Quantification of Chronic Functional Mitral Regurgitation By Automated 3-D Peak and Integrated Proximal Isovelocity Surface Area and Stroke Volume Techniques Using Real-Time 3-D Volume Color Doppler Echocardiography: In Vitro and Clinical Validation

Thavendiranathan et al: Automated 3D Quantification of Mitral Regurgitation

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Abstract

Background—The aim of this study was to test the accuracy of an automated 3-D PISA (in vitro and patients) and stroke volume technique (patients) to assess MR severity using real-time 3-D volume color flow Doppler transthoracic echocardiography (RT-VCFD).

Methods and Results—Using an in vitro model of MR, the effective regurgitant orifice area (EROA) and volume (RVol) were measured by the PISA technique using 2-D and 3-D (automated “true” 3-D PISA) transthoracic echocardiography. The mean anatomical regurgitant orifice area (0.35±0.10cm²) was underestimated to a greater degree by the 2-D (0.12±0.05cm²) than the 3-D method (0.25±0.10cm²), p<0.001 for both. Compared to the flowmeter (40±14ml), the RVol by 2-D PISA (20±19ml) was underestimated (p<0.001), but the 3-D peak (43±16ml) and integrated PISA (38±14ml) based RVol were comparable (p>0.05 for both). In patients (n=30, functional MR), 3-D EROA correlated well with cardiac magnetic resonance (CMR) RVol r=0.84 and regurgitant fraction r=0.80. Compared to CMR RVol (33±22ml), the integrated PISA RVol (34±26ml, p=0.42) was not significantly different, however, the peak PISA RVol was higher (48±27ml, p<0.001). In addition, RVol calculated as the difference in automated mitral and aortic stroke volumes by RT-VCFD was not significantly different from CMR (34±21ml vs. 33±22ml, p=0.33).

Conclusions—Automated RT-VCFD 3-D PISA is more accurate than 2-D PISA method to quantify MR. In patients with functional MR the 3-D RVol by integrated PISA is more accurate than a peak PISA technique. Automated 3-D stroke volume measurement can also be used as an adjunctive method to quantify MR severity.

Key Words: mitral regurgitation, real time 3-D volume color flow Doppler echocardiography, 3-D integrated PISA, 3-D peak PISA, automated 3-D stroke volume, cardiac magnetic resonance imaging
Abbreviations

PISA – proximal isovelocity surface area
PFCR – proximal flow convergence region
LOA – level of agreement
RT-VCFD – Real time volume color flow Doppler
2-D TTE – 2-D transthoracic echocardiography
EROA – effective regurgitant orifice area
RVol – regurgitant volume
RF – regurgitant fraction
MR – mitral regurgitation
SV - stroke volume
Current recommendations underscore the use of qualitative and quantitative echocardiographic methods to grade severity of chronic mitral regurgitation (MR)^1,2. A commonly used quantitative method is the proximal isovelocity surface area (PISA) technique which has several limitations when performed by 2-D echocardiography^3,4. Although 3-D echocardiography based PISA techniques have been shown to be more accurate^5-9, it is not widely used due to limitations of gated acquisitions, the need for time consuming manual interaction with the data, and ongoing need for shape assumptions. Also, current 3-D PISA techniques don’t account for the dynamic nature of the regurgitant orifice which is especially important in functional MR^10,11.

The use of an automated 3-D method to quantify the “true” 3-D peak PISA using non-gated real time 3-D volume color flow Doppler transthoracic echocardiography (RT-VCFD) in mostly degenerative MR was recently illustrated^12 in comparison to echocardiographic reference standards. However, this method has not been validated in an in vitro model of MR under controlled hemodynamic conditions or tested against an external reference standard such as cardiac MRI (CMR). Furthermore, a single peak PISA measurement does not account for the dynamic nature of the regurgitant orifice especially in functional MR^10,11 and can result in inaccuracy in calculation of the regurgitant volume^10,13. Also, the PISA method may be limited as in multiple MR jets and when PISA is constrained by adjacent wall.

Using RT-VCFD, the aims of this study were to first assess the feasibility and accuracy of the automated “true” 3-D PISA technique for quantification of MR using an integrated PISA approach in comparison to the peak PISA method in an in vitro model and in patients with functional MR. Secondly to assess the accuracy of an automated 3-D color Doppler based stroke volume quantification method^14 which uses trans-mitral and aortic stroke volumes (SV) to
quantify MR severity in the same patient population. Cardiac MRI (CMR) was used as the reference standard in patients.

**Methods**

**In Vitro Validation of Automated 3-D Surface Area Computation**

The accuracy of the automated algorithm for computation of the 3-D surface area (similar to PISA) was first tested using a hollow sphere submerged in a solution with consistency similar to chest wall tissue (Supplemental Figure I). This sphere was imaged with the SC2000 platform (2.8MHz frequency, 14 cm depth) and the surface area was computed for 5 separate trials of acquisition and segmentation.

**In Vitro Flow Model and Echocardiographic Acquisition**

The *in vitro* validation was performed using a pulsatile cardiac flow model previously described. The model consisted of a circulatory loop, regurgitant circuit, a flow-meter, and an ultrasound imaging chamber. Pulsatile flow was driven through the regurgitant loop incorporating a divider plate containing a modifiable orifice. Orifices evaluated included 0.15cm² and 0.4cm² circle, 0.4cm² arc, and 0.39cm² rectangle (Figure 1). Regurgitant flow through the orifices ranged between 12 - 57 ml/beat at a heart rate of 60 beats/min.

Using 2-D transthoracic echocardiography (Siemens ACUSON SC2000™ with 4v1 2-D, 2.25-4.25MHz transducer) color and continuous wave (CW) Doppler data were acquired for every orifice/flow condition (total of 11 conditions, Figure 1) with the transducer aligned parallel to the regurgitant flow using an “apical” window. The Nyquist limit was adjusted to achieve a clear PISA. The 2-D color Doppler frame rate was 14-24 Hz at a Doppler scan depth of 12-16cm. For each of the 11 orifice/flow conditions, 5 different PISA measurements were made.
resulting in 55 measurements. For each condition 3 CW Doppler signals and the corresponding flowmeter measurements of the RVol (reference standard for RVol) were also recorded for a total of 33 measurements.

With the same flow conditions and imaging window, RT-VCFD acquisitions were made immediately after the 2-D acquisition with acquisition of 3 consecutive non-stitched 3-D volumes. The depth and color Doppler sector size were optimized resulting in an acquisition volume rate between 12-43 VPS. As for the 2-D data the visualization of the 3-D PISA was optimized during acquisition. Similar to the 2-D acquisition, 55 PISA and 33 CW and flowmeter recordings were made.

**RT-VCFD acquisition - Patients**

Consecutive patients age > 18 years, referred to the CMR laboratory with incidental finding of at least mild functional MR were recruited to participate in an Institutional review board approved study. Exclusion criteria included: atrial fibrillation, intra-cardiac shunts, concomitant aortic valve disease, and poor echocardiographic windows (inability visualize ≥2 myocardial segments or image the entire ventricle without dropouts or artifacts).

RT-VFCD of MR in the apical 4-chamber view optimized for PISA was acquired (Figure 2) with 3-D B-mode and color Doppler volume sector adjusted for the left ventricle and mitral valve (Movie 1). Three 3-D volumes were recorded from 3 consecutive cardiac cycles. Post-acquisition, offline analysis was performed using custom software (described below) for quantification of the effective regurgitant orifice area (EROA) and regurgitant volume (RVol).

Subsequently, a second set of 3-D volumes were acquired from 3 consecutive cycles in the apical 3 or 5 chamber view incorporating the LVOT and mitral valve in a single volume (Movie 2, Figure 3B-D). The color Doppler box was placed to cover both the mitral and aortic...
valves simultaneously. The depth and space-time settings were optimized, and the highest possible color Doppler velocity scale was used to minimize aliasing\textsuperscript{14}. The total acquisition time for each dataset was 3-5 seconds. Using this technique the mitral RVol and fraction (RF) were quantified using previously validated custom software\textsuperscript{14,16} described below.

**Automated Measurement of 3-D PISA**

The algorithm used to recognize, segment, and quantify 3-D PISA has been previously described\textsuperscript{12,17}. The essential steps are summarized in Figure 2 and Movie 1. The user first selects the aliasing velocity and identifies the PISA on the appropriate systolic frames (Figure 2B). Then the direction of the MR jet is identified in the same frame (Figure 2B). The algorithm generates an isovelocity segmentation in the voxel-based 3-D space representing the 3-D geometry (free of specific geometric assumptions) of the PISA (Figure 2C-G) using an optimized Random Walker method with a directed graph approach\textsuperscript{17}. The segmentation results were automatically smoothened by 3-D Gaussian kernel and an isovelocity surface mesh was computed using a marching cube algorithm. No modifications were made to the automatically generated surface areas. The mesh vertexes were transformed from acoustic to Cartesian space for computation of 3-D PISA.

**EROA and RVol Calculations**

Two dimensional PISA (*in vitro*) was calculated using the hemispheric assumption\textsuperscript{1}. EROA was derived as (PISA x aliasing velocity)/peak MR velocity, and RVol as EROA x VTIMR, where VTI is the velocity-time integral of the trans-orifice CW Doppler\textsuperscript{1}. The 3-D EROA was calculated using the largest systolic 3-D PISA (Figure 3A). With RT-VCFD, for the *in vitro* and
patient study the RVol was calculated in 2 ways. First the EROA was multiplied by the VTImR and we refer to this as the “peak PISA RVol”. Secondly, RVol was calculated for each systolic frame as: (3-D PISA from that frame)x(color Doppler aliasing velocity)/(frame rate)^10 and summed for the entire systolic period to obtain an “integrated PISA RVol” (Figure 2G, 3A, Movie 1). EROA and RVol were obtained from 3 separate 3-D volumes and averaged for each patient.

**Automated Quantification of Aortic and Mitral Stroke Volume (Patients)**

A detailed mathematical basis of the automated stroke volume quantification algorithm for the measurement of mitral inflow and aortic outflow SV was previously described and validated^{14, 16}. Briefly, the algorithm incorporated the color Doppler velocities and the sampling region (mitral valve or left ventricular outflow tract [LVOT]) to calculate SV without assumptions of geometry or uniformity of flow velocities (Figure 3B-D). Automated de-aliasing algorithm was applied as necessary as described previously^{14, 17}. The difference between mitral inflow and LVOT SV was the mitral RVol while the regurgitant fraction (RF) was the RVol divided by the mitral inflow SV.

**CMR Data Acquisition and Analysis**

CMR data acquisition and analysis is described in the Supplement^{18}. Briefly, the aortic SV determined by phase contrast imaging was subtracted from left ventricular SV (LV SV) obtained by planimetry of short axis cines to obtain mitral regurgitant volume (RVol) and regurgitant fraction (RF) was RVol divided by the LV SV.
Grading Severity of MR and Eccentricity

The CMR RF was used to categorize the patients into mild, mild to moderate, moderate to severe, and severe MR (Grades 1-4 MR) based on RF thresholds from ASE guidelines\(^1\). The MR jets were also categorized as central or eccentric with eccentric jets defined as the majority of the MR jet not being parallel to the long axis of the left atrium in standard views.

Reproducibility

The inter-observer variability for the PISA technique was assessed for all patients while the intra-observer variability was performed in 50%. The inter- and intra-observer variability for the SV technique was also tested in all patients. In 10 patients test re-test reproducibility was assessed for the PISA and SV techniques by first obtaining a 3D acquisition, followed by repositioning the patient and the transducer and obtaining a second data-set by a different sonographer.

Statistical Analysis

Continuous data are expressed as mean ± SD while categorical data as frequency or percentage. The \textit{in vitro} EROA were skewed and hence Spearman Rho and Wilcoxon tests were used. Linear regression analysis, Pearson’s correlation coefficient, Lin’s Concordance Correlation Coefficient (CCC), and ANOVA with Bonferroni post-hoc analyses were used for RVol comparisons. Analysis of covariance was used to assess the impact of regurigitant orifice or MR eccentricity on EROA or RVol measurements.

All patient data were normally distributed as determined by Kolmogorov-Smirnov test and visual inspection of the data using normal plots. We assessed for the presence of outliers in
our data using the Outlier Labeling Rule, however, none were identified. Linear regression analysis and Pearson’s correlation coefficient were used to test strength of relationships. For the linear regression analysis P-P plots of the residuals were used to assess for normality. Paired t-test was used to test difference between measurements. Agreement between methods and reproducibility were examined using Bland-Altman analysis and CCC. The kappa statistic was used to assess agreement in categorizing MR severity. Receiver operating characteristic (ROC) curve was used to identify the 3D EROA to differentiate severe from non-severe MR. MedCalc (11.4.2.0, Mariakerke, Belgium) and SPSS (ver 19.0.0, IBM Corporation, Chicago, IL) were used for statistical analysis.

**Results**

**In Vitro Validation of Automated 3-D Surface Area Computation**

There was no statistically significant difference between the caliper measured mean ± SD surface area and that obtained by automated 3-D segmentation (49.1 ± 0.2cm² vs was 49.9 ± 2.1cm², p = 0.31, Supplement Figure I) illustrating the accuracy of the 3-D segmentation algorithm with grey scale images.

**In Vitro 2-D and 3-D PISA based EROA**

3-D PISA was successfully quantified for all orifice/flow conditions with typical quantification time of <20 seconds for the peak PISA technique and 60 seconds for the integrated PISA technique for an average of 8±2 systolic frames.

The flow model had anatomic regurgitant orifices (AROA) ranging from 0.15cm² to 0.4cm² (mean ± SD: 0.35±0.10cm²). There was modest correlation between the 2-D PISA EROA
and AROA (Rho=0.74, p<0.001), the values were however underestimated (mean ± SD: 0.12±0.05cm², range 0.04–0.25cm², p<0.001). The 3-D EROA had a good correlation with the AROA (Rho=0.83, p<0.001), but although larger than 2-D PISA EROA (mean ± SD: 0.25±0.10cm², range 0.07–0.38, p<0.001), it was smaller than the AROA (p<0.001). The mean EROA for each orifice was underestimated by both techniques (Supplement Figure II). When the large circle versus arc and rectangle (non-circular) orifices were compared there was a significant difference in the measurement of EROA by 2-D TTE (p=0.01, ANCOVA), but not with RT-VCFD (p=0.43, ANCOVA).

**In Vitro 2-D and 3-D PISA Based RVol**

There was good correlation between the flow meter and 2-D TTE measurements of RVol (r=0.73, p<0.001; CCC 0.26) and integrated (r=0.93, p<0.001; CCC 0.92) and peak PISA (r=0.91, p<0.001, CCC 0.88) measurements of RVol (Figure 4). There were significant differences in the RVol between the techniques (p<0.001, ANOVA). On post-hoc analysis, compared to the flowmeter RVol (40 ± 14ml [range 12-58ml], there was no statistically significant difference in RVol determined by 3-D peak PISA (43 ± 16ml [range 12-72]) or integrated PISA (38 ± 14ml [range 9-57]) methods (p>0.05 for both), however, the 2-D PISA based RVol (20 ± 9ml [range 5-33ml]) was underestimated (p<0.001). When regurgitant orifice shapes were compared (large circle, arc, and rectangle) there was a significant difference in the measurement of RVol by 2-D TTE (p=0.02), but not with RT-VCFD integrated or peak PISA RVol (p=0.73 and 0.11 respectively).
Patient Population

Amongst 35 patients evaluated, 30 (Table) had adequate RT-VCFD studies for analysis (n=2 for drop outs of ≥2 segments, n=3 for artifacts). The mean RT-VCFD volume rate was 22 ± 6 VPS (range 13-38 VPS). MR was functional in all patients, 11 had eccentric MR while 19 had central MR. Using CMR RF, 9 patients had grade 1 MR, 8 grade 2, 6 grade 3, and 7 grade 4 MR. All RT-VCFD studies were completed within 30 minutes of the CMR study. Heart rate and blood pressure were not different between studies (80±18 versus 83±17 beats/minute; 124±18 / 74±11 versus 127±17 / 74±11mmHg).

Automated 3-D Peak PISA Based EROA (patients)

Quantification of EROA using automated 3-D peak PISA surface area was feasible in all included patients. There was good correlation between 3-D EROA and the CMR MR severity grade (Rho=0.86, p<0.001), RVol (r=0.84, p<0.001), and RF (r=0.80, p<0.001)(Figure 5).

Automated 3-D Peak and Integrated PISA Based RVol (patients)

The time duration for the peak PISA calculation for one cardiac cycle was 15 ± 4seconds, while for the integrated PISA technique it was 1.7 ± 0.7minutes. The mean number of systolic frames used for integrated PISA was 7 ± 4. CMR quantification took 6-8minutes. There was a good correlation between RVol obtained by CMR and peak (r=0.87, p<0.001, Figure 6, CCC 0.71) and integrated PISA (r=0.92, p<0.001, Figure 6, CCC 0.92) methods. Compared to CMR RVol (33 ± 22ml), the mean peak PISA RVol (48 ± 27ml) was higher (p<0.001), however, the integrated PISA RVol (34 ± 26ml) was not significantly different (p=0.42). Also, the RVol quantification in comparison to CMR was not affected by the eccentricity of the MR for both peak (p=0.89) and integrated PISA (p=0.09).
RVol Measurements by Automated 3-D Stroke Volume Technique

The time required to obtain 3-D mitral and aortic SV for 3-5 cardiac cycles was 30-60 seconds. The RVol computed by the automated SV technique correlated significantly with those obtained by CMR (r=0.91, p<0.001, Figure 7; CCC 0.91). There was no significant difference in the mean RVol by RT-VCFD and CMR (34 ± 21ml vs 33 ± 22ml, p=0.33). Similarly the RF by RT-VCFD and CMR had good correlation (r=0.92, p<0.001, Figure 7; CCC 0.91) with no significant difference (37 ± 17% versus 36 ± 19%, p=0.81).

Classification of MR Severity

The agreement between the integrated PISA or SV technique and CMR classification of MR (Grade 1-2 versus 3-4) was compared. For the PISA technique using the integrated RVol, there was substantial agreement with CMR severity categories (κ=0.79, p<0.001). When MR grades 1-4 were examined individually, the integrated PISA RVol method categorized 5 patients into lower and 3 into higher MR grades when compared with CMR RVol. However, none were reclassified from clinically insignificant (grades 1-2) to clinically significant (grades 3-4) MR or vice versa. If the peak PISA RVol was used, in comparison to the integrated PISA method, 50% of the patients would have been classified into higher MR severity by at least 1 grade. Using ROC curves the 3-D PISA EROA that best differentiated severe (Grade 4) from non-severe (grades 1-3) MR was 0.51cm² with a sensitivity and specificity of 86% (95% CI: 42-100%) and 96% (95% CI: 78-100%) respectively and an area under the curve of 0.91 (95% CI: 0.75-0.98). This cut-off had a sensitivity and specificity of 100% (95% CI: 83-100%) and 86% (95% CI: 52-86%) respectively to differentiate severe MR (grade 4) from moderate MR (grade 2 and 3). For the SV technique using RF, there was substantial agreement with CMR MR severity (Grade 1-2 versus 3-4) categories (κ=0.73, p<0.001). For MR grades 1-4, compared to CMR the SV
technique categorized 3 patients into higher and 1 into lower MR grade. Amongst these patients one was re-classified from grade 2 to 3 MR while the other from 3 to 2 MR.

**Reproducibility**

The inter- and intra-observer variability (mean±2SD) for the EROA in the clinical study was 0.02±0.14cm² and 0.01 ± 0.08cm² (respective CCC were 0.93 and 0.98) and for the RVol by integrated PISA was 2.2ml±15.0ml and 0.7±6.7ml (CCC 0.95 and 0.99). With the SV technique inter-observer variability (mean±2SD) for RVol and RF were 0.9 ± 11.5ml and 0.2 ± 10.9% respectively (CCC 0.96 and 0.93), while the intra-observer variability was 0.6 ± 7.6ml and 0.5 ± 6.9% (CCC 0.98 and 0.97).

On test re-test analysis the mean±2SD absolute difference in EROA and integrated PISA RVol were 0.0±0.13cm² and 2.1±13.7ml (CCC 0.91 and 0.94). With the volumetric technique the difference in RVol and RF were 1.2±8.8ml and 1.6±9.7% respectively (CCC 0.97 and 0.95).

**Discussion**

This study illustrates the accuracy and reproducibility of real-time (non-stitched) 3-D transthoracic echocardiography acquisition by two automated methods to quantify MR. In an in vitro model an automated 3-D PISA method (without geometric assumptions) was more accurate for the quantification of EROA and RVol than the 2-D PISA method. The estimation of RVol by the 3-D peak or integrated PISA methods were not significantly different. In patients with functional MR the 3-D PISA determined EROA had good correlation with CMR MR severity. However, due to the dynamic nature of the regurgitant orifice, the integrated PISA method was more accurate than the peak PISA method for RVol quantification. An additional approach using automated mitral and aortic stroke volumes calculated using a 3-D color Doppler based...
technique was accurate for quantification of MR RVol and RF. In patients, both techniques had substantial agreement with CMR for differentiating moderate to severe versus less severe MR and had good inter-, intra- and test-retest reproducibility. The feasibility of 86% (similar to 2-D PISA\textsuperscript{10}), and advantages of automation may further promote the clinical adoption of these techniques.

Automated 3-D PISA based EROA and RVol

Among other factors, the need for shape assumptions and the inability to account for the dynamic regurgitant orifices are technical limitations to 2-D and 3-D PISA-based EROA and RVol measurements. The latter is specifically important in patients with functional MR where the regurgitant orifice is thought to be largest at the beginning and end of systole and smallest in the middle\textsuperscript{10,13}. Although previous studies have shown that 3-D methods are more accurate than 2-D PISA methods for MR quantification, these studies either required significant manual interaction with the data or ongoing geometric assumptions\textsuperscript{5-9}, and were unable to account for the dynamic nature of the regurgitant orifice\textsuperscript{5-9,12}.

In this study the 3-D PISA was measured in an automated method without making any specific geometric assumptions. Using this technique, in the \textit{in vitro} model, there was smaller underestimation of the anatomical regurgitant orifice area (AROA) by the 3-D method as compared to the 2-D PISA method. An important reason for underestimation by both techniques is the boundary effects of pulsatile flow through a rigid sharpened edge orifice resulting in flow contraction at the EROA by up to 40% compared to the AROA\textsuperscript{21}. In addition, for the 3-D method the velocity component of the 3-D PISA that is not radial to the transducer cannot be detected with color Doppler and can cause underestimation of the “true” 3-D PISA. However the
latter is a limitation of ultrasound technology as opposed to the 3-D technique. The 3-D underestimation is unlikely related to the segmentation algorithm which was accurate in the grey scale images of a hollow sphere where Doppler velocities were not an issue. The relatively smaller EROA by 2-D compared to 3-D method is likely due to greater underestimation of EROA for two large asymmetric orifices by 2-D PISA and the errors in 2-D PISA radius measurements due to the challenges in identifying the center of the regurgitant orifices particularly in the in vitro model.

Also similar to previous 2-D studies but different from all previous 3-D PISA publications, this study compared the use of 3-D peak and integrated PISA methods to quantify MR RVol. In the in vitro model the RVol by the 3-D peak and integrated PISA methods was accurate while the 2-D RVol was underestimated by 50%. The degree of underestimation of RVol by 2-D TTE is similar to previous studies including a study with the same in vitro model. However, unlike the 50 and 16% underestimation of EROA and RVol seen in this latter study using gated 3-D acquisition, our study with RT-VFCFD showed less underestimation. The difference between flowmeter and both 2-D and 3-D methods for RVol calculation was smaller for the EROA versus AROA comparison because the CW Doppler measures velocities at the EROA and not the AROA. The comparable RVol measurements by the 3-D integrated and peak PISA methods in the in vitro model illustrates that when the regurgitant orifice is static both methods are accurate. Also, the measurements of both the EROA and RVol using automated 3-D PISA were not affected by orifice geometry.

In patients with functional MR although a good correlation was seen between EROA and MR severity measures as determined by CMR, the EROA measurements in the various severity categories were higher than expected by ASE guidelines. This is due to the use of the largest
systolic PISA for EROA measurement in patients with functional MR\textsuperscript{10,13}, the use of the 3-D surface area which results in larger proximal flow convergence region (PFCR) surface area than that determined using a hemispheric or hemi-ellipse assumption (Supplemental Results and Supplemental Figure III), and limitations of lateral resolution of the 3-D acquisition at the site of the PFCR. As a consequence a peak PISA EROA cut-off of 0.51cm\textsuperscript{2} (compared to the traditional 2D EROA of 0.4cm\textsuperscript{2}) was necessary to differentiate severe from non-severe MR. Also due to the larger EROA, the peak PISA technique based RVol was overestimated compared to CMR. This was overcome by using the integrated PISA method where the dynamic variation in the EROA is accounted for. This finding is in concordance with the better accuracy of 2-D integrated PISA-based RVol compared to peak PISA-based RVol especially in functional MR\textsuperscript{10}. But, unlike this manual 2-D TTE study our data demonstrated overestimation (not underestimation) of peak PISA-based RVol because the 3-D PISA is usually larger than 2-D formulations of PISA (Supplemental Results and Supplemental Figure III). Similar to the previous 3-D PISA studies the eccentricity of the MR jet did not affect the accuracy of MR RVol quantification\textsuperscript{5,12}.

Also, the integrated PISA RVol had good agreement with CMR to differentiate patients with Grade 1-2 versus 3-4 MR. This is particularly relevant, as intervention may be considered in asymptomatic patients with grade 3-4 MR while those with grade 1-2 may be followed clinically. Finally, the EROA and integrated RVol measurements had good inter- and intra-observer reproducibility as well as test re-test reproducibility.

Automated 3-D Stroke Volume Technique Based RVol and RF

We previously demonstrated the accuracy (compared to CMR) and reproducibility of automated measurement of mitral and aortic SV using RT-VFCD imaging in patients without valvular heart
disease\textsuperscript{14}. We extended this approach in this study to quantify RVol and RF in patients with functional MR in a rapid and accurate manner with acceptable reproducibility. The use of this 3-D technique for MR quantification has not been previously demonstrated. This method is unique as compared to any previous 3-D studies SV quantification\textsuperscript{24} or 2-D pulsed wave Doppler (PW) method\textsuperscript{1} as the mitral inflow and aortic outflow SV are obtained from the same cardiac cycle from the same 3-D volume data. Also the velocities from the entire mitral annulus or LVOT are used to quantify SV as opposed to assumption of uniformity of velocities across the orifice as with 2-D PW methods. This approach may be particularly useful in the presence of multiple MR jets or in cases where the PISA technique would be inaccurate and also to verify the accuracy of quantification using a second complementary method.

Limitations

The limitations of the \textit{in vitro} model have been previously described\textsuperscript{15,23}. In the current PISA algorithm, although the peak PISA technique was automated, the integrated PISA technique required manual addition of RVol from each frame. However, this is more practical than any other integrated PISA method described in the literature\textsuperscript{10}. The number of systolic frames used in our study for integrated PISA method reflects the limitation of the 3-D temporal resolution, however, was not different from 2-D studies of integrated PISA\textsuperscript{10}. Although the number of patients studied was not large, we had patients with the entire range of MR severity. Also, although we do not have an independent assessment of orifice shape in our patients, the preponderance of non-circular orifice shape in functional MR has been well described\textsuperscript{25}.

The use of CMR as the reference standard has limitations as RVol and RF quantification require the use of several measurements (LV EDV, ESV and, aortic SV) - each of which can
have measurement variability ranging from 3-9%\textsuperscript{18}. However, other CMR techniques for MR quantification also have similar limitations. Therefore, the wider level of agreement for RVol and RF between CMR and the 3-D techniques in our study is at least partially due to this limitation. This is further supported by the fact that the variability in RVol when compared to flow-meter measurements in the \textit{in vitro} study was small (Figure 4) and that classification of patients into Grade 1-2 versus 3-4 MR using these techniques was good. Unfortunately good external reference standards for MR quantification are limited, and CMR measurements have been compared with invasive cardiac catheterization\textsuperscript{18} and used as reference standard in other MR studies where the variability in RVol was also as high\textsuperscript{10}. Finally there are no societal guidelines as to the appropriate threshold criteria to grade MR severity by CMR and hence we used the ASE RF criteria.

**Conclusions**

Automated measurement of 3-D PISA based EROA and RVol is feasible, accurate and reproducible using RT-VCFD. The integrated PISA method is more accurate than peak PISA method in patients with functional MR. Automated measurement of mitral and aortic SV can be used to calculate RVol/RF and provides a novel approach to assess MR severity from a single volumetric data set. Combined with the work-flow advantages of automation it may now be possible to accurately quantify MR in routine clinical echocardiography using RT-VCFD imaging.
Disclosures

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Dr. Stephen Little: Research support- Siemens Medical Imaging.
Saurabh Datta is an employee of Siemens Medical Solutions.
None of the other authors have any disclosures.

References


Table. Patient demographic and hemodynamic data

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<td>Male/Female, %</td>
<td>37/63</td>
</tr>
<tr>
<td>MR Severity by CMR</td>
<td></td>
</tr>
<tr>
<td>Mild or Mild to Moderate MR, n</td>
<td>17</td>
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<tr>
<td>Moderate to Severe or Severe, n</td>
<td>13</td>
</tr>
<tr>
<td>Cardiac MRI</td>
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</tr>
<tr>
<td>LV end-diastolic volume, ml</td>
<td>247 ± 116</td>
</tr>
<tr>
<td>LV end-systolic volume, ml</td>
<td>158 ± 104</td>
</tr>
<tr>
<td>LV total stroke volume, ml</td>
<td>88 ± 23</td>
</tr>
<tr>
<td>LV EF (%)</td>
<td>40 ± 14</td>
</tr>
<tr>
<td>Aortic SV by phase contrast, ml</td>
<td>54 ± 21</td>
</tr>
<tr>
<td>RVol, ml</td>
<td>33 ± 22</td>
</tr>
<tr>
<td>RF, %</td>
<td>36 ± 19</td>
</tr>
<tr>
<td>RT-VCFD</td>
<td></td>
</tr>
<tr>
<td>EROA, cm²</td>
<td>0.36 ± 0.21</td>
</tr>
<tr>
<td>RVol by integrated PISA, ml</td>
<td>34 ± 26</td>
</tr>
<tr>
<td>RVol by peak PISA, ml</td>
<td>48 ± 27</td>
</tr>
<tr>
<td>RVol by stroke volume technique, ml</td>
<td>34 ± 21</td>
</tr>
<tr>
<td>RF by stroke volume technique ,%</td>
<td>37 ± 17</td>
</tr>
</tbody>
</table>

*Applies to all parameters except for the number of patients with the various MR severity categories. CMR, cardiac magnetic resonance imaging; LV, left ventricle; MR, mitral regurgitation; ml, millilitres; EF, ejection fraction; SV, stroke volume; RVol, regurgitant volume; RF, regurgitant fraction; RT-VCFD, real time volume color flow Doppler; EROA, effective regurgitant orifice area; PISA, proximal isovelocity surface area.
**Figure Legends**

**Figure 1.** Orifice size, geometry, and flow rates used in the *in vitro* study.

**Figure 2.** Steps in PISA quantification. (A) 3-D VCFD, (B) Initialization: the PFCR was optimized, the base and direction of the PFCR was defined (yellow dot and arrow). (C-F) Segmentation: PFCR was automatically identified, modeled, and displayed in a green (arrow) (D) Confirmation: the user may review and confirm the PISA segmentation. EROA and peak PISA RVol are automatically calculated from the largest PISA. (G) Integrated RVol is calculated by adding the RVol from each systolic frame.

**Figure 3.** Illustration of the PISA and stroke volume (SV) technique and CMR RVol for the same patient. (A) PISA technique - the surface area for each frame is illustrated above the PISA and RVol at the bottom. The first frame was the peak PISA. (B-D) SV technique mitral inflow (B), LVOT flow (C), and flow volume curves (D) for 3 cardiac cycles. (E-G) CMR end diastolic (E) and systolic (F) volumes by planimetry and aortic stroke volume by phase contrast imaging (G). There was good agreement in RVol.

**Figure 4.** Linear regression and Bland-Altman analysis for comparison of 2-D TTE, 3-D integrated and peak PISA regurgitant volumes (RVol) and flowmeter measured RVol.

**Figure 5.** Relationship between EROA determined by 3-D peak PISA and CMR MR severity grade, regurgitant volume (RVol), and regurgitant fraction (RF).
**Figure 6.** Comparison of CMR Rvol and RT-VCFD RVol calculated both using integrated and peak PISA techniques.

**Figure 7.** Comparison of regurgitant volume (RVol) and fraction (RF) between CMR and 3-D stroke volume quantification technique using linear regression and Bland-Altman Analysis.
<table>
<thead>
<tr>
<th>Orifice Geometry</th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>Size (cm²)</td>
<td>0.15</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Flow (ml/beat)</td>
<td>12, 18</td>
<td>35, 45, 54</td>
<td>40, 49, 57</td>
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<tr>
<td>Systolic pressure differential (mmHg)</td>
<td>63, 152</td>
<td>68, 97, 161</td>
<td>53, 96, 118</td>
</tr>
</tbody>
</table>

3-D PISA Geometry

2-D PISA Geometry
Peak PISA = Largest PISA \times MR_{VTI}

Integrated PISA = \sum_{i}^{n} 3D PISA_\text{n} \times V_{\text{n}} / \text{Volume Rate}
Integrated PISA $R_{vol}$ (sum of all frames) = 56.49ml
Peak PISA EROA = 0.43cm$^2$, $R_{vol} = 73.5$ml

LV EDV 630ml
LV ESV 502ml
Regurgitant Volume = (630 - 502) - 68 = 60ml

Flow
79ml
76ml
72ml
Rvol = 137 - 76ml = 61ml
$Y = 1.1 + 0.47x$
P < 0.001
$r = 0.73$

$Y = 1.6 + 0.9x$
P < 0.001
$r = 0.93$

$Y = 2.1 + 1.0x$
P < 0.001
$r = 0.91$
Spearman's Rho = 0.86
p < 0.001

r = 0.84
p < 0.001

CMR RVol (ml)

CMR RF (%)
Quantification of Chronic Functional Mitral Regurgitation By Automated 3-D Peak and Integrated Proximal Isovelocity Surface Area and Stroke Volume Techniques Using Real-Time 3-D Volume Color Doppler Echocardiography: In Vitro and Clinical Validation

Paaladinesh Thavendiranathan, Shizhen Liu, Saurabh Datta, Sanjay Rajagopalan, Thomas Ryan, Stephen R. Igo, Matthew S. Jackson, Stephen H. Little, Nathalie De Michelis and Mani A. Vannan

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SUPPLEMENTAL MATERIAL
Supplemental Methods

CMR Data Acquisition and Analysis

CMR was performed using a 1.5T magnet (MAGNETOM Avanto, Siemens Medical Solutions) using a 12-channel phased array coil. LV short axis cines were acquired using: segmented balanced steady state free precession (bSSFP) sequence, TR/TE 3/1.3ms, bandwidth 930Hz/pixel, flip angle 65°, effective temporal resolution of 20-25 frames per cardiac cycle, 8 mm short axis slices. LV end-diastolic (LVEDV) and end-systolic volumes (LVESV) were quantified as per guidelines(1). LV SV was calculated by subtracting LVESV from LVEDV (Figure 3 E-F of main manuscript). Aortic SV was obtained using through-plane phase contrast imaging 1-2cm above the aortic valve (Figure 3G main manuscript): TR/TE =53.3ms/2.1ms, bandwidth 401Hz/pixel, flip angle 25°, 6mm slice thickness, 20 frames per cardiac cycle, with aliasing velocity set at 150cm/s. An ROI was also placed on the chest wall closest to the aortic valve and propagated through all slices and used for phase offset error correction. The aortic SV was subtracted from LV SV to obtain mitral regurgitant volume (RVol) and regurgitant fraction (RF) was RVol divided by the LV SV(2-4).
Supplemental Results

Hemisphere versus Hemi-ellipse versus 3-D PISA

In a subgroup of 10 patients (moderate MR=5, severe MR =5) using the RT-VCFD acquisition we measured PISA by 3 methods: 2-D PISA using hemispheric and hemi-ellipse assumption, and the “true” 3-D PISA method. The major and minor dimensions and radius for the 2-D PISA methods were obtained by multiplanar reformat of the 3-D data (Figure III). The hemispheric surface area was calculated using the standard formula while the hemi-ellipse area was calculated using MATLAB which generated a mesh for the hemi-ellipse by numerically solving the hemi-ellipse equation and computing the surface area by integration of the mesh elements. The mean PISA measured with the hemispheric(0.27 ± 0.11cm²) and hemi-ellipse techniques(0.39 ± 0.20 cm²) were significantly smaller than that determined by 3-D surface area method (0.46 ± 0.22 cm²) (p=0.001 and 0.04 respectively).
Supplemental References

Movie Legends

Movie 1. An illustration of the quantification of mitral regurgitation using an automated 3-D PISA technique.

Movie 2. An illustration of the automated mitral inflow and aortic outflow stroke volume quantification for calculation of mitral regurgitant volume and fraction.
Supplemental Figures

Figure I. Validation of the automated surface area segmentation algorithm.
**Figure II.** *In vitro* measurements of EROA by 2-D TTE and RT-VCFD in comparison to the true orifice area for each orifice tested. The EROA was underestimated for each orifice by both techniques, however, the underestimation was much higher with 2-D TTE than RT-VCFD.
**Figure III.** Comparison of (B) 3-D surface area (C) hemispheric and (D) hemi-ellipse assumption based surface area to compute EROA. Based on the EROA, the patient would be categorized as having mild MR based on hemispheric PISA, moderate MR by hemi-ellipse PISA, and severe MR by 3-D. CMR severity was grade 3 (moderately severe), and CMR RVol was 56.1ml, RF 47