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Original Article

Proteomics of pediatric heart failure: from traditional biomarkers to new discovery strategies*

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Abstract Heart failure in children is a complex clinical syndrome with multiple aetiologies. The underlying disorders that lead to heart failure in children differ significantly from those in adults. Some clinical biomarkers for heart failure status and prognosis appear to be useful in both age groups. This review outlines the use and the present status of biomarkers for heart failure in paediatric cardiology. Furthermore, clinical scenarios in which development of new biomarkers might address management or prognosis are discussed. Finally, strategies for proteomic discovery of novel biomarkers and application to practice are described.

Keywords: Biomarker; heart failure; proteomics; post-translational modification

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Heart failure is challenging to assess and manage in children. Advances in molecular diagnosis, classification, and prognosis have improved through individual and multi-institutional efforts, such as the Pediatric Cardiomyopathy Registry.[1] In addition, devices and transplantation, modelled on approaches to heart failure in adults, have been successfully implemented.[2] Nevertheless, there are many gaps in knowledge about the diagnosis, prognosis, predictive testing, and treatment in paediatric heart failure. The discovery process for new proteomic biomarkers also has the potential to provide better disease assessment and management and importantly may uncover biological insights leading to new therapies. This review will focus on the present clinical applications of biomarkers in heart failure and the development of new biomarkers for paediatric heart failure using proteomics.

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Heart failure can be defined broadly as a clinical syndrome in which there is impaired ability of the heart to fill and eject blood from the ventricles.[2,3] The main causes of heart failure in children that lead to hospitalisation include CHD, various forms of cardiomyopathy, and myocarditis.[2] In spite of many advances in the diagnosis and treatment of paediatric heart failure, there are many ongoing challenges to paediatric cardiologists and their colleagues in allied specialties who care for these children.

The definition of biomarkers

Biomarkers of disease can be helpful in many aspects of disease management. The National Cancer Institute at the National Institutes of Health has provided a definition of biomarkers as "A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition".[4]

Biomarkers for paediatric heart failure should offer one or more characteristics including accurate diagnostic information, risk stratification and monitoring of therapeutic effects and clinical status, and should be available in a relatively short timescale so as to inform

clinical care. As described by Braunwald[5], biomarkers of heart failure may detect one or more of the several processes involved in heart failure, including inflammation, oxidative stress, extracellular-matrix remodelling, neurohormonal activation, and myocyte injury or stress. In addition, it is likely that novel biomarkers discovered by unbiased approaches may uncover cellular regulatory pathways not previously linked to heart failure. These novel markers can be followed-up in laboratory studies that can result in a new biological insight about heart failure.

Classical biomarkers in cardiology and contributions to paediatric and clinical care

A number of biomarkers that are well-established in adult cardiology have been studied in paediatric heart failure. Examples of these include brain natriuretic peptide or amino terminal [NT]-pro brain natriuretic peptide, troponins, and norepinephrine.[6] Brain natriuretic peptide is a protein secreted in response to ventricular or atrial stretch.[7] Troponins I and T are small sarcomere proteins with cardiac-specific isoforms, and are useful as biomarkers for cardiac myocyte necrosis, but as assays have become more sensitive it is apparent that there is troponin leak even in situations without apparent myocyte necrosis.[5] Other biomarkers such as arginine vasopressin, epinephrine, and norepinephrine are associated with the activation of the renin–angiotensin–aldosterone system and the sympathetic nervous system.[5] The interleukins and C-reactive protein indicating inflammation may offer some valuable clues to the paediatric cardiologist.[5] Syndecan-1 and Galectin-3 indicating cardiac fibrosis are also useful as biomarkers for heart failure.[8,9] The following sections of the review will address the applications of some of the established biomarkers of paediatric heart failure. Published reviews discuss a range of established and novel biomarkers relevant to heart failure in adults.[5,10]

Markers of neurohormonal activation in paediatric heart failure

Heart failure triggers the activations of several components of the neurohormonal system, specifically the activation of the renin–angiotensin–aldosterone system and the sympathetic nervous system. Neurohormonal activation results in compensatory changes in water and sodium retention, tachycardia, arterial vasoconstriction, and venoconstriction, which augment the cardiac preload and afterload.[11] These compensatory responses are ultimately deleterious in the failing heart.[11] Angiotensin-converting enzyme inhibitors and β-blockers can inhibit the two systems, respectively, and are mainstays in modern therapy of heart failure. Norepinephrine is a biomarker for heart failure. Ross et al[12,13] reported that elevated plasma norepinephrine levels were present in paediatric heart failure patients and correlated with the severity of heart failure as well as with normalising after resolution of heart failure. Current therapeutic strategies in paediatric heart failure follow along the lines of adult heart failure treatment using medications, which antagonise the neurohormonal activation,[3] although typically rather than following hormone levels per se, clinical signs correlating with neurohormonal activation serve as the measures.

Troponins

Assays for cardiac troponin I and T have been widely used in the diagnosis of adult myocardial infarction.[14] In paediatric practice, elevation of troponin is a useful marker for acute myocarditis, cardiac contusion injury, and cardiac toxicity from chemotherapy in children.[15–17] In addition, studies have shown that cardiac ischaemia/injury leads to elevated troponin levels in children.[18,19] Checchia et al[18] reported that the ejection fraction and circumferential fibre shortening of survivors from cardiac arrest are inversely correlated with troponin I level. Although troponin assays provide valuable information about cardiac injury and myocarditis, overuse of troponin screening in children with chest pain provides minimal benefits and is associated with increased resource utilisation, unless patients have symptoms that may suggest myocarditis, such as fever and/or electrocardiographic abnormalities.[20]

Another setting in which troponin assays may be helpful is in the monitoring for rejection after cardiac transplantation. Troponins may be elevated in acute rejection after heart transplantation.[21,22] A recent pilot study examined the use of a sensitive troponin T assay along with [NT]-pro brain naturetic peptide levels in children with suspected acute rejection and correlated these markers with rejection on biopsy;[21] however, this will require further study in larger populations to assess whether biomarkers could replace endomyocardial biopsy, which is the current standard of care.[21] Earlier studies have suggested that elevated troponin levels might also be useful as a measurement of potential cardiac graft status in organ donors.[23] More recently, a study demonstrated that troponin I levels in a potential transplant donor heart were not correlated with post-transplant hospital length of stay and graft status.[24]

High-sensitivity troponin assays are defined differently by different groups, although some in the biomarker community advocate a definition of these assays as assays that detect troponin in the blood of 50% or more of apparently healthy adults.[25]

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Potter et al[26] also found that transient elevations of high-sensitivity troponin T did not correlate with any cardiac disease in healthy young children. It is notable that the presence of troponin in highly sensitivity assays shows promise to suggest risk for all-cause mortality in adults presenting to the emergency care even when acute myocardial infarction is ruled out at initial presentation.[27] Further investigations are required before the value of high-sensitivity troponin markers is determined in paediatric heart failure.

Brain natriuretic peptides in paediatric heart failure and CHD

Brain natriuretic peptide and amino terminal [NT]-pro brain natriuretic peptide are biomarkers correlated with myocyte stress.[5] Elevated levels of brain natriuretic peptide and amino terminal [NT]-pro brain natriuretic peptide in paediatric heart failure correlate with symptoms and adverse events, including the need for mechanical cardiac support.[28–30] Children with single ventricle have a high risk of developing heart failure. Lowenthal et al[31] reported that the elevation of plasma brain natriuretic peptide and [NT]-pro brain natriuretic peptide was correlated with Ross scores in young children with single ventricle and were helpful tests to detect clinical heart failure in young children with single ventricle, regardless of the stage of palliation. A study of a cohort of children who had the Fontan procedure evaluated in the outpatient setting demonstrated that the majority had normal levels of brain natriuretic peptide and elevation of the levels had only a modest correlation with poor outcome.[32] Book et al[33] reported that adult patients with right ventricular heart failure due to CHD had elevated plasma brain natriuretic peptide levels. A group of patients aged 19.7 ± 4.0 years after the atrial switch operation showed elevated brain natriuretic peptide levels and correlated with the indices of right ventricular function.[34] Nevertheless, another group of patients aged 9.6–37.7 years with systemic right ventricle had normal plasma brain natriuretic peptide levels, but increases were noted with poor clinical status.[35] Furthermore, these authors found that the brain natriuretic peptide level was positively correlated with the severity of tricuspid regurgitation.[35] As reviewed by Kim and Januzzi[36], combined with standard clinical assessments of heart failure, assays of brain natriuretic peptide or [NT]-pro brain natriuretic peptide have been shown to be valuable in the diagnosis of acute decompensated heart failure, and the use of these assays longitudinally may be associated with improved clinical outcomes of heart failure in adults.

Caveats in the extension of adult heart failure biomarkers to children

The experience with biomarkers in adult heart failure should be extended to paediatric heart failure with caution. The aetiologies of heart failure in children differ significantly.[2] In addition, there are significant differences in the normal ranges of some assays between children and adults. Data have shown that the baseline level of troponins and brain natriuretic peptide in neonates are higher than that of teenagers and adults.[37,38]

Opportunities for the development of additional biomarkers for paediatric heart failure

There are numerous other opportunities for the development of biomarkers in paediatric heart failure to address gaps in the present clinical approaches. Biomarkers may prove to be useful in the assessment and prognosis of foetal CHD and cardiomyopathy. Biomarkers may provide useful information for the timing of surgical revision, sequential palliative procedures, and heart transplantation for cyanotic CHD and end-stage heart failure – for example, one study demonstrated that in addition to clinical risk factors peak serum lactate and peak vasoactive inotrope scores may be useful in stratifying risk for extracorporeal membrane oxygenation after the Norwood procedure.[39] A recent review outlined that the use of some novel biomarkers such as ubiquitin C-terminal hydrolase 1, phosphorylated axonal neurofilament heavy chain, tissue plasminogen activator, plasminogen activator inhibitor 1, and glial fibrillary acidic protein may help identify children at high risk following paediatric heart surgery.[40] On the other hand, there is a paucity of data, and this is an important area for further research.

Surrogate markers predicating the event of sudden cardiac death may be useful in cardiomyopathic diseases in paediatrics, as current preventative strategies with internal cardiac defibrillators are associated with higher complication rates in children.[41] Hypertrophic cardiomyopathy is a leading cause of sudden cardiac death in young athletes. A novel model reported by O'Mahony et al[42] demonstrated that clinical factors can predict risk of sudden cardiac death and provide accurate individualised estimates for the probability of sudden cardiac death in patients with hypertrophic cardiomyopathy aged 16 years and above using easily collected clinical parameters. Although this strategy does not include serum biomarkers, validation of biomarkers that can track the risk of sudden death may be useful. In particular, it would be useful to validate markers associated with late gadolinium enhancement as surrogate imaging

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markers for fibrosis, which has been associated with the risk of ventricular arrhythmia.[43]

Discovery strategies to develop new biomarkers for paediatric heart failure

In order to develop new biomarkers for paediatric heart failure, it is necessary to define some of the gaps in knowledge in paediatric heart failure. The United States National Heart, Lung, and Blood Institute recently convened a working group of experts who recommended strategies to enhance progress in understanding and treating paediatric heart failure.[44] In a similar effort, during the Second International Conference on Paediatric Cardiomyopathy, Kantor et al[45] reviewed biomarkers of heart failure to highlight the current knowledge and future directions of biomarker usage in paediatric cardiomyopathy and heart failure.

The National Heart Lung and Blood working group acknowledged the lack of progress in paediatric heart failure and the need to focus on mechanisms that may be specific to paediatric heart failure. This group encouraged a number of specific studies including mechanistic studies, development of better disease models, and partnering with industry and other groups to advance therapies such as improved mechanical support, development of cellular therapies, and cellular models of disease.[44] Development of protein, DNA, and microRNA biomarkers was also encouraged. This group also suggested ongoing development of registries and databases, including linking genotype, genomics, and protein biomarkers to disease outcome;[44] however, the development of new biomarkers and specifically those linked to paediatric heart failure remains a work in progress.

Pathways for biomarker development

A potential developmental strategy in paediatric heart failure is the development of new biomarkers using proteomics approaches. About 15 years ago, the proteomics methods were heavily based on two-dimensional gel electrophoresis technology, but the field has been transformed by the application of newer methodologies using mass spectrometry. Increasingly, mass spectrometer is the instrument of choice to profile tissue and body fluid proteins and metabolites in health and disease.

Mass spectrometry-based proteomics is a powerful technique based on detecting the physical–chemical properties – namely, mass-to-charge ratios – of whole proteins or more commonly proteins digested with enzymes – typically trypsin – to peptides. After peptide mixtures are further separated by liquid chromatography, they are injected into the mass spectrometer where they undergo a series of coupled process that include the following: ionisation, acceleration, deflection by the application of an electric or magnetic field, and detection. The application of this rapidly progressing technology has transformed the discovery-based studies of many diseases. Although the field is advancing rapidly, extensive details about the use of mass spectrometry-based protein profiling in cardiovascular disease are beyond the scope of this review but are described in recent reviews.[46–49]

The process of proteomic biomarker discovery starts by defining a small, relatively homogeneous patient cohort along with an age-matched control population. Of course it is also necessary to define the type of samples that will be utilised for the study – for example, tissue-derived versus body fluids. Standard operating procedures for sample collection and clinical phenotype must be carefully defined. Logically, preference is given to biomarkers detectable in samples obtained via less-invasive means, such as saliva, urine, or serum. Subsequently, the results should be validated in a larger, separate set of samples, before involving large pharmaceutical companies or even "startup" smaller biotechnology firms. Ultimately industry involvement is necessary in the process of translating the discovery efforts to large well-characterised study populations and the final development of a clinical assay platform. Figure 1 provides a schematic representation of this process.

Several aspects of assay development must be considered. An early consideration when developing a

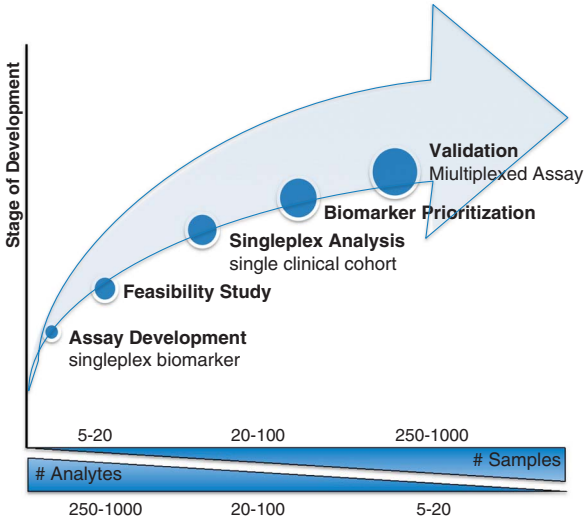

Figure 1.
A schematic representation of the development pipeline for proteomic biomarkers from assay development to validation, and the potential cohort numbers of samples as well, and the number of analytes measured at various stages of the process.

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biomarker is the optimisation of the methods that will be used to isolate the proteins and digestions of the proteins to peptides from the collected sample. Automated methodologies for sample preparation and digestion to peptides are emerging and available for β testing or commercially to assure a low coefficient of variation in sample measurements. In some instances, when the protein of interest is on the lower end of protein abundance in the biological samples, strategies are used to enrich the proteins of interest. For those cases, techniques to deplete the most abundant protein, such as albumin in serum samples,[50] organelle specific,[46] or phosphorylated peptides enrichment,[51,52] are implemented in the sample preparation to overcome the problem of dynamic scale.

Generally speaking, proteomics discovery can be defined as the application of techniques that can broadly detect proteins, or their modifications, associated with a disease state. Proteomics techniques can be applied directly to the tissue of interest, such a heart biopsy, or applied to a body fluid that reflects acute or chronic protein change in diseased tissue, such as the classical example of cardiac troponins partial proteolysis leakage to the serum during cardiac ischaemic injury. Measuring protein rather than gene, microRNAs, or non-coding RNA expression changes has been, in general, more useful to correlate with diseases states; however, efforts in the area of genomics are being made to push these molecules as useful biomarkers for diagnosis or prognosis.[53,54] Perhaps, even better than focussing on a given protein, previous work measuring post-translational modifications, such as site-specific phosphorylation, better correlate with a disease state like heart failure.[51,52,55–57]

A general problem with discovery-based approaches is that the experiments result in large amount of information, thus data analysis and data mining for biological relevance is time consuming and complex. For this and other reasons, many published laboratory findings never progress to clinical application.[56,57] A notable exception to this limitation is a recent study where they identified quiescin Q6 as a biomarker for acutely decompensated adult heart failure.[48] The key to success in that study was the method development tailored to small-abundance proteins and sophisticated software algorithms to screen for biologically significant molecules that were further validated in an animal model, and combined with a gold standard biomarker, such as B-type natriuretic peptide. This is when hypothesis-based efforts, or targeted proteomics, can yield more promising candidates;[49,55] however, the drawbacks are the limitation to available biological knowledge, which is particularly relevant to paediatric heart failure, and prior knowledge can limit new insights into disease processes.

In summary, a number of studies using small patient cohorts have supported the use of biomarkers such as brain natriuretic factor and troponins in paediatric heart failure. Assessment of biomarkers in larger cohorts such as in established registries or in patients involved in clinical trials is one opportunity to learn more about the correlation of biomarkers with disease. Application of discovery-based proteomic approaches may well contribute to the future care of children with heart failure.

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Conflicts of Interest

None.

Ethical Standards

This work was performed in accordance to the ethical standards and AAUP, Policy Documents and Reports 11th edition (Baltimore: Johns Hopkins University Press, 2015), 91–93.

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