Disturbed myocardial connexin 43 and N-cadherin expressions in hypoplastic left heart syndrome and borderline left ventricle

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Objectives: Borderline left ventricle is the left ventricular morphology at the favorable end of the hypoplastic left heart syndrome. In contrast to the severe end, it is suitable for biventricular repair. Wondering whether it is possible to identify cases suitable for biventricular repair from a developmental viewpoint, we investigated the myocardial histology of borderline and severely hypoplastic left ventricles.

Methods: Postmortem specimens of neonatal, unoperated human hearts with severe hypoplastic left heart syndrome and borderline left ventricle were compared with normal specimens and hearts from patients with transposition of the great arteries. After tissue sampling of the lateral walls of both ventricles, immunohistochemical and immunofluorescence stainings against cardiac troponin I, N-cadherin, and connexin 43, important for proper cardiac differentiation, were done.

Results: All severely hypoplastic left hearts (7/7) and most borderline left ventricle hearts (4/6) showed reduced sarcomeric expressions of troponin I in left and right ventricles. N-cadherin and connexin 43 expressions were reduced in intercalated disks. The remaining borderline left ventricle hearts (2/6) were histologically closer to control hearts.

Conclusions: Four of 6 borderline left ventricle hearts showed myocardial histopathology similar to the severely hypoplastic left hearts. The remainder were similar to normal hearts. Our results and knowledge regarding the role of epicardial-derived cells in myocardial differentiation lead us to postulate that an abnormal epicardial–myocardial interaction could explain the observed histopathology. Defining the histopathologic severity with preoperative myocardial biopsy samples of hearts with borderline left ventricle might provide a diagnostic tool for preoperative decision making. (J Thorac Cardiovasc Surg 2012;144:1315–22)

Hypoplastic left heart syndrome (HLHS) is a spectrum of morphologic abnormalities of the left heart first described by Noonan and Nadas.¹ At the severe end, the left ventricle is vestigial, in combination with aortic and mitral atresia. In cases with mitral stenosis, the hypoplastic left ventricle can show endocardial fibroelastosis with aortic atresia or severe aortic stenosis.² In contrast to the original definition, the term *HLHS* has often been used to refer only to the severe end of this spectrum. In this report, we use the term *HLHS* as originally described. The term *hypoplastic left heart complex* was introduced to refer to a subset at the mild end of the HLHS spectrum, comprising small but functional left ventricles without endocardial fibroelastosis and no valvular atresia or severe stenosis.³ The term *borderline*

left ventricle (BLV) has been used to indicate this favorable end of the HLHS spectrum.⁴ In this study we use the term *hypoplastic left heart (HLH)* to indicate the phenotype of the left ventricle at the severe end of the HLHS spectrum and *BLV* to indicate the phenotype of the left ventricle at the favorable end.

Different therapeutic strategies have been described that can be applied to neonates with HLHS.⁵ Cardiac transplantation and univentricular surgical correction are the 2 possibilities for the HLH phenotype. For the BLV phenotype, both univentricular and biventricular repairs have been described.^{5,6} Determination of whether a left ventricle is adequate to maintain the systemic circulation is, however, a major challenge in the decision making before the operative correction. Despite reports in the literature of the use of multiple diagnostic tools to aid in this decision making, a conclusive diagnostic tool is still missing.^{6–8} A possible solution could be found in a different developmental background of the myocardium of the hearts with BLV and HLH phenotypes. In particular, very little is known about the histology of left ventricular myocardium across the HLHS spectrum.^{2,9–11}

Recent studies on cardiogenesis have provided new insight into the origin of the ventricular myocardium,

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Abbreviations and Acronyms

BLV	=	borderline left ventricle
Cx43	=	connexin 43
EPDC	=	epicardium-derived cell
HLH	=	hypoplastic left heart
HLHS	=	hypoplastic left heart syndrome
ICD	=	intercalated disk
PBS	=	phosphate-buffered saline solution

enabling a better approach to understanding the developmental background of the myocardium in HLHS. The myocardium of the left and right ventricles is derived from different progenitor cell populations, the first and the second heart fields, respectively.¹² The epicardium originates from the second heart field and gives rise to epicardium-derived cells (EPDCs). These EPDCs migrate into the myocardial wall and, through epicardial–myocardial interactions, exert an essential role in establishing myocardial architecture.^{13,14}

Studies in the past 2 decades showed that electromechanical coupling between the cardiomyocytes is essential for myocardial differentiation.^{14–16} In particular, the intercalated disk (ICD) has a major role in the regulation of the electromechanical transduction, incorporating adherence junctions, desmosomes, and gap junctions. Adherence junctions and desmosomes provide N-cadherin–mediated intercellular adhesion and function as a platform for cytoskeletal anchorage, whereas gap junctions composed of connexin 43 (Cx43) are involved in cell–cell exchange.^{15,16} Recently, we investigated the role of EPDCs in myocardial maturation in the mouse¹⁴ by studying the expression patterns of troponin I, N-cadherin, and Cx43. Several EPDC-depletion models during development show myocardial abnormalities, including ventricular noncompaction and downregulation of myocardial N-cadherin and Cx43 expressions.¹⁴

In this study, we investigated normal human postmortem hearts and compared these with specimens with HLH and BLV phenotypes by using the aforementioned markers involved in myocardial differentiation and ICD function. Additionally, we evaluated the staining of these markers in hearts from patients with transposition of the great arteries to ensure that the observations made in HLH and BLV were specific to these malformations. We hypothesized that BLV and HLH might present with myocardial histopathologic abnormalities in varying degrees of severity, which might be caused by abnormal myocardial development and maturation regulated by EPDCs. Moreover, we hypothesized that our findings might be helpful in providing an additional diagnostic tool for surgical decision making.

MATERIALS AND METHODS

Selection

Twenty-two human heart specimens from patients who had not undergone operation, comprising 6 with normal anatomy, 7 with HLH phenotype, 6 with BLV phenotype, and 3 with transposition of the great arteries, were studied. The hearts were selected according to the definition criteria in the literature, as explained previously. In the HLH group, hearts with mitral and aortic valve atresia as well as hearts showing mitral valve stenosis and aortic valve atresia were included. Hearts with BLV phenotype were defined as those in which the apex was formed by the right ventricle and the left ventricular lumen was at least a third of the base–apex distance of the right ventricle in the absence of right-sided abnormalities. The left ventricle in BLV was small but functional, without macroscopic myocardial abnormalities, valvular atresia, or severe stenosis. In the normal hearts, the left ventricle was larger than the right ventricle and the apex was formed by the left ventricle without other morphologic abnormalities.

Morphologic measurements of cardiac structures were performed to refine the selection. The length of the left ventricular lumen was measured from the mid aortomitral continuity to the compact myocardium at the apex, whereas the right ventricular lumen was measured from the base of the pulmonary valve to the compact myocardium at the apex. A Z score for the morphologic measurements was calculated (Table 1).

The hearts were obtained from the Leiden Collection of malformed hearts (Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, The Netherlands). This collection includes hearts preserved in ethanol and glycerin, dating from the early 1950s to the recent era. This study was undertaken in accordance with the Dutch regulation for the proper use of human tissue for medical research purposes.

Sampling

Transmural tissue blocks of approximately 5 × 5 mm were taken from the free walls of both ventricles. The site of sampling was standardized from the proximal third of ventricular base–apex length. The embedding was standardized, and the samples were collected in 70% ethanol. The samples were routinely processed for paraffin embedding and immunohistochemical investigation. Transverse sections (5 μ m) in the craniocaudal direction were mounted on albumin and glycerin-coated glass slides in order from 1 through 10.

Immunohistochemical Examination

After deparaffinization and rehydration of the slides, microwave antigen retrieval was applied by heating 12 minutes at 98°C in a citric acid buffer (0.01 mol/L in distilled water, pH 6.0). Inhibition of endogenous peroxidase was performed for 20 minutes with a solution of 0.3% hydrogen peroxide in phosphate-buffered saline solution (PBS). The slides were incubated overnight with the primary antibody anti-troponin I (1/200), dissolved in PBS and polysorbate 20 with 1% bovine serum albumin. Between subsequent incubation steps, all slides were rinsed in PBS (2 times) and PBS and polysorbate 20 (1 time). The slides were incubated 60 minutes with the secondary antibody goat anti-rabbit biotin (1/200) in goat serum (1/66) in PBS and polysorbate 20. Subsequently, all slides were incubated 45 minutes with avidin–biotin complex reagent. For visualization, the slides were incubated 10 minutes with 400 μ g/mL 3-3'-diaminobenzidine tetrahydrochloride dissolved in tris(hydroxymethyl)aminomethane maleate buffer (pH, 7.6) to which 20 μ L hydrogen peroxide was added. Counterstaining was performed with 0.1% hematoxylin for 5 seconds, followed by rinsing 10 minutes with tap water. All slides were dehydrated, mounted with Entellan (Merck & Co, Inc, Whitehouse Station, NJ), and studied under light microscopy.

Immunofluorescence

Double stainings for N-cadherin (1/500 mouse monoclonal antibody GC-4; Sigma-Aldrich Co LLC, St Louis, Mo) and Cx43 (1/200 rabbit

TABLE 1. Cardiac phenotype and postmortem (postfixation) measurements

Age (d)	Phenotype	RV	Z*	LV	Z*	TV	Z*	MV	Z*	PV	Z*	AV	Z*	Asc Ao	Z*	Ao arch
Hypoplastic left heart																
3	MA, AA, PDA, PFO	32	0	10	-5	13	3	0	-5	9	2	0	-6	2	-5	Hypoplasia
11	MA, AA, PDA, PFO	30	0	10	-5	10	0	0	-5	7	0	0	-6	0.5	-7	3, coarctation
21	MA, AA, PDA, PFO	34	1	8	-5	13	3	0	-5	9	2	0	-6	1.5	-6	2.5, coarctation
2	MA, AA, PDA, PFO	36	1	18	-3	15	5	0	-5	8	1	0	-6	3	-4	Resected
1	MS, AA, PDA, PFO, EFE	28	-1	10	-5	8	-2	2.5	-3	6	-1	0	-6	1	-6	Hypoplasia
0	MS, AA, PDA, PFO, EFE	36	1	10	-5	13	3	2	-4	9	2	0	-6	0.5	-7	Resected
4	MS, AA, PDA, PFO	36	1	12	-4	13	3	3.5	-3	7	0	0	-6	1	-6	3, coarctation
Borderline left ventricle																
2	PDA, PFO, AV and sub-Ao stenosis	31	0	27	-2	10	0	5	-2	7	0	2.5	-4	3.5	-4	Hypoplasia
75	PDA, PFO, bicuspid AV	39	2	33	-1	13	3	6	-2	9	2	6	0	6	-1	3, coarctation
17	PDA, sub-Ao stenosis, VSD	35	1	29	-1	9	-1	6	-2	6	-1	4	-2	4	-3	3, coarctation
11	PDA, small MV, VSD	36	1	35	0	13	3	6	-2	9	2	6	0	7	0	Hypoplasia
8	PFO	37	1	36	0	10	0	8	-1	7	0	6	0	6	-1	2, coarctation
0†	PDA, PFO	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Normal hearts																
49	PDA	35	1	43	1	11	1	9	0	8	1	6	0	7	0	5, normal
0	PDA, PFO	31	0	34	0	9	-1	8	-1	6	-1	6	0	6	-1	Resected
3	PDA, PFO	31	0	33	-1	11	1	9	0	8	1	6	0	7	0	5.5, normal
68	PFO	36	1	45	1	11	1	10	1	7	0	7	1	7	0	5.5, normal
22	PFO	21	-2	28	-1	8	-2	6	-3	6	-1	4.5	-2	5.5	-2	4.5, normal
1	No PFO, no PDA	29	0	36	0	10	0	10	1	6	-1	6	0	7	0	5, normal
Transposition of the great arteries																
9	PDA, PFO	33	0	42	1	9	-1	8	-1	6	-1	6	0	6	-1	5, normal
1	No PFO, no PDA	34	1	37	0	10	0	8	-1	6	-1	6	0	7	0	Resected
1	No PFO, no PDA	28	-1	29	-1	8	-2	7	-1	5	-2	5	-1	6	-1	5, normal

All measurements are in millimeters. RV, Right ventricular lumen; LV, left ventricular lumen; TV, tricuspid valve; MV, mitral valve; PV, pulmonary valve; AV, aortic valve; Asc, ascending; Ao, aorta; MA, mitral valve atresia; AA, aortic valve atresia; PDA, patent ductus arteriosus; PFO, patent foramen ovale; MS, mitral valve stenosis; EFE, endocardial fibroelastosis; VSD, ventricular septal defect. *Z scores are calculated as follows: (raw score—mean for normal hearts)/SD for normal hearts. †This heart was no longer available at the time of measurement.

polyclonal antibody C6219; Sigma-Aldrich) were performed. Sections were pretreated as described previously and incubated overnight with the primary antibodies. Appropriate secondary antibodies for Cx43 (Alexa Fluor 488–conjugated donkey antirabbit IgG (heavy and light chains) highly crossabsorbed, A11029; Invitrogen-Molecular Probes, Breda, The Netherlands) and for N-cadherin (Alexa Fluor 568–conjugated goat anti-mouse IgG1, A21124; Invitrogen-Molecular Probes) were used for visualization with a Leica microscope (DM 5500B; Leica Microsystems GmbH, Wetzlar, Germany).

RESULTS

Study of the histology revealed myocardial abnormalities in all cases of HLH. Four of 6 hearts with BLV phenotype showed abnormalities similar to the HLH cases. The remaining 2 BLV hearts showed histologic characteristics similar to those of normal hearts. Surprisingly, the histologic abnormalities observed in the left ventricles of the hearts in the BLV and HLH groups were also detected in the right ventricle.

Histology of Normal Hearts and BLV, and HLH Groups

Troponin I. In the normal hearts, the cardiomyocytes were visualized by troponin I expression, which showed a clear

banded pattern corresponding to the sarcomeric organization. There were no differences in expression between the left and right ventricles (Figure 1, A and B). In 2 of 6 hearts with BLV phenotype, most of the cardiomyocytes showed troponin I sarcomeric expression, and the sarcomeres were properly organized (Figure 1, C and D). In the remaining 4 of 6 hearts with BLV phenotype, the banded pattern shown by troponin I expression in the sarcomeres was almost completely absent (Figure 1, E and F). These hearts with BLV phenotype were similar to the hearts with HLH phenotype, in which troponin I expression in the sarcomeres was also almost absent (Figure 1, G and H).

N-cadherin. N-cadherin expression in the normal hearts was present in the properly organized ICDs (adherence junctions) of all cardiomyocytes (Figure 2, A and B). Similar to the normal hearts, in 2 of 6 hearts with BLV phenotype, N-cadherin was expressed in the well-organized ICDs of the cardiomyocytes (Figure 2, C and D). The remaining 4 of 6 (severely affected) hearts in the BLV group demonstrated disorganized ICDs, and expression of N-cadherin in the ICDs was rare (Figure 2, E and F). Similar abnormalities were observed in all hearts with HLH phenotype (Figure 2, G and H).

FIGURE 1. Immunohistochemical expression of troponin I visualizing the sarcomeres. In the normal hearts (A and B), the troponin I expression demonstrates a clear banded pattern corresponding to the sarcomeric organization (*arrowheads*). In 2 of 6 hearts with borderline left ventricle (BLV) phenotype (C and D), most of the cardiomyocytes show troponin I sarcomeric expression, and the sarcomeres are properly organized (*arrowheads*). In the remaining 4 of 6 hearts with borderline left ventricle phenotype (severely affected; E and F), the banded pattern is almost completely absent (compare with C and D). These hearts are more similar to the hearts with hypoplastic left heart (HLH) phenotype (G and H), in which the troponin I expression in the sarcomeres is seen in few cells (compare *arrowheads* with those in E and F). No major differences are seen in expression pattern between the left and right ventricles. N, Nucleus; BLV, borderline left ventricle; HLH, hypoplastic left heart. Scale bars represent 10 μ m.

Connexin 43. In the normal hearts, the expression of Cx43 was concentrated at the lateral borders and ICDs of the cardiomyocytes (gap junctions; Figure 2, A and B). In 2 of 6

hearts with BLV phenotype, Cx43 expression was seen in the ICDs and lateral borders of the cardiomyocytes (Figure 2, C and D), similar to the normal hearts. The

FIGURE 2. Immunofluorescent expression patterns of N-cadherin (red) and connexin 43 (Cx-43; green) visualizing, respectively, the adherence junction and the gap junctions. In the normal hearts (A and B), N-cadherin expression is observed in the nicely organized intercalated disks (ICDs) of all cardiomyocytes (arrows). In 2 of 6 hearts with the borderline left ventricle (BLV) phenotype (C and D), N-cadherin is expressed in the intercalated disks of all cardiomyocytes, and the intercalated disks are well organized (arrows). The severely affected hearts with borderline left ventricle phenotype (E and F) demonstrate more disorganized intercalated disks, and the expression of N-cadherin in the intercalated disks is diminished relative to controls (compare with A–D). Similar observations are made in the hearts with hypoplastic left heart (HLH) phenotype (G and H). In the normal hearts (A and B), the expression of connexin 43 is concentrated at the lateral borders (arrowheads) and intercalated disks (arrows), similar to the intercalated disks (arrows) and lateral borders (arrowheads) of 2 of 6 hearts with borderline left ventricle phenotype (C and D). The severely affected hearts with borderline left ventricle phenotype (E and F) show almost complete lack of connexin 43 expression in the intercalated disks (arrows) and lateral borders (arrowheads), with only a few cells expressing connexin 43 in these locations. The expression pattern in the hearts with more severe borderline left ventricle phenotype is similar to that in hearts with hypoplastic left heart phenotype (compare E and F with G and H): most of the cells show lack of connexin 43 in intercalated disks and lateral borders (arrows and arrowheads, respectively). No major differences in expression patterns of N-cadherin and connexin 43 are observed between the left and right ventricles. In all images, 4',6-diamidino-2-phenylindole (DAPI) staining (blue) is shown as a reference. N, Nucleus; ICD, intercalated disk; BLV, borderline left ventricle; HLH, hypoplastic left heart. Scale bars represent 10 μ m.

FIGURE 3. Immunohistochemical expression patterns (A and B) of troponin I and immunofluorescent expression patterns (B and C) of connexin (Cx-43; green) and N-cadherin (red) in the cardiomyocytes of the hearts with transposition of the great arteries (TGA). Troponin I expression in both left (A) and right (B) ventricles shows a clear banded pattern corresponding to the sarcomeric organization (black arrowheads). In both left (C) and right (D) ventricles, N-cadherin is expressed in the intercalated disks (ICD; arrows), whereas connexin 43 is seen at the lateral borders (white arrowheads) and intercalated disks (arrows) of the cardiomyocytes, resembling the expression pattern in the normal hearts. In C and D, 4',6-diamidino-2-phenylindole (DAPI) staining (blue) is shown as a reference. N, Nucleus; TGA, transposition of the great arteries; ICD, intercalated disk. Scale bars indicate 10 μ m.

remaining 4 of 6 (severely affected) hearts in the BLV group showed almost complete lack of Cx43 expression in the ICDs and lateral borders of the cardiomyocytes (Figure 2, E and F) and were thus similar to the hearts with HLH phenotype (Figure 2, G and H).

Histology of Hearts With Transposition of the Great Arteries

Similar to the normal hearts, the cardiomyocytes in the hearts with transposition of the great arteries were visualized by troponin I expression as showing a clearly organized sarcomeric banded pattern in both ventricles (Figure 3, A and B). In addition, in the cardiomyocytes of the hearts with transposition of the great arteries, both the N-cadherin and the Cx43 expression patterns were similar to those observed in the normal hearts. N-cadherin was expressed in the ICDs (Figure 3, C and D), whereas Cx43 was seen at the lateral borders and ICDs of the cardiomyocytes (Figure 3, C and D).

DISCUSSION

Several studies have focused on various hemodynamic^{6,7,11} and genetic⁸ mechanisms to elucidate the origin

of the HLHS. Earlier studies reported on the histopathology of the coronary arteries,^{9,10} aortic arch,² and mitral valve¹⁷ in hearts with the HLH phenotype. Data on myocardial organization and possible pathology, however, have been lacking. In this study, we observed myocardial pathology in the left and right ventricles of hearts with both HLH and BLV phenotypes. The latter observation is of interest, because it indicates that the origin of the myocardium, being for the left ventricle the first heart field and for the right ventricle the second heart field,¹² cannot provide a conclusive answer for understanding the hypoplasia of the left ventricle and the histopathology in both ventricles.

We previously attempted to link the observed myocardial histopathology with abnormal myocardial differentiation and found several arguments for a role of the epicardium and EPDCs.¹³ The EPDCs have an important role in myocardial and valve differentiation, as well as in coronary artery maturation.¹⁸ Disruption of the genes involved in the second heart field at the venous pole leads to altered epicardial and EPDC formation and to EPDC-related cardiac malformations, including altered atrioventricular cushion development, myocardial abnormalities, and anomalous coronary vasculature formation.¹⁸ The latter observation

is of relevance because coronary artery pathology has been reported in a subset of cases with HLHS and endocardial fibroelastosis.^{2,9,10}

The histopathology of the myocardium of both ventricles has been found to be related to the sarcomeric organization and the ICDs. During the postnatal differentiation of the ventricular myocardium, the ICD has a primary role in the electromechanical signal transduction between the cardiomyocytes, with the expression of Cx43 and N-cadherin becoming more organized and concentrated in the ICD.¹⁶ Dysregulation of these proteins in the ICD leads to several forms of myocardial pathology. In the left ventricle of human hearts with congestive heart failure, reductions in Cx43 and N-cadherin expressions have been reported,¹⁹ a finding recently confirmed by *in vitro* and *in vivo* experiments in rat hearts.¹⁵ Abnormalities in expression patterns of Cx43 and N-cadherin in ICDs were observed in human hearts with hypertrophic cardiomyopathy and ventricular arrhythmias leading to sudden cardiac death.²⁰ The observed pathology reported as disorganized ICD and reduced expression of Cx43 and N-cadherin in the ICD is similar to our findings in hearts with HLH and severe BLV phenotypes.

Recently, the roles of Cx43, N-cadherin, and troponin I in maturation, alignment, and proliferation of the myocardium have been reported.¹⁴ It was shown, in both *in vivo* and *in vitro* experiments, that the EPDCs can stimulate the maturation, alignment, and proliferation of the cardiomyocytes by affecting the electromechanical transduction and the signaling pathways related to the extracellular matrix.¹⁴ In the *in vitro* experiments, the depletion of EPDCs resulted in downregulation of Cx43 and N-cadherin expressions. In addition, these cardiomyocytes showed also dysregulation of the cellular array.

On the basis of the observations regarding the role of EPDCs in myocardial development and the supporting results of the studies mentioned in the literature, we postulate that an abnormal epicardial–myocardial interaction through altered electromechanical signal transduction might provide an explanation for the observed myocardial pathology in hearts with HLH and BLV phenotypes. The presence of Cx43 and N-cadherin can be essential to support myocardial function in a nonchallenged state. It has been reported that Cx43 expression can be downregulated by 80% before functional arrhythmogenic consequences are seen.²¹ The observed pathology in some of the BLV group hearts could be possibly linked, however, to being less favorable for biventricular repair.

Histopathology as a Diagnostic Tool for Preoperative Decision Making

With regard to the treatment of the BLV phenotype, both univentricular and biventricular repairs have been described.⁴ The operative decision is complicated, however,

by the lack of a proper definition of BLV. Several semiquantitative methods and criteria have been developed in the last 2 decades to determine whether the left ventricle in BLV is adequate to maintain the systemic circulation after biventricular repair; however, none appear to be conclusive. Because an inadequate decision may result in severe complications and even in death, a conclusive diagnostic tool for decision making is desirable.^{22–24}

In the “gray zone” of the pathologic spectrum, it is extremely important that the surgical decision between a biventricular and a univentricular operation is taken properly. If the decision to undertake a biventricular repair turns out to be wrong, the repercussions will be permanent, including irreversible pulmonary hypertension. On the other hand, incorrectly choosing a univentricular palliation does not necessarily imply that (if the left ventricle functions better than thought before) biventricular repair is out of the question. In addition, a recent study of Hoashi and colleagues²⁵ has demonstrated acceptable outcomes in patients who were initially treated with the intent to perform single-ventricle palliation but had conversion to “1½- or 2-ventricle” physiology.

Currently, in part because of the recent implementation of hybrid Norwood techniques,^{26,27} the decision for univentricular versus biventricular management can be postponed for several months. After the hybrid Norwood procedure, it may be possible to perform myocardial biopsies, which may prove to be a helpful tool in future decision making.

In this study, we demonstrated in 2 of 6 hearts with macroscopic BLV phenotype a histologic similarity to normal hearts, while the other 4 of 6 showed a histologic similarity to hearts with the HLH phenotype. We have also shown that the observed downregulation of Cx43 and N-cadherin as well as the lack of cardiac troponin I, demonstrating sarcomeric disorganization, can be linked to myocardial pathology seen in cardiac failure and cardiomyopathy. This implies that less than optimal function is to be expected in time in hearts with the HLH phenotype and the severe type of BLV. Defining the myocardial phenotype of the latter category by preoperative histologic study of myocardial biopsy specimens might provide a valuable diagnostic tool for preoperative decision making in the choice of univentricular or biventricular repair.

Limitations and Perspectives

Although the results are very promising, the number of studied hearts is too limited to draw statistically significant conclusions. The data presented in this study are based on the retrospective study of postmortem unoperated hearts. A prospective, multicenter study will provide more reliable results not only by comparing the myocardium of the unoperated BLV and HLH phenotypes but also by comparing the myocardium of the monoventricular versus biventricular

repair of the BLV phenotype. Such a study will provide clinical data that can conclusively support or rule out the hypothesis of this study.

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