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Design and Implementation of a Prospective Adult Congenital Heart Disease Biobank

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Abstract

Background—Adults with congenital heart disease (ACHD, CHD) comprise a growing, increasingly complex population. The Boston Adult Congenital Heart Disease Biobank is a program for the collection and storage of biospecimens to provide a sustainable resource for scientific biomarker investigation in ACHD.

Methods—We describe a protocol to collect, process and store biospecimens for adults with CHD or associated diagnoses developed based on existing literature and consultation with cardiovascular biomarker epidemiologists. The protocol involves collecting urine and ~48.5 ml of blood. A subset of the blood and urine undergoes immediate clinically relevant testing. The remaining biospecimens are processed soon after collection and stored at –80°C as aliquots of EDTA and lithium heparin plasma, serum, red cell and buffy coat pellet, and urine supernatant. Including tubes with diverse anti-coagulant and clot accelerator contents will enable flexible downstream use. Demographic and clinical data are entered into a database; data on biospecimen collection, processing and storage are managed by an enterprise laboratory information management system.

Results—Since implementation in 2012, we have enrolled more than 650 unique subjects (aged 18–80 years, 53.3% women); the Biobank contains over 11,000 biospecimen aliquots. The most common primary CHD diagnoses are single ventricle status-post Fontan procedure (18.8%),

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repaired tetralogy of Fallot with pulmonary stenosis or atresia (17.6%) and left-sided obstructive lesions (17.5%).

Conclusions—We describe the design and implementation of biospecimen collection, handling and storage protocols with multiple levels of quality assurance. These protocols are feasible and reflect the size and goals of the Boston ACHD Biobank.

Keywords

biomarkers; biobank; biospecimen; organization & administration; collection; processing; storage; congenital heart disease; adult congenital heart disease

INTRODUCTION

A subset of patients with congenital heart disease (CHD) survives to adulthood without medical intervention. The advent of congenital heart surgery in the 1940s followed by additional advances in the care of infants and children with previously fatal congenital heart defects has created a new population of adults with repaired congenital heart disease. There are an estimated 1.5 million adults in the United States living with repaired or unrepaired CHD;¹ this population and its use of healthcare continues to grow.^{1–3} These patients have an increased risk of diverse adverse outcomes including arrhythmia, hypertension, pregnancy complications, heart failure, thromboembolism, kidney disease, liver cirrhosis, hospitalization and premature death.^{3–10}

While there are many adults with CHD, this population is exceptionally diverse. ACHD is comprised of dozens of diagnoses and many diagnoses have an array of possible treatments with the preferred approach based on specific patient characteristics and often evolving over time. There is much greater phenotypic heterogeneity in ACHD than in other fields of adult cardiology, such as heart failure or coronary artery disease. This poses major challenges to systematic investigation. Clinical care in ACHD, therefore, often requires consideration of data from related fields or extrapolation from fundamental physiologic principles. While the choice of therapy is the most evident example, diagnostic tests validated in general adult cardiology or pediatric CHD are often credulously applied to ACHD patients with limited validation or exploration of potential pitfalls in this complicated group. While there have been a number of attempts to understand how best to apply clinical biomarker testing in this burgeoning population, these efforts have been hampered by modest sample sizes and the remarkable diversity of underlying disease and historical approaches to management.

The aim of this project is to develop an adult congenital heart disease biobank and thereby to facilitate research on biomarkers in adults with CHD, with a focus on prospective clinical risk prediction and mechanistic pathophysiology among diverse classes of CHD.

METHODS

STUDY SAMPLE DESCRIPTION AND RECRUITMENT

All patients who present to Boston Children's Hospital or Brigham and Women's Hospital for evaluation of suspected or known CHD (see "Outside Recruitment and Enrollment")

section below for details on a subset of participants enrolled via an alternative pathway). There are 2 inclusion criteria: age ≥ 18 years-old and known or suspected CHD or associated diagnoses including pulmonary hypertension or connective tissue disease (e.g., Marfan syndrome). Patients with severe anemia felt, in the opinion of caregivers, to preclude phlebotomy are not approached for participation. Starting February 2015, we have excluded patients who were hospitalized for any reason and discharged within the prior 4 weeks (see below). There are no requirements regarding fasting state, medication use or recent exercise.

Patients are usually approached during regularly scheduled clinic visits with assent of the primary provider for that encounter. An informational brochure is provided and a research team member explains the study, answers any questions and obtains written informed consent. Patients who are seen at Boston Children's Hospital in specific clinics receive a letter informing them of their eligibility 4 weeks prior to the appointment to allow time for consideration.

REGULATORY STATUS

The protocol has been reviewed and approved by Boston Children's Hospital's Institutional Review Board, with a formal reliance agreement between the Partners Healthcare/Brigham and Women's Hospital and the Boston Children's Hospital Institutional Review Boards and adheres to The Code of Ethics of the World Medical Association. The informed consent process includes discussion of broad possible uses for the samples. The written consent form notes that the main aim of this study is to store blood samples for adults who have congenital heart disease to study which blood tests can predict specific problems in the future. These samples may also be used to study the scientific reasons certain events happen, as well as to explore the genetic basis of congenital heart disease. It is also noted that the samples may be used for diverse types of genetic research and for research questions other than those directly related to adults with congenital heart disease. Participants are provided a check box to opt in or out of genetic testing; that is, patients may choose to participate but not allow genetic testing on their samples.

BIOSPECIMEN COLLECTION, PROCESSING, STORAGE AND TESTING

At the time of enrollment, ~48.5 mL of blood is drawn from a peripheral venous puncture using a 21g butterfly needle with luer attachment, after the patient has been resting in a seated position for at least 5 minutes. BD Vacutainer® (Becton, Dickinson and Company, Franklin Lakes, NJ) tubes are drawn, in the standard order of draw, as follows: 2 × 8.5mL serum separator tube (SST), 1 × 6mL lithium heparin, 1 × 3mL ethylenediaminetetraacetic acid (EDTA), 2 × 10mL EDTA, and 1 × 2.5mL Paxgene Blood RNA tube (Figure 1). Prior to collection all tubes are stored at room temperature; when a subject enrolls, tubes are labeled with a hospital barcode label indicating patient name, gender, date of birth, and date of service. EDTA tubes are inverted 8–10 times after drawing to prevent clot formation. In addition to blood, ~20–30 mL of urine is also obtained using a sterile collection container.

Following collection, labeled blood and urine biospecimens are immediately transported at room temperature to an internal laboratory for processing, with a maximum delay of 30 minutes. Processing and storage are performed manually (Table 1).

Contemporary analysis is conducted on 3mL EDTA, one 8.5mL SST, and the unprocessed urine biospecimen. The contents of the 3mL EDTA tube are used for complete blood count with differential and one of the 8mL SST tubes (after processing, see below) is used to perform a comprehensive metabolic panel, lipid panel, high-sensitivity C-reactive protein (hsCRP) and uric acid (Table 2). An unprocessed urine biospecimen is used to perform urinalysis, urine albumin and urine creatinine concentration (and urine albumin-to-creatinine ratio).

The remaining biospecimens are stored after processing. The 10mL EDTA tubes are centrifuged at 1300g for 10 minutes at 4°C and separated into cryogenic aliquots of plasma (1mL each), and buffy coat/red blood cell combination (1mL each). The lithium heparin plasma tube is also centrifuged at 1300g for 10 minutes at 4°C and the supernatant is transferred into cryogenic aliquot tubes (1mL each). The blood from the SST tubes is allowed to clot for 30–60 minutes at room temperature post-draw. Following this, the SST tubes are then centrifuged at 1300g for 10 minutes at 4°C; one of these tubes is sent to LabCorp (Laboratory Corporation of America, Burlington, NC; www.labcorp.com) for analysis; the supernatant of the other SST is transferred into cryogenic aliquot tubes (1mL each). Lastly, 5mL of urine is transferred from the collection container to a Cryovial® (Thermo-Fisher Scientific, Waltham, MA), self-standing, sterile tube and centrifuged at 1300g for 10 minutes at 4°C. Following centrifuging, 4mL of the supernatant is transferred into 2 × 2mL cryogenic aliquot tubes. All aliquots are labeled with cryogenic labels that include subject ID, date of collection, biospecimen type, biospecimen volume, and study protocol number. The aliquots are transferred to a locked freezer at –80°C until future use. The Paxgene tube is allowed to come to room temperature for 2 hours before being transferred to –20°C for 24 hours and then stored at –80°C.

In February 2015, the standard storage protocol was revised to reflect changes in amount of EDTA plasma biospecimen stored in each cryogenic aliquot. Previously, EDTA plasma was aliquoted into as many 1mL aliquots as possible (median 11, 25th–75th percentiles 9–12 aliquots per subject); this has been changed so that the first 4 aliquots contain 250µL of plasma each, and additional aliquots contain 1mL of plasma (Figure 1, asterisk). Storing smaller volumes of plasma will help minimize thaw/freeze cycles. The same brand and size of aliquot tubes will be used for the new storage protocol.

Biospecimens are stored in the Boston Children's Hospital Biobank Core Lab, a biospecimen custodian facility service provided to researchers and affiliates of Boston Children's Hospital for tracking, processing, storage, and retrieval of biospecimens. Upon receipt, each biospecimen is entered into an institutional Laboratory Information Management System (Bio-specimen Storage Tracking and ORganization, BioSTOR, Boston Children's Hospital, Boston, MA),¹¹ assigned a unique barcode ID, and added to the Biobank Core Lab inventory (see below for details). Following submission, authorized users may view and manage the biospecimen inventory and data, but do not have direct access to biospecimens. Any biospecimen handling and processing is performed by trained Biobank Core Lab staff to maintain biospecimen integrity and guarantee best practices.

Financial assistance from the Harvard Catalyst has allowed for basic clinically relevant blood tests to be performed on part of the collected biospecimens (Figure 1). The tests are performed by LabCorp, a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory. Test characteristics are described in Supplemental Tables 1 and 2.

RESULTS OF TESTING AND DISSEMINATION OF RESEARCH FINDINGS

Results of the LabCorp laboratory testing (Table 2) are shared with each participant's cardiologist and included in the electronic medical record. As described in the consent form, we will not share individual results of future research testing with study participants. We will, though, communicate aggregated scientific results via three potential avenues: if patients request to review findings with investigators, through discussion of study results with clinical providers to facilitate direct dissemination of information from provider to patient, and through a newsletter or website for community accessibility (http://www.childrenshospital.org/centers-and-services/programs/a_-_e/adult-congenital-heart-service-program/research-and-innovation/bachbank or www.tinyurl.com/ACHDBiobank).

DATA COLLECTION

Following enrollment, data are collected on variables that have been previously identified as likely to be useful for planning derivative research studies (e.g., diagnosis, related diagnostic testing, and functional class). Variables pertain to demographics, general medical history, type of congenital heart disease, genetic syndromes, medications, systemic ventricle function, hemodynamics, arrhythmia, physical exam, functional class and exercise testing (Table 3). Most variables, such as functional class and physical examination variables, are defined according to clinical data obtained on the same day as enrollment and sample collection; clinical testing results are collected from the most recent prior test and the date is recorded. All data are extracted from the study participant's medical record.

Data are subsequently collected on pre-determined outcomes of interest to better understand event rates and aid in future study design. Information on such events is extracted from the medical record at follow-up visits and other interim clinical communications. Specific outcomes of interest include: functional deterioration, elective and non-elective hospitalization including cause, and death.

DATA MANAGEMENT AND QUALITY CONTROL

Basic clinical and demographic data as outlined above is recorded on paper case reporting forms and then entered manually into a REDCap (Research Electronic Data Capture) database in conjunction with a unique study ID. For this exploratory database, we employ single data entry followed by two methods of review: (1) identification of unlikely values and extreme observations via automated alerts during data entry and also post-hoc review of distributions and (2) individual review by a second investigator of each observation. Data collected and stored in the REDCap database undergoes regular quality control. We will use double data entry for studies using assay results or involving additional assays of collected biospecimens; we will include collection of variables already included in the exploratory database, introducing an additional level of review for potential data entry errors.

BioSTOR, the internal laboratory information management system, is used to log data on date of specimen collection, biospecimen quantity, available aliquots with location, chain-of-custody, and other relevant biospecimen-specific data such as prior freeze-thaw cycles or observed hemolysis at time of processing. In addition, data on biospecimen collection and available aliquots are entered into the REDCap database, including description of incomplete collections (e.g., if only urine was collected) and of any issues during collection, processing or storage such as hemolysis or an unplanned thaw cycles.

Access to the data is limited to investigators and research staff directly involved in data entry. All access to these databases is logged (requiring user login) with the potential for audits.

COMPARISON GROUP

Healthy individuals without known congenital heart disease or associated conditions are recruited to volunteer as controls for the ACHD Biobank. This group of study participants will serve as a comparison group to improve the scientific validity of future testing and results; this is particularly important for the patient population of interest since normal values may vary with age and normative ranges may not be available for younger age groups for a subset of biomarkers. The comparison group will include interested volunteers, patients' friends or family members (whether or not kindred-related, noted in database with relationship to subject) who meet the following criteria: 18 years-old, no current tobacco use, no known congenital heart disease and no history of diabetes mellitus, chronic kidney disease or prior myocardial infarction or cerebrovascular accident. These comparison subjects will be frequency matched to cases on important variables (e.g., age, sex, season) based on specific scientific rationale for each individual project. The protocol for phlebotomy timing and technique, processing and preparation (e.g., no restriction on recent food intake) are the same as for the ACHD cases. Volunteers are recruited through enrolled patients at clinical visits and through the Boston Children's Hospital internal website, "Children's Today" (Boston Children's Hospital Intranet, <http://web2.tch.harvard.edu/>). The same protocol for collection, handling, processing and storage is followed for all participants. There is currently no remuneration or other extrinsic incentive provided for control participation.

SCIENTIFIC ADVISORY BOARD

A scientific advisory board has been appointed to guide and provide oversight for the ACHD Biobank regarding overarching goals, optimal future specimen use, and protocols for requesting use of biospecimens. The board met for the first time in December 2014 and will continue to meet semi-annually to review protocols and progress.

PROTOCOLS AND PUBLICATIONS COMMITTEE

The success of the Boston ACHD Biobank depends on the quality and importance of the resulting scientific publications and presentations. We have developed a set of preliminary publication and presentations policies to encourage and facilitate analyses and ensure appropriate, ethical, expeditious, consistent, efficient use of biorepository data while preventing redundancy or authorship disputes. A Publications and Presentations Committee

will include 5 members approved by the scientific advisory board, and will review investigator proposals for the use of Boston ACHD Biobank data or biospecimens. The current policies and committee structure were modeled after the approaches used for publically funded large cardiovascular epidemiology cohorts,^{12–14} with modification for the specific circumstances of the current project. Application to use data and samples will be open to any interested investigator, irrespective of prior involvement with the Biobank or institutional affiliation. In addition to scientific merit, use of data and samples will require investigators to contribute substantially to enhancing the value and/or sustainability of the Biobank. Such contributions may take various forms, such as funding or additional data collection, depending on available resources.

CONFIDENTIALITY AND DATA SECURITY

No identifying data is included with collected biospecimens; biospecimens are identified with unique subject and biospecimen IDs. A password-protected study enrollment log identifies subjects based on subject ID; this is located on the Boston Children's Hospital encrypted server and is accessible only to study personnel. Each specimen submitted to the Biobank Core Lab is identified by a unique subject alias and stored securely in the locked facility. All databases are strictly password-protected and identifying information is only available to authorized study personnel; user access and data entry and editing are logged and available for future review.

PROTOCOL REVISIONS

In February 2015, the Biobank protocol was amended to 1) include repeat biospecimen collection for previously enrolled subjects every 2 years (18–30 months) and 2) exclude collection of biospecimens from patients who had been discharged for any reason within the prior 4 weeks. This exclusion was added to increase the likelihood that biospecimens would be collected at times of clinical stability. A small subset, 7.4%, of the first 487 subjects enrolled would have been excluded for this reason. Eligible patients will also receive a letter prior to their appointment informing them of the comparison group and encouraging them to bring friends and family to the clinic if they are interested.

OUTSIDE RECRUITMENT AND ENROLLMENT

The Boston ACHD Biobank piloted an alternative approach to recruitment at the Adult Congenital Heart Association's 7th National Conference (Chicago, Illinois) in September 2014. The Adult Congenital Heart Association is a patient advocacy and education organization whose mission is to improve and extend the lives of patients born with heart defects through education, advocacy and the promotion of research. The attendees of this conference included medical providers, investigators, patients and others. Eligible subjects were able to enroll in the conference's dedicated research room. Biospecimens were collected according to standard protocol and processing was done on-site in the research room. Biospecimens were stored on-site at –80°C using cryogenic shippers. These shippers were then transported back to Boston Children's Hospital overnight and entered into long-term storage. Biospecimens to be clinically tested according to protocol were picked up by LabCorp at the conference location in Chicago. We will continue to consider additional

opportunities for enrollment at public educational or advocacy events and multicenter collaboration.

PARTICIPANT DESCRIPTION

The Boston ACHD Biobank currently includes biospecimens from more than 650 subjects including over 600 adults with CHD or associated diagnoses and almost 50 controls. The median [25th–75th percentile] number of 1mL aliquots for EDTA plasma, heparin plasma, serum, and 2mL aliquots for cells and urine are 11 [9–12], 3 [2–3], 4 [3–4], 2 [2–2] and 2 [2–2] respectively. Boston Adult Congenital Heart and Pulmonary Hypertension Service clinicians see an average of more than 60 outpatients per week at Boston Children's Hospital and Brigham and Women's Hospital. Because of logistical and resource limitations, only a subset can be approached. On a representative week, approximately 5–10 patients and 1–2 controls consent to participate and provide biospecimens. Few (<5%) patients who are approached decline to participate in the study, though a larger minority are unwilling or unable to provide biospecimens at the time of initial discussion because of logistical considerations.

To date, the median age of ACHD Biobank subjects is 37 [25th–75th percentile, 28–51; range 18–80] years, 53.3% are women and 75.7% are identified as Non-Hispanic White. The most common comorbid chronic medical problems are systemic hypertension (16.8%) and dyslipidemia (14.0%). A diagnosis of diabetes mellitus is present in 5.2%. About 1 in 6 (16.3%) subjects have ever used tobacco; few are current smokers (3.6%). The vast majority of subjects, 88.8%, have CHD while the remaining participants have associated diagnoses including pulmonary hypertension and connective tissue disease. Of those subjects with CHD, the following most common primary diagnostic groups account for >5% of the total sample: single ventricle status-post Fontan procedure (18.8%), repaired tetralogy of Fallot with pulmonary stenosis or pulmonary atresia (17.6%), left-sided outflow obstructive lesions (17.5%) and D-loop transposition of the great arteries after atrial switch procedure (5.8%).

LIMITATIONS

The study subjects, as currently described, will be recruited mainly from a single referral ACHD center and data from this sample may not be generalizable to the experience in other centers, especially for diagnoses with between-center heterogeneity in management. The current design will not produce a random sample of ACHD patients seen at this clinic; it may preferentially sample more complex patients with more frequent clinical visits. While this precludes prevalence estimates, it does focus limited resources on the subset of patients of most interest and importance in terms of the burden of premature morbidity and mortality. Likewise, subjects and controls are included irrespective of recent food intake, recent exercise or time of day. Some biomarkers will be affected by time of draw or fasting status; this will increase variance associated with measurements resulting in lower statistical power. Because timing of draw and protocols are equivalent for controls and cases and because these characteristics are unlikely to be associated with differential measurement error (e.g., fasting status is unlikely to be associated with specific clinic characteristics or outcome), this should tend to bias results towards the null hypothesis.

Despite efforts to obtain medical records, it is likely that clinical data and follow-up will be less complete for subjects not followed at the primary center (e.g., those enrolled at patient advocacy conferences). The consequent missing data could require imputation procedures or limit inclusion of a subset of samples in specific studies, but, handled correctly, this should not bias results (e.g., observations will be censored for survival analysis). Future collaborations with other ACHD centers will require us to address how best to ensure complete, consistent clinical data collection and follow-up. The comparison group may not derive from the same catchment population as the ACHD population. It is difficult to identify a sampling method that could provide a comparison group reflecting those who would be at risk for CHD and received care in this ACHD program. CHD is present at birth and referral to this specific ACHD program is not simply defined by a geographic area or age group (e.g., average age varies markedly between diagnoses) or limited to patients cared for in childhood in Boston. Given these limitations, use of the comparison group data will focus mainly on understanding normal assay ranges as performed on our biospecimens and in a group with a similar age- and sex-distribution. Recruitment of family and friends may enhance the likelihood of a shared environment, but will not fully address this issue. The inclusion of family members may also facilitate genetic studies, though this may be limited because family recruitment will not be systematic.

DISCUSSION

In this paper, we have described the design and implementation of a biobank for blood and urine biospecimens in adults with congenital disease and associated diagnoses.

Circulating biomarkers are a burgeoning field of diagnostic medicine and several paradigm shifting biomarkers have been introduced into adult cardiology practice in the past 3 decades including cardiac troponin and B-type natriuretic peptide (BNP).^{15–19} The use of circulating biomarkers is now ubiquitous in general clinical medicine for diagnosis (e.g. troponin for myocardial infarction, fasting glucose for diabetes mellitus, serum creatinine and urine albumin for chronic kidney disease) and prognosis (e.g. BNP for heart failure; creatinine, bilirubin and INR for cirrhosis). This has transformed the diagnosis of treatable disease and has facilitated research and application of research results to clinical medicine. There has been little investigation on urinary biomarkers in ambulatory ACHD patients;²⁰ circulating biomarkers have been more extensively studied, with most research focusing on markers previously validated in acquired heart disease.^{21–32} The clinical interpretation and impact of these studies have been limited by relatively small sample sizes and heterogeneous samples. For example, a recent review summarizing research on biomarkers in heart failure associated with ACHD listed 41 studies in adults with single ventricle physiology, classified into 6 distinct diagnostic subgroups ranging from Eisenmenger syndrome to the Fontan circulation; these studies had an average sample size of 46 and only 2 studies included >100 subjects.²² The first research report using the Biobank described in this manuscript, a study of circulating plasma galectin-3 in 70 patients with single ventricle Fontan circulation, was recently published;³³ a second manuscript in the same group of patients investigated glomerular filtration rate and urine biomarkers is accepted for publication as well.³⁴ There are now >110 patients with Fontan circulation enrolled in the Biobank.

In addition to direct diagnostic and prognostic clinical application, circulating biomarkers can provide mechanistic insights. Prospective biobanks that collect, process and store blood samples and other biologic material have been established for the general adult population and an array of diseases-specific populations including oncology and cardiovascular disease.^{35–38} Systematic study of biomarker profiles, using both clinically relevant markers and investigational assays, has identified mechanisms of disease development and disease progression. In addition, biomarkers can play a role as a surrogate of a favorable outcome (e.g. decreasing BNP predicting favorable ventricular remodeling) and therefore may be able to facilitate coherent research in seemingly dissimilar diseases or those difficult to phenotype by standard methods (e.g. single ventricle or systemic right ventricle disease).

The study protocol reflects a pragmatic balance between the goals of tight control of pre-analytical variables and collection of biospecimens in such a way to facilitate diverse types of future research, with the constraints of clinical workflow and available resources. Blood is collected in several types of tubes so that most biomarkers, including most validated cardiovascular biomarkers, will be able to be measured. We have not had the resources, however, to isolate the buffy coat or extract DNA at the time of processing and rather have stored aliquots of combined cell pellet. The anticipated influences of pre-analytical variables will vary widely between studies using this resource, depending on the specific biomarker, assay, sample type, study design, and research question. The protocol described will support research on many validated cardiovascular biomarkers, such as NT-BNP and ST2, which are stable in EDTA plasma stored at -80°C .^{39–41} Other markers will be more difficult to interpret such as triglyceride and glucose concentrations (since the subjects were not necessarily fasting). Investigators will need to consider the relevance of pre-analytical variables in the larger context of each analysis.

It is important to distinguish the Biobank from the studies that will use the Biobank specimens. The Biobank is not a research study intended to test a specified hypothesis. Participants enroll with an understanding that the samples may be used to study a wide variety of biomarkers and research questions. This is not entirely dissimilar from the use of autopsy tissue donated to institutional cardiac pathology registries. Neither the patients' families who donated nor the cardiac pathologists who accepted those hearts through the middle of the 20th century could have anticipated that they would be used to develop novel percutaneous septal defect closure devices in the 1980s.⁴² With that caveat, we anticipate several likely avenues of inquiry for the Biobank samples. First, the most straightforward application will be to validate extant biomarkers used in other fields for adults with CHD. For example, high sensitivity troponin levels have been shown to specify a population at higher risk of cardiovascular adverse events in the general population and in those with acquired heart disease.^{43, 44} A second use will be discovery of novel clinical biomarkers in this population, whether circulating proteins or microRNAs or gene expression profiles, to predict specific outcomes. Both validated previously described biomarkers and novel biomarkers may help us describe disease phenotype in ways to allow more coherent categorization of clinical disease than is currently possible with a diagnosis and imaging based approaches. Third, these biospecimens will support mechanistic research to help explore the reasons patients develop certain adverse outcomes. For example, is there evidence of increased inflammation prior to development of a specific type of arrhythmia?

Or are there specific characteristics that predispose patients with thrombogenic conditions, such as a Fontan circulation,⁴⁵ to clinical thromboembolic complications? Finally, validated, readily measured circulating and urinary biomarkers strongly linked to pathophysiology and important clinical outcomes could serve as reliable surrogate endpoints for future research on specific interventions for ACHD.

As outlined above, the number of patients with any specific type of CHD is often low, and the event rates are generally lower than in patients with acquired heart disease. Therefore, the absolute number of outcomes in any ACHD study is usually small, making it difficult to answer pressing clinical questions.^{46, 47} Development of robust, continuous surrogate endpoints would be a major step towards being able to study ACHD interventions in rigorous randomized trials with adequate statistical power.

For a number of reasons, multi-center collaboration would increase the impact of the initiative described. This could take the form of a combined biobanking effort or distinct institutional programs in parallel. Either would provide a larger catchment population to enhance sample size and could help address inter-institutional variability in disease severity and clinical approaches. Because of these issues, validation in independent cohorts may be especially relevant to ACHD. Our hope is that the methods here described will encourage other centers to engage in similar focused efforts using standardized protocols that will facilitate future collaboration.

The biospecimens collected and the methods by which they are handled, processed, and stored, as described here, will determine the validity and generalizability of data deriving from this ACHD Biobank. The ultimate value of this program, however, will depend on effective collaboration among an array of committed investigators with diverse expertise.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Boston Adult Congenital Heart Disease Biobank Biospecimen Collection, Analysis and Storage

Approximately 48.5cc of venous blood and 30cc of urine are collected. A 3cc EDTA tube and 8.5cc SST tube are processed and sent to LabCorp for testing as listed above. Details of the processing and storage of the remaining sample are described in detail in Table 1.

Abbreviations: CBC=complete blood count; cc=cubic centimeter;

EDTA=ethylenediaminetetraacetic acid; SST= serum separator tube; hsCRP= high-sensitivity C-reactive protein.

Table 1

Description of use and long term storage of biospecimens collected.

Vacutainer Tube	Use of Biospecimen	Use Post-Collection
EDTA, 10mL (× 2)	Plasma storage Red blood cell and buffy coat storage	Plasma stored in 1mL cryogenic aliquots at –80°C; first 1mL stored in 4 × 250 µL aliquots Pellet (buffy coat with red blood cells) stored in 2 × 2mL cryogenic aliquots at –80°C
EDTA, 3mL	Clinical testing	Sent to LabCorp for complete blood count with differential
SST with clot activator, 8.5mL (× 2)	Serum storage and clinical testing	Serum from 1 tube stored in 1mL cryogenic aliquots at –80°C 1 tube used for comprehensive metabolic panel, lipid panel, hsCRP and uric acid
PST (lithium heparin), 6mL (× 1)	Plasma storage	Stored in 1mL cryogenic aliquots at –80°C
PAXgene Blood RNA, 2.5mL (× 1)	Blood messenger RNA stabilization	Stored as is at –20°C for 24 hours and then at –80°C
Sterile urine container, 30mL, (× 1)	Urine storage and clinical testing	Supernatant stored in 2 × 2mL cryogenic aliquots at –80°C Remaining unprocessed sample is sent to LabCorp for urinalysis

Abbreviations: EDTA=ethylenediaminetetraacetic acid, SST= serum separator tube, PST=plasma separator tube, RNA= ribonucleic acid, hsCRP= high-sensitivity C-reactive protein.

Table 2

Laboratory testing performed on fresh samples.

Description of specific laboratory tests performed on unfrozen samples at the time of enrollment.

On whole blood from the 3 mL EDTA Vacutainer: WBC($\times 10^3/\mu\text{L}$) with differential RBC($\times 10^6/\mu\text{L}$) Hemoglobin (g/dL) Hematocrit (%) MCV(fL) MCH (pg) MCHC (g/dL) RDW (%) Platelets ($\times 10^3/\mu\text{L}$)
On serum from the 8.5 mL SST Vacutainer: Glucose (mg/dL) Blood urea nitrogen (mg/dL) Creatinine (mg/dL) Sodium (mmol/L) Potassium (mmol/L) Chloride (mmol/L) Carbon dioxide (mmol/L) Calcium (mg/dL) Total protein (g/dL) Albumin (g/dL) Globulin (g/dL) Total bilirubin (mg/dL) Alkaline phosphatase (IU/L) Aspartate aminotransferase (IU/L) Alanine aminotransferase (IU/L) Cholesterol, Total (mg/dL) Triglycerides (mg/dL) HDL cholesterol (mg/dL) VLDL cholesterol (mg/dL) LDL cholesterol, calculated (mg/dL) High-sensitivity C-reactive protein (mg/L) Uric acid (mg/dL)
On urine: Standard urinalysis Creatinine (mg/dL) Albumin (ug/dL)

Abbreviations: EDTA=ethylenediaminetetraacetic acid; WBC=white blood cell count; RBC=red blood cell count; MCV=mean corpuscular volume; MCH=mean corpuscular hemoglobin; MCHC=mean corpuscular hemoglobin concentration; RDW=red cell distribution width; SST=serum separator tube; HDL=high-density lipoprotein; LDL=low-density lipoprotein; VLDL=very low-density lipoprotein.

Table 3**Data Collected at Time of Enrollment**

Description of data elements collected upon subject enrollment.

Demographics Date of birth, sex, race/ethnicity
Medical History <i>Medical comorbidities:</i> systemic hypertension, diabetes mellitus, obstructive sleep apnea, atherosclerotic coronary artery disease, dyslipidemia, history of sub-acute bacterial endocarditis, pulmonary hypertension, asthma, chronic obstructive pulmonary disease, pregnancy history, history of tobacco use (current, former, never) with quantification of pack years <i>Current medications</i>
Primary Congenital Heart Disease Diagnosis or Associated Diagnosis Primary CHD diagnosis (or designation of pulmonary hypertension, connective tissue disease as qualifying diagnosis) plus classification as simple, moderately complex or severely complex CHD
Genetics Known genetic syndrome or congenital association
Imaging Data Systemic ventricular ejection fraction and subjective assessment of systolic function; severity of systemic atrioventricular valve regurgitation
Catheterization and Arrhythmia Catheterization of electrophysiology study within the prior 5 years, hemodynamic data, history of cardiac arrest, placement of pacemaker or implantable cardioverter defibrillator, arrhythmia diagnoses
Current Clinical Status <i>Physical examination</i> Height, weight, blood pressure, heart rate, resting O ₂ saturation by pulse oximetry <i>Functional class</i> New York Heart Association functional class
Exercise Testing <i>Cardiopulmonary exercise test</i> Type of study (cycle ergometer or treadmill), peak respiratory exchange ratio, peak VO ₂ (mL/kg/min and % predicted), V _E /VCO ₂ slope, presence of O ₂ desaturation (decrease by >3% from rest) <i>6-minute walk test</i> Distance walked, presence of O ₂ desaturation (decrease by >3% from rest)
Visit Information Type of visit at which draw was completed, possible confounding events (e.g., recent procedure with use of anesthesia, iodinated contrast, or gadolinium)
Sample Collection Number of and type of tubes collected, number of aliquots of each sample type, any issues related to sample collection or processing or storage, urine collected

Abbreviations: CHD=congenital heart disease; O₂=oxygen; V_E=minute ventilation; VCO₂= carbon dioxide production; VO₂= oxygen consumption