PRE-INTERVENTION MYOCARDIAL STRESS IS A GOOD PREDICTOR OF AORTIC VALVOLUPLASTY OUTCOME FOR FETAL CRITICAL AORTIC STENOSIS AND EVOLVING HLHS

Laura Green[1], Wei Xuan Chan[1,2], Indumita Prakash[1], Andreas Tulzer[3,4], Gerald Tulzer[3,4], Choon Hwai Yap[1]

[1] *Department of Bioengineering, Imperial College London, London, United Kingdom*

[2] *BHF Centre of Research Excellence, Imperial College London, London, United Kingdom*

[3] *Department of Pediatric Cardiology, Children's Heat Center Linz, Kepler University Hospital, Linz, Austria*

[4] *Medical Faculty, Johannes Kepler University Linz, Altenberger Strasse 69, 4040 Linz, Austria*

Correspondence: *Choon Hwai Yap,* c.yap@imperial.ac.uk

KEY POINTS

- Predicting the morphological birth outcomes (uni-ventricle versus biventricle) of fetal aortic valvuloplasty for fetal aortic stenosis with evolving HLHS is important for accurate patient selection, parental counseling and management decisions.
- Computational simulations show that a biomechanics parameter, pre-intervention peak systolic myofiber stress, is uniquely robust in distinguishing between such outcomes, outperforming all echo parameters.
- An empirical equation was developed to quickly compute peak systolic myofiber stress from routine echo measurements, and was the best predictor of outcomes among a wide range of parameters tested.

ABSTRACT

Fetal critical aortic stenosis with evolving hypoplastic left heart syndrome (CAS-eHLHS) causes biomechanical and functional aberrations, leading to a high risk of progression to HLHS at birth. Fetal aortic valvuloplasty (FAV) can resolve outflow obstruction and may reduce progression risk. However, it is currently difficult to accurately predict which patients will respond to the intervention and become functionally biventricular (BV) at birth, as opposed to becoming univentricular (UV), which is important for patient selection, parental counselling, and surgical planning. Therefore, we investigated whether biomechanics parameters from pre-FAV image-based computations could robustly distinguish between CAS-eHLHS cases with BV or UV outcomes in a retrospective cohort. To do so we performed image-based finite element biomechanics modelling of 9 CAS-eHLHS cases undergoing intervention and 6 healthy fetal control hearts, and found that a biomechanical parameter, peak systolic myofiber stress, showed a uniquely large difference between BV and UV cases, which had a larger effect magnitude than echocardiography parameters, as seen in Abstract Figure 1. A simplified equation was derived for quick and easy estimation of myofiber stress from echo measurements via principal component analysis. When tested on a retrospective cohort of 37 CAS-eHLHS cases, the parameter outperformed other parameters

in predicting UV versus BV outcomes, and thus have a high potential of improving outcome predictions if incorporated into patient selection procedures. Physiologically, high myocardial stresses likely indicate a healthier myocardium that can withstand high stresses and resist pathological remodeling, which can explain why it is a good predictor of BV outcomes.

Keywords: cardiac biomechanics, evolving hypoplastic left heart syndrome, fetal aortic valvuloplasty, fetal critical aortic stenosis, finite element modelling

INTRODUCTION

In selected fetal hearts with critical aortic stenosis (CAS), natural history studies showed there to be a 73% likelihood of progression to hypoplastic left heart syndrome (HLHS) by the time of birth, and for this reason, was named CAS with evolving HLHS (CAS-eHLHS) (Mäkikallio *et al.*, 2006; Gardiner *et al.*, 2016). Progression to HLHS was especially likely for cases with retrograde transverse aortic arch flow, left-to-right flow across the foramen ovale, and monophasic mitral inflow. The aortic obstruction in these cases leads to high left ventricular (LV) pressure, globular LV shapes, hypertrophy of LV walls, diminished LV contractile deformation, high aortic valve (AV) velocity, endocardial fibroelastosis and mitral valve regurgitation (MVr), which also causes high left atrial (LA) pressure and LA distension (Tulzer & Arzt, 2013). These abnormalities are likely detrimental to the growth of the LV and cause of subsequent hypoplastic development which leads to a univentricular (UV) outcome.

In such cases, fetal aortic valvuloplasty (FAV) was found to be promising, by reducing the risk of progression to HLHS (Mäkikallio *et al.*, 2006; Gardiner *et al.*, 2016; Friedman *et al.*, 2018; Tulzer *et al.*, 2022*a*). FAV is a catheter-based intervention which widens the aortic obstruction to improve several of the above abnormal features, which will likely restore LV growth to prevent a UV outcome. However, high volume institutions have shown FAV to carry with it a 4-8% chance of fetal demise (Pickard *et al.*, 2020; Tulzer *et al.*, 2022*b*). A recent cost-benefit analysis showed that FAV confers a modest medium-term (age 6) survival benefit from 72% to 82% (Pickard *et al.*, 2020).

Current patient selection criteria for FAV includes the presence of LV systolic and diastolic dysfunction, retrograde bidirectional transverse aortic arch flow, a LV long axis Z-score<-2, dilated LV, left to right shunting through the foramen ovale and MVr or aortic stenosis gradient >20 mmHg (Tulzer & Arzt, 2013; Friedman *et al.*, 2018). However, a substantial proportion of fetuses selected for FAV did not avoid progression to UV outcome, even with a successful intervention, suggesting a current inability to accurately predict outcomes, and that current selection criteria for the intervention can be refined to more accurately identify patients that will benefit from the intervention. More accurate prediction can better inform decisions on whether to proceed with the intervention, given that there are significant procedural risks to fetuses. It may also lead to the identification of cases that are likely to respond to the intervention, but that are outside of the current selection criteria. For this reason, there has been continued investigation into biomarkers that are indicative of outcomes (Friedman *et al.*, 2018; Prosnitz *et al.*, 2018; Beattie *et al.*, 2020; Tulzer *et al.*, 2022*a*).

Biomechanics evaluation of the CAS-eHLHS fetal heart, or the investigation of its force dynamics in relation to its motion and deformation, is important. The CAS-eHLHS heart has drastically different biomechanical characteristics from the healthy heart, the FAV intervention is essentially a mechanical intervention in nature, and the heart is likely to be mechanosensitive in its growth, where abnormal biomechanics may play a significant role in the progression to malformation. Cardiac Finite Element (FE) computational modelling is a good approach for investigating myocardial biomechanics and has been very

useful in improving our understanding of multitude of heart diseases in the past (Dewan *et al.*, 2017; Ong *et al.*, 2020; Wisneski *et al.*, 2020; Shavik *et al.*, 2021). For example, we have previously used such a model to elucidate the effects of how various CAS-eHLHS characteristics impact cardiac function and biomechanics (Ong *et al.*, 2020). Further, a previous FE model coupled with a myocardial growth mathematical model showed that reduced strain stimuli can potentially explain the gestational development of some HLHS cases (Dewan *et al.*, 2017). FE modelling performs an assessment of the physics of the heart's function and can provide biomechanics parameters that are difficult to directly obtain from clinical measurements, such as myocardial stresses and the extent of active tension generation, which may be useful for predicting outcomes.

Here, we performed image-based, patient-specific FE modelling of CAS-eHLHS and healthy hearts to elucidate the biomechanical characteristics of the disease. We hypothesize that the biomechanics parameter of myocardial stress, obtained at a pre-interventional stage for CAS-eHLHS cases undergoing FAV, may show a better association with UV or BV outcomes compared with clinical measurements, and thus has the potential of being a better predictive biomarker.

MATERIALS AND METHODS

Image Acquisition

4D echocardiography images of 9 fetal hearts with CAS-eHLHS undergoing FAV and 6 healthy hearts were acquired using the GE Voluson E10 (GE Healthcare, Chicago, IL, USA) ultrasound machine. Images were taken in the spatio-temporal image correlation mode, using the standard settings of sweeps of 10-15s and capture rate of 70-90 frames per second. The 9 CAS-eHLHS datasets and 1 healthy dataset were obtained with informed consent from the Kepler University Hospital, Austria, under Institutional Review Board protocol 1009/2017. the remaining healthy datasets were obtained from National University Hospital Singapore under Domain Specific Review Board protocol 2014/00056. These images were used for biomechanics analysis and for developing an empirical equation to estimate myocardial stresses.

2D echocardiography and Doppler measurements for a further 28 CAS-eHLHS fetuses undergoing intervention, acquired with the Vivid E95, were also obtained from Linz Hospital, Austria and were further used together with the first 9 CAS-eHLHS cases for analysis on predictive capabilities of various parameters. Since the original 9 CAS-eHLHS cases were used to develop the empirical equation without referencing their BV versus UV outcomes, they are still eligible for use when testing for how well parameters can predict outcomes.

Patient Characteristics

Healthy control cases were from ages of 21+2 wks+days to 32+0 wks+days gestation. At the time of study all CAS-eHLHS patients that had complete datasets with clear 4D volume images were used. The CAS-eHLHS patients were selected for FAV intervention at Linz Hospital, Austria, if they exhibited the following characteristics: dilated and poorly contracting LV, left to right shunting at atrial level, retrograde aortic arch flow, LV length Z-score >0 or RV/LV ratio >1.1 and no signs of AV atresia. The CAS-eHLHS patients ranged from 22+4 wks+days to 32+0 wks+days gestation. Age matched healthy control cases for CAS-eHLHS patients were not available, so a range of healthy cases were selected that encapsulated the age range of CAS-eHLHS patients.

Characteristics of patients included in the study are given in Table 1, including age, disease features and postnatal outcomes and postnatal procedures. Throughout the study, the disease cases are

denoted by a 'D' in front of the ID number and healthy cases by a 'H'. A BV circulation postnatal outcome was defined as when the LV was the only source of systemic circulation and where there was an absence of pulmonary hypertension at 1 year of age. Diseased cases outside of this definition were designated to be a UV outcome. Analyses were performed to determine which pre-interventional features were correlated to outcomes and have a potential to be used for outcome predictions. Postnatal procedures for cases with BV outcomes included AV dilation or the Ross-Kono procedure, while that for cases with UV outcomes included the Norwood procedure or a Ross-Kono with subsequent BV-UV conversion.

Image Processing of Fetal Echocardiography

From the fetal echo images, morphometric measurements of the heart chambers, including LV and RV longitudinal length, posterior wall thickness (PWT), LV inner diameter at diastole (LVID), and relative wall thickness (RWT) were performed in accordance with previous approaches (Tulzer *et al.*, 2022*a*), by 2 independent observers, repeated twice per person, and averaged. RWT is calculated as,

$$RWT = \frac{2 \times PWT}{LVID} \quad \text{Equation 1}$$

and aspect ratio was calculated as,

$$Aspect\ ratio = \frac{LVID}{LV\ Length} \quad \text{Equation 2}$$

Patient-specific reconstruction of 3D computational models of fetal hearts were obtained using methods previously described (Ong *et al.*, 2020). Briefly, segmentation of the myocardium was performed from 4D fetal echocardiography images to reconstruct the heart chambers at one or two time points. A validated cardiac motion estimation algorithm (Wiputra *et al.*, 2020) was then used to extract cardiac motions, which was used to animate the heart chambers over the cardiac cycle and obtain reconstructions and chamber volumes for all other time points (Figure 1). Further details are given in Supplementary Material 1.

LV myocardial engineering strains (Equation 3) were computed from the images in both the longitudinal and circumferential directions along a mid-wall line, identified by averaging the epicardial and endocardial boundaries, from the 4-chamber or transverse echo view, using previously documented techniques (Ren *et al.*, 2023). The mid-wall line was discretized into 100 elements, its motion was tracked, and changes in lengths of elements from end diastole to end systole were computed and spatially averaged,

$$Engineering\ Strain = \frac{Length_{systole} - Length_{diastole}}{Length_{diastole}} \quad \text{Equation 3}$$

The engineering strains measured will be reported as circumferential and longitudinal strains throughout the remainder of the study.

Computational Modelling Methods

Image-based, patient-specific FE Modelling of fetal LV myocardial biomechanics was performed in accordance with our previous methodologies (Ong *et al.*, 2020; Green *et al.*, 2022). The FE model was connected to a lumped parameter model to enable ventricular-vascular coupling.

LV myocardium models reconstructed from echo images were used for FE modelling and were meshed into a minimum of 2,500 quadratic tetrahedral elements (Figure 2A), which was sufficient for mesh convergence as shown in our previous study (Ong *et al.*, 2020). A transversely isotropic Fung type passive stiffness model informed by experimental measurements (Guccione *et al.*, 1991), and the

Guccione model of active tension generation (Guccione *et al.*, 1993) were adopted for the model, as explained in Supplementary Material 2. For all models, the myocardium helix angle (HA) was assumed to vary linearly from the epicardium to endocardium, from -52° to +71° respectively (example in Figure 2B), which was the average HA configuration reported in 3 previous fetal LV tissue studies (Ohayon *et al.*, 1999; Garcia-Canadilla *et al.*, 2018; Nishitani *et al.*, 2020). Before computations, the unloaded state of the LV, or the theoretical geometry of the heart at zero LV cavity pressure, was calculated and assumed to be the stress-free condition. This was achieved via Finsberg et al.'s backward displacement method (Finsberg *et al.*, 2018): the pressure loading from the load-free state to the end diastolic state was iteratively simulated until the targeted end-diastolic pressure and volume was achieved, and at every iteration, the load-free state was adjusted by changing the pressure for an inverse of the loading deformation. The targeted end diastolic pressure was set as 5 mmHg for healthy hearts (Johnson *et al.*, 2000), but this was adjusted incrementally upwards in 1 mmHg intervals if negative pressures were encountered during early diastole. For diseased cases, end-diastolic pressure was assigned by the end-diastolic left atrium (LA) pressure, obtained by assuming a linear relationship between LA size and pressure (LA compliance) (Matsuda *et al.*, 1990). This models the abnormal LA pressurization in diseased cases.

The formal FE simulation was performed by minimizing the weak formulation of a Lagrangian functions described by Shavik et al. (Shavik *et al.*, 2018), which enforces tissue stress equilibrium, incompressibility, and a specific cavity volume to yield cavity pressure, using the Newton Solver in the FEniCS software. The boundary conditions were like that previously reported (Shavik *et al.*, 2018), with the basal plane of the LV constrained in the longitudinal direction and a weak 90 Pa spring applied to the entire epicardium at the load-free state, to simulate interactions with surrounding tissues and to constrain translational motion of the model.

A lumped parameter model (Figure 2C) was used for 1D modelling of fetal circulation and was coupled to the LV FE model. The lumped parameter model was based on Pennati et al.'s work (Pennati *et al.*, 1997; Pennati. & Fumero., 2000), but underwent minor recalibration to more recent measurements of human fetal intracardiac pressure (Johnson *et al.*, 2000), and descending aorta pulse pressure (Versmold *et al.*, 1981), as explained in our previous modelling study (Green *et al.*, 2022), where all model parameters are given. The model is scalable to a range of gestational ages, through a series of allometric equations. The model was executed for 30 cycles, to ensure steady state was achieved.

Tuning Model for Patient Specific Match

The lumped parameter coupled FE modelling was iteratively performed by altering valve flow resistances and myocardial contractility, to best match each computational model with clinical measurements. Through this matching process, the patient-specificity of the model can be maintained, and the biomechanics parameters can be back-computed.

For healthy fetal hearts, the lumped parameter model was first scaled to the specific gestational age. Iterative FE and lumped parameter simulations were then performed while varying myocardial contractility ($T_{0,LV}$ in Supplementary Material 2, Equation S2), until the simulated stroke volume matched that measured from the image, thus back-computing the contractility. 60 kPa was used as the initial guess of active tension magnitude, based on the upper limit of experimental measurements of 49.9 ± 9.3 kPa of fetal myocardium at 18+4 wks+days gestation (Racca *et al.*, 2016).

For CAS-eHLHS hearts, the lumped parameter model was again scaled to the specific gestational age. The patient specific matching process, however, was more extensively performed. First, iterative simulations were performed while varying flow resistances of the AV, mitral valve (MV) inflow and MVr, until simulations matched the expected valvular pressure gradients, which were calculated from clinical

valvular Doppler velocities, using the simplified Bernoulli's equation. Next, iterative simulations were performed while adjusting myocardial contractility, until the simulated stroke volume matched that measured from images. A loop over the two types of iterative simulations was performed, until both valvular pressure gradients and stroke volume matched measurements from echo images. This thus allowed the back-computation of myocardial contractility.

A simple gradient descent algorithm was used to reach convergence for all iterative simulations, and parameter matches were optimized to minimize errors as much as possible, to less than 10% error. Where a satisfactory match could not be obtained, the age scaling of the lumped parameter was adjusted and the optimization process was repeated, until a match could be achieved.

Statistical Analysis

Z-scores for echo measurements parameters were computed using healthy population data from Luewan et al. for LVID and PWT (Luewan *et al.*, 2011), from Devore et al. for LV and RV longitudinal length, end-diastolic volume (EDV), stroke volume and ejection fraction (DeVore *et al.*, 2017; Devore *et al.*, 2019) and Chen et al. for ascending aorta diameter dimensions (Chen *et al.*, 2022). For biomechanics parameters from computational outputs, no population data is currently available in literature, to eliminate the age dependent variation, therefore, when a computational parameter showed a trend of variability with age, it was normalized with the regression line of our healthy cohort before being analysed.

The Mann-Whitney U test performed on all data comparisons due to small sample sizes. Statistical significance assumed when $P < 0.05$. To estimate the effect size of differences in specific parameters between the BV and UV groups the Cohen's D value was calculated as,

$$d = \frac{\bar{x}_{BV} - \bar{x}_{UV}}{s_p} \times corr \qquad \text{Equation 4}$$

where $\bar{x}$, is the mean value of the parameter for the UV or BV group as indicated by the subscript, s_p is the pooled standard deviation and $corr = 0.7839$ and is the correction factor for when $N < 50$.

Principal Component and Regression Analysis

A simple linear mathematical equation was developed for rapid computation of peak-systolic volume-averaged myocardial stress from clinically measurable parameters. Principal component analysis was first used to seek modes or linear combinations of parameters that best described the variability between diseased fetal cases. The number of principal components needed for a cumulative representation of >90% of the variability was noted, and then regression was performed to determine the coefficients for these modes, so that a weighted sum of these modes could be used to estimate peak systolic myofiber stress.

Receiver Operating Characteristic (ROC) Analysis

To determine which pre-FAV image-based characteristic had the greatest capability in distinguishing BV versus UV outcomes, ROC analysis was performed on retrospective images (n=37). The 9 cases used to develop the mathematical equation to compute myocardial stress were used here as well, as part of the 37 samples. This is appropriate as the equation was developed only to approximate myocardial stress, and this was done without any reference to outcomes of the cases. Outcomes data was only used in the ROC analysis. Samples with incomplete datasets were not included here. The analysis was performed on parameters within this study, and for RV/LV longitudinal length ratio and estimated peak LV pressure, which were identified by Tulzer et al. (Tulzer *et al.*, 2022*a*) and Friedman et al. (Friedman *et al.*, 2018) as promising pre-FAV parameters for indicating post-FAV outcomes.

RESULTS

Echocardiography Anatomic and Functional Measurements

Echocardiography cardiac anatomic and functional measurements for pre-FAV and healthy control hearts are shown in Figure 3. Pre-FAV peak valvular velocity measurements (Figure 3A-C) showed no statistical significance between the BV and UV disease sub-groups, although both AV and MV antegrade velocities were substantially higher than healthy hearts. Both BV and UV disease sub-groups had elevated EDV Z scores from healthy controls, but the difference was only significant between BV and healthy groups, and not between BV and UV groups (Figure 3D). Both BV and UV groups had significantly lower stroke volume and ejection fraction Z scores than healthy groups but again, there was no significant difference between BV and UV groups (Figure 3E-F). The BV group had significantly higher LV and RV longitudinal length than controls (Figure G-H), and although BV LV longitudinal length was generally higher than the UV group, the difference was not significant. Similarly, the BV group generally had higher LVID (Figure 3J), lower RWT (Figure 3M), and higher aspect ratio (Figure 3K), than the UV group and healthy controls, but again significance was found only between the BV and control group. No significance was observed across all groups in RV/LV length ratio (Figure 3I) and PWT (Figure 3L). For ascending aorta diameter Z score, healthy patient specific data was not available for ascending aorta calculations and therefore the standard deviation of normative data has been included in Figure 3N (Chen *et al.*, 2022). No statistical significance was found between BV and UV groups, but both BV and UV groups were significantly greater than healthy controls. In terms of strains, both BV and UV groups were significantly smaller than healthy controls, but again, no significance was observed between the BV and UV groups (Figure 3O-P).

Overall, the data demonstrated that diseased hearts were larger than healthy hearts and had lower strains and stroke volume. Between the BV and UV groups, BV hearts tended to have larger average dimensions and higher average strains than UV hearts. However, there were consistently significant overlaps in measurements between the two groups, suggesting that it may not be easy to distinguish between the two groups with echo measurements alone.

Image-Based Simulations of Cardiac Biomechanics

Figure 4 shows representative results from the image-based FE simulations, showing that the resulting pressure-volume (P-V) loops and spatially averaged myofiber stress over the cardiac cycle were reasonably physiologic. Plots for other cases are given in Supplementary Material 5.

From the trends of computed results, LV work done (the area within the P-V loop), and myocardial contractility of healthy models increased with gestational age from regression analysis (Figure 5B and D). Peak LV pressure was known to increase with gestational age according to previous invasive measurements (Johnson *et al.*, 2000). To account for the age dependency of these biomechanics' parameters, the parameters were normalized by the expected value of a healthy heart of the same age from the regression analysis, before comparisons between groups were performed. However, peak systolic myocardial stress in the myofiber direction appeared to have little change over age, and as such, no normalization was done.

From the normalized quantifications, diseased hearts were found to have generally higher LV pressures compared to the healthy controls and the UV group, but no statistical significance was found (Figure 5E). Diseased hearts had significantly lower work done than healthy hearts, and the BV hearts produced higher work than the UV hearts, but no significance was found between BV and UV groups (Figure 5F). In terms of peak systolic myofiber stress, UV hearts had very similar values to healthy hearts, and the BV hearts had a drastic elevation (more than two-fold), which was significantly higher than both

the UV and healthy groups, with no data overlap between the groups (Figure 5G). For myocardial contractility, both diseased groups were significantly lower than healthy controls, and a significant difference was observed between BV and UV groups. However, there was some overlap between the UV and BV data.

Overall, the data demonstrated that diseased hearts had significantly altered biomechanics, including elevated cavity pressures and decreased circulatory work done and lower myocardial contractility compared to healthy hearts. Further, BV but not UV LVs experienced higher myocardial stresses than controls. Between the BV and UV groups, both contractility and peak systolic myofiber stress were significantly different. The difference in peak systolic myofiber stress between BV and UV hearts was especially large with no overlap between the two groups. This was thus potentially a good parameter for distinguishing the two groups, for predicting FAV outcomes.

Effect Size of Parameter Differences Between UV and BV Groups

To determine which of the parameters investigated had stronger ability to distinguish between UV and BV groups, we computed the Cohen's D standardized mean difference (Abstract Figure 1 and Supplementary Data Spreadsheet) for all CAS-eHLHS cases modelled. The Cohen's D value quantified how different parameter values between the UV and BV groups were. Results showed that myofiber stress had the highest Cohen's D, which was much higher than Cohen's D for any other parameters. This was followed by echo-measured ventricular size parameters such as LV longitudinal length and LVID, which were close to the biomechanics parameters, normalized work done and contractility.

Relationship Between Echocardiographic Parameters and Biomechanics Parameters

To understand the dependencies of the peak systolic myofiber stress, we performed correlation analysis between stress and echo parameters. Results (in Supplementary Material 6) showed that myocardial stress had a good positive correlation between LV size parameters (EDV, longitudinal length and LVID), and MVr, and a good negative correlation with RWT. The correlations are logical from the theoretical standpoint. Myocardial stress was determined via a force balance between the cavity blood pressure and tensile forces in the myocardial walls, and was thus higher with greater systolic pressure, which was the driver of regurgitation. Myofiber stress thus correlated positively with MVr. Further, myocardial wall stresses could be approximated by myocardial wall forces divided by cross-sectional area, which was determined by wall thickness. Myofiber stress thus correlated negatively with RWT. The relationship between cavity pressure and wall tension was further governed by the shape of the LV chamber, as could be illustrated by the hoop stress theory, which stated that larger chambers had higher wall tension. Myofiber stress thus correlated positively with LV size. These theories thus suggested that it is possible to derive a parameter that can be easily calculated with measurable echo parameters to theoretically represent peak systolic myofiber stress. This will enable fast and easy estimation of myofiber stress from echo scans without a need for time consuming simulations, for the prediction of FAV outcomes.

To derive this parameter, we performed principal component analysis of 8 echo parameters (listed in Supplementary Material 7, Equation S8b) and used the top 3 modes (Supplementary Material 7, Equation S8c) for regression with peak systolic myofiber stress results from FE simulations, as the 3 modes could account for 93.05% of variability between cases. The resulting regression equation enabled estimations of peak systolic myofiber stress close to FE outputs, as shown in Supplementary Material 7, Figure S4. The final function for the estimation of peak systolic myofiber stress is,

$$\begin{aligned} &Estimated\ Peak\ Systolic\ Myofiber\ Stress \\ &= -24.9240 + 0.9550V_1 + 4.2687V_2 - 0.1952V_3 + 1.1530V_4 \\ &+ 0.1677V_5 - 1.4611V_6 - 0.004771V_7 + 4.8968V_8 \end{aligned} \qquad \text{Equation 5}$$

where the echo parameters included are, V_1 = AV velocity (m/s), V_2 = MVr velocity (m/s), V_3 = LV longitudinal length (mm), V_4 = RV longitudinal length (mm), V_5 = LVID (mm), V_6 = RWT, V_7 = LV EDV (ml) and V_8 = stroke volume (ml).

Possible Post-FAV Outcome Predictors, A Retrospective Analysis

ROC analysis was performed to determine the ability of estimated peak systolic myofiber stress in classifying BV versus UV outcomes, to compare it with that of other known image-based pre-FAV predictors (Friedman *et al.*, 2018; Tulzer *et al.*, 2022*a*) and of individual parameters within Equation 5. Measurements used to compute Figure 6 are provided in the Supplementary Data Spreadsheet. The results show estimated peak systolic myofiber stress to have the largest area under the curve (AUC) of 0.91, and therefore the greatest capability in distinguishing BV versus UV outcomes. The next best performing parameters were LV longitudinal length Z score (AUC=0.84) and EDV Z score (AUC=0.83).

DISCUSSION

We performed a retrospective analysis of CAS-eHLHS fetal hearts to identify pre-FAV parameters that have a high likelihood of having the ability to predict UV versus BV outcomes, after FAV intervention. To do this, we investigated differences in various parameters between UV and BV groups. Our study is different from previous such works in that we performed advanced patient-specific biomechanics simulations to obtain biomechanics parameters, to add on to echocardiographic parameters in our analysis. Our simulations enabled careful back-computation of the myocardial contractility and LV pressures, while adhering to echocardiographic image features and detailed biomechanics formulations, and it provided information on myocardial stresses, which are not measurable clinically. Importantly, our results showed that myocardial stress is uniquely large in the BV group and is potentially a better parameter for predicting UV versus BV outcomes than all currently proposed clinical parameters.

Overall, our measurements agreed well with previous investigations on differences between UV and BV groups. Firstly, our data showed that BV groups had generally higher mean circumferential and longitudinal strains than UV groups, which were significantly lower than control groups. Ishii et al. (Ishii *et al.*, 2014) reported in a larger cohort study (n=57) that there were similarly diminished strain magnitudes in diseased hearts, and found significant differences between UV and BV groups for circumferential strains in 1 out of 6 segments of the LV and for longitudinal strains in 3 out of 6 segments of the LV. In our study, however, as our sample sizes are smaller (n=15), UV-versus-BV statistical significances were not found.

Secondly, our data showed that the BV group had higher EDVs (Figure 3D), LV longitudinal lengths (Figure 3G), RV longitudinal lengths (Figure 3H) and LVIDs (Figure 3J) than the UV group, but differences were not statistically significant. This aligns with previous studies, which analyzed retrospective data and found cases that went on to be born as BV after intervention generally had larger LV long axis Z scores (Friedman *et al.*, 2018) and larger left heart structures (including, aortic valve Z score, LV short axis Z score and LV long axis Z score) (McElhinney *et al.*, 2009) at the pre-FAV time point. Again, our small sample size had likely prevented a statistical significance, but our data generally corroborated with literature on the direction of the UV versus BV differences.

Thirdly, our simulation results showed LV pressures were generally higher in BV hearts compared to UV hearts, but differences were not significant. Friedman et al. and McElhinney et al. estimated LV pressure from valve Doppler measurements and found that BV cases had significantly higher LV pressures compared with UV cases at the time of intervention (McElhinney *et al.*, 2009; Friedman *et al.*, 2018), which corroborated our observations. Our data thus corroborated Friedman et al. and McElhinney et al.'s, and the lack of significance could again be attributable to our small sample size. However, we noted that

Friedman et al. and McElhinnery et al. did not factor in pressure variation with gestational age (Johnson *et al.*, 2000) when analyzing LV pressure calculations, as we did. When we switched to an analysis of LV pressures without the age-normalization (Supplementary Data Spreadsheet), we found that differences between UV and BV groups were larger than if normalization was done (lower p value).

Our main finding was that peak systolic myofiber stress was the best pre-FAV parameter to distinguish between UV and BV cases, and this outperformed all other parameters, including several that were found to have good predictive values in previous studies (Ishii *et al.*, 2014; Friedman *et al.*, 2018; Tulzer *et al.*, 2022*a*). For example, Tulzer et al. showed that RV-to-LV length ratio had strong predictive influence (Tulzer *et al.*, 2022*a*), Friedman et al. reported a high probability of a BV outcome in cases where peak LV pressure was >47 mmHg and ascending aorta Z-score was ≥0.57 (Friedman *et al.*, 2018), while Ishii et al. reported higher LV longitudinal and circumferential strains in BV compared to UV cases (Ishii *et al.*, 2014). We made the comparison of these individual parameters to peak systolic myofiber stress and found that the latter still performed better. A limitation of our study is that sample sizes were smaller than previous reports, nonetheless, given that the effect size of myofiber stress is much higher than other parameters (Abstract Figure 1), results are likely to repeatable with larger samples. Thus, myofiber stress has a good potential of enhancing predictions of UV versus BV outcomes. It can be used to enhance predictive framework such as the CART analysis proposed by Tulzer et al., which uses a combination of RV-to-LV length ratio, LV pressure (calculated from peak MVr velocity) and gestational age for prediction (Tulzer *et al.*, 2022*a*).

We believe that there are physiological explanations for why hearts with stronger contractility and higher stresses are likely to reach a BV outcome instead of a UV outcome. Stronger hearts are likely to support better contractile deformations after FAV intervention, providing better biomechanical stimuli for growth of the LV and thus supporting better growth and development before birth. Further, higher myocardial stresses in BV cases suggested that the myocardium in these cases resisted pathological hypertrophy and dilation remodeling despite high tissue stresses, while retaining a strong functional ability to generate pumping pressures. This might suggest that the myocardium is healthier and could elicit better growth and remodeling responses after intervention. Generally, our results suggest that biomechanically "stronger" LVs are more likely to respond positively to FAV, by progressing to a successful BV outcome at one year of age.

Our results suggest that biomechanical simulations of fetal hearts can improve predictive capabilities of outcomes, and this is likely useful for clinical decisions. However, presently, biomechanical simulations are computationally expensive, and involve multiple processing steps including the computational reconstruction of the heart's structure and motion. We have thus proposed a simplified equation to quickly estimate the peak systolic myocardial stress from echo parameters with ease, as shown in Equation 5. Although this equation will not be as accurate as the FE simulations, we showed in Supplementary Material 7 Figure S4 that it retained its ability to clearly distinguish BV and UV groups. The equation can be updated in future for accuracy when more data becomes available, or to reduce the number of dependent parameters to reduce error propagation. There is also potential for using Physics-Informed Neural Networks to achieve accurate, real-time estimations of myocardial stresses (Buoso *et al.*, 2021).

When we performed validation on 37 CAS-eHLHS cases, we found that the estimated peak systolic myofiber stress parameter remained the best predictor of UV versus BV post-FAV outcomes, judging from its high AUC in the ROC analysis. Here, however, it is likely that errors involved in estimating myocardial stresses via a simple linearized equation had resulted in some reduction of predictive performance. In future, biomechanics simulations may be possible in real-time via machine learning, particularly with

physics informed neural networks, to resolve this issue. We believe that such future work is warranted. From the ROC analysis, a potentially useful peak systolic myofiber threshold can be obtained for use in predictions. The threshold of stress = 17.4 kPa provides 100% sensitivity, and approximately 73% specificity (thus correctly predicting all BV cases), the threshold of stress = 26.1 kPa provides approximately 41% sensitivity and 100% specificity (thus correctly predicting all UV cases), while the threshold of stress = 17.4 kPa corresponds to the highest accuracy (average of sensitivity and selectivity weighted by prevalence), which correctly predicts 100% of BV cases and approximately 73% of UV cases. Presently, it is unclear which is the most clinically beneficial threshold to use, and further risk versus benefit analysis should be conducted. For example, recent thought about FAV is that even for UV outcomes, the FAV could have reduced disease severity and improved survival benefits, and such a notion warrants further studies.

There are several limitations of this study. Firstly, our sample size is small, and future studies with larger sample sizes are likely to enhance the current analysis and produce positive refinement to our prediction model in Equation 5. Secondly, all CAS-eHLHS patients analyzed in the study had already been selected for FAV intervention and the sample size was small due to limited data availability, which imposed bias, it would be recommended to widen the study, to help mitigate this limitation. Thirdly, our biomechanics computations required idealizations, such as the assumption of the same HA spatial variability configuration, lumped parameters model values, and myocardial stiffness for all cases. Also, during the optimisation loops, the best possible match with imaged parameters was obtained, but some match errors remained. Finally, it must be acknowledgement that CAS-eHLHS data was only obtained from 1 institute and BV or UV outcome largely depends on the institutional experience and management algorithm, which is not standardized between centers.

CONLCUSIONS

Biomechanics parameters, including peak systolic myofiber stress and back-computed myocardial contractility seemed to be promising metrics to differentiate between UV and BV cases. Importantly, peak-systolic myofiber stress had a uniquely large difference between UV and BV cases, suggesting that it has the potential of being a better indicator of UV versus BV outcome than all other current clinical measurements. A simplified equation for estimating myocardial stress showed that this parameter has better predictive capabilities compared to echo measurements. We speculate that this parameter can be helpful in clinical evaluation for a more accurate patient selection for FAV.

ADDITIONAL INFORMATION

Data Availability Statement

Computational module available here - https://github.com/WeiXuanChan/heartFEM

Competing Interests

The authors have no conflict of interest to declare.

Author Contributions

Laura Green – Designed research, developed methodologies, performed simulations, analyzed data, interpreted data, prepared manuscript, and vetted manuscript.

Dr Wei Xuan Chan – Designed research, developed modelling formulation, interpreted data, vetted manuscript.

Indumita Prakash – Performed simulations and analyzed data.

Dr Andreas Tulzer – Performed clinical intervention, collected clinical data, analyzed data, interpreted data, vetted manuscript.

Dr Gerald Tulzer – Performed clinical intervention, collected clinical data, interpreted data, vetted manuscript.

Dr Choon Hwai Yap – Designed research, developed methodologies, analyzed data, interpreted data, prepared manuscript, vetted manuscript.

Funding

The Study was supported by Imperial College PhD scholarship (Green), and BHF Centre of Research Excellence grant (RE/18/4/34215, Chan).

REFERENCES

Beattie MJ, Friedman KG, Sleeper LA, Lu M, Drogosz M, Callahan R, Marshall AC, Prosnitz AR, Lafranchi T, Benson CB, Wilkins-Haug LE & Tworetzky W (2020). Late gestation predictors of a postnatal biventricular circulation after fetal aortic valvuloplasty. *Prenat Diagn* **41,** 479–485.

Buoso S, Joyce T & Kozerke S (2021). Personalising left-ventricular biophysical models of the heart using parametric physics-informed neural networks. *Med Image Anal* **71,** 1–11.

Chen F, Zeng S, Yi A, Chen L, Zhou D, Liu Y & Yao L (2022). Z-score model of foetal ascending aorta diameter distensibility. *Front Cardiovasc Med* **9,** 1–7.

DeVore GR, Klas B, Satou G & Sklansky M (2017). Evaluation of the right and left ventricles: An integrated approach measuring the area, length, and width of the chambers in normal fetuses. *Prenat Diagn* **37,** 1203–1212.

Devore GR, Klas B, Satou G & Sklansky M (2019). Evaluation of fetal left ventricular size and function using speckle-tracking and the simpson rule. *Journal of Ultrasound in Medicine* **38,** 1209–1221.

Dewan S, Krishnamurthy A, Kole D, Conca G, Kerckhoffs R, Puchalski MD, Omens JH, Sun H, Nigam V & McCulloch AD (2017). Model of human fetal growth in hypoplastic left heart syndrome: Reduced ventricular growth due to decreased ventricular filling and altered shape. *Front Pediatr* **5,** 1–15.

Finsberg H, Xi C, Tan J Le, Zhong L, Genet M, Sundnes J, Lee LC & Wall ST (2018). Efficient estimation of personalized biventricular mechanical function employing gradient-based optimization. *Int J Numer Method Biomed Eng* **34,** 1–20.

Friedman KG, Sleeper LA, Freud LR, Marshall AC, Godfrey ME, Drogosz M, Lafranchi T, Benson CB, Wilkins-Haug LE & Tworetzky W (2018). Improved technical success, postnatal outcome and refined predictors of outcome for fetal aortic valvuloplasty. *Ultrasound in Obstetrics and Gynecology* **52,** 212–220.

Garcia-Canadilla P, Dejea H, Bonnin A, Balicevic V, Loncaric S, Zhang C, Butakoff C, Aguado-Sierra J, Vázquez M, Jackson LH, Stuckey DJ, Rau C, Stampanoni M, Bijnens B & Cook AC (2018). Complex

Congenital Heart Disease Associated With Disordered Myocardial Architecture in a Midtrimester Human Fetus. *Circ Cardiovasc Imaging* **11,** 1–10.

Gardiner HM et al. (2016). Natural history of 107 cases of fetal aortic stenosis from a European multicenter retrospective study. *Ultrasound in Obstetrics and Gynecology* **48,** 373–381.

Green L, Chan WX, Ren M, Mattar C, Chuan L, Choon L & Yap H (2022). The dependency of fetal left ventricular biomechanics function on myocardium helix angle configuration. *Biomech Model Mechanobiol*; DOI: 10.1007/s10237-022-01669-z.

Guccione JM, McCullock AD & Waldman LK (1991). Passive material properties of intact ventricular myocardium determined from a cylindrical model. *J Biomech Eng* **113,** 42–55.

Guccione JM, Waldman LK & McCulloch AD (1993). Mechanics of active contraction in cardiac muscle: Part II—cylindrical models of the systolic left ventricle. *J Biomech Eng* **115,** 82–90.

Ishii T, McElhinney DB, Harrild DM, Marcus EN, Sahn DJ, Truong U & Tworetzky W (2014). Ventricular strain in fetuses with aortic stenosis and evolving hypoplastic left heart syndrome before and after prenatal aortic valvuloplasty. *Fetal Diagn Ther* **35,** 18–26.

Johnson P, Maxwell DJ, Tynan MJ & Allan LD (2000). Intracardiac pressures in the human fetus. *Heart* **84,** 59–63.

Luewan S, Yanase Y, Tongprasert F, Srisupundit K & Tongsong T (2011). Fetal cardiac dimensions at 14-40 weeks' gestation obtained using cardio-STIC-M. *Ultrasound in Obstetrics and Gynecology* **37,** 416–422.

Mäkikallio K, McElhinney DB, Levine JC, Marx GR, Colan SD, Marshall AC, Lock JE, Marcus EN & Tworetzky W (2006). Fetal aortic valve stenosis and the evolution of hypoplastic left heart syndrome: Patient selection for fetal intervention. *Circulation* **113,** 1401–1405.

Matsuda Y, Toma Y, Matsuzaki M, Moritani K, Satoh A, Shiomi K, Ohtani N, Kohno M, Fujii T, Katayama K, Matsuda M & Kusukawa R (1990). Change of left atrial systolic pressure waveform in relation to left ventricular end-diastolic pressure. *Circulation* **82,** 1659–1667.

McElhinney DB, Marshall AC, Wilkins-Haug LE, Brown DW, Benson CB, Silva V, Marx GR, Mizrahi-Arnaud A, Lock JE & Tworetzky W (2009). Predictors of technical success and postnatal biventricular outcome after in utero aortic valvuloplasty for aortic stenosis with evolving hypoplastic left heart syndrome. *Circulation* **120,** 1482–1490.

Nishitani S, Torii N, Imai H, Haraguchi R, Yamada S & Takakuwa T (2020). Development of helical myofiber tracts in the human fetal heart: Analysis of myocardial fiber formation in the left ventricle from the late human embryonic period using diffusion tensor magnetic resonance imaging. *J Am Heart Assoc*; DOI: 10.1161/JAHA.120.016422.

Ohayon J, Usson Y, Jouk PS & Cai H (1999). Fibre orientation in human fetal heart and ventricular mechanics: A small perturbation. *Comput Methods Biomech Biomed Engin* **2,** 83–105.

Ong CW, Ren M, Wiputra H, Mojumder J, Chan WX, Tulzer A, Tulzer G, Buist ML, Mattar CNZ, Lee LC & Yap CH (2020). Biomechanics of Human Fetal Hearts with Critical Aortic Stenosis. *Ann Biomed Eng*; DOI: 10.1007/s10439-020-02683-x.

Pennati G, Bellotti M & Fumero R (1997). Mathematical modelling of the human foetal cardiovascular system based on Doppler ultrasound data. *Med Eng Phys* **19,** 327–335.

Pennati. G & Fumero. R (2000). Scaling approach to study the changes through the gestation of human fetal cardiac and circulatory behaviors. *Ann Biomed Eng* **28,** 442–452.

Pickard SS, Wong JB, Bucholz EM, Newburger JW, Tworetzky W, Lafranchi T, Benson CB, Wilkins-Haug LE, Porras D, Callahan R & Friedman KG (2020). Fetal Aortic Valvuloplasty for Evolving Hypoplastic Left Heart Syndrome: A Decision Analysis. *Circ Cardiovasc Qual Outcomes* **13,** 32–41.

Prosnitz AR, Drogosz M, Marshall AC, Wilkins-Haug LE, Benson CB, Sleeper LA, Tworetzky W & Friedman KG (2018). Early hemodynamic changes after fetal aortic stenosis valvuloplasty predict biventricular circulation at birth. *Prenat Diagn* **38,** 286–292.

Racca AW, Klaiman JM, Pioner JM, Cheng Y, Beck AE, Moussavi-Harami F, Bamshad MJ & Regnier M (2016). Contractile properties of developing human fetal cardiac muscle. *Journal of Physiology* **594,** 437–452.

Ren M, Chan WX, Green L, Armstrong AK, Tulzer A, Tulzer G, Buist ML & Yap CH (2023). Contribution of Ventricular Motion and Sampling Location to Discrepancies in 2D versus 3D Fetal Ventricular Strain Measures. *Journal of the American Society of Echocardiography*; DOI: 10.1016/j.echo.2022.12.024.

Shavik SM, Jiang Z, Baek S & Lee LC (2018). High spatial resolution multi-organ finite element modeling of ventricular-arterial coupling. *Front Physiol*; DOI: 10.3389/fphys.2018.00119.

Shavik SM, Wall S, Sundnes J, Guccione JM, Sengupta P, Solomon SD, Burkhoff D & Lee LC (2021). Computational Modeling Studies of the Roles of Left Ventricular Geometry, Afterload, and Muscle Contractility on Myocardial Strains in Heart Failure with Preserved Ejection Fraction. *J Cardiovasc Transl Res* **14,** 1131–1145.

Tulzer A, Arzt W, Gitter R, Sames-Dolzer E, Kreuzer M, Mair R & Tulzer G (2022*a*). Valvuloplasty in 103 fetuses with critical aortic stenosis: outcome and new predictors for postnatal circulation. *Ultrasound in Obstetrics and Gynecology* **59,** 633–641.

Tulzer A, Arzt W, Scharnreitner I, Hochpoechler J, Bauer C & Tulzer G (2022*b*). Complications associated with fetal cardiac interventions – prevalence and management: experience from 213 procedures. *Fetal Diagn Ther*; DOI: 10.1159/000527121.

Tulzer G & Arzt W (2013). Fetal cardiac interventions: Rationale, risk and benefit. *Semin Fetal Neonatal Med* **18,** 298–301.

Versmold HT, Kitterman JA, Phibbs RH, Gregory GA & Tooley WH (1981). Aortic blood pressure during the first 12 hours of life in infants with birth weight 610 to 4,220 grams. *Pediatrics* **67,** 607–613.

Wiputra H, Chan WX, Foo YY, Ho S & Yap CH (2020). Cardiac motion estimation from medical images: a regularisation framework applied on pairwise image registration displacement fields. *Sci Rep*; DOI: 10.1038/s41598-020-75525-4.

Wisneski AD, Wang Y, Deuse T, Hill AC, Pasta S, Sack KL, Yao J & Guccione JM (2020). Impact of Aortic Stenosis on Myofiber Stress: Translational Application of Left Ventricle-Aortic Coupling Simulation. *Front Physiol* **11,** 1–8.

FIGURE LEGENDS

Abstract Figure 1. Cohen's D values for various parameters to describe how different parameters are between the BV and UV groups.

Figure 1. [Top row] Example of patient specific reconstructed LV geometries for one diseased case with BV outcome, with UV outcome and a healthy case. Geometries to scale, scale bar shown under H3. [Middle row] Reconstructed geometries superimposed onto associated echo image, green outline highlighting geometry proportion close to current image plane. [Bottom row] Volume over time variation of each geometry output from cardiac motion estimation methods.

Figure 2. [A] Example of mesh applied to H3. [B] Example of fibers applied to H3, with negative fiber angle at epicardial surface and linear transmural variation to positive fiber angle at endocardial surface. (C) Schematic of lumped parameter model with H3 geometry. Modelling parameters are given in Supplementary Material 2, Table S1.

Figure 3. Imaged-based measurements overlayed onto mean and standard deviation bar plots, for the BV and UV diseased groups, with data for healthy controls included where appropriate. * $p<0.05$. sample sizes were n=5 for BV, n=4 for UV and n=6 for healthy controls. For ascending aorta diameter, literature data was used (Chen *et al.*, 2022) as measurements could not be clearly obtained from some of our images.

Figure 4. Computational biomechanics outputs for a BV model (D2), UV model (D7) and healthy model (H3).

Figure 5. Biomechanical parameters output from FE simulations for the BV (n=5) and UV (n=4) diseased groups and healthy controls (n=6). [A-D] Parameters plotted with respect to gestational age. The expected normative values (dotted line) were obtained from measurements by Johnson et al. *(Johnson* et al.*, 2000)* for [A], and from regression of our healthy control data for [B-D]. [E-H] The same biomechanics parameters after normalization, by the expected normative values for the matching gestational age, expressed as data points superimposed on mean and standard deviation bar plots. * $p<0.05$.

Figure 6. ROC curves computed from retrospective data (n=37) for the individual classes of Equation 5, estimated peak systolic myofiber stress and literature-based pre-FAV predictors of post-FAV outcomes. AUC values for each ROC included in legend.

TABLES

Table 1. Patient characteristics of CAS-eHLHS cases before intervention. In diseased cases age, is the fetal age at the pre-FAV scans, which generally occurs 1-3 days before FAV intervention.

Case	Postnatal Circulation	Age (wks+days)	Multiple FAVs	Bradycardia	Pericardial Effusion	LV Thrombus	Hydrops	Postnatal Procedures
D1	BV	24+6	N	Y	N	N	N	Dil.
D2	BV	29+0	Y	N	N	N	Y	RK
D3	BV	29+3	N	N	N	N	N	RK
D4	BV	29+6	N	Y	N	N	N	N/A
D5	BV	30+1	N	N	N	N	N	-
D6	UV	22+4	N	N	N	Y	N	NW
D7	UV	24+6	N	Y	N	N	N	NW
D8	UV	27+2	N	N	N	N	N	NW
D9	UV	29+1	Y	N	N	N	N	NW, RK, Conv.
H1	-	21+2	-	-	-	-	-	-
H2	-	21+3	-	-	-	-	-	-
H3	-	21+4	-	-	-	-	-	-
H4	-	24+0	-	-	-	-	-	-
H5	-	28+0	-	-	-	-	-	-
H6	-	32+0	-	-	-	-	-	-

Y - yes, N - no, Dil – balloon dilation of the AV, RK - Ross-Kono procedure, NW - Norwood Procedure, Conv - BV to UV conversion. N/A – no procedure as parents chose comfort care at an alternative centre; procedure planned for this patient was Ross-Konno surgery, with patient believed to progress to a BV outcome classification, therefore, for the purpose of this study the patient was classified as BV.