Research Article

Reproducibility of intracardiac and transpulmonary biomarkers in the evaluation of pulmonary hypertension

Ken Monahan[1], Theresa A. Scott[2], Yan Ru Su[1], Carrie G. Lenneman[1], David X. Zhao[1], Ivan M. Robbins[3], and Anna R. Hemnes[3]

[1]Division of Cardiovascular Medicine, [2]Department of Biostatistics, and [3]Division of Pulmonary and Critical Care Medicine, Vanderbilt Medical Center, Vanderbilt University, Nashville, Tennessee, USA

ABSTRACT

Intracardiac and transpulmonary levels of natriuretic peptides (NPs) and cyclic guanosine monophosphate (cGMP) provide insight into the pathophysiology of pulmonary hypertension (PH) secondary to left-heart failure but have not been evaluated in established or suspected pulmonary arterial hypertension (PAH). Demonstrating adequate reproducibility of these markers is an important precursor to further study. We hypothesized that the reproducibility of intracardiac and transpulmonary NPs and cGMP is similar to the reproducibility of these markers sampled from the peripheral venous circulation. In outpatients undergoing right-heart catheterization for PH, blood samples were obtained from a peripheral venous site, superior vena cava, inferior vena cava, coronary sinus, pulmonary artery, and pulmonary capillary wedge position. At each site, a repeat sample was collected approximately 60 seconds after the initial measurement. Reproducibility was assessed using the slope of the regression line between initial and follow-up levels. We enrolled 10 patients: Six had PAH, two had pulmonary venous hypertension, and two had normal pulmonary pressure. At all sites, the slopes of the regression lines for BNP were close to identity. BNP was generally more reproducible than NT-pro-BNP. For the NPs and cGMP, reproducibility at intracardiac and transpulmonary sites was similar to the peripheral venous site. Reproducibility of NPs was not influenced by PH severity, access site, or time between measurements. The two patients with the highest transpulmonary pressure gradients had high transpulmonary BNP uptake, but their transpulmonary cGMP gradients were negative. In patients evaluated for PH, reproducibility of NPs and cGMP at intracardiac and transpulmonary sites is high and is comparable to that of peripheral venous measurements.

Key Words: cyclic guanosine monophosphate, natriuretic peptides, right-heart catheterization

Pharmacologic treatment of pulmonary arterial hypertension (PAH) targets key pathways implicated in the pathogenesis of the disease, but initial therapy is largely empiric. Knowledge of the important mechanism(s) of pulmonary vascular disease for a given patient could help tailor treatment. Measurement of biomarkers from the cardiac and pulmonary circulations may be useful in this regard, but it is important to quantify the reproducibility of these markers at these sites prior to further investigation. In this study, we assess the reproducibility of natriuretic peptides (NPs) and cyclic guanosine monophosphate levels taken from intracardiac and transpulmonary sites in patients undergoing hemodynamic evaluation for PAH.

Address correspondence to:
Dr. Ken Monahan
1215, 21st Ave, 5th floor MCE
Nashville, TN 37232, USA
Email: ken.monahan@vanderbilt.edu

Naturietic peptides (NPs; B-type natriuretic peptide [BNP] and N-terminal-pro-BNP [NT-pro-BNP]) obtained from the peripheral venous circulation are elevated in pulmonary arterial hypertension (PAH).[1] Levels of these markers have been shown to correlate with the degree of right-ventricular impairment,[2] functional and hemodynamic parameters,[3,4] and overall prognosis[5,6] in PAH. Intracardiac and transpulmonary NP measurements are available at right-heart catheterization (RHC) and may

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aid in the assessment of patients undergoing pulmonary hypertension (PH) evaluation.

Intracardiac and transpulmonary NP levels, coupled with transpulmonary cyclic guanosine monophosphate (cGMP), have been used to investigate the mechanism of elevated pulmonary vascular resistance (PVR) in patients with PH due to left-heart failure,[7] which involves preserved BNP uptake by the lungs, but impaired pulmonary cGMP production. This approach may be useful in establishing the contribution to PVR of the NP-cGMP pathway in those with PAH but has not been examined in this patient population. Knowledge of the precision, or reproducibility, of intracardiac and transpulmonary NP and cGMP measurements in PH patients is an important precursor to further study in this area, but few data are available.

Therefore, we hypothesized that in patients undergoing evaluation for PH with RHC, (1) NPs and cGMP sampled from an intracardiac or transpulmonary location are highly reproducible; and (2) the degree of reproducibility from these sites is similar to that observed in the peripheral venous circulation.

MATERIALS AND METHODS

Study cohort

Adult patients referred from our institution's pulmonary vascular center to the cardiac catheterization laboratory (CCL) for RHC as part of their clinical care for PH were approached for enrollment. Women of child-bearing age underwent urine testing to exclude pregnancy on the day of their procedure. Subjects were not eligible to participate if significant anemia was present (hemoglobin < 10 g/dL and hematocrit [HCT] < 30); no other exclusion criteria were applied. All participants signed informed consent and the protocol was approved by the institutional review board.

Standard demographic, clinical, and hemodynamic data were collected for each participant to facilitate analysis of determinants of biomarker reproducibility.

CCL procedure

Participants underwent standard RHC using the internal jugular or femoral approach for venous access and a 7-French Swan-Ganz catheter (Edwards Life Science; Irvine, Calif., USA) for hemodynamic measurements. For each participant, approximately 5-10 mL of whole blood were collected from the distal port of the catheter at each of the following locations: Site of peripheral venous access, superior vena cava (SVC), inferior vena cava (IVC), main pulmonary artery (PA), and pulmonary capillary wedge (PCW) position. PCW position was confirmed by oxygen saturation more than 95%. Coronary sinus (CS) samples were collected with a dedicated CS catheter (Biosense Webster; Diamond Bar, Calif., USA) via the same venous access. For approximately 60 seconds after the initial sample was obtained at each site, the catheter was not manipulated, no measurements were recorded, and no medications were given to the patients. A 5-10 mL follow-up sample was then collected from the same site. For participants that had concurrent left-heart catheterization (LHC), a pair of systemic arterial samples was collected from the site of arterial access (femoral or radial) in a similar fashion.

NP and cGMP measurements

Blood samples were collected in ethylenediaminetetraacetic acid-containing Vacutainer tubes, packed in wet ice, and immediately transported for centrifuging and processing. BNP was measured by the Triage BNP assay (Biosite Inc; San Diego, Calif., USA), which has a nominal precision of less than 10%. NT-pro-BNP was measured by enzyme-linked immunosorbent assay ([*ELISA*]; ALPCO; Salem, N.H., USA) with a nominal precision of 6-7%. For cGMP, an ELISA assay (IBL-America; Minneapolis, Minn., USA) with a nominal precision of 4-8% was used. NP levels were obtained at all available sites; cGMP levels were limited to the peripheral venous site, PA and PCW position.

cGMP ''responsiveness'' to BNP

We calculated cGMP ''responsiveness'' to BNP for illustrative purposes; this parameter has been qualitatively described in a population with PH due to left-heart failure.[7] We define it here as the following:

cGMP responsiveness to BNP = transpulmonary cGMP production/transpulmonary BNP uptake; where
transpulmonary cGMP production = $[cGMP_{PCW}] - [cGMP_{PA}]$
transpulmonary BNP uptake = $[BNP_{PA}] - [BNP_{PCW}]$

Statistical analysis

Reproducibility for the NPs and cGMP at each site was evaluated using the slope of the regression line (point estimate, 95% cardiac index [CI]) between initial and follow-up levels. If the markers were perfectly reproducible, all points would fall on the identity line and the slope of the associated regression line would be unity. Deviations of the slope from unity can, therefore, be compared across sites for the same marker and across markers for each site.

Linear regression was used to assess the impact of the following factors on NP reproducibility at each site: Venous access location, time between measurements, and PH severity (as indicated by mean PA pressure [mPAP], PVR, mean right atrial pressure, cardiac index CI, and 6-Minute Walk Distance). For these analyses, the absolute and relative differences between initial and follow-up NP levels were used as indicators of reproducibility. The relative difference of a given marker at a given site was defined as [(follow-up level)-

(initial level)|]/initial level. *P* values less than 0.05 were considered statistically significant. The R statistical software package (R Development Core Team; Vienna, Austria; www.r-project.org) was used for all statistical analyses.

RESULTS

Ten patients were enrolled in the study. Three patients had concurrent LHC, each with a pair of systemic arterial samples. SVC, IVC, and PA sample pairs were available from all participants. Due to technical limitations in locating the CS ostium and obtaining a sufficient volume of blood from the wedge position, CS samples were obtained in six patients and two sets of PCW samples were obtained in five patients; one complete set of PA and PCW samples for BNP and cGMP were available for seven patients. There were no procedural complications in any of the study patients and obtaining the extra blood samples added a negligible amount of time to the overall procedure.

The characteristics of the study cohort are presented in Table 1. The cohort is composed primarily of middle-aged obese females with dyspnea on minimal exertion. LV systolic function was normal in all participants. Six patients were diagnosed with PAH (three idiopathic, two portopulmonary, and one connective tissue disorder), two with pulmonary venous hypertension, and two had normal PA pressure.

Table 1: Demographic, clinical, and hemodynamic characteristics of the study cohort

	Mean	Standard deviation
Age (years)	54	9
Female (%)	70	
BMI (kg/m^2)	35	6
NYHA class	2.7	0.5
LVEF (%)	58	
Diastolic dysfunction (%)	50	
PAH/PVH/no-PH	6/2/2	
Idiopathic	3	
Portopulmonary	2	
Connective tissue disorder	1	
6-minute walk (m)	272	155
Mean RAP (mmHg)	9	5
Mean PAP (mmHg)	42	14
Mean PCW pressure (mmHg)	13	6
Trans-pulmonary gradient (mmHg)	29	15
Cardiac index (L/min/m^2; Fick)	2.6	0.8
Pulmonary vascular resistance (WU)	7	4

BMI: body mass index; **LVEF:** left ventricular ejection fraction; **NYHA:** New York Heart Association; **PAH:** pulmonary arterial hypertension; **PAP:** pulmonary artery pressure; **PCW:** pulmonary capillary wedge; **PVH:** pulmonary venous hypertension; **PVR:** pulmonary vascular resistance; **RAP:** right atrial pressure; **WU:** wood units

The slopes of the regression lines between initial and follow-up NP and cGMP levels are displayed in Table 2. At all intracardiac sites, the slopes of the regression lines for BNP are near identity and are comparable to those for the peripheral venous site. For NT-pro-BNP, the slopes are further from identity with wider CIs. Reproducibility of cGMP in the PA and the PCW position is comparable to reproducibility at the peripheral venous site.

The initial and follow-up levels for each participant at each site are presented in graphical form for the NPs in Figure 1 for BNP (A) and NT-pro-BNP (B). There are very few outliers in these data sets, even for the sites from which it was the most technically challenging to obtain samples (i.e., CS and wedge).

Transpulmonary gradients of BNP and cGMP, along with cGMP "responsiveness" to BNP, are shown in Table 3. In general, BNP decreased and cGMP increased across the pulmonary vascular bed. However, there were two individuals in whom the transpulmonary cGMP gradient was negative, a finding also noted in prior work in this area.[7] These same individuals had among the highest transpulmonary pressure gradients in the cohort, which is consistent with the presence of impaired cGMP production in severe PH.

Regression analyses showed that reproducibility of NPs is not influenced by access site, time between measurements, or PH severity (as indicated by mean RA pressure, CI, mean PA pressure, PVR, or 6-Minute Walk Distance).

DISCUSSION

The findings of this study support the hypothesis that the reproducibility of intracardiac and transpulmonary

Table 2: Reproducibility of intracardiac levels of BNP, NT-pro-BNP, and cGMP

Site	No. sets	BNP			NT-pro-BNP			cGMP		
		Regression line slope	95% LCL	95% UCL	Regression line slope	95% LCL	95% UCL	Regression line slope	95% LCL	95% UCL
Venous	6	1.03	0.95	1.11	0.61	−0.30	1.53	0.76	0.46	1.07
SVC	8	1.00	0.96	1.04	0.67	0.57	0.77			
IVC	8	1.00	0.86	1.13	0.69	0.26	1.12			
CS	6	1.16	0.95	1.36	0.91	0.72	1.10			
PA	10*	1.02	0.97	1.07	0.91	0.65	1.17	0.87	0.37	1.37
PCW	5	1.02	0.97	1.07	1.07	0.57	1.58	1.04	0.88	1.19

*5 sets for cGMP; **BNP:** B-type natriuretic peptide; **cGMP:** cyclic guanosine monophosphate; **CS:** coronary sinus; **IVC:** inferior vena cava; **LCL:** lower confidence limit; **NT:** N-terminal; **PA:** pulmonary artery; **PCW:** pulmonary capillary wedge; **SVC:** superior vena cava; **UCL:** upper confidence limit

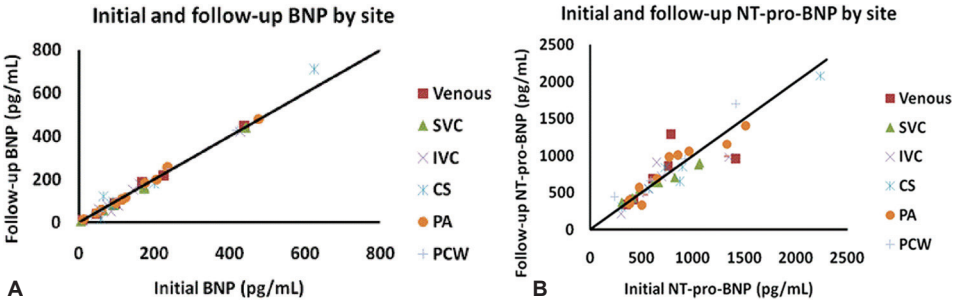

Figure 1: Natriuretic peptide reproducibility by site. Initial and follow-up levels of **(A)** BNP and **(B)** NT-pro-BNP are displayed. While most points fall near the identity line, there is more scatter for NT-pro-BNP than for BNP, suggesting lower reproducibility of the former compared with the latter.

Table 3: Transpulmonary gradients and cGMP responsiveness to BNP

Diagnosis	TPPG (mm Hg)	BNP (pg/mL)			cGMP (pM/mL)			Responsiveness (pM/pg)
		PA	PCW	TPG	PA	PCW	TPG	
PAH	36	256	233	23	76	67	−9	−0.4
PAH	26	59	58	1	119	141	22	22
No PH	7	45	34	11	127	147	20	1.8
PAH	41	477	420	57	133	123	−10	−0.2
PVH	10	124	96	28	76	90	14	0.5
PAH	30	15	13	2	99	117	18	9
PAH	33	98	53	45	164	252	88	2

BNP: B-type natriuretic peptide; **cGMP:** cyclic guanosine monophosphate; **PA:** pulmonary artery; **PAH:** pulmonary arterial hypertension; **PCW:** pulmonary capillary wedge; **PVH:** pulmonary venous hypertension; **TPG:** transpulmonary gradient; **TPPG:** transpulmonary pressure gradient

levels of NPs and cGMP are comparable to peripheral venous measurements. In addition, the reproducibility at all sites was high. Therefore, further evaluation of these markers in the PH population should not be limited by concerns regarding their short-term variability. BNP appeared more reproducible than NT-pro-BNP, suggesting that the former may be preferable to the latter in subsequent work in this area. While high reproducibility of these markers may have been predicted based on the coefficients of variation reported by the individual assay kits, the nonstandard location of the sampling sites and the potential impact on marker levels of the many variables inherent to a CCL procedure justified formal investigation of reproducibility in a more ''real-world'' setting.

The reasons for the difference in reproducibility between NPs are not entirely clear. Studies of peripheral venous BNP and NT-pro-BNP have shown them to have similar reproducibility over longer time frames (days and weeks) than were evaluated in the present study.[8] Differences in biologic variability (i.e., secretion and clearance) are less likely to account for the difference in reproducibility over the short interval between sets of measurements. Therefore, a difference in analytic variability (i.e., the assay employed and its precision) is a more likely explanation. The BNP and NT-pro-BNP samples were processed in separate laboratories at our institution and that may account for at least some of the observed differences.

In a study of intracardiac and transpulmonary BNP and transpulmonary cGMP in patients undergoing CCL evaluation for suspected cardiovascular disease,[9] site-specific reproducibility was not assessed formally for either marker. This cohort had normal pulmonary pressures (determined invasively) and transpulmonary BNP uptake was negligible; in contrast, BNP generally decreased across the pulmonary vascular bed in our study. This difference raises the possibility that, in the face of increased PVR, the lungs may seek to capture more BNP in order to stimulate cGMP production with the goal of affecting further pulmonary vasodilation. Interestingly, this study also showed that transpulmonary cGMP production increased after administration of a neutral endopeptidase inhibitor, suggesting that altering pathways involved with BNP degradation can impact transpulmonary cGMP production as well. A transpulmonary NT-pro-BNP gradient was demonstrated in a small group of patients with end-stage parenchymal lung disease (n = 6) and a separate subgroup with idiopathic PAH (n = 4).[10] Site-specific reproducibility of NT-pro-BNP was not evaluated. Those with a transpulmonary NT-pro-BNP gradient had elevated pulmonary pressures, but there was no gradient in the rest of the cohort, which had much lower PA pressures. Although the small sample sizes limit formal analyses, both this study and our work suggest that NP uptake by the lungs may be driven partially by pulmonary hemodynamics.

The ''responsiveness'' parameter has a wide range in this small cohort and is an oversimplification of the complex interactions between BNP and cGMP across the pulmonary vascular bed. Nonetheless, it may provide a useful summary measure of this pathway during the

initial assessment of pulmonary vascular status as well as in gauging the response to PH therapies, particularly phosphodiesterase (PDE) inhibitors. The two patients with negative transpulmonary cGMP gradients had high BNP uptake and high transpulmonary pressure gradients. It is possible that the negative cGMP gradients could be explained by variations in the cGMP assay, although we have demonstrated a high degree of precision for this assay in our cohort. This finding could also indicate significant pulmonary ''NP resistance''[7] (i.e., low ''responsiveness'' of cGMP to BNP) in these individuals with advanced PAH, defined as impairment in pulmonary cGMP production (and potential ''consumption'' of cGMP by the lungs) despite high pulmonary BNP uptake, which would ordinarily be expected to provide a stimulus for cGMP production.[11] Given the increase in pulmonary cGMP production in response to PDE inhibition demonstrated in those with left-heart failure and high-PVR, we speculate that (1) PDE inhibitors may act to overcome low responsiveness of cGMP to BNP in PAH as well; and (2) demonstration of low-responsiveness of cGMP to BNP at RHC may provide an individualized rationale for treatment with PDE inhibitors.

It is possible that analogous parameters could be applied to other pathways implicated in the pathogenesis of PAH, such as the cyclic adenosine monophosphate-prostacyclin axis; evaluation of how ''responsiveness'' changes with a vasodilator challenge may also be of use.

As mentioned above, the small cohort size is a limitation of the study. In that context, the mixture of PAH, PVH, and no-PH participants further limits the applicability of the findings to any one group but does suggest some degree of generalizability across the spectrum of patients referred to a specialty center for PH evaluation.

In summary, in patients undergoing hemodynamic evaluation for PH, NPs and cGMP levels measured within the heart and across the lungs are highly reproducible; for these markers, the degree of reproducibility is similar to that obtained from peripheral venous samples. The utility of transpulmonary biomarkers in the work-up and management of those with suspected or known PH warrants further investigation.

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