

Two-dimensional speckle tracking imaging to assess the hazards of left ventricular function and ventricular wall motion disorders in children with pre-excitation syndrome and the efficacy of radiofrequency ablation treatment

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Abstract. – OBJECTIVE: Our study aimed at analyzing the echocardiographic multi-indicator evaluation of the risk of Wolff-Parkinson-White syndrome (WPW) on the left ventricular function and ventricular wall motion disorders, as well as the effect of radiofrequency ablation treatment.

PATIENTS AND METHODS: The clinical data of 55 WPW patients treated with radiofrequency (RF) ablation at the Children's Hospital of Nanjing Medical University between January 2018 and December 2022 were retrospectively analyzed and included in the observation group, while other 50 healthy children were included in the control group during the same time. We analyzed the echocardiographic indices of the patients, assessed the effects of the disease on left ventricular myocardial function and ventricular wall motion disorders, and evaluated the effects of radiofrequency ablation treatment on the myocardium of the left ventricle. The echocardiographic parameters were analyzed to assess the effect of the disease on left ventricular myocardial function and ventricular wall dyskinesia.

RESULTS: Of the 55 patients with pre-excited syndrome, 20 had type A bypass and 35 had type B bypass. Ten patients had pre-excited dilated cardiomyopathy with significant enlargement of the left ventricular cavity, reduced left ventricular systolic function, and a significant impairment of ventricular wall motion; the other 5 patients had basal segmental septal motion incoordination. Compared to the control group, patients with left ventricular end-diastolic diameter (LVEDD) (42.9 ± 5.0 mm vs. 39.2 ± 3.0 mm), peak strain dispersion (PSD) (38.8 ± 15.3 ms vs. 21.7 ± 2.2 ms), maximum peak time difference (MPTD) (200.2 ± 92.8 ms vs. 89.5 ± 9.8 ms) and interventricular mechanical delay (IVMD) (36.2 ± 13.7 ms vs. 21.2 ± 2.1 ms) before RF ablation were increased. Left ventricular ejection fraction (LVEF) ($57.1 \pm 9.1\%$ vs. $65.9 \pm 2.6\%$), E/A (1.1 ± 0.2 vs. 1.8 ± 0.2) and global longitudinal strain (GLS) ($-18.7 \pm 2.2\%$

vs. $-22.4 \pm 0.5\%$) decreased, with statistically significant differences ($p < 0.05$). All 55 patients had a successful procedure, and all postoperative echocardiographic parameters were found to be improved, compared to the preoperative period. The results of the postoperative review after 3 months showed differences in E/A, PSD, MPTD, and IVMD compared to the healthy group, suggesting that left ventricular diastolic function and synchrony had not fully returned to normal.

CONCLUSIONS: Echocardiography can better evaluate myocardial motion and function in patients with Wolff-Parkinson-White syndrome and monitor the effect and progress of disease treatment, and has high clinical application value.

Key Words:

Wolff-Parkinson-White syndrome, Echocardiography, Pre-excitation cardiomyopathy, Two-dimensional speckle tracking imaging.

Introduction

Wolff-Parkinson-White syndrome (WPW) is a common cardiac arrhythmia. With an incidence of approximately 0.1-0.3%¹, it is the most common cause of cardiac dyskinesia in children². Episodic tachycardia is the main clinical symptom. Some children have reduced cardiac function and a very small number may have enlarged left ventricles and reduced systolic function in the absence of a tachycardia episode. The diagnosis of WPW is currently made based on electrocardiographic findings. However, the current electrocardiogram (ECG) examination does not allow for the evaluation of myocardial motion and function.

The two-dimensional speckle tracking image (2D-STI) is an echocardiographic technique for quantitative analysis that has been developed in the last decade. 2D-STI is independent from the angle of the acoustic beam and has good accuracy and reproducibility in assessing myocardial function and motion⁴. In this study, the risk of myocardial function and wall motion in the left ventricle was evaluated by conventional echocardiography and 2D-STI in WPW children. We aim to increase clinical awareness and improve the diagnosis of the disease, and to further evaluate myocardial motion after radiofrequency ablation to provide an objective basis for clinical management of the prognosis of the disease.

Patients and Methods

Subjects

This study was approved by the Ethics Committee of the Children's Hospital of Nanjing Medical University (No. 202206111). All children's guardians signed informed consent forms. Fifty-five WPW children who underwent radiofrequency ablation between January 2018 and December 2022 in the Children's Hospital of Nanjing Medical University were retrospectively included in the observation group.

Observation group inclusion criteria

- (1) Body surface electrocardiogram (ECG) findings met the diagnostic criteria of WPW⁵: manifested by shortened P-R interval and widened QRS wave groups with pre-excitation waves.
- (2) Received radiofrequency ablation treatment.
- (3) Clear echocardiographic images and comprehensive data.

Observation group exclusion criteria

- (1) Congenital heart disease.
- (2) Cardiomyopathy, atrial fibrillation, sustained supraventricular tachycardia, fractional interventricular pre-excitation, hyperthyroidism, electrolyte disorders, liver and kidney dysfunction, and transarterial conduction to the ventricles *via* the collateral pathway during acute infection.
- (3) All children were off antiarrhythmic drugs for at least 5 half-lives before ablation.

Periodic 24-h ambulatory electrocardiography was performed in each child to determine the presence of acute tachycardia episodes and the presence of supraventricular tachycardia lasting 12 h or tachycardia lasting 12 h.

Other 50 healthy children were selected as the control group.

Control group inclusion criteria

- (1) Normal echocardiographic and electrocardiographic findings in the children.
- (2) No abnormal lesions in other systems.

Control group exclusion criteria

- (1) Normal cardiovascular system examination, but the children had clinical symptoms.
- (2) Previous history of taking medication for cardiovascular diseases.

The observation group was divided into the type A bypass group and the type B bypass group, according to the location of the bypass during the radiofrequency ablation. Type A WPW atrioventricular bypass was mainly located in the left ventricle, and type B WPW atrioventricular bypass was mainly located in the right ventricle. The observation group was divided into a pre-operative group, a 1-week post-operative group, and a 3-month post-operative group according to the time points of pre- and post-operative ECG.

Instruments and Methods

The GE Vivid E95 cardiovascular ultrasound system (General Electric Company, CT, USA) was used for the ECG. The probe was either an M5SC-D or 6S-D phased-array probe (General Electric Company, CT, USA). The width of the sweep sector and image depth were optimized, and the frame rate was adjusted (>60 fps). The ECG gating was then connected. The examined child was placed in a flat or left lateral position with full chest exposure. The examination was performed in a quiet state with a stable heart rate. For infants who were uncooperative, chloral hydrate 5% (1 ml/kg, Tefeng Pharmaceutical Co., Ltd., Urumqi, China) was administered orally or by a reserved enema to sedate the child. Standard views were taken, and data were measured for routine ECG. 2D-STI was performed using the aortic spectrum to determine aortic valve closure time, tracing the left ventricular endocardium in parasternal, apical four-chamber view, apical three-chamber view, and apical two-chamber view, respectively. The software automatically calculated longitudinal strain parameters for the left ventricular myocardium. All patients were echocardiography by the same cardiovascular sonographer before ablation, 1 week after ablation, and 3 months after ablation. Each echocardiographic parameter was calculated as the mean of three cardiac cycles. To avoid subjective bias, the physician reviewer of the ECG was unaware of the patient grouping.

Conventional echocardiographic parameters: (1) left ventricular end-diastolic diameter (LVEDD). The left ventricular end-diastolic diameter is measured on an M-mode image at the level of the mitral valve cusps in a parasternal long-axis view or a short-axis view of the left ventricle. (2) Left ventricular ejection fraction (LVEF). This was obtained using the biplane Simpson's method. (3) Left ventricular diastolic function. The E and A peaks of the mitral orifice flow spectrum were measured, and the E/A ratio was calculated. (4) M-mode observation of the left ventricular wall for isotropic motion. (5) Two-dimensional observation of the segments of abnormal ventricular wall motion in each section.

An indicator of biventricular synchrony is the interventricular mechanical delay (IVMD). The time from the onset of the QRS waveform to the onset of the aortic and pulmonary valve flow spectra was measured separately using pulsed Doppler flow spectral images. The IVMD=left ventricular pre-ejection time - right ventricular pre-ejection time.

2D-STI parameters: EchoPAC (Version 201, GE Healthcare, Horten, Norway) workstation was used for offline image processing and analysis. These included (1) a bull's-eye view of the longitudinal peak strain and peak strain time; 2) peak strain dispersion (PSD) of each segment of the LV myocardium; (3) maximum peak time difference (MPTD); (4) global longitudinal strain (GLS) of the left ventricular myocardium.

Statistical Analysis

The data were analyzed using SPSS 25.0 statistical software (IBM Corp., Armonk, NY, USA). Measurement data that conformed to a normal distribution were expressed as mean \pm standard deviation (SD). Ultrasound measurements in each group at different time points and a comparison of each parameter of ultrasound between the type A bypass group and type B bypass group, and the normal control group were analyzed using one-way ANOVA. Counting data were expressed as numbers (percentages). $p < 0.05$ was considered a statistically significant difference.

Results

General Clinical Information

In the observation group, there were 55 cases, 24 females and 31 males, with a ratio of 1:1.3; age ranged from 1 year and 3 months to 16 years and 2 months, with an average age of 9.3 ± 3.3 years. In the control group, there were 50 cases, 21 females

and 29 males, with a ratio of 1:1.4; aged 1 year and 9 months to 15 years and 8 months, with an average age of 8.7 ± 2.7 years. There was no statistically significant difference between the two groups in terms of age and gender ($p > 0.05$). All children developed postoperative complications such as hemorrhage, infection, cardiac arrhythmia, and kidney injury. After symptomatic treatment, all complications were significantly improved.

General Information Between WPW Subtypes

In the observation group, there were 20 patients with type A and 35 patients with type B, a ratio of 1:1.79. LVEDD and LVEF did not differ from the control group in type A patients ($p > 0.05$), while all other parameters differed from the control group ($p < 0.05$). Fourteen of the patients with type B had abnormal ventricular wall motion and 13 had basal segment thinning during systole with paradoxical motion (Figures 1 and 2). 10 cases met the diagnostic criteria for dilated cardiomyopathy. Based on the complaints, frequency of supraventricular tachycardia, and periodic 24-h ambulatory ECG to exclude cardiomyopathy and other causes, the children presented with clinical signs and symptoms of chronic congestive heart failure. Analysis of the longitudinal peak strain bull's-eye view of the left ventricular myocardium showed little involvement of the apical segment in patients with WPW. Type A bypass involved mainly the inferior and anterior lateral walls of the left ventricle (Figure 3), while type B involved mainly the anterior, anterior, and inferior septum and posterior wall (Figure 4) (Table I).

Comparison of Ultrasound Parameters in the Observation Group Before and After Radiofrequency Ablation and in the Control Group (Table II)

All parameters of preoperative ultrasound in the observation group deviated from the control group, with statistically significant differences between the groups ($p < 0.05$). On review 1 week after surgery, the abnormalities of ventricular wall motion disappeared and were significantly better than all parameters preoperatively (Figures 5-7, $p < 0.05$). Only LVEDD was not different from the control group ($p > 0.05$), while the remaining parameters were all different from the control group ($p < 0.05$). The differences between LVEDD, LVEF, and GLS in the 3-month postoperative group and the control group were not statistically significant ($p > 0.05$), while the remaining parameters were still different from the control group ($p < 0.05$).

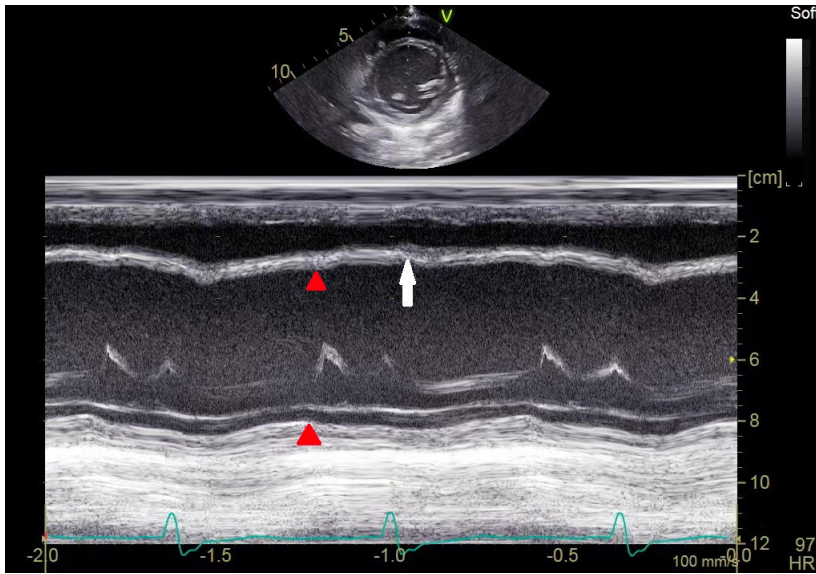


Figure 1. Patient, male, 7 years old. An electrocardiogram shows pre-excitation syndrome. The preoperative M-mode echocardiogram shows the septum moving in the same direction as the posterior wall of the left ventricle (arrow), and the septum is partially convex to the right ventricle (arrow).

Figure 2. Patient, female, 7 years old. Pre-excitation syndrome. A preoperative echocardiogram of the parasternal four-chambered heart shows an enlarged left ventricle with a deviation of the septum towards the right ventricle and localized bulging of the basal segment (arrow). There is paradoxical motion with the ventricular septum as a whole.

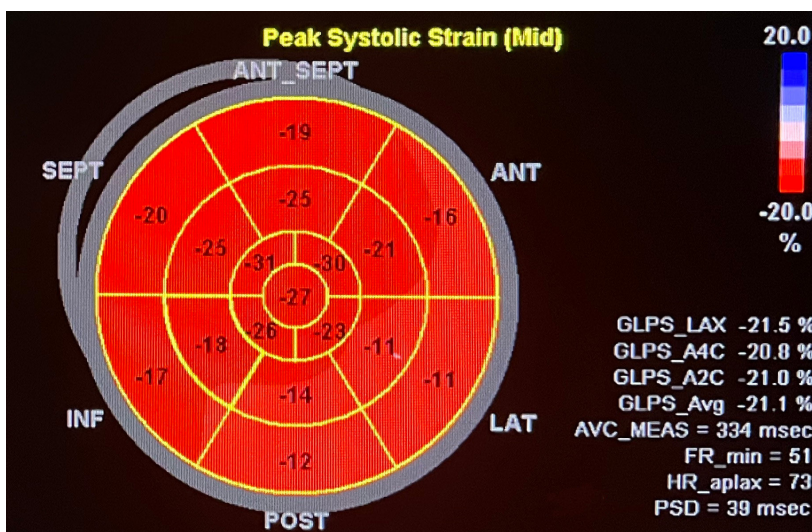
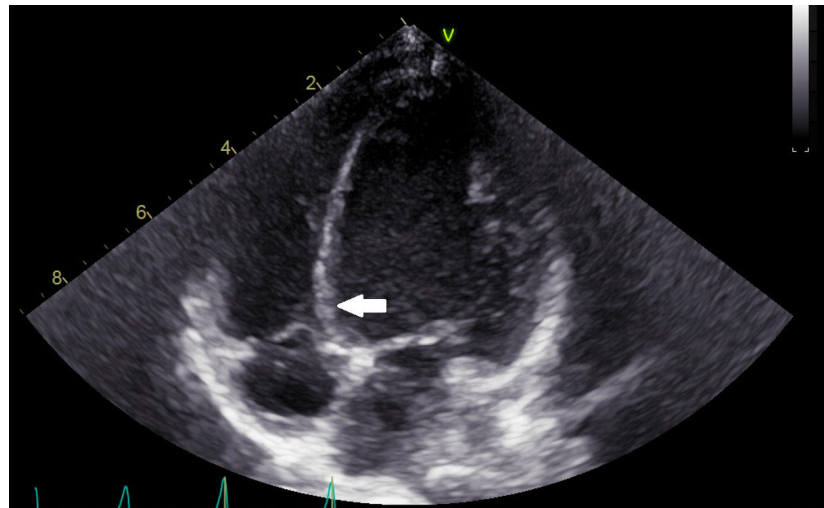


Figure 3. Patient, male, 11 years old. Treated with radiofrequency ablation for pre-excitation syndrome. The intraoperative localization bypass was the left bypass. Preoperative 2D speckle tracking imaging bull's eye view of longitudinal strain in the myocardium shows left ventricular inferior wall (-12%), inferior lateral wall (-11%), and anterior lateral wall (-16%). The strain values were below normal and were dominated by the basal segment.

Figure 4. Patient, female, 10 years old. Treated with radiofrequency ablation for pre-excited cardiomyopathy. Intraoperative electrophysiology clarifies type B right-sided bypass. Preoperative 2D speckle tracking imaging of longitudinal strain in the left ventricular myocardium bull's-eye image shows anterior wall (-14%), anterior septum (-9%), inferior septum (-15%) and inferior wall (+9%). The strain values are lower than normal, predominantly at the base, with the inferior wall showing a contradiction with the overall motion.

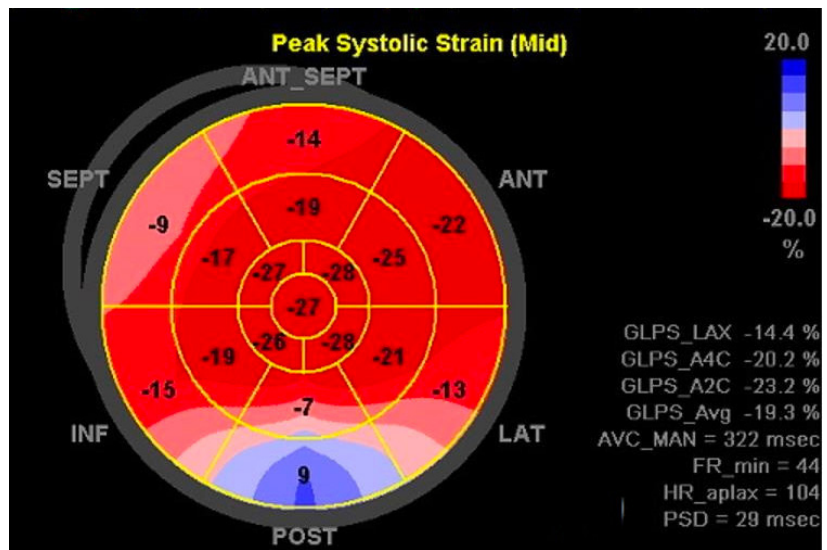


Table I. Comparison of ultrasound parameters between type A bypass and type B bypass groups and control group.

Types	n	LVS		LVSF		LVDF		Ventricular synchrony		
		LVEDD (mm)	LVEF (%)	GLS (%)	E/A	PSD (ms)	MPTD (ms)	IVMD (ms)		
Type A bypass	20	40.6±4.0 ^c	62.3±1.7 ^c	-20.1±0.7 ^{ac}	1.2±0.2 ^{ac}	28.4±3.5 ^{ac}	139.5±20.3 ^{ac}	27.2±2.8 ^{ac}		
Type B bypass	35	44.2±5.1 ^{ab}	54.2±10.2 ^{ab}	-18.2±2.4 ^{ab}	1.0±0.1 ^{ab}	44.6±16.3 ^{ab}	228.2±95.8 ^{ab}	41.2±14.8 ^{ab}		
Control group	50	39.2±3.0 ^c	65.9±2.6 ^c	-22.4±0.5 ^{bc}	1.8±0.2 ^{bc}	21.7±2.2 ^{bc}	89.5±9.8 ^{bc}	21.2±2.1 ^{bc}		
<i>F</i>		17.183	39.113	272.798	61.370	65.091	88.70 ⁶	56.504		
<i>p</i>		0.006	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		

Compared with the control group, ^a*p*<0.05; Compared with the A-type bypass group, ^b*p*<0.05; Compared with the B-type bypass group, ^c*p*<0.05. LVS, left ventricular size; LVSF, left ventricular systolic function; LVDF, left ventricular diastolic function; VS, ventricular synchrony; PSD, peak strain dispersion; IVMD, interventricular mechanical delay; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; MPTD, maximum peak time difference; GLS, global longitudinal strain.

Table II. Comparison of ultrasound parameters in the observation group before and after radiofrequency ablation and in the control group.

Types	n	LVS		LVSF		LVDF		Ventricular synchrony		
		LVEDD (mm)	LVEF (%)	GLS (%)	E/A	PSD (ms)	MPTD (ms)	IVMD (ms)		
Preoperative group	55	42.9±5.0 ^{abc}	57.1±9.1 ^{abc}	-18.7±2.2 ^{abc}	1.1±0.2 ^{abc}	38.8±15.3 ^{abc}	200.2±92.8 ^{abc}	36.2±13.7 ^{abc}		
Postoperative 1-week group	55	40.0±3.8 ^d	63.5±2.4 ^{acd}	-21.1±0.8 ^{acd}	1.5±0.1 ^{acd}	25.1±5.5 ^{acd}	139.3±43.2 ^{acd}	23.8±5.1 ^{acd}		
Postoperative 3-month group	55	39.2±3.6 ^d	65.7±1.7 ^{cd}	-22.1±0.4 ^{cd}	1.7±0.1 ^{abd}	20.3±2.2 ^{abd}	111.2±14.8 ^{abd}	19.6±2.0 ^{abd}		
Control group	50	39.2±3.0 ^d	65.9±2.6 ^{cd}	-22.4±0.5 ^{cd}	1.8±0.2 ^{bcd}	21.7±2.2 ^{ab}	89.5±9.8 ^{bcd}	21.2±2.1 ^{bcd}		
<i>F</i>		11.04	36.81	98.39	210.68	55.79	45.22	54.27		
<i>p</i>		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		

Compared with the control group, ^a*p*<0.05; Compared with the postoperative 3-month group, ^b*p*<0.05; Compared with the postoperative 1-week group, ^c*p*<0.05; Compared with the preoperative group, ^d*p*<0.05. LVSF, left ventricular systolic function; LVDF, left ventricular diastolic function; VS, ventricular synchrony; PSD, peak strain dispersion; IVMD, interventricular mechanical delay; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; MPTD, maximum peak time difference; GLS, global longitudinal strain.

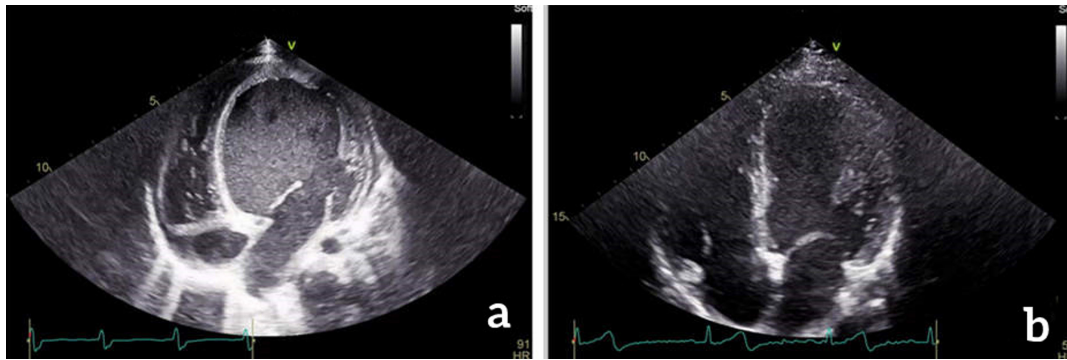


Figure 5. Patient, male, 8 years old. He was treated with radiofrequency ablation for pre-excitation syndrome. Type B bypass, preoperatively (a), the left ventricle of the parasternal four-chamber heart was spherically dilated, and the septum was markedly convex to the right ventricle. On review 1 week postoperatively (b), the left ventricle was significantly smaller than before surgery. The four-chambered heart shows a harmonious right-to-left ratio

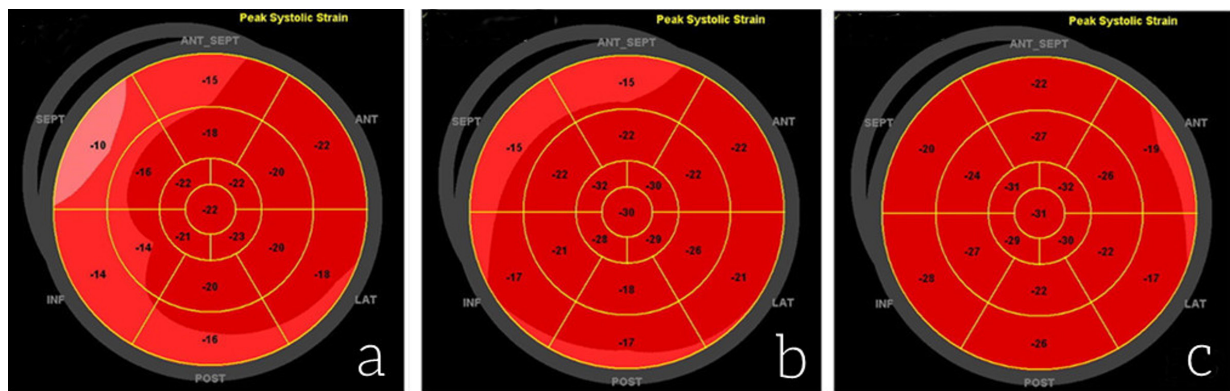


Figure 6. Patient, female, 11 years old. Intraoperative diagnosis of type B bypass. Preoperative (a), postoperative 1-week (b), and postoperative 3-month (c) bull's-eye views of longitudinal strain in the left ventricular myocardium show progressively higher strain values in the anterior wall, anterior septum, inferior septum, and inferior wall. This has returned to normal on review at 3 months postoperatively.

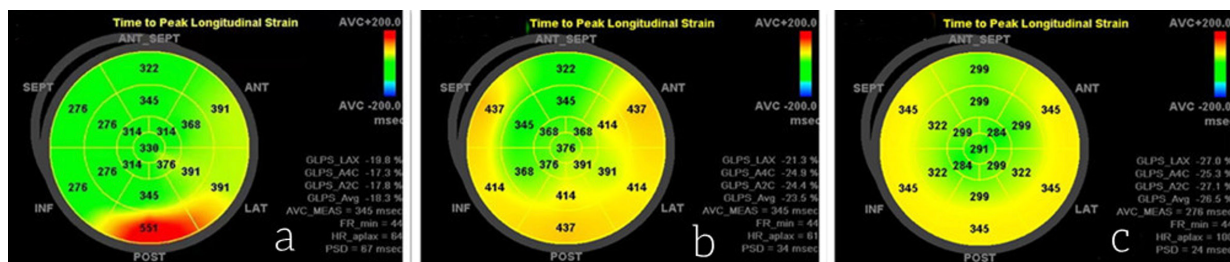


Figure 7. Patient, male, 10 years old, type B bypass. Preoperative (a), postoperative 1-week (b), and postoperative 3-month (c) peak time strain bull's-eye diagrams show a decrease in PSD from 67 ms to 34 ms and finally 24 ms, returning to normal values.

Discussion

Mechanisms of WPW Occurrence and Regression

In the normal physiological state, the AV node is the only structure for electrical conduction between the atria and the ventricles. When there is an abnormal bypass between the atria and ventricles, the impulses from the sinoatrial node, in addition to traveling down the normal conduction pathway, also excite the part of the myocardium earlier through the faster conduction bypass, causing arrhythmias, called ventricular pre-excitation⁶. The more common clinical symptom is episodic tachycardia. Patients experience significant precordial discomfort, pain, palpitations, and a range of other symptoms. After radiofrequency ablation eliminates the abnormal target, cardiac conduction returns to normal, and the clinical symptoms of the child may disappear⁷.

An Exploration of the Differences Between the Various Subtypes of WPW

Type C is the rarer of the three subtypes, and type B is more common than type A⁸. The results of this study showed that all cases with abnormal ventricular wall motion and the formation of pre-excited cardiomyopathy were type B bypasses, which is consistent with the current findings⁹. Very few patients with type B collateral tract progressed to pre-excited cardiomyopathy. Ten patients in this study presented with dilated cardiomyopathy, including an enlarged left ventricle and reduced LVEF, referred to as pre-excited dilated cardiomyopathy¹⁰. The B bypass is a right-sided atrioventricular bypass that excites a relatively early septum in an otherwise normal heart, resulting in asymmetric excitation throughout the left ventricle¹¹. As the early-excited ventricular wall site undergoes systolic motion before the heart is fully diastolic, the preload on the ventricular wall is reduced, and the resistance to cardiac contraction is reduced. As a result, local ventricular wall work is reduced, leading to a long-term reduction in coronary blood supply, wall thinning, and reduced wall stress¹². Fourteen patients in the observation group with type B WPW showed abnormal ventricular wall motion, manifesting as the contradictory motion of the basal segment of the septum locally to the septum as a whole. Type M showed the isotropic motion of the septum to the posterior left ventricular wall. The combination of abnormal electrical and mechanical conduction mediates left ventricular dysfunction and re-

modeling, resulting in paradoxical septal motion, myocardial thinning, failure of the left ventricle to achieve maximal filling, decreased myocardial contractility and work, and ultimately left ventricular enlargement and reduced LVEF, with the most severe manifestations of dilated cardiomyopathy¹³. The prognosis for this disease is better than for primary dilated cardiomyopathy. Normal electrophysiology is restored by ablation of abnormal bypass targets by radiofrequency ablation, LV myocardial contraction is synchronized, and LV systolic function is fully restored^{13,14}. In patients with type A WPW and some type B WPW that have not yet resulted in ventricular remodeling, conventional echocardiographic parameters LVEDD and LVEF do not differ from the normal group, and only mitral E/A values are lower than in normal children. This suggests that diastolic function is impaired before abnormal systolic function in children with WPW, consistent with the findings of Tomaske et al¹⁵.

2D-STI and Ventricular Synchrony Analysis in WPW Patients

2D-STI has matured as a technique for studying myocardial function, and this technical index is more sensitive in responding to myocardial work^{16,17}. Longitudinal strain bull's-eye views of the left ventricle reveal the localized reduced motion of the ventricular wall in almost all patients. In severe cases, the localized myocardium showed contradictory motion to the overall myocardium. In contrast, two-dimensional echocardiography revealed abnormal ventricular wall motion in only 14 cases. Further analysis of the longitudinal strain curve of the ventricular septum showed two peak contractions of the affected septum, the earlier one being an earlier contraction with a smaller peak. Rebound motion occurred when the aortic valve closed, followed by peak systolic motion again with posterior wall motion. Bull's-eye diagrams of peak strain dispersion in each segment of the left ventricular myocardium show peak times in each segment, corresponding to longitudinal strain bull's-eye diagrams, with longer peak times in segments with decreasing strain values, and left ventricular systolic asynchrony manifested as differences in peak times in each segment, further elaborating the pattern of myocardial excitation in WPW from objective indicators¹⁸. All WPW patients in this study had biventricular motion and overall left ventricular motion asynchrony, manifested by early contraction of the right ven-

tricle and post-contraction of the left ventricle. This may be because the affected ventricular septum in patients with WPW contradicts the left ventricular motion and projects towards the right ventricle during contraction, participating in the contraction of the right ventricle, resulting in a faster rise in right ventricular pressure and a relatively early opening of the pulmonary valve. In contrast, the left ventricle cannot be filled to its maximum extent, resulting in delayed aortic valve opening and a vicious circle between abnormal ventricular wall motion and synchronization of biventricular motion, which accelerates the impairment of myocardial function¹⁹.

Ultrasound Indicators to Monitor the Effectiveness of Radiofrequency Ablation of WPW

Radiofrequency ablation is the first treatment option for patients with WPW. Most patients with WPW can be treated regularly with anti-arrhythmic drugs, which can reduce the occurrence of paroxysmal supraventricular tachycardia to some extent. However, the recovery of left ventricular function and ventricular wall motion is only satisfactory with successful ablation therapy¹⁵. In this study, only 1 week after undergoing the procedure, all echocardiographic parameters were significantly improved compared to the preoperative period, and LVEDD returned to normal levels. The LVEDD, LVEF, and GLS had all returned to normal levels by 3 months after the procedure but left ventricular diastolic function had not yet returned to normal, and the left ventricular time to peak and biventricular synchrony was slightly reduced compared to normal. The study confirmed that radiofrequency ablation is an effective treatment for WPW and that left ventricular function and ventricular wall motion returned to normal at the 3-month review. Therefore, WPW is an indication of radiofrequency ablation with a good prognosis, in agreement with the study by Uhm et al²¹ and others^{22,23}. Radiofrequency ablation is usually used to treat some heart diseases, such as premature ventricular complex-induced cardiomyopathy and medically refractory paroxysmal and persistent atrial fibrillation. Echocardiography provides better monitoring of the post-ablation outcome and provides objective clinical data on the prognosis of patients with WPW.

There are shortcomings of this study. (1) The 2D-STI index was not comprehensive and failed to explore myocardial damage in further depth,

and the comprehensiveness of the study will be emphasized in future follow-up studies; (2) no C-type cases were included, and the study of the disease was not complete.

Conclusions

Echocardiography can better evaluate myocardial motion and function in patients with Wolff-Parkinson-White syndrome and monitor the effect and progress of disease treatment and has high clinical application value.

Ethics Approval

This study was approved by the Ethics Committee of the Children's Hospital of Nanjing Medical University (No. 202206111).

Informed Consent

All guardians of the children have signed informed consent forms.

Funding

This study was supported by the Jiangsu Province Maternal and Child Health Research Project (F202023), the National Natural Science Foundation of China (No. 81970265), and the 13th Five-Year Nanjing Health Youth Talents Training Project Fund (No. QRX17171).

Authors' Contributions

Jun Chen is responsible for the guarantor of integrity of the entire study, definition of intellectual content, and manuscript review; Zhen Cheng is responsible for study concepts, manuscript preparation, and manuscript editing; Hao Liu is responsible for study design, experimental studies, and statistical analysis; Shanliang Zhu is responsible for the literature research; Ye Chen is responsible for the data acquisition; Shiwei Yang is responsible for the data analysis.

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Conflict of Interest

All authors declare no conflict of interest.

Availability of Data and Materials

All data and material can be obtained by the email of the corresponding author.

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