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Children as Biomarker Orphans: Progress in the Field of Pediatric Biomarkers

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Biomarkers are ubiquitously incorporated into clinical practice as surrogate endpoints of physiologic and pathologic processes that cannot be directly measured. For example, a lipid panel is used to evaluate cardiovascular disease, but does not directly measure vascular health. Biomarkers monitor health, detect exposures, diagnose disease, predict disease severity and outcome, and monitor treatment effects. The potential utility of biomarkers in pediatrics is high. Children are not only at risk of developing unique illnesses, but prevalent chronic diseases previously thought of as “adult” illness, including obesity, cardiovascular disease, and type 2 diabetes, have their origins in childhood.(1, 2) In an era where precision, or personalized, medicine is becoming a priority, testing that leads to early intervention and curbs disease severity may have an immense impact on population health and the utilization of health care resources. The Table highlights examples of commonly used and recent biomarkers in pediatrics. Despite this potential, biomarker discovery in pediatrics has been limited. In 2010, the barriers to pediatric biomarker discovery were reviewed.(3) In this updated review, we will discuss the progress, as well as remaining challenges specific to pediatrics, and potential strategies for moving forward.

Definition

Biomarkers are defined by the Institute of Medicine as tests “that are objectively measured and evaluated as indicators of normal biologic processes, pathologic processes, or pharmacologic responses to therapeutic intervention.”(22) Therefore, biomarkers may include biofluids (urine, blood, saliva, stool, cerebral spinal fluid), other tissue (including hair or nails), radiographic, genetic, or any other physiologic testing. The arena of biomarker discovery is moving toward proteins and other small molecule targets, as they provide sensitivity and specificity of current and emerging health status that genetic approaches cannot match.

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Desirable Characteristics of Biomarkers

Several desirable characteristics have been defined for biomarkers.(23) New biomarkers need to be highly correlated with the current gold standard, and should offer an improvement over what is already available. The sample must be both easy to obtain (referring to specimen collection) and easy to process and analyze in the laboratory. Both aspects are necessary for serial measurements to follow trends. Ideally, tests should also have high specificity and sensitivity and be inexpensive to perform.

New methods of non-invasive surrogate testing as an alternate to blood are especially important in pediatrics. Painful procedures are anxiety producing for both patients and parents and often require sedation. Even imaging such as magnetic resonance imaging (MRI) requires sedation in young children. Sedation adds cost and risk, making testing more difficult for patients and next to impossible to perform on healthy control subjects. Although invasive procedures may still be needed for confirmatory testing in some individuals, reliable non-invasive biomarkers from saliva, urine, stool, or hair may help avoid unnecessary procedures in many others.

Interest in Pediatric Biomarkers

Systematic literature searches were performed within the PubMed database to capture published pediatric clinical studies on biomarkers using biospecimens and biobanks. The specimen categories were Cerebrospinal, Urine, Blood, Serum, Plasma, Saliva, Stool, Hair and General/Miscellaneous, and the search strategies included MeSH vocabulary terms. The search query for biomarkers was combined with queries for all specified body fluids and for clinical studies. Searches were limited to the pediatric population and publication years 2012–2016. The biomarker queries were limited by language; biobank queries were not limited by language or publication status. Detailed search strategies and the complete list of references are provided in the Appendix (available at www.jpeds.com).

The search identified 1,126 publications. Although many diseases have overlapping biological system involvement, the publications were broadly categorized (Figure 1), with Infection/Sepsis, Metabolic/Nutrition, and Kidney/Urologic as the three most common systems. This is partially driven by the availability of high quality inflammatory pathway multiplex immunoassays (including products from Meso Scale Discovery, Gaithersburg, MD, and Luminex Corporation, Toronto, ON, Canada) and the recent discovery of acute kidney injury biomarkers with clinical utility.(24–26) The majority of publications (75%) described diagnostic biomarkers, recognizing, however, that biomarkers may have both diagnostic and prognostic utility; 70 publications (6%) were specifically dedicated to the discovery of new biomarkers for diagnosing and monitoring pediatric diseases. The most common biofluid used was blood (Figure 2). The number of open and closed pediatric biomarker trials registered on ClinicalTrials.gov increased to 568 as of December 2016.(27) Even though the increase in publications and trials is encouraging, the pace of pediatric studies pales in comparison with adult studies, where the number of annual biomarker publications in 2010 was approaching 25,000, and is heavily influenced by the fields of oncology and genomics.(28)

Challenges for Pediatrics

The biomarker development process has been critically developed by the Early Detection Research Network (EDRN), an initiative through the National Cancer Institute that facilitates translation of oncology biomarker research into clinical application, but is applicable to multiple specialties.(29) An idea evolves from a basic science discovery and ultimately into clinical use through five phases: 1) Exploratory/Discovery (promising new molecules are identified), 2) Diagnostic Evaluation (assay detects established disease), 3) Early Detection Validation (assay detects pre-clinical disease), 4) Prospective application (detection and false test rates determined), and 5) Clinical Utility (impact on population disease burden).

The hurdles to biomarker development are multifaceted. Although some barriers are specific to each phase of development, some themes exist across the spectrum and can be generalized into three broad categories: 1) lack of clinician scientists and infrastructure, 2) lack of patient samples and 3) lack of control samples.

Challenges with identification/discovery of new target molecules

Relatively few clinician scientists are working on pediatric biomarkers, as evidenced by the number of publications. Training programs and institutional support for clinical investigation are limited, and the federal funding climate is prohibitive for many would-be clinician scientists. There is also little interest from industry in supporting pediatric trials, as they comprise a small portion of the market share.

Compared with adults, few children participate in research studies in general, and few large pediatric trials routinely collect biofluids that can be leveraged for ancillary biomarker studies or as control samples. Parental reluctance to participate in trials is one limiting factor. Many childhood diseases are quite rare, and care is often focused only at certain academic centers. Finally, even common diseases have a low prevalence compared with adults.

Painful or invasive procedures for sample collection are difficult to perform even in routine clinical care, and are even more challenging to get approval for research studies outside of life-threatening illness. In studies that do collect samples, variability in timing, collection method, biofluid type, and storage can be confounding factors that make utilization of samples problematic. There are also age-related restrictions on research blood volumes, which limits the number of exploratory tests that can be performed on any one sample.

Obtaining control samples is even more problematic, and appropriate age-matching is critical to determine normal reference values. Normal ranges are highly dependent upon developmental age and can dramatically change from one age group to the next. Significant physiologic differences exist between developmental groups (premature, neonatal, toddler, school age, adolescent); therefore, extrapolating data from adults or even other pediatric age groups is inadequate. The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) is a nation-wide, multi-center collaborative project carried out by several children's hospitals across Canada. The goal of CALIPER is to develop normal reference ranges by age and sex stratification using blood samples collected from healthy community

children between 0 and 18 years of age. Testing included multiple specialty areas, such as cardiology, nephrology, immunology, critical care, and oncology (www.sickkids.ca/Caliperproject). CALIPER investigators found that comprehensive chemistry values among healthy individuals demonstrated complex partitions across age groups, requiring age correction for every clinical measure studied.(30) The discrepancy across age groups was also born out in analysis of endocrine, immunoassay-based chemistry, and hematologic markers.(31) Another example is fecal calprotectin, which is routinely used as a marker of intestinal inflammation in adults and older children. There is great interest in using this non-invasive test as a marker of necrotizing enterocolitis in infants. However, normal ranges vary significantly by gestational age among preterm infants, making cut-off values difficult to establish.(10, 32)

Unlike the case in adults where biobanks of normal specimens are available for use in studies, repositories for pediatric studies are rare. Biobanks that span the spectrum of developmental stages are extremely limited. Therefore, the reference proteomes and metabolomes, catalogs of all known proteins and metabolites produced by a specific tissue, that are being developed for adults, do not yet include pediatric patients. Additionally, biobanks are expensive to maintain and require resources to coordinate sample collection and expeditious processing to maintain the integrity of the sample. Depending upon the type of molecule being investigated, the “shelf life” prior to processing and once stored can be variable.

Challenges with pre-clinical and clinical validation testing

Once a molecule has been identified as being clinically useful in preliminary clinical trials, similar obstacles must be overcome to perform adequate validation testing to understand the sensitivity, specificity, and false positive and negative rates. Validation must be performed for each specific outcome; it cannot be presumed that cutoff criteria for one population can be extrapolated to another without additional testing.(33) Enrolling sufficient sample size at one institution is near impossible; such studies require multi-site trials, which adds another layer of complexity for coordination and funding. Similar stratification by age group and sex is again necessary, and applies to control subjects as well. Realistically, it may take several years to accumulate sufficient sample sizes for both study and control subjects.

Challenges with monitoring disease control/treatment effect

After incorporating a test into clinical practice, ongoing monitoring of efficacy must be measured. Short-term outcome data is relatively easy to monitor; however long term impact may be harder to measure. Depending upon the type of intervention (diagnosis, change in treatment, etc.) and the age when it occurred, pediatric subjects may need to be followed for 20–30 years before it is known if the risk for complications in adulthood has been modified. A final consideration is the potential ethical risk of identifying genetic abnormalities or pre-dispositions that could impact the child and family both psychologically and financially; this risk is significantly less with protein markers.

Recent Progress in Pediatric Collaboration

Several collaborations aimed at collecting broad clinical data to compliment biospecimen collection are emerging, but have been slow to materialize in pediatrics. The National Children's Study was intended to be a large-scale, long-term child and parent prospective cohort study planned by the NIH to investigate environment influences on development in response to the Children's Health Act of 2000 (www.nichd.nih.gov/research/NCS). Five thousand of the target 100,000 subjects were enrolled from 40 sites, but insurmountable flaws in design and methods, along with funding restraints, caused the study to close in 2014.

An alternative project funded by the NIH is the Environmental Influences on Child Health Outcomes (ECHO), which has current requests for application (RFAs) through several U mechanisms (www.nih.gov/echo). The overarching goal is to coordinate longitudinal studies that evaluate demographic, epigenetic (changes in gene expression without an alteration in the genome), and environmental influences on four key pediatric conditions: upper and lower airway disease, obesity, peri- and post-natal outcomes, and neurodevelopment (including social and behavioral development). Centers can participate as data coordinating and/or data analysis centers. Another component of the ECHO study is the IDeA (institutional developmental award) States Pediatric Clinical Trials Network, which gives an opportunity for children in rural and medically underserved locations to participate in clinical trials. Additional infrastructure and support includes the Children's Health Exposure Analysis Resource (CHEAR). CHEAR is comprised of the National Exposure Assessment Laboratory Network, which provides state-of-the-art analysis of biological samples, a data repository and analysis center, and a data coordinating center. Their intent is to create a public resource for childhood exposures and to provide researchers with tools to fully assess environmental exposures for their ongoing epidemiologic or clinical studies. While the collection of biospecimens is not directly mentioned, the infrastructure is being built to support such ventures.

Disease-specific biobanks are also forming that include pediatric data. The National Biological Sample and Data Repository for Pulmonary Arterial Hypertention (PAH Biobank) is a NIH funded biobank that stores biological samples, genetic data, and clinical data (www.PAHBiobank.org). The samples are housed at Cincinnati Children's Hospital, and interested researchers can apply to have access to data and samples. The National Cancer Institute has Specimen Resource Locator database to help researchers gain access to data and specimens, and includes some pediatric studies (www.specimens.cancer.gov). The NeuroBioBank is also NIH-supported and includes post-mortem brain tissue, clinical data sets, information technology experts, and access to advocacy groups and potential donors. The pediatric studies have had a particular focus on autism spectrum disorders, epilepsy, and traumatic brain injury.

Internationally, the field of pediatric biomarkers is also moving forward. The Newborns and Genotoxic Exposure Risk (NewGeneris) project is a European study utilizing questionnaires and blood derived biomarkers from six different mother-child cohorts to evaluate associations between maternal diet, fetal exposure to dietary contaminants, and early health

effects on the child, such as oncologic or immune disorders.(34) Subsequent publications have demonstrated that carcinogens can cross the placenta.(35, 36) Future outcome studies are anticipated.

The mission of the European National Institute of Environmental Health Sciences is to develop the entire human “exposome,” which is a measure of all exposures over a lifetime that may impact health, including early childhood and nutritional exposures. Their goal is to translate research findings into public health applications through the Human Exposome Project, which includes the international Human Early-Life Exposome, focused on maternal-fetal exposures (www.projecthelix.eu).

The Human Proteome Organization (HUPO) is an international collaborative project that aims to foster advances in technology and map the entire human proteome in order to study biological processes and disease (<https://www.hupo.org>). The Human Pediatric Proteomic Project (PediOme) is an initiative within HUPO that strives to 1) characterize the normal proteome across the span of pediatric ages from birth to adulthood 2) broaden the understanding of the pathophysiology of pediatric diseases, and 3) facilitate collaboration among scientists, clinicians, and industry (www.hupo.org/HPP-PediOme; www.biomedcentral.com/collections/pediome).

Strategies for Improving Pediatric Biomarker Discovery and Development

Pediatric networks need to make specimen collection for biomarker development a priority within their clinical trials. Moving the field of pediatric biomarker development forward is both imperative and possible, but alternative strategies will need to be employed. To summarize the key elements, pediatric studies must be collaborative and ultimately multi-centered to accrue sufficient sample size and resources, have appropriate age-matched controls, and focus on non-invasive techniques for obtaining biospecimens.

Strategies for developing a team

Teams should bridge basic and clinical science disciplines in order to ask the appropriate clinical questions and employ the best strategies for answering them. Many academic centers now have clinical investigation institutes to assist with coordinating collaboration, biostatistics and informatics support, core laboratories, and may include assistance with funding. Bioengineering is another discipline intimately involved in biomarker development that should be considered for partnering.

Strategies for finding funding and other resources

While NIH funding has shrunk, there are still funding opportunities, as well as other resources for collaboration, guidelines for establishing a biorepository, and links to other studies and biobanks. Familiarizing oneself with the NIH website and program officers is crucial for navigating the various resources, as there is much overlap between institutes. There are currently over 30 NIH RFAs that include pediatric biomarkers. Funding may also be found through pediatric foundations, particularly those focused on rare disorders. These foundations may also provide partnership with patient and family focus groups that can

facilitate enrollment of both subjects and control family members, and may have additional industry support that can be leveraged.

Trial networks provide additional resources. These networks pool study design tools and datasets, provide links to patient advocacy groups, related studies, and clinical guidelines, and may potentially have funding opportunities as well. Examples include the Pediatric Trials Network (www.pediatrictrials.org), the Pediatric Heart Network, (www.pediatricheartnetwork.org), the Collaborative Pediatric Critical Care Research Network (www.cpccrn.org), and the Neonatal Research Network (neonatal.rti.org). Whenever possible, investigators are encouraged to participate in biospecimen collection in the various trial networks, which is often a missed opportunity to both contribute to and take advantage of pooled resources.

There is a reduced impetus to fund biomarker discovery. Only 6% of the pediatric biomarker publications from the last five years focused on biomarker discovery, which may in part be related to intellectual property and patent law. Biomarkers, once discovered and clinical utility demonstrated, are very difficult to patent. A change in patent law following two recent landmark cases with *Prometheus* and *Myriad Genetics* occurred when the United States Supreme Court ruled that natural phenomena are not patentable unless some human alteration has been made.⁽³⁷⁾ Without intellectual property protection, there is little incentive for industry to invest in clinical implementation of new biomarkers, especially in pediatrics. In the end, rather than removing restraints on new biomarker development, the reverse is true.

Strategies for study design and data collection

Institutional Review Board (IRB) approved protocols should be designed to collect remnant specimens on patients who are having clinical samples (blood, tissue, or CSF) collected as part of their routine care, especially if the patients are likely to need additional procedures (premature infants, trauma or oncology patients, etc.) or have rare disorders of interest. Collection of non-invasive specimens should simultaneously be obtained for comparison with other specimen types and control samples. These samples can be stored to build a biorepository. Investigators will need to work with their own clinical laboratories to standardize a procedure for collecting, processing, transporting and storing samples. In order to collect control samples for blood, researchers can work with community practitioners and laboratories to take advantage of preventative screening laboratories (lead at 1 year of age, lipid panel at age 12, etc). Urine, saliva and stool can be easier to collect in outpatient settings, but still require planning, as samples may need to be collected at home.

Strategies for qualifying biomarkers

Many pediatric biomarker studies measure a number of common proteins, such as cytokines (interleukins, tumor necrosis factor, etc.), brain proteins (GFAP, S100B, UCHL1, etc), or kidney injury proteins (KIP1, NGAL, etc). Although high quality commercial assays are available, there has been no effort to qualify these assays across multiple vendors to ensure reproducibility. In pediatrics, where sample volumes are often limited and irreplaceable, particularly in rare and small cohorts, replicating study results or comparing results across

studies is not possible. Efforts to qualify specific assays have been very successful in adult biomarker studies for Alzheimer and Parkinson disease. Similar efforts to qualify additional assays would benefit both pediatric and adult studies. The Federal Drug Administration (FDA), which regulates the use of biomarkers, has developed a Biomarker Qualification Program through the Center for Drug Evaluation and Research (CDER). The goals of the program are to provide a framework for assessing the qualification of newly developed biomarkers and integrate the qualified biomarkers into the regulatory review process to reduce redundancy and facilitate adoption of new biomarkers by the scientific community (www.fda.gov).

Strategies for putting it all together

Ideally, investigators should work together to organize funding and resources to synergize research programs that are not simply repetitions of similar work. Specific clinical questions related to either diagnosis or prognosis should be triaged. Coordinated efforts that target a small number of the most pressing diseases at a time are more likely to be successful than multiple independent efforts. Once novel or discovery molecules are identified, resources should be devoted to continuing with validation studies. Involving the FDA and industry early in the process can facilitate trial expansion and ultimately bring the test to market.

Summary

The challenges of pediatric biomarker development are complex. While progress has been made, many obstacles still exist, particularly around specimen acquisition and allocation. Investigators should continue to lobby their own institutions, the NIH, and industry to work together to close the gap between adult and pediatric medicine. The phrase “valley of death” was used by Butler in 2008 to describe the significant divide that exists from bringing a basic science discovery to a clinically useful application.⁽³⁸⁾ The field of pediatrics will need to push forward on several fronts to coordinate investigator and clinician cooperation, to incorporate routine biospecimen collection into trials, to establish accessible biobanks, and to think creatively about the hurdles stemming from intellectual property shortcomings and industry reluctance to participate in pediatric trials. Otherwise, the “valley of death” from discovery to clinical use will continue to widen for children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
The proportion of publications by body system from 2012–2016

Figure 2.

The number of pediatric biomarker publications by body fluid or tissue type from 2012–2016. Multiple tissue types were used in some manuscripts, which are represented more than once in the graph.

Table 1

Examples of pediatric biomarkers in clinical use

Test	Screening/Diagnostic Utility	Comments	Biospecimen	Reference
Anti-liver kidney microsomal antibody (LKM)	Autoimmune hepatitis, chronic hepatitis C	Positive in Type 2 autoimmune hepatitis and in 10% of hepatitis C	Serum/blood	(4, 5)
Anti-nuclear antibody (ANA)	Multiple rheumatologic & autoimmune disorders: systemic lupus erythematosus, rheumatoid arthritis, dermatomyositis, idiopathic thrombocytopenic purpura, autoimmune hepatitis and thyroiditis	Low titers in up to 20% healthy children; Positive in >90% of connective tissue disorders; elevations can occur with drug reactions and some viral infections	Serum/blood	(6, 7)
Brain natriuretic peptide (BNP)	Heart disease and failure	Normally elevated in the first few days of life then decline; levels correlate with pulmonary-to-systemic flow and vascular resistance ratios, mean pulmonary artery pressure, obstructive lesions, and allograft disease after transplant	Serum/blood	(8)
Calprotectin	Intestinal inflammation	Used for screening and treatment response in inflammatory bowel disease; may also be elevated with intestinal infection, cystic fibrosis, and juvenile polyps. Normal values are highly variable in premature infants	Stool	(9, 10)
C-peptide	Diabetes Mellitus	Approximates endogenous insulin production, helps differentiate type	Serum/blood	(11)
C-reactive protein (CRP)	Acute and chronic inflammation from severe bacterial infection, tissue injury, autoimmune disease	Highly sensitive, but not specific, rises more quickly compared to ESR	Serum/blood	(7)
Creatine kinase (CK)	Muscle injury	Elevated in rhabdomyolysis, myopathies, and myositis	Serum/blood	(12, 13)
Ferritin	Iron deficiency anemia (low) Hemophagocytic lymphohistiocytosis (high)	May be elevated as an acute phase reactant in sepsis and shock	Serum/blood	(7, 14)
Fractional exhaled nitric oxide (FENO)	Asthma	Marker of eosinophilic inflammation used for diagnosis and treatment response	Breath test	(15, 16)
Galactomannan	Aspergillus infection (immunocompromised patients)	Less sensitive for other fungal infections	Serum/blood	(17)
Hemoglobin A1C	Diabetes mellitus	Used for diagnosis and to guide dosing of insulin	Serum/blood	(18)
Lactate	Ischemia, sepsis, lactic acidosis/metabolic crisis	Elevation may predict mortality in acutely ill or trauma patients	Serum/blood	(19)
Procalcitonin	Sepsis/severe bacterial infection, shock	More specific to bacterial sepsis than CRP in children	Serum/blood	(7, 20)
Sedimentation rate (ESR)	Acute and chronic inflammation from autoimmune disease or infection	Acute phase reactant with high sensitivity but low specificity for any one disease, rises more slowly compared to CRP	Serum/blood	(7)
Tissue transglutaminase (TTG)	Celiac disease	Highly sensitive and specific; used for screening and treatment response	Serum/blood	(21)