Rationale and design of Long-term Outcomes and Vascular Evaluation after Successful Coarctation of the Aorta Treatment study

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ABSTRACT

Background : **Coarctation of the aorta (CoA) can be treated using surgery, balloon angioplasty, or stent implantation. Although short-term results are excellent with all three treatment modalities, long-term cardiovascular (CV) morbidity and mortality remain high, likely due to persistently abnormal vascular function. The effects of treatment modality on long-term vascular function remain uncharacterized. The goal of this study is to assess vascular function in this patient population for comparison among the treatment modalities.**

Methods : **We will prospectively assess vascular Afunction in large and small arteries fusing multiple noninvasive modalities and compare the results among the three groups of CoA patients previously treated using surgery, balloon angioplasty, or stent implantation after frequency matching for confounding variables. A comprehensive vascular function assessment protocol has been created to be used in 7 centers. Our primary outcome is arterial stiffness measured by arterial tonometry. Inclusion and exclusion criteria have been carefully established after consideration of several potential confounders. Sample size has been calculated for the primary outcome variable.**

Conclusion : **Treatment modalities for CoA may have distinct impact on large and small arterial vascular function. The results of this study will help identify the treatment modality that is associated with the most optimal level of vascular function, which, in the long term, may reduce CV risk.**

Keywords : Arterial stiffness, cardiac magnetic resonance imaging, coarctation of the aorta, long-term outcomes, pulse wave velocity, vascular function

INTRODUCTION

Current treatment techniques are equally effective at eliminating the stenosis in CoA patients.[1] However, a

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good anatomical result does not preclude late systemic hypertension in office visits (12%–65%),[1-6] at peak exercise (10%–47%),[3-5,7,8] or during ambulatory blood pressure (BP) monitoring (30%–59%).[7-10] Furthermore, treated patients have reduced life expectancy [Figure 1],[2] mostly due to cardiovascular (CV) complications[2,11-14] and stroke.[15]

Successfully treated CoA patients have stiffer large arteries[16-21] and compromised vascular reactivity in small arteries,[8,10,22-26] their arterial pressure waveform is altered,[9,10,23,27,28] have imbalances in vascular function biomarkers,[24,25,27,29,30] and increased left ventricular (LV) mass.[8,9,19-21,26,31,32] Vascular dysfunction is associated with older age at treatment,[2,19,22,29,31,33] but early treatment does not guarantee normal vascular function.[16,22]

Different treatment modalities may have varying effects on the stiffness of the repaired arterial segment:[34] Surgical repair results in a focal scar in the anastomosis; stenting creates a short, rigid segment; and balloon dilation (BD) produces a controlled tear of the intima and part of the media. Although it is possible that these differences translate into differences in vascular dysfunction, this has not been systematically compared. The largest, albeit observational and nonrandomized, comparison between the three modalities showed a lower BP in patients treated with BD versus those treated with stenting or surgery.[1] A small retrospective study showed less frequent exercise-induced hypertension in BD patients compared with other treatment types.[33] Conclusions drawn from these prior studies are hampered by methodological limitations and limited focus. In the general population, arterial stiffness is associated with major CV events.[35] Thus, choosing the CoA treatment option that optimizes vascular function is crucial for long-term outcomes in CoA.

Aim and hypothesis

The Long-term Outcomes and Vascular Evaluation after Successful Coarctation of the Aorta Treatment study aims to determine whether surgery, BD, and stenting are associated with differences in arterial stiffness in optimally treated patients. Our hypothesis is that patients who underwent successful BD will have better vascular function than patients who underwent successful surgical repair or stenting since this modality may least likely damage the biomechanical properties of the aortic wall.

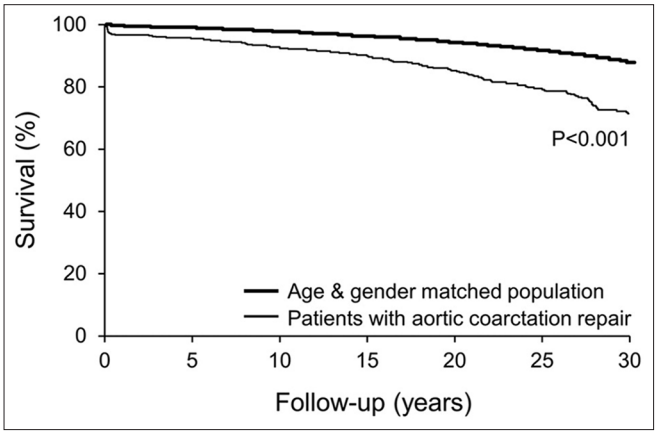

Figure 1: Survival after treatment of coarctation of the aorta. Survival curves of 819 surgical patients for over 60 years (reprinted with permission from Elsevier, license number 4131890880395)

METHODS

Study overview

This study is a cross-sectional prospective observational study of patients with CoA previously treated using one of three treatment modalities. Patients will be recruited at seven large pediatric cardiac centers from Europe and the United States of America [Appendix 1]. The study procedures will occur in a 1- or 2-day visit [Figure 2].

Recruitment

Selection criteria are depicted in Table 1. The study protocol was approved by Institutional Review Boards. Recruitment occurred between June 2013 and December 2017. The study data are collected and managed using REDCap software, hosted at Children's Hospital Boston.[36]

Study procedures

A list with the main clinical and study tests variables are depicted in Tables 2 and 3. The comprehensive list of study variables is in Appendix 7

Arterial stiffness

CoA treatments alter the biomechanics of the isthmus and may increase arterial stiffness. The velocity of the pulse wave velocity (PWV) travel in the arterial tree increases with arterial stiffness. Carotid–femoral PWV (cfPWV) is extensively validated in large studies a marker of aortic stiffness, and an independent predictor of CV events.[37] We will measure cfPWV with applanation tonometry, using either the NIHem (CV Engineering, Inc., Norwood, MA USA) or the SphygmoCor (AtCor Medical, West Ryde, NSW, Australia) devices.[37] This technique assumes a homogenous stiffness across the aorta and may potentially not accurately estimate the true carotid-to-femoral artery length. Cardiovascular magnetic resonance (CMR) measurements of PWV, on the other way, enables the detection of more subtle changes in segmental aortic PWV, above versus below the CoA site, and uses real aortic travel paths.[38] We will also use CMR to measure aortic area change during the cardiac cycle, paired with BP measurements, to quantify local arterial strain, compliance, distensibility, and the β-stiffness index [Appendix 2a and b].

Endothelial function

In CoA, the loss of central aortic pulsatility, which buffers systole, generates chronic shear stress

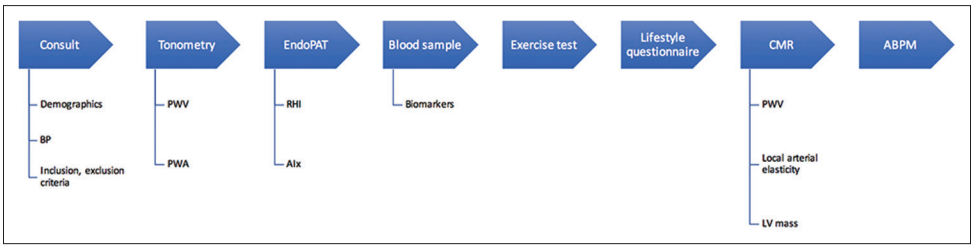

Figure 2: Long-term Outcomes and Vascular Evaluation after Successful Coarctation of the Aorta Treatment study workflow. ABPM: Ambulatory blood pressure monitoring, AIx: Augmentation index, BP: Blood pressure, CMR: Cardiac magnetic resonance imaging, PWA: Pulse wave analysis, PWV: Pulse wave velocity, RHI: Reactive hyperemia index

Table 1: Inclusion and exclusion criteria

Criteria	Definitions and comments
Inclusion criteria	
Coarctation of the aorta	
Current age 8-35 years	Lower age to allow facilitate the completion of the study tests and higher age to avoid overlap with aging-related vascular dysfunction[58]
Treatment for CoA after 1994	Date after which all three modalities were in clinical use.
Exclusion criteria	
Residual CoA	Systolic upper-to-lower extremity BP gradient >20 mmHg.* Residual gradient is a confounder since it impacts vascular function. 8
Atypical CoA	Mid-thoracic or abdominal coarctation.
Severe transverse aortic arch hypoplasia	Transverse arch diameter z-score at initial echocardiogram <-4†
Treatment of CoA at age <1y	A more severe disease subset, essentially amenable to surgery
Clinically significant associated cardiac defects that may affect independently vascular function	Mitral stenosis (echocardiographic mean inflow Doppler gradient >6 mmHg) aortic stenosis (echocardiographic mean Doppler gradient >20 mmHg); ventricular septal defect (>3 mm in diameter); atrial septal defect (required surgical or percutaneous closure other than a patent foramen ovale); other cardiac lesions that required medical, surgical or interventional treatment
Use of two treatment modalities for CoA	This does not include balloon dilation and subsequent stent placement at the same catheterization procedure
History of known vasculopathy with vascular dysfunction	Examples: Kawasaki disease, Takayasu's arteritis, Raynaud's disease
Genetic syndromes with diffuse arteriopathy	Examples: Williams syndrome, juvenile rheumatoid arthritis
Known traditional cardiovascular risk factors	Severe obesity (body mass index >95% for age and sex in children and >40 Kg/m^2 for adults); diabetes (fasting plasma glucose ≥126 mg/dl or random (non-fasting) glucose ≥200 mg/dl); hyperlipidemia (triglycerides ≥250 mg/dl; fasting LDL ≥190 mg/dl; HDL ≤30 mg/dl, currently taking statins or first degree relatives with familial hypercholesterolemia); smoking

Legend: y=years; BP=blood pressure; CoA=coarctation of the aorta; LDL=low-density lipoprotein cholesterol; HDL=high-density lipoprotein cholesterol; *using highest lower extremity systolic blood pressure; †using previously published normative values[57]

Table 2: List of main clinical variables

Variables	Comments or definitions
Medical history	
Minimum transverse arch diameter Z-score on initial echo	Using published normative values*
Isthmus z score on initial echo	Using published normative values*
Initial Doppler coarctation gradient	mmHg
Bicuspid/Bicommisural Aortic Valve?	Yes/No
Initial arm-leg systolic BP gradient	mmHg
Visit BP	
Residual systolic BP gradient	Supine and automated mmHg
Right arm BP	Seated and manual mmHg

Legend: BP=blood pressure; *using previously published normative values[57]

downstream in smaller arteries, creating endothelial dysfunction, which is associated with CV events.[39] We will measure endothelial function with the reactive hyperemia index using endothelial pulse amplitude tonometry (endo-PAT), a novel noninvasive and reproducible technique that measures changes in pulsatile arterial volume with a fingertip probe.[40] Analysis of the pulse waveform allows for automated calculation of endothelial function in one arm, while the contralateral serves as control, making this is a patient standardized method [Appendix 3].

Pulse waveform analysis

In CoA, the stiff aorta and repaired isthmus may be important reflecting sites that impact the pulse waveform. Its analysis is an important clinical tool for monitoring of vascular function and predicting CV events.[37] We will measure three variables that express pulse waveform: central aortic pressure (CAP), pulse pressure (PP), and augmentation index (AIx; ratio of the amplitude of the reflected wave in the ascending aorta and the PP).[37] CAP, PP, and AIx can be measured noninvasively using applanation tonometry (and Endo-PAT for AIx), calibrated by the peripheral diastolic and mean arterial pressure.[37] There is a lack of consensus regarding the optimal method to estimate the CAP with tonometry. The NIHem system assumes that carotid artery pulse waveform accurately

Table 3. List of main study test variables

Variables	Comments or definitions
Applanation tonometry	
Central systolic blood pressure	mmHg
Central pulse pressure	mmHg
Carotid-femoral PWV	meters/second
Augmentation index at HR75	%
CMR	
Left ventricular mass indexed to BSA	g/m^2
Ascending Ao - Descending Ao PWV (Ascending Ao to proximal, mid and distal descending Ao)	Meters/second
Type of arch	Romanesque; Gothic; Crenel
Aortic strain (Ascending, Proximal, Mid and Distal Ao)	
Aortic Distensibility (Ascending, Proximal, Mid and Distal Ao)	mmHg-1
Endo-PAT	
Reactive hyperemia index (RHI)	
Augmentation index at 75 bpm	%
ABPM	
24 h Average systolic and diastolic BP	mmHg
24 h systolic and diastolic load	%
Exercise test	
Pre-Exercise SBP gradient	mmHg
Peak exercise BP	mmHg
Biomarkers	
NOx	ug/ml
ADMA	ng/L
High Sensitivity CRP	mg/L
VCAM-1	ng/ml
IL-1β	pg/ml
TFG-β	
MMP-2/Gelatinase A	ng/ml
MMP-9/Gelatinase B	ng/ml

Legend: ADMA=Asymmetric Dimetilarginine; Ao=Aorta; BP=blood pressure; BP=Blood Pressure; BSA=Body Surface Area; CMR=Cardiac magnetic resonance; DBP=Diastolic Blood Pressure; HDL=High-density lipoprotein cholesterol; Hs-CRP=High sensitivity C-Reactive Protein; IL-1β = Interleukin 1 beta; LDL=Low-density lipoprotein cholesterol; MMP-2=Matrix Metalloproteinase-2; MMP-9=Matrix Metalloproteinase-9; NOx=Nitric Oxide; PWV=Pulse Wave Velocity; LV=Left Ventricle; SBP=Systolic Blood Pressure; TFG-β = Transforming Growth Factor beta; VCAM-1=Vascular Cell Adhesion Molecule 1; * using previously published normative values[57]

reflects the central aortic waveform and the pulsed wave analysis is automatically calculated from the carotid waveform. The SphygmoCor device uses a generalized transform function to generate a central aortic PP curve from the radial or carotid pressure tracings, which has not been validated in children. Considering our largely pediatric group and need to maintain consistency between data acquired on each device, we use the nonprocessed, signal-averaged SphygmoCor carotid tracing as the central aortic tracing which will be then digitized to calculate the CAP, following previously published approach [Appendixes 2a and 3].[41]

Blood pressure phenotype

BP phenotype is abnormal despite successful treatment of CoA. Office hypertension is a known risk factor for CV disease and the BP response during the ET is predictive of future development of resting hypertension in the general population.[42] Ambulatory blood pressure monitoring (ABPM) is superior to the office measurement in its ability to distinguish patients at the highest risk for target-organ damage.[43] We will assess BP phenotype with the manual auscultation technique to measure the right arm office BP; supine four extremity oscillometric BP measurement to assess for residual coarctation; ABPM to measure the circadian BP profile; and ET to assess the BP response to exercise and exercise-induced arm to leg BP gradient. Based on the office BP and ABPM results, we will classify our patients according to Table 4 [Appendix 4].

Biomarkers

We will measure asymmetric dimetilarginine (ADMA; NO's inhibitor),[44] and nitrite and nitrate (NOx, stable by-product of NO), biomarkers of endothelial function. Arterial stiffness is associated with increased systemic inflammation markers, which we will quantify with high-sensitivity C-reactive protein (hs-CRP) and local inflammatory cytokines of vascular wall function vascular adhesion molecule 1 (VCAM-1) and interleukin-1 beta (IL-1β).[25,45] We will finally assess the molecular mechanisms of aortic wall response to vascular dysfunction, with matrix metalloproteases (MMP-2 and MMP-9),[46] and transforming growth factor beta-1 (TGF-β1, a smooth cell growth-modulating factor involved in the arterial wall response to hypertension).[30] NOx will be determined by chemiluminescence (Sievers NOAnalyzer 280i) and all remaining measurements will be performed with enzyme-linked immunosorbent assay kits: ADMA (Sunred Biological Technology, Shanghai, China); hs-CRP (BoosterBio, Pleasanton, USA); VCAM-1; IL-1β; matrix metalloproteases (MMP)-9; MMP-2; and TGFβ-1 (RayBiotech, Inc. Norcross, USA) [Appendix 5].

Left ventricular mass

The altered BP phenotype that persists after CoA treatment represents an increase in afterload that leads to LV hypertrophy, strongly related to high BP and carrying a grave prognosis for cardiac events.[47] We will quantify LV mass by CMR, a well-established method for its calculation [Appendix 2b].

Cardiovascular health assessment

Patients with CoA experience increased CV disease compared to the general population. Literature in the general population has demonstrated that risk of cardiometabolic disease and accelerated atherosclerosis is mitigated by ideal CV health (ICVH),[48] defined as having optimal levels of health factors (BP, total cholesterol, and plasma glucose) and behaviors (smoking, body mass index, physical activity, and diet). We will implement a questionnaire to assess family history of CV disease and ICVH according to the guidelines of the American Heart Association [Appendix 6].[48]

Table 4: Classification of BP Phenotype by ABPM

Classification	Office BP SBP or DBP*	24h Mean ABPM SBP or DBP†
Non-hypertensive	Pediatric: <95th %tile Adults: <140/90 mmHg	Pediatric: <95th %tile Adults: <135/85 mmHg
White Coat Hypertension	Pediatric: ≥95th %tile Adults: >140/90 mmHg	Pediatric: <95th %tile Adults: <135/85 mmHg
Masked Hypertension	Pediatric: <95th %tile Adults: <140/90 mmHg	Pediatric: >95th %tile Adults: >135/85 mmHg
Ambulatory Hypertension	Pediatric: >95th %tile Adults: >140/90 mmHg	Pediatric: >95th %tile Adults: >135/85 mmHg

Legend: AMBP=Ambulatory blood pressure monitoring; BP=Blood Pressure; ABPM=Ambulatory Blood Pressure Monitoring; Pediatric patients have age <18yo and adult patients age ≥18yo; %tile=percentile; BP=blood pressure; DBP=diastolic blood pressure; and SBP=systolic blood pressure. *For pediatric patients, based on the National High Blood Pressure Education Program Task Force normative data[54]; for adult patients, based on the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure report.[55] †For pediatric patients, based on normative pediatric ABPM values from the American Heart Association Atherosclerosis, Hypertension and Obesity in Youth Committee of the Council on Cardiovascular Disease in the Young;[43] for adult patients, based on the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research report[59]

Statistical considerations

Adjustment for confounders

We will adjust our treatment groups for three main documented confounders: (a) age at treatment; (b) current age; and (c) bicuspid aortic valve (associated with impaired aortic elasticity).[49] During recruitment, we will attempt to frequency match the three treatment groups. During analysis, the treatment groups will be compared for each of these three confounding variables and adjustments will be made using multivariable modeling with linear and logistic regression models.

Analytic plan

Our primary outcome variable will be cfPWV assessed by tonometry. Differences across groups will be explored using one-way analysis of variance. If differences in matching variables are detected among the groups, adjustment will be made using analysis of covariance. *Post hoc* analyses will be performed as necessary. Sample size estimates were obtained based on prior data that show that arch PWV measured by CMR is 3.3 ± 0.6 m/s in normal patients and 4.7 ± 1.1 m/sec after CoA surgery.[20,50] Sample size estimates for comparison of PVW between three equal-sized treatment groups (assuming overall significance level = 0.05 and power = 0.8) are shown in Table 5. We plan on recruiting 24–30 patients in each group for a total sample size of 72–90.

DISCUSSION

Methodological considerations

We chose a multicenter design to overcome recruitment challenges secondary to restrictive enrollment criteria (particularly the lower treatment age limit of 1 year, which excludes a majority of CoA patients that present in infancy, mostly managed by surgery) and need for matching treatment groups for confounders.

cfPWV is our primary outcome variable because it is validated as an accurate and reproducible measure of arterial stiffness with proven association to hard CV outcomes that can be reliably measured by applanation tonometry and CMR. We chose other parameters to complete a complementary and comprehensive assessment of vascular function in small and large arteries.

Importance of knowledge to be gained

This work will be the first systematic and comprehensive comparison of vascular function between three different treatment modalities in CoA patients. We postulate that the integrity of the arterial wall is best preserved with balloon dilatation, compared to stenting or surgery. We are aware that our population is highly selected, but believe that this is the only way to compare the three treatment types. The results of our selected population may be relevant when several modalities are applicable to one patient. Currently, the preservation of vascular function is not considered when choosing between treatment modalities. Ultimately, the results of our study may help clinicians choose treatment modalities based not only on relief of anatomic stenosis but also on their ability to preserve long-term vascular health.

Study limitations

Our results will reflect vascular function in a selected group of optimally treated CoA patients and may not be generalizable to all CoA patients. We will compare vascular function after treatment but not before the treatment. Variation in antihypertensive medication protocols between different institutions may affect vascular parameters.

CONCLUSION

There is ample evidence to suggest that CoA is a systemic arterial disease and not merely a focal stenosis of the aortic isthmus. However, the current management paradigm continues to focus on alleviating the anatomic stenosis. Our study aims to refine this treatment paradigm by adding the preservation of vascular function to the goals of successful treatment. The strengths of this study include its multicenter design and the use of multiple noninvasive modalities to perform a comprehensive and prospective assessment of vascular function and CV health.

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Table 5: Sample size estimation

Smallest mean PWV (m/s) among groups	Largest mean PWV (m/s) among groups	Standard deviation	Sample size for each group	Total sample size
4.0	4.8	1.0	30	90
4.0	4.8	1.1	36	108
4.0	4.8	1.2	43	129
4.4	5.3	1.0	24	72
4.4	5.3	1.1	29	87
4.4	5.3	1.2	34	102

Legend: PWV=Pulse wave velocity

Conflicts of interest

There are no conflicts of interest.

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APPENDICES

Appendix 1: Core laboratories, clinical sites, investigators for the LOVE-COARCT atudy, funding

Study Principal Investigators

Institution	Name
Department of Pediatric Cardiology Hospital de Santa Marta Centro Hospitalar de Lisboa Central Lisbon, PORTUGAL	José D. Martins, MD
Department of Cardiology Boston Children's Hospital Harvard Medical School, Boston, MA USA	Ashwin Prakash, MD

Site Investigators of the LOVE Pediatric Consortium

Institution	Name
Department of Pediatric Cardiology Hospital de Santa Marta Centro Hospitalar de Lisboa Central Lisbon, PORTUGAL	José D. Martins, MD (Study PI, Site PI) Fátima F. Pinto, MD, PhD (Study Advisor) Marta António, MD Lurdes Medroa Teresa Mateus Mafalda Selas, RN
Department of Cardiology Boston Children's Hospital Harvard Medical School Boston, USA	Ashwin Prakash, MD (Study PI, Site PI) Sarah de Ferranti, MD Kimberlee Gauvreau, ScD Tal Geva, MD Jonathan Rhodes, MD Cara Hass, BS (Study Coordinator) Jeffrey Reichman (Study Coordinator) James E. Lock, MD (Study Advisor) Jane Newburger, MD, MPH (Study Advisor)
Joint Division of Pediatric Cardiology Children's Hospital and Medical Center University of Nebraska College of Medicine Omaha, USA	Shelby Kutty, MD (Site PI)
Division of Pediatric Cardiology Department of Pediatrics Lucile Packard Children's Hospital Stanford University Palo Alto, USA	Elif Seda Selamet Tierney, MD (Site PI) Angela Chen
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Serviço de Cardiologia Pediátrica Hospital Pediátrico de Coimbra Coimbra, PORTUGAL	António Marinho, MD (Site PI) Eduardo Castela, MD
Division of Pediatric Cardiology Texas Children's Hospital Baylor College of Medicine Houston, USA	Shaine Morris, MD (Site PI) Justin Zachariah, MD
CEDOC Chronic Diseases Nova Medical School Lisbon PORTUGAL	Miguel Mota Carmo, MD PhD (Director) Maria Guarino, MD PhD
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Core Laboratories

Core Lab	Institution
Cardiac Magnetic Ressoance (Ashwin Prakash) Preventive Cardiology (Sarah de Ferranti) Biostatistics (Kimberlee Gauvreau)	Department of Cardiology Boston Children's Hospital Harvard Medical School Boston, USA

Contd...

Appendix 1: Contd...

Core Laboratories	
Institution	**Name**
Tonometry and BP Assessment (Justin Zachariah)	Division of Pediatric Cardiology Texas Children's Hospital Baylor College of Medicine Houston, USA
Biomarkers (Maria Guarino)	CEDOC Chronic Diseases Nova Medical School Lisbon PORTUGAL
Endothelial Function (Elif Seda Selamet Tierney)	Division of Pediatric Cardiology Department of Pediatrics Lucile Packard Children's Hospital Stanford University Palo Alto, USA
Data Coordination	
Data Coordination (Jose Martins, Ashwin Prakash, Cara Hass, Jeffrey Reichman)	Department of Pediatric Cardiology Hospital de Santa Marta Centro Hospitalar de Lisboa Central Lisbon, PORTUGAL and Department of Cardiology Children's Hospital Boston Harvard Medical School Boston, USA

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Appendix 2a: Applanation tonometry manual of operations

For applanation tonometry, some centers will use the NIHem system (Cardiovascular Engineering, Inc., Norwood, MA USA) and others the SphygmoCor device (AtCor Medical, West Ryde, NSW, Australia). The technology is similar and the results comparable.

The patient demographics and brachial blood pressure (BP) are entered into the system. First, the tonometer is placed over the right carotid artery, just lateral to the thyroid cartilage. The location is adjusted and pressure applied as needed to optimize waveform. After ensuring that the tracings are optimal, the tracing is recorded. The carotid site is marked. Then, the tonometer is placed over the right femoral artery and the same process for obtaining an optimal curve recording is followed. The femoral site is marked. Finally, in the centers that use the SphygmoCor device, a third recording of the radial artery is performed, in the same fashion. A caliper is used to measure the distance from the suprasternal notch to the carotid site and from the suprasternal notch to the femoral site. Both distances are entered in the system.

For pulse wave velocity and augmentation index calculation, both systems analyze the curves and supply the data with the proprietary software package, without any input from the examiner.

For pulse wave analysis (central aortic pressure and pulse pressure), the analysis procedure differed slightly between systems. The analysis from the NIHem system is done by the system's software. In the centers that used SphygmoCor, the signal averaged carotid pulse wave is digitalized and calibrated according to a published approach:[41,51] The brachial diastolic and mean pressures are used and the same diastolic and mean pressures are assigned to the averaged carotid pulse. Moreover, the radial pressure waveform is used to retrieve the correspondent time instants of diastolic and mean pressures. Given the two pressure values and the correspondent time instants, it is possible to calibrate each averaged carotid pressure waveform. This process allows a quantitative analysis of the pulse waveform.

Appendix 2b: Cardiac magnetic resonance imaging manual of operations

Cardiac magnetic resonance (CMR) will be performed using commercially available whole-body 1.5 T scanners (Achieva; Philips Healthcare, Best, the Netherlands; Signa 1.5T or GE Medical Systems, Milwaukee, WI, USA). Electrocardiography (ECG)-gated steady-state free precision (SSFP) localizers will be used in sagittal, coronal, and axial planes during free breathing. Ventricular function will be assessed from short-axis stack to cover ventricles from base to apex, acquired using the following imaging parameters: slice thickness 5–8 mm, slice gap 0–1 mm, slice number 12–14, cardiac phases 30, retrospective gating with breath-holding. In patients unable to breath-hold 3 signal

averages during free-breathing will be used. SSFP cine imaging will also be performed in two orthogonal long-axis planes of the left ventricular outflow tract (during breath-hold), short axis of the ascending aorta (AAO), and in the long axis of the aortic arch (free-breathing, used as reference for pulse wave velocity measurements), proximal descending aorta (DAO, 2–3 cm distal to the isthmus, sufficiently distal to dephasing jets), mid-DAO (diaphragmatic level), and distal DAO (just above iliac bifurcation). ECG-gated through-plane phase-contrast flow measurements will be performed at the AAO (5 mm distal to the sinotubular junction) and in proximal-, mid-, and distal-DAO segments (matched to location of the cine SSFP acquisitions) using the following imaging parameters: signal averages = 2, cardiac phases 100 (TFE factor/views per segment/ = 1 [to maximize temporal resolution]), and velocity encoding 200–250 cm/s (higher if needed to avoid aliasing). ECG and respiratory navigator-gated three-dimensional SSFP MRA of the aortic arch will be performed in the sagittal plane.

The patient's right arm BP while on scanner table and length of time since last meal and content of last meal will be recorded. Images will be analyzed by a single observer (A.P.) in the CMR core lab using a commercial computer workstation (Extended Workstation; Philips Healthcare) and using commercially available analysis software (QMass and QFlow, Medis, The Netherlands). Ventricular function and mass will be calculated using standard techniques. Cross-sectional areas of the AAO and proximal, mid, and distal DAO will be directly planimetered at peak systole and mid-diastolic frames to calculate parameters of segmental aortic stiffness as previously described.[52] Pulse wave velocity will be measured using the transit-time method.[20] Pulse wave velocity will be calculated for the entire aorta (AAO to distal DAO), as well as in the following segments: AAO to proximal DAO, proximal DAO to mid-DAO, and mid-DAO to distal DAO. Aortic arch shape will be classified and the aortic arch index calculated as previously described.[53]

Appendix 3: Endothelial pulse amplitude testing manual of operations

The testing room will be arranged to provide a quiet, restful environment with a comfortable temperature of 22°C to 23°C. Before testing, patients will be asked to fast overnight for 12 h, except for the consumption of water. Unless the patients are taking a daily vitamin, they will be asked to refrain from taking vitamin pills and over-the-counter medications; in the case that an over-the-counter medication is used, it will be documented.

The Endo-PAT (Itamar Medical Ltd, Caesarea, Israel) testing protocol[40] will be performed in the morning (starting time between 8 and 11 am) and fasting. Any restrictive clothing that could interfere with blood flow to the arms or fingers will be removed, including heavy coats or clothes with thick sleeves, watches or rings or other jewelry on the hands and fingers, and long fingernails shortened with a fingernail clipper.

Noninvasive pneumatic probes will be placed on the index fingers of both hands. The pulse wave amplitude will be recorded continuously from both index fingers. Reactive hyperemia will be performed by achieved by occlusion of the brachial artery of one arm with a BP cuff for 5 min (to 200–220 mmHg). The tracing in the nonoccluded arm will serve as a control for changes in overall physiologic state. The Endo-PAT data will be analyzed with the proprietary software package, without any input from the examiner. The Endo-PAT index is defined as the ratio of the average pulse amplitude during the 1 minute period beginning after exactly 90 s of reactive hyperemia compared with the average pulse amplitude during the 210-s preocclusion baseline period.

Appendix 4a: Right arm, auscultatory blood pressures measurement manual of operations

The patient will be seated with the feet flat on the floor, with the knees at 90° and the back supported. After 5 min of resting quietly, with no conversation or television, the auscultatory BP will be obtained in the right arm. For cuff choice, the length of the bladder encircled no <80% and no more than 100%, of the bicep and the width of bladder encircled no <40% and no more than 50%, of the circumference of patient's arm circumference, measured at the widest area of bicep, midway between the tip of the patient's shoulder and the tip of the patient's elbow. The patient's right arm will be at placed at heart level, supported at the level of the nipple by resting arm on a table or chair arm or propped on a pillow.

The stethoscope's bell will be placed over patient's brachial pulse. The cuff will be inflated up to 140 mmHg and deflated slowly while listening for the Korotkoff sounds, systole being number when the sound is first heard consistently and diastole when the last pulsation is heard or when it muffles. If pulsations are immediately audible, the cuff will be deflated entirely and the patient allowed to sit quietly for 1 minute. Then, the cuff will be again inflated to 160 mmHg (or higher) and the steps above will be followed. This procedure will be repeated until the blood pressure (BP) is not immediately audible.

Three BPs will be obtained, allowing 1 min between deflation and reinflation of cuff for each measurement. The average of the 2nd and 3rd measurements will be considered the final right arm BP and interpreted according to the published guidelines for children[54] and adults.[55]

Appendix 4b: Four extremity, automated blood pressures (Dinamap) measurement manual of operations

While the patient is supine, two sets of four extremity blood pressure (BP) will be measured, with the automated BP monitor (Dinamap).

The BP pressure gradient will be registered, between the second systolic right arm measurement and the highest of the two legs systolic second measurements. In the presence of an aberrant right subclavian artery that originates distal to the Coarctation of the aorta site, seen by cardiovascular magnetic resonance, we will use the second left systolic arm measurement for the residual gradient.

Appendix 4c: Ambulatory blood pressure (BP) monitor methods of operations

The patient data will be recorded. The choice of the cuff will follow the same guidelines described for manual auscultation of right arm BP. Cuff inflation will be programmed for 15–20-min intervals. During nighttime, intervals are wider, but not fewer than one per hour and preferably more. The patient will record the sleep time, wake time, and any periods of vigorous exercise. The patient will be instructed to avoid direct contact of the monitor with water and participation in activities that could damage it.

The study will be considered adequate if there is a record of at least 1 reading per hour, i.e., no more than 1 h between consecutive readings for a full 24-h study. If less than 12 h are recorded, the ambulatory blood pressure monitoring data will be considered inadequate. Diurnal pattern will be determined by the patient diary. Vigorous exercise periods will be excluded.

The data on 24-h systolic BP load, 24-h diastolic BP load, diurnal systolic dipping, diastolic dipping and 24 h, daytime and nighttime mean systolic BP, and mean diastolic BP will be recorded. Patients will be staged as having ambulatory hypertension, masked hypertension, white coat hypertension or normotensive, according to the age-based normative tables based on statements for children and adolescents[43] and adults.[56] Patients currently on antihypertensive medication are also classified into the hypertensive group [Table 4].

Appendix 4d: Exercise test: Manual of operations

The patient information will be entered per equipment specification and the study identifier on the datasheet and the date of the test. For patient safety issues, medical history, medications, activity level, and symptoms will be reviewed and the exercise stress test protocol wil be explained. Antihypertensive medications will be continued the day of testing.

The patient will be asked to lay supine, and a right arm and right or left leg blood pressure measured using a commercial oscillometric and appropriate sized cuff bladders and recorded as preexercise blood pressure (BP) values and gradient. The patient then will step onto the treadmill and instructed to hold the handlebar throughout the test. We will use the standard Bruce treadmill protocol and, when available, a Met Cart. As the patient exercises, their symptoms and electrocardiography (ECG) will be continuously monitored. At 2-min of each stage, a BP will be taken in the right arm by having the patient take their hand off the treadmill and hold onto the arm of the person performing the test. The test will be terminated when the patient can no longer continue the exercise, reaches a systolic BP higher than 240 mmHg, has clinically relevant symptoms or ECG changes. Immediately after the exercise ended, BP in the right arm and the left leg will be recorded in a supine position. For the recovery period, the patient will sit upright in a chair, and right arm BP will be recorded at 1, 3, 5, and 7 min of recovery, at which time the test is ended.

The data on exercise duration, baseline and exercise right arm BP, pre- and post-exercise systolic BP gradient, patient symptoms, ECG changes and, when available, cardiorespiratory physiological data will be documented. We will label exercise-induced hypertension when the systolic BP is ≥220 mmHg.

Appendix 5: Biomarkers manual of operations

The patients will follow a low-nitrate diet for 3 days before the blood sample collection, which avoids of a list of foods with a high content in nitrites that influence nitric oxide determination, including bacon, beets, broccoli, canned food, cauliflower, celery, Chinese cabbage, corned beef, ham, hot dogs, lettuce, old cheese, radish, salami, sausages, smoked fish, spinach, and turnip. After an overnight fast (for 12 h), samples will be collected by venipuncture from catheters maintained with saline only, since heparin interferes with accuracy of the biomarkers assessed. The first 5–10 mL of blood will be discarded and 2.7 ml of venous blood will be collected into 3.2% sodium citrate (light-blue) tubes (BD Vacutainer®), and into plastic microtubes (Safe-Lock Eppendorf). Within 3 h of collection, samples will be centrifuged for 20 minutes at 3000g (4ºC). Aliquots of 250 μl of the supernatant will be collected into 14 labeled microtubes of 1.5 ml and immediately stored at − 80ºC until shipping to the Biomarkers Core Laboratory.

Aliquots for NOx analysis will be deproteinized using cold ethanol precipitation methodology. Ethanol will be refrigerated to 0ºC and added to the plasma sample in a 1:3 proportion. After letting it stand at 0ºC for 30 min, the sample will

be centrifuged at 14,000 rpm for 10 min. The supernatant will be then removed for analysis. The quantification of plasma NO levels will be carried out using a nitric oxide analyzer, the Sievers Instruments NOA 280i™, a high sensitivity detector of that allows determination of NO based on a chemiluminescence reaction between NO and ozone.

Plasma asymmetric dimetilarginine (ADMA); vascular cell adhesion molecule 1 (VCAM-1); high-sensitivity C-reactive protein (hs-CRP) interleukin-1-beta (IL-1β); MMP-2 and MMP-9 will be quantified using the following double-antibody sandwich enzyme-linked immunosorbent assay ELISA kits: Human asymmetrical dimethylarginine, ADMA (Sunred Biological Technology, Shangai, China); hs-CRP (BoosterBio, Pleasanton, USA); VCAM-1; IL-1β; MMP-9/Gelatinase A; MMP-2/Gelatinase B; and transforming growth factor beta (RayBiotech, Inc. Norcross, USA).

Appendix 6: Cardiovascular health assessment manual of operations

The following questionnaires will be used.

Lifestyle questionnaire:

- On an average weekday, how many hours do you watch TV?
- On an average weekday, how many hours do you play video/computer games or use a computer for something that is not school/work related?
- In the past week, how many days were you/was your child physically active for a total of at least 30 min/day?
- In the past week, how many days did you/your child eat breakfast? In the past week, how many days did you/your child eat food from a fast food restaurant?
- In the past week, how many days did all or most of your family sit down and eat dinner at home?
- On an average weekday, how many hours of sleep do you get a night?
- Have you smoked one or more cigarettes in the past month? If yes, please quantify.
- Were you previously a smoker?
- Do you live in a household with a smoker?

Family history questionnaire:

For all the following questions, the possible answers will be "no," "parents/siblings," "grandparents/aunts/uncles," and "both"

- Biological relatives of you/your child with overweight/obesity
- Biological relatives of you/your child with type 2 diabetes
- Biological relatives of you/your child with high blood pressure
- Biological relatives of you/your child with high cholesterol
- Biological relatives of you/your child with heart disease/stroke
- All answers had the following options: Parents/siblings/grandparents/aunts/uncles.

Appendix 7. Comprehensive List of Study Variables

	Variables	Comments or Definitions
Medical History	BSA at Initial Echocardiogram	using Haycock's Formula; m^2
	Minimum Transverse Arch Diameter Z-score on Initial Echo	Calculated with Boston z-scores
	Isthmus z score on Initial Echo	Calculated with Boston z-scores
	Initial Doppler coarctation gradient	mmHg
	Bicuspid/Bicommisural Aortic Valve?	Yes/No
	Initial arm-leg systolic BP gradient	mmHg
	Type of Initial Treatment	Balloon/Stent/Surgery
	Currently daily medications?	Yes/No. If yes, please specify.
Local blood results	Total Cholesterol	mg/dL
	LDL	mg/dL
	HDL	mg/dL
	Triglycerides	mg/dL
	Plasma Glucose	mg/dL
	Insulin	uIU/mL
	Hemoglobin A1C	%
Applanation tonometry	Central Systolic Blood Pressure	mmHg
	Central Pulse Pressure	mmHg
	Heart Rate	bpm
	Carotid Femoral PWV	meters/second
	Augmentation Index (%)	%
	Augmentation Index at HR75	%

Contd...

Appendix 7. Contd...

	Variables	Comments or Definitions
CMR	LV End-Diastolic Volume indexed to BSA	ml/m^2
	LV End-Systolic Volume indexed to BSA	ml/m^2
	LV Ejection Fraction	%
	LV Mass indexed to BSA	g/m^2
	Ascending Ao - Descending Ao PWV (Ascending Ao to proximal, mid and distal descending Ao)	$\frac{\text{Distance (Asc Ao to Desc Ao)}}{\text{Time Delay (Asc Ao to Desc Ao)}}$ meters/second
	Type of arch	Romanesque; Gothic; Crenel
	Aortic diameter (Ascending, Proximal, Mid and Distal Ao)	mm/mm^2
	Aortic strain (Ascending, Proximal, Mid and Distal Ao)	$\frac{\text{Sistolic Area - Diastolic Area}}{\text{Diastolic Area}}$
	Aortic compliance (Ascending, Proximal, Mid and Distal Ao)	$\frac{\text{Ao Area Sist - Ao Area Diast}}{\text{SBP} - \text{DBP}}$ $cm^2/mmHg$
	Aortic Distensibility (Ascending, Proximal, Mid and Distal Ao)	$\frac{\text{Ao strain}}{\text{SBP} - \text{DBP}}$ mmHg-1
	Aortic stiffness β index (Ascending, Proximal, Mid and Distal Ao)	$\frac{\ln (\text{SBP} / \text{DBP})}{\text{Strain}}$
	Loss of pulse amplitude	$100 \times \frac{\text{flow (AscAo} - \text{DescAo)}}{\text{flow (AscAo)}}$
	Aorta Young's modulus (Ascending, Proximal, Mid and Distal Ao)	$\frac{(\text{SBP - DBP})\,\text{Ao diameter diastole}}{\text{Ao diameter}\,(\text{systole - diastole})\,\text{Ao wall thickness}}$
	Arterial elastance (Ea)	$\frac{\text{End Systolic Pressure}}{\text{Sroke Volume}}$ mmHg/ml
	LV end-systolic elastance (Ees)	$\frac{\text{End Systolic Pressure}}{\text{LV end Systolic Volume}}$ mmHg/ml
Endo-PAT	Reactive Hyperemia Index (RHI)	
	Augmentation Index	%
	Augmentation Index at 75 bpm	%
BP during the patient's visit	Residual SBP gradient (between right arm and highest of the legs)	Supine. Automated. Two sets of measurements; mmHg
	Right arm BP	Seated. Manual. Three sets of measurements; mmHg
ABPM	24 Average Systolic BP	mmHg
	24 Hour Average Diastolic BP	mmHg
	Daytime Average Systolic BP	mmHg
	Daytime Average Diastolic BP	mmHg
	Nighttime Average Systolic BP	mmHg
	Nighttime Average Diastolic BP	mmHg
	24h systolic load	%
	24h diastolic load	%
	Diurnal Systolic Variation	%
	Diurnal Diastolic Variation	%
Exercise test	Exercise Duration	Minutes
	Pre-exercise right arm BP	mmHg
	Pre-exercise leg BP	mmHg
	Pre-Exercise SBP gradient	mmHg
	Post-exercise right arm BP	mmHg
	Post-exercise leg BP	mmHg
	Pre-Exercise SBP gradient	mmHg
	Peak exercise BP	mmHg
Biomarkers	NOx	ug/ml
	ADMA	ng/L
	High Sensitivity CRP	mg/L
	VCAM-1	ng/ml
	IL-1beta	pg/ml

Contd...

Appendix 7. Contd...

	Variables	Comments or Definitions
	TFG-Beta	
	MMP-2/Gelatinase A	ng/ml
	MMP-9/Gelatinase B	ng/ml

Legend: ADMA = Asymmetric Dimetilarginine; Ao = Aorta; BP = blood pressure; BP = Blood Pressure; BSA= Body Surface Area; CMR = Cardiac magnetic resonance; DBP = Diastolic Blood Pressure; HDL = High-density lipoprotein cholesterol; Hs-CRP = High sensitivity C-Reactive Protein; IL-1β = Interleukin 1 beta; LDL = Low-density lipoprotein cholesterol; MMP-2 = Matrix Metalloproteinase-2; MMP-9 = Matrix Metalloproteinase-9; NOx = Nitric Oxide; PWV = Pulse Wave Velocity; LV = Left Ventricle; SBP = Systolic Blood Pressure; TFG-β = Transforming Growth Factor beta; VCAM-1 = Vascular Cell Adhesion Molecule 1; † using previously published normative data.[57]