RESEARCH

Impact of 3D echocardiography on grading of mitral stenosis and prediction of clinical events

C Bleakley MD MRCP, M Eskandari MD FRACP, O Aldalati MD MRCP, K Moschonas MD MRCP, M Huang, A Whittaker and M J Monaghan PhD FRCP(Hon) FACC FESC

Cardiology Department, Kings College Hospital, London, UK

Correspondence should be addressed to M J Monaghan: mark.monaghan@nhs.net

Abstract

Background: The mitral valve orifice area (MVOA) is difficult to assess accurately by 2D echocardiography because of geometric assumptions; therefore, 3D planimetry may offer advantages. We studied the differences in MVOA measurements between the most frequently used methods, to determine if 3D planimetry would result in the re-grading of severity in any cases, and whether it was a more accurate predictor of clinical outcomes.
Methods: This was a head-to-head comparison of the three most commonly used techniques to grade mitral stenosis (MS) by orifice area and to assess their impact on clinical outcomes. 2D measurements (pressure half-time (PHT), planimetry) and 3D planimetry were performed retrospectively on patients with at least mild MS. The clinical primary endpoint was defined as a composite of MV balloon valvotomy, mitral valve repair or replacement (MVR) and/or acute heart failure (HF) admissions.
Results: Forty-one consecutive patients were included; the majority were female (35; 85.4%), average age 55 (17) years. Mean and peak MV gradients were 9.4 (4) mmHg and 19 (6) mmHg, respectively. 2D and 3D measures of MVOA differed significantly; mean 2D planimetry MVOA was 1.28 (0.40) cm^2, mean 3D planimetry MVOA 1.15 (0.29) cm^2 ($P=0.003$). Mean PHT MVOA was 1.43 (0.44) cm^2 ($P=0.046$ and $P<0.001$ in comparison to 2D and 3D planimetry methods, respectively). 3D planimetry reclassified 7 (17%) patients from mild-to-moderate MS, and 1 (2.4%) from moderate to severe. Overall, differences between the two methods were significant (X^2, $P<0.001$). Only cases graded as severe by 3D predicted the primary outcome measure compared with mild or moderate cases (odds ratio 5.7).
Conclusion: 3D planimetry in MS returns significantly smaller measurements, which in some cases results in the reclassification of severity. Routine use of 3D may significantly influence the management of MS, with a degree of prediction of clinical outcomes.

Introduction

2D echocardiography, including Doppler, is the most commonly used method of assessment of mitral stenosis (MS) and is the basis of current guidelines on its management (1, 2). However, the mitral valve is a complex structure with a geometrically irregular appearance, especially in the presence of significant calcification

or rheumatic infiltration. It is therefore difficult to correctly bisect the leaflet tips using 2D transthoracic echocardiography (TTE). Other 2D methods derive the mitral valve orifice area (MVOA) from calculations involving the velocity of blood flow through the stenotic orifice, such as the pressure half-time (PHT) method. These are subject to haemodynamic variables such as loading, left ventricular compliance and coexisting valve disease and must therefore be interpreted in the context of potential confounders.

Given these inherent issues with 2D, the use of 3D echocardiography is becoming more established. 3D TTE offers an adjustable dataset that can be manipulated with multi-plane reconstruction to more accurately align with the true mitral valve orifice and has been validated against the data obtained by Gorlin's equation at invasive catheterisation (3). The present retrospective cohort study was designed to investigate how 3D TTE planimetry would compare with established 2D MVOA measures, and whether it would have additional predictive value with respect to clinical outcomes in MS.

Methods

This was a head-to-head comparison of the three most commonly used techniques to grade MS by orifice area. 3D measurements of the MVOA were performed retrospectively in 41 consecutive patients with at least mild MS by traditional 2D estimates (PHT and 2D planimetry MVOA). 2D and 3D measures were performed separately by blinded operators. The clinical primary endpoint was defined as a composite of MV balloon valvotomy, mitral valve repair or replacement (MVR) and/or acute heart failure (HF) admissions. This study was performed as part of an approved, retrospective, quality control audit within the department. Therefore, ethical approval was not sought or required.

Procedures

2D measures

All TTE imaging was acquired using commercial ultrasound systems (Philips EPIQ and X5-1 matrix array transducer, 3040 elements, frequency 1–5 MHz; GE Vivid E95 and M5Sc-D transducer, frequency 1.4–4.6 MHz). Standard TTE imaging was performed from the parasternal and apical windows. 2D planimetry of the mitral valve was acquired from the zoomed parasternal short axis view *en face* to leaflet opening, with harmonic imaging,

optimal gain and compression settings. The MVOA was manually traced along the inner edge of the leaflet tips at the point of maximal excursion during diastole, taking care to reduce the gain in order to avoid underestimation (Fig. 1). The PHT was calculated from continuous wave Doppler using alignment through the leaflet tips in mid-diastole in the apical four-chamber view. The MVOA was then calculated using the formula 220 divided by the PHT, providing that there was not more than moderate mitral or aortic regurgitation (4).

3D MVOA

3D image acquisition was taken from either the parasternal long axis (PLAX) or apical four-chamber window, depending on best image quality, in a zoomed view. 3D imaging was acquired in 3D zoom mode with the 2D image firstly optimised to ensure that the annulus and leaflet tips were included within the reference volume and the gain corrected. Acquisition was made in a single beat with a volume rate of at least 16 volumes/second. Measurement of the MVOA was performed offline on a dedicated workstation (Philips Q-Lab version 10.2 software; GE Healthcare Vivid-E95) by two operators blinded to the 2D acquisition measures. Multiplanar reconstruction was used to identify the maximal opening of the mitral valve leaflet tips by moving frame by frame through the cardiac cycle. Perpendicular planes were then aligned to bisect the mitral orifice, taking care to position the axial plane to include both leaflet tips. The plane was then moved iteratively through the orifice to identify the smallest opening. Planimetry was performed by tracing this area, with careful consideration of commissural fusion. It should be highlighted that this does not constitute an actual 3D volumetric representation of the mitral orifice, the measurement obtained by 3D analysis is still based on 2D dimensions; the difference lies in the ability to manipulate the image into a more correct alignment using 3D. In order to assess intra- and inter-observer variability, seven randomly selected cases were analysed separately by two independent observers. The outcome data were obtained by a trained practitioner who was blinded to both the echo data and the final analysis. The analysis of the outcome data is described in the 'Methods' section.

Statistical analysis

This retrospective cohort study was designed to compare 2D and 3D planimetry MVOA measurements to those obtained by PHT (the reference method).
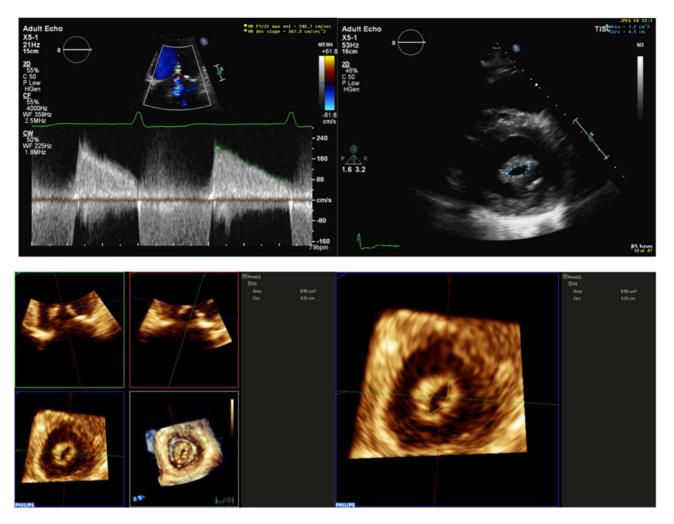
Figure 1

Images obtained from a single patient demonstrating the significant differences between measurements. This patient was assigned a grading of moderate MS based on 2D and spectral Doppler imaging, but falls into the severe category when 3D analysis is used. Panel A: Pressure half-time (PHT) measurement = MVOA 1.4 cm²; panel B: 2D planimetry obtained from the parasternal view = MVOA 1.2 cm²; panel C: alignment of 3D datasets obtained from the apical four-chamber view = MVOA 0.95 cm²; panel D: zoomed view of 3D planimetry.

Having demonstrated satisfactory normality of distribution, paired sample *t* tests were used to compare groups separately. Significance was considered to have been reached at the 5% level. Results are presented as mean values together with standard deviation (s.d.), while echocardiographic differences at baseline are presented as mean values, s.d. and 95% CIs. The sample size in this study was small, as was the number of cases meeting the primary outcome (a total of 14 cases). In order to form an acceptable multivariate logistic regression model, we therefore categorised cases into a binary variable of severe MS (yes/no) as per each method of MVOA assessment. We then calculated the Charlson comorbidity index (CCI) for each case to account for potential confounders in the regression model. This index is a well-validated tool to predict mortality from comorbid diagnoses, each of

which is weighted according to its potential influence on the outcome (5, 6). The regression model was formed of these two independent variables only (severe MS (yes/no) and the CCI) to predict the primary outcome. SPSS (IBM SPSS Statistics for Windows, version 24.0. Armonk, NY, USA: IBM Corp.) was used for all analyses.

Results

Baseline characteristics

The majority of patients were female (85.4%), mean age 55 (17) years. AF was present in 58%, otherwise the most common comorbidity was hypertension (30%) (Table 1). Echocardiography data are as described in Table 2. Average mean and peak Doppler gradients were 9.4 (4.3) mmHg

and 19.2 (6.3) mmHg, respectively. Left atrial (LA) volume and right ventricular systolic pressure (RVSP), traditional indicators of MS severity, were both significantly raised (111 (36.7) mL and 50 (24) mmHg, respectively).

2D and 3D measures of MVOA

Assuming the PHT technique as the method of reference, the mean MVOA was different between the three groups (*P*=0.046 and *P*<0.001 in comparison to 2D and 3D planimetry methods, respectively). The PHT method consistently returned the largest estimate of MVOA, while 3D assessment was significantly smaller than both PHT and 2D planimetry. The mean 2D planimetry MVOA was 1.28 (0.40) cm² and mean 3D planimetry MVOA 1.15 (0.29) cm² (*P*=0.003). The mean PHT was 168 (65) ms with a mean MVOA of 1.43 (0.44) cm² according to PHT MVOA estimation (*P*=0.046 and *P*<0.001 in comparison to 2D and 3D planimetry methods, respectively).

Reclassification of MS severity by 3D echo

Cases were classified into mild, moderate and severe MS in 9 (22%), 22 (53.7%) and 10 (24.4%), respectively, according to 2D planimetry. Only 2 (5%) patients were classified as

Table 1 Baseline characteristics.

Variable	Outcome (%)	Number of cases
Age (years)	55.5 (2.67)	41
CCI	2.17 (0.3)	41
Female	35 (85.4)	35/41
DM	16	6/37
Hypertension	30	11/37
Active smoking	13.5	5/37
Hypercholesterolaemia	16.2	6/37
CKD	7.5	3/40
Previous MI	5.4	2/37
Previous TIA or stroke	24.3	9/37
Previous cardiac surgery	2.7	1/37
AF	58	22/38
Pulmonary disease	40	12/30
NYHA at time of Echo		
I	39	14/36
II	28	10/36
III	28	10/36
IV	5.1	2/36
NYHA – most recent		
I	56.7	17/30
II	33.3	10/30
III	10	3/30
IV	0	0

AF, atrial fibrillation; CCI, Charlson comorbidity index; CKD, chronic kidney disease; DM, diabetes mellitus; MI, myocardial infarction; NYHA, New York Heart Association; TIA, transient ischaemic attack.

Table 2 Echocardiographic characteristics.

Variable	Mean (s.d.)	95% CI
Mean gradient (mmHg)	9.4 (4.3)	8–10.8
Peak gradient (mmHg)	19.2 (6.3)	17.2–21.2
PHT (ms)	168	147–189
MVOA by PHT (cm²)	1.43 (0.44)	1.3–1.57
MVOA by 2D planimetry (cm²)	1.28 (0.40)	1.15–1.41
MVOA by 3D planimetry (cm²)	1.15 (0.29)	1.06–1.24
E (cm/s)	1.93 (0.45)	1.77–2.09
A (cm/s)	1.73 (0.57)	1.49–1.98
E/A	1.05 (0.3)	0.9–1.1
E′ (cm/s)	5 (1.27)	4.4–5.6
LA area (cm²)	31 (7.5)	28.8–33.7
LA volume (mLs)	111 (36.7)	99.8–123.1
RVSP (mmHg)	50 (24)	41.8–58.7

A, mitral inflow A wave; cm², centimetres squared; E, mitral inflow E wave; E′, tissue Doppler E′ wave (averaged from the septal and lateral mitral annulus); LA, left atrium; mL, millilitres; mmHg, millimetres of mercury; ms, milliseconds; PHT, pressure half-time; RVSP, right ventricular systolic pressure.

having mild MS by 3D planimetry, 30 (73%) as moderate and 9 (22%) as severe. In comparison to 2D measures, 3D planimetry reclassified seven patients from mild-to-moderate MS, and one from moderate to severe. On the other hand, 2D reclassified two 3D-measured cases from moderate to severe (Fig. 2). Overall, the differences between the two methods were significant (chi-square test, *P*<0.001).

Prediction of clinical outcomes

As Table 3 shows, only the cases classified as severe by 3D were able to significantly predict the primary
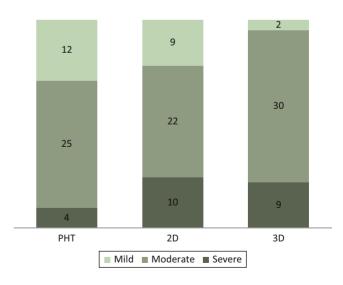

Figure 2
Chart depicting the numbers of patients assigned into each category of mitral stenosis by each of the methods of mitral valve orifice area assessment.

Table 3 Multivariate logistic regression models summary for predicting clinical outcomes.

Variable	OR	95% CI		P value	Accuracy of the model at classifying cases (%)
MVOA by PHT					
CCI	1.17	0.85	1.61	0.314	65.9
Severe MS (PHT)	0.67	0.06	7.2	0.741	
MVOA by 2D					
CCI	1.18	0.83	1.66	0.340	65.9
Severe MS (2D)	4.29	0.93	19.7	0.061	
MVOA by 3D					
CCI	1.15	0.81	1.63	0.420	65.9
Severe MS (3D)	5.7	1.13	28.7	0.035	

outcome; severe MS cases, which were classified by 3D as such, had an odds ratio (OR) of 5.7 of meeting the primary outcome compared to mild or moderate cases. While this is a rather elementary regression model, necessitated by the small sample size, it shows a clear signal that 3D is likely to be a better predictor of clinical outcomes. Primary outcome measures were met in the following number of cases: valvotomy ($n=4$); repair or replacement ($n=13$); acute HF admission ($n=10$); overlapping cases (acute HF admission and repair/replacement $n=7$, valvotomy, acute HF admission and repair $n=2$).

Table 3 summarises the models accordingly.

Correlation with haemodynamic variables

3D measures correlated well with both the right ventricular systolic pressure (RVSP) (Spearman's $=-0.504$, $P=0.002$) and LA volume (-0.354, $P=0.023$). 2D measurements correlated only with RVSP (-0.48, $P=0.003$), while MVOA by PHT did not correlate with any of the haemodynamic variables (Table 4).

Reproducibility analysis

We randomly selected seven cases (17% of cases) to test the inter-observer variability (reproducibility) of our cohort. Observers were asked to assess MVOA by 3D planimetry. The following results demonstrate good-to-excellent inter-observer variability (Table 5).

Discussion

This study demonstrated that 3D planimetry of the MVOA results in significantly smaller measurements compared with either 2D planimetry or PHT derived area, which in some cases results in the reclassification of MS severity. Perhaps more importantly, the study has also demonstrated that 3D planimetry is predictive of clinical outcomes in MS (including mitral valve surgery and acute HF admissions) and correlates well with haemodynamic markers of MS severity. Relying on traditional measures of valve disease severity is becoming increasingly difficult to defend with the development of technologies that offer clear advantages over 2D imaging. In relation to MS, there have been a number of recent advancements with respect to 3D technology including the introduction of modelling (7), semi-automated valve area tracing (8) and analysis of proximal flow convergence (9, 10). These 3D methods offer potential gains over standard imaging as the irregularity of the mitral valve means that during diastole, its leaflet tips cut across single planes making it difficult to ensure correct alignment. This study analysed the comparative differences between some of the most commonly used 2D and 3D MVOA measures.

Planimetry

There were significant differences between 2D planimetry and 3D planimetry for the assessment of the MVOA, with significantly smaller measurements obtained by 3D. This

Table 4 Summary of the correlation results between the MVO area and the haemodynamic parameters.

Haemodynamic variable	MVO by PHT	P value	MVO by 2D	P value	MVO by 3D	P value
RVSP	−0.279*	0.100	−0.480*	0.003	−0.504*	0.002
LA volume	−0.122*	0.448	−0.261*	0.099	−0.354*	0.023
LA area	0.040*	0.809	0.009*	0.957	−0.089*	0.589

*Spearman's correlation.
LA, left atrium; RVSP, right ventricular systolic pressure.

Table 5 Reproducibility analysis.

Inter-observer variability				
Mean difference	Value	95% CI		*P* value
	0.10	−0.099	0.119	0.831
ICC	Cronbach's alpha	95% CI		*P* value
	0.805	−0.134	0.967	0.033
S.E.M.	0.118			

ICC, interclass correlation; s.e.m., standard error measurement (cm²).

discrepancy is not unexpected and has been demonstrated in previous small studies (11, 12, 13), due to the adjustable 3D dataset allowing manipulation of the multiplanar reconstruction to more accurately intersect the valve orifice. Additionally, the degree of commissural fusion can be difficult to gauge on TTE, with one study finding commissural assessment feasible in only 60% of cases using 2D alone (13). However, post-processing of a 3D image not only allows adjustment of gain and brightness, but also the ability to assess the commissures from multiple different aspects throughout the cardiac cycle. It is likely that the consistent oversising of the MVOA by 2D planimetry with respect to 3D TTE seen in this study is at least in part due to difficulties in delineating the degree of commissural contribution to stenosis.

3D planimetry and PHT derived MVOA

The measures which differed most significantly in this study were the PHT derived MVOA and 3D planimetry. A well-established method of deriving the MVOA in MS, PHT is calculated as the time taken for the pressure drop across the stenotic valve to halve; the valve area is derived by dividing 220 by the PHT (4, 14). The half-time is directly proportional to net chamber compliance and the square root of the initial transmitral gradient, making it susceptible to haemodynamic variability (15). In our study, there was relatively poor correlation between PHT-derived area and 3D measures suggesting that baseline factors such as LA and LV compliance, as well as concomitant valve disease led to overestimates of the MVOA by the PHT. While planimetry is largely unaffected by surrounding haemodynamics, PHT is not so protected.

Prediction of clinical events and haemodynamic variables

One of the most interesting aspects of this study was the signal of clinical prediction from 3D planimetry, suggesting that it may better identify those with more clinically relevant disease. This was reinforced by the correlation of 3D with traditional haemodynamic markers of MS severity, namely the RVSP and LA volume. The agreement demonstrated between 3D planimetry and these important haemodynamic variables indicate that 3D provides a more complete picture of clinically relevant valve disease. In clinical practice, a method capable of accurately identifying the true degree of stenosis may allow clinicians to risk stratify those with MS who as yet may not have developed complications. As outlined above, the results of the regression analysis suggest that 3D planimetry is likely to predict clinical outcomes in this group of patients. This is interesting, as it suggests that 3D could be used in future models to provide a quantitative analysis of valvular health. Additionally, 3D planimetry is particularly attractive as a marker of valvular function as it provides a single metric which is easily appreciated by the clinician. To the authors' knowledge, this is the first study to link 3D planimetry to clinical outcomes. However, while innovative and interesting, the association is tentative given the small sample size and limited statistical modelling. A larger study would be required to more fully interrogate this encouraging development in the assessment of MS.

Limitations

This was a small retrospective study, constrained by the usual potential confounders of limited sample size. In particular, the causal link with clinical outcomes should be interpreted with some caution as the applied regression modelling will have been less robust than with a larger sample. Additionally, due to the retrospective study design, it is not clear whether the decision to operate would have been significantly influenced by 3D measurements. These were performed retrospectively by operators blinded to both 2D measures and clinical outcomes, and it is therefore not possible to link clinical decisions to 3D valve areas. It would be interesting to prospectively study the association between 3D measures and clinical decision making in a larger study.

Conclusion

The assessment of MS has traditionally relied on 2D measures, techniques that can be influenced by image orientation and surrounding haemodynamics. 3D planimetry does not require assumptions of normal chamber behaviour and is easily manipulated to correctly identify the true mitral orifice. This study demonstrates

that 3D planimetry generally provides smaller MVOA measurements that resulted in the reclassification of MS severity in some cases. More importantly, the study also shows a signal of clinical prediction from 3D that was not seen with comparable 2D methods and that 3D correlated with haemodynamic markers of MS severity. These findings are of considerable potential interest as clinicians look to more accurately risk stratify those with MS, indicating that 3D echocardiography provides a complete picture of clinically relevant valve disease.

References

1 Baumgartner H, Falk V, Bax JJ, De Bonis M, Hamm C, Holm PJ, Iung B, Lancellotti P, Lansac E, Rodriguez Muñoz D, *et al.* 2017 ESC/EACTS Guidelines for the management of valvular heart disease. *European Heart Journal* 2017 **2017** 2739–2791. (https://doi.org/10.1093/eurheartj/ehx391)

2 Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP, Fleisher LA, Jneid H, Mack MJ, McLeod CJ, O'Gara PT, *et al.* 2017 AHA/ACC focused update of the 2014 AHA/ACC guideline for the management of patients with valvular heart disease: a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. *Journal of the American College of Cardiology* 2017 **2017** 252–289. (https://doi.org/10.1161/CIR.0000000000000503)

3 Binder TM, Rosenhek R, Porenta G, Maurer G & Baumgartner H. Improved assessment of mitral valve stenosis by volumetric real-time three-dimensional echocardiography. *Journal of the American College of Cardiology* 2000 **36** 1355–1361. (https://doi.org/10.1016/S0735-1097(00)00852-4)

4 Hatle L, Angelsen B & Tromsdal A. Noninvasive assessment of atrioventricular pressure half-time by Doppler ultrasound. *Circulation* 1979 **60** 1096–1104. (https://doi.org/10.1161/01.CIR.60.5.1096)

5 Charlson ME, Pompei P, Ales KL & MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies:

development and validation. *Journal of Chronic Diseases* 1987 **40** 373–383. (https://doi.org/10.1016/0021-9681(87)90171-8)

6 Quan H, Li B, Couris CM, Fushimi K, Graham P, Hider P, Januel JM & Sundararajan V. Updating and validating the Charlson comorbidity index and score for risk adjustment in hospital discharge abstracts using data from 6 countries. *American Journal of Epidemiology* 2011 **173** 676–682. (https://doi.org/10.1093/aje/kwq433)

7 Mahmood F, Owais K, Taylor C, Montealegre-Gallegos M, Manning W, Matyal R & Khabbaz KR. Three-dimensional printing of mitral valve using echocardiographic data. *JACC: Cardiovascular Imaging* 2015 **8** 227–229. (https://doi.org/10.1016/j.jcmg.2014.06.020)

8 Mahmoud Elsayed HM, Hassan M, Nagy M, Amin A, Elguindy A, Wagdy K & Yacoub M. A novel method to measure mitral valve area in patients with rheumatic mitral stenosis using three-dimensional transesophageal echocardiography: feasibility and validation. *Echocardiography* 2018 **35** 368–374. (https://doi.org/10.1111/echo.13786)

9 Sampaio F, Ladeiras-Lopes R, Almeida J, Fonseca P, Fontes-Carvalho R, Ribeiro J & Gama V. Three-dimensional proximal flow convergence automatic calculation for determining mitral valve area in rheumatic mitral stenosis. *Echocardiography* 2017 **34** 1002–1009. (https://doi.org/10.1111/echo.13558)

10 de Agustin JA, Mejia H, Viliani D, Marcos-Alberca P, Gomez de Diego JJ, Nuñez-Gil IJ, Almeria C, Rodrigo JL, Luaces M, Garcia-Fernandez MA, *et al.* Proximal flow convergence method by three-dimensional color Doppler echocardiography for mitral valve area assessment in rheumatic mitral stenosis. *Journal of the American Society of Echocardiography* 2014 **27** 838–845. (https://doi.org/10.1016/j.echo.2014.04.023)

11 Min S-Y, Song J-M, Kim Y-J, Park H-K, Seo M-O, Lee M-S, Kim DH, Kang DH & Song JK. Discrepancy between mitral valve areas measured by two-dimensional planimetry and three-dimensional transoesophageal echocardiography in patients with mitral stenosis. *Heart* 2013 **99** 253–258. (https://doi.org/10.1136/heartjnl-2012-302742)

12 Pérez de Isla L, Casanova C, Almería C, Rodrigo JL, Cordeiro P, Mataix L, Aubele AL, Lang R & Zamorano JL. Which method should be the reference method to evaluate the severity of rheumatic mitral stenosis? Gorlin's method versus 3D-echo. *European Journal of Echocardiography* 2007 **8** 470–473. (https://doi.org/10.1016/j.euje.2006.08.008)

13 Schlosshan D, Aggarwal G, Mathur G, Allan R & Cranney G. Real-time 3D transesophageal echocardiography for the evaluation of rheumatic mitral stenosis. *JACC: Cardiovascular Imaging* 2011 **4** 580–588. (https://doi.org/10.1016/j.jcmg.2010.12.009)

14 Hatle L, Brubakk A, Tromsdal A & Angelsen B. Noninvasive assessment of pressure drop in mitral stenosis by Doppler ultrasound. *British Heart Journal* 1978 **40** 131–140. (https://doi.org/10.1136/hrt.40.2.131)

15 Thomas JD, Wilkins GT, Choong CY, Abascal VM, Palacios IF, Block PC & Weyman AE. Inaccuracy of mitral pressure half-time immediately after percutaneous mitral valvotomy. Dependence on transmitral gradient and left atrial and ventricular compliance. *Circulation* 1988 **78** 980–993. (https://doi.org/10.1161/01.CIR.78.4.980)