Review Article

Insights and perspectives into clinical biomarker discovery in pediatric heart failure and congenital heart disease—a narrative review

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Background and Objective: Heart failure (HF) in the pediatric population is a multi-factorial process with a wide spectrum of etiologies and clinical manifestations, that are distinct from the adult HF population, with congenital heart disease (CHD) as the most common cause. CHD has high morbidity/mortality with nearly 60% developing HF during the first 12 months of life. Hence, early discovery and diagnosis of CHD in neonates is pivotal. Plasma B-type natriuretic peptide (BNP) is an increasingly popular clinical marker in pediatric HF, however, in contrast to adult HF, it is not yet included in pediatric HF guidelines and there is no standardized reference cut-off value. We explore the current trends and prospects of biomarkers in pediatric HF, including CHD that can aid in diagnosis and management.

Methods: As a narrative review, we will analyze biomarkers with respect to diagnosis and monitoring in specific anatomical types of CHD in the pediatric population considering all English PubMed publications till June 2022.

Key Content and Findings: We present a concise description of our own experience in applying plasma BNP as a clinical biomarker in pediatric HF and CHD (tetralogy of fallot *vs.* ventricular septal defect) in the context of surgical correction, as well as untargeted metabolomics analyses. In the current age of Information Technology and large data sets we also explored new biomarker discovery using Text Mining of 33M manuscripts currently on PubMed.

Conclusions: (Multi) Omics studies from patient samples as well as Data Mining can be considered for the discovery of potential pediatric HF biomarkers useful in clinical care. Future research should focus on validation and defining evidence-based value limits and reference ranges for specific indications using the most up-to-date assays in parallel to commonly used studies.

Keywords: Pediatric heart failure (HF); congenital heart disease (CHD); biomarker discovery; narrative review

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Introduction

Background and history of pediatric congenital heart disease

Heart failure (HF) in pediatric congenital patients is a multi-factorial process with a wide spectrum of etiologies and clinical manifestations that are very distinct from the adult HF population (1-4). With the effective disappearance of native rheumatic heart disease in the US due to improved hygiene and the application of antibiotics with symptoms, the most common cause of pediatric HF has become congenital heart disease (CHD) (5-7). Worldwide, CHD still has a very high mortality with an increasing prevalence with nearly 60% developing HF in the first 12 months of life (8-10). Hence, early discovery and diagnosis of CHD in neonates is pivotal. For infants as well as children with heart conditions, HF is the most common indication to receive medical treatment and 50% of the referrals with significant failure eventually leading to the evaluation for heart transplantation (11). Children with CHD who eventually receive heart transplantation, have a different in outcome from those with cardiomyopathy (12). The overall mortality is similar, but patients with cardiomyopathy may have fewer morbidities. Nevertheless, the incidence as well as the prevalence of pediatric HF is still not known, due to the many types and etiologies, without an accepted universal classification (5-7).

However, a simple, reliable, and quantifiable systematic test to classify and standardize pediatric HF patients, especially in CHD, has been lacking. There are still many patients who have mal-adaptive anatomical substrate or residual lesions that may lead to HF over time. Clinical trials involving invasive studies can be difficult due to the sensitive research issues and barriers involving children. The potential need to corroborate findings with invasive studies such as frequent blood tests or invasive testing such as indwelling catheters can decrease parental acceptance for research studies. Consequently, most diagnosis and therapy in pediatric HF patients is still based on subjective observations along with standard testing. Due to the intense variability with CHD, classification schema are complicated and a method to standardize and quantitatively gauge HF is difficult. Translating conclusions from adult studies into the pediatric realm has also become commonplace.

Several biomarkers for HF including the extensively investigated atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), C-reactive protein (CRP) and tumor necrosis factor alpha (TNF-α) have been identified and are applied clinically in the adult population to assess the diagnosis, severity and management of the HF condition before and after treatment (13-17). BNP as a clinical biomarker in the assessment of pediatric HF and CHD is straightforward and can be a significant factor in the diagnosis and treatment (7,12,18). More recently, additional reports have indicated that plasma BNP, sometimes in parallel to Troponin-I (19), can be used as suitable diagnostic and prognostic biomarkers in pediatric HF (15,16,20). However, in contrast to adult HF, plasma BNP as clinical biomarker is not yet included in the pediatric HF guidelines and there is no standardized reference cut-off value yet (16,21). Furthermore, in a wide variety of (multi)-Omics studies, several constituents are noteworthy and becoming increasingly popular such as Galectin-3 (22,23), Lipids (24) and Free Fatty Acids (25,26). We searched for biomarkers with respect to diagnosis and monitoring in specific anatomical types of CHD in the pediatric population, together with a concise description of our own experience in applying plasma BNP as a clinical biomarker in pediatric HF and CHD in the setting of surgical correction. We present the following article in accordance with the Narrative Review reporting checklist (available at: https://cdt.amegroups.com/article/view/10.21037/cdt-22-386/rc).

Methods

A query of the literature using the online search engines PubMed and Google scholar was conducted for pediatric HF and CHD (*Table 1*). Accordingly, no data restrictions were incorporated (from origin to July 2022), and only publications in English were considered. We queried a broad search using definitions and key words such as "children", "heart failure", "congenital heart disease", "biomarkers", "genes", "proteins", and "metabolites". Lastly, we also combined key words "BNP and multi-omics and metabolomics" to search for BNP studies integrated with multi-omics. We encountered many manuscripts reporting biomarkers in pediatric patients also with CHD. As part of a narrative review, we focused on the most notable proteins and metabolites reported over the last 5 years. We will discuss further experiences and developments of the use of the known biomarkers BNP and N-terminal ProBNP (NT-ProBNP) in pediatric HF observing an increasing number of reports with positive application in diagnosis and management, especially in recent years.

In a pilot study performed in Long Beach, we conducted

Table 1 Summary and overview of the literature search approach

Items	Specification
Date of search	07/1/2022–07/16/2022
Databases and other sources searched	PubMed was the primary database used, Google Scholar was also used but far less extensive than PubMed
Search terms used	"Children" [All Fields] AND "Pediatric" [All Fields] AND "Heart Failure" [All Fields]
	"Children" [All Fields] AND "Heart Failure" [All Fields] AND "Congenital"
	"Children" [All Fields] AND "Heart Failure" [All Fields] AND "Biomarkers" [All Fields]
	"Children" [All Fields] AND "Heart Failure" [All Fields] AND "Proteins" [All Fields] AND "Metabolites" [All Fields] AND "Genes" [All Fields]
	"Children" [All Fields] AND "Heart Failure" [All Fields] AND "B-type natriuretic peptide" [All Fields] AND "BNP" [All Fields]
Timeframe	All published manuscripts up to July 2022 were considered in our basic query
	There was a focus on "recent" review studies (published from 2010 onwards) that directly addressed Pediatric Heart Failure
Inclusion and exclusion criteria	Focus was placed on published original papers and reviews in English. Non English manuscript not translated were excluded
	Manuscripts were excluded that were not relevant for the scope of the paper, or that addressed adult heart failure
Selection progress	The queries were conducted independently by DL, MC, SS

Metabolic Profiling of Blood Plasma before and after surgical correction of CHD in neonates in HF: Employing a multiple-reaction-monitoring (MRM) based mass spectrometry (MS) quantitative methodology, we examined 9 quantification panels of plasma metabolites [P180, Energy Metabolism, Free Fatty Acids, Eicosanoids and other oxidation products of polyunsaturated fatty acids (PUFAs), Lipids, Steroids, Neurotransmitters, Bile acids, Oxidative status assays] using service offered by Biocrates Life Science AG (Innsbruck, Austria). We aimed for a total of 610 plasma metabolites; the absolute abundance levels of plasma metabolites were quantified for following computational analyses. All children were between 3–6 months old. For statistical analysis of metabolites, we conducted a differential analysis. A multiple testing correction was applied to the P values calculated by the *t*-tests, using the Benjamin-Hochberg method to reduce the false positive rate. A threshold of false discovery rate (FDR) <0.05 and ±1.5 Fold-change was applied to detect metabolites with significant changes. The figure displaying neonatal metabolomics pilot data was made in r-programming. Plasma BNP levels before and after surgery were compared with paired *t*-test. The study on the infants is approved by the Institutional Review Board (IRB) of the Miller's Children Hospital in Long Beach (IRB No. 086-12). The IRB is overseeing all ethical and safety aspects of the patients. Dr. Setty has had annual meetings with the IRB to discuss and report safety and privacy aspects. All blood withdrawals as part of the pilot study were conducted during routine clinical care of the patients via their already present central line. Accordingly, the patients were not subjected to any additional risk or harm during the study. For each patient, parental informed consent was received before inclusion into the study. The study carried out conforms to the Declaration of Helsinki (as revised in 2013), available at: https://www.wma.net/wp-content/uploads/2016/11/DoH-Oct2013-JAMA.pdf.

We conducted a text mining algorithm for 8,325 heart related proteins over 33M publications on PubMed to quantify these according to their correlation in CHD and created a knowledge graph. Detailed description and methods are described previously (27,28).

Pathophysiology of pediatric congenital HF

HF is a complex interplay of neuro-hormonal, molecular-cellular, and circulatory hemodynamic factors rather than

Table 2 Congenital heart disease overview

Pathophysiology	Incidence (% of all CHD)	Shunt	Obstructive
Acyanotic			
VSD	25	L→R	
ASD	10	L→R	
PDA	10	L→R	
AVSD	5	Mixing	
Coarctation of the aorta	10		√
Aortic stenosis	10		√
Pulmonary valve stenosis	10		√
Cyanotic			
TOF	10	R→L	√
Transposition great vessels	5	R→L	
Tricuspid atresia	1-2	R→L	√
Truncus arteriosus	1	Mixing	
Hypoplastic left ventricle	1	Mixing	
Pulmonary atresia	1		√
TAPVR	1	Mixing	√

Basic overview of structural defects, acyanotic and cyanotic with a sample representation of some anatomical configurations, incidence, shunt type (either from left-to-right, from right-to-left, or mixed), and obstructive nature. VSD, ventricle septal defect; ASD, atrial septal defect; PDA, patent ductus arteriosus; AVSD, atrial ventricle septal defect; TOF, tetralogy of fallot; TAPVR, total anomalous pulmonary venous return; CHD, congenital heart disease.

a simple dysfunction of the heart (29,30). Although the majority of CHD is based on a variety of factors, some cases, such as specific cardiomyopathies, are only associated with a chromosomal abnormality, a single gene defect, teratogens, or maternal metabolic syndrome. In contrast to adult HF, CHDs in pediatric HF patients, especially based on structural heart defects, are extremely heterogeneous with a wide variety of types and subclasses each with distinct pathophysiology (31). The structural heart defect affects the pressures in all four heart chambers and the blood flow containing oxygen and carbon dioxide. The heterogenic situation depends on whether an obstruction is present

in the left or right heart which subsequently determines dependency on duct/shunt. A very important element in this complex pathophysiology is the occurrence of pulmonary overflow or underflow of blood which affects its oxygen levels. It is very likely that all known clinical biomarkers such as BNP, CRP, TNF-α and any other potential multi-omics markers are strongly affected by the heterogenic condition in CHDs.

Structural defects in CHD can be separated into three general main pathophysiological groups: (I) left-to-right shunts, (II) right-to-left shunts, and (III) obstructive stenotic lesions on both the right and left sides (*Table 2*). Acyanotic CHD includes the left-to-right shunts with increased pulmonary blood flow but normal saturations. Right-to-left lesions can decrease pulmonary blood flow and desaturate blood on the systemic side, while obstructive lesions can cause a combination of the two depending on the specific anatomy.

Definition and classification of pediatric HF

HF can be defined as a clinical and pathophysiologic syndrome resulting from ventricular dysfunction, volume or pressure overload or a mixture of these pathologies. According to The International Society for Heart and Lung Transplantation, pediatric HF can be defined as both a clinical and pathophysiological syndrome initiated by insufficiency of the ventricle and volume or pressure overload, either alone, or a mixture of both (32). Since pediatric HF has several different triggers, it has a variety of clinical presentations and symptoms that also depend on the age of the patient (10,33,34). In infants, the earliest clinical manifestations are usually subtle and typically present with feeding difficulties due to a baseline dyspnea (35). Eventually, infants that are affected by HF will classically present with failure to thrive mainly observed by poor weight gain by following growth curves. Additional findings during the physical examination in infants who have HF include mild-to-severe retractions with respirations, tachypnea, or dyspnea with grunting (a form of positive end-expiratory pressure), tachycardia, a gallop rhythm (S3, S4), cardiomegaly and hepatomegaly. Toddlers and older may present with exercise intolerance, somnolence, anorexia, or with other signs & symptoms such a shortness of breath, crackles or wheezing, and cough. In both infants and past the toddler age, the physical examination may present with a gallop-rhythm and hepatomegaly as well as peripheral edema and jugular venous distention.

To better understand the specific patient-based management and response to pediatric HF therapy (medical and/or surgical) as well as its complex pathophysiology, a comprehensive knowledge supported by evident laboratory and clinical tests are crucial. Accordingly, pulse oximetry is beneficial in determining cyanosis, electrocardiogram (EKG) is important to assess arrhythmias that can induce cardiac dysfunction, the chest X-ray is used to determine cardiomegaly indicated by the cardiothoracic index, venous congestion and pulmonary edema. Lastly, echocardiography is pivotal for determining CHD anatomy, pressure gradients, cardiac function and identifying chamber dimensions. BNP is one of several biomarkers for HF that have been identified to assess the diagnosis, severity, and management of the cardiac condition before and after treatment in adult HF. Both TNF-α and CRP are sensitive markers for systemic inflammation in adult HF and myocardial infarction (36). In the majority of studies, volume and pressure loading and cardiac dysfunction show elevated levels of BNP regardless of the presence of a congenital heart condition as the underlying etiology which indicates that BNP alterations are more related to the physiologic state (e.g., hemodynamics) rather than the condition or type of treatment. Troponin-I and T can be used to discern ongoing myocardial damage or stress in this setting especially with the heightened sensitivity of the newer assays (18). There have been studies assessing potential biomarker parameters for pediatric CHD readmissions, but these are not routinely used clinically (15,20).

In the adult HF population, the New York Heart Association Classification (NYHA I-IV) is applied as a standard gradation method, but this has never been validated in the pediatric HF population, although it was applied to pediatric HF until 1987. The NYHA classification does not measure the HF severity but rather indicates the functional capacity of a patient. Since the functional capacity during a state of HF is profoundly different in children as in comparison to adults, the use of the NYHA classification did not translate well into children, particularly to infants. Moreover, pediatric HF patients have a significantly different clinical presentation, pathophysiology, and compensatory mechanism (37), and most importantly, a very different etiology of HF with structural CHD as the most common cause. Consequently, Ross *et al.* (38), developed a symptom-based numerical classification comparable to the NYHA classification, applying more age-related variables such as feeding difficulties, growth problems (failure to thrive) and exercise intolerance. The ROSS classification was initially developed to indicate an overall assessment of the HF severity in infants, but has been updated several times to pertain to all pediatric ages, and to incorporate heart rate and respiratory rate and the size of the liver (39). Assignments with the original ROSS classification have been updated with either no symptoms in class I, and continuous symptoms such as tachypnea, retractions and diaphoresis during rest in class IV (38). However, ROSS class II and III are more ambiguous and subjective and often overlap, with mild tachypnea or diaphoresis during feeding in class II and profound tachypnea and diaphoresis during feeding in class III. Additionally, the classification of failure to thrive is subjective to the judgment by the clinician much like the NYHA classification in adults and is often the only manifestation observed in pediatric HF. Furthermore, failure to thrive may also have a non-cardiac etiology such as gastrointestinal, genetic, and/or metabolic causes. This is where a specific HF biomarker in the pediatric population would be able to differentiate between the various clinical etiologies. Ideally, a specific pediatric HF classification should accurately predict the disease risk, so that clinical managing can be custom-made to the HF condition. Pediatric patients who are classified as "No risk" would not need immediate treatment, "Mild risk" would require close observation with medical treatment, "Moderate risk" would provoke rigorous management, while "Severe risk" would prompt utmost therapy and consideration for organ transplantation.

More recently, Connolly *et al.* (37) developed and introduced a novel grading-system named the New York University Pediatric Heart Failure Index for children (NYU-PHFI) and adolescents based on many features during the physical examination in the ROSS classification, but also incorporating a balanced score with medical therapy for HF treatment, and the presence of a single left ventricle (LV). The NYU-PHFI is a weighted, linear combination of scores centered around physical signs & symptoms, as well as the medications. With this index, healthy children have a very low score (0–2) while the maximum score of 30 indicates severe HF (37). The physical signs and symptoms are mainly HF markers that can be identified through routine history, physical exam and diagnostic tests such as chest X-ray, EKG and echocardiogram. Because an underlying disease state can present without symptoms, the index also includes current medical therapy items. The addition of HF medication increases the NYU-PHFI index, which compensates for the reduction in signs and symptoms, to account for patients whose symptoms are relieved by HF medical therapy. The HF medication list

include digoxin, diuretics, angiotensin-converting enzyme (ACE) inhibitors, β-blockers, anticoagulants, and anti-arrhythmics. Drug therapies associated with severe HF such as anticoagulants and higher anti-HF medication dosages are correlated with higher index scores. As a physiologic indicator, a single ventricle anatomical configuration was also included as a separate constituent because single ventricle patients with progressive changes may not be apparent by clinical observations as well as technical measurements. For example, pediatric patients with single ventricle Fontan palliation may not have symptoms with normal aerobic capacity according to their age and body size and would not get higher index scores.

BNP as a clinical biomarker in pediatric HF and CHD

BNP is a member of the natriuretic peptide hormone family and is mainly secreted by cardiomyocytes from the ventricles in response to pressure overload, volume expansion and increased ventricular wall stress. Upon release, BNP induces diuresis, natriuresis and vasodilation to the circulatory system. The precursor hormone, NT-ProBNP is stored together with BNP in atrial granules and they are released simultaneously (40). In adult HF, BNP as well as NT-proBNP are established guideline biomarkers for diagnosis and monitoring of management (41,42), and a high plasma BNP level has been associated with a more severe condition of the disease, while a normal BNP level (i.e., <100 pg/mL) excludes HF (13,43). In pediatric patients, trends may be valuable in this capacity but also requires multiple visits and phlebotomy draws. As most biomarkers are considered individually, a multi-marker strategy may be valuable in refining HF risk stratification and HF classification. For example, the use of BNP in conjunction with Troponin-I in the adult patient population results in a better risk stratification in acute coronary syndromes than with either marker individually (13,43). The accuracy of risk prediction in HF was amplified when the ANP level was combined with additional biomarkers such as CRP and myeloperoxidase (13,43). Moreover, when the four biomarkers BNP, Troponin-I, CRP and cystatin C were coupled together, the risk stratification for mortality from cardiovascular causes among older men was significantly enhanced (13,43).

Studies reporting that plasma BNP is a suitable diagnostic and prognostic biomarker in pediatric patients with CHD have increased in recent years. However, in contrast to adult HF, plasma BNP as a clinical biomarker is not yet included in the pediatric HF guidelines, including CHD. As aforementioned, this is likely due to the heterogeneity and complex pathophysiology of CHDs. Despite growing studies on the ability of BNP to diagnose HF and monitor treatment algorithms, there are also contrasting reports pointing to hemodynamic changes in the pediatric and neonatal population that may be affecting BNP levels. An early literature study by Eindhoven et al. (44) indicated that plasma levels of BNP and NT-proBNP don't necessarily correlate with the severity of the disease, and are not only an expression of hemodynamic overload, but also of non-cardiac confounders such as the neuro-hormonal system or organ function. Most of the literature is from retrospective single center studies including several neonatal conditions including CHD (16,45,46).

The number one cause of HF in neonates is CHD in which the type of cardiac malformation affects the risk and degree of occurrence (47). Consequently, earlier discovery and diagnosis of CHD in neonates is pivotal. BNP as a clinical biomarker in the assessment of the degree of HF is important in treatment (18). Moreover, to diagnose neonatal CHD, the age of the newborns is very important (45,46,48-50). In healthy full-term neonates, the BNP levels are increased immediately after birth, peaking at 2 days, and then slowly decreasing to reach a stable level at 1 month (51). It is not clear why BNP levels sharply increase after birth, and this is likely due to multiple factors (16). For instance, the perinatal circulation and redistribution of blood from the placenta to the lungs have a very significant effect on the volumes in the ventricles and pressure (52,53). Moreover, during the neonatal period, both lung maturation and renal development can affect hemodynamics and volume distribution resulting in BNP plasma level changes (54). Plasma BNP levels may also be affected by the growth and development of fetus, gestational age, and preterm or full-term status (16).

Overall, the plasma BNP concentrations are elevated during CHDs in the pediatric population, with the majority of studies indicating a correlation between plasma BNP levels with RV dilation and the severity of pulmonary regurgitation. In tetralogy of fallot (TOF), the BNP values were significantly higher compared to age and gender matched control patients (44,55), although the studies only take patients into account at an age between 4 to 30 years and did not include neonates. Most studies in TOF patients exhibited a positive correlation between the BNP levels and the severity of pulmonary valve regurgitation (56)

and the RV end-diastolic volume (57), while a negative correlation to exercise capacity and peak oxygen uptake was reported (58). None of the studies reported a link between BNP and LV function or end-diastolic volume (56,57). It also serves to ask the question of whether elevated BNP values mean different things in uncorrected or corrected CHD states. Biomarkers, such as BNP, have been reported to be used to help stratify risk for readmission. They have also been used as an additional variable in grading volume overload in certain populations, but the overall utility is not defined and often used as an adjunct tool along with subjective clinical assessment. Although the clinical value of plasma BNP in pediatric HF is clear, there is still no standardized cut-off value for diagnosis and for therapy monitoring in the pediatric guidelines as is the case in adult HF. Moreover, neonates undergo many different phases in their development with hemodynamic and even structural changes that can strongly affect BNP levels. Plasma BNP in combination with other detection methods such as Chest-X ray, EKG, and echocardiography, or in parallel with other blood plasma (multi) omics panels (e.g., genomics, proteomics, metabolomics) may greatly increase its clinical biomarker value.

Perspectives on novel biomarker development in pediatric HF and CHD

We have witnessed several revisions in our understanding of the syndrome of HF both in the adult as well as in the pediatric population, and it is clear that the development and progression of HF does not only result from simple changes in cardiac function, but is rather a complex interface of hemodynamic, neuro-hormonal, molecular cellular and genetic constituents (7,59). The clinical evaluation of the severity and course of HF in the pediatric population is often concealed by the subjective nature of the diagnostic parameters, leading to diverse opinions and perceptions by the physicians. Currently, several enzymes, hormones, and other markers of cardiac stress, injury and dysfunction have emerged as clinically important biomarkers to support in the diagnosis, decision making, prognosis and classification in cardiovascular diseases. Biomarkers in HF can be divided into six categories: (I) markers of cardiomyocyte stress; (II) markers of inflammation; (III) markers of neurohormonal activation; (IV) markers of cardiomyocyte injury; (V) markers of extracellular matrix remodeling; (VI) markers of metabolism and nutrition. Three criteria for the assessment of biomarkers in clinical application can be

outlined (60). First, biomarkers must exhibit reliable repeated measurements at reasonable costs. Second, the marker must offer additional information that is not yet acquired from a cautious clinical evaluation. Third, knowing the measured level of the marker ought to help with the clinical management and decisions (60).

The assets of biomarkers may be the following (18): (I) they are objectively quantified as numerical values; (II) they are reproducible; (III) they can be repeatedly measured over time and follow-up; (IV) they are less invasive and safe; and (V) they straightforward with relatively low costs. The application of biomarkers in pediatric HF and CHD is likely to become more common. Considering defined clinical needs, sample collection and recruitment, statistical evaluation, and the selection of an analytical platform, the workflow in biomarker development can be divided into three main phases: (I) discovery, (II) verification and (III) validation. With regards to these three phases, plasma BNP in pediatric HF and CHD may still require more validation in different strata and underlying etiologies of pediatric HF including those with CHD. Accordingly, additional (multi) Omics panels integrated with plasma BNP levels should be considered to aid in diagnosis and monitoring of management.

Omics studies for biomarker discovery in CHD

In the pediatric population, human samples for biomarker studies and validation can be retrieved as whole blood, saliva, urine or as cardiac biopsies during surgical procedures (61). Multi-Omics studies in human samples may uncover new biomarkers (in addition to BNP) for clinical stratification and outcome prediction (62-65). Accordingly, we consider several venues that may advance the development of novel potential biomarkers in CHD: (I) Genomic and transcriptomic analyses to identify specific genes and DNA sequence variants correlated to CHD (14,66-68). (II) Proteomic analyses because cardiac dysfunction in CHD and HF are likely to result from underlying gene alterations and protein expression levels (69-71), protein-interactions and protein post-translational modifications. Surprisingly, relatively few proteomic studies have been reported on CHD. (III) Metabolic analyses (72-75).

Within these 3 venues we encountered several noteworthy proteomic constituents that are becoming increasingly popular in adult HF as well as the pediatric population. For instance, the mild-regional proadrenomedullin which is a neuro-hormone responsive to myocardial stretch similar

to BNP, was evaluated on admission in children (average age 24 months) presenting with HF. The study reported a high diagnostic value of plasma BNP, MR-proADM, and Troponin-I levels as a triple-panel upon admission showing a correlation between the clinical severity and disease progression of pediatric HF (76). Furthermore, Galactin-3 which is a carbohydrate-binding lectin that is released by macrophages to regulate inflammatory functions in immunity, and cancer, has been proposed as a suitable tool for the diagnosis and risk stratification of chronic HF in children (40), however it has not yet been established as in adult HF. Interestingly, Galectin-3 is secreted by many cell types, including macrophages and is involved in a variety of processes such as inflammation, apoptosis, fibrosis and inhibition of antioxidation (23). In 12–18-month-old children diagnosed with CHD and HF, plasma levels of Growth Differentiation Factor 15 (aka, GDF-15) and beta2-microglobulin (aka, beta2-MG) were positively correlated to the severity of their cardiac function comparing HF *vs.* healthy controls (77). As connective tissue growth factors play an important role in several biological such as cell proliferation, angiogenesis and extracellular matrix production, the plasma-N-terminal growth factor was found significantly higher in children with HF compared to healthy volunteers and may be a promising biomarker (78). Another noteworthy protein, the cardiac-myosin-binding-protein-c (aka cMyBP-C), which is a thick-filament associated protein and a specific cardiomyocyte determinating factor of the structure and integrity of sacromeres, as well as contractile function. Plasma concentrations of cardiac-myosin-binding-protein-c may be a good clinical biomarker for diagnosis of HF in children (average age of 16 months) with a sensitivity of 100% and a specificity of 96% at 45 ng/mL as a cutoff value. Furthermore, the value for the prediction of adverse outcomes in HF patients was acquired by a ROC curve with a sensitivity of 90% and specificity of 93% at a cutoff value of 152 ng/mL cMyBP-C at admittance (79). Other proteins that are known for their association to cardiac tissue injury such as neutrophil gelatinase-associated lipocalin (79), and Heart-Type Fatty Acid Binding Protein (aka, H-FABP) (26), both show increased plasma levels in children with HF as compared to healthy controls.

Metabolomic studies, including those in adult HF (2,80), are a way of highlighting biochemical pathways and signatures correlated to the underlying pathophysiology that may aid in the diagnosis or management of pediatric HF and CHD (81,82). A wide range of metabolites in a variety of CHDs have been reported (83-85), notably acylcarnitines,

triacylglycerides, amino-acid related compounds, lipids, and free fatty acids. Accordingly, lipids play an important role in a wide range of cardiovascular diseases, inflammatory processes, atherosclerosis, myocardial infarction, cardiac function, ventricular remodeling, and metabolic diseases that can start at a juvenile age (24). Lipidomic analyses is an expanding field and should also be explored in pediatric HF and CHD (25). The role of free fatty acids in atherosclerosis, myocardial infarction and HF has been described without clear consensus (86), and may also be considered in the pediatric patient population.

BNP plasma levels integrated with other potential omics biomarkers for clinical guidance or monitoring

Clinically interesting multi-omics panels integrated with plasma BNP levels may aid in diagnosis and monitoring of management in CHD (as well as in adult HF). Nevertheless, clinical studies integrating plasma BNP with multi-omics are still very scarce and are currently more described in animal models and not always in the setting of HF, and not in CHDs. A study in a mouse model deficient of natriuretic peptide receptor (for the binding of BNP) showed significant metabolic differences with the control group (87). This suggests that BNP is indeed correlated to other metabolic panels and may be integrated with a panel of metabolomics. Moreover, a mouse model of alcoholic cardiomyopathy (88) revealed a correlation between increased BNP levels and 297 differentially expressed metabolites were identified which were involved in KEGG pathways related to the biosynthesis of unsaturated fatty acids, vitamin digestion and absorption, oxidative phosphorylation, pentose phosphate, and purine and pyrimidine metabolism. A clinical study in 136 HF patients over 4 stages (A-D) conducted untargeted metabolomics and lipidomics on their blood plasma (89). The investigators reported that a set of specific molecules, such as lysophosphatidylcholine 18:2, cholesteryl ester 18:1, alanine, choline, and Fructose, were associated with BNP or left ventricular ejection fractions. Lastly, a machine learning (ML) approach to analyze multi-omics in HF patients and their EKGs and echocardiography was performed. However, the pilot-study is still descriptive and concluded that multi-omics pathways still need to be revealed in future studies (88).

In an attempt to simplify CHD, we divided Pediatric CHD and HF into cyanotic and acyanotic groups which

both may leave distinct metabolomic signatures that can be explored to predict clinical outcomes or therapeutic efficacy (90). This led us to explore metabolomics pilot studies in cyanotic CHD (TOF) *vs.* acyanotic CHD (VSD). Preliminary data performed and collected from a pilot study at Miller Children's Hospital in Long Beach demonstrated interesting results. Perioperative data collected in the pilot study demonstrated that 10 TOF and 15 VSD infants aged less than 6 months had plasma BNP and Troponin-I analyzed at two-time intervals (pre-op and 2 days post-surgery) along with two different clinical evaluations for pediatric HF, ROSS and NYU-PHFI. On average, BNP levels were normal or slightly elevated in both groups prior to surgery (mean plasma BNP: 145.4±122 pg/mL in VSD as indicated in *Figure 1A*, and 132.0±138 pg/mL in TOF as indicated in *Figure 1B*). All patients in both groups showed an increase of their BNP levels 2 days post-operatively (mean plasma BNP 545.9±247 pg/mL in VSD and 779.4±371 pg/mL in TOF). All patients in both groups showed a further increase of their BNP levels, 2 days after surgical correction, but in parallel, every patient was clinically improved from HF symptoms and both the ROSS classification (*Figure 1C* for VSD patients, *Figure 1D* for TOF patients) and NYU-PHFI scores (*Figure 1E* for VSD patients, *Figure 1F* for TOF patients) were normalized in the majority of patients off inotropic support. Before surgical correction, the patients were exhibiting early signs of HF as evidenced by their clinical ROSS and NYU-PHFI evaluations. Since BNP mainly relies on pressure and stretch of the heart chambers, a hypothesis is that the acute ventricular wall stress of closing shunts and increasing volume loading on ventricles caused an acute rise in the BNP. However, with the different types of CHD, this could signify that the BNP value is just a physiological parameter responding to pressure and stretch of the LV rather than a clinical marker with diagnostic and gradation abilities as observed in the adult population. The role of BNP (in conjunction with or without Troponin-I, CRP or TNF-α) in pediatric CHD is not completely clear and not yet defined in the pediatric HF guidelines (14). Even though this was a limited pilot study with a relatively small number of neonatal patients, it highlights the question how to interpret BNP in this population before and after surgical correction and if there are better avenues in which to study potential biomarkers for HF in CHD. To better illuminate the importance of BNP as a diagnostic or prognostic marker, or as a mean to stratify the HF status in CHD, larger and well-designed prospective studies are warranted.

At the same time periods in the same patients, preliminary perioperative data of targeted metabolites demonstrated that these infants exhibited disparate metabolic signatures before and after surgical correction (*Figure 2*). An MRM-based MS spectrometry method distinguished and measured the absolute abundances of a total 610 metabolites each allocated to 20 biological classes. The TOF patients displayed 16 metabolites (out of 610) that changed 2 days after surgery, with 10 metabolites upregulated post-surgery and 6 metabolites downregulated (*Figure 2*). The upregulated metabolites were 2 Ceramides, 2 dihydroceramides, and 6 phosphatidylethanolamines. The 6 downregulated metabolites were 2 biogenic amines, 3 phosphatidylcholines and 1 phosphatidylethanolamine. The VSD patients displayed 28 metabolites that changed 2 days after surgical correction, with 16 metabolites upregulated and 12 metabolites downregulated. The upregulated metabolites were 1 glycerophospholipid, 11 phosphatidylethanolamines, 2 ceramides, and 2 dihydroceramides. The downregulated metabolites were 4 amino acids, 3 biogenic amines, 3 glycerophospholipids, and 2 phosphatidylethanolamines. Interestingly, in both subgroups the majority of metabolites remained unchanged, suggesting that the changed metabolites were not solely related to the impact of the surgical procedure (e.g., release of inflammatory cytokines) and may be potential biomarkers with clinical value.

Bioinformatics, data mining & ML in the age of information technology

Most CHDs remain poorly understood and appear to have multiple causal factors, with genetic or environmental factors. There are several knowledge gaps in our understanding of CHD, and traditional means of statistical analyses are often not sufficient to estimate the wide spectrum of genetic and environmental factors leading to CHD. Hence, to study the roles of a wide range of multiple factors in the prediction and prevention CHDs in the age of Information Technology Machine Learning is an efficient venue to increase our understanding and treatment of CHD (91,92). Compared to traditional models, ML algorithms often have significantly better performance for prediction and are robust in handling collinearity problems and outliers (93,94). ML is growing into a popular method to discover unseen patterns in sizeable datasets of heart sounds, images (95), EKGs, and omics data for early and more accurate predictions of CHD (96,97). ML has already
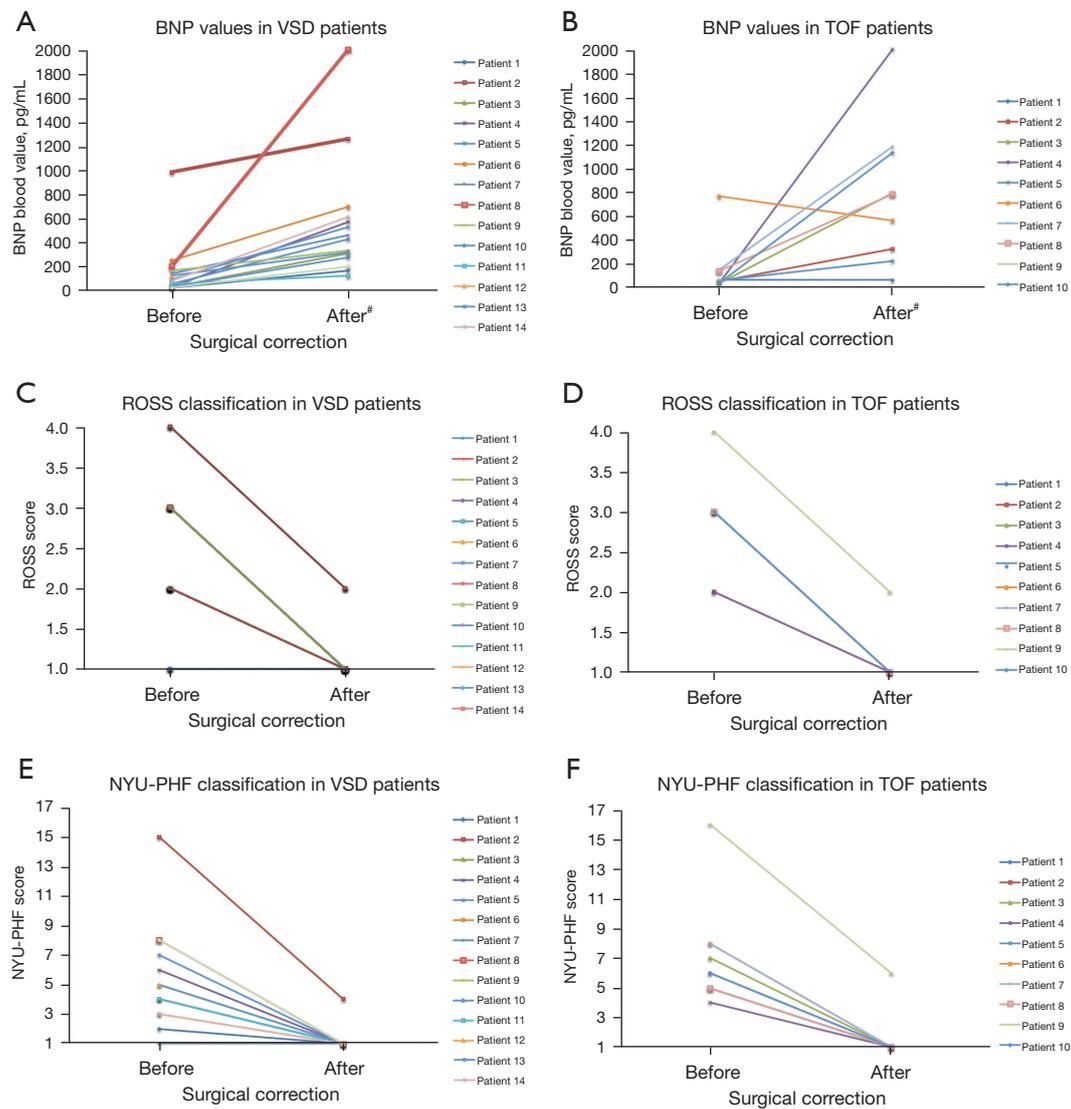
Figure 1 BNP values in Patients with VSD and TOF. BNP from blood plasma were determined in parallel with their clinical evaluation signified by the ROSS score and NYU-PHF index in 15 neonates with VSD (non-cyanotic CHD) and in 10 neonates with TOF (cyanotic CHD), right before and after surgical correction. Before surgical correction, the patients were exhibiting early signs of HF as evidenced by their clinical ROSS and NYU-PHFI evaluations. Nevertheless, all patients in both groups showed a further increase of their BNP levels, after surgical correction, but in parallel, every patient was clinically improved from HF symptoms and both the ROSS classification and NYU-PHFI were normalized in almost all patients. This indicates that in CHD of the pediatric population, the BNP value is just a physiological parameter responding to pressure and stretch of the LV rather than a clinical marker with diagnostic and gradation abilities as observed in the adult population, and that clinical implications should be drawn with caution during the perioperative period. Moreover, all patients were neonates aged between 3–6 months and still in their development and growth stage which also have an effect on plasma BNP. Plasma BNP levels were compared before and after surgical correction by paired *t*-test, #, P<0.05. BNP, B-type natriuretic peptide; VSD, ventricle septal defect; TOF, tetralogy of fallot; HF, heart failure; NYU-PHF, New York University Pediatric Heart Failure; NYU-PHFI, New York University Pediatric Heart Failure Index; CHD, congenital heart disease; LV, left ventricle.
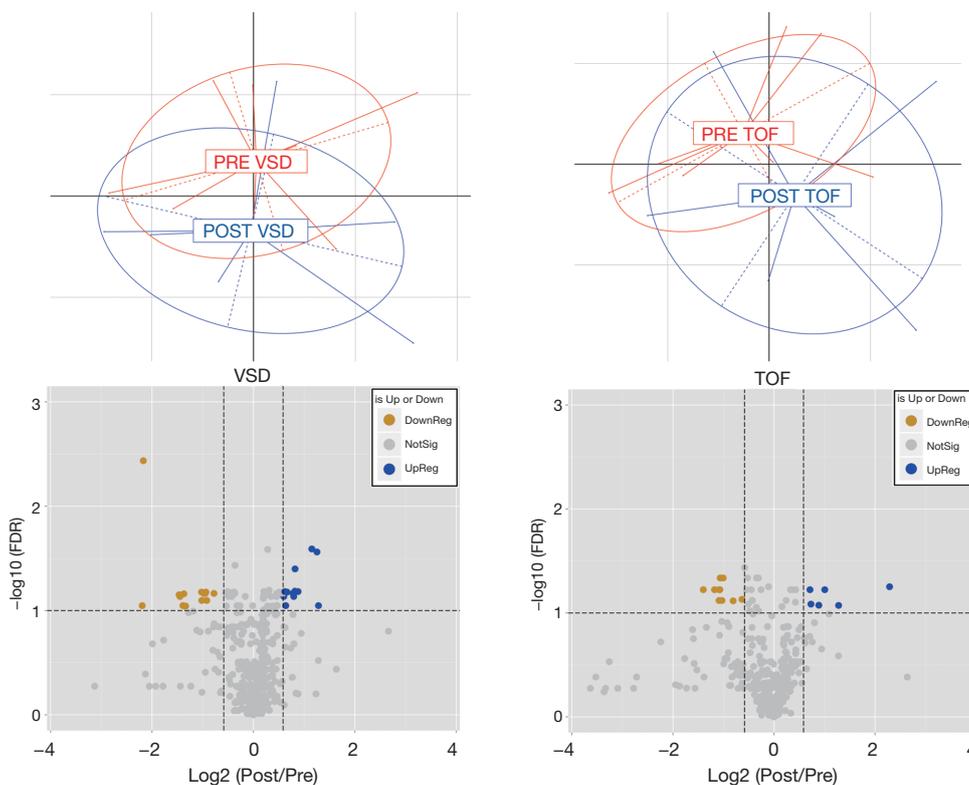
Figure 2 Targeted Metabolomic Pilot study in TOF and VSD. We explored a targeted metabolomics pilot study in cyanotic CHD (TOF) *vs.* acyanotic CHD (VSD). The TOF patients displayed 16 metabolites (out of 610) that changed 2 days after surgery, with 10 metabolites upregulated post-surgery and 6 metabolites downregulated. The VSD patients displayed 28 metabolites that changed 2 days after surgical correction, with 16 metabolites upregulated and 12 metabolites downregulated. Interestingly, in both subgroups the majority of metabolites remained unchanged, suggesting that the changed metabolites were not solely related to the impact of the surgical procedure (e.g., release of inflammatory cytokines) and may be potential biomarkers with clinical value. A differential analysis was conducted. A multiple testing correction was applied to the P values calculated by the *t*-tests, using the Benjamin-Hochberg method to reduce the false positive rate. A threshold of FDR <0.05 and ±1.5 Fold-change was applied to detect metabolites with significant changes. VSD, ventricle septal defect; TOF, tetralogy of fallot; CHD, congenital heart disease; FDR, false discovery rate.

been applied to enable early detection and discovery of CHD and to successfully identify risk factors followed by optimum management plans. By analyzing over 44,000 medical records for 10,019 patients, Diller *et al.* devised a Deep Learning algorithm to predict primary clinical diagnostics, the complexity of the disease, and the most optimal treatment regimens (98). In parallel, ML algorithms can also be applied to study large Omics data from animal models or patients, or text data from patient notes and clinical reports (99-101). To address the knowledge gaps above, our group conducted a study applying a ML based Text Mining algorithm to computationally quantify 8,325 proteins known to be related to the heart according to their frequency, integrity, and distinctiveness in CHD.

Hence, a vast amount of unstructured text data in over 33M publications (PubMed) was mined (~400 manuscripts/sec) according to a Text Mining algorithm to calculate protein-disease relationships developed by our collaborator (27,28,64). Out of 8,325 heart related protein, 937 proteins associated to CHD were identified. A Knowledge Graph (*Figure 3*) of the proteins associated to CHD indicated that fibrilline-1 had the highest association with CHD (largest radius in the KG) followed by somatotropin, alpha-fetoprotein, protein-jagged-1, and insulin-like growth factor. Both fibrilline-1 and somatotropin had the most cooccurrences with other proteins (i.e., these proteins were often described together in CHD) as indicated by the number of edges to each protein node. Altogether, we
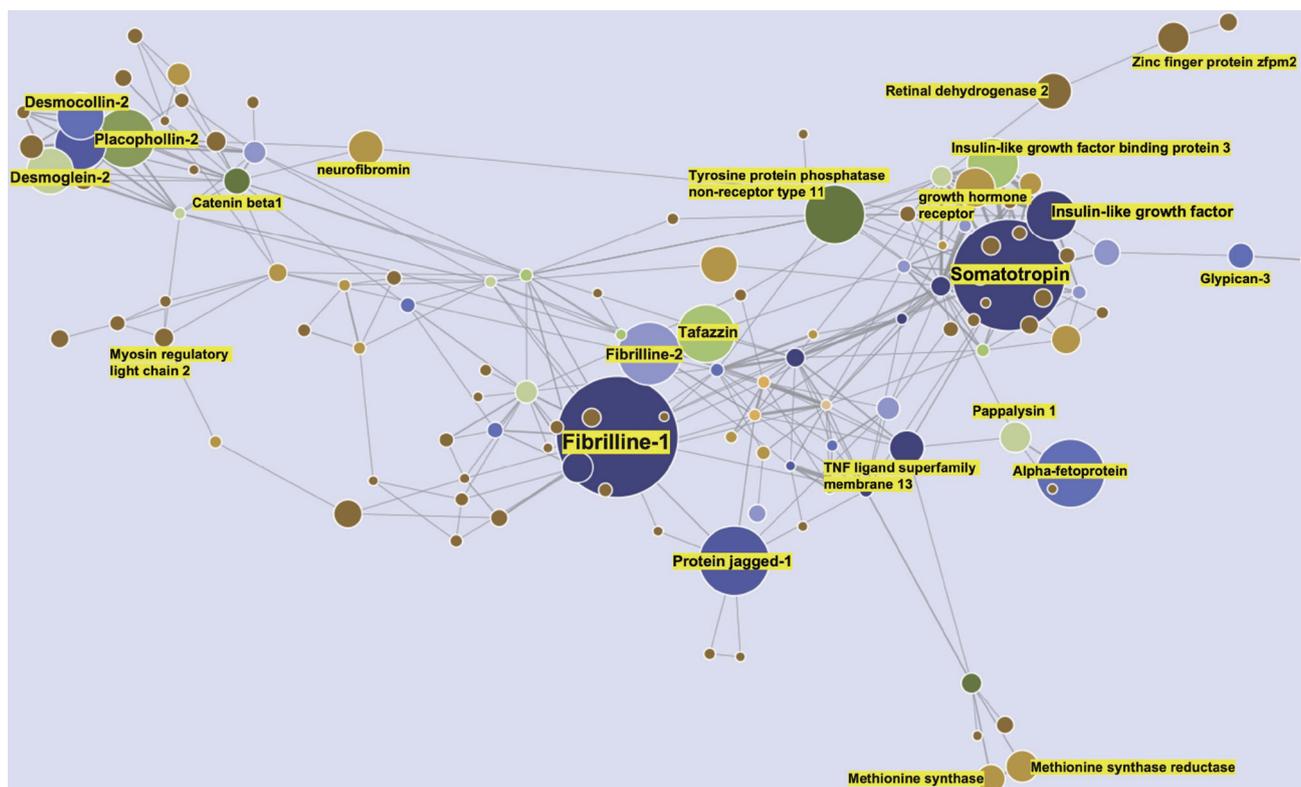
Figure 3 Proteomic analyses by ML based Text Mining. We conducted a pilot study applying a ML based Text Mining algorithm to computationally quantify the association of 8,325 proteins to CHD. Hence, 937 proteins associated to CHD were identified in over 33M publications (PubMed). A Knowledge Graph of the proteins associated to CHD indicated that fibrilline-1 had the highest association with CHD (largest radius in the Knowledge Graph) followed by somatotropin, alpha-fetoprotein, protein-jagged-1, and insulin-like growth factor. Both fibrilline-1 and somatotropin had the most cooccurrences with other proteins (i.e., these proteins were often described together in CHD) as indicated by the number of edges to each protein node. ML, machine learning; CHD, congenital heart disease.

believe that our ML based Text Mining was able to unveil protein-CHD associations (in over 33M publications) that may be suitable biomarker candidates. Targeted proteomic investigations in patient's blood and tissue samples will shed more light on these protein candidates in CHD biomarker discovery.

Conclusions

Pediatric HF, including those with CHD, do not have a standard guideline for any clinical biomarker as a diagnostic or therapeutic mean. Even BNP and NT-ProBNP, although very popular in both adult and less often is pediatric clinical practice, has not been included in the standard guidelines for pediatric HF. New biomarker discovery (that may be integrated with BNP) to enhance diagnosis and therapy monitoring in pediatric HF and CHD can be unveiled from

whole blood or from myocardial biopsies. In the current age of Information Technology and large data sets, we consider (multi) Omics studies from patient samples as well as Data Mining and ML studies for the discovery of potential biomarkers in pediatric HF and CHD. Hence, future research should focus on validation and defining evidence-based cut-offs and reference ranges for specific indications using the most up-to-date assays.

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Footnote

Reporting Checklist: The authors have completed the

Narrative Review reporting checklist. Available at: https://cdt.amegroups.com/article/view/10.21037/cdt-22-386/rc

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