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Late Causes of Death After Pediatric Cardiac Surgery
A 60-Year Population-Based Study

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ABSTRACT

BACKGROUND Comprehensive information regarding causes of late post-operative death following pediatric congenital cardiac surgery is lacking.

OBJECTIVES The study sought to analyze late causes of death after congenital cardiac surgery by era and defect severity.

METHODS We obtained data from a nationwide pediatric cardiac surgery database and Finnish population registry regarding patients who underwent cardiac surgery at <15 years of age at 1 of 5 universities or 1 district hospital in Finland from 1953 to 2009. Noncyanotic and cyanotic defects were classified as simple and severe, respectively. Causes of death were determined using International Classification of Diseases diagnostic codes. Deaths among the study population were compared to a matched control population.

RESULTS Overall, 10,964 patients underwent 14,079 operations, with 98% follow-up. Early mortality (<30 days) was 5.6% (n = 613). Late mortality was 10.4% (n = 1,129). Congenital heart defect (CHD)-related death rates correlated with defect severity. Heart failure was the most common mode of CHD-related death, but decreased after surgeries performed between 1990 and 2009. Sudden death after surgery for atrial septal defect, ventricular septal defect, tetralogy of Fallot, and transposition of the great arteries decreased to zero following operations from 1990 to 2009. Deaths from neoplasms, respiratory, neurological, and infectious disease were significantly more common among study patients than controls. Pneumonia caused the majority of non-CHD-related deaths among the study population.

CONCLUSIONS CHD-related deaths have decreased markedly but remain a challenge after surgery for severe cardiac defects. Premature deaths are generally more common among patients than the control population, warranting long-term follow-up after congenital cardiac surgery. (J Am Coll Cardiol 2016;68:487–98) © 2016 by the American College of Cardiology Foundation.
follow-up. However, a limited number of studies exist on this subject. In this study, we investigated the nationwide causes of patient deaths up to 60 years after their first cardiac operation, and compared them to deaths in the general population to identify areas that require special attention during the long term.

**METHODS**

The Finnish Ministry of Social Affairs and Health granted permission for this study, and the ethical committee approved the research protocol. We included all pediatric cardiac operations performed between 1953 and 2009 in Finland on children who were under 15 years of age at the time of their first operation. Follow-up began after the first cardiac surgery of the patient, and ended at death, emigration, or on December 31, 2012. The only procedures excluded were patent ductus arteriosus (PDA) closures in children <1 month of age, and pacemaker implantations in patients for whom this was the only procedure.

Each patient was assigned 1 primary diagnosis from a severity-based hierarchical list of cardiac defects: PDA, atrial septal defect (ASD), coarctation of the aorta (COA), ventricular septal defect (VSD), TOF, TGA, HLHS, and UVH. All remaining cardiac defects were collectively referred to as miscellaneous. For patients with several cardiac defects, we chose the hierarchically more severe condition. For the sake of comparison, we dichotomized defect severity into simple (PDA, ASD, COA, and VSD) and severe (TOF, TGA, HLHS, and UVH) defects according to the lack or presence of cyanosis, respectively.

**MORTALITY AND CAUSES OF DEATH.** We excluded all early deaths (within 30 days post-operatively) from analyses regarding causes of death and incidence of death. For patients that emigrated, we marked the day of emigration as the last day of follow-up in survival analyses. Unclear causes of death were confirmed by autopsy. We obtained diagnosis codes for all deceased patients from Statistics Finland. Causes of death were categorized into CHD-related and non-CHD-related deaths. CHD-related deaths were classified as diseases within the Q20 to Q28 range from the International Classification of Diseases-10th Revision diagnosis system (and International Classification of Diseases-9th Revision numbers 745 to 747 for older cases).

We further categorized all CHD-related deaths into heart failures, sudden deaths, post-reoperative early deaths (<30 days), and cardiovascular deaths according to a previous classification system (2). Sudden deaths included all unwitnessed deaths that occurred during sleep and cardiovascular-related deaths occurring within 1 h of onset (or the significant worsening) of symptoms. Post-reoperative early death spanned all early operative deaths (within 30 days) after the patients’ subsequent reinterventions. We classified the remaining causes of death not covered by other classes as cardiovascular.

We compared survival and the incidence of different CHD-related deaths by decade of operation to assess advances in late surgical outcome. Due to the small number of patients undergoing surgery for HLHS before the 1990s, we excluded such cases from the comparison of time periods.

Statistics Finland supplied us with age-, sex-, time of birth-, and hospital district–matched control subjects from the general population for each study patient. We then compared the modes of non-CHD-related deaths of study patients to those among the general population, presenting results as rate ratios (RRs).

**STATISTICS.** We analyzed mortality by Poisson regression models utilizing a Lexis-type data structure with 3 time scales, vis-à-vis age, calendar year, and time since surgery (3). We report RRs on the basis of the Poisson regression with 95% confidence intervals (CIs). In the estimation of hazard rate curves, we used splines to produce smoothed curves with a 95% confidence envelope. Standard deviations are reported with mean values. Survival data is presented in the form of Kaplan-Meier plots, and 2-tailed p values obtained with the log-rank test; p values <.05 were considered significant. All data analyses were carried out using the R program (R Development Core Team, Vienna, Austria, 2011) and IBM SPSS Statistics version 23.0 (SPSS, Inc., Chicago, Illinois).
RESULTS

PATIENTS. A total of 10,964 patients underwent 14,079 operations between 1953 and 2009. Follow-up ended prematurely for 177 patients (1.6%) who emigrated, and we excluded 95 patients (0.9%) from the study due to incomplete follow-up data, resulting in a total follow-up coverage of 97.5% (n = 10,692). Male patients dominated the study population across all groups, except PDA and ASD (Table 1).

MORTALITY. Early mortality (<30 days) occurred in 5.6% (n = 613) of the study population, and was greatest among patients with severe cardiac defects including TOF, TGA, HLHS, and UVH (Table 2).

Late mortality reached 10.4% (n = 1,129) among the entire study population (Table 2). Of the late deceased patients, 58% were male and 42% were female. The majority of deaths among early survivors with severe cardiac defects occurred within 10 years of their operation, whereas those with simple defects died more than 20 years after their operation. Late survival of 30-day-survivors at 40 years after the operation was 87% (95% CI: 86% to 89%) and 65% (95% CI: 61% to 69%) for patients with simple and severe cardiac defects, respectively (severe vs. simple hazard ratio [HR]: 3.7; 95% CI: 3.3 to 4.2; p < 0.0001 for log rank) (Central Illustration). Late survival of 30-day-survivors at 15 years after the operation was 92% (95% CI: 90% to 94%) for simple defects and 72% (95% CI: 70% to 74%) for severe defects operated on from 1953 to 1989, with an increase in 20-year survival from 92% (95% CI: 90% to 94%) to 96% (95% CI: 95% to 97%) for severe defects operated on from 1953 to 1989 and 1990 to 2009, respectively. Heart failure was the leading cause of CHD-related death among the majority of defect groups, occurring at a mean of 14 ± 16 years of age (Table 3, Figure 1B). We found a bimodal distribution for the hazard for fatal heart failure among all defect groups undergoing surgery between 1953 and 1989, with higher rates for severe cardiac defects (Tables 3 and 4, Figure 2). For patients with VSD and TGA undergoing operations between 1990 and 2009, however, the incidence and hazard for fatal heart failure diminished significantly, particularly late after surgery (Table 3, Figure 2). Fifty-two patients (17% of all fatal heart failures) that died due to heart failure were diagnosed with pulmonary hypertension and died on average 8 years (range of age at death: 0.1 to 48.7 years) after their first operation.

CAUSES OF CHD-RELATED DEATHS. Collectively, 721 patients (6.6%) died due to CHD-related causes. The cause of CHD-related death was identified for all but 7 patients. The majority of deaths among patients with PDA, ASD, and COA were unrelated to their cardiac defect, whereas patients with TOF, TGA, UVH, and HLHS died more often as a result of CHD-related issues (Figure 1A).

The 40-year freedom from fatal heart failure was 98% after surgery for simple defects and 89% after surgery for severe defects (severe vs. simple HR: 1.6; 95% CI: 1.5 to 1.7; p < 0.0001), with an increase in 20-year survival from 92% (95% CI: 90% to 94%) to 96% (95% CI: 95% to 97%) for severe defects operated on from 1953 to 1989 and 1990 to 2009, respectively. Heart failure was the leading cause of CHD-related death among the majority of defect groups, occurring at a mean of 14 ± 16 years of age (Table 3, Figure 1B). We found a bimodal distribution for the hazard for fatal heart failure among all defect groups undergoing surgery between 1953 and 1989, with higher rates for severe cardiac defects (Tables 3 and 4, Figure 2). For patients with VSD and TGA undergoing operations between 1990 and 2009, however, the incidence and hazard for fatal heart failure diminished significantly, particularly late after surgery (Table 3, Figure 2). Fifty-two patients (17% of all fatal heart failures) that died due to heart failure were diagnosed with pulmonary hypertension and died on average 8 years (range of age at death: 0.1 to 48.7 years) after their first operation.
The 40-year freedom from sudden death was 99% (95% CI: 98.5% to 99.5%) after surgery for simple defects and 91% (95% CI: 90% to 92%) after surgery for severe defects (severe vs. simple HR: 9.9; 95% CI: 6.7 to 14.6; p < 0.0001). The incidence and hazard for sudden death decreased to zero among patients with ASD, VSD, TOF, and TGA that underwent surgery between 1990 and 2009 (Table 3, Figure 2). Five patients with UVH who died suddenly had tricuspid atresia, 4 presented with double-inlet left ventricle, and 3 presented with isomerism. The most common known reason for sudden death among patients undergoing operations for COA was aortic rupture/dissection, with 6 patients (32% of all sudden deaths among patients with COA) undergoing operations between 1953 and 1989. Two of these patients experienced aortic rupture/dissection within 6 months after their first operation. The remainder died an average of 25 years after their first surgery, with the latest death occurring in 2007. In addition, 6 patients (32%) with COA (all but 1 operated on between 1953 and 1989) died suddenly. These patients presented with aortic valve stenosis at the post-mortem examination and died due to unknown/arrhythmia-based causes. Seven of the 13 patients (53%) in the VSD group that died suddenly suffered from pulmonary hypertension. The most common causes of sudden death were arrhythmia and myocardial infarction, however the majority of deaths remaining unexplained (Table 4). The mean and median age at sudden death were 21 and 19.6 years of age (range 0.1 to 58.5 years of age), respectively.

Early deaths after reoperation were more common among patients with severe defects (Tables 3 and 4, Figure 1B). The incidence of post-reoperative early deaths decreased significantly among patients undergoing surgery for VSD and TOF between 1990 and 2009 (Table 3). Twenty-four (86%) of 28 post-reoperative early deaths among patients with VSD occurred after secondary VSD closure, with the most common prior procedure being pulmonary-artery banding and COA repair. Of the 23 post-reoperative early deaths among TOF patients, 20 (87%) occurred among patients with primary palliation. Thirteen of these deaths occurred after the subsequent TOF repair surgery. Nine (30%) of 30 post-reoperative early deaths among UVH patients occurred after Fontan surgery. The incidence of cardiovascular death was greatest among patients with HLHS and UVH, and lowest among patients with PDA and ASD (Table 3, Figure 1B). Patients were on average 13 ± 16 years of age at the time of cardiovascular death (Figure 1B). The incidence of cardiovascular death decreased significantly only among VSD patients that underwent surgery between 1990 and 2009 compared to those undergoing surgeries between 1953 and 1989 (Table 3). The hazard of cardiovascular deaths revealed a bimodal distribution among the majority of defect groups (Figure 2). The most common cardiovascular cause of death was cerebral infarction (18 deaths, 20% among cardiovascular deaths) (Table 4), with myocardial infarction ranking second (10 deaths, 11%) (Table 4), with a higher incidence among patients with severe cardiac defects. Nine patients that died due to cardiovascular reasons suffered from pulmonary hypertension, 5 of which died due to cerebral infarction.

CAUSES OF NON-CHD-RELATED DEATHS. In total, 408 patients (4%) died due to non-CHD-related causes. The most common mode of known non-CHD-related death was respiratory disease (n = 56) (Online Table 1), specifically pneumonia (n = 41). More than one-half of all deaths due to pneumonia occurred among patients with a mental disability (n = 23; 56% of all pneumonia deaths). The rate of respiratory disease among the study population was significantly higher than that among the general population (RR: 11.00; 95% CI: 6.7 to 17.0; p < 0.0001) (Figure 3A). This held true when comparing late (1953 to 1989) and early (1990 to 2009) time periods to the general population (RR: 6.10; 95% CI: 3.6 to 10.4; RR: 120.00; 95% CI: 16.4 to 888.6; p < 0.0001 for both, respectively) (Figure 3B).

Patients died significantly more often due to neurological and infectious diseases than the general population (neurological disease RR: 3.72; 95% CI: 2.6 to 5.4; infectious disease RR: 6.47; 95% CI: 2.6 to 16.1; p < 0.0001 for both) (Figure 3A). However, for infectious diseases, the statistical significance diminished when comparing early and late time periods.
CENTRAL ILLUSTRATION Causes of Late Death After Pediatric Cardiac Surgery: Long-Term Survival

(A) Long-term Kaplan-Meier-depicted survival of patients operated on from 1953 to 2009 according to disease severity, excluding early mortality but including emigrated patients. (B) Long-term Kaplan-Meier-depicted survival of patients according to disease severity and decade of operation, excluding early mortality but including emigrated patients. All p values have been obtained by the log-rank test, and represent tests of significance between the simple and severe cardiac defect groups in A and between era of operation for each severity group in B.
respectively to the general population matched for time (Figure 3B). Cerebral hemorrhage (n = 14) and epilepsy (n = 8) represented the 2 most common modes of neurological death (Online Table 1). Also, deaths due to neoplasm were more common among study patients compared to the general population, particularly among female patients operated on between 1990 and 2009 (RR: 1.60; 95% CI: 1.3 to 1.9; p = 0.002) (Figure 3A). This was especially true among female patients operated on from 1953 to 1989 (RR: 2.60; 95% CI: 1.3 to 5.2; p = 0.005) (Online Figure 1). Moreover, alcohol-related deaths were more common among the

Deaths due to accidents occurred slightly but significantly more often among the study population than the general population, causing death among patients at an average age of 29 years of age (RR: 1.60; 95% CI: 1.2 to 2.2; p = 0.002) (Figure 3A). This was especially true among female patients operated on from 1953 to 1989 (RR: 2.60; 95% CI: 1.3 to 5.2; p = 0.005) (Online Figure 1). Moreover, alcohol-related deaths were more common among the
patient population, especially among male patients operated on from 1953 to 1989 (RR: 1.80; 95% CI: 1.1 to 2.7; \( p = 0.012 \)) (Figure 3A, Online Figure 1). In addition, the suicide rate was similar between the study population and the general population (Figures 3A and 3B), with 37 suicides among those operated on from 1953 to 1989 (Online Table 1).

**DISCUSSION**

In this study, we adopted a population-based approach and investigated the causes of death among patients with common congenital cardiac defects. The unique comprehensive Finnish national registry enabled this type of population-based study with an extensive long-term follow-up. Furthermore, we compared results from 2 different time periods to analyze changes in patterns for both CHD-related and non–CHD-related deaths among patients, and used a statistical model that allowed us to examine the simultaneous effect of age, cohort, and period of operation on long-term mortality (3). This type of approach of examining multiple time scales simultaneously— with similar results—has previously been described, albeit using a different statistical method (4).

**MORTALITY AND FOLLOW-UP.** Both early and late results have improved significantly during recent decades. This is to a great degree attributed to advances in perioperative treatment and surgical techniques. Moreover, earlier treatment of patients has generally resulted in improved results during recent decades.

In Finland, all surgical procedures were centralized at Helsinki University Central Hospital from the mid-1990s. Patient follow-up until 18 years of age was organized either at 1 of the 5 university hospitals across Finland or at a central hospital equipped with a visiting cardiologist from 1 of the university hospitals. All invasive diagnostic procedures were centralized to Helsinki, along with the follow-up of the majority of patients with UVH or TGA. This centralization of treatment has undoubtedly positively influenced both early and late results.

As expected, patients with severe cardiac defects were on average younger at the time of death compared to those with simple defects. Compared to the often single-staged correction of simple defects, surgically staged palliation or correction of severe cardiac defects places greater strain on the heart, and predisposes patients to operative death after subsequent reinterventions or early cardiovascular deaths.

**CHD-RELATED DEATHS.** Nearly one-fifth of patients who died due to heart failure suffered from pulmonary hypertension. The link between persistent postoperative pulmonary hypertension and mortality has previously been described (5,6). However, the incidence of fatal heart failure decreased among all patients undergoing surgery between 1990 and 2009. This finding was most notable among patients operated on for TGA, owing mostly to the shift from the Mustard and Senning procedures to the arterial switch operation (ASO) to correct TGA in the 1990s (7,8). Heart failure also markedly decreased among patients receiving surgery for VSD. In fact, VSD patients emerged as the only group of patients witnessing a significant reduction in all CHD-related deaths during the later time period. Earlier intervention has led to a decline in the incidence of pulmonary hypertension among these patients, whereby the occurrence of late heart failure has diminished.

Patients in all defect groups who underwent surgery from 1953 to 1989 experienced a bimodal hazard for fatal heart failure after their first operation. The
The risk for fatal heart failure was collectively highest 40 years after the surgery and lowest about 15 to 20 years post-operatively. This is largely due to the natural progression of heart failure, as scar tissue, small residual defects, or even a slightly high pulmonary pressure strain the surgical heart faster than a normal healthy heart. Furthermore, age-related increases in the incidence of hypertension and arteriosclerosis contribute to an increased mortality among older patients. Interestingly, the point of lowest hazard coincides approximately with the shift from being a pediatric to adult patient, after which the hazard for death begins to increase again. This raises the question of whether the shift to adult healthcare possibly affects the quality and frequency of follow-up.

Moreover, issues related to the maturation process and hormonal changes occurring among patients may influence their commitment to follow-up and treatment during adolescence.

One important observation focuses on the marked decrease in the number of sudden deaths during the later time period, as we found no deaths among the patients with ASD, VSD, TOF, and TGA undergoing surgery between 1990 and 2009. The incidence of sinus node dysfunction and sudden death is well documented among patients with UVH, including late after surgery (9,10). Accordingly, in our material, the HR for sudden death among patients undergoing surgery for UVH exhibited an increasing trend among patients receiving surgery between 1990 and 2009.
We compared patients undergoing surgery during 1953 to 1989 and 1990 to 2009, the early and late time periods, to determine the change in the hazard rate for death. \( n \) – number of patients in each defect group and era of operation, excluding early mortality (<30 days) and emigrated patients. CHD – congenital heart defect; CI – confidence interval; HR – hazard ratio.
contrast, the incidence of sudden death among surgical patients for TGA decreased to zero between 1990 and 2009, attributed mainly to the increasing employment of the atrium-sparing ASO technique (8,11–13). Aortic stenosis represents a common co-morbidity among patients with COA, especially among patients with a bicuspid aortic valve (14,15). We found a similar trend in our study, with one-third of patients that underwent surgery for COA experiencing aortic stenosis at the time of sudden death.

Recurrent post-operative hypertension commonly occurs among patients undergoing surgery for COA, which stands as a major risk factor for aortic rupture/dissection, the most common reason for sudden death among such patients in our study (14,15).

The decrease in the incidence of post-reoperative early deaths among patients with TOF operated on from 1990 to 2009 may be explained by the shift from staged palliative surgery to primarily 1-time operations during recent decades. Eighty-one percent of all TOF surgeries were primary repair during the 2000s, compared to only 14% during the 1960s. This was supported by the finding that the majority of post-reoperative early deaths occurred among TOF patients that underwent a primary palliative approach for their defect. From the year 2000 forward, a greater number of patients with VSD underwent primary VSD closure, whereas during previous decades it was common for patients to undergo pulmonary banding or aortic arch reconstruction prior to their VSD correction. This, along with the aforementioned advances in perioperative treatments and younger age at operation, has most likely decreased the number of post-reoperative early deaths among VSD patients between 1990 and 2009.

Cerebral infarction stood as the major cause of cardiovascular death, which occurred most commonly during the first year post-operatively either after the first or subsequent operations (9 of 16; 56%), and most commonly affected patients with TOF (6 of 18; 33%) and UVH (4 of 18; 22%). Myocardial infarctions were not specific to any defect group. Patients undergoing an ASO for TGA previously exhibited a higher risk of coronary artery-related complications (16–18). This, however, was not the

![FIGURE 3 RR for Different Causes of Non-CHD-Related Deaths](image-url)

Rate ratio (RR) (A) for all patients and (B) by decade. We compared the rate of death for each patient to 4 age-, sex-, and time-matched control subjects from the general population from the corresponding operative time period. CHD = congenital heart defect; CI = confidence interval.
case in our study, because only 2 patients undergoing surgery for TGA after ASO died due to myocardial infarction. Coronary artery-related complications, however, may occur during the immediate postoperative period after ASO (17,19–21). In our study, 13 of 29 deaths that occurred within 30 days after the ASO procedure occurred due to coronary artery-related complications.

NON-CHD-RELATED DEATHS. Respiratory disease—in particular, pneumonia—emerged as the most common form of non-CHD-related death among the study population, especially among patients with genetic disorders. Moreover, most cases listed as deaths due to anomalies represented complications associated with pneumonia. Respiratory death remained significantly more common among patients treated surgically between 1990 and 2009 compared to the general population. This may be partially explained by the marked decrease in the incidence of death due to pneumonia among the general population in recent decades.

The rate of death due to neoplasms was greater among the study population than the general population. This has been observed previously, and has been mainly attributed to genetic factors—particularly Down syndrome—and to possible catheter- and imaging-related radiation (22–25). The role of radiation as an etiologic factor for neoplasms in this study may be supported by the high incidence of radiation-prone tissue neoplasms, such as cancer of hematolymphoid tissue, intrathoracic organs, and breast tissue.

The rate of death due to neurological diseases was higher among the study population than the general population, consisting mostly of cerebral hemorrhage. These events occurred on average 30 years post-operatively, mainly among patients with simple defects. Four of these patients (29%) had confirmed intracranial aneurysms from post-mortem examinations.

To our surprise, the rate of accidental deaths was higher among the study population than the general population, specifically among female patients undergoing surgery from 1953 to 1989. Alcohol abuse is a major public health issue in Finland, and was reflected through the relatively high numbers of alcohol cases involved in suicides and accidents, as well as alcohol-related diseases. Chronic depression was listed as a contributory cause of death among only 5 patients that committed suicide and 1 patient that died in an accident. Nonetheless, chronic depression is an underdiagnosed disorder that should be taken into account during the follow-up of these patients. However, the incidence of alcohol-related diseases and suicides was less common among the study population than the control population for those operated on from 1990 to 2009.

STUDY LIMITATIONS. Although we have previously employed the current hierarchy-based assignment of diagnoses, it has certain limitations (1). The most prominent of these is that some lower ranked defects may go unnoticed in the presence of 1 or multiple higher ranked defects, despite a potentially important effect on the outcome. Prime examples of this are the presence of COA and PDA among patients with VSD, and aortic stenosis among patients with COA. Similarly, the dichotomization of defects into simple and severe forms may oversimplify results. Also, we decided to categorize TOF as a severe defect due to the cyanotic nature of the disease, which may skew the results towards an over-optimistic outcome. Moreover, the low number of terminal events among the patient population and accompanying wide confidence intervals hamper the accuracy and interpretation of the fitted hazard curves in Figure 2, particularly among patients operated on from 1990 to 2009. However, the rationale for implementing the current time model was to offer the practicing clinician an idea on possible terminal events among adults with CHDs operated on decades ago.

CONCLUSIONS

The number of late CHD-related deaths decreased among patients undergoing surgery for congenital cardiac defects. The risk of late sudden death among surgical patients during the later study time period decreased to zero among patients with ASD, VSD, TOF, and TGA, but remained an ongoing challenge among patients undergoing surgery for UVH. Late heart failure also decreased substantially, presenting a minimal risk among patients with simple cardiac defects. Patients undergoing surgery for severe cardiac defects remain at risk for late heart failure, warranting intensive follow-up decades after surgery. Respiratory disease, particularly pneumonia, remains a significant cause of non-CHD-related death among patients, and should be identified and treated in a timely manner.
Causes of Late Death After Pediatric Cardiac Surgery

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Late mortality, particularly sudden death after surgery for congenital heart disease, has diminished with advances in surgical techniques and perioperative management, but patients with severe defects remain at risk for many years after surgery and require intensive long-term surveillance.

TRANSLATIONAL OUTLOOK: Additional studies are needed to identify prophylactic strategies that reduce late mortality in patients at greatest risk, such as those born with univentricular defects.

REFERENCES


APPENDIX For a supplemental figure and table, please see the online version of this article.