European Journal of Echocardiography (2010) **11**, 27–37
doi:10.1093/ejechocard/jep152

Right ventricle in pulmonary arterial hypertension: haemodynamics, structural changes, imaging, and proposal of a study protocol aimed to assess remodelling and treatment effects

Luigi P. Badano[1]*, Carmen Ginghina[2], Jacob Easaw[3], Denisa Muraru[2], Maria T. Grillo[1], Patrizio Lancellotti[4], Bruno Pinamonti[5], Gerry Coghlan[6], Martina Perazzolo Marra[7], Bogdan A. Popescu[2], and Salvatore De Vita[8]

[1]Department of Cardiopulmonary Sciences, University Hospital S Maria della Misericordia, P.le S Maria della Misericordia 15, 33100 Udine, Italy; [2]Prof. Dr C.C. Iliescu' Institute of Cardiovascular Diseases, Bucharest, Romania; [3]Royal United Hospital, Bath, UK; [4]Department of Cardiology, University Hospital Sart Tilman, Liege, Belgium; [5]Cardiovascular Department, Ospedali Riuniti and University of Trieste, Trieste, Italy; [6]FRCP Department, Royal Free Hospital, London, UK; [7]Department of Cardiothoracic and Vascular Sciences, University of Padua, Padua, Italy; and [8]Chair of Reumatology, Udine University P.le S Maria della Misericordia 15, 33100 Udine, Italy

Received 21 December 2008; accepted after revision 10 September 2009; online publish-ahead-of-print 7 October 2009

Although right ventricular (RV) failure is the main cause of death in patients with pulmonary arterial hypertension (PAH), there is insufficient data about the effects of PAH treatment on RV geometry and function mainly because the RV assessment has been hampered by its complex crescentic shape, large infundibulum, and its trabecular nature. Echocardiography is a widely available imaging technique particularly suitable for follow-up studies, because of its non-invasive nature, low cost, and lack of ionizing radiation or radioactive agent. Real-time three-dimensional echocardiography (RT3DE) has been shown to be accurate in assessing RV and left ventricular (LV) volumes, stroke volumes, and ejection fractions in comparison with cardiac magnetic resonance imaging. In this review, we describe RV structural and functional changes which occur in patients with PAH and strengths and weaknesses of current non-invasive imaging techniques to assess them. Finally, we describe an ongoing multicentre, prospective observational study involving seven centres expert in treating patients with PAH from four different countries. Investigators will use conventional and advanced echo parameters from RT3DE and speckle-tracking echocardiography to assess the extent of LV and RV remodelling before symptom onset and during pharmacological treatment in patients with PAH. Seventy patients who will survive for at least 1 year will be recruited. All the participating institutions will perform comprehensive standard 2D and Doppler as well as RT3DE examinations with a pre-defined imaging protocol. Measurements will be performed at the core echocardiography laboratory by experienced observers who will be unaware of each patient's treatment assignment and whether the examination was a baseline or a follow-up study. Enrolment duration is expected to be 1 year.

Keywords Right ventricle • Right ventricular function • Right ventricular remodelling • Speckle tracking • Treatment • Therapy • Echocardiography • Three-dimensional echocardiography • Pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a progressive and seriously debilitating disease characterized by pathological lesions of the pulmonary arteries progressing from early medial hypertrophy to end-stage plexiform fibrosis and leading to an increase in pulmonary artery pressure that may exceed systemic levels. Although pulmonary pressure rise is the distinctive characteristic of this disease, the level of pulmonary artery pressure itself has only modest prognostic significance in patients with PAH. It is rather the ability of the right ventricle (RV) to cope with the progressive increase in pulmonary arterial pressure that mainly

* Corresponding author. Tel: +39 0432 554557; fax: +39 0432 482353. *E-mail address*: lbadan@tin.it; badano.luigi@aoud.sanita.fvg.it

determines both the patients' functional capacity and survival.[1–4] However, in patients with PAH, RV dysfunction has not received the same scientific interest as the mechanisms of PAH.

Thus, the aims of our paper are: (i) to review the alterations of cardiac morphology and function associated with PAH; (ii) to outline the strengths and weaknesses of currently available non-invasive imaging technologies (mainly echocardiography) in assessing RV size and function; and (iii) to present a study protocol aimed at assessing the extent of RV remodelling that occurs in asymptomatic patients with PAH and in those treated with current disease-targeted therapies.

Right ventricle in pulmonary hypertension

The geometry of the normal RV is complex. It consists of an inflow (sinus) and an outflow (conus) portions separated by the crista supraventricularis. It is crescent shaped in cross-section, formed by the concave RV free wall opposite to the convex interventricular septum. Functionally, the RV pumps the same stroke volume as the left ventricle (LV), but using 25% of the stroke work because of the low resistance of the pulmonary vasculature. Therefore, the RV wall is thinner and more compliant than the LV wall. Normal RV contraction acts in a sequential manner, as a peristaltic wave directed from inflow to infundibulum. Spatially, RV wall deformation consists of three components (inward, longitudinal, and circumferential traction due to LV contraction), of which longitudinal shortening is the major contributor to the overall RV performance.[5] Right ventricular free wall and interventricular septum contribute equally to RV performance. Right ventricle is closely connected to the LV: they share a wall (interventricular septum); the RV free wall is attached to the anterior and posterior interventricular septum; and they have mutually encircling epicardial fibres and share the same intrapericardial space.

Significant morphological and functional adaptative changes of the RV develop in patients affected by PAH.[6]

The first adaptation that occurs is myocardial hypertrophy,[7] followed by progressive contractile impairment. Possible mechanisms involved in the progression of RV myocardial contractile dysfunction include: ischaemia,[8] changes in gene expression of the sarcomere proteins,[9] and activation of myocardial renin–angiotensin system.[10] However, afterload mismatch remains the main determinant of RV dysfunction in patients with PAH.

Right ventricle dilatation occurs in order to compensate a reduced fractional shortening by an increase in preload, so that stroke volume is maintained. As contractile dysfunction progresses, RV failure occurs and it is characterized by high RV filling pressures, diastolic dysfunction,[11] and reduced cardiac output. The decrease in cardiac output is also due to the functional tricuspid regurgitation caused by tricuspid annular dilatation and impaired leaflet coaptation. Augmented RV volume is the result of chamber remodelling, owing to the increase in cardiac myocyte length as a consequence of newly synthesized sarcomeres assembled in series. With hypertrophy and dilatation, the RV progressively becomes more spherical, its cross-sectional area enlarges, and the interventricular septum flattens, causing also LV diastolic dysfunction.[12]

Diastolic dysfunction is the most frequent type of LV impairment in patients with PAH. Usually, patients with PAH show a delayed relaxation pattern of LV filling and a small end-diastolic LV volume.[12] The reduced LV end-diastolic volume contributes to the decrease in stroke volume.[13] Although reduction of LV ejection fraction may occur in patients with PAH, it is a rather uncommon finding.

The exact mechanisms leading to the development of RV failure in patients with PAH are still unclear. Several mechanisms have been hypothesized: RV myocardial ischaemia, microvascular endothelial cell dysfunction, and myocyte apoptosis. In severe end-stage PAH, the RV changes its shape from the normal conformation to a more spherical one,[14,15] and RV wall stress increases because RV wall thickness does not increase proportionally.[16]

Imaging the right ventricle and assessing its geometry and function

A variety of two-dimensional echocardiographic (2DE) measurements and Doppler parameters have been described for the assessment of RV dimensions and function, although none so far has demonstrated superiority over cardiac catheterization or cardiac magnetic resonance (CMR). Major challenges for RV assessment by conventional 2DE derive from (i) complex asymmetric geometry; (ii) heavily trabeculated inner contour with poor endocardial definition; (iii) separate inflow and outflow which may be adequately visualized only from separate views; (iv) load-dependency and lower accuracy of most conventional echo parameters in comparison with invasive measures.[17]

Probably the best non-invasive correlates of RV function are ejection fraction (EF) and RV volumes, but these parameters are rather difficult to accurately obtain by conventional echocardiographic methods. Cardiac magnetic resonance is currently the most reliable non-invasive tool for this purpose, mostly due to its unique spatial resolution and 3D imaging capacity. Cardiac magnetic resonance is, however, inapplicable in certain patients (e.g. with claustrophobia or implanted metallic devices). In addition, its wide use is precluded by cost, long scan and analysis time, limited availability, and dedicated expertise. Moreover, CMR has limited ability to assess load-independent parameters (such as myocardial contractility) in patients with long-standing pressure overloaded RV.[18]

Unfortunately, echocardiographic assessment of RV in clinical practice is rather elusive and mostly qualitative. A multiparameter approach to compensate for each other flaws is time-consuming and not clinically feasible.

Recent recommendations from the 4th World Symposium on PAH from Dana Point, California[19] underline that a PAH-targeted therapy guided by RV function correlates may be superior to the traditional strategies outlined by previous guidelines.[20] Therefore, there is a compelling need for non-invasive assessment of accurate, simple, and reproducible parameters of RV geometry and function: (i) to outline the subtle changes in early disease stages or the

response to treatment in order to guide specific therapy; (ii) to compare measurements between patients or serially within a given patient; and (iii) to improve communication between clinicians.

The following paragraphs briefly review the various echocardiographic parameters proposed for RV geometry and function assessment, for an increased awareness of their pitfalls and of the obvious need for newer techniques to complement standard approaches.

Right ventricular linear dimensions

Right ventricle free wall thickness and dimensions can be easily measured from parasternal, apical four-chamber, or subcostal view. Cut-off values of RV diameters[21] may identify RV dilation independently or relatively to LV size. However, relative comparison to LV size may be misleading when LV dilation coexists. Right ventricular diameters are inherently 1D estimates and cannot describe by themselves the complex shape of RV. Although M-mode measurements are time-saving, widely applicable, and practical, even for less experienced operators, they are highly dependent on image quality, insonation angle, and load. Diameter measurement inconsistency is even higher when performed regardless of the respiratory phase. In addition, significant variability related to probe or patient position during examination renders them unreliable to identify mild RV abnormalities. For instance, simply rolling patient from supine to left lateral position increases measured RV diameter up to 40%.[22] Of note, progressive RV dilation is also accompanied by changes in heart position and by rotation in the thorax, which impact on the interpretation of serial measurements.

Right ventricular areas and right ventricular fractional area change

In patients with a dilated RV, the four-chamber view often displays a foreshortened cavity, since it often fails defining anatomical landmarks for the RV and may not intersect the RV crescent at its maximal dimension. Right ventricular area measurements are grossly affected by inherent manual tracing pitfalls, particularly in PAH patients with hypertrophied, heavily trabeculated RV. Although significantly correlated with angiographically measured RV volumes,[23] there is a significant overlap of the four-chamber view diameters and areas between normal and volume overloaded RV.[22] Right ventricular fractional area change (RVFAC) is calculated from the RV end-diastolic and end-systolic areas measured from four-chamber view as: RVFAC = 100 × (RV end-diastolic area − RV end-systolic area)/RV end-diastolic area. The RVFAC is a rather simple method of assessing RV function that has been shown to correlate well with CMR data.[21] When compared with other 2DE measures of RV systolic function [including tricuspid annular plane systolic excursion (TAPSE) and transversal fractional shortening], FAC was best correlated with CMR-determined EF.[24] Suboptimal RV endocardial definition, especially at the anterior RV wall, limits the accuracy and reproducibility of RVFAC.[25,26]

Right ventricular ejection fraction and right ventricular volumes by two-dimensional echocardiography

In clinical practice, probably the most intuitive parameters of RV size and contractile function are RV volumes and RVEF, same as for LV quantitation. Right ventricular ejection fraction is widely used as a noninvasive measure of RV performance, despite its well-known load dependency. Several methods are used to assess RV volumes and EF, including 2DE and real-time three-dimensional echocardiography (RT3DE), radionuclide angiography, and CMR. Selection of the best method and interpretation of its results hamper the adequate use of these indices.[27] Depending on the type of modality used to assess the RV, EF can vary from 40 to 76%. Currently, magnetic resonance imaging (MRI) is the gold standard for calculating RV volumes and EF. Cardiac MRI has shown that normal RVEF is 61 ± 7%, ranging from 47 to 76%.[17]

Several methods have been used to calculate RV volumes, such as the Simpson's method and the area–length method assuming the RV to be an ellipsoid or a pyramid. Four-chamber RV area and maximal short-axis diameter offered the best estimation of RV volumes with 2DE.[21] Volumes derived from calculations based on 2D measurements (diameters and areas) are based on geometric assumptions which are inaccurate in describing the complex RV shape. This explains the variable correlation of different RV models with angiographic or radionuclide studies. Difficulties in obtaining two standardized orthogonal RV views and failure to include the RV infundibulum (which may account for up to 25% of RV volume) are additional causes for 2DE limitations.[22] Image quality and operator experience may further impact on the results.

Tricuspid annular plane systolic excursion

This is a simple M-Mode measure from the apical four-chamber view of the longitudinal excursion of the lateral tricuspid annulus towards the RV apex, with demonstrated prognostic value in PAH.[26] Tricuspid annular plane systolic excursion is used as a correlate of RV systolic function, since longitudinal displacement of RV base accounts for the greater proportion of total RV volume change[28] in comparison with radial shortening in normal ventricles. A reasonable correlation between TAPSE and RVEF assessed by radionuclide angiography was found.[29]

However, TAPSE ignores the outlet portion and the septal contribution to RV ejection,[30] which may become important to maintain overall RV function,[25] especially when the longitudinal component decreases. In fact, TAPSE in patients after mitral valve repair surgery was discrepantly reduced in contrast with unchanged RVEF measured by RT3DE, demonstrating its reduced accuracy to estimate true RV performance after cardiac surgery.[31] Although simple, widely available, and feasible also in patients with poorly visualized RV endocardial borders, TAPSE is 1D and angle-dependent. Regional RV myocardial abnormalities are also neglected when using TAPSE as a single measure. Since it is measured with external reference point, it also takes into account the overall heart motion and not only the longitudinal excursion of RV base.[22] Moreover, its load dependency needs to be considered in case of significant tricuspid regurgitation, when

RV base active excursion may inaccurately reflect overall RV contractile function. Not only RV but also LV systolic performance may influence TAPSE value due to ventricular interdependence.[32]

Eccentricity index

Eccentricity index (EI) described by Ryan *et al.*[33] is a relatively easy-to-use measure of septal shift towards LV in overloaded RV. Eccentricity index is calculated on routine 2D short-axis view of LV and may discriminate between RV volume overload and pressure overload states. A high EI is an important echocardiographic predictor of mortality in PAH.[34] Improvement in EI is seen with PAH-targeted treatment.[13] However, there is the problem of different level measurements impacting on the results. Again, slightly off-axis images can result in artefactually flattened septum, giving rise to wrong EI. Other possible problem is identifying end-diastole and end-systole, which could also affect the measurements for EI.[33]

Myocardial performance (Tei) index

Myocardial performance index (MPI) is calculated from time intervals obtained from Doppler recordings of tricuspid and pulmonary flow. It incorporates both measures of systolic and diastolic function, so it represents an estimate of global RV performance independent of geometric assumptions. The normal value of this index for the RV is 0.28 ± 0.04 and increases with increasing RV dysfunction.[17] A significant advantage is that MPI is relatively independent of RV loading conditions and heart rate. Moreover, its prognostic value was ascertained in PAH patients[35] and its accuracy was also demonstrated in congenital heart disease,[36] myocardial infarction,[37] and chronic respiratory diseases.[38] The calculation of this index based on short intervals requires very good quality Doppler tracings with special care for optimal visualization of isovolumic event timing. Shortcomings derive from its calculation based on different cardiac cycle measurements and being adversely affected by rhythm and conduction disturbances. In addition, the Tei index may suffer a 'pseudonormalization' effect due to a shortened isovolumic relaxation time when increased right atrial pressure coexists.[30] Yet, colour-tissue Doppler enables the time measurements to be obtained from the same cardiac cycle in a more practical and time-saving manner.

Since newer echocardiographic technologies have been made available, novel indices of RV dimensions and function have been proposed and tested.

Tissue Doppler myocardial velocities

Right ventricular free wall sampling allows quantitative assessment of both RV systolic and diastolic function. Myocardial systolic velocity (*S*) by pulsed tissue Doppler imaging (TDI) is a relatively simple and accurate non-invasive correlate of RV systolic function, although several cut-off values have been described to predict RV systolic dysfunction in various studies ($<9.5-11.5$ cm/s).[39–42] It is important to mention that peak S-wave value with pulsed TDI and with colour-TDI cannot be used interchangeably, since the former measures peak myocardial velocities, whereas the latter measures mean myocardial velocities, which are ~20% lower.[5] Tissue Doppler imaging velocities may also be affected by overall cardiac motion and by tethering of the adjacent segments. Poor spatial resolution due to active systolic motion of RV base is problematic for the RV.

Isovolumic myocardial acceleration is a new TDI-derived parameter, less affected by preload or afterload. It is calculated by dividing the maximal isovolumic myocardial velocity by the time-to-peak velocity.[43] It was the most reliable contractility index among various velocity parameters in an experimental study.[44] Further validation of this new index is warranted for clinical use.[17]

Right ventricular deformation imaging

Tissue Doppler imaging has been extensively studied and validated for its ability to quantify myocardial deformation. Myocardial strain represents the percentage of myocardial fibre shortening and hence this could be used as a 'true' segmental systolic performance, being less dependent on preload. Strain and strain-rate abnormalities of RV are seen in pulmonary hypertension, as well as in amyloidosis, congenital heart diseases, and arrhythmogenic RV dysplasia. Doppler-derived strain and strain-rate may identify subtle changes in response to vasodilator treatment[45] and may outline early signs of RV involvement in the PAH course. Drawbacks of the TDI method are angle-dependency, 1D estimation, and low signal-to-noise ratio. It requires a high frame rate, for which separate wall imaging is usually accomplished with pulsed TDI. In contrast, colour TDI may facilitate simultaneous segmental recordings for comparative analysis of event timings.

Speckle-tracking echocardiography (STE) is a relatively new, non-invasive method for the assessment of myocardial deformation from grey-scale 2DE images. The unique speckle pattern in each region represents a 'fingerprint' which can be tracked frame-by-frame throughout the cardiac cycle and consequently derive myocardial deformation in 2D. Therefore, this technique has a great promise in assessing regional and global RV deformation in different directions, i.e. longitudinal, radial, and circumferential, in terms of both amplitude and timing (*Figure 1*). However, STE has been criticized for its lower temporal resolution and dependence on optimal image quality. Recently, this new method demonstrated its value to outline changes in RV systolic function, as assessed by 2D strain and strain-rate, in proportion to the severity of pulmonary pressure rise in PAH patients.[46] Also, it proved to be sensitive enough to detect early alterations of RV function in patients with systemic sclerosis and normal pulmonary pressures.[47]

Real-time three-dimensional echocardiography

Complex anatomy is ideally displayed using 3D reconstruction techniques. Therefore, the assessment of RV volume and function for clinical use may represent the most justified application of RT3DE, as the awareness of 2DE drawbacks in this regard and the advancements in ultrasound technology are fastly growing.

Accurate volume analysis independent of RV size and shape, without foreshortened views and geometric assumptions ensure the superiority of RT3DE for RV quantitation over the conventional echocardiographic methods. Good correlation between RT3DE and CMR for calculating RV volumes and EF was reported.[48] Compared with CMR data, RV volumes calculated from 3D

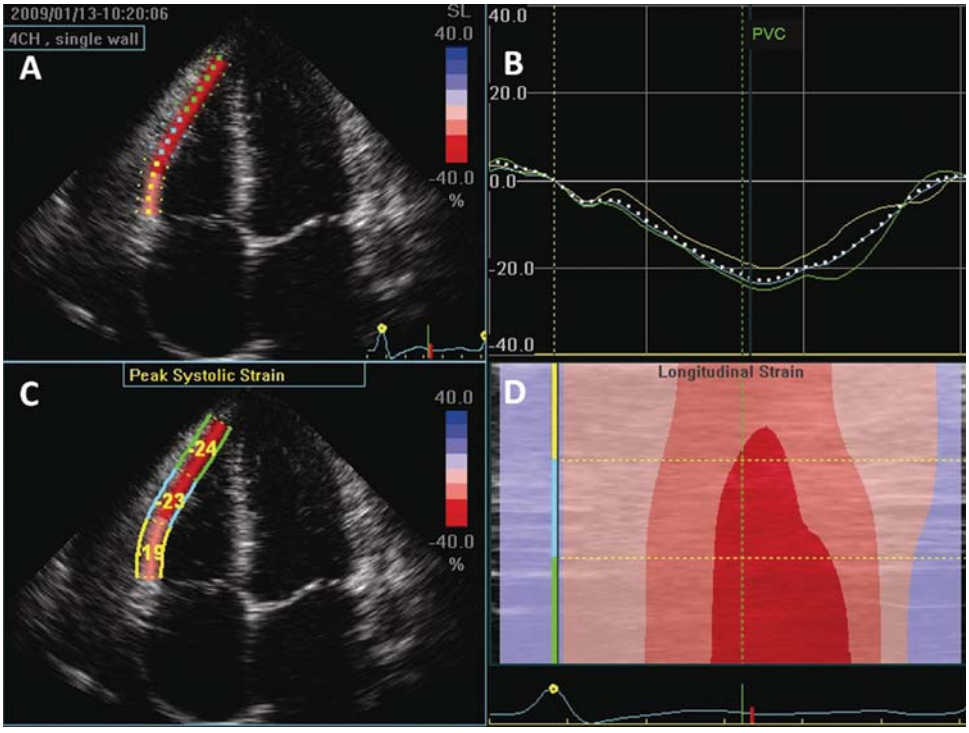

Figure 1 Longitudinal strain of the right ventricular free-wall assessed using the speckle-tracking modality. Apical four-chamber 'quad' view. (*A*) Tracked apical loop with colour coding of the three myocardial segments. (*B*) Average segmental strain graphically displaced. Each colour line corresponds to the same colour-coded myocardial segment. (*C*) Colour display of peak systolic strain. Colour scale shown on the right corner. (*D*) M-mode representation of peak systolic strain. Myocardial segments are colour-coded, strain colour scale same as in (*C*).

echocardiography showed significantly better agreement and lower intra- and interobserver variability than 2D echocardiography.[49] Real-time three-dimensional echocardiography enables unique views to outline and better understand particular causes of pulmonary hypertension, such as septal defects or complex congenital pathology.[50] Real-time three-dimensional echocardiography quantitation of RV end-diastolic volume was recently demonstrated to be feasible and superior to 2DE-derived value in the subset of PAH patients.[51] The capability to complement RV assessment with geometric data on tricuspid valve tenting in tricuspid regurgitation secondary to pulmonary hypertension confirms the unique value of RT3DE to comprehensively address PAH patients.[52]

Wide-angle full-volume acquisitions to accommodate enlarged RVs are presently feasible with good image quality in a time-saving manner. The apical approach is generally used to acquire full-volumetric data sets from four to seven ECG-triggered subvolumes. However, a key advantage of RT3DE is the ability to acquire full-volume RV data sets from any traditional parasternal, apical, or subcostal view to fit each patient's best imaging window. Then, post-processing analysis with electronic cropping, slicing, rotating, and translating tools allows the identification of pre-defined RV views to start quantitative analysis workflow. Current semi-automated softwares for offline analysis of RV based on RT3DE data sets use border-detection algorithms to obtain a dynamic surface-rendered RV cast which enables the evaluation of RV geometry and contraction from any perspective (*Figure 2*). Qualitative assessment is complemented by time–volume curve display and a panel of quantitative data derived from actual volumetric measurements and not simple calculations. Digitally archived 3D data sets may also be post-processed retrospectively for comparison during serial follow-up.

Yet, no imaging technique is ideal and RT3DE makes no exception. Patient's inability to cooperate for breathhold, arrhythmias, the larger footprint and size of matrix-array transducer, dependence on optimal acoustic quality, and need for specific training are among the most incriminated drawbacks of this method. To date, limited data are available regarding normal reference values for RV volumes and EF using RT3DE[49] and further extensive studies are required in this area.

Study protocol

Currently, several disease-targeted therapies are available such as endothelin receptor antagonists (ERA), phosphodiesterase-5 inhibitors, and prostacyclins that improve exercise capacity and cardiopulmonary haemodynamics, as shown in several large-scale clinical trials.[53] Two studies have shown that compared with placebo, short-term treatment with bosentan[13] or prostacyclin[54] prevented further enlargement of RV area. However, there is a lack of available data on the effect of long-term treatment of PAH on RV function and geometry.[1,55]

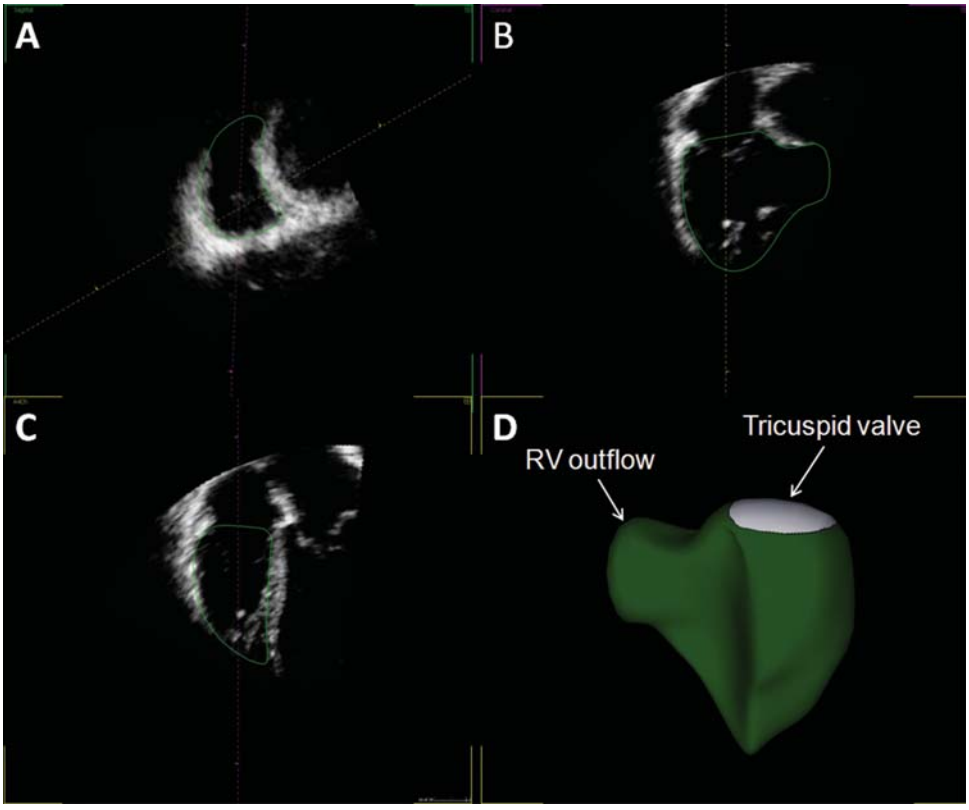

Figure 2 Real-time three-dimensional echocardiographic assessment of right ventricular volumes and ejection fraction. The right ventricular analysis programme displays three orthogonal planes: sagittal (*A*), coronal (*B*), and four-chamber (*C*). Results of three-dimensional right ventricular reconstruction are shown in (*D*).

Real-time three-dimensional echocardiography has been shown to be accurate in assessing RV and LV volumes, stroke volumes, and EFs in comparison with CMR imaging.[48]

Methods

Study objectives

The study has five main objectives:

(i) to investigate the effect of treatment on RV and LV remodelling and function using RT3DE (treated arm);
(ii) to assess the effect of treatment on RV and LV myocardial function using STE to measure maximal longitudinal 2D strain (treated arm);
(iii) to correlate results obtained from RT3DE and STE with changes in New York Heart Association (NYHA) functional class, 6 min walk test (6MWT), quality of life (QoL), and cardiac biomarkers in PAH patients (both treated and non-treated arms);
(iv) to compare results obtained from RT3DE and STE with conventional 2DE and Doppler indices of RV and LV function (both treated and non-treated arms);
(v) to assess the relationship between RV geometry and function with patient's clinical status irrespective of being in the disease-targeted group (treated arm) or in the conventional treatment group (non-treated arm).

Study design

This is a multicentre, prospective observational study involving seven centres: three Italian (Udine, Trieste, and Padua), two British (Bath and London), one Belgian (Liege), and one Romanian (Bucharest). All these centres are expert in treating patients with PAH. According to the current recommendations, PAH will be defined as a mean pulmonary artery pressure >25 mmHg at rest in the setting of a normal pulmonary arterial wedge pressure ≤ 15 mm Hg.[19,56] Two distinct groups of patients will be evaluated: Group 1 (treated arm), those in whom active therapy is required (ERA, phosphodiesterase-5 inhibitors, prostacyclins or, if acute vasoreactivity has been demonstrated, calcium channel blockers); and Group 2 (non-treated arm), patients with confirmed PAH at cardiac catheterization, but, for any reason (low functional class, contraindications, or patient refusal to assume targeted therapies), will receive only supportive therapy (i.e. patients on any or in combination with oxygen/diuretics/digoxin/anticoagulants). Treatment will be prescribed according to clinical criteria by the clinician taking care of the patient.[3]

Seventy patients in the treated arm ($\sim$10 per centre) who will survive for at least 1 year will be recruited. This sample volume will give a 90% power of showing a $\geq 15\%$ increase in the RV MPI (an echocardiographic parameter which is independently correlated with survival in patients with PAH[35]). The study inclusion and exclusion criteria are summarized in *Table 1*.

Table 1 Summary of the inclusion and exclusion criteria

All the following criteria need to be fulfilled for study inclusion:
Idiopathic PAH, associated with collagen vascular disease or due to chronic thrombo-embolic disease not suitable for thromboendarterectomy
A resting mean pulmonary artery pressure >25 mmHg at rest on right heart catheterization
Sinus rhythm
Age >18 years
Adequate acoustic window for speckle-tracking analysis and possibility to acquire 3D data sets for both right and left ventricular volume assessment
Any of the following criteria excludes the patient from the study:
Patients on active treatment for PAH or PAH secondary to congenital heart disease
Patients who are severely disabled and will not be able to complete the study
Significant lung disease as shown by FVC < 70% predicted or FEV1/FVC < 50%
HIV, portopulmonary hypertension, haemolytic anaemia associated PAH, anorexigen associated PAH
WHO Group 2 PAH as evidenced by pulmonary wedge pressure >15 mmHg or elevated LV end-diastolic pressure
WHO Group 5 PAH
Life expectancy <1 year due to severe PAH or any other forms of terminal disease
Pregnant women
Refusal to give informed consent

Study protocol

Patients with PAH confirmed by cardiac catheterization, who agree to be enrolled, will undergo the following evaluation before starting therapy:

(i) clinical evaluation including clinical examination, NYHA functional class assessment, Borg dyspnoea score,[57] Euro QoL questionnaire, and a 6MWT;[58]
(ii) resting electrocardiogram;
(iii) blood sampling for N-terminal pro-brain natriuretic peptide and troponin;
(iv) a representative 2DE, Doppler, and RT3DE study.

All clinical and echocardiographic examinations will be repeated at 3, 6, and 12 months on patients recruited to the treated arm for PAH. Patients in the non-treated arm for PAH will undergo the same evaluation at 6 and 12 months (*Figure 3*).

Two-dimensional and Doppler echocardiography

A comprehensive transthoracic 2D and Doppler echocardiography will be performed using second-harmonic imaging from parasternal and apical windows, with the patient in the left lateral position. Loops from three cardiac cycles will be acquired during breathhold. Particular attention will be paid to avoid foreshortening of apical views. Apical views of the LV and RV will be recorded with the sampling frequency of 60–80 Hz and sector width adjusted accordingly for subsequent analysis using STE (frame rate 60–80/s). Loops and tracings will be digitally stored for offline analysis.

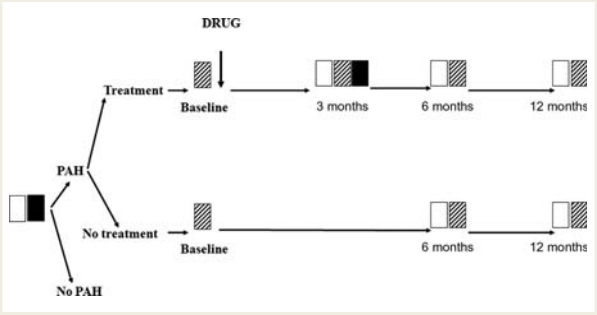

Figure 3 Study protocol. PAH, pulmonary artery hypertension. White boxes indicate clinical evaluation with NYHA class, quality of life assessment, cardiac troponin and N-terminal pro-brain natriuretic peptide dosage, 6 min walk test, and comprehensive two-dimensional and Doppler echocardiographic study performance; dashed boxes indicate three- and two-dimensional strain echocardiography.

Tissue velocity imaging

Pulsed tissue velocity imaging (TVI) will be performed using spectral pulsed-Doppler signal filters, adjusting the Nyquist limit to15–20 cm/s and using the minimal optimal gain. In the apical four-chamber view, a 5 mm pulsed Doppler sample volume will be placed at the level of the lateral mitral annulus and lateral tricuspid annulus. The apical view will be chosen to obtain a quantitative assessment of the regional wall motion almost simultaneously to the Doppler inflow and outflow and to minimize the insonation angle between the Doppler beam and the longitudinal wall motion. Pulsed TVI is characterized by a myocardial systolic wave (S_m) and two diastolic waves—early (E_m) and atrial (A_m). The myocardial peak velocity S_m (cm/s) and the time from Q-wave to S_m onset (ms) will be measured as systolic indices. E_m and A_m peak velocities (cm/s) and the E_m/A_m and E/E_m ratios will be determined as diastolic measurements.[59]

Real-time three-dimensional echocardiography

Harmonic RT3DE will be performed from the apical window immediately following 2DE and Doppler imaging, using a matrix-array transducer to obtain two pyramidal data sets with standard sector field and line density in the full-volume acquisition mode: one containing the entire LV cavity and a second one containing the RV cavity. In the full-volume acquisition mode, four wedges will be obtained over four consecutive cardiac cycles during a breathhold with electrocardiographic gating. Data sets will be digitally stored for offline analysis.

The ECG, respiratory rate, and blood pressure will be recorded throughout the imaging process for quantitative evaluation of echocardiographic data.

Echocardiographic image analysis

Recordings will be analysed at the core echocardiography laboratory by experienced observers who will be unaware of each patient's treatment assignment and whether the examination was at baseline or during follow-up. Two-dimensional echocardiography and Doppler measurements will be made on three representative beats and the results will be averaged. All heart chamber dimension measurements will be indexed to body surface area calculated using the DuBois method.

Echocardiographic variables

The following echocardiographic variables will be measured:

(i) left ventricular end-diastolic and end-systolic volumes;[21]
(ii) right ventricular fractional area change;[21]
(iii) tricuspid annular plane systolic excursion;[60]
(iv) eccentricity index will be calculated by the method of Ryan *et al.*;[33]
(v) maximal diameter and inspiratory collapse of the inferior vena cava during respiration will be measured from subcostal images to obtain an estimated right atrial pressure;[61]
(vi) pericardial effusion size will be determined from parasternal long- and short-axis views. Effusions will be graded and assigned a score as follows: absent (score = 0), trace (score = 1; separation of pericardial layers in both systole and diastole), small (score = 2; diastolic separation <1 cm), moderate (score = 3; diastolic separation of 1–2 cm), or large (score = 4; diastolic separation >2 cm);[54]
(vii) linear RV dimensions will be taken as per previously published guidelines.[21]

Doppler parameters

The following Doppler parameters will be measured:

(i) right ventricular (pulmonary artery) acceleration time;[62]
(ii) right ventricular ejection time will be measured from the RV outflow pulsed-wave Doppler signal as the interval from the onset of forward flow to pulmonic valve closure;
(iii) Doppler LV and RV indices, as global measures of LV and RV function, respectively, will be calculated as described by Tei *et al.*[63]
(iv) peak tricuspid regurgitant jet velocity;[64]
(v) cardiac output will be calculated from pulsed-wave Doppler recordings of the LV outflow tract velocity profile;
(vi) left ventricular filling will be assessed from pulsed-wave Doppler mitral inflow signals. Quantification will include measurements of mitral E- and A-wave peak velocities, and the E-wave deceleration time.

Two-dimensional speckle-tracking image analysis

Speckle-tracking analysis will be carried out using a commercially available echo analysis software (EchoPAC, BT08, GE Healthcare, Horten, Norway) equipped with Automatic Function Imaging analysis package. The software allows automated identification of natural acoustic markers within the myocardium[65,66] and subsequent tracking of their movements during the cardiac cycle. After manual marking of subendocardium, the endocardial and epicardial LV and RV borders will be automatically tracked and delineated. After manual adjustment (if necessary) or approval of the computer-generated myocardial wall delineation, the automated speckle-tracking analysis will be performed in the apical views, in which the LV myocardium will be automatically divided into six regions of interest (ROI) and the RV into three ROI (*Figure 1*). For each ROI thus identified, maximal longitudinal strain during cardiac cycle will be obtained.

Real-time three-dimensional echocardiographic right ventricular analysis

Real-time three-dimensional echocardiography RV analysis will be undertaken with a commercially available offline system (4D RV-Function 1.1, TomTec Imaging Systems GmbH, Unterschleissheim, Germany). Each RV volume data set will be imported into the application and manipulated by rotating, angulating, and slicing in any of the three displayed orthogonal planes, which will simultaneously reset views in the other two planes. A central reference point will be selected by moving through any plane. Secondary rotation is also possible. In this way, the clearest possible views will be obtained to start the full-volume analysis. All images will be viewed in sagittal (to outline the tricuspid valve in the best possible view), four-chamber (to outline the apex), and coronal (to outline the RV outflow tract) views, in the best possible view containing a full-volume set (*Figure 2*). Contrast, zoom, shading, and colour will be used to improve delineation of the endocardium. The end-diastolic and end-systolic frames are chosen in all views by visual inspection of cine loops. The endocardial border of the RV has to be drawn in end-diastole and at end-systole in every plane (sagittal, coronal, and four-chamber views); dependent on the quality of the data set, 5 up to 12 points will be sufficient for subsequent automatic contour detection. Three-dimensional images will be generated with the possibility for manual correction in every frame of the cardiac cycle (*Figure 4*). The interventricular septum will be excluded from RV volume assessment. Trabeculations will be included in the endocardial rim, but the apical component of the RV moderator band will be excluded from the cavity.

Real-time three-dimensional echocardiographic left ventricular analysis

Left ventricular volumes and EF will be measured with dedicated offline computers and software (4D LV analysis 2.6, TomTec Imaging Systems GmbH). The data set will be aligned in two orthogonal planes along the long axis of the single ventricle with clear depiction of the atrioventricular valve (*Figure 4*). Brightness and contrast will be adjusted to optimize signal-to-noise ratio. End-diastole will be chosen as the largest chamber size and/or the frame before mitral valve closure. End-systole will be chosen as the smallest chamber size and/or the frame before mitral valve opening. Using still and motion frames, the endocardial and epicardial borders will be identified and manually traced in sequential cross-sectional planes.

Statistical methods

The null hypothesis of the study is that in patients enrolled in the treated arm, there will be no change in echocardiographic and Doppler parameters of RV geometry and function from the baseline. Right ventricular Doppler MPI has been considered the primary parameter because of favourable changes shown in a previous pharmacological trial in patients with PAH[13] and its correlation with survival in these patients.[35] As a consequence, the sample size of the study was based on these changes. The changes from baseline to treatment values of each echocardiographic and Doppler parameter will be calculated for individual patients in whom technically adequate studies will be available at baseline, 3, 6 months, and 1 year. Statistical analysis will be based on the intention-to-treat population (full analysis set). Continuous data will be expressed as mean $\pm$ SD. Baseline data will be compared by means of the χ^2 test for categorical variables and unpaired *t*-test for continuous variables. Analysis of variance with the Tukey *post hoc* test will be used to analyse repeated measures. Multiple stepwise regression analyses will be performed to identify among the echocardiographic and Doppler parameters the predictors of the increase in the 6 min walking distance or of the QoL score improvement. Statistically significant variables in univariate analysis will be entered in the multivariate models. A value of $P < 0.05$ will be considered statistically significant. Statistical analyses will be performed using SPSS 13.0 (Chicago, IL, USA) for Windows.

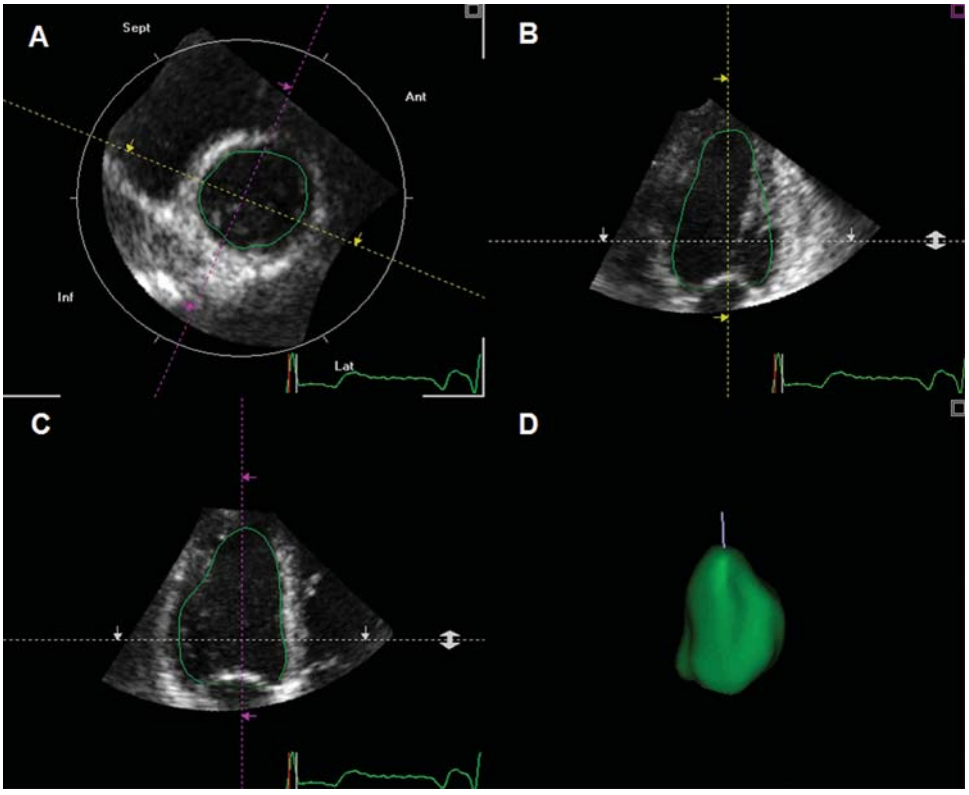

Figure 4 Real-time three-dimensional echocardiographic assessment of left ventricular volumes and ejection fraction. The left ventricular analysis programme displays three orthogonal planes: sagittal (*A*), four-chamber (*B*), and two-chamber (*C*). Results of three-dimensional left ventricular reconstruction are shown in (*D*).

Discussion

The present study has been designed to provide additional information that may help in understanding the pathophysiology of PAH and PAH-targeted treatment effects on RV geometry and function. Compared with current literature, this study presents many innovative aspects, summarized as follows:

(i) relatively long follow-up (up to 1 year);
(ii) use of RT3DE to assess changes in size and function of RV and LV;
(iii) assessment of RV geometry and function in PAH patients in NYHA class I and II;
(iv) assessment of RV myocardial function in PAH patients and its changes with and without therapy using 2D STE;
(v) explore the relationship between changes in RV and LV function during therapy, if any, with changes in patient functional class.

Conflict of interest: none declared.

Funding

D.M. was supported by a scientific grant from University Hospital S Maria della Misericordia. The work has been supported by an unrestricted grant from Actelion Pharmaceuticals Italia, s.r.l.

References

1. Chin KM, Kim NH, Rubin LJ. The right ventricle in pulmonary hypertension. *Coron Artery Dis* 2005;**16**:13–8.
2. D'Alonzo GE, Barst RJ, Ayres SM, Bergofsky EH, Brundage BH, Detre KM *et al.* Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. *Ann Intern Med* 1991;**115**:343–9.
3. McLaughlin VV, Sitbon O, Badesch DB, Barst RJ, Black C, Galiè N *et al.* Survival with first-line bosentan in patients with primary pulmonary hypertension. *Eur Respir J* 2005;**25**:244–9.
4. Sandoval J, Bauerle O, Palomar A, Gómez A, Martínez-Guerra ML, Beltrán M *et al.* Survival in primary pulmonary hypertension. Validation of a prognostic equation. *Circulation* 1994;**89**:1733–44.
5. Kukulski T, Hubbert L, Arnold M, Wranne B, Hatle L, Sutherland GR. Normal regional right ventricular function and its change with age: a Doppler myocardial imaging study. *J Am Soc Echocardiogr* 2000;**13**:194–204.
6. Galie N, Manes A, Palazzini M, Negro L, Romanazzi S, Branzi A. Pharmacological impact on right ventricular remodelling in pulmonary arterial hypertension. *Eur Heart J* 2007;**9**:H68–74.
7. Dias CA, Assad RS, Caneo LF, Abduch MC, Aiello VD, Dias AR. Reversible pulmonary trunk banding. II: an experimental model for rapid pulmonary ventricular hypertrophy. *J Thorac Cardiovasc Surg* 2002;**124**:999–1006.
8. Gómez A, Bialostozky D, Zajarias A, Santos E, Palomar A, Martínez ML *et al.* Right ventricular ischemia in patients with primary pulmonary hypertension. *J Am Coll Cardiol* 2001;**38**:1137–42.
9. Lowes BD, Minobe W, Abraham WT, Rizeq MN, Bohlmeyer TJ, Quaife RA *et al.* Changes in gene expression in the intact human heart. Downregulation of alpha-myosin heavy chain in hypertrophied, failing ventricular myocardium. *J Clin Invest* 1997;**100**:2315–24.
10. Abraham WT, Raynolds MV, Gottschall B, Badesch DB, Wynne KM, Groves BM *et al.* Importance of angiotensin-converting enzyme in pulmonary hypertension. *Cardiology* 1995;**86**:9–15.

11. Chen EP, Craig DM, Bittner HB, Davis RD, Van Trigt P. Pharmacological strategies for improving diastolic dysfunction in the setting of chronic pulmonary hypertension. *Circulation* 1998;**97**:1606–12.
12. Louie EK, Lin SS, Reynertson SI, Brundage BH, Levitsky S, Stuart S. Pressure and volume loading of the right ventricle have opposite effects on left ventricular ejection fraction. *Circulation* 1995;**92**:819–24.
13. Galiè N, Hinderliter AL, Torbicki A, Fourme T, Simonneau G, Pulido T *et al.* Effects of the oral endothelin-receptor antagonist bosentan on echocardiographic and Doppler measures in patients with pulmonary arterial hypertension. *J Am Coll Cardiol* 2003;**41**:1380–6.
14. Bristow MR, Zisman LS, Lowes BD, Abraham WT, Badesch DB, Groves BM *et al.* The pressure-overloaded right ventricle in pulmonary hypertension. *Chest* 1998; **114**:101–6S.
15. Boxt LM, Katz J, Kolb T, Czegledy FP, Barst RJ. Direct quantitation of right and left ventricular volumes with nuclear magnetic resonance imaging in patients with primary pulmonary hypertension. *J Am Coll Cardiol* 1992;**19**:1508–15.
16. Quaife RA, Chen MY, Lynch D, Badesch DB, Groves BM, Wolfel E *et al.* Importance of right ventricular end-systolic regional wall stress in idiopathic pulmonary hypertension: a new method for estimation of right ventricular wall stress. *Eur J Med Res* 2006;**11**:214–20.
17. Haddad F, Hunt SA, Rosenthal DN, Murphy DJ. Right ventricular function in cardiovascular disease. Part I. *Circulation* 2008;**117**:1436–48.
18. Biederman RW. Cardiovascular magnetic resonance imaging as applied to patients with pulmonary artery hypertension. *Int J Clin Pract* 2009;**63**:20–35.
19. Badesch DB, Champion HC, Sanchez MA, Hoeper MM, Loyd JE, Manes A *et al.* Diagnosis and assessment of pulmonary artery hypertension. *J Am Coll Cardiol* 2009;**54**:S55–66.
20. Galiè N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA *et al.* Guidelines for the diagnosis and treatment of pulmonary hypertension: The Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). *Eur Heart J*. 2009;**30**: in press
21. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA *et al.* Recommendations for chamber quantification. *Eur J Echocardiogr* 2006;**7**:79–108.
22. Jiang L, Levine RA, Weyman AE. Echocardiographic assessment of right ventricular volume and function. *Echocardiography* 1997;**14**:189–205.
23. Watanabe T, Katsume M, Matsukubo H, Furukawa K, Ijichi H. Estimation of right ventricular volume with two-dimensional echocardiography. *Am J Cardiol* 1982;**49**: 1946–53.
24. Anavekar NS, Gerson D, Skali H, Kwong RY, Yucel EK, Solomon SD. Two-dimensional assessment of right ventricular function: an echocardiographic-MRI correlative study. *Echocardiography* 2007;**24**:452–6.
25. Popescu BA, Antonini-Canterin F, Temporelli PL, Giannuzzi P, Bosimini E, Gentile F *et al.* Right ventricular functional recovery after acute myocardial infarction: relation with left ventricular function and interventricular septum motion. GISSI-3 Echo Substudy. *Heart* 2005;**91**:484–8.
26. Forfia PR, Fisher MR, Mathai SC, Housten-Harris T, Hemnes AR, Borlaug BA *et al.* Tricuspid annular displacement predicts survival in pulmonary hypertension. *Am J Respir Crit Care Med* 2006;**174**:1034–41.
27. Kovalova S, Necas J, Vespalec J. What is a 'normal' right ventricle? *Eur J Echocardiogr* 2005;**6**:15–23.
28. Smith JL, Bolson EL, Wong SP, Hubka M, Sheehan FH. Three-dimensional assessment of two-dimensional technique for evaluation of right ventricular function by tricuspid annular motion. *Int J Cardiovasc Imaging* 2003;**19**:189–97.
29. Ueti OM, Camargo EE, Ueti Ade A, de Lima-Filho EC, Nogueira EA. Assessment of right ventricular function with Doppler echocardiographic indices derived from tricuspid annular motion: comparison with radionuclide angiography. *Heart* 2002; **88**:244–8.
30. Lindqvist P, Calcutteea A, Henein M. Echocardiography in the assessment of right heart function. *Eur J Echocardiogr* 2008;**9**:225–34.
31. Tamborini G, Muratori M, Brusoni D, Celeste F, Maffessanti F, Caiani EG *et al.* Is right ventricular systolic function reduced after cardiac surgery? A two- and three-dimensional echocardiographic study. *Eur J Echocardiogr* 2009;**10**:630–4.
32. Lopez-Candales A, Rajagopalan N, Saxena N, Gulyasy B, Edelman K, Bazar R. Right ventricular systolic function is not the sole determinant of tricuspid annular motion. *Am J Cardiol* 2006;**98**:973–7.
33. Ryan T, Petrovic O, Dillon JC, Feigenbaum HF, Conley MJ, Armstrong W. An echocardiographic index to for separation of right ventricular volume and pressure overload. *J Am Coll Cardiol* 1985;**5**:918–24.
34. Raymond RJ, Hinderliter AL, Willis PW, Ralph D, Caldwell EJ, Williams W *et al.* Echocardiographic predictors of adverse outcomes in primary pulmonary hypertension. *J Am Coll Cardiol* 2002;**39**:1214–9.
35. Yeo TC, Dujardin KS, Tei C, Mahoney DW, McGoon MD, Seward JB. Value of a Doppler-derived index combining systolic and diastolic time intervals in predicting outcome in primary pulmonary hypertension. *Am J Cardiol* 1998;**81**:1157–61.
36. Eidem BW, Tei C, O'Leary PW, Cetta F, Seward JB. Nongeometric quantitative assessment of right and left ventricular function: myocardial performance index in normal children and patients with Ebstein anomaly. *J Am Soc Echocardiogr* 1998;**11**:849–56.
37. Yoshifuku S, Otsuji Y, Takasaki K, Yuge K, Kisanuki A, Toyonaga K *et al.* Pseudonormalized Doppler total ejection isovolume (Tei) index in patients with right ventricular acute myocardial infarction. *Am J Cardiol* 2003;**91**:527–31.
38. Yamaguchi K, Miyahara Y, Yakabe K, Kiya T, Nakatomi M, Shikuwa M *et al.* Right ventricular impairment in patients with chronic respiratory failure on home oxygen therapy- non-invasive assessment using a new Doppler index. *J Int Med Res* 1998;**26**:239–47.
39. De Castro S, Cavarretta E, Milan A, Caselli S, Di Angelantonio E, Vizza Carmine D *et al.* Usefulness of tricuspid annular velocity in identifying global RV dysfunction in patients with primary pulmonary hypertension: a comparison with 3D echo-derived right ventricular ejection fraction. *Echocardiography* 2008;**25**:289–93.
40. Meluzín J, Spinarová L, Bakala J, Toman J, Krejcí J, Hude P, Kára T *et al.* Pulsed Doppler tissue imaging of the velocity of tricuspid annular systolic motion: a new, rapid, and non-invasive method of evaluating right ventricular systolic function. *Eur Heart J* 2001;**22**:340–8.
41. Miller D, Farah MG, Liner A, Fox K, Schluchter M, Hoit BD. The relation between quantitative right ventricular ejection fraction and indices of tricuspid annular motion and myocardial performance. *J Am Soc Echocardiogr* 2004;**17**:443–7.
42. Saxena N, Rajagopalan N, Edelman K, Lopez-Candales A. Tricuspid annular systolic velocity: a useful measurement in determining right ventricular systolic function regardless of pulmonary artery pressures. *Echocardiography* 2006;**23**:750–5.
43. Vogel M, Derrick G, White PA, Cullen S, Aichner H, Deanfield J *et al.* Systemic ventricular function in patients with transposition of the great arteries after atrial repair: a tissue Doppler and conductance catheter study. *J Am Coll Cardiol* 2004;**43**:100–6.
44. Vogel M, Schmidt MR, Kristiansen SB, Cheung M, White PA, Sorensen K *et al.* Validation of myocardial acceleration during isovolumic contraction as a novel noninvasive index of right ventricular contractility: comparison with ventricular pressure-volume relations in an animal model. *Circulation* 2002;**105**:1693–9.
45. Borges AC, Knebel F, Eddicks S, Panda A, Schattke S, Witt C *et al.* Right ventricular function assessed by two-dimensional strain and tissue Doppler echocardiography in patients with pulmonary arterial hypertension and effect of vasodilator therapy. *Am J Cardiol* 2006;**98**:530–4.
46. Pirat B, McCulloch ML, Zoghbi WA. Evaluation of global and regional right ventricular systolic function in patients with pulmonary hypertension using a novel speckle tracking method. *Am J Cardiol* 2006;**98**:699–704.
47. Matias C, Isla LP, Vasconcelos M, Almería C, Rodrigo JL, Serra V *et al.* Speckle-tracking derived strain and strain-rate analysis: a technique for the evaluation of early alterations in right ventricle systolic function in patients with systemic sclerosis and normal pulmonary artery pressure. *J Cardiovasc Med (Hagerstown)* 2009; **10**:129–34.
48. Niemann PS, Pinho L, Balbach T, Galuschky C, Blankenhagen M, Silberbach M *et al.* Anatomically oriented right ventricular volume measurements with dynamic three-dimensional echocardiography validated by 3-Tesla magnetic resonance imaging. *J Am Coll Cardiol* 2007;**50**:1668–76.
49. Gopal AS, Chukwu EO, Iwuchukwu CJ, Katz AS, Toole RS, Schapiro W *et al.* Normal values of right ventricular size and function by real-time 3-dimensional echocardiography: comparison with cardiac magnetic resonance imaging. *J Am Soc Echocardiogr* 2007;**20**:445–55.
50. Hansalia S, Manda J, Pothineni KR, Nanda NC. Usefulness of live/real time three-dimensional transthoracic echocardiography in diagnosing acquired left ventricular-right atrial communication misdiagnosed as severe pulmonary hypertension by two-dimensional transthoracic echocardiography. *Echocardiography* 2009;**26**:224–7.
51. Amaki M, Nakatani S, Kanzaki H, Kyotani S, Nakanishi N, Shigemasa C *et al.* Usefulness of three-dimensional echocardiography in assessing right ventricular function in patients with primary pulmonary hypertension. *Hypertens Res* 2009;**32**: 419–22.
52. Sukmawan R, Watanabe N, Ogasawara Y, Yamaura Y, Yamamoto K, Wada N *et al.* Geometric changes of tricuspid valve tenting in tricuspid regurgitation secondary to pulmonary hypertension quantified by novel system with transthoracic real-time 3-dimensional echocardiography. *J Am Soc Echocardiogr* 2007;**20**:470–6.
53. Chin KM, Rubin LJ. Pulmonary arterial hypertension. *J Am Coll Cardiol* 2008;**51**: 1527–38.
54. Hinderliter AL, Willis PW 4th, Barst RJ, Rich S, Rubin LJ, Badesch DB *et al.* Effects of long-term infusion of prostacyclin (epoprostenol) on echocardiographic measures of right ventricular structure and function in primary pulmonary

hypertension. Primary Pulmonary Hypertension Study Group. *Circulation* 1997;**95**: 1479–86.
55. Coghlan JG, Davar J. How should we assess right ventricular function in 2008? *Eur Heart J Suppl* 2007;**9**:H22–8.
56. McLaughlin VV, Archer SL, Badesch DB, Barst RJ, Farber HW, Lindner JR *et al.* ACCF/AHA 2009 expert consensus document on pulmonary hypertension a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association developed in collaboration with the American College of Chest Physicians; American Thoracic Society, Inc.; and the Pulmonary Hypertension Association. *J Am Coll Cardiol* 2009;**53**:1573–619.
57. Borg GAV. Psycho-physical bases of perceived exertion. *Med Sci Sports Exerc* 1982;**14**:377–81.
58. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. *Am J Resp Crit Care Med* 2002;**166**:111–7.
59. Isaaz K, Thompson A, Ethevenot G, Cloez JL, Brembilla R, Pernot C. Doppler echocardiographic measurement of low velocity motion of the left ventricular posterior wall. *Am J Cardiol* 1989;**64**:66–75.
60. Hammarstrom E, Wranne B, Pinto FJ, Puryear J, Popp RL. Tricuspid annular motion. *J Am Soc Echocardiogr* 1991;**4**:131–9.
61. Kircher BJ, Himelman RB, Schiller NB. Noninvasive estimation of right atrial pressure from the inspiratory collapse of the inferior vena cava. *Am J Card* 1990;**66**:493–6.
62. Kitabatake A, Inoue M, Asao M, Masuyama T, Tanouchi J, Morita T *et al.* Noninvasive evaluation of pulmonary hypertension by a pulsed Doppler technique. *Circulation* 1983;**68**:302–9.
63. Tei C, Dujardin KS, Hodge DO, Bailey KR, McGoon MD, Tajik AJ *et al.* Doppler echocardiographic index for assessment of global right ventricular function. *J Am Soc Echocardiogr* 1996;**9**:838–47.
64. Currie PJ, Seward JB, Chan KL, Fyfe DA, Hagler DJ, Mair DD *et al.* Continuous wave Doppler determination of right ventricular pressure: a simultaneous Doppler-catheterization study in 127 patients. *J Am Coll Cardiol* 1985;**6**:750–6.
65. Reisner SA, Lysyansky P, Agmon Y, Mutlak D, Lessick J, Friedman Z. Global longitudinal strain: a novel index of left ventricular systolic function. *J Am Soc Echocardiogr* 2004;**17**:630–3.
66. Leitman M, Lysyansky P, Sidenko S, Shir V, Peleg E, Binenbaum M *et al.* Two-dimensional strain—a novel software for real-time quantitative echocardiographic assessment of myocardial function. *J Am Soc Echocardiogr* 2004;**17**:1021–9.