG, Bagiella E, et al. Sex hormones are associated with right ventricular structure and function: The MESA-right ventricle study. Am J Respir Crit Care Med. 2011;183(5):659–67.
48. Ventetuolo CE, Mitra N, Wan F, Manichaikul A, Barr RG, Johnson C, et al. Oestradiol metabolism and androgen receptor genotypes are associated with right ventricular function. Eur Respir J [Internet]. 2016;47(2):553–63. Available from: http://dx.doi.org/10.1183/13993003.01083-2015
49. Lahm T, Albrecht M, Fisher AJ, Selej M, Patel NG, Brown JA, et al. 17β-Estradiol attenuates hypoxic pulmonary hypertension via estrogen receptor-mediated effects. Am J Respir Crit Care Med. 2012;185(9):965–80.
50. Liu A, Schreier D, Tian L, Eickhoff JC, Wang Z, Hacker TA, et al. Direct and indirect protection of right ventricular function by estrogen in an experimental model of pulmonary arterial hypertension. Am J Physiol Circ Physiol. 2014;307(3):H273–83.
51. Triposkiadis F, Butler J, Abboud FM, Armstrong PW, Adamopoulos S, Atherton JJ, et al. The continuous heart failure spectrum: moving beyond an ejection fraction classification. Eur Heart J [Internet]. 2019;(January):1–11. Available from: https://academic.oup.com/eurheartj/advance-article/doi/10.1093/eurheartj/ehz158/5431175
52. Wang W, Hu X, Liao W, Rutahoile WH, Malenka DJ, Zeng X, et al. The efficacy and safety of pulmonary vasodilators in patients with Fontan circulation: a meta-analysis of randomized controlled trials. Pulm Circ. 2019;9(1).
53. Ridderbos F-JS, Hagdorn QAJ, Berger RMF. Pulmonary vasodilator therapy as treatment for patients with a Fontan circulation: the Emperor's new clothes? Pulm Circ. 2018;8(4):2045894018811148.
54. Thompson SG, Pocock SJ. Can meta-analyses be trusted? Lancet (London, England). 1991 Nov;338(8775):1127–30.

55. Schmidt M, Rothman KJ. Mistaken inference caused by reliance on and misinterpretation of a significance test. Int J Cardiol [Internet]. 2014;177(3):1089–90. Available from: http://dx.doi.org/10.1016/j.ijcard.2014.09.205
56. Wasserstein RL, Schirm AL, Lazar NA. Moving to a World Beyond "p < 0.05." Am Stat [Internet]. 2019 Mar 29;73(sup1):1–19. Available from: https://doi.org/10.1080/00031305.2019.1583913
57. Petersen SE, Abdulkareem M, Leiner T. Artificial Intelligence Will Transform Cardiac Imaging — Opportunities and Challenges. Front Cardiovasc Med. 2019;6(September):1–6.
58. Litjens G, Ciompi F, Wolterink JM, de Vos BD, Leiner T, Teuwen J, et al. State-of-the-Art Deep Learning in Cardiovascular Image Analysis. JACC Cardiovasc Imaging. 2019;12(8):1549–65.
59. Leiner T, Rueckert D, Suinesiaputra A, Baeßler B, Nezafat R, Išgum I, et al. Machine learning in cardiovascular magnetic resonance: basic concepts and applications. J Cardiovasc Magn Reson. 2019;21(1):1–14.
60. Benjamins JW, Hendriks T, Knuuti J, Juarez-Orozco LE, van der Harst P. A primer in artificial intelligence in cardiovascular medicine. Netherlands Hear J. 2019;27(9):392–402.
61. Benjamins JW, van Leeuwen K, Hofstra L, Rienstra M, Appelman Y, Nijhof W, et al. Enhancing cardiovascular artificial intelligence (AI) research in the Netherlands: CVON-AI consortium. Netherlands Hear J. 2019;27(9):414–25.
62. Samad MD, Wehner GJ, Arbabshirani MR, Jing L, Powell AJ, Geva T, et al. Predicting deterioration of ventricular function in patients with repaired tetralogy of Fallot using machine learning. Eur Hear J - Cardiovasc Imaging [Internet]. 2018;19:730–8. Available from: https://academic.oup.com/ehjcimaging/advance-article/doi/10.1093/ehjci/jey003/4930735
63. Diller G-P, Lammers AE, Babu-Narayan S, Li W, Radke RM, Baumgartner H, et al. Denoising and artefact removal for transthoracic echocardiographic imaging in congenital heart disease: utility of diagnosis specific deep learning algorithms. Int J Cardiovasc Imaging [Internet]. 2019; Available from: https://doi.org/10.1007/s10554-019-01671-0
64. Ruiz VM, Saenz L, Lopez-Magallon A, Shields A, Ogoe HA, Suresh S, et al. Early prediction of critical events for infants with single-ventricle physiology in critical care using routinely collected data. J Thorac Cardiovasc Surg [Internet]. 2019;158(1):234-243.e3. Available from: https://doi.org/10.1016/j.jtcvs.2019.01.130
65. Diller GP, Kempny A, Babu-Narayan S V., Henrichs M, Brida M, Uebing A, et al. Machine learning algorithms estimating prognosis and guiding therapy in adult congenital heart disease: Data from a single tertiary centre including 10 019 patients. Eur Heart J. 2019;40(13):1069–77.
66. Meyer A, Cypko MA, Eickhoff C, Falk V, Emmert MY. Artificial intelligence-assisted care in medicine: a revolution or yet another blunt weapon? Eur Heart J. 2019 Oct;40(40):3286–9.