ORIGINAL ARTICLE
DOI 10.4070 / kcj.2010.40.8.391

Print ISSN 1738-5520 / On-line ISSN 1738-5555

Open Access

Dual Pulsed-Wave Doppler Tracing of Right Ventricular Inflow and Outflow: Single Cardiac Cycle Right Ventricular Tei Index and Evaluation of Right Ventricular Function

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ABSTRACT

Background and Objectives: The reliability and usefulness of the right ventricular (RV) Tei index (RTX) remains controversial because it has not been possible to simultaneously measure RV inflow and outflow. However, dual pulsed-wave Doppler (DPD) enables flow velocities to be obtained at different sampling sites simultaneously. In this study we evaluated the feasibility and reliability of RTX values obtained by DPD (RTX_{DPD}). **Subjects and Methods:** Forty-one patients who underwent cardiac catheterization and echocardiography for RV volume or pressure overloading conditions were evaluated. Symptom-limited exercise treadmill testing with expired gas analysis was performed and maximal exercise capacity was measured. **Results:** RTX by conventional flow Doppler (RTX_{CFD}, 0.262±0.164) was similar to RTX_{DPD} (0.253±0.117, p=NS), whereas RTX by tissue Doppler echocardiography (RTX_{TDE}, 0.447±0.125) was significantly larger than RTX_{DPD} (p<0.001). Based on multiple regression analysis, maximal exercise capacity was independently related to RTX_{DPD} (β=-0.60, p<0.001), mid-RV dimension (β=-0.26, p=0.012), left ventricular ejection fraction (β=0.22, p=0.023), and early diastolic tricuspid annular velocity (β=0.21, p=0.048). **Conclusion:** It is feasible and reliable to evaluate RV function using RTX_{DPD} values. However, to evaluate the clinical usefulness of RTX_{DPD}, additional studies are required with a large number of patients and long-term follow-up. **(Korean Circ J 2010;40:391-398)**

KEY WORDS: Echocardiography; Echocardiography, Doppler, pulsed; Cardiac function; Right ventricle.

Introduction

The Tei index (also known as the myocardial performance index), has been reported to reflect both systolic and diastolic ventricular function.[1-5] However, there are some concerns about the reliability of the Tei index since it cannot be calculated in a single cardiac cycle, particularly for the right ventricle (RV).[6)7)] Moreover, this shortcoming has seriously limited the application of the RV Tei index (RTX) in the presence of substantial heart rate fluctuations. Efforts have been made to overcome this limitation by using tissue Doppler echocardiography (TDE) to determine Tei indices,[7)8)] but the values measured by conventional flow Doppler (CFD) and TDE differ slightly.[9)] Recently, dual pulsed-wave Doppler (DPD) echocardiography was introduced, which allows flow velocities at different points to be measured using two independent sample volumes.

Using this technique, one can measure the parameters required to calculate RTX values in a single cardiac cycle (RTX_{DPD}), which might overcome the limitations of CFD. Accordingly, we evaluated the feasibility and reliability of RTX_{DPD} versus RTX by CFD (RTX_{CFD}) and TDE (RTX_{TDE}). In addition, we also investigated the clinical usefulness of RTX_{DPD} by correlation analysis using invasively-measured RV pressures and exercise capacity.

Received: December 28, 2009
Revision Received: February 19, 2010
Accepted: March 10, 2010
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Subjects and Methods

Study subjects

This study was approved by the Ethical Review Board of Samsung Medical Center in Seoul, Korea. Forty-one patients who underwent both right cardiac catheterization and echocardiography for RV volume or pressure overloading conditions, or for congenital heart diseases with cardiac shunts were evaluated. The study patients also underwent treadmill exercise testing with expired gas analysis. Patients meeting any of the following criteria were excluded: atrial fibrillation or hemodynamic instability, age <10 years, echocardiographic windows too poor for analysis purposes, and unwillingness to participate in the study. Fifteen healthy persons were also evaluated as the normal healthy control group for comparison of echocardiographic parameters, including RTX values.

Echocardiographic examinations

Comprehensive conventional two-dimensional (2-D) echocardiographic examinations were performed with an Accuvix XQ® cardiovascular ultrasound system (Medison, Seoul, Korea). The ultrasound examinations included measurements of the mid- and basal-transverse RV diameters, and the longitudinal diameter in an apical 4-chamber view, according to the recommendations of the American Society of Echocardiography.[10] Left atrial volumes were calculated using the ellipsoidal method and indexed with respect to body surface area.[11] The peak early diastolic mitral inflow velocity (E) and mitral annular velocity (E'_{MV}) were measured in the apical 4-chamber view and the E/E'_{MV} ratio was calculated.

Annular velocities of the tricuspid valve (TV) were obtained in the apical 4-chamber views. The tricuspid annular velocities included the peak systolic TV annular velocity (S'_{TV}), peak early diastolic velocity (E'_{TV}), and late peak diastolic velocity (A'_{TV}).[12] Tricuspid inflows and pulmonary ejection flows were measured in the parasternal short axis view and used to determine the RTX_{CFD} and RTX_{DPD} values (Fig. 1A, B and C). The RTX was defined as the sum of the isovolumic contraction time (ICT) and relaxation time (IRT) divided by the pulmonary ejection time (PET), as follows: RTX=(ICT+IRT)/PET. To

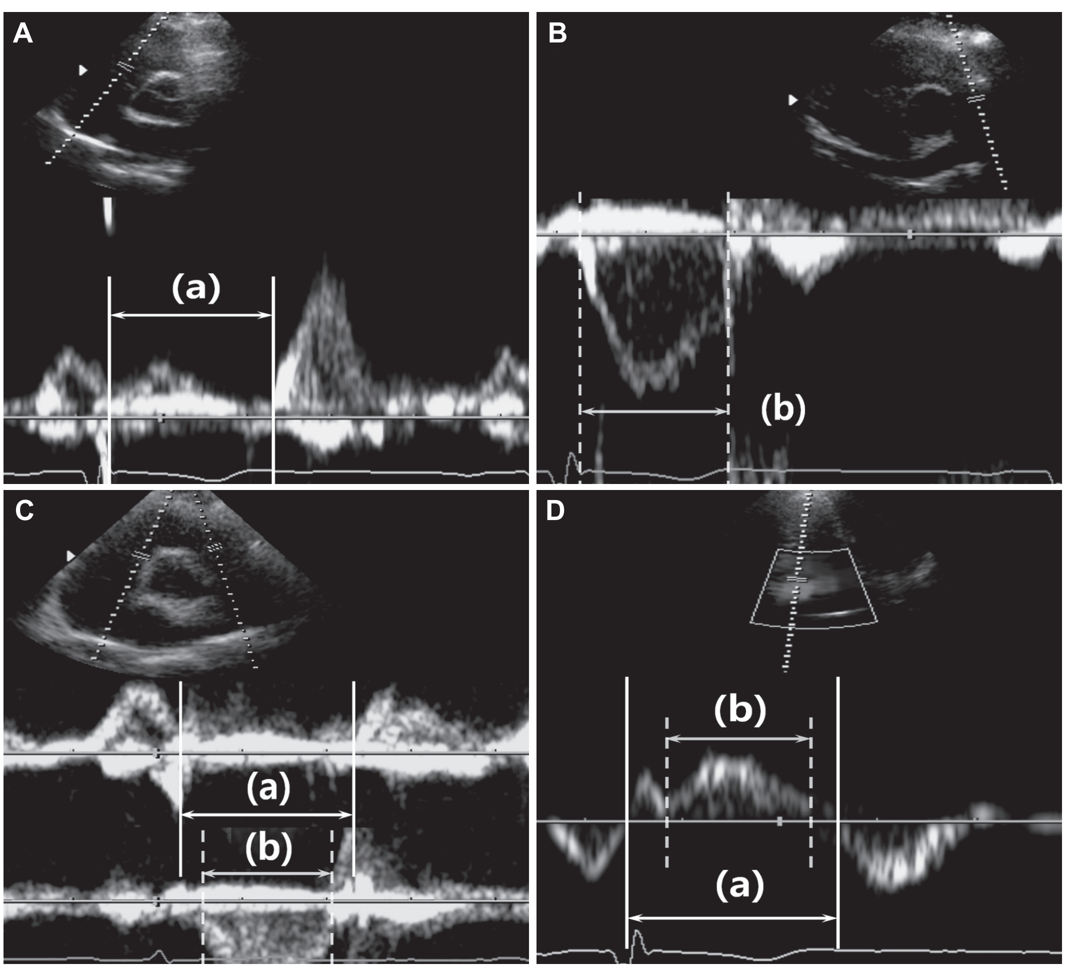

Fig. 1. Measurement of right ventricular Tei index (RTX) by (A and B) the conventional flow Doppler method (RTX_{CFD}), (C) the dual pulsed-wave Doppler method (RTX_{DPD}), and by (D) tissue Doppler echocardiography (RTX_{TDE}). RTX was defined as [(a)-(b)]/(b), where (a) is the time from tricuspid valve inflow cessation to onset for RTX_{CFD} and RTX_{DPD} and time from the end of A'_{TV} to the onset of E'_{TV} for RTX_{TDE}, and (b) is the pulmonary ejection time for RTX_{CFD} and RTX_{DPD} or the duration of S'_{TV} for RTX_{TDE}. A'_{TV}: late diastolic tricuspid annular velocity, E'_{TV}: early diastolic tricuspid annular velocity, S'_{TV}: systolic tricuspid annular velocity.

derive the sum of the ICT and IRT, the PET was subtracted from the time between the cessation to onset of tricuspid valve inflow.[7)]

To calculate the RTX_{TDE}, the sum of the ICT and IRT was derived by subtracting the S'_{TV} duration from the time interval between the end of the A'_{TV} and the onset of the E'_{TV} (Fig. 1D). Each of these parameters was measured using three consecutive beats and then averaged.

Right cardiac catheterization and cardiopulmonary exercise testing

Right cardiac catheterization was performed using a balloon-tipped pulmonary artery catheter in all patients. The RV systolic pressure (RVSP) was measured for three consecutive beats and then averaged. The study subjects were grouped according to the RVSP values using a cutoff value of 40 mmHg as follows: group A with a high RVSP (≥40 mmHg, n=18) and group B with a normal RVSP (<40 mmHg, n=23).

A symptom-limited exercise treadmill test with expired gas analysis was performed in all 41 study subjects. The peak O_2 consumption rate (VO_2 max) was measured at peak exercise. The VO_2 max was indexed versus body weight and peak exercise capacity {metabolic equivalents (METs)} was calculated by dividing the measured VO^2 max values by 3.5 mL/kg/min.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 (SPSS Interactive Graphics, version 17.0; SPSS, Inc., Chicago, IL, USA). P<0.05 were considered statistically significant. Data are presented as the means±SD or as frequencies. Continuous variables were compared via one-way analysis of variance with post-hoc test using Bonferroni's correction method, and categorical data was analyzed using a Chi-squared or Fisher's exact test. Comparison between the mean values of the RTX measured using different methods was done with a paired t-test. The 2-tailed Pearson method was used to evaluate correlations between the RTX and other echocardiographic parameters.

In addition, stepwise multiple linear regression models were developed to predict exercise capacity. To investigate intra- and inter-personal measurement variability, measurements were performed off-line by two investigators on 20 randomly selected cases. The intraclass correlation coefficient of the RTX_{DPD} for intra- and inter-observer measurements was 0.93 (n=20; p<0.001; 95% CI, 0.84-0.97) and 0.83 (n=20; p<0.001; 95% CI, 0.62-0.93).

Table 1. Baseline clinical data of study patients according to the right ventricular systolic pressure

Group	High RVSP (Group A)	Normal RVSP (Group B)	Healthy control group	Total	p*
N	18	23	15	56	
Male (%)	6 (33)	9 (39)	6 (40)	21 (38)	0.905
Age (year)	40±17†	33±10	29±3	34±12	0.021
Weight (kg)	61±10	60±11	62±12	61±10	0.868
Height (cm)	162±8	163±7	168±9	164±8	0.078
Body mass index (kg/m²)	23.4±3.7	22.4±2.9	21.8±2.3	22.6±3	0.303
Body surface area (m²)	1.64±0.14	1.64±0.17	1.70±0.20	1.66±0.17	0.553
Heart rate (bpm)	69±14	78±14†	67±10	72±14	0.019
Systolic blood pressure (mmHg)	126±16†	115±16	113±11	118±16	0.023
Diastolic blood pressure (mmHg)	70±12	70±10	68±7	70±10	0.814
RVSP (mmHg)	70±30	28±4	-	47±29	<0.001
Diagnosis, n (%)					
ASD	8 (44)	14 (61)	-	22 (54)	0.295
Large ASD (≥1 cm)	8 (44)	12 (52)		20 (49)	
Small ASD (<1 cm)	0 (0)	2 (9)		2 (5)	
Patent ductus arteriosus (%)	1 (6)	8 (35)	-	9 (22)	0.054
Pulmonary regurgitation s/p TOF (%)‡	1 (6)	1 (4)	-	2 (5)	1.000
Idiopathic PAH (%)	4 (22)	0 (0)	-	4 (10)	0.030
RVOT obstruction (%)	4 (22)	0 (0)	-	4 (10)	0.030
Valvular pulmonary stenosis (%)	2 (11)	0 (0)		2 (5)	
Ventricular septal defect (%)	2 (11)	0 (0)		2 (5)	

Data are presented as the means±SD or as numbers (%). *p were calculated using an independent t-test or Chi-squared test between groups A and B. Fisher's exact tests were used when applicable. One-way analysis of variance with post-hoc analysis using the Bonferroni correction method was also used for comparison of the parameters between three groups, †p<0.05 compared with the healthy control group in post-hoc analysis, ‡Patients that developed chronic pulmonary regurgitation after surgical treatment of tetralogy of Fallot (TOF). RVSP: right ventricular systolic pressure, ASD: atrial septal defect, PAH: pulmonary arterial hypertension, RVOT: right ventricular outflow tract

Results

Baseline characteristics of the study population

The baseline clinical characteristics and the diagnoses of the study subjects are shown in Table 1. The mean age of the 56 enrolled subjects was 34±12 years, and 21 subjects (38%) were males. In group A (i.e., a RVSP ≥40 mmHg), the mean RVSP was 70±30 mmHg, which was significantly greater than group B (28±4 mmHg, p<0.001). No significant differences in baseline characteristics were observed between these two groups, except for a slightly higher heart rate and lower systolic blood pressure in group B (p=0.073 and p=0.065, respectively). The clinical diagnoses in group B were mainly atrial septal defects (n=14, 61%) or patent ductus arteriosus (n=8, 35%). However, patients with idiopathic pulmonary arterial hypertension (n=4, 22%) as well as RV outflow tract obstruction (n=4, 22%) were also included in group A.

Echocardiographic data and cardiopulmonary function testing

Echocardiographic data are presented by patient group in Table 2. No significant differences were observed between groups A and B in terms of cardiac chamber size or ejection fraction, although the mid-RV dimension measured in the apical 4-chamber view was significantly larger in group A (44±11 mm vs. 37±8 mm, p=0.028). In contrast, the S'_{TV} and E'_{TV} were significantly lower in group A (11.2±2.9 cm/sec and 10.1±3.9 cm/sec vs. 14.8±3.9 cm/sec, and 14.6±4.5 cm/sec, p=0.003 and p=0.001, respectively), whereas the RTX_{CFD} and RTX_{DPD} were significantly higher in group A (0.353±0.202 vs. 0.192±0.076, p<0.001, and 0.326±0.139 vs. 0.196±0.047, p<0.001). However, the RTX_{TDE} values were not significantly different (p=0.160). Moreover, patients in group A had a shorter duration of exercise and a lower maximal exercise capacity (7.1±3.2 minutes vs. 10.2±1.4 minutes, p<0.001, and 6.3±2.4 METS vs. 9.6±1.7 METS, p<0.001, respectively).

Correlation analysis between the right ventricular Tei index and other parameters

The mean RTX_{CFD} (0.262±0.164) was similar to the mean RTX_{DPD} (0.253±0.117, p=0.440), whereas the mean RTX_{TDE} (0.447±0.125) was significantly higher than the mean RTX_{DPD} (p<0.001) by paired t-tests. The RTX_{CFD} and RTX_{DPD} values agreed and correlated well with each other by Pearson's cor-

Table 2. Echocardiography and cardiopulmonary function test results

Group	High RVSP (Group A)	Normal RVSP (Group B)	Healthy control group	Total	p*
N	18	23	15	56	
LV end-diastolic dimension (mm)	43.6±9.3	47.2±8.5	47.1±4.1	46.0±7.9	0.302
LV end-systolic dimension (mm)	28.3±8.3	29.1±5.9	27.0±3.1	28.3±6.2	0.593
LV ejection fraction (%)	58±10†	62±7	67±3	62±8	0.004
Left atrial volume index (mL/m^2)	23.9±11.6	20.6±5.1	18.3±3.0	21.1±7.7	0.110
Mitral E velocity (m/sec)	0.68±0.24†	0.79±0.20	0.89±0.16	0.78±0.21	0.021
E'_{MV} (cm/sec)	11.7±4.6†‡	16.8±4.5	17.3±2.0	15.3±4.7	<0.001
E/E'_{MV}	6.2±2.2‡	4.9±1.5	5.2±1.1	5.4±1.7	0.049
Right atrial size (mm)	49±13†	43±8†	34±2	43±11	<0.001
RV long-axis dimension (mm)	84±14†	80±9	71±8	79±11	0.003
Mid RV dimension (mm)	44±11†‡	37±8	31±4	37±10	<0.001
Basal RV dimension (mm)	38±10†	34±6†	28±3	34±8	0.001
S'_{TV} (cm/sec)	11.2±2.9†‡	14.8±3.9	15.1±2.5	13.7±3.7	0.001
E'_{TV} (cm/sec)	10.1±3.0†‡	14.6±4.5†	18.3±3.4	14.1±4.9	<0.001
A'_{TV} (cm/sec)	13.6±5.9	13.9±4.9	10.5±2.6	12.9±4.9	0.082
RTX by CFD	0.353±0.202†‡	0.192±0.076	0.132±0.071	0.227±0.156	<0.001
RTX by TDE	0.486±0.154†	0.416±0.088	0.389±0.084	0.431±0.117	0.040
RTX by DPD	0.326±0.139†‡	0.196±0.047	0.157±0.068	0.227±0.114	<0.001
Exercise duration (min)	7.1±3.2	10.2±1.4	-	8.8±2.8	<0.001
VO_2 max (mL/min)	22±8.3	33.7±6	-	28.5±9.2	<0.001
Exercise capacity (METS)	6.3±2.4	9.6±1.7	-	8.2±2.6	<0.001

Data are presented as the means±SD or as numbers (%). *p calculated using the independent t-test or one-way analysis of variance with post-hoc analysis by the Bonferroni correction method, †p<0.05 compared with the healthy control group, ‡p<0.05 compared with group B. RVSP: right ventricular systolic pressure, LV: left ventricle, E: early diastolic mitral inflow velocity, E'_{MV}: early diastolic mitral annular velocity, E/E'_{MV}: E to E'_{MV} ratio, RV: right ventricle, S'_{TV}: peak systolic tricuspid annular velocity, E'_{TV}: peak early diastolic tricuspid annular velocity, A'_{TV}: peak late diastolic tricuspid annular velocity, RTX: RV Tei index, CFD: conventional flow Doppler, TDE: tissue Doppler echocardiography, DPD: dual pulsed-wave Doppler, VO_2 max: peak oxygen consumption rate, METS: metabolic equivalents relation analysis (r=0.90, p<0.001) and Altman and Bland curve analysis (Fig. 2). However, there was only a weak relationship between the RTX_{TDE} and RTX_{DPD} (r=0.48, p=0.001). While the RTX_{CFD} and RTX_{DPD} correlated moderately with the S'_{TV} (r=0.57, p=0.001 and r=0.59, p=0.001), there was no correlation between the RTX_{TDE} and S'_{TV} (Fig. 3). Moreover, the RTX_{CFD} and RTX_{DPD} correlated well with maximal exercise capacity (r=0.62, p<0.001 and r=0.65, p<0.001), whereas the RTX_{TDE} was only correlated weakly (r=0.45, p=0.004) (Fig. 3).

Multiple linear regression analysis and prediction of maximal exercise capacity

Multiple stepwise linear regression analyses were performed to identify those parameters that independently predicted maximal exercise capacity (Table 3). The mid-RV dimension, S'_{TV}, E'_{TV}, RVSP, and RTX_{DPD} were related to maximal exercise capacity by simple linear regression analysis, and subsequent multiple linear regression analysis showed that male gender (β=0.45, p<0.001), LV ejection fraction (β=0.22, p=0.023), E'_{TV} (β=0.30, p=0.003), mid-RV dimension (β=-0.26, p=0.012), and RTX_{DPD} (β=-0.60, p<0.001) were independently related to maximal exercise capacity (adjusted R^2=0.67). Based on a multiple regression model, including the RTX_{CFD} and other independent variables, the RTX_{CFD} was also independently related to maximal exercise capacity (β=-0.56, p<0.001, adjusted R^2=0.64). However, based on multiple regression analysis including the RTX_{TDE} as an independent variable, the RTX_{TDE} was independently related to the exercise capacity, but the relationship was not as strong (β=-0.31, p=

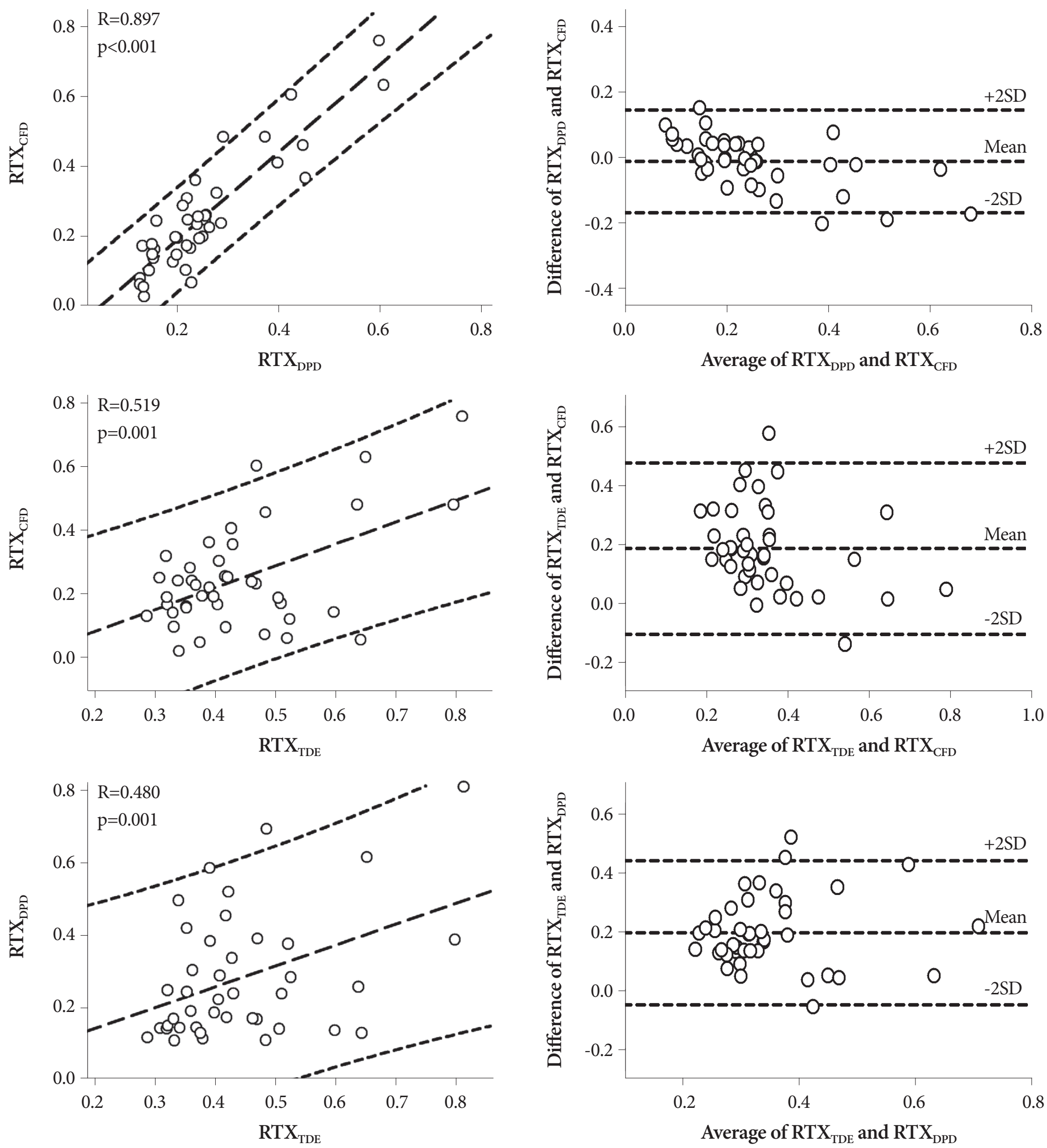

Fig. 2. Correlation (left column) and Altman-Bland plots (right column) between right ventricular Tei indexes (RTX) using conventional flow Doppler (CFD) and dual pulsed-wave Doppler (DPD; upper row); RTX using CFD and tissue Doppler (TDE; mid-row); and RTX using the DPD and TDE methods.

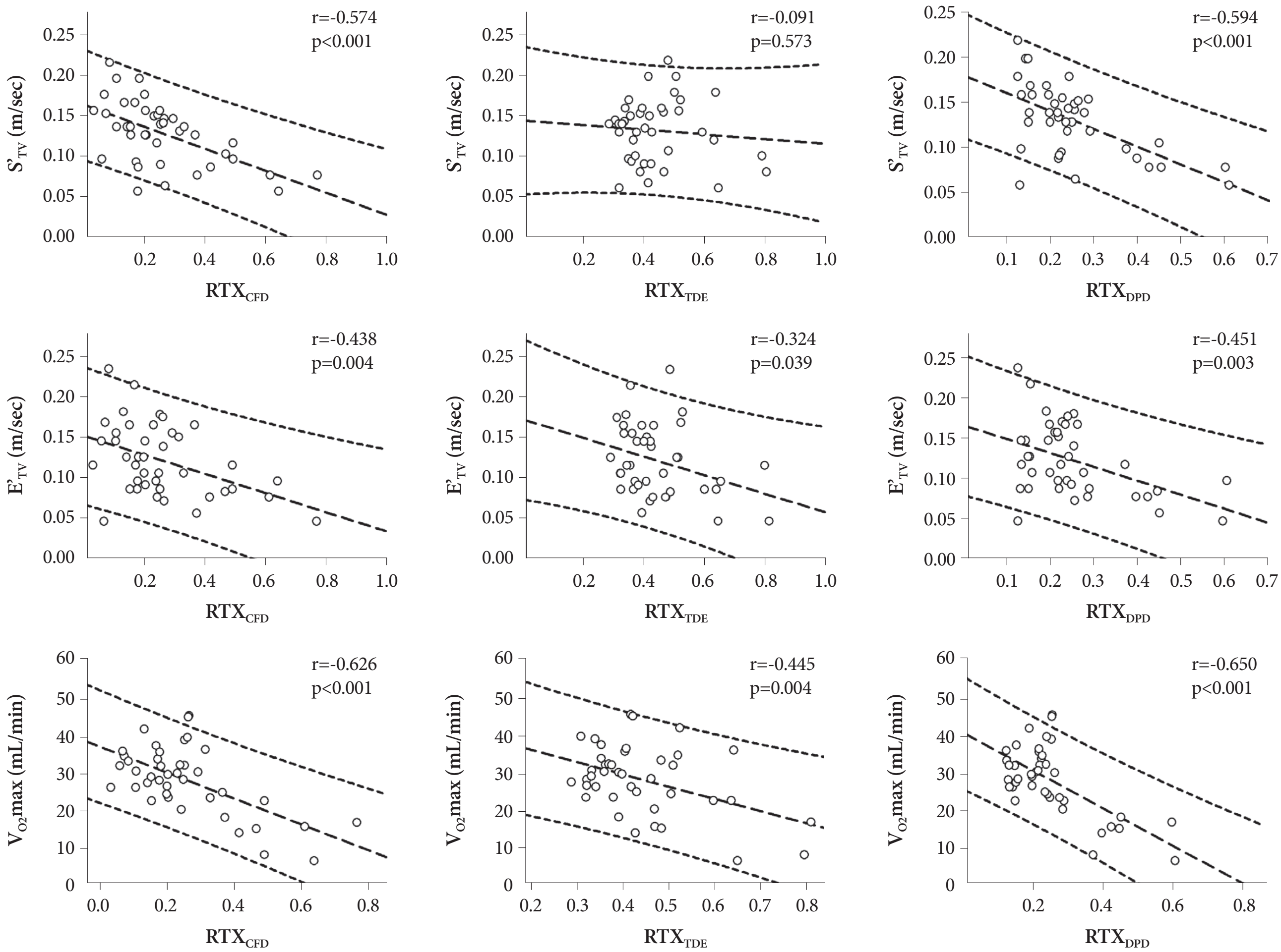

Fig. 3. Correlation plots of the right ventricular Tei index (RTX) vs. the S'_{TV} (upper row), E'_{TV} (mid-row), and maximal exercise capacity (lower row). Horizontal axes in the leftmost column represent RTX values determined using conventional flow Doppler (RTX_{CFD}); the middle column represents RTX values determined using tissue Doppler echocardiography (RTX_{TDE}); and the right column RTX values determined using the dual pulsed-wave Doppler method (RTX_{DPD}). S'_{TV}: systolic tricuspid annular velocity, E'_{TV}: early diastolic tricuspid annular velocity.

Table 3. Multiple linear regression analysis and the prediction of maximal exercise capacity (METS)

	Univariate			Multivariate*		
	r	R^2	p	B±SE	β	p
Constant	-	-	-	7.7±2.2		0.001
Male gender	0.22	0.05	0.088	2.44±0.52	0.453	<0.001
Age (year)	-0.21	0.04	0.097			
LV ejection fraction (%)	0.24	0.06	0.067	0.068±0.028	0.217	0.023
E'_{MV} (cm/sec)	0.53	0.29	<0.001			
E/E'_{MV} ratio	-0.20	0.04	0.107			
Mid RV dimension (cm)	-0.30	0.09	0.029	-0.066±0.025	-0.256	0.012
S'_{TV} (cm/sec)	0.37	0.14	0.009			
E'_{TV} (cm/sec)	0.46	0.22	0.001	12.2±5.9	0.208	0.048
RV systolic pressure (mmHg)	-0.64	0.41	<0.001			
RTX_{DPD}	-0.65	0.42	<0.001	-13.5±2.4	-0.604	<0.001
RTX_{CFD}*	-0.62	0.39	<0.001			
RTX_{TDE}*	-0.45	0.20	0.001			

N=41, dependent variable as METS. R^2=0.79, Adjusted R^2=0.75, standard error of the estimate=1.32. The backward stepwise approach was used to select best model fits to predict the METS. *RTX_{CFD} and RTX_{TDE} were excluded for the multiple regression model in this table (see text for details). LV: left ventricle, E'_{MV}: early diastolic mitral annular velocity, E/E'_{MV}: E to E'_{MV} ratio, RV: right ventricle, S'_{TV}: systolic tricuspid annular velocity, E'_{TV}: early diastolic tricuspid annular velocity, RTX: RV Tei index, DPD: dual pulsed-wave Doppler, CFD: conventional flow Doppler, TDE: tissue Doppler echocardiography

0.025, adjusted R^2=0.45).

Discussion

The primary findings of this study were that the RTX_{DPD} is correlated well with the RTX_{CFD}, but not with the RTX_{TDE}, in patients with a RV volume or pressure overloading condition, and the RTX_{DPD} can be reliably measured during single cardiac cycles. Furthermore, the RTX_{DPD} was an independent predictor of exercise capacity by multiple regression analysis.

RTX_{CFD} determinations assume that each cardiac cycle has the same cardiac length, and thus these determinations are limited in patients with significant beat-to-beat variability or atrial fibrillation. Using the DPD method, we were able to obtain a flow signal at two independent sites simultaneously, and this method allowed precise determinations of ICT, IVT, and ET during single cardiac cycles. Thus, this method overcomes the limitation of the RTX_{CFD}. In a previous study, the RTX_{TDE} was shown to be reliable for evaluating RV function in pediatric patients.[7] However, in the present study, the RTX_{DPD} did not concur with the RTX_{TDE}, although excellent concordance existed between the RTX_{DPD} and RTX_{CFD}. In a recent study performed in children, slight differences were found between the RTX_{TDE} and RTX_{CFD}, especially in larger and older children.[9] Because the patients enrolled in this study were >10 years of age, our results support the opinion that the RTX_{TDE} cannot substitute for the RTX_{CFD} in these patients and age factors should be considered during the clinical use of the RTX_{TDE}.

The finding that the RTX_{TDE} was related to the E'_{TV}, but not the S'_{TV}, was interesting in that only the tricuspid annulus was used to determine the RTX_{TDE}, which might partially explain why the RTX_{TDE} values differed from the RTX_{CFD} and RTX_{DPD} values (Fig. 3). The RTX_{TDE} was determined using tricuspid annular velocity tracing, which is associated with motion of the RV inlet. However, the structure and function of the RV inlet was primarily associated with RV diastolic properties. Additionally, in the RV the inflow and outflow tracts were separated unlike those in the left ventricle. Therefore, the fact that the RTX_{TDE}, which is obtained only using the tricuspid annular velocity, is unrelated to RV systolic properties is understandable.

This is the first study to compare the RTX_{DPD} with RTX_{CFD} and maximal exercise capacity in patients with various RV loading conditions. In the present study, the RTX_{DPD} was found to be related to the maximal exercise capacity of patients with a RV volume or pressure overload independent of age, gender, left ventricular ejection fraction, RVSP, and other RV echocardiographic parameters. This finding may be consistent with previous observations concerning the clinical usefulness of the RTX, and thus we are confident that the RTX_{DPD} offers a feasible and useful means of evaluating the cardiac performance. Moreover, the excellent correlation between the RTX_{DPD} and RTX_{CFD} further supports the reliability of the RTX_{CFD}, when measured in subjects with a regular cardiac rhythm. In addition, the DPD method could prove to be a useful clinical tool for measuring the time intervals, such as the ICT, IRT, or Tei indices, even in patients with atrial fibrillation, in which various parameters are non-determinable because of an irregular cardiac cycle.

Study limitation

The present study had several limitations that should be noted. The sample size was relatively small, and therefore the statistical power might be low. The RV pressure measurements were performed using fluid-filled catheters and not catheter-tipped manometers, and we did not evaluate invasive RV parameters, such as dP/dt. However, the RV dP/dt increases paradoxically as the peak RV pressure increases until RV contractile dysfunction becomes evident, and therefore these derivatives of time-pressure curves might not represent RV function. The study group was heterogeneous as the patients enrolled for the RTX_{DPD} analysis had various diseases. However, since RV function was shown to be one of the most important clinical predictors in the majority of study patients, the finding that RTX_{DPD} independently predicts maximal exercise capacity supports its clinical usefulness in patients with RV volume or pressure overload. We used the RV dimension or E'_{TV} as surrogate parameters of RV function instead of RV ejection fraction, which might be a gold standard of RV function if measured by a cardiac magnetic resonance imaging technique. The RTX_{DPD} was not superior, but similar to the RTX_{CFD} in terms of the correlation with the maximal exercise capacity. However, we measured the RTX_{DPD} in the same cardiac cycle reliably and this method could make the RV Tei index potentially useful in the patients with an irregular heart rate, such as atrial fibrillation.

Conclusions

The RTX_{DPD} is a feasible and reliable method for evaluating RV function. We recommend that a further study with a larger number of patients (including patients with atrial fibrillation) be conducted to determine the relationship between the RTX_{DPD} and clinical outcome.

Acknowledgments

This study was supported by a grant from the IN-SUNG Foundation for Medical Research.

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