Biomarkers of Pulmonary Hypertension Are Altered in Children with Down Syndrome and Pulmonary Hypertension

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Objective To evaluate the performance of pulmonary hypertension (PH) biomarkers in children with Down syndrome, an independent risk factor for PH, in whom biomarker performance may differ compared with other populations.

Study design Serum endostatin, interleukin (IL)-1 receptor 1 (ST2), galectin-3, N-terminal pro hormone B-natriuretic peptide (NT-proBNP), IL-6, and hepatoma-derived growth factor (HDGF) were measured in subjects with Down syndrome and PH (n = 29), subjects with Down syndrome and resolved PH (n = 13), subjects with Down syndrome without PH (n = 49), and subjects without Down syndrome with World Symposium on Pulmonary Hypertension group I pulmonary arterial hypertension (no Down syndrome PH group; n = 173). Each biomarker was assessed to discriminate PH in Down syndrome. A classification tree was created to distinguish PH from resolved PH and no PH in children with Down syndrome.

Results Endostatin, galectin-3, HDGF, and ST2 were elevated in subjects with Down syndrome regardless of PH status. Not all markers differed between subjects with Down syndrome and PH and subjects with Down syndrome and resolved PH. NT-proBNP and IL-6 levels were similar in the Down syndrome with PH group and the no Down syndrome PH group. A classification tree identified NT-proBNP and galectin-3 as the best markers for sequentially distinguishing PH, resolved PH, and no PH in subjects with Down syndrome.

Conclusions Proteomic markers are used to improve the diagnosis and prognosis of PH but, as demonstrated here, can be altered in genetically unique populations such as individuals with Down syndrome. This further suggests that clinical biomarkers should be evaluated in unique groups with the development of population-specific nomograms. *(J Pediatr 2022;241:68-76)*.

Children with Down syndrome, or trisomy 21, have an elevated risk of pulmonary hypertension (PH). The prevalence of PH in children with Down syndrome is as high as 6% at age 1 year and 15% at age 10 years.[1] Although individuals with Down syndrome have a shorter life expectancy compared with individuals without Down syndrome, those with Down syndrome and PH have an even higher risk of death, with an OR for mortality nearly 4-fold higher than that in those without PH.[2]

PH is classified into 5 etiologies according to the World Symposium on Pulmonary Hypertension (WSPH).[3] Children with Down syndrome commonly have multiple conditions predisposing them to the development of pulmonary vascular disease. Congenital heart disease (CHD), pulmonary hypoplasia, obstructive sleep apnea,[4-6] chronic aspiration,[7] and recurrent respiratory infections[8,9] are all associated with the development of pulmonary vascular disease and occur much more frequently in children with Down syndrome.[2] In addition, chromosomal abnormalities, such as trisomy 21, may cause abnormal angiogenic

AUROC	Area under the receiver operating curve
CHD	Congenital heart disease
HDGF	Hepatoma-derived growth factor
IL	Interleukin
NT-proBNP	N-terminal pro-hormone B-natriuretic peptide
PAH	Pulmonary Arterial Hypertension
PH	Pulmonary hypertension
ST2	Interleukin-1 receptor 1
WSPH	World Symposium on Pulmonary Hypertension

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signaling. Altered angiogenic signaling may be an additional independent risk factor for disease in individuals with Down syndrome, predisposing them to the development of pulmonary vascular disease.

Serum biomarkers, which are noninvasive and objective, are being investigated as markers of PH diagnosis and prognosis and are being considered as surrogate endpoints for clinical trials. We have shown the association of multiple markers, including interleukin (IL)-6, galectin-3, IL-1 receptor 1 (known as ST2), endostatin, and hepatoma-derived growth factor (HDGF), with PH severity and survival in both adults and children.[10-15] These markers, along with N-terminal pro-hormone B-natriuretic peptide (NT-proBNP), may have utility as markers of PH severity. Their performance in children with Down syndrome is not well characterized. If these biomarkers are to be considered as surrogates for invasive testing and as potential therapeutic targets, changes in serum levels and performance as diagnostic tools in this unique and vulnerable population must be understood.

Dysregulation of vascular proteins, including angiogenic and inflammatory proteins, as well as proteins involved in cardiomyocyte and smooth muscle function, has been implicated in PH and may be particularly relevant in children with Down syndrome. Angiostatic proteins, such as collagen 18A1 (Col18a1) and its breakdown product endostatin, found on chromosome 21, have been of specific interest in Down syndrome complicated by PH. Other markers, such as IL-6, ST2, galectin-3, and HDGF, have been implicated in PH in children as markers of inflammation (IL-6), pulmonary vascular dysfunction (ST2 and HDGF), and cardiac fibrosis (ST2 and galectin-3) but have not been explicitly explored in children with Down syndrome.

In the present multicenter study, we sought to evaluate the levels of PH-relevant serum biomarkers in children with WSPH group I pulmonary arterial hypertension and children with Down syndrome both with and without PH. The overall goal was to evaluate whether levels of the markers NT-proBNP, ST2, IL-6, galectin 3, endostatin, and HDGF differed substantially in children with PH and children with Down syndrome with and without PH.

Methods

This study was approved by the Institutional Review Boards at all participating centers, with informed consent provided for all subjects. Study cohorts included subjects with Down syndrome and current PH (Down syndrome PH group), subjects with Down syndrome with resolved PH (Down syndrome resolved PH group), subjects with Down syndrome with no history of PH (Down syndrome no PH group), and subjects with PH but without Down syndrome (no Down syndrome PH group). PH was defined according to the WSPH criteria at time of data collection as a mean pulmonary artery pressure >25 mmHg with a pulmonary capillary wedge pressure <15 mmHg and a pulmonary vascular resistance >3 WU by cardiac catheterization or a tricuspid regurgitant velocity >2.9 m/s by echocardiography without valvar obstruction, right ventricular systolic pressure over one-half the systemic pressure, or estimated mean pulmonary artery pressure by pulmonary valve regurgitant velocity.[1,3,16] All subjects (both those with Down syndrome and those without Down syndrome) with active PH were evaluated by cardiac catheterization before study entry, and those with resolved PH or no PH were screened by echocardiography. Subjects were classified into their respective groups (Down syndrome PH, Down syndrome resolved PH, Down syndrome no PH, and no Down syndrome PH) based on their clinical status at the time of blood sampling (**Figure 1**). All subjects with CHD had undergone primary repair before enrollment.

Subjects with Down Syndrome

Subjects with Down syndrome were enrolled from the University of Colorado Pediatric Pulmonary Hypertension Study and the Sie Center for Down syndrome at Children's Hospital Colorado. For this study, 29 children with Down syndrome and current PH and 24 children with Down syndrome and no PH were enrolled. Subjects with Down syndrome were also enrolled from Boston Children's Hospital and included 13 subjects with Down syndrome with resolved PH and 25 subjects with Down syndrome and no history of PH. Most subjects with Down syndrome and resolved PH had CHD, with improvement in PH seen after repair of heart disease. Data available for all subjects included demographic information, CHD type and surgical repair, history of prematurity, and medication use. Information on obstructive sleep apnea and other pulmonary diseases common in children with Down syndrome was not consistently available; thus, although the Down syndrome PH and Down syndrome resolved PH groups met the criteria for WSPH group 1 PH, they may have a mixed phenotype of group 1 PH as well, as group 3 PH as common in children with Down syndrome.[17]

Subjects without Down Syndrome

For comparison, subjects with WSPH group I PH but without Down syndrome (ie, the no Down syndrome PH group) were enrolled from the National Biological Sample and Data Repository for Pulmonary Arterial Hypertension (PAH Biobank).[10,15] The PAH Biobank is a National Heart, Lung, and Blood Institute–funded resource of biological samples, genetic data, and clinical data maintained at Cincinnati Children's Hospital Medical Center under the direction of Dr William Nichols (www.pahbiobank.org). Currently, 5 pediatric enrolling centers from around the US participate in the PAH Biobank. A total of 173 subjects with current PH but without Down syndrome were enrolled in this study.

Laboratory Analysis

A custom electrochemiluminescent immunosorbent assay was developed (MesoScale Discovery) by robotic printing

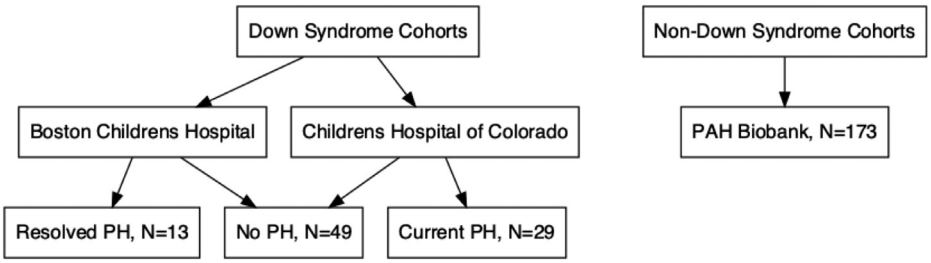

Figure 1. Diagram of enrollees by group.

capture antibodies (R&D Systems, DIY1098; part 841455) and paired with an endostatin, IL-6, ST2, NT-proBNP, and galectin-3 detection antibody and assay standards (R&D Systems, DIY1098; part 841456) to quantify serum endostatin, IL-6, ST2, NT-proBNP, and galectin-3, respectively. Interassay percent coefficient of variations were endostatin, 2.38%; NT-proBNP, 3.0%; IL-6, 6.5%; galectin-3, 5.8%; and ST2, 6.8%. Biomarkers that were below the lower limit of detection were imputed as one-half the lower limit of detection. Further details of the enzyme-linked immunosorbent assay have been published previously.[10,14,15] Laboratory samples were collected concurrently with the recording of clinical data.

Statistical Analyses

Descriptive statistics are presented as median with IQR or mean ± SD as appropriate based on normality of the data. Data available included age, sex, PH subtype, race, ethnicity, and type of CHD. Only subjects with repaired CHD were included, although subjects may have had residual shunts or other residual lesions. Subjects were categorized as having Down syndrome with PH, Down syndrome with a history of resolved PH, Down syndrome without PH, or no Down syndrome with PH. Differences between cohorts were evaluated using the Fisher exact test, Pearson χ^2 test, or Kruskal–Wallis rank-sum test as appropriate depending on variable type, normality, and sample size; the Fisher exact test was used preferentially for small sample sizes. Each biomarker was evaluated based on category (Down syndrome and PH status).

Subgroup analysis was performed for subjects with CHD and those without CHD and for shunt type (pre-tricuspid vs post-tricuspid shunt). Area under the receiver operating curve (AUROC) analysis was performed to evaluate whether each biomarker could distinguish PH in the setting of Down syndrome. A classification tree was created to identify the most useful markers and cutoff values to discriminate PH in subjects with Down syndrome. AUROC and classification tree analyses included only subjects with Down syndrome, because biomarkers are not necessary to identify Down syndrome in current clinical practice. A 2-sided P value < .05 was considered statistically significant. Statistical analyses were conducted with R version 3.6.1 (R Foundation for Statistical Computing).

Results

Demographic data for each cohort at enrollment and at blood sampling are presented in **Table I**. There were a total of 264 enrollees, 91 of whom had Down syndrome. Overall, the cohort was 55% female, which was consistent across subcategories. Subjects with Down syndrome and PH were the youngest, with a median age of 5 years (IQR, 1-11 years); median age in the other groups was 10.5 years (IQR, 7.8-13.5 years) in the Down syndrome resolved PH group, 7 years (IQR, 3.7-14.4 years) in the Down syndrome no PH group, and 13 years (IQR, 9-17 years) in the no Down syndrome PH group. Most of the participants in all cohorts self-identified as white.

CHD was extremely prevalent in the subjects with Down syndrome, present in 21 subjects (72%) in the Down syndrome PH group, in 12 subjects (92%) in the Down syndrome resolved PH group, and in 25 subjects (51%) in the Down syndrome no PH group. Comparatively, 62 subjects (35.8%) in the no Down syndrome PH group had CHD. The most common CHD diagnoses were atrial septal defect, ventricular septal defect, and complete AV canal, with most subjects having a post-tricuspid shunt.

All subjects with PH were receiving PH therapy, typically with phosphodiesterase inhibitors (86%) and endothelin receptor antagonists (59%). A higher proportion of subjects in the no Down syndrome PH group were receiving a prostacyclin agonist, particularly an intravenous or subcutaneous prostacyclin agonist (**Table I**).

Biomarkers

Endostatin. Endostatin levels were highest in the Down syndrome PH group (median, 101.8 ng/mL; IQR, 67-124 ng/mL) and the Down syndrome resolved PH group (median, 97.5 ng/mL; IQR, 77-102 ng/mL) and lower in the Down syndrome no PH group (median, 60.6 ng/mL; IQR, 44-82 ng/mL) (**Figure 2**, A). All subjects with Down syndrome, regardless of PH status, had significantly higher endostatin levels compared with the no Down syndrome PH group.

Galectin-3. Galectin-3 levels were higher in all 3 groups of subjects with Down syndrome compared with those without Down syndrome (**Figure 2**, B). Among the groups with Down syndrome, galectin-3 level trended higher in the

Table I. Demographic data and biomarker levels of all cohorts at enrollment

Variables	DS PH (N = 29)	DS PH Hx (N = 13)	DS no PH (N = 49)	No DS PH (N = 173)	*P* value*
Trisomy 21, n (%)	29 (100)	13 (100)	49 (100)	0 (0)	<.001
Current PH, n (%)	29 (100)	0 (0)	0 (0)	173 (100)	<.001
History of PH, n (%)	29 (100)	13 (100)	0 (0)	173 (100)	<.001
Sex, n (%)					.2
Male	13 (45)	7 (54)	28 (57)	71 (41)	
Female	16 (55)	6 (46)	21 (43)	102 (59)	
Age at sample, y, median (IQR)	5.0 (1.0-11.0)	10.5 (7.8-13.5)	7.0 (3.7-14.4)	13.0 (9.0-17.0)	<.001
Weight, kg, median (IQR)	19 (15-31)	NA	16 (13-21)	22 (14-47)	.4
Height, cm, median (IQR)	107 (92-120)	NA	94 (86-114)	120 (97-153)	.07
BSA, m^2, median (IQR)	0.71 (0.58-0.96)	NA	NA	0.80 (0.58-1.29)	.5
Race, n (%)					
White	26 (89.7)	12 (92.3)	39 (79.6)	130 (75.1)	
Black	1 (3.4)	1 (7.7)	3 (6.1)	15 (8.7)	
Asian	2 (6.9)	0 (0)	0 (0)	15 (8.7)	
Biracial	0 (0)	0 (0)	5 (10.2)	5 (2.9)	
Native American/Alaskan	0 (0)	0 (0)	0 (0)	5 (2.9)	
Unknown	0 (0)	0 (0)	2 (4.1)	3 (1.7)	
Endostatin, ng/mL, median (IQR)	101.8 (67-124)	97.5 (77-102)	60.6 (44-82)	28.8 (22-36)	<.001
Galectin-3, ng/mL, median (IQR)	20.2 (13.1-25.2)	29.8 (25.9-34.2)	15.5 (10.6-22.7)	8.4 (7.6-10.8)	<.001
NT-proBNP, pg/mL, median (IQR)	237 (169-1277)	221 (183-350)	89 (42-143)	194 (76-409)	<.001
ST2, ng/mL, median (IQR)	6.1 (3.7-8.0)	8.7 (6.3-10.3)	3.8 (2.9-6.8)	3.0 (2.1-4.4)	<.001
IL-6, pg/mL, median (IQR)	2.1 (0.9-3.4)	0.1 (0.09-3.5)	0.1 (0.09-2.2)	1.4 (0.8-2.2)	<.001
HDGF, ng/mL, median (IQR)	1.4 (1.0-2.4)	1.1 (0.9-3.9)	2.4 (1.6-3.3)	0.8 (0.5-1.2)	<.001
CHD, n (%)	21 (72)	12 (92)	25 (51)	62 (35.8)	<.001
Pre/post-tricuspid shunt, n (%)					.5
Post-tricuspid	11 (38)	10 (77)	16 (32)	26 (15)	
Pre-tricuspid	8 (28)	2 (15)	6 (12)	9 (5)	
ASD, n (%)	13 (45)	4 (31)	11 (22)	15 (8.7)	<.001
VSD, n (%)	11 (38)	2 (15)	5 (10)	16 (9.2)	.001
CAVC, n (%)	2 (6.9)	6 (46)	3 (6.1)	4 (2.3)	<.001
PDA, n (%)	1 (3.4)	1 (7.7)	14 (29)	4 (2.3)	<.001
Tetralogy of Fallot, n (%)	0 (0)	4 (31)	1 (2.0)	0 (0)	<.001
Other lesion, n (%)	0 (0)	0 (0)	9 (18)	7 (4.1)	.004
Phosphodiesterase 5 inhibitor, n (%)	15 (52)	0 (0)	0 (0)	159 (92)	.9
Endothelin receptor antagonist, n (%)	8 (28)	0 (0)	0 (0)	112 (65)	.5
Prostacyclin analog, n (%)	2 (6.9)	0 (0)	0 (0)	87 (50)	.2
IV/SC prostacyclin analog, n (%)	1 (3.4)	0 (0)	0 (0)	60 (35)	.2
Soluble guanylate cyclase stimulator, n (%)	0 (0)	0 (0)	0 (0)	2 (1.2)	.9
Calcium channel blocker, n (%)	3 (10)	0 (0)	0 (0)	29 (17)	.4

ASD, atrial septal defect; *BSA*, body surface area; *CAVC*, complete atrioventricular canal; *DS*, Down Syndrome; *IV*, intravenous; *NA*, not available; *PDA*, patent ductus arteriosus; *SC*, subcutaneous; *VSD*, ventricular septal defect.
*Fisher exact test, Pearson χ^2 test, or Kruskal–Wallis rank-sum test.

Down syndrome resolved PH group (median, 29.8 ng/mL; IQR, 25.9-34.2 ng/mL) but was not significantly different from that in the Down syndrome PH group (median, 20.2 ng/mL; IQR, 13.1-25.2 ng/mL) or Down syndrome no PH group (median, 15.5 ng/mL; IQR, 10.6-22.7 ng/mL). Although galectin-3 levels were higher in the Down syndrome PH group compared with the Down syndrome no PH group, the difference was not statistically significant.

NT-proBNP. NT-proBNP levels were highest in the Down syndrome PH group (median, 237 pg/mL; IQR, 169-1277 pg/mL) and Down syndrome resolved PH group (median, 221 pg/mL; IQR, 183-350 pg/mL) compared with the no Down syndrome PH group (median, 194 pg/mL; IQR, 76-409 pg/mL) (**Figure 2**, C). Among the subjects with Down syndrome, levels were not different between the Down syndrome PH and Down syndrome resolved PH groups. Levels in the Down syndrome no PH group (median, 89 pg/mL; IQR, 42-143 pg/mL) were similar to known levels in healthy children (ie, children with no genetic syndromes and no cardiopulmonary disease; range, 6-190 pg/mL).[18]

ST2. ST2 levels were elevated in all subjects with Down syndrome compared with the no Down syndrome PH group (**Figure 2**, D). ST2 levels were higher in the Down syndrome PH group (median, 6.1 pg/mL; IQR, 3.7-8 pg/mL) and Down syndrome resolved PH group (median, 8.7 ng/mL; IQR, 6.3-10.3 pg/mL) compared with the no Down syndrome PH group (median, 3.0 ng/mL; IQR, 2.1-4.4 pg/mL). ST2 levels were higher in the Down syndrome PH group compared with the Down syndrome no PH group, but the difference was not statistically significant.

IL-6. IL-6 levels were highest in the Down syndrome PH group (median, 2.1 pg/mL; IQR, 0.9-3.4 pg/mL) and no Down syndrome PH group (median, 1.4 pg/mL; IQR, 0.8-2.2 pg/mL), but not statistically different (**Figure 2**, E). Among the subjects with Down syndrome, IL-6 levels were significantly higher in the Down syndrome PH group (median, 2.1 ng/ml; IQR, 0.9-3.36 pg/mL) compared with the Down syndrome no PH group (median, 0.1 ng/mL; IQR, 0.09-2.2 pg/mL), but were not different from those in the Down syndrome resolved PH group. IL-6 levels were

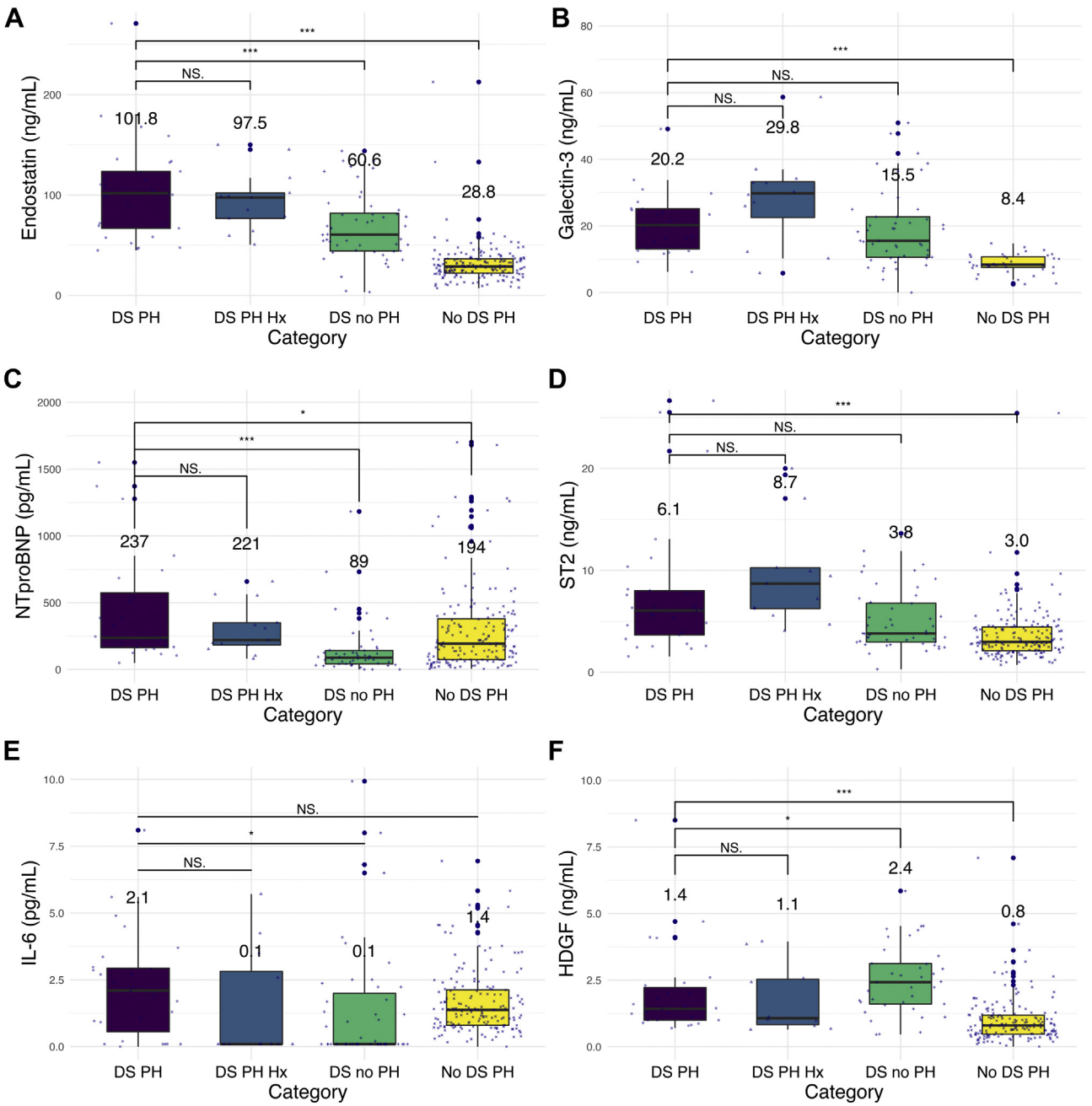

Figure 2. Boxplots of biomarkers in subjects with Down syndrome with PH, subjects with Down syndrome with a history of PH, subjects with Down syndrome without PH, and subjects without Down syndrome with PH. **A,** Endostatin. **B,** galectin-3. **C,** NT-proBNP. **D,** ST2. **E,** IL-6. **F,** HDGF. *$P < .05$; **$P < .01$; ***$P < .0001$.

elevated only in groups with PH, irrespective of Down syndrome status.

HDGF. HDGF levels were increased in subjects with Down syndrome compared with the no Down syndrome PH group (Figure 2, F). HDGF levels were higher in the Down syndrome PH group compared with the no Down syndrome PH group (median, 0.8 ng/mL; IQR, 0.5-1.2 pg/mL). Among subjects with Down syndrome, HDGF levels were higher in the Down syndrome no PH group compared with the Down syndrome PH group (median, 2.4 pg/mL [IQR, 1.6-3.3 pg/mL] vs 1.4 pg/mL [IQR, 1-2.4 pg/mL]).

Biomarkers in CHD

CHD was frequent in the subjects with Down syndrome and is known to be associated with PH even without Down syndrome. Biomarkers were evaluated by shunt type in the

subjects with CHD. A total of 120 subjects (45%) had CHD, all of whom had undergone repair of CHD. Among the subjects with Down syndrome and CHD, 21 subjects had current PH, 12 had a history of resolved PH, and 25 had no PH (**Table II**; available at www.jpeds.com). Most subjects had a post-tricuspid shunt. Although there was a trend toward higher endostatin, NT-proBNP, and HDGF levels in subjects with a post-tricuspid shunt, this did not reach significance in any group (**Figure 3**, A-E; available at www.jpeds.com). ST2 levels were notably higher in subjects in the Down syndrome resolved PH group with a post-tricuspid shunt (median, 9.1 ng/mL vs 4.4 ng/mL; $P = .03$), although the sample size was small. Galectin-3 and IL-6 levels varied across the groups based on shunt type.

Biomarkers That May Differentiate PH in Children with Down Syndrome

All biomarkers were assessed by AUROC analysis in subjects with Down syndrome with PH and without PH to assess for possible diagnostic utility for PH in the setting of Down syndrome. **Figure 4**, A shows the receiver operating characteristic curve for each biomarker, excluding patients with Down syndrome and resolved PH. Endostatin had the best discriminatory capability, with an AUROC of 79%, followed by NT-proBNP (75.5%) and galectin-3 (72.8%). IL-6, ST2, and HDGF had equivocal discriminatory capability, with AUROC values of 58.4%, 59.0%, and 53.7%, respectively. When subjects with a history of resolved PH but no current PH were included, endostatin, NT-proBNP, and galectin-3 still performed relatively well, with AUROC values of 75.0%, 66.8%, and 64.1%, respectively (**Figure 5**; available at www.jpeds.com).

A classification tree was created to evaluate whether certain markers could better distinguish between subjects with Down syndrome and current PH, a history of resolved PH, and no PH. After model testing, NT-proBNP and galectin-3 were used as the classifiers (**Figure 4**, B). Subjects with an NT-proBNP level ≤146 pg/mL were classified as no PH, with 77% correctly classified by NT-proBNP alone in this cohort. Among the subjects with an NT-proBNP level >146 pg/mL, those with a galectin-3 level ≤26 ng/mL were classified as current PH (86% correctly classified in this cohort), and those with a galectin-3 level >26 ng/mL were classified as history of resolved PH (82% correctly classified in this cohort).

Discussion

Proteomic approaches to PH offer the promise of noninvasive diagnosis, more accurate risk stratification and prognosis, and possibly even new therapeutic targets. This could be especially helpful for children with Down syndrome, who are uniquely predisposed to PH both from a genetically based predisposition and as a complication of other comorbidities, including CHD and multiple types of lung disease.[2] This study shows that current clinical biomarkers of PH have different profiles in children with Down syndrome regardless of whether they currently have or have had PH. Specifically, endostatin, ST2, galectin-3, and HDGF levels were elevated in subjects with Down syndrome regardless of the presence of PH, and NT-proBNP and IL-6 levels were relatively similar in subjects with Down syndrome and those without Down syndrome.

Our findings imply that these biomarkers, and indeed other disease markers, need to be evaluated in genetically unique populations such as children with Down syndrome, in whom altered angiogenic and cellular signaling may result in differing expression of proteins and thus require a unique interpretation of proteomic markers in disease.

Cardiac markers, including NT-proBNP and galectin-3, were elevated in children with Down syndrome and PH and ultimately proved to be the best biomarkers for distinguishing PH, history of resolved PH, and no PH. The classification scheme suggests that elevated NT-proBNP is a marker of PH, with extremely elevated galectin-3 (highest levels) identifying those with resolved PH. Similarly, ST2 was also highest in subjects with Down syndrome with history of resolved PH. All 3 of these markers have some cardiac specificity, with NT-proBNP released in response to cardiac stretch and ST2 and galectin-3 involved in cardiac fibrosis. NT-proBNP, which is a good marker of cardiac stretch, can identify patients with cardiopulmonary disease, but a second marker is needed to isolate those with current heart disease but resolved pulmonary vascular disease from those with heart disease and pulmonary vascular disease. Galectin-3 has not performed as well as a biomarker for PH in the absence of either Down syndrome or CHD, so it is interesting to see the extremely elevated levels in these children.[12] In children with Down syndrome, in whom there is an extremely high prevalence of CHD, protein markers with significant cardiac effects, such as NT-proBNP and galectin-3, may be more important than pulmonary vascular markers. Although CHD may influence galectin-3 levels, it is notable that when limiting our analysis to just those subjects without CHD, galectin-3 levels were still extremely elevated in all groups with Down syndrome compared with those without Down syndrome, also suggesting altered expression in subjects with Down syndrome. Future studies will also need to investigate how these markers change in a larger cohort of subjects with Down syndrome and resolved PH without CHD.

Endostatin was expected to be elevated in subjects with Down syndrome owing to an increased copy number of the collagen 18a1 gene on chromosome 21.[19] Bush et al reported that abnormal endostatin levels, as well as dysregulated angiopoietin 1 and angiogenin, distinguished children with Down syndrome and PH, suggesting an antiangiogenic phenotype in trisomy-21.[19] However, they found that endostatin alone did not adequately distinguish PH from no PH.[19] Galambos found up-regulated endostatin in the lung tissue of subjects with Down syndrome along with reduced vascular density and increased vessel wall thickness.[20] The current study, which found that endostatin was able to distinguish PH in subjects with Down syndrome, made a specific

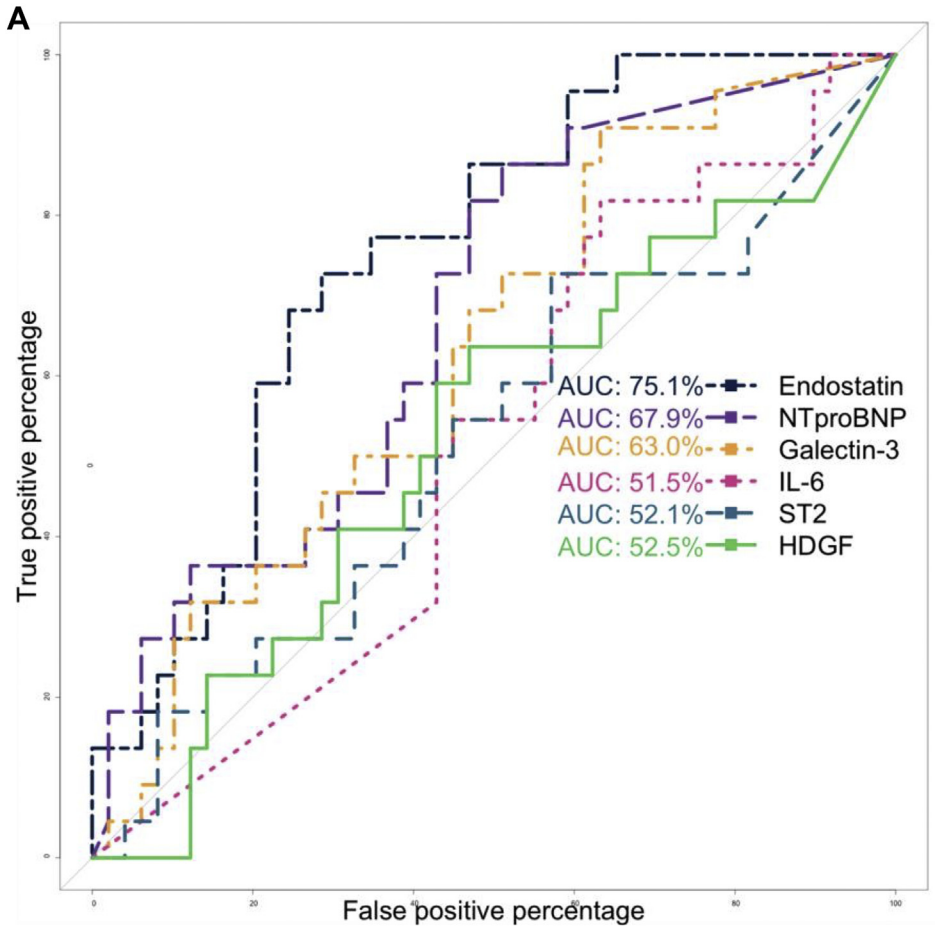

Figure 4. **A,** AUROC analysis of each biomarker comparing subjects with Down syndrome and PH and those with Down syndrome without PH. *Subjects with a history of PH are excluded. **B,** Classification tree of biomarkers to distinguish subjects with Down syndrome (DS) with current PH (DS PH), with a history of PH (DS resolved PH), and without PH (DS no PH). Nodes/leaves show classifier.

distinction among subjects with current PH, subjects with a history of resolved PH, and subjects who never had PH. Interestingly, in subjects with Down syndrome and a history of resolved PH, endostatin levels were still elevated, at levels similar to those subjects with Down syndrome and current PH. Ultimately, endostatin was not used in our classification tree, owing to its inability to distinguish PH from resolved PH. Although our prior work showed that endostatin levels decrease over time with improved hemodynamics, it is clear that Down syndrome is a state of chronically elevated endostatin at baseline, and that increased expression from PH does not diminish with resolution of PH.[21] It is intriguing to hypothesize that chronically high endostatin levels could partially explain the high susceptibility of PH in children with Down syndrome and comorbidities such as CHD, obstructive sleep apnea, or other lung diseases. It is conceivable that trending endostatin levels longitudinally may provide some insight into the risk for development of PH.

ST2, another marker that has shown efficacy as a prognostic marker in pediatric PH, was also extremely elevated in our subjects with Down syndrome. ST2 is known to bind to the IL-33 receptor and to promote cardiac fibrosis. Previous studies have demonstrated that it is produced by the pulmonary vascular endothelium and has good performance as a prognostic marker.[14,15] Our subjects with Down syndrome had elevated ST2 levels compared with those without Down syndrome, again regardless of PH status. Those with PH or a history of resolved PH had the highest levels. This may again be influenced by CHD, particularly in those with a history of resolved PH.

Limitations of this study include the small sizes of each cohort and the diversity of PH and CHD types. The presence of CHD confounds interpretation of some of these markers, especially because information about residual shunts and lesions was not available. Details regarding obstructive apnea and other lung disease also were not available, and thus our subjects could not be assessed for WSPH group 3 disease, a common finding concurrent with WSPH group 1 PH in children with Down syndrome.[17] Thus, although all subjects met the criteria for WSPH group 1 PH, they may have had mixed disease. Nonetheless, given the prevalence of CHD and other comorbidities in children with Down syndrome and children with PH, it is important to evaluate these markers in both the presence and absence of CHD. This study is of a single cohort without validation of the classification scheme. Future studies should validate the classification scheme in a larger cohort of subjects with Down syndrome both with and without CHD. The lack of clinical data about PH severity, particularly information from concurrent cardiac catheterization or echocardiographic data, and timing from diagnosis and repair, limits interpretation of these markers, particularly as severity or prognostic markers. Future work should evaluate these markers in relation to PH severity and should look at longitudinal changes in biomarker levels to better establish trends in children with Down syndrome.

This study demonstrates that cardiac and angiogenic proteins, which have utility as markers of PH, are distinctly different in children with Down syndrome. Multiple proteins, including endostatin, ST2, HDGF, and galectin-3 are elevated in children with Down syndrome. Despite these changes, these markers are still able to discriminate PH from no PH in the setting of Down syndrome using group-specific threshold values. Using NT-proBNP and galectin-3 in combination we were able to effectively discriminate PH from a history of resolved PH and no PH in children with Down syndrome. Our data suggest that these biomarkers are still useful in children with Down syndrome with PH; however, population-specific cutoff values need to be established. ■

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Data Statement

Data sharing statement available at www.jpeds.com.

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50 Years Ago in *THE JOURNAL OF PEDIATRICS*

Virus-Induced Suppression of Cellular Immunity

Lischner HW. Viral suppression of delayed hypersensitivity (Editorial). J Pediatr 1972;80:174-7.

At the time of this editorial, it was known that infection by certain viruses and the administration of certain live attenuated virus vaccines suppressed cutaneous hypersensitivity to tuberculin or other antigens. It also was clear that in the case of natural hepatitis and measles, and vaccine measles infection, delayed hypersensitivity to antigens to which the child previously had been sensitive was depressed. In addition, depressed delayed hypersensitivity skin response was associated with transient defective lymphocyte function and vulnerability to re-activation of previously quiescent tuberculosis or other infections in which cell-mediated immunity plays a prominent protective role. Following this knowledge, tuberculin skin testing (offered universally at 12 months of age) was recommended to be performed prior to measles immunization. For other viral infections, such as uncomplicated varicella and vaccinia, depression of delayed skin hypersensitivity occurred, but there was not a detectable effect on resistance to infection. The original science publication that led to this editorial related to the effect of mumps and mumps vaccine on delayed hypersensitivity and cellular immunity, for which there were conflicting data at the time. Dr Lischner concluded that the preponderance of data at hand would make it prudent to perform tuberculin testing, if indicated, prior to mumps vaccination.

The applications and recommendations have changed as tuberculin testing is now performed only on the basis of risk (rather than universally) and mumps vaccine is available only as a combination measles-mumps-rubella vaccine with or without varicella vaccine. Lischner's advice of 1972 remains current, however. If tuberculin testing is indicated, this should be performed prior to measles-mumps-rubella vaccination.

Review of this editorial reminds this writer of a critical mentor in infectious diseases. At that time, Harold Lischner was the wicked-smart immunologist at St. Christopher's Hospital for Children. He was the immunology brain engaged in the discovery of DiGeorge syndrome. Dr Lischner's approach to any uncertainty was to follow every lead to literature on all primary experimentation on the topic, even when it was just hours before the deadline of a grant submission and the bulk of the specifics of the proposed study or trial were as yet unwritten. Although this thwarted achievement of short-term goals, it was a lesson in the foundations of discovery and expansion of knowledge. Thanks, Dr Lischner, for your preservation of science and truth.

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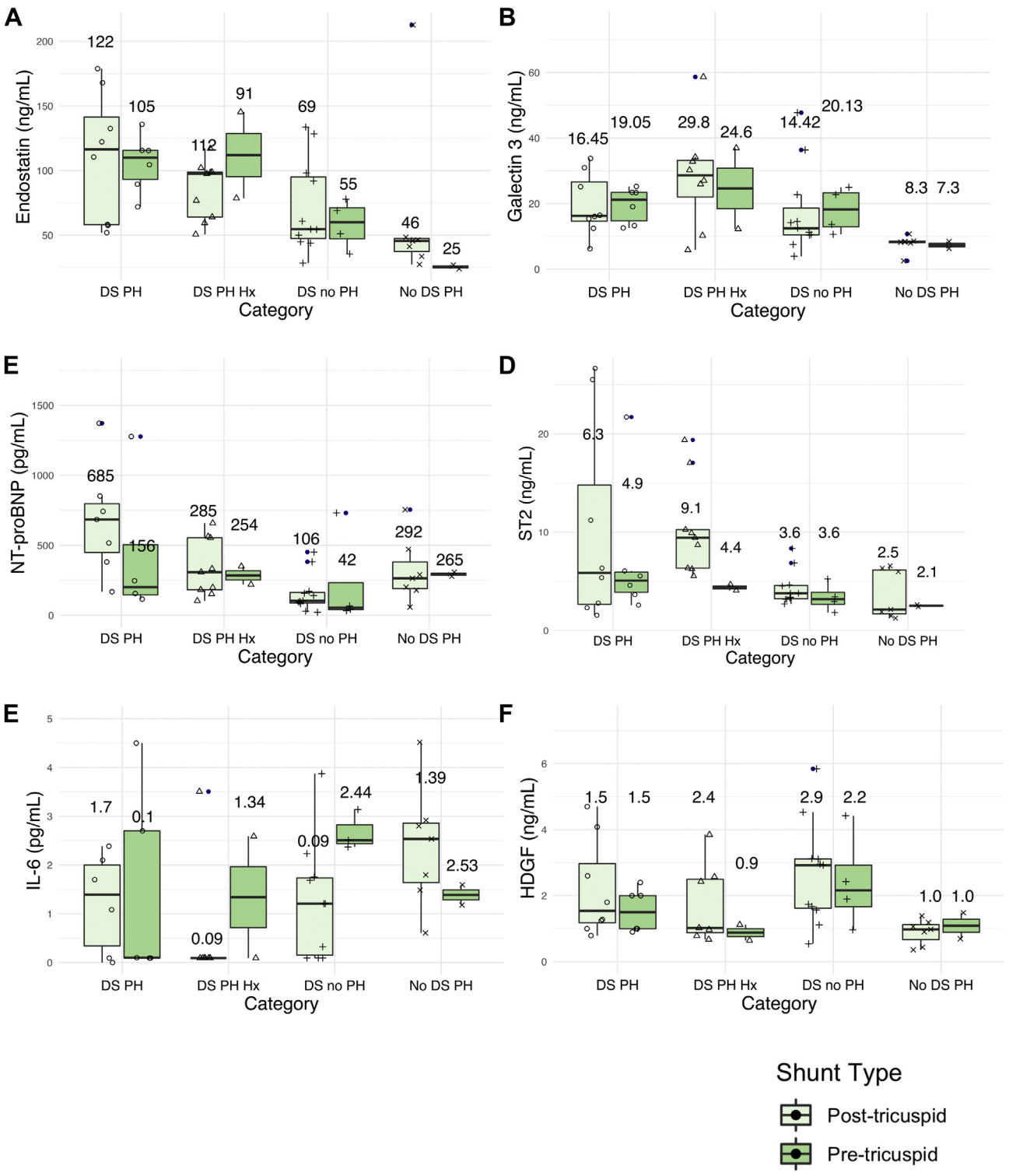

Figure 3. Boxplots of biomarkers in subjects with CHD, by pre-tricuspid shunt vs post-tricuspid shunt. Shown are subjects with Down syndrome with PH, subjects with Down syndrome with a history of PH, subjects with Down syndrome without PH, and subjects without Down syndrome with PH.

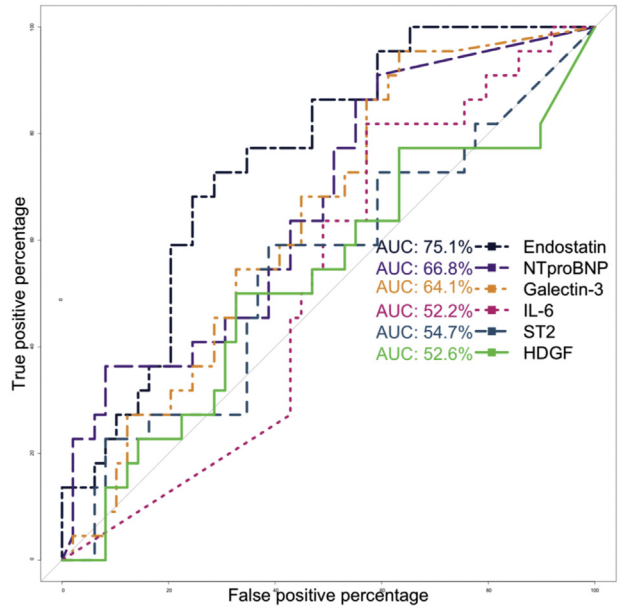

Figure 5. AUROC analysis of each biomarker comparing subjects with Down syndrome and PH and those with Down syndrome without PH. *Subjects with a history of PH but no current disease are classified as no PH.

Table II. Biomarkers by category in subjects with CHD

Variables	DS PH (N = 21)	DS PH Hx (N = 12)	DS no PH (N = 25)	No DS PH (N = 62)	*P* value*
Endostatin, ng/mL, median (IQR)	89 (58-122)	91 (74-100)	58 (49-82)	33 (24-40)	<.001
ST2, ng/mL, median (IQR)	5.3 (2.79-10.4)	7.95 (6.1-10)	3.8 (3.1-5.3)	2.85 (2.14-4.46)	<.001
NT-proBNP, pg/mL, median (IQR)	517 (169-1372)	265 (190-401)	102 (55-148)	296 (175-467)	<.001
Galectin-3, ng/mL, median (IQR)	17.75 (13.1-25.2)	29.79 (22.5-34.9)	14.9 (12.2-23.3)	8.2 (6.62-8.4)	<.001
IL-6, pg/mL, median (IQR)	2.1 (0.10-2.97)	0.09 (0.09-2.82)	1.26 (0.09-2.51)	1.70 (1.05-2.90)	.09
HDGF, ng/mL, median (IQR)	1.28 (1.00-2.20)	1.12 (0.88-3.21)	2.44 (1.62-3.11)	0.80 (0.48-1.25)	<.001
Trisomy 21, n (%)	21 (100)	12 (100)	25 (100)	0 (0)	<.001
Pre/post-tricuspid shunt, n (%)					.5
Post-tricuspid	11 (52)	10 (83)	16 (64)	26 (42)	
Pre-tricuspid	8 (38)	2 (17)	6 (24)	9 (15)	
ASD, n (%)	13 (62)	4 (33)	11 (44)	15 (24)	.01
VSD, n (%)	11 (52)	2 (17)	5 (20)	16 (26)	.07
PDA, n (%)	1 (5)	1 (8)	14 (56)	4 (6.5)	<.001
CAVC, n (%)	2 (10)	6 (50)	3 (12)	4 (6.5)	.002
Tetralogy of Fallot, n (%)	0 (0)	4 (33)	1 (4.0)	0 (0)	<.001
Other lesion, n (%)	0 (0)	0 (0)	7 (28)	3 (5)	<.001

ASD, atrial septal defect; *CAVC*, complete atrioventricular canal; *DS*, Down Syndrome; *PDA*, patent ductus arteriosus; *VSD*, ventricular septal defect.
*Kruskal–Wallis rank-sum test, Pearson χ^2 test, or Fisher exact test.