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Original Article

Myocardial histopathology in late-repaired and unrepaired adults with tetralogy of Fallot

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ABSTRACT

Survival of patients after repair of tetralogy of Fallot (TOF) is worse than for the general population. We aimed to assess the time-related effects of surgical repair on right (RV) and left ventricle (LV) myocardium by quantifying hypertrophy and fibrosis. Cardiomyocyte transverse diameter and percent of fibrosis were measured in 8 adult heart specimens with late-repaired TOF, 6 with unrepaired TOF, and 11 normal hearts (controls). The RV and LV mean and median cardiomyocyte diameter and percent of fibrosis were significantly greater than controls in both repaired and unrepaired hearts. The mean RV inferior wall myocyte diameter in unrepaired hearts was significantly greater at average age at death than in repaired hearts (24.9 ± 2.5 vs. 16.4 ± 1.3 μm, $P = .015$), but not the mean RV anterior wall myocyte diameter (21.5 ± 2.2 vs. 17 ± 1.2 μm, $P = .09$) or the mean LV myocyte diameter (19.7 ± 1.5 vs. 16.7 ± 0.8 μm, $P = .10$). Of the RV myocyte diameter measurements, only the RV anterior wall myocyte diameter for repaired hearts correlated with age at death, while LV myocyte diameter for both repaired and unrepaired hearts correlated with age at death. None of the measures of myocyte diameter correlated with age at repair. The mean RV anterior wall, inferior wall, and LV percent fibrosis were all significantly greater in unrepaired hearts at average age at death compared with repaired hearts (16.3 ± 1.3 vs. 13.0 ± 0.7%, $P = .04$; 18.1 ± 1.9 vs. 12.7 ± 1.0%, $P = .03$; 15.7 ± 0.8 vs. 11.6 ± 0.4%, $P = .004$, respectively). There was a significant correlation between RV percent fibrosis (both locations) and age at death for repaired hearts but not for unrepaired hearts, while LV wall percent fibrosis correlated significantly with age at death for both groups. RV percent fibrosis was not significantly correlated with age at repair, while LV percent fibrosis was negatively correlated with age at repair. Hypertrophy and fibrosis in RV and LV of late-repaired TOF hearts progress during follow-up despite a good repair. These could be the substrate of ventricular dysfunction and arrhythmia seen clinically late after correction.

1. Introduction

Tetralogy of Fallot (TOF) can be repaired in infancy with low operative mortality, and more than 90% of repaired patients are expected to be alive 25 years after repair [1,2]. Long-term survival, however, is not equal to that of the general population, with increasing morbidity and mortality rates beginning in the third to fourth decades of life [3,4]. Over the past 15 years, several risk factors for less favorable outcomes have been identified in adults with repaired TOF including patient history (e.g., older age at repair), electrophysiological markers (e.g., prolonged QRS duration, atrial tachyarrhythmias) [5–8], hemodynamic abnormalities resulting from chronic pulmonary regurgitation (PR) [e.g., right ventricular (RV) dilation, RV and/or left ventricular (LV)] dysfunction], and myocardial scar [9,10]. Recently, RV hypertrophy [RV mass/volume ratio ≥0.3 detected with cardiac magnetic resonance imaging (CMRI)] has been identified as an additional, independent risk factor for death and sustained ventricular tachycardia in repaired TOF patients [7].

There has been considerable interest in the histopathology of the myocardium in unrepaired TOF patients since the late 1960s. Studies have shown cardiomyocyte hypertrophy and disarray, varying degrees and types of fibrosis, and cardiomyocyte degeneration, thought to be the consequence of high RV pressure and hypoxemia [11–15]. More recent studies have shown hypertrophy and fibrosis of both the RV

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and LV in unrepaired TOF [16] that increase with age [17] and are associated with cardiac dysfunction and a worse outcome early after repair [18,19]. However, little is known about myocardial histopathology after repair of TOF. It is unknown if repair changes the natural history of the myocardium, which is important information for the management of many older patients followed in adult congenital heart disease clinics.

The aims of this study are to (a) quantify hypertrophy (cardiomyocyte diameter) and fibrosis (percent) in the RV and LV myocardium of repaired patients with TOF as a function of time after repair and (b) compare hypertrophy and fibrosis between repaired and unrepaired patients at comparable ages to assess the effects of surgery on the natural history of the myocardium.

2. Methods

2.1. Specimen selection

The database of the Cardiac Registry at Boston Children's Hospital was searched to identify all heart specimens with TOF. The following were excluded: (a) malformation syndromes except for Down and DiGeorge syndromes and (b) hearts with complex forms of TOF including TOF with pulmonary atresia, TOF with absent pulmonary valve, and TOF with common atrioventricular canal.

Next, the specimens were divided into unrepaired hearts (including hearts from patients that died within 48 h of surgery) and hearts from patients that had undergone complete repair and survived for at least 48 h. Hearts were reviewed to find good quality specimens with well-preserved anatomic landmarks and firm, intact myocardium.

Unrepaired hearts were included if the patient had survived to at least 10 years of age and had never undergone intracardiac surgery or died acutely within 2 days of surgical repair (considered unrepaired for the purpose of assessing chronic changes within the myocardium). Hearts were eligible for inclusion if a systemic-to-pulmonary artery shunt had been performed but were excluded if any intracardiac procedure had previously been performed (e.g., Brock procedure).

Repaired hearts were included if the patient had survived at least 1 year following surgical repair and if postoperative hemodynamic data were available. Patients with a suboptimal hemodynamic result (RV pressure > ½ systemic, pulmonary-to-systemic flow ratio >1.5) were excluded.

A control group of normal hearts from 11 adults who died of noncardiac causes and who had no history of significant heart disease, normal heart weight for body size and age, and no ventricular hypertrophy was selected from the Department of Pathology at Brigham and Women's Hospital. Controls were matched with TOF patients for age at death (within each decade).

All specimens were preserved in 10% neutral buffered formalin. All families had provided written consent for the specimens to be maintained in the Cardiac Registry or the Pathology Department at Brigham and Women's Hospital and for them to be used for research and teaching according to hospital policy in force at the time of collection.

The computer database of the Department of Cardiology at Boston Children's Hospital was searched for demographic data, operative reports, catheterization data, echo and MRI reports, and autopsy data.

2.2. Histology

From the selected repaired and unrepaired TOF specimens, we took two samples of myocardium from the RV (inferior wall and anterior wall from the apical ½ of the ventricle) and one sample from the LV anterior wall. One myocardial sample was available from the RV anterior wall from five of the normal hearts, and one sample from the LV anterior wall from the remaining six normal hearts. The tissue sample was cut perpendicular to the surface of the endocardium and epicardium, producing a transmural section. Care was taken to obtain samples away from surgical sites to avoid a surgical scar.

Samples were embedded in paraffin, cut into 5-μm sections, and mounted. Sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome (MT) stain. Twelve fields per H&E-stained section were photographed at 40× magnification for measurement of cardiomyocyte size, and four fields per MT-stained section were photographed at 10× magnification for measurement of fibrosis. Images were obtained and stored digitally using a high-resolution camera (Olympus DP72; Olympus Corporation, Munster, Germany) attached to a light microscope (Olympus BX51; Olympus Corporation, Munster, Germany).

For measurement of the cardiomyocyte size, we selected sections in which myocardial fibers were cut longitudinally or obliquely, and the short-axes dimension of fibers containing nuclei was measured with a computer system (cellSens 1.7; Olympus Corporation, Munster, Germany). Nuclei are located near the center of myocytes so that a section of a myocyte containing the nucleus is likely to approximate the central diameter of the myocyte. The thin sections used in this study minimized the problem of fiber overlap. We measured all cells meeting these criteria but excluded fibers if the boundaries were not clear or degenerative changes were prominent. The fibers at or near the edges of areas of massive fibrosis or adjacent to interstitial spaces containing large blood vessels were also excluded.

For measurement of percent fibrosis, we subdivided the wall into subepicardial and subendocardial regions and excluded papillary muscles and trabeculations. The cross-sectional area of fibrosis was measured semiautomatically using a multipurpose color image analyzer (ImageJ 1.47v, http://imagej.nih.gov/ij/). The percent area of fibrosis was obtained by dividing the sum of the fibrotic areas of each field by the total tissue area of the field. Star-shaped microscars (replacement fibrosis) and collagen around arterioles were included in this analysis. Collagen in the intima and media of vessels, the endocardium, and obvious artifacts were excluded. The myocyte diameter and percent fibrosis in TOF patients were compared with the age-matched normal control group. To address if and how repair of TOF might modify the natural history of the myocardium, the relationships between cardiomyocyte diameter and percent fibrosis and age at death were examined for both repaired and unrepaired specimens.

2.3. Statistical analysis

Data are expressed as mean value at average age at death (± 1 S.E.) and range for myocyte diameter and percent fibrosis. Median and range, absolute and relative frequency, and percentage were used for clinical data.

For each location (RV anterior wall, RV inferior wall, and LV anterior wall), regression models were used to estimate the association between the myocyte diameter and percent fibrosis outcomes and age at death and age at repair (for repaired TOF subjects) in each group (normal controls, unrepaired TOF, and repaired TOF). The models include terms for age at death, group (with normal controls as the reference group), the age at death by group interaction, and the age at repair by repaired TOF group interaction. In the results section, group comparisons are made at the mean age of death for the sample (34.3 years) and the average age of repair for repaired TOF patients (12.9 years). Standard linear regression was used to analyze myocyte diameter since one observation per subject was collected. Linear mixed-effects models were used to analyze percent fibrosis since two observations per subject were collected (one from the endocardium and one from the epicardium). A random intercept was used to account for the correlation between observations from the same individual. The associations between the RV and LV anterior myocyte diameter and between the RV and LV anterior percent fibrosis for the sample were estimated using Pearson correlation coefficient. All analyses were performed using SAS version 9.3; tests were two-sided at the .05 significance level.