Diffuse myocardial fibrosis in the systemic right ventricle of patients late after Mustard or Senning surgery: an equilibrium contrast cardiovascular magnetic resonance study

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Aims

After atrial redirection surgery (Mustard–Senning operations) for transposition of the great arteries (TGA), the systemic right ventricle (RV) suffers from late systolic failure with high morbidity and mortality. Mechanisms of late RV failure are poorly characterized. We hypothesized that diffuse interstitial expansion representing diffuse fibrosis is greater in systemic RVs of patients following Mustard–Senning surgery and that it would be associated with other markers of heart failure and disease severity.

Methods and results

We used equilibrium contrast cardiovascular magnetic resonance (CMR) imaging to quantify extracellular volume (ECV) in the septum and RV free wall of 14 adults presenting to a specialist clinic late after surgery for TGA (8 Mustard, 6 female, median age 33). These were compared with 14 age-and sex-matched healthy volunteers. Patients were assessed with a standardized CMR protocol, NT-brain natriuretic peptide (NT-proBNP), and cardiopulmonary exercise (CPEX) testing.

The mean septal ECV was significantly higher in patients than controls (0.254 ± 0.036 vs. 0.230 ± 0.032; P = 0.03). NT-proBNP positively related to septal ECV (P = 0.04; r = 0.55). The chronotropic index (CI) during CPEX testing negatively related to the ECV (P = 0.04; r = −0.58). No relationship was seen with other CMR or CPEX parameters.

RV free wall ECV was difficult to measure (heavy trabeculation, sternal wires, blood pool in regions of interest) with high and poor inter-observer reproducibility: this analysis was abandoned.

Conclusion

Septal interstitial expansion is seen in adults late after atrial redirection surgery for TGA. It correlates well with NT-proBNP and CI and may have a role in the development of RV systolic impairment. Measuring interstitial expansion in the RV free wall is difficult using this methodology.

Keywords

Congenital heart disease • Systemic right ventricle • Cardiovascular magnetic resonance imaging • Diffuse fibrosis

Introduction

Although intra-atrial repair (Mustard and Senning operations) of transposition of the great arteries (TGA) has been largely been superseded by the arterial switch operation, there remains a sizeable population of adults under a regular follow-up who underwent such early palliative surgery. With the morphologic right ventricle (RV) supporting the systemic circulation, there is a high burden of early morbidity and mortality from heart failure and sudden cardiac death.1 Survival rates have been calculated at 76% by 20 years of age2 with a mean age at death of 27 years.3 Long-term outcome in this cohort relates to the function of the systemic RV.4

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Cardiovascular magnetic resonance (CMR) imaging is an accurate and reproducible method for assessing systolic function of the systemic RV and is widely regarded as the best available imaging modality for this purpose.5,6

Other markers of heart failure severity, including high circulating brain natriuretic peptide (NT-proBNP) levels and impaired cardiopulmonary exercise (CPEX) performance, have also been described in adults with congenital heart disease.7–9 Both measures have been shown to relate to long-term prognosis in mixed cohorts with congenital heart disease (CHD) and in those with a systemic RV.10–15

Myocardial fibrosis is considered to be a marker of disease severity and its presence is used to monitor disease progression and regression.16 In those with previous Mustard or Senning surgery, focal fibrosis, assessed using the late gadolinium enhancement (LGE) technique, has been found to correlate with systolic function of the systemic RV and with documented arrhythmia.17 However, the LGE method does not allow the evaluation of diffuse fibrosis and this latter has been found to correlate with systemic RV end-diastolic volume and ejection fraction in a small cohort described by Broberg et al.18 Our group has previously validated equilibrium contrast CMR (EQ-CMR) against cardiac biopsy determination of diffuse fibrosis for the assessment of left ventricular interstitial expansion (a surrogate marker of diffuse fibrosis in most disease states) in patients with severe aortic stenosis and hypertrophic cardiomyopathy.19 The technique is similar to that of Broberg, but the use of EQ removes uncertainties regarding contrast kinetics in heart failure or renal impairment and does not require an assumption of pseudo-equilibrium.

In this prospective study, we used EQ-CMR to evaluate diffuse fibrosis in adults late after atrial redirection surgery for TGA, comparing the results to both normal subjects and also other heart failure parameters to determine its usefulness as a measure of disease severity.

**Methods**

**Study population**

Fourteen adult patients with previous Senning or Mustard surgery for TGA, followed up in a dedicated congenital heart disease heart failure clinic at our Institution, were prospectively recruited between June 2009 and June 2010. All patients underwent the measurement of NT-pro BNP, CPEX testing, and CMR imaging within a 3-month period. None required admission for decompensated heart failure during this time. These patients were compared with 14 age- and gender-matched normal subjects, as there is some evidence to suggest an increase in extracellular volume (ECV) with age.20 All normal subjects underwent cardiac history and examination, 12-lead ECG and CMR to ensure they did not have any underlying cardiovascular disease. The study had institutional approval and all patients and normal subjects gave informed consent to participate in the study.

**NT-pro brain natriuretic peptide**

The peripheral venous blood was collected from each patient after they had rested for a minimum of 20 min. Samples were centrifuged and immediately analysed via sandwich immunoassay, using electrochemiluminescence (E 170 Module, Roche Diagnostics, Basel, Switzerland).

**CMR**

CMR was performed using a 1.5T Siemens Avanto scanner (Siemens Medical Systems, Erlangen, Germany), with the assessment of ventricular volumes as previously described.21,22 Manual segmentation of the ventricles, with the exclusion of the major RV trabeculae from the blood pool volume, was undertaken. Quantification of aortic forward flow volume, using phase contrast volumetry, provided an internal quantitative guide to the RV stroke volume and improved the accuracy of volumetric quantification in the presence of tricuspid valve regurgitation.23 Volumes were indexed to the body surface area.

**Equilibrium contrast CMR**

EQ-CMR, as previously validated against histology in aortic stenosis and hypertrophic cardiomyopathy,17 is an add-on to a standard clinical scan. We quantified the tissue volume of distribution, a marker of the ECV, of the routine clinical contrast agent, Gadodiamidemeglumine (gadolinium-DOTA, marketed as Dotarem © Guerbet S.A., France), which partitions freely between the plasma and interstitial space but does not enter cells. Interstitial tissue volume is primarily determined by the amount of extracellular matrix. EQ-CMR involves three steps: (i) a standard gadolinium bolus followed by constant infusion to eliminate contrast kinetic effects and achieve an EQ state throughout the body; (ii) signal intensity (T1) measurement pre- and post-contrast equilibrium using CMR; and (iii) a direct measure of blood volume of contrast distribution, by taking a complete blood count, and equating the blood volume of contrast distribution to one minus the haematocrit. The ECV is then calculated as15:

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ECV = \frac{(1 - \text{haematocrit}) \times (1/T_{1\text{myocardiumpost-contrast}} - 1/T_{1\text{myocardiumpre-contrast}})}{(1/T_{1\text{bloodpost-contrast}} - 1/T_{1\text{bloodpre-contrast}})}
\]

In this study, T1 mapping was performed using the same method as previously described, fast low angle single shot inversion recovery (FLASH IR) at increasing inversion times, in a mid-short axis slice.17 The blood T1 was assessed using a region of interest within the LV cavity. The myocardial T1 was assessed using a region of interest within the interventricular septum and also within the RV free wall.

**Cardiopulmonary exercise testing**

CPEX was performed on an electronically braked ergometer cycle (Lode, Groningen, Holland) with computerized breath-by-breath ventilator gas analysis (MedGraphics, MN, USA). Prior to exercising, height and weight were measured and all sensors calibrated. Patients wore a full-face mask connected to an expiratory limb pneumotachograph, allowing sampling of mouth tidal PCO2 and PO2. A 12-lead ECG was recorded at rest, continuously during exercise and into recovery.

Patients were encouraged to exercise to full capacity on an incremental protocol. Workload increased at 15–25 W depending on the underlying fitness level; however, the ramping protocol included an initial loadless 3 min of cycling to allow equilibration. Criteria for ending the test included clinical need or patient exhaustion; having aimed for, or achieved a respiratory exchange rate >1.01. A period of active recovery (slow pedalling) followed maximal exertion.

Cuff blood pressure was checked every 2 min, and transvenous oxygen saturations were measured continuously via a probe positioned over the right supraorbital artery. Peak oxygen uptake (pVO2), anaerobic threshold (AT), and ventilatory response to CO2 production (VE/VCO2) were derived from the respiratory gas analysis during maximal exertion. VE/VCO2 was measured as the ratio at the AT. In turn, AT was determined by using the modified V-slope
method. The chronotropic index (CI) was calculated as has been previously described\textsuperscript{13} as: (peak HR – resting HR) over (220-age-resting HR).

Statistical analysis
The Kolmogorov–Smirnov test was used to assess normality. Values are expressed as mean ± SD, median (25–75th percentile values), or percentages, as appropriate. Univariate comparisons were performed by Student’s t-test, Mann–Whitney U test, and χ\textsuperscript{2} test for normally distributed, non-normally distributed and categorical variables respectively. Pearson’s or Spearman’s correlation coefficients were used as appropriate to look for univariate associations between continuous variables (SPSS, Inc., Chicago, USA and GraphPad Software, Inc., La Jolla, USA). Differences were considered to be significant at a P-value <0.05.

Results
Patient and control subject characteristics
The fourteen patients (six females) had a mean age of 33.7 ± 6.5 years and the median age at palliation of 9.7(7–20) months (Table 1). Six (43%) had Senning palliation. Details of the 14 normal subjects (six females, median age 32, range 24–49) are also in Table 1.

CMR
The mean indexed RV end-diastolic volume (RVEDVi) was 79 ± 13 mL/m\textsuperscript{2}, mean indexed RV end-systolic volume (RVESVi) 35 ± 4 mL/m\textsuperscript{2}, and mean RV ejection fraction (RVEF) 59 ± 5% (Table 1). No patients had any focal, macroscopic areas of LGE. The mean septal ECV in patients was 0.254 ± 0.036, which was significantly higher than that of normal subjects (0.230 ± 0.032; P = 0.03). There was no correlation between CMR measures of systemic RV volume or systolic function to septal ECV.

| Table 1 | EDVi and ESVi refer to indexed ventricular volumes during diastole and systole, respectively |
|---------|-------------------------------------------------------------------------------------------------
|         | d-TGA (n = 14) | Controls (n = 14) | P-value |
| Age, years | 34 ± 7 | 32 (24–49) | 0.64 |
| Age at surgery, median (range), months | 10 (7–20) | -- | -- |
| Male/female | 8/6 | 8/6 | -- |
| NYHA functional class | 12/2 | 14/0 | -- |
| CMR: systemic ventricular | | | |
| EDVi, mL/m\textsuperscript{2} | 79 ± 13 | 70 ± 15 | 0.11 |
| ESVi, mL/m\textsuperscript{2} | 35 ± 4 | 25 ± 8 | 0.002 |
| EF, % | 59 ± 5 | 66 ± 5 | 0.002 |
| Septal ECV | 0.254 ± 0.036 | 0.23 ± 0.032 | 0.03 |

Right ventricular free wall ECV
We were unable to accurately assess the ECV in the right ventricular free wall (or any other right ventricular segment away from the septum) using the current method. This was mainly due to trabeculation that resulted in myocardial regions of interest partial voluming with the blood. The presence of blood in the region of interest will falsely elevate the ECV. The problem is illustrated in Figure 1. This assessment was therefore abandoned and only septal ECV is reported.

EQ-CMR and NT-proBNP
The median NT-proBNP was 19.5 (14–45) pmol/L. NT-proBNP showed a positive relationship with septal ECV, P = 0.04; r = 0.55 (Figure 2).

Discussion
This study suggests that diffuse fibrosis is more pronounced in the myocardium of the systemic RV of adults late after palliation of TGA compared with controls. The extent of interstitial expansion was related to known markers of disease severity and outcome parameters (NT-proBNP and chronotropic index) suggesting a potential causative role.

Lifelong maintenance of the systemic circulation by the morphological RV results in a heart failure phenotype that is associated with significant early morbidity and premature mortality.\textsuperscript{2,3} Evidence is emerging that myocardial fibrosis, determined histologically or by CMR, is a key factor in the development of ventricular dysfunction in patients with both acquired and congenital heart disease where pressure overload is the principal insult.\textsuperscript{17,18} Further, LGE on CMR has been linked to prolongation of ECG parameters associated with inhomogenous electrical activation associated with sudden cardiac death\textsuperscript{17}. Quantifying myocardial fibrosis in patients with systemic RVs also provides an additional measure of disease severity. Further studies are needed to investigate the clinical usefulness of EQ-CMR in predicting the outcome in this group.

We have previously validated EQ-CMR as a novel non-invasive technique to quantify diffuse myocardial fibrosis in adults with left ventricular disease.\textsuperscript{19} In the present prospective study, we used a similar method to identify and quantify diffuse myocardial fibrosis in adults with atrial switch palliation of TGA. Our results are similar to those of Broberg et al.\textsuperscript{18} who used a bolus technique as opposed to EQ-CMR. There are a number of T\textsubscript{1} mapping-based techniques in development by CMR currently.\textsuperscript{25} The use here of an infusion of gadolinium, to reach contrast equilibrium, eliminates contrast kinetics, which can alter in an individual over time (for example, due to changes in body weight or renal function) which may permit more precise evaluation of changes over time in any
individual, important both in the clinical and research settings. The technique has been validated against histology in aortic stenosis and hypertrophic cardiomyopathy. Although the bolus gadolinium technique is perhaps simpler, the total CMR scan time in our cohort remained at \( \approx 1 \) h for all patients, as we gave the bolus and infusion early in the protocol while continuing to scan other parameters during equilibration. Despite the differences in study method, the results obtained in both small studies are similar, and suggest that CMR diffuse fibrosis assessment is a useful biomarker in this cohort of patients.

Earlier work has confirmed that focal myocardial scar, as represented by localized LGE of the myocardium on CMR imaging, can be present in patients with atrial switch palliated TGA, and relates to the systolic function of the systemic RV. This finding most likely reflects localized surgical insult or coronary insufficiency (due to RV hypertrophy or abnormal coronary circulation). In
our study, we did not find that the ECV related to measures of RV size or systolic function. This may reflect the small cohort size involved, or may suggest early pathophysiological detection of disease such that diffuse fibrosis is a precursor to measurable RV dysfunction. It is worth noting that the mean RVEF of this study cohort was within the perceived normal range. It is possible that such diffuse fibrosis may relate to diastolic dysfunction in the systemic RV. In our study, diastolic function was not assessed with MRI, as echocardiographic techniques for diastology have a temporal resolution and sensitivity that far surpasses that of current MR techniques. Echocardiographic correlation may be crucial for further investigation of this issue.

Interestingly, diffuse fibrosis was found to relate to NT-proBNP. This is an established neurohormonal marker of disease in the general cardiology population. It is also increasingly being recognized as a valuable tool in CHD, and specifically in TGA, where it correlates to systemic RV function as determined by CMR. In the general cardiology population NT-proBNP has an established role in detecting asymptomatic left ventricular dysfunction in high-risk populations and in predicting cardiovascular events in those with preserved ventricular function. The finding of diffuse fibrosis on CMR imaging in the setting of preserved RV systolic function may represent a similar early warning sign.

Septal ECV also showed a negative correlation to the chronotropic index, but no relation to pVO₂ or VE/VCO₂—both established independent markers of the outcome in CHD. Giardini et al. studied adults with a systemic RV and previous Mustard or Senning palliation and proposed a VE/VCO₂ slope cut-off >35.4 as being predictive of 4-year mortality or cardiac-related admission, suggesting that our cohort was quite well managed at the time of study. Similarly, values for pVO₂ in this cohort were above the levels previously published as predictors of adverse events in congenital cohorts. Adults with a systemic RV often describe themselves as well and asymptomatic, despite objective evidence to the contrary. Formal exercise testing has, however, found that the majority with a systemic RV have chronotropic incompetence and furthermore, that in a proportion of those studied, such inability to increase heart rate was unrelated to metabolic parameters, suggesting that this alone may be causing exercise intolerance. Whether our finding represents early detection of ensuing RV dysfunction remains to be determined; however, it is likely that any underlying cause is multifactorial. It is of interest to note that a study of adults with hypertrophic cardiomyopathy also found a relationship between LGE on CMR and blunted heart rate response on exercise. Such LGE has been found to be an independent predictor of deficient exercise capacity in such cohorts, however, relationship to outcome and underlying mechanism is not yet established.

It is important to note here the small but a significant annual incidence of bradyarrhythmias and complete heart block that occur in our population. Such arrhythmias increase with time during the follow-up, and published frequency of pacemaker insertions varies from 5 to 11%. It is well recognized that sick sinus syndrome is associated with a diffuse fibrotic process in the sinoatrial node and indeed a recent murine model has recapitulated this. Furthermore, the patients presented in this paper develop increased atrial wall stress and stretch which will promote local hypertrophy and fibrosis in conjunction with the effects of corrective surgery. Akoum et al. has recently demonstrated significant correlations between atrial fibrosis using LGE and sinus node disease supporting pathological studies and recent murine models of the process.

The septal fibrosis seen on EQ-CMR may therefore underlie the conduction pathway problems that develop in such patients, possibly due to myocardial disarray. It will be interesting to see whether the degree of abnormality can predict future development of conduction block and the need for pacing.

Limitations

Our study is limited in the size and further larger-scale investigation is needed. Although the results show the potential for the technique to be useful, newer methods for T₁ mapping have recently become available and show the potential for higher resolution whole heart mapping in three breath holds. These could allow more accurate assessment of the RV free wall, which we were unable to assess in this study. Further, this study was limited in that no echocardiographic diastolic data were acquired—this will need further study and inclusion in future work.

Despite the presence of RV hypertrophy in the patient cohort, the assessment of diffuse fibrosis in the anterior free wall of the RV was technically problematic. The ubiquitous presence of trabeculation within the systemic RV free wall leaves very few areas of the compacted RV myocardium during diastole that are without blood pool contamination. If zones of the blood pool are included in the myocardial analysed region of interest, the ECV would be falsely elevated. Metal artefact from sternal wires also contributed to artefact in this zone in many patients.

Conclusion

This is a proof of a concept study to investigate and quantify diffuse fibrosis in the myocardium of adults with a systemic RV using a non-invasive CMR technique. We find that diffuse fibrosis is present in the septum of adults with a systemic RV, late after Senning, or Mustard palliation for TGA. The extent of diffuse fibrosis showed association with NT-proBNP measurement and may aid in the identification of patients at risk of developing bradyarrhythmia. Larger and longer-term studies are needed to determine whether such diffuse fibrosis is of prognostic significance and whether its extent can be modified with therapeutic intervention.

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