Assessment of N-terminal prohormone B-type natriuretic peptide as a measure of vascular and ventricular function in pediatric pulmonary arterial hypertension

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Abstract: Pulmonary arterial hypertension (PAH) is a progressive disease that puts excessive mechanical loads on the ventricle due to a gradual increase in pulmonary vascular impedance. We hypothesize that the increase in right ventricular (RV) afterload is reflected in the concentration of circulating biochemical markers of ventricular strain and stress (B-type natriuretic peptide [BNP] and N-terminal prohormone BNP [NT-proBNP]). We retrospectively analyzed right heart catheterization (RHC) and serum biochemical analysis data ($n = 56$) for a pediatric PAH cohort with no sign of left ventricular dysfunction. Using RHC data, we computed an estimate of pulmonary vascular resistance (PVR), compliance, and ventricular-vascular coupling. We also compared how the early onset of interventricular decoupling (characterized as septal flattening) impacts serum NT-proBNP concentrations. Our data revealed correlated NT-proBNP expression with both the resistive and reactive components of RV afterload, an estimate of ventricular-vascular coupling, and a significant increase in biomarker expression in patients with a flattened interventricular septum. Furthermore, the strong correlation between PVR and NT-proBNP appears to break down under flat septum morphology. Over 80% of resistive RV afterload variance is reflected in serum NT-proBNP concentration in pediatric patients with PAH with no sign of left ventricular dysfunction. Reactive afterload appears to contribute to myocardial NT-proBNP release at advanced stages of PAH. Therefore, in mild-to-moderate PAH, resistive afterload is likely the greatest contributor to RV wall stress. These findings could also be used to estimate invasive RHC measurements from serum biochemical analysis, but more work is needed to improve correlations and overcome the issue of interventricular decoupling.

Keywords: pulmonary hypertension, biomarker, NT-proBNP, afterload.

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Pulmonary arterial hypertension (PAH) is a progressive disease that presents several prognostic challenges. The disease is officially diagnosed by performing right heart catheterization (RHC) and evaluating the mean pulmonary arterial pressure (mPAP) threshold (mPAP <25 mmHg).¹ RHC is an invasive procedure that requires anesthesia for pediatric patients and would be unsuitable for regular prognosis. Therefore, it is simply not a practical option for periodic screening,² which is necessary to properly assess treatment efficacy and gauge disease progression. However, RHC is currently the gold standard in patient monitoring because it can be used to obtain a myriad of other critical measurements that have been correlated with outcome and provide an overall picture of right ventricle (RV) and pulmonary artery (PA) function (e.g., cardiac output [CO], right atrial pressure, pulmonary capillary wedge pressure [PCWP], pulmonary vascular resistance [PVR], and capacitance [C]). Given the logistical limitations of periodically obtaining patient RHC data, particularly in children, plasma protein biomarkers have been recognized as a potential surrogate for direct RV functional measurements.^{3,4}

B-type natriuretic peptide (BNP) is a neurohormone secreted in both the left ventricle (LV) and RV in response to volume expansion⁵ and pressure overload.⁶ N-terminal prohormone BNP (NT-proBNP) is a cleaved byproduct of BNP,⁷ which has a longer in vivo half-life⁸ and offers numerous logistical advantages for clinical monitoring.² Serum biomarker expression has been shown to be a better indicator of outcome than mPAP,³ with the diagnostic value of BNP and NT-proBNP well established in symptomatic and asymptomatic⁹ heart failure.¹⁰ These biochemical markers have also revealed enormous prognostic value in pulmonary hypertension^{7,11} with the potential for assessing treatment efficacy, because serum levels have been shown to decrease with functional improvement.^{6,12} In the pediatric pulmonary hypertension (PH) population, these peptides are currently used for disease management and have been associated with several functional hemodynamic markers.¹³ However, in PAH, RV biomarker expression has not been directly associated with the resistive and reactive components for afterload, which would establish a direct link between progressive vascular dysfunction and ventricular response. Nevertheless, directly

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correlating afterload with NT-proBNP expression requires additional consideration in children, who naturally decrease the amount of circulating biomarker with age,^{10,14,15} making it an influential variable.

The overall objective of our research group is to maximize the clinical utilization of easily measurable serum biochemical properties to offer insight into RV-PA hemodynamics and functional state. The objective of this study was to (1) establish correlations between functional phenotype and RV myocardial NT-proBNP expression in the pediatric PH population and (2) investigate the role of interventricular septal flattening on biomarker expression.

METHODS

Patient demographic and study acceptance criteria

Retrospective RHC and biomarker data for 36 patients with PH (mPAP >25 mmHg confirmed by RHC), ranging in age from infancy to young adulthood (Fig. 2), were analyzed for our study. Among the 36 patients, some offered biannual follow-up measurements that were each considered as an individual data set within the sample, resulting in a total data set of 56 sets of data. Longitudinal disease progression was not considered for this study. In addition to RHC, each patient underwent same-day echocardiography to assess ventricular function. Only RHC data sets that corresponded to no LV dysfunction or morphological abnormalities, decreed by a trained cardiologist, were included in the study, leading to the reasonable assumption that the measured serum biomarker expression originates from the RV myocardium. Additional sample patient demographic characteristics are included in Figure 2.

PA function phenotyping (afterload)

The resistive and reactive components of afterload of the PA vasculature is ideally characterized using three-element Windkessel model.¹⁶ The distal resistance ($PVR - Z_c$) and C can be found from typical RHC data,

$$PVR = \frac{mPAP - PCWP}{CO}, \quad (1)$$

$$C = \frac{CO}{HR \times PP}. \quad (2)$$

In equations (1) and (2), mPAP is the mean pulmonary arterial pressure, PCWP is the pulmonary capillary wedge pressure approximated as the pressure in the left atrium, CO is the cardiac output, HR is the heart rate, and PP is the pulse pressure. The characteristic impedance (Z_c) can be found using a technique that has been outlined in detail in Kheyfets et al.¹⁷ In short, Z_c is computed from an estimate of PA distensibility (D ; eq. 3), where $PVR' = 50 \times PVR$ is considered PA resistance under zero pressure:^{18,19}

$$mPAP = \frac{[(1 + D \times PCWP)^5 + 5D \times PVR' \times CO]^{1/5} - 1}{D}. \quad (3)$$

The final characteristic impedance is found in equation (4), where ρ is the blood density (1.06 g/mL) and R is the lumen radius assumed proportional to body surface area (BSA),

$$Z_c = \frac{\rho}{\pi R^2} \sqrt{\frac{1}{\rho D}}. \quad (4)$$

A measure of afterload that combines all the Windkessel parameters can be computed as the arterial elastance (E_a):

$$E_a = \frac{PVR}{t_s + \tau \times (1 - e^{-\tau/t_d})}. \quad (5)$$

In equation (5), t_s and t_d are the systolic and diastolic times, assumed to be 30% and 50% of the cardiac cycle, respectively. The diastolic pressure decay is defined as follows: $\tau = (PVR - Z_c) \times C$.

Phenotyping RV-PA coupling

Ideally, RV-PA coupling would be computed with the aid of ventricular pressure-volume loops,²⁰ which were not available for the present data set. Therefore, we introduce an alternative nondimensional parameter symbolizing the ratio of ventricular to vascular function:

$$VVCR_2 = \frac{\text{ventricular metric}}{\text{vascular metric}} = \frac{(PAP_{\text{sys}}/SV)^2(\text{age})}{PVR/C} \quad (6)$$

The numerator in equation (6) represents the ventricular end-systolic function (assuming that PA pressure at end systole [PAP_{sys}] is equal to RV pressure at end systole [RVP_{sys}]), normalized by the displaced volume. As disease progresses, the stroke volume (SV) decreases, whereas $RVP_{\text{sys}} = PAP_{\text{sys}}$ increases, thus increasing the numerator. The denominator of equation (6) represents the ratio of resistive to reactive afterload. As the disease progresses, the denominator also increases, causing the following scenario: when afterload increases at a considerably faster rate than ventricular accommodation, the overall ratio will decrease.

Biochemical phenotyping

BNP and NT-proBNP plasma concentrations were measured within 5 days of RHC at Children's Hospital Colorado (Aurora, CO). BNP analysis was done on blood samples collected in ethylenediaminetetraacetic acid tubes, whereas NT-proBNP analysis was performed on blood collected in a serum sample tube. The minimum measurable BNP values were bound to 15 pg/mL, which was assayed using the i-STAT system with a two-site enzyme-linked immunosorbant assay (Abbott Laboratories, Chicago, IL). Serum NT-proBNP was measured using an electrochemiluminescence immunoassay (ProBNP II, Roche Diagnostics, Indianapolis, IN) at Mayo Medical Laboratories (Rochester, MN).

Statistical analysis

We present two types of statistical data: (1) linear correlations and (2) comparisons of the mean of two samples (PH patients with flattened septum vs. normal morphology). For both tests, each data set was confirmed to be normally distributed (Gaussian distribution) with logarithmic operations performed to improve normality (Fig. 1).

Figure 1. Example of data normalization applied to N-terminal prohormone B-type natriuretic peptide (NT-proBNP) measurements and any data set with a nonnormal distribution. A histogram (A) and quantile-quantile (Q-Q) plot (B) of the nonnormalized NT-proBNP expression are shown, alongside a histogram (C) and Q-Q plot (D) of the same data normalized using a log transform.

An unpaired Student *t* test was performed to compare the means of two sample groups. An *F* test was used to assess variance equivalence. For both statistical tests, a 95% confidence interval was considered to be significant, suggesting a 95% probability that the sample result is reflective of the population.²¹

RESULTS

Data set demographic characteristics

Figure 2 shows relevant study data set demographic and hemodynamic characteristics. Given the large age range considered in our study and the fact that NT-proBNP expression has been shown to vary in development,¹⁴ all of the following correlations are normalized by $\log_{10}(\text{age})$.

The characteristic impedance (Z_c) was found using equation (6), combined with the relationship between pulse wave velocity and distensibility.¹⁷ Figure 3 shows a comparison between Z_c and PVR, compliance, and NT-proBNP. The close relationship between C and Z_c offers an indirect validation of the technique, because the derivation for Z_c did not include any aspect of the compliance equation (eq. [2]). As expected, the characteristic impedance appears to increase with increasing PVR and decreasing compliance, reflecting disease progression. Furthermore, Z_c explains 36% of the variability in NT-proBNP expression, with both variables increasing concurrently.

BNP vs. NT-proBNP expression

Earlier studies have reported on the relationship between BNP and NT-proBNP expression.^{10,22} In this article, we show that our data set is consistent with earlier findings (see Fig. 4). Given the

relationship between the two biomarkers and the aforementioned clinical advantages of measuring NT-proBNP over BNP, the following discussion will focus on NT-proBNP.

NT-proBNP expression and PA functional phenotype

Figure 5 shows normalized NT-proBNP correlated against resistive (A) and reactive (B) components of impedance, a multivariate model of PVR and compliance (C), and arterial elastance (D). For both the resistive and reactive components of impedance, afterload is associated with an increase in NT-proBNP expression, but the log of PVR index (PVRi, defined as PVR/BSA) explains approximately 75% of the variability in NT-proBNP. A multivariate regression, combining PVRi and C index, results in the following expression: $\log(\text{NT} - \text{proBNP})/\log(\text{age}) = (2.0) + 0.29 \times \text{PVR} + 0.24 \times C + (8.36e - 6) \times (\text{PVR}/C)$ ($R^2 = 0.70$, $P < 0.001$, by two-tailed test). Furthermore, the P values for all three terms are statistically significant ($P < 0.05$, by two-tailed test). It is noteworthy that the coefficient of the last term is small, but it must be considered in terms of the unit increase in the ratio, and its inclusion improves the statistical significance of the other two terms.

NT-proBNP expression and RV-PA coupling

Because no RV-PA coupling is available for this retrospective data set, a nondimensional estimate was developed (using Buckingham π theorem) to indicate RV response to afterload increase (ventricular-vascular coupling ratio [VVCR2]). Figure 6 shows a linear correlation between VVCR2 and NT-proBNP expression, which suggests that a decrease in the coupling ratio corresponds to an increase in biomarker expression.

Figure 2. Quintile plots showing minimum, lower, median, upper, and maximum quintiles of patient demographic characteristics and right heart catheterization hemodynamic data. BPM: beats per minute; BSA: body surface area; CO: cardiac output; HR: heart rate; mPAP: mean pulmonary arterial pressure.

NT-proBNP expression and interventricular coupling (septal flattening)

The interventricular septum is known to flatten in response to a decreased pressure difference between the LV and the RV. Here, we show that interventricular septal flattening occurs concurrently with RV-PA functional degradation and RV distress biomarker expression, before a notable decrease in pump (RV pumping) function. Within the entire data set considered, the sample was divided into two groups, (1) moderate-to-severe septal flattening and (2) mild-to-normal septal flattening, which were categorized on the basis of echocardiographic findings within 24 h of RHC by a trained cardiologist. Figure 7 shows bar plots (with error bars indicating standard deviation) comparing differences between patients with a no-

tably flat interventricular septum and normal septal motion. As expected, an increased mPAP and PVR cause a flattened septum, which does not appear to contribute to a decreased CO. However, patients with a notably flattened septum do have a significantly higher plasma NT-proBNP concentration. It is worth noting that the group with a flattened septum, which showed an increased PVR and NT-proBNP, did not reveal any difference in systemic vascular resistance when compared with the normal septal morphology group.

Considering only patients with a normal interventricular septum

Performing the same correlations as above while excluding patients with a flattened septum significantly improves most of the

Figure 3. Relationship between estimated characteristic impedance (Z_c) and right heart catheterization measured afterload and N-terminal prohormone B-type natriuretic peptide (NT-proBNP) expression ($P < 0.0001$, by two-tailed test). Z_c appears to reflect both the reactive and resistive components of afterload and is increasing concurrently with NT-proBNP expression. C: capacitance; PVR: pulmonary vascular resistance.

Figure 4. Relationship between log B-type natriuretic peptide (BNP) and log N-terminal prohormone BNP (NT-proBNP) in the considered data set ($P < 0.0001$, by two-tailed test) in patients with no evidence of left ventricular morphological or functional abnormalities. Several cardiologists recorded BNP to be <10 pg/mL, which still corresponded to a large variation in NT-proBNP.

presented correlations, notably the correlations between PVR and NT – proBNP ($R^2 = 0.81$), C and NT – proBNP ($R^2 = 0.81$), and $\log(\text{NT – proBNP})/\log(\text{age}) = (2.04) + 0.30 \times \text{PVR} + 0.24 \times C + (8.83e - 6) \times (\text{PVR}/C)$ ($R^2 = 0.75$). However, the correlation between PVR and C revealed an $R^2 = 0.94$ (plots not shown).

DISCUSSION

Note on study demographics and data manipulation

The patients considered in this study vary in age from infants several months old to young adults. Nevertheless, pediatric PH patients were the primary target for this study, and an explicit comparison should be done involving adults to confirm the resulting trends. In fact, NT-proBNP expression has been reported to be lower in pediatric patients, compared with adults.¹¹

Due to the fact that NT-proBNP appeared to change with age in the pediatric population, we divided measured concentration levels by age. PVR, a computation that is a function of a volume measurement (CO) that is proportional to size, is traditionally indexed by multiplying BSA.²³ However, in this study, we found that it better correlated with NT-proBNP when dividing it by BSA. This

Figure 5. A, Normalized N-terminal prohormone B-type natriuretic peptide (NT-proBNP) correlated against resistive afterload. B, Normalized NT-proBNP correlated against reactive afterload. C, Nonlinear multivariate regression function of reactive and resistive components of pulmonary vascular impedance correlated against normalized NT-proBNP. D, Normalized NT-proBNP correlated against arterial elastance (E_a). BSA: body surface area; C: capacitance; PVR: pulmonary vascular resistance.

Figure 6. Relationship between age-normalized N-terminal prohormone B-type natriuretic peptide (NT-proBNP) expression and approximated ventricular-vascular coupling ratio (VVCR2; $P < 0.0001$, by two-tailed test).

is not an attempt to change convention, but rather simply to show that NT-proBNP is indeed governed by a function of (1) resistive afterload and (2) a variable indicative of patient body size, which is a simple, noninvasive measurement. Therefore, if the present results are corroborated in larger future studies and the consider-

ations presented in this article are accounted for (septal inversion, discussed below), circulating biomarkers could be used to compute afterload and other important clinical characteristics.

Relationship between RV NT-proBNP expression and vascular functional phenotype

Gradual degradation of vascular function in PH is characterized by distal remodeling,^{24,25} attributed to a complex network of biochemical pathways and hemodynamically appearing as an increase in PVR and a decrease in vascular compliance (increasing the reactive component of impedance). The increased transmural pressure leads to an elevation in vascular circumferential stress in the proximal PA,²⁶ thus triggering an up-regulation of load-bearing extracellular matrix proteins. Unlike the adult vasculature, which sees an up-regulation of collagen, a stiff protein with a short half-life, the developing circulation sees an increase in elastin. Elastin is more compliant than collagen, has a half-life on the order of decades,²⁷ and allows for a more uniform stress distribution within the vessel wall.²⁸ This effectively reduces the reactive afterload acting on the RV, which would undoubtedly impact biomarker expression.

We attempted to correlate NT-proBNP expression against four measures of afterload: (1) PVR, (2) C , (3) E_a , and (4) multivariate

Figure 7. Bar plots (with standard deviation represented by error bars) showing statistical difference in mean pulmonary arterial pressure (mPAP; A), resistive afterload (B), ventricular pump function (C), and N-terminal prohormone B-type natriuretic peptide (NT-proBNP; D) expression between patients with normal and flattened interventricular septums. In scenarios of increased afterload and mPAP, with preserved cardiac output (CO), right ventricular distress biomarker expression is significantly increased in patients with a flattened interventricular septum. PVR: pulmonary vascular resistance.

regression, considering both the resistive and reactive components of afterload. NT-proBNP correlated against the resistive component of afterload (PVR) produced a better association than E_a , compliance, or the multivariate regression. In the multivariate regression, the predictive capability of the model was close to that of PVR alone, but C did not appear to have a significant impact on NT-proBNP variability other than in several patients with extremely stiffened PAs. This is supported by (1) the three-dimensional mesh plot, which shows a sudden increase at extremely low compliance but is otherwise flat across the $\log(C/BSA)$ axis, and (2) in the linear regression, which shows $\log(C)/BSA$ vs. NT-proBNP. In the latter, the strong correlation is mostly driven by patients with extremely low compliance, with the remainder of the sample poorly distributed. The resistive component of afterload explains over 70% of the variability in NT-proBNP expression. Interestingly, the association found here in the pediatric population is notably more significant than that found in the adult population.²⁹ PVR is a measure of the dissipated energy of blood flow,³⁰ resulting from distal constriction, that must be replaced by a source. Given the aforementioned remodeling differences between pediatric and adult patients, it is possible that the reactive component of impedance plays a lesser role in pediatrics, with PVR contributing to the majority of ventricular distress.

An increased PVR also decreases the normal pressure difference between the ventricles and is partially responsible for the flattened septum. In fact, PVR offers the most notable difference between patients with normal and flat interventricular septal walls and would be a rational explanation for the RV-PA pressure buildup. Within the current sample, patients with moderate to severe septal flattening presented elevated serum biomarker expression, with relatively preserved CO. Therefore, the physical shift of the septum does not directly impact CO, but it could be directly influencing increased LV pressure and falsifying NT-proBNP readings. Within our data set, the strength of the correlation between PVRi and NT-proBNP expression is notably decreased in patients isolated with a flat interventricular septum. However, it is increased when considering only those patients with a normal septum. This could be explained in two ways: (1) the septal shift works to absorb the increased afterload and take stress off the myocardial wall, thus decreasing distress biomarker expression; and/or (2) the septal shift causes an increase in LV myocardial stress, which could be resulting in LV myocardium biomarker expression, thus supplying blood with circulating peptides that originated from both ventricles. The latter explanation is likely more descriptive of the current findings, given that the overall level of biomarker expression increased in the group with a flattened septum. Therefore, serum NT-proBNP levels in patients with even mild LV dysfunction or interventricular septal flattening might not be ideal estimates of PA vascular function.

Relationship between RV NT-proBNP expression and ventricular-vascular coupling

Ventricular-vascular coupling is typically computed as the ratio of end-systolic elastance to E_a .^{20,31} Initiatively, it is a measure of the

RV functional reserve, beginning to decrease in manifest PH as the RV is no longer able to accommodate an increase in afterload through adaptive remodeling mechanisms. Ventricular-vascular coupling has been shown to decrease concurrently with BNP in a developing PH pig model³² but has not been adequately investigated in pediatric patients with regard to RV-PA decoupling. Properly computing ventricular-vascular coupling requires a simultaneous measure of ventricular pressure and volume under baseline and challenge conditions. In animal studies, this can be achieved with a vena-cava occlusion, with is obviously not an option for human studies. Numerous estimates have been proposed to circumvent this challenge,³³ but validating these techniques remains an ongoing process. Regardless, the current retrospective data set considered for this study did not offer adequate data for reliably computing ventricular-vascular coupling. Therefore, we sought to arrive at a nondimensional equation that relates the available hemodynamic measurements of ventricular function to vascular function. For this purpose, we considered ventricular function to be represented as the maximum pressure generated by the RV, normalized by the volume of blood displaced in each stroke. As the ventricle remodels, it is able to generate higher pressures with frequent strokes of decreased volume, suggesting that the numerator of equation (6) would increase with disease. It is worth noting that some studies have used the ratio of $(\text{end} - \text{systolic vascular pressure})/SV$ to estimate systemic vascular afterload (E_a),³⁴ whereas a recent review suggested the estimate was using mPAP²⁰ in the pulmonary circulation. However, we feel systolic PAP is more characteristic of ventricular performance, because it is more reflective of the maximum RV pressure. In our estimate (eq. [6]), vascular performance was taken as the ratio of resistive to reactive afterload (PVR/C). As the disease progresses, PVR increases while C decreases, thus increasing the ratio of the two measurements and the denominator of equation (6). In this study, we find that the ratio of RV ventricular function increase to the rate of PA vascular function increase is significantly correlated to ventricular NT-proBNP expression. In other words, as the rate of ventricular accommodation to a deteriorating vascular function is decreased, the concentration NT-proBNP is increased. Interestingly, the cardiac marker is not correlated to NT-proBNP expression and is relatively constant within our sample.

Clinical and research translation and future work

The basic research implications of this work suggest that, in the mild-to-moderate phases of the disease, the resistive component is primarily responsible for elevated RV wall stress (characterized by an increase in NT-proBNP). However, as the PA becomes extremely stiff, this appears to cause a sudden increase in NT-proBNP expression (probably accompanied by RV wall stress). Future work will utilize computational modeling to confirm that NT-proBNP release correlates with RV myocardial stress in pediatric PAH.

The clinical implications of this work suggest that noninvasive NT-proBNP measurements could be used to approximate PVR at early stages of the disease and possibly to approximate vascular stiffness and ventricular-vascular coupling. However, additional work is needed to arrive at a multivariate algorithm that adequately pre-

dicts the variability of vascular (and vascular-ventricular) function from noninvasive serum biochemical analysis. The implications of such an algorithm would be invaluable, particularly for pediatric PAH patients, who must be placed under general anesthesia during RHC.

CONCLUSIONS

The overall objective of this study was to show preliminary correlations between serum NT-proBNP measurements and ventricular-vascular function in pediatric PH. Assuming little-to-no circulating NT-proBNPs originating from the LV, afterload explains more than 80% of the variability in RV NT-proBNP expression, mostly dominated by the resistive components of impedance. However, the reactive component of afterload does appear to contribute to biomarker expression at extreme stiffness, which might further be compromised due to NT-proBNP expression from the LV. Finally, NT-proBNP appears to correlate with an estimate of RV-PA decoupling, but additional work is needed to compute coupling directly.

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