Updating clinical endpoint definitions

Paul M. Hassoun[1], Sylvia Nikkho[2], Erika B. Rosenzweig[3], Gail Moreschi[4], John Lawrence[4], John Teeter[5], Christian Meier[2], Ardeshir H. Ghofrani[6], Omar Minai[7], Paula Rinaldi[8], Evangelos Michelakis[9], and Ronald J Oudiz[10]

[1]Johns Hopkins University, Baltimore, Maryland, USA; [2]Bayer Pharma AG, Berlin, Germany; [3]Columbia University, New York; [4]US Food, and Drug Administration, Silver Spring, Maryland, USA; [5]Pfizer Incorporated, New London, Connecticut, USA; [6]University of Giessen, Giessen, Germany; [7]Cleveland Clinic, Cleveland, Ohio; [8]Novartis Pharmaceutical Corporation, East Hanover, New Jersey, USA; [9]University of Alberta, Edmonton, Alberta, Canada; and [10]David Geffen School of Medicine, University of California, Los Angeles, California, USA

ABSTRACT

The 6-Minute Walk Distance (6-MWD) has been the most utilized endpoint for judging the efficacy of pulmonary arterial hypertension (PAH) therapy in clinical trials conducted over the past two decades. Despite its simplicity, widespread use in recent trials and overall prognostic value, the 6-MWD has often been criticized over the past several years and pleas from several PAH experts have emerged from the literature to find alternative endpoints that would be more reliable in reflecting the pulmonary vascular resistance as well as cardiac status in PAH and their response to therapy. A meeting of PAH experts and representatives from regulatory agencies and pharmaceutical companies was convened in early 2012 to discuss the validity of current as well as emerging valuable endpoints. The current work represents the proceedings of the conference.

Key Words: end-points, clinical trials, pulmonary arterial hypertension

Clinical trials to assess the efficacy of drug therapy for pulmonary arterial hypertension (PAH) have routinely used the 6-Minute Walk Distance (6-MWD) as a validated primary endpoint since the first controlled trial over 15 years ago.[1] Although reflecting functional status and predicting survival in PAH, this simple and practical measure has certain limitations and therefore there have been recent calls for alternate endpoints[2] that are clinically significant, have pathophysiological relevance to the disease and are sensitive enough to be subjected to statistical analysis. The following is a summary from a roundtable discussion on clinical endpoints that brought together experts in PAH and representatives from Pharma and from regulatory agencies (i.e., the US Food and Drug Administration), whose task was to review the strengths and limitations of current endpoints, along with recommendations for improvement.

TRADITIONAL ENDPOINTS

Functional class

Functional classification (FC) is widely used as a marker of disease severity in cardiovascular disease and is strongly predictive of mortality.[1,3-6] In pulmonary hypertension (PH), it provides a measure of the limits imposed on a patient by the disease.[7,8] Regulatory agencies include FC in their labeling of PAH-specific therapies. Published treatment guidelines include FC in their recommendations for the evaluation and treatment of patients,[7,8] and FC is commonly employed as an endpoint in clinical studies of PH therapies. In addition, FC correlates with quality of life (QoL) assessment, enforcing its usefulness as an intermediate endpoint.[9]

New York Heart Association and World Health Organization Classification

The New York Heart Association (NYHA) Functional Classification System was primarily developed and validated in heart failure studies.[10] In 1998, the World Health Organization (WHO) expert panel amended the NYHA diagnostic classification system specifically for patients with PH in order to include symptoms such as dyspnea, fatigue and chest pain, as well as syncope and

Address correspondence to:
Dr. Paul M. Hassoun
Johns Hopkins University
Division of Pulmonary and Critical Care Medicine
1830 East Monument Street
Baltimore, MD 21205, USA
Email: phassoun@jhmi.edu

Access this article online	
Quick Response Code:	**Website:** www.pulmonarycirculation.org
	DOI: 10.4103/2045-8932.109920
	How to cite this article: Hassoun PM, Nikkho S, Rosenzweig EB, Moreschi G, Lawrence J, Teeter J, et al. Updating clinical endpoint definitions. Pulm Circ 2013;3:206.

near syncope more relevant to patients with PH.[11] Patients who have experienced syncope are generally assigned to WHO FC IV (although this is not explicitly stated in the WHO Functional Classification System). Due to similarities between the two classification systems, many clinicians refer to them collectively as NYHA/WHO Functional Classification.[12]

Prognostic value of functional classification

NYHA/WHO FC is a powerful predictor of survival in PH.[4,6] In untreated patients with idiopathic PAH (IPAH) or heritable PAH, the median survival was six months for WHO FC IV, two and a half years for WHO FC III and six years for WHO FC I and II.[13] In the REVEAL registry, patients who were NYHA/WHO FC IV at baseline had a significantly lower one-year survival rate than those with better functional class.[4] In observational studies of patients receiving epoprostenol therapy, survival was significantly longer in patients at NYHA FC III, compared with those with NYHA FC IV.[5] In a follow-up of patients receiving subcutaneous treprostinil, worse FC at baseline was associated with lower survival rates.[14] There was an association between increased mortality and baseline NYHA FC IV in a three-year follow-up of patients receiving oral bosentan.[6]

Functional classification as an endpoint in clinical trials

FC is an important endpoint in clinical trials of PAH therapy as it reflects patient well-being. The key advantages of FC as an endpoint are that changes can be easily measured and assessed within three months of therapy and are predictive of mortality. Therefore, FC can be used to trigger treatment adjustment.[11] It has also been included in the parameters defining goal-oriented treatment strategies.[15] Several clinical trials of prostacyclins, endothelin receptor antagonists and phosphodiesterase-5 inhibitors have shown improvements in FC [Table 1]. In the BREATHE-1 study, 42% of the bosentan-treated patients and 30% of the placebo-treated patients were in a better WHO FC at Week 16 than at baseline, which coincided with improvements in exercise capacity, Borg dyspnea score and reduced time to clinical worsening (TTCW).[16] In the SUPER study, sildenafil significantly improved WHO FC in addition to exercise capacity and hemodynamics.[17] In some clinical trials, improvements in FC were not evident despite improvements in other clinical endpoints. This may be related in part to the background treatments being used in these trials, making it more difficult to improve FC.[20,21]

Table 1: Improvements in new York Heart Association/ World Health Organization functional classification shown in pulmonary arterial hypertension clinical trials

Study	Treatments	Pts with FC improvement of ≥1 class	Other improved endpoints
BREATHE-1[16]	Bosentan Placebo	42% 30% (*P*=NS)	Exercise capacity, Borg dyspnea score, time to clinical worsening
SUPER-1[17]	Sildenafil 40 mg Placebo	36% 7% (*P*<0.001)	Exercise capacity, hemodynamics
Primary PH Study[18]	Epoprostenol Conventional therapy	40% 3% (*P*<0.02)	Exercise capacity, QoL, hemodynamics
AIR-1[19]	Iloprost Placebo	23.8% 12.7% (*P*=0.03)	Combined endpoint (NYHA FC+6-MWD), hemodynamics, dyspnea, QoL
TRIUMPH-1[20]	Treprostinil Placebo (add-on to oral therapy)	No significant improvement	Exercise capacity, QoL, NT-pro BNP
PHIRST[21]	Tadalafil Placebo	No significant improvement	Exercise capacity, QoL, time to clinical worsening

FC: functional classification; **BNP:** blasma brain natriuretic peptide; **PH:** pulmonary hypertension

Advantages/disadvantages of FC as a clinical endpoint

Strengths of FC as a clinical endpoint:

- Convenience
- Ease of classification
- Widely and broadly used
- Can be predictive of survival as well as QoL

Weaknesses of FC as a clinical endpoint:

- Self-reporting is required by patients
- The subjective nature of functional classification results in great variability in how classifications are judged between physicians[9]
  - Multiple factors not mentioned in NYHA/WHO definitions may be used
  - Definition of symptoms may differ widely among clinicians and are not reliable in children
  - A questionnaire could be used to aid standardization (AIR study, unpublished)
- Inconsistencies make inter-trial comparisons difficult
- The simplistic nature of this endpoint may mean that this classification is poorly discriminating and that subtle changes in clinical status will not be detected[12]
- The reliability and validity of this measure is not clearly established.

Recommendations

- FC will continue to be an important secondary endpoint in future clinical trials as well as a component of primary composite endpoints
- It provides a useful indicator of survival, physical capacity and well-being
- It is recognized in guidelines and by regulatory authorities
- However, as PH treatment improves, the focus of clinicians may shift to early detection of PH in patients

with less severe disease. Therefore, it may prove challenging to use FC as an endpoint when a greater proportion of patients are in FC I/II and it may become difficult to determine improvement from FC II to I
- Development of tools to promote a uniform approach to NYHA/WHO Functional Classification is an important step in helping to standardize the clinical care of patients with PAH and in performing and interpreting clinical studies
- A questionnaire for standardization to harmonize understanding of FC may be useful
- There may be scope for development of a tighter or subdivided functional classification system[12]
- NYHA/WHO Functional Classification may not serve as a single primary endpoint in clinical trials but may be useful as an important part of a composite endpoint. Indeed, it has been utilized successfully as part of a combined primary endpoint previously.[19]

Exercise capacity

The 6-Minute Walk Distance test

The use of exercise capacity as a measure of disease severity and treatment response is common in PH clinical trials and also in clinical practice. The most commonly used measure of exercise capacity is the 6-MWD test. It is not uncommon to enhance the 6-MWD test with a dyspnea rating at the end of the test, using either the Borg Dyspnea Index (BDI) or the Mahler dyspnea index. Adjunctive measures such as pulse oximetry (SpO_2) and heart rate (HR) can also be added to further characterize exercise performance during the 6-MWD test.

Two major strengths of the 6-MWD test include its simplicity and its widespread use and validation in PAH. It is a test that reflects activities of daily living and, to the extent that 6-MWD can be improved, it is a worthwhile metric. In addition, the 6-MWD distance has been shown to predict survival in several cardiopulmonary disorders including PAH.[1,22] Baseline 6-MWD, as well as thresholds of 6-MWD distance reached under treatment, has been shown to correlate with patient outcome.[4,23-25] In contrast, the change in 6-MWD, either in response to treatment or as patients deteriorate, has not been shown to correlate well with outcome,[25,26] although results have been variable depending on the length of observation.[27] A recent study, using both distributional and anchor-based methods and a large cohort of PAH patients, determined the minimal important difference (MID) in the 6-MWD test to be approximately 33 m.[28] Limitations of the 6-MWD test include a lack of ability to account for physical patient characteristics such as stride length and weight, a learning effect and inability to provide information on the physiologic response to exercise. Recent data suggest that 6-MWD test alone is not sufficient to define the clinical status of the patient.[29]

Recommendations

- The 6-MWD test should continue to be used as part of the clinical assessment of PH patients; however, it should not be considered a mandatory test
- Performance of the 6-MWD test should be standardized and should follow American Thoracic Society (ATS) guidelines[30]
- Patients must be developmentally able to perform the 6-MWD test and should not have physical or mental comorbidities that could influence the performance of the test
- Adjunctive measurements such as HR and SpO_2 can be used to enhance the interpretation of the test
- The use of the 6-MWD test as a primary endpoint alone in PH clinical trials should be restricted to instances whereby the results are projected to be both statistically and clinically significant

Cardiopulmonary exercise testing (CPET)

The physiologic response to exercise can be assessed with a comprehensive evaluation of several key exercise variables known to be affected by pulmonary vascular disease. These include HR and blood pressure, submaximal oxygen consumption (anaerobic threshold, AT), peak oxygen consumption (VO_2), ventilatory inefficiency (VE/VCO_2, $PETCO_2$), rest and exercise blood pressure and exercise and recovery patterns of these variables.

Strengths of CPET include its ability to evaluate physiologic severity, its prognostic use and its highly reproducible nature.[31-33] Limitations relative to the 6-MWD test include the need for technical expertise and longer time for administration and interpretation. The use of CPET in clinical trials has been discouraged due to the technical expertise required; however, this has not been the case in studies of other cardiopulmonary disorders such as heart failure, where CPET has often been the gold standard reference test.

Recommendations

- CPET should continue to be used as part of the clinical assessment of PH patients; however, it should not be considered a mandatory test
- Performance of CPET should be standardized and should follow ATS guidelines[34]
- Patients must be developmentally able to perform CPET and should not have physical or mental comorbidities that could influence the performance of the test
- As drug development with new targets and combination therapies emerges, the use of CPET as a primary endpoint in PH clinical trials should be reconsidered
- A core CPET lab must be used to systematically interpret all physiologic data captured at clinical recruiting sites
- Recruiting sites charged with obtaining CPET data for clinical trials must operate using standardized

validation procedures and must be validated by the core CPET lab.

Hemodynamics

Use of hemodynamic endpoints to assess response to therapy in clinical trials is justified and reasonable as hemodynamic alterations are integral to the causal pathway of PAH. The significance of baseline hemodynamic alterations has long been recognized. For instance, elevated right atrial pressure (RAP) and decreased cardiac index (CI) are strong predictors of death and/or lung transplantation.[13,35,36] However, traditional hemodynamic measures of disease severity, such as CI and RAP are inconsistently associated with outcomes in certain PAH groups such as scleroderma-associated PAH (SSc-PAH).[37-39] This may be related to differential responses to cardiac loads between SSc-PAH and IPAH as demonstrated in studies utilizing pressure-volume relationships, suggesting decreased mean ventricular pressure at any given afterload in SSc-PAH.[40] Other hemodynamic measurements such as pulmonary arterial capacitance (as estimated by stroke volume divided by pulmonary artery pulse pressure) independently predict survival in SSc-PAH.[38] Further, stroke volume index (SVI), perhaps a more specific measure of right ventricle (RV) function compared to CI, is also strongly predictive of outcome in this cohort; neither CI nor RAP independently predicted survival in this specific group of patients.[36]

Changes in hemodynamic values have also been examined in more recent studies. Reductions in RAP and mean pulmonary arterial pressure (mPAP) and increases in CI after intravenous epoprostenol therapy are associated with improved survival.[5] A failure of pulmonary vascular resistance (PVR) to decrease after therapy with bosentan or epoprostenol is a harbinger of poor prognosis.[25,27] Low CI and elevated RAP and PVR after 3 months of therapy with inhaled iloprost are associated with an increased risk of death.[41] Some of these hemodynamic endpoints have clearly been validated as surrogate markers in controlled trials. Significant reductions in mPAP and PVR and increases in CI, have been shown after a 12-week treatment with intravenous epoprostenol, the only controlled study in PAH that has shown improved survival with treatment,[1] and in response to sildenafil therapy.[17] Improved hemodynamics was also recently shown in a randomized double-blind, placebo-controlled, dose ranging study of sildenafil in treatment-naïve children with PAH.[42] However, the FDA recently recommended against the use of this drug in this population since there was an increased risk of death in the high- versus low-dose groups. Hemodynamic changes (decreased mPAP and PVR) in response to intravenous epoprostenol have also been shown in SSc-PAH, although there was no change in survival in this group.[43] Other trials, however, have shown little or no change in hemodynamics between drug and placebo.[19,44] Hemodynamic values are not currently accepted as endpoints by regulatory authorities.[45]

Strengths

- Hemodynamic data are accurate, reproducible and highly reflect the disease as integrated cardiopulmonary function
- They are done routinely in all referral centers and have been standardized
- They have baseline prognostic values and change in response to therapy (at least in IPAH).

Weaknesses

- They are invasive and time-consuming and may not necessarily represent a direct benefit to the patient
- They are usually obtained at baseline at rest and may not accurately reflect alterations related to exercise
- Optimal hemodynamic endpoint has not been defined
- They have not changed consistently in various recent trials; however, this may be related to the short time frame (e.g., 12 weeks), patient composition (e.g., SSc-PAH patients unlikely to show hemodynamic changes with current therapy) and add-on therapy trial.

Recommendations

- Hemodynamic data may be considered as primary endpoints (e.g., PVR, SVI, stroke volume/pulse pressure [SV/PP]) in select trials (e.g., children trials where other endpoints may be less reliable) and in randomized controlled trial (RCT) greater than four to six months, although missing values for patients who drop out or refuse a repeat catheterization represent a significant limitation.

OTHER CLINICAL ENDPOINTS

Clinical endpoints for PAH have undergone an evolution from the straight 6-MWD test to more comprehensive endpoints that reflect disease progression and/or medical failure. Amongst these endpoints, death and transplantation remain relatively clear, but the definition of "time to clinical worsening" has not always been consistent between trials.

Health-related quality of life in pulmonary arterial hypertension

Ideally, therapeutic interventions in PAH should improve symptoms, prolong survival and enhance QoL. Of these three therapeutic goals, the impact of therapeutic interventions on PAH-specific QoL is least well characterized. Most available health-related QoL (HR-QoL) data in PAH has been derived from existing generic (e.g., SF-36, EQ-5D) or condition-specific heart failure instruments such

as the Congestive Heart Failure Questionnaire directly employed or adapted for use as secondary endpoints in pharmaceutical-sponsored studies.[46,47] In this setting, instrument domains related to physical functioning appear to be the most sensitive to therapeutic interventions demonstrated to improve other functional parameters, such as the 6-MWD. However, the minimal change indicative of clinically meaningful improvement in PAH has not been determined for any instrument and the lack of consistency of HR-QoL instruments employed in therapeutic trials has made between-study comparisons difficult.

More recently, the Cambridge Pulmonary Hypertension Outcome Review (CAMPHOR) has been developed as a PAH-specific HR-QoL instrument.[48] This instrument was derived and validated from separate cohorts of PAH patients in the UK. The instrument has been validated outside of the UK (US),[49] although its performance in response to therapeutic interventions has yet to be determined. At least one additional PAH-specific HR-QoL instrument is currently in development.

The lack of a disease-specific instrument to assess HR-QoL in PAH in response to therapeutic interventions is currently an unmet need. The committee supports the development of specific and fully validated instruments for assessing HR-QoL in PAH.

All-cause mortality

Survival is the most meaningful clinical endpoint when evaluating new therapies; however, it can require the study of more than a thousand patients, which is not feasible in an orphan disease such as PAH. All-cause mortality is one endpoint that is easily measured but may overestimate the number of deaths attributable to PAH. Another option is to use "disease-related mortality" which would only include deaths due to PAH. This would require a clinical events committee to determine whether a death was due to PAH. Unfortunately, this is not always clear and may compromise the integrity of a trial. Further, mortality alone would not be a suitable endpoint because of the low event rate and inability to power a study adequately for a short-term trial.

Lung transplantation

Whether or not a patient undergoes lung transplantation is also clear to capture; however, there is still room for error here. There are likely center-specific patterns in lung transplant referral which may relate to the presence of a robust transplant program, success rates and average wait times, in addition to patient's disease severity and projected prognosis. Therefore, the likelihood of listing and actual transplantation during the course of a clinical trial may differ between centers. One may account for this in part by noting at the trial baseline whether a patient is "actively listed" or not. This information should be routinely included in baseline data collection. It seems reasonable to analyze the "time to transplant" for those "actively listed" at trial onset separately from those who are not. For the patients who were not previously listed, the need for "new transplant listing" should be the worsening event and the transplant a censored event. This is still not without bias based on center-specific practices.

Composite endpoints: TTCW

Since single surrogate endpoints such as the 6-MWD test are not ideal for clinical trials in PAH, composite endpoints have been proposed.[50] Therefore, composite endpoints have been used to increase the overall event rate and thereby reduce the number of patients needed for a trial. Indeed, the European Medicines Agency (EMA) encourages the use of a composite endpoint such as TTCW as the primary endpoint in PAH clinical trials (Table 2; EMA, 2009). TTCW has emerged as a frequently used secondary endpoint in recent long-term PH clinical trials. However, as McLaughlin et al.[45] emphasize in the Dana Point recommendations from 2009, "time to clinical worsening" has not been entirely consistent in its definition in various recent trials, but certainly has value as a composite endpoint. This endpoint was designed to

Table 2: Definition of time to clinical worsening in different trials

Component	BREATHE-1 & 351 (5,41)	EARLY (42)	STRIDE-1 (35)	STRIDE-2 (22)	ARIES-1 (43)	ARIES-2 (43)	SUPER-1 (21)	STEP (23)	PACES (44)
Death	√	√	√	√	√	√	√	√	√
Hospital stay	√	√		√	√	√	√	√	√
Lung transplantation	√		√	√	√	√	√	√	
Atrial septostomy	√		√	√	√	√			
Symptomatic progression (NYHA/WHO FC)	√	√		√	√	√			
Lack of improvement or worsening PAH (±6-min walk)	√	√		√	√	√		√	
Need for additional PAH therapy	√		√	√	√	√	√	√	√
P value	<0.05	<0.05	NS	NS	NS	<0.05	NS	<0.05	<0.005

CW: clinical worsening; **NYHA/WHO FC:** New York heart association/world health organization functional class; **PAH:** pulmonary arterial hypertension

be a more comprehensive analysis of disease progression, but has suffered from inconsistencies in definition, making trial comparisons more difficult. The most commonly used components of TTCW include events such as (1) all-cause mortality, (2) need for an interventional procedure including transplant or septostomy, (3) PAH-related hospitalization, and (4) some additional measures of clinical worsening which may include WHO FC progression, decline in 6-MWD by at least 15%, signs of worsening right heart failure and/or need for additional PAH-targeted therapies. Table 1[45] reports the various definitions used for TTCW in recent clinical trials. Composite endpoints include those that measure disease progression (e.g., TTCW) and those that assess improvement in a patient's physical capacity and well-being.

Endpoints measuring disease progression and deterioration

The impact of a treatment on disease progression associated with PAH can be measured by TTCW. This endpoint is viewed as clinically relevant by clinicians and regulatory agencies and has been used in several clinical trials as a secondary, or more recently a primary endpoint.[51] The composition of this endpoint varies from study to study. The main components include the following:

- Change in physical capacity, such as a 10-20% decrease in 6-MWD
- Deterioration in NYHA FC
- Significant clinical events such as need for hospitalization or additional therapy, transplantation, or mortality.

Endpoints measuring improvement of patient's physical capacity and well-being

Despite the usefulness of endpoints measuring disease progression and deterioration, from the perspective of both the patient and the treating physician, it may be more relevant to assess improvement in physical capacity and well-being. A composite endpoint was selected as the primary endpoint in the AIR study in order to give a more rigorous assessment of the efficacy of iloprost.[19] It included (1) an increase of at least 10% in the 6-MWD, (2) improvement in the NYHA/WHO FC and (3) absence of a deterioration in the clinical condition or death. A significant effect of treatment in favor of iloprost (P = 0.007) with an estimated odds ratio of 3.97 (95% CI 1.47-10.75) was found. Nearly 40% of patients showed increased 6-MWD by at least 10%. Approximately 20% of patients showed improvement in FC. Not all patients with improved FC had a 10% increase in 6-MWD. Thus, a larger proportion than met the primary endpoint met lesser criteria for clinical improvement to warrant continuation of therapy. Despite the usefulness of composite endpoints in measuring physical capacity and well-being and the obvious limitations of single surrogate endpoints, to date, the AIR study is the only large clinical trial that has employed such an endpoint.

Advantages/disadvantages of composite endpoints

Composite endpoints are derived from a combination of individual endpoints and have been validated in heart failure trials. They have several advantages over single endpoints:[52] (1) precision (and therefore statistical power) increases with event rate; (2) a composite endpoint can make it easier to detect a therapeutic benefit compared with analyzing each component separately, without requiring an increase in sample size (the higher the number of events, the smaller the sample size required based on more power to detect any treatment effect); and (3) besides mortality, clinically relevant components such as 6-MWD or NYHA/WHO FC may be incorporated, offering a more global assessment of the patient and their clinical condition. For both patients and physicians, it is more relevant to assess improvement over a short period of time rather than waiting for deterioration or death. Use of composite "improvement" endpoints allows individual responders to be identified, lowers the placebo response and thereby also lowers the number of patients needed. It also permits the investigation of a drug effect in a shorter period of time.

However, the use of composite endpoints in clinical trials also has several disadvantages: (1) for TTCW, the event rate may vary and is sometimes hard to predict at the start of a study. To mitigate this, more recent trials are "event-driven," that is, they keep patients enrolled until an endpoint occurs, which sometimes leads to considerable adjustments of the sample size and duration of the study;[51] (2) an individual component may confound the entire composite endpoint;[53] (3) outcomes such as hospitalization can be driven by social and nonmedical factors and need to be defined as disease driven;[54] (4) the inclusion of individual endpoints with country-specific availability, that is, lung transplantation, may pose an imbalance in multinational studies; (5) a composite endpoint assumes that each of the components has equal implications to the patient and the physician. This may not always be the case. For example, TTCW may be driven by deterioration in 6-MWD as opposed to death; and (6) due to the rigorousness of a composite "improvement" endpoint, the responder rate may be viewed as low even though a high proportion of patients may have benefitted in their clinical well-being overall.

Recommendations

- Appropriately designed and validated composite endpoints can provide a clinically relevant and valid means of investigating new treatments in trials
- Should a composite endpoint such as TTCW or improvement be incorporated into a trial, the individual components of such an endpoint should be clinically relevant, of prognostic value and ideally standardized across clinical trials in PAH[50]

- For non-PAH indications, the composition of such an endpoint may read differently and should be developed according to the underlying disease
- The successful design and implementation of composite endpoints into clinical trials will require a consensus to be reached between PH experts, pharmaceutical companies and regulatory authorities

The group of experts from the Dana Point 4th World Symposium proposed the following:

- A uniform definition of TTCW should be used in future pivotal (Phase III) RCTs in PAH. In the definition of TTCW, hard events would include the following:
  - All-cause mortality
  - Nonelective hospital stay for PAH (with predefined criteria, usually for initiation of intravenous prostanoids, lung transplantation, or septostomy)
  - Disease progression defined as a reduction from baseline in the 6-MWD by 15%, confirmed by two studies done within two weeks plus worsening FC (except for patients already in FC IV)

- The consensus was that when TTCW is used in an RCT, there would be an infrastructure required to adjudicate events in question. This will be necessary particularly with respect to "worsening PH" events. Other insights from the FDA on the use of TTCW as an endpoint have been that while acceptable, perhaps a numerical value assignment to each component would further enhance the reliability of this endpoint. In addition, considering capturing the total number of events would also provide a broader, more inclusive endpoint. A numerical system which would include multiple events for a given patient could be designed.

BIOMARKERS

BNP and NT-pro BNP

Plasma brain natriuretic peptide (BNP)[55-57] and its terminal prohormone (NT-pro BNP)[58] are secreted mainly by the ventricular myocytes in response to volume overload and increased wall stress. Hesselstrand et al.[59] showed that natriuretic peptide levels were related to the transtricuspid gradient in 227 consecutive patients with scleroderma. Several studies have evaluated BNP and NT-pro BNP as biomarkers of prognosis in patients with PAH.[55-58] Various cutoff levels of BNP have been associated with poor outcomes compared to patients with lower levels.[60] BNP and NT-proBNP have also been used in patients with PH in the setting of chronic parenchymal lung disease,[61,62] congenital systemic-to-pulmonary shunts,[63] and in acute and chronic thromboembolic disease.[55,64,65]

Uric acid

Serum uric acid (UA) is a marker of impaired oxidative metabolism and is elevated in several chronic conditions such as heart failure and chronic obstructive pulmonary disease (COPD). In a study of 99 IPAH patients, Nagaya et al.[66] showed that serum UA levels were elevated, correlated with pulmonary hemodynamics, had strong association with long-term mortality and decreased with vasodilator therapy.

Renal function

Decreased renal function as measured by elevated blood urea nitrogen levels[67] or increased serum creatinine and decreased glomerular filtration[68] have been shown to be associated with a worse hemodynamic profile and were independent predictors of mortality in patients with PAH.

Other circulating markers

Markers of endothelial dysfunction are of great interest in PAH. Endothelin-1 (ET-1) is a potent vasoconstrictor produced by endothelial cells[69] and has shown some promise as a biomarker for PAH. A small study[70] found that active ET-1 and its precursor, big ET-1, correlated with cardiopulmonary hemodynamics and 6-MWD and were strong prognostic markers for patients with IPAH. In a recent study of PAH patients,[71] ET-1/ET-3 ratio had a strong correlation with RAP, mixed venous oxygen saturation, WHO FC and 6-MWD.

D-dimer is elevated in patients with IPAH compared with controls and is associated with disease severity and 1-year survival.[72] Synthesized mainly in endothelial cells, plasma von Willebrand factor (vWF) plays a role in platelet aggregation and adhesion at sites of vascular injury, is elevated in severe PAH and changes in parallel with improvements in hemodynamics in response to prostacyclin therapy.[73] In a retrospective cohort study of PAH patients, increased vWF levels at baseline and follow-up were associated with reduced survival.[74] Elevated plasma vWF antigen (vWF: Ag) has also been found in PAH and baseline vWF: Ag correlated with the risk of death in the subsequent year.[75,76]

Several markers of inflammation, such as C-reactive protein,[77] growth differentiating factor-15,[78] and certain interleukins[79] have been shown to have potential for prognostic information as well; however, these require further study and validation. Cardiac troponin-T is a sensitive and specific marker for myocardial injury and can be detected in the setting of acute RV failure from acute pulmonary embolism.[80] Preliminary information suggests that detection of cardiac troponins may be markers of poor prognosis in patients PAH.[81] Very little information is available regarding changes in any of these biomarkers in response to therapy. A recent study showed an improvement in levels of angiopoietin 2, matrix metalloproteinase 9 and

vascular endothelial growth factor with the addition of intravenous treprostinil.[82]

There is very little experience with using blood biomarkers to assess response to therapy in PAH and thus there is little data on their utility regarding response to therapy in clinical practice.

Recommendations regarding blood biomarkers

- We should determine which biomarker (e.g., BNP vs. NT-pro BNP) should be used in clinical trials to ensure adequate validation of that variable
- All clinical trials going forward should, at a minimum, include BNP or NT-pro BNP as an exploratory measure of outcome
- All clinical trials should include at least two other biomarkers as exploratory outcome measures for future validation
- Blood and tissue repositories should be created in association with all clinical trials going forward so that if new biomarkers become available in the future, their validity may be determined objectively

Imaging of the RV in pulmonary arterial hypertension clinical trials

Despite the tremendous attention that left ventricular (LV) failure has received, RV failure has remained understudied both at the preclinical and clinical level, although in patients with PAH, the status of the RV is the most important predictor of both morbidity and mortality.[83-85]

RV function can be affected by experimental therapies that target the pulmonary circulation. For example, the PDE 5 inhibitors have direct effects on the hypertrophied (but not normal) RV.[86] The other two classes of currently approved drugs for PAH were both initially developed to treat LV diseases and both failed clinical trials with potentially increased mortality, suggesting possible adverse effects on the myocardium. Thus, the possibility that a negative response to an experimental therapy may be due to a suppression of RV function (while there are still beneficial effects on the pulmonary vessels) needs to be considered as it could completely alter the interpretation of the results.

Echocardiography

This is used widely in the assessment of patients with PAH and RV disease, although it remains inferior to magnetic resonance imaging (MRI) for overall assessment of RV function (mostly due to the complex, crescent-like shape of the RV). However, recently, two methods have emerged as reliable indices of RV function and contractility. Tricuspid Annular Plane Systolic Excursion (TAPSE) reflects the longitudinal systolic excursion of the lateral tricuspid valve annulus toward the apex. It is usually measured using M-mode imaging in the 4-chamber view and studies showed good correlation between TAPSE and RV ejection fraction measured by radionuclide angiography.[87-89] Another noninvasive index of contractility based on the myocardial isovolumic acceleration (IVA) assessed by tissue Doppler has been described; IVA reflects RV myocardial contractile function and is less affected by preload and afterload within a physiologic range when compared to either dP/dt max or elastance and has been extensively validated clinically.[90-92] Both methods are used clinically and can be standardized for clinical trials.

Magnetic resonance imaging

Cardiac MRI (cMRI) is the gold standard for evaluating right heart structure and function. The complex 3D structure of the RV can be directly evaluated with MRI in order to measure RV volume, mass and function (e.g., ejection fraction)[93,94] without the need for computational assumptions; values for RV mass and volume in normal cohorts have also been reported.[95] Recent studies using MRI have demonstrated the prognostic value of RV mass and end-diastolic volumes assessed by MRI in PAH.[96] MRI has a very high inter-study reproducibility of all methods for measurement of chamber volumes and mass,[97,98] making it an important tool for clinical trials.

Pulmonary angiography may also be performed using MRI and pulmonary blood flow can be quantified in patients with PAH.[99] In addition, RV stress (e.g., adenosine) perfusion protocols can be added in a manner similar to those applied for LV ischemia.[7,100,101] Recent studies showed evidence of MRI-measured ischemia in the RV of SSc-PAH patients.[102] If RV ischemia is considered as a contributor to RV failure, this technique may allow protocols to directly measure perfusion. In addition, if experimental therapies to modulate angiogenesis are tested in PAH, their potential effect on the RV should be considered. MRI offers the ability to measure lung parenchyma and RV free wall ischemia in the same setting. MRI's ability to offer "single stop shop" comprehensive assessment of the "RV-pulmonary circulation" unit is increasingly being recognized.[86]

Metabolic and molecular imaging

There is some evidence that the metabolism of the RV which changes as it hypertrophies is etiologically involved in RV failure and can be therapeutically targeted.[103] This means that it could be followed by imaging tools like positron emission tomography (PET). There are still many questions that need to be resolved with mechanistic studies (e.g., whether a switch in metabolism might be related to transition from compensated to de-compensated RV function). In addition, the performance of appropriate PET studies is difficult to standardize as it is also possible that some patients with PAH may have a generalized metabolic disturbance (e.g., insulin resistance[104]). Overall, the use of PET is promising, but its inclusion in clinical trials may be premature.

Recommendations

- cMRI is the gold standard test for assessment of RV function and remodeling. Therefore, some cMRI parameters (e.g., RV mass and RVEF) should be validated as endpoints for clinical trials.
- All clinical trials should include imaging sub-studies which would allow validation of valuable imaging endpoints.
- TAPSE should be validated as a reliable endpoint in response to therapy.

CONCLUSIONS

In this document, we have reviewed the evidence related to the validity of current and emerging endpoints in clinical trials for PAH. We believe there is at this time an urgent need to identify and validate novel endpoints that reliably reflect the disease status (both from a pulmonary vascular and RV standpoint) and its response to therapy. Composite endpoints seem to be most valuable at this time although defining the best objective endpoints (including survival and lung transplantation) to be included into a composite score may be challenging. As treatment of the disease is slowly moving to more effective targeted therapy, this effort at defining reliable endpoints should be rewarding.

REFERENCES

1. Barst RJ, Rubin LJ, Long WA, McGoon MD, Rich S, Badesch DB, et al. A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension. The Primary Pulmonary Hypertension Study Group. N Engl J Med 1996;334:296-302.
2. Peacock A, Naeije R, Galie N, Reeves JT. End points in pulmonary arterial hypertension: The way forward. Eur Respir J 2004;23:947-53.
3. Benza RL, Gomberg-Maitland M, Naeije R, Arneson CP, Lang IM. Prognostic factors associated with increased survival in patients with pulmonary arterial hypertension treated with subcutaneous treprostinil in randomized, placebo-controlled trials. J Heart Lung Transplant 2011;30:982-9.
4. Benza RL, Miller DP, Gomberg-Maitland M, Frantz RP, Foreman AJ, Coffey CS, et al. Predicting survival in pulmonary arterial hypertension: Insights from the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL). Circulation 2010;122:164-72.
5. McLaughlin VV, Shillington A, Rich S. Survival in primary pulmonary hypertension: The impact of epoprostenol therapy. Circulation 2002;106:1477-82.
6. McLaughlin VV, Sitbon O, Badesch DB, Barst RJ, Black C, Galie N, et al. Survival with first-line bosentan in patients with primary pulmonary hypertension. Eur Respir J 2005;25:244-9.
7. Galie 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;30:2493-537.
8. Rubin LJ: Diagnosis and management of pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. Chest 2004;126:4S-6.
9. Taichman DB, Shin J, Hud L, Archer-Chicko C, Kaplan S, Sager JS, et al. Health-related quality of life in patients with pulmonary arterial hypertension. Respir Res 2005;6:92.
10. Bennett JA, Riegel B, Bittner V, Nichols J. Validity and reliability of the NYHA classes for measuring research outcomes in patients with cardiac disease. Heart Lung 2002;31:262-70.
11. McLaughlin VV, McGoon MD. Pulmonary arterial hypertension. Circulation 2006;114:1417-31.
12. Hoeper MM, Oudiz RJ, Peacock A, Tapson VF, Haworth SG, Frost AE, et al. End points and clinical trial designs in pulmonary arterial hypertension: Clinical and regulatory perspectives. J Am Coll Cardiol 2004;43:48S-55.
13. 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.
14. Barst RJ, Galie N, Naeije R, Simonneau G, Jeffs R, Arneson C, et al. Long-term outcome in pulmonary arterial hypertension patients treated with subcutaneous treprostinil. Eur Respir J 2006;28:1195-203.
15. Hoeper MM, Markevych I, Spiekerkoetter E, Welte T, Niedermeyer J. Goal-oriented treatment and combination therapy for pulmonary arterial hypertension. Eur Respir J 2005;26:858-63.
16. Rubin LJ, Badesch DB, Barst RJ, Galie N, Black CM, Keogh A, et al. Bosentan therapy for pulmonary arterial hypertension. N Engl J Med 2002;346:896-903.
17. Galie N, Ghofrani HA, Torbicki A, Barst RJ, Rubin LJ, Badesch D, et al. Sildenafil citrate therapy for pulmonary arterial hypertension. N Engl J Med 2005;353:2148-57.
18. Battle RW, Davitt MA, Cooper SM, Buckley LM, Leib ES, Beglin PA, et al. Prevalence of pulmonary hypertension in limited and diffuse scleroderma. Chest 1996;110:1515-9.
19. Olschewski H, Simonneau G, Galie N, Higenbottam T, Naeije R, Rubin LJ, et al. Inhaled iloprost for severe pulmonary hypertension. N Engl J Med 2002;347:322-9.
20. McLaughlin VV, Benza RL, Rubin LJ, Channick RN, Voswinckel R, Tapson VF, et al. Addition of inhaled treprostinil to oral therapy for pulmonary arterial hypertension: A randomized controlled clinical trial. J Am Coll Cardiol 2010;55:1915-22.
21. Galie N, Brundage BH, Ghofrani HA, Oudiz RJ, Simonneau G, Safdar Z, et al. Tadalafil therapy for pulmonary arterial hypertension. Circulation 2009;119:2894-903.
22. Miyamoto S, Nagaya N, Satoh T, Kyotani S, Sakamaki F, Fujita M, et al. Clinical correlates and prognostic significance of six-minute walk test in patients with primary pulmonary hypertension. Comparison with cardiopulmonary exercise testing. Am J Respir Crit Care Med 2000;161:487-92.
23. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, et al. Survival in patients with idiopathic, familial and anorexigen-associated pulmonary arterial hypertension in the modern management era. Circulation 2010;122:156-63.
24. Nickel N, Golpon H, Greer M, Knudsen L, Olsson K, Westerkamp V, et al. The prognostic impact of follow-up assessments in patients with idiopathic pulmonary arterial hypertension. Eur Respir J 2012;39:589-96.
25. Sitbon O, Humbert M, Nunes H, Parent F, Garcia G, Herve P, et al. Long-term intravenous epoprostenol infusion in primary pulmonary hypertension: Prognostic factors and survival. J Am Coll Cardiol 2002;40:780-8.
26. Savarese G, Paolillo S, Costanzo P, D'Amore C, Cecere M, Losco T, et al. Do changes of 6-minute walk distance predict clinical events in patients with pulmonary arterial hypertension?: A meta-analysis of 22 randomized trials. J Am Coll Cardiol 2012;60:1192-201.
27. Provencher S, Sitbon O, Humbert M, Cabrol S, Jais X, Simonneau G. Long-term outcome with first-line bosentan therapy in idiopathic pulmonary arterial hypertension. Eur Heart J 2006;27:589-95.
28. Mathai SC, Puhan MA, Lam D, Wise RA. The minimal important difference in the 6-minute walk test for patients with pulmonary arterial hypertension. Am J Respir Crit Care Med 2012;186:428-33.
29. Gabler NB, French B, Strom BL, Palevsky HI, Taichman DB, Kawut SM, et al. Validation of 6-minute walk distance as a surrogate end point in pulmonary arterial hypertension trials. Circulation 2012;126:349-56.
30. ATS statement: Guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111-7.
31. Hansen JE, Sun XG, Yasunobu Y, Garafano RP, Gates G, Barst RJ, et al. Reproducibility of cardiopulmonary exercise measurements in patients with pulmonary arterial hypertension. Chest 2004;126:816-24.
32. Wensel R, Opitz CF, Anker SD, Winkler J, Hoffken G, Kleber FX, et al. Assessment of survival in patients with primary pulmonary hypertension: Importance of cardiopulmonary exercise testing. Circulation 2002;106:319-24.
33. Oudiz RJ, Barst RJ, Hansen JE, Sun XG, Garofano R, Wu X, et al. Cardiopulmonary exercise testing and six-minute walk correlations in pulmonary arterial hypertension. Am J Cardiol 2006;97:123-6.
34. Ross RM. ATS/ACCP statement on cardiopulmonary exercise testing. Am

J Respir Crit Care Med 2003;167:1451.
35. Sandoval J, Bauerle O, Palomar A, Gomez A, Martinez-Guerra ML, Beltran M, et al. Survival in primary pulmonary hypertension. Validation of a prognostic equation. Circulation 1994;89:1733-44.
36. Glanville AR, Burke CM, Theodore J, Robin ED: Primary pulmonary hypertension. Length of survival in patients referred for heart-lung transplantation. Chest 1987;91:675-81.
37. Fisher MR, Mathai SC, Champion HC, Girgis RE, Housten-Harris T, Hummers L, et al. Clinical differences between idiopathic and scleroderma-related pulmonary hypertension. Arthritis Rheum 2006;54:3043-50.
38. Campo A, Mathai SC, Le Pavec J, Zaiman AL, Hummers LK, Boyce D, et al. Hemodynamic predictors of survival in scleroderma-related pulmonary arterial hypertension. Am J Respir Crit Care Med 2010;182:252-60.
39. Condliffe R, Kiely DG, Peacock AJ, Corris PA, Gibbs JS, Vrapi F, et al. Connective tissue disease-associated pulmonary arterial hypertension in the modern treatment era. Am J Respir Crit Care Med 2009;179:151-7.
40. Overbeek MJ, Lankhaar JW, Westerhof N, Voskuyl AE, Boonstra A, Bronzwaer JG, et al. Right ventricular contractility in systemic sclerosis-associated and idiopathic pulmonary arterial hypertension. Eur Respir J 2008;31:1160-6.
41. Opitz CF, Wensel R, Winkler J, Halank M, Bruch L, Kleber FX, et al. Clinical efficacy and survival with first-line inhaled iloprost therapy in patients with idiopathic pulmonary arterial hypertension. Eur Heart J 2005;26:1895-902.
42. Barst RJ, Ivy DD, Gaitan G, Szatmari A, Rudzinski A, Garcia AE, et al. A randomized, double-blind, placebo-controlled, dose-ranging study of oral sildenafil citrate in treatment-naive children with pulmonary arterial hypertension. Circulation 2012;125:324-34.
43. Badesch DB, Tapson VF, McGoon MD, Brundage BH, Rubin LJ, Wigley FM, et al. Continuous intravenous epoprostenol for pulmonary hypertension due to the scleroderma spectrum of disease. A randomized, controlled trial. Ann Intern Med 2000;132:425-34.
44. Simonneau G, Barst RJ, Galie N, Naeije R, Rich S, Bourge RC, et al. Continuous subcutaneous infusion of treprostinil, a prostacyclin analogue, in patients with pulmonary arterial hypertension: A double-blind, randomized, placebo-controlled trial. Am J Respir Crit Care Med 2002;165:800-4.
45. McLaughlin VV, Badesch DB, Delcroix M, Fleming TR, Gaine SP, Galie N, et al. End points and clinical trial design in pulmonary arterial hypertension. J Am Coll Cardiol 2009;54:S97-107.
46. Chen H, Taichman DB, Doyle RL. Health-related quality of life and patient-reported outcomes in pulmonary arterial hypertension. Proc Am Thorac Soc 2008;5:623-30.
47. Rubenfire M, Lippo G, Bodini BD, Blasi F, Allegra L, Bossone E. Evaluating health-related quality of life, work ability and disability in pulmonary arterial hypertension: An unmet need. Chest 2009;136:597-603.
48. McKenna SP, Doughty N, Meads DM, Doward LC, Pepke-Zaba J. The Cambridge Pulmonary Hypertension Outcome Review (CAMPHOR): A measure of health-related quality of life and quality of life for patients with pulmonary hypertension. Qual Life Res 2006;15:103-15.
49. Gomberg-Maitland M, Thenappan T, Rizvi K, Chandra S, Meads DM, McKenna SP. United States validation of the Cambridge Pulmonary Hypertension Outcome Review (CAMPHOR). J Heart Lung Transplant 2008;27:124-30.
50. Ventetuolo CE, Benza RL, Peacock AJ, Zamanian RT, Badesch DB, Kawut SM. Surrogate and combined end points in pulmonary arterial hypertension. Proc Am Thorac Soc 2008;5:617-22.
51. Galie N, Simonneau G, Barst RJ, Badesch D, Rubin L. Clinical worsening in trials of pulmonary arterial hypertension: Results and implications. Curr Opin Pulm Med 2010;16 :S11-9.
52. Cannon CP. Clinical perspectives on the use of composite endpoints. Control Clin Trials 1997;18:517-29.
53. Skali H, Pfeffer MA, Lubsen J, Solomon SD. Variable impact of combining fatal and nonfatal end points in heart failure trials. Circulation 2006;114:2298-303.
54. Lubsen J, Kirwan BA. Combined endpoints: Can we use them? Stat Med 2002;21:2959-70.
55. Nagaya N, Nishikimi T, Okano Y, Uematsu M, Satoh T, Kyotani S, et al. Plasma brain natriuretic peptide levels increase in proportion to the extent of right ventricular dysfunction in pulmonary hypertension. J Am Coll Cardiol 1998;31:202-8.
56. McNairy M, Gardetto N, Clopton P, Garcia A, Krishnaswamy P, Kazanegra R, et al. Stability of B-type natriuretic peptide levels during exercise in patients with congestive heart failure: Implications for outpatient monitoring with B-type natriuretic peptide. Am Heart J 2002;143:406-11.
57. Lubien E, DeMaria A, Krishnaswamy P, Clopton P, Koon J, Kazanegra R, et al. Utility of B-natriuretic peptide in detecting diastolic dysfunction: Comparison with Doppler velocity recordings. Circulation 2002;105:595-601.
58. Yandle TG. Biochemistry of natriuretic peptides. J Intern Med 1994;235:561-76.
59. Hesselstrand R, Ekman R, Eskilsson J, Isaksson A, Scheja A, Ohlin AK, et al. Screening for pulmonary hypertension in systemic sclerosis: The longitudinal development of tricuspid gradient in 227 consecutive patients, 1992-2001. Rheumatology (Oxford) 2005;44:366-71.
60. Nagaya N, Nishikimi T, Goto Y, Miyao Y, Kobayashi Y, Morii I, et al. Plasma brain natriuretic peptide is a biochemical marker for the prediction of progressive ventricular remodeling after acute myocardial infarction. Am Heart J 1998;135:21-8.
61. Bozkanat E, Tozkoparan E, Baysan O, Deniz O, Ciftci F, Yokusoglu M. The significance of elevated brain natriuretic peptide levels in chronic obstructive pulmonary disease. J Int Med Res 2005;33:537-44.
62. Leuchte HH, Neurohr C, Baumgartner R, Holzapfel M, Giehrl W, Vogeser M, et al. Brain natriuretic peptide and exercise capacity in lung fibrosis and pulmonary hypertension. Am J Respir Crit Care Med 2004;170:360-5.
63. Nagaya N, Nishikimi T, Uematsu M, Kyotani S, Satoh T, Nakanishi N, et al. Secretion patterns of brain natriuretic peptide and atrial natriuretic peptide in patients with or without pulmonary hypertension complicating atrial septal defect. Am Heart J 1998;136:297-301.
64. Nagaya N, Sasaki N ando M, Ogino H, Sakamaki F, Kyotani S, et al. Prostacyclin therapy before pulmonary thromboendarterectomy in patients with chronic thromboembolic pulmonary hypertension. Chest 2003;123:338-43.
65. Kucher N, Printzen G, Goldhaber SZ. Prognostic role of brain natriuretic peptide in acute pulmonary embolism. Circulation 2003;107:2545-7.
66. Nagaya N, Uematsu M, Satoh T, Kyotani S, Sakamaki F, Nakanishi N, et al. Serum uric acid levels correlate with the severity and the mortality of primary pulmonary hypertension. Am J Respir Crit Care Med 1999;160:487-92.
67. Batal O, Khatib OF, Dweik RA, Hammel JP, McCarthy K, Minai OA. Comparison of Baseline Predictors of Prognosis in Pulmonary Arterial Hypertension in Patients Surviving </=2 Years and Those Surviving >/=5 Years After Baseline Right-Sided Cardiac Catheterization. Am J Cardiol 2012;109:1514-20.
68. Shah SJ, Thenappan T, Rich S, Tian L, Archer SL, Gomberg-Maitland M. Association of serum creatinine with abnormal hemodynamics and mortality in pulmonary arterial hypertension. Circulation 2008;117:2475-83.
69. Giaid A, Yanagisawa M, Langleben D, Michel RP, Levy R, Shennib H, et al. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. N Engl J Med 1993;328:1732-9.
70. Rubens C, Ewert R, Halank M, Wensel R, Orzechowski HD, Schultheiss HP, et al. Big endothelin-1 and endothelin-1 plasma levels are correlated with the severity of primary pulmonary hypertension. Chest 2001;120:1562-9.
71. Montani D, Souza R, Binkert C, Fischli W, Simonneau G, Clozel M, et al. Endothelin-1/endothelin-3 ratio: A potential prognostic factor of pulmonary arterial hypertension. Chest 2007;131:101-8.
72. Shitrit D, Bendayan D, Bar-Gil-Shitrit A, Huerta M, Rudensky B, Fink G, et al. Significance of a plasma D-dimer test in patients with primary pulmonary hypertension. Chest 2002;122:1674-8.
73. Veyradier A, Nishikubo T, Humbert M, Wolf M, Sitbon O, Simonneau G, et al. Improvement of von Willebrand factor proteolysis after prostacyclin infusion in severe pulmonary arterial hypertension. Circulation 2000;102:2460-2.
74. Kawut SM, Horn EM, Berekashvili KK, Widlitz AC, Rosenzweig EB, Barst RJ. von Willebrand factor independently predicts long-term survival in patients with pulmonary arterial hypertension. Chest 2005;128:2355-62.
75. Lopes AA, Maeda NY. Circulating von Willebrand factor antigen as a predictor of short-term prognosis in pulmonary hypertension. Chest 1998;114:1276-82.
76. Friedman R, Mears JG, Barst RJ. Continuous infusion of prostacyclin normalizes plasma markers of endothelial cell injury and platelet aggregation in primary pulmonary hypertension. Circulation 1997;96:2782-4.
77. Quarck R, Nawrot T, Meyns B, Delcroix M. C-reactive protein: A new predictor of adverse outcome in pulmonary arterial hypertension. J Am Coll Cardiol 2009;53:1211-8.
78. Nickel N, Kempf T, Tapken H, Tongers J, Laenger F, Lehmann U, et al. Growth differentiation factor-15 in idiopathic pulmonary arterial hypertension. Am J Respir Crit Care Med 2008;178:534-41.
79. Soon E, Holmes AM, Treacy CM, Doughty NJ, Southgate L, Machado RD, et al. Elevated levels of inflammatory cytokines predict survival in idiopathic

and familial pulmonary arterial hypertension. Circulation 2010;122:920-7.
80. Becattini C, Vedovati MC, Agnelli G. Prognostic value of troponins in acute pulmonary embolism: A meta-analysis. Circulation 2007;116:427-33.
81. Torbicki A, Kurzyna M, Kuca P, Fijalkowska A, Sikora J, Florczyk M, et al. Detectable serum cardiac troponin T as a marker of poor prognosis among patients with chronic precapillary pulmonary hypertension. Circulation 2003;108:844-8.
82. Hiremath J, Thanikachalam S, Parikh K, Shanmugasundaram S, Bangera S, Shapiro L, et al. Exercise improvement and plasma biomarker changes with intravenous treprostinil therapy for pulmonary arterial hypertension: A placebo-controlled trial. J Heart Lung Transplant 2010;29:137-49.
83. Bogaard HJ, Abe K, Vonk Noordegraaf A, Voelkel NF. The right ventricle under pressure: Cellular and molecular mechanisms of right-heart failure in pulmonary hypertension. Chest 2009;135:794-804.
84. Haddad F, Doyle R, Murphy DJ, Hunt SA. Right ventricular function in cardiovascular disease, part II: Pathophysiology, clinical importance and management of right ventricular failure. Circulation 2008;117:1717-31.
85. Barst RJ, Gibbs JS, Ghofrani HA, Hoeper MM, McLaughlin VV, Rubin LJ, et al. Updated evidence-based treatment algorithm in pulmonary arterial hypertension. J Am Coll Cardiol 2009;54 (1 Suppl):S78-84.
86. Nagendran J, Archer SL, Soliman D, Gurtu V, Moudgil R, Haromy A, et al. Phosphodiesterase type 5 is highly expressed in the hypertrophied human right ventricle and acute inhibition of phosphodiesterase type 5 improves contractility. Circulation 2007;116:238-48.
87. Hammarstrom E, Wranne B, Pinto FJ, Puryear J, Popp RL. Tricuspid annular motion. J Am Soc Echocardiogr 1991;4:131-9.
88. 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.
89. 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.
90. Tiruppathi C, Freichel M, Vogel SM, Paria BC, Mehta D, Flockerzi V, et al. Impairment of store-operated Ca_2^+entry in TRPC4(-/-) mice interferes with increase in lung microvascular permeability. Circ Res 2002;91:70-6.
91. Vogel M, Cheung MM, Li J, Kristiansen SB, Schmidt MR, White PA, et al. Noninvasive assessment of left ventricular force-frequency relationships using tissue Doppler-derived isovolumic acceleration: Validation in an animal model. Circulation 2003;107:1647-52.
92. Paria BC, Vogel SM, Ahmmed GU, Alamgir S, Shroff J, Malik AB, et al. Tumor necrosis factor-alpha-induced TRPC1 expression amplifies store-operated Ca_2^+influx and endothelial permeability. Am J Physiol Lung Cell Mol Physiol 2004;287:L1303-13.
93. 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.
94. Katz J, Whang J, Boxt LM, Barst RJ. Estimation of right ventricular mass in normal subjects and in patients with primary pulmonary hypertension by nuclear magnetic resonance imaging. J Am Coll Cardiol 1993; 21:1475-81.
95. Lorenz CH, Walker ES, Morgan VL, Klein SS, Graham TP, Jr. Normal human right and left ventricular mass, systolic function and gender differences by cine magnetic resonance imaging. J Cardiovasc Magn Reson 1999;1:7-21.
96. van Wolferen SA, Marcus JT, Boonstra A, Marques KM, Bronzwaer JG, Spreeuwenberg MD, et al. Prognostic value of right ventricular mass, volume and function in idiopathic pulmonary arterial hypertension. Eur Heart J 2007;28:1250-7.
97. Grothues F, Moon JC, Bellenger NG, Smith GS, Klein HU, Pennell DJ. Interstudy reproducibility of right ventricular volumes, function and mass with cardiovascular magnetic resonance. Am Heart J 2004;147:218-23.
98. Semelka RC, Tomei E, Wagner S, Mayo J, Caputo G, O'Sullivan M, et al. Interstudy reproducibility of dimensional and functional measurements between cine magnetic resonance studies in the morphologically abnormal left ventricle. Am Heart J 1990;119:1367-73.
99. Ohno Y, Hatabu H, Murase K, Higashino T, Nogami M, Yoshikawa T, et al. Primary pulmonary hypertension: 3D dynamic perfusion MRI for quantitative analysis of regional pulmonary perfusion. AJR Am J Roentgenol 2007;188:48-56.
100. Axel L. Tissue mean transit time from dynamic computed tomography by a simple deconvolution technique. Invest Radiol 1983;18:94-9.
101. Jerosch-Herold M, Wilke N, Stillman AE. Magnetic resonance quantification of the myocardial perfusion reserve with a Fermi function model for constrained deconvolution. Med Phys 1998;25:73-84.
102. Shehata ML, Basha TA, Tantawy WH, Lima JA, Vogel-Claussen J, Bluemke DA, et al. Real-time single-heartbeat fast strain-encoded imaging of right ventricular regional function: Normal versus chronic pulmonary hypertension. Magn Reson Med 2010;64:98-106.
103. Ihlen H, Amlie JP, Dale J, Forfang K, Nitter-Hauge S, Otterstad JE, et al. Determination of cardiac output by Doppler echocardiography. Br Heart J 1984;51:54-60.
104. Zamanian RT, Hansmann G, Snook S, Lilienfeld D, Rappaport KM, Reaven GM, et al. Insulin resistance in pulmonary arterial hypertension. Eur Respir J 2009;33:318-24.

Source of Support: Nil, **Conflict of Interest:** None declared.