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Vascular structure and stiffness in pediatric Mulibrey nanism using ultra-high frequency ultrasound

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Authors' contributions: TS, MLN, HJ, and EJ designed the study. TS was responsible for all study visits, data collection, data analyses, and writing of the manuscript. All authors contributed to the writing process and finalization of the manuscript. All the authors have read and approved the final version of the manuscript and agreed to be held accountable for all aspects of the work.

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Ethics approval and consent to participate: the study conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Helsinki University Hospital ethics board for women, children and psychiatry (269/13/03/03/2015/227).

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Abstract

Mulibrey nanism (MUL) is a disorder with growth delay and congestive heart failure determining prognosis. We aimed to delineate arterial and venous morphology, and arterial stiffness in a representative pediatric MUL cohort.

Twenty-three MUL and 23 individually sex and age-matched healthy controls were prospectively assessed in a cross-sectional study with ultra-high frequency ultrasound (48-70 MHz).

Heart failure was present in 7 MUL patients, with severe congestive heart failure in 2. Pericardiectomy had been performed in 6 MUL. Arterial lumen diameters and arterial wall layer thickness (intima-media thickness and adventitia thickness) were smaller in MUL patients, but appropriate for body size when compared with controls. Systolic and diastolic blood pressure, aortic and carotid compliance, stiffness as well as central aortic pulsed wave velocity were all similar in MUL compared with controls. Plasma pro-BNP levels were variably elevated (>300 ng/L) in 9/23 MUL patients and in 4/18 MUL patients older than 5 years of age. Internal jugular vein (mean difference 0.054 mm, CI95% 0.024-0.084) and cubital vein (0.046 mm, CI95% 0.013 - 0.078) total wall thickness was elevated in MUL compared with controls. There were no statistically significant relations between vascular parameters and clinical or laboratory signs of heart failure or pericardiectomy.

Arterial lumen, wall layer thickness and stiffness are appropriate for body size in MUL, and like healthy controls. Mild venous wall thickening in the upper body region may be due to increased venous pressures related to remodelling caused by diastolic heart failure.

Introduction

Mulibrey (muscle - liver - brain - eye) nanism (short stature) (MUL; OMIM #253250) is a multiorgan peroxisomal disease caused by a monogenic recessive disorder by mutations in the TRIM37 gene.[1,2] The disease is particularly prevalent in the Finnish population. In autopsy studies and in biopsies from different organs, including the lungs, MUL patients display significant abnormalities, including a high frequency of tumors and large, dilated, and cystic blood vessels indicative of disturbed angiogenesis.[3,4] The patients suffer from severe growth disturbance, cardiac pericardial constriction, ventricular hypertrophy, and congestive hepatopathy.[5-12] Cardiac manifestations are, however, variable but a major determinant of MUL patient outcomes. There are, to date, no published studies on vascular structure and function in pediatric MUL disease, although disturbances in body growth, pericardial constriction, and cardiomyopathy-related chronic heart failure may impact vascular growth and maturation as well as vascular function.

Arterial morphology, including lumen diameter and wall layer thickness dimensions, is related to body anthropometrics, sex, and blood pressure in both healthy children and children with cardiovascular disease.[13,14] Furthermore, studies report a thickened venous wall in teenage patients adapted to the Fontan circulation with inherently low cardiac output and elevated central venous pressures.[15,16] These studies indicate that long-term alterations in the vascular milieu lead to significant remodeling of the vascular wall in the growing child. Thus, the present study was designed to address knowledge gaps in pediatric MUL patient vascular morphology and function. We designed to study vascular morphology and function in MUL, especially in relation to body growth and hemodynamic abnormalities, with the aim of increasing our understanding of the cardiovascular challenges in the management of these patients.

Our hypothesis was that both the arterial and venous wall morphology, as well as arterial function, are altered in MUL patients, and related with disturbances in body growth, altered hemodynamics and variable degrees of heart failure. To differentiate body size and age-related changes, we performed a case-control study including age and sex matched healthy controls as a comparison group. The objective was to study the venous and arterial wall morphology, and arterial stiffness in relation to age, body size and cardiac function among MUL patients.

Materials and Methods

This cross-sectional case-control study included 23 pediatric MUL patients in Finland aged 1-16 years. The studies were performed in 2015-2017 by one investigator (TS) on outpatient visits at the Children's Hospital, Helsinki University Hospital, Finland. Inclusion criteria for MUL patients were i) genetic diagnosis of MUL and ii) willingness to participate in the study. The Finnish major mutation (c.493-2A>G) was seen in all patients, with one being compound heterozygous for a Fin-minor (c.2212delG) mutation. Twenty-three of all 27 MUL patients, aged 1-16 years, participated. Healthy controls, individually matched for age and sex, were recruited for every patient. Exclusion criteria for controls were a current disease of any kind, a hereditary cardiovascular disorder in a parent or sibling, regular use of medication, and significant arrhythmia, hypertension, or respiratory disease during study assessment. The study conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Helsinki University Hospital ethics board for women, children and psychiatry (269/13/03/03/2015/227). Informed consent was obtained at recruitment. The cardiac findings from this study cohort have recently been published.[11]

Patient hospital charts were reviewed to collect data on previous and current diagnoses, disease history, medications, and interventions including invasive hemodynamic catheterization, pericardiectomy and other operations. Current signs of respiratory or heart failure symptoms, medications and other treatments were recorded during the study visit. Both MUL and control subject´s clinical status was prospectively assessed by TS during the study visits using a standard data collection form. Information documented included presence and characteristics of heart murmurs, right internal jugular vein height from estimated right atrial level (mamilla) in sitting position (estimated central venous pressure in mmHg calculated as height divided by 1.36), peripheral pulse strength and presence of pulsus paradoxus, resting heart, and respiratory rate, size of liver (distance from costal margin), presence of ascites or other fluid collections, as well as detailed anthropometric measures of weight and height, and dimensions of thorax, abdomen, hip, head, upper and lower limb. A standard 12-lead ECG was recorded. Heart failure was graded using the modified Ross classification in children.[17] Subject height was assessed with an electronic stadiometer to the nearest 0.1 cm, and weight with an electronic scale to the nearest 0.1 kg (Seca GmbH 770 & Co. KG, Hamburg, Germany). All other anthropometric and jugular vein height measures were assessed with a tape measure to the nearest 0.1 cm. Z-scores for child height, weight, and BMI were derived using recent Finnish reference data.[18] Overweight and obesity were defined using IOTF criteria.[19] Lean Body Mass (LBM) was derived using the previously DXA-based validated formula,[20] and fat mass was calculated as LBM subtracted from total body weight. Fat mass percentage was calculated as fat mass divided by total body weight.

Blood Pressure

Blood Pressure (BP) was assessed according to NHBPEP 4th report guidelines using three repeat oscillometric measurements (Dinamap ProCare 200, GE Healthcare, Chicago, USA) at rest in a sitting position. A difference of <5 mmHg between measures was deemed appropriate. The mean of the two lowest readings was used in analyses. SBP and DBP z-scores were generated using the 4th report reference.[21]

Laboratory parameters

A fasting blood venous blood sample was obtained during morning hours from all MUL patients, and total cholesterol, LDL and HDL cholesterol, triglycerides, blood glucose, HbA1c, insulin, alanine aminotransferase, hs-CRP, and pro-BNP were assessed according to the Helsinki University Hospital clinical laboratory standards.

Vascular assessments

Vascular Ultra-High Frequency ultrasound (UHF) cine-clips were acquired using Vevo 770 (VisualSonics, 2005, Toronto, Canada) equipped with RMV710B, RMV712, and RMV708 transducers (center frequencies 25,35 and 55 mmHz) for initial 5 MUL patients, and Vevo MD (VisualSonics, 2016, Toronto, Canada) equipped with UHF22, UHF48 and UHF70 transducers (center frequencies 15MHz, 30MHz and 50MHz) for all other study subjects. All images were acquired by one experienced investigator (TS) as previously validated.[15,22] The highest frequency transducer to visualize the vascular far wall without compression was used. Veins were imaged with a few millimeters layer of ultrasound gel, avoiding contact between the transducer and skin. The common carotid artery and internal jugular vein were assessed 1 cm proximal to the carotid bulb,

radial and ulnar arteries 1 cm proximal to the palma manus, brachial artery and cubital vein 3 cm proximal to the cubital fold, femoral artery distal to the inguinal fold and 1 cm proximal to the bifurcation, and dorsal tibial artery and vein at the medial malleolar level. Bilateral imaging was performed in the neck and groin regions, with the mean of right and left measurements used in analyses. Vessel Lumen Diameter (LD), and far wall Intima-Media Thickness (IMT), and Intima-Media-Adventitia Thickness (IMAT) were assessed in end-diastole.[15,21] Adventitial Thickness (AT) was calculated as the difference between IMAT and IMT. The mean of three measurements per site was used in final analyses. The intra-observer Coefficients of Variations (CV) for different arterial VHRU measurements were 1.2-2.9% for LD, 6.9-9.8% for IMT, and 7.6-28.6% for AT, and inter-observer CVs were 1.5-4.6% for LD, 6.0-10.4% for IMT, and 5.9-20.5% for AT.

The abdominal aorta was imaged using Vivid 7 ultrasound system (GE Medical Systems, Horten, Norway) with 3- to 7-MHz transducers, and measurements were made offline using the EchoPac (version 113) workstation. Abdominal aortic (1cm proximal to coeliac trunk bifurcation) and common carotid artery local stiffness and distensibility were calculated using the following formulas: stiffness index $\beta=\ln(SBP/DBP)/((LDs–LDd)/LDd)$ and distensibility $=(LDs–LDd)/ LDd\ (SBP–DBP)$, where SBP is right arm SBP, DBP is right arm DBP, LDs is systolic lumen dimension, and LDd is diastolic lumen dimension. The mean of two measurements was used in analyses. Our intraobserver, interobserver, and test-retest CVs were 15%, 19%, and 25%, for the carotid stiffness index and 15%, 17%, and 22%, for carotid distensibility. Similarly, intra- and interobserver CVs were 25% and 26% for aortic stiffness index, and 26% and 29% for aortic distensibility.

The transit time between distal transverse aortic and femoral artery pulse waves was measured by pulsed-wave Doppler by calculating the time difference between foot of pulse-waves in relation to ECG R-wave. The direct distance between recording sites (jugulum to femoral) was measured using

a tape measure to the nearest 0.1 cm. This distance was multiplied by 0.8. Central aortic Pulse Wave Velocity (PWV) as a measure of regional aortic stiffness was then calculated by dividing distance by transit time. The mean of two recordings was used in analyses. Intra-observer CV based on the two PWV measurements was 5.4%.

Data analysis

Sample size calculations or power analyses were not performed as the MUL condition is rare, and the aim was to include the complete Finnish pediatric MUL cohort alive at the time of recruitment. Categorial data are presented with numbers and proportions, and continuous data is reported as mean±SD or median (range), as appropriate. Continuous variables were assessed for normal distribution using histograms and Shapiro-Wilks test. Differences between MUL and controls were assessed using t-tests or Mann-Whitney U-tests, as appropriate. ANCOVA was used to calculate the mean difference (and 95% CIs) between MUL and controls adjusting for anthropometric measures. Associations between different parameters were assessed with scatter plots, following Pearson correlation and Spearman rank-order correlation, as appropriate. Regression analyses were performed to study associations between cardiovascular parameters and gender, age, and body size, as well as to assess associations between the different cardiovascular parameters. Data analyses were performed with SPSS version 25, and graphs generated with GraphPad Prism 8.

Results

Background and clinical characteristics

MUL patients were significantly smaller in body size compared with controls both for more general anthropometric measures such as height and weight, but also for more detailed regional measures including head and limb circumference as well as upper and lower limb length (Table 1). No abnormalities in heart sounds were noted. Heart rate was higher, and systolic blood pressure was lower in MUL patients as compared with controls. No difference was, however, observed in BP z-scores between groups accounting for body size. The modified Ross score for heart failure in children was elevated (>0) in 16 MUL patients. Among these, the score was low (1-2) in nine, moderate (3-4) in five and high (6 and 13) in two MUL patients. Among two patients with high modified Ross scores, both had a history of premature birth, RDS/BPD during infancy, severe growth restriction, and failure to thrive, with one patient requiring long-term intermittent Continuous Airway Pressure Support (CPAP). In 18 MUL patients, the liver margin was palpable and enlarged, and the jugular vein was visible above the clavicle in the upright position, indicating elevated central venous pressures. Among patients with observable jugular veins, the jugular venous pressure height was 10±4 cm (range 2-18 cm) corresponding to 7±3 mmHg with no apparent relation with age. Jugular veins were non-observable in all healthy control children. There were no signs of ascites or pulsus paradoxus. Plasma pro-BNP levels were variably elevated (>300 ng/l) in 9/23 MUL patients and in 4/18 MUL patients older than 5 years of age. Serum alanine aminotransferase was mildly elevated (>40 U/l) in 6 MUL patients. Fasting blood lipid, glucose, insulin levels were normal, and hs-CRP levels were also in the normal range among MUL patients.

Five MUL patients had a history of pericardiectomy, and one was pericardiectomized two years after study evaluation. Elevated (mean 12-20 mmHg) and equalized right and left atrial pressures with the absence of pulmonary hypertension (mean pressure <25 mmHg) were confirmed on diagnostic catheterization performed during general anesthesia in all 6 MUL patients prior to pericardiectomy.

Other cardiac diagnoses included one ASD secundum closed with a device, one moderate size ASD secundum without intervention showing inspiratory interatrial right to left shunting and desaturation on exercise, and two WPWs.

Vascular dimensions and function

Arterial and venous dimensions among MUL and control patients are outlined in Table 2 with mean difference and 95CIs reported adjusted for BSA and organ circumference.

Sample images of common carotid, femoral, brachial, and radial arteries, as well as internal jugular and cubital veins from MUL patients with UHF ultrasound, are provided in Figure 1.

Strong positive associations between anthropometric measures and arterial and venous lumen and wall layer thickness were found among both MUL and control groups, as expected (results not shown). Most differences in dimensions were attenuated to non-significant levels when adjusting for body size. Aortic, common carotid, brachial, radial, ulnar, femoral, and dorsal tibial arterial lumen diameters were all similar between study groups. Arterial adventitia thickness was also similar between groups. No consistent differences in combined intima-media thickness among the arteries was found except for the femoral artery that was statistically thicker among MUL patients when accounting for difference in body size. A trend for increased abdominal aortic compliance among MUL compared with controls was found, but there was no statistically significant difference in aortic stiffness index or aortic pulse wave velocity. No significant relations between vascular dimensions or function and pericardiectomy status, Ross heart failure scores, or pro-BNP levels were found (results not shown).

Internal jugular and cubital vein combined intima-media-adventitia thickness were increased among MUL compare with controls when adjusting for difference in body size, but no statistically significant difference in dorsal tibial vein wall layers were found (Table 2). There were no differences in venous lumen diameters between groups. Age was positively correlated with cubital vein and dorsal tibial vein combined intima-media-adventitia thickness both among MUL (r=0.56, p=0.005 and r=0.56, p=0.008, respectively) and controls (r=0.48, p=0.021 and r=0.72, p=0.0002, respectively) as outlined in Figure 2. No statistically significant association between estimated jugular venous pressure and venous lumen diameter or venous wall layer thickness was found.

Discussion

Cardiovascular dimensions are widely known to be related with body size in a growing child. However, with significant aberrancy in body growth and alterations in the hemodynamic milieu, vascular dimensions may not follow normal healthy body growth patterns. The present study, however, shows that the arterial and venous structure and different measures of arterial stiffness are mostly appropriate for body size and, thus, in the normal range in pediatric MUL disease when compared with age matched healthy children. This adds significant novel information to the existing literature on the rare MUL disease.

MUL disease presents with variable growth delay that can be severe. The study shows that, not only height and weight for age may be substantially decreased in MUL disease, but also detailed anthropometric measures of upper and lower body parts are significantly smaller when compared with healthy children. Our findings are in line with the original clinical descriptions of MUL patients including triangular craniofacial features with bulging forehead, general body gracility, and short and

thin extremities.[6] Our comprehensive set of anthropometrics and vascular assessments show that vascular dimensions in MUL are largely congruent with both body and regional anthropometrics and, thus, not different from healthy children. One could argue that age could have an impact on vascular morphology in MUL, but we were unable to show any abnormalities in the pediatric age range. Even in the MUL patients with the most severe growth restriction (*e.g.*, 14 y.o. girl with height 98 cm and weight 12.2 kg) the vascular morphology was consistent with body anthropometrics and consistent with vascular growth and dimensions being closely related to organ size. Our findings are well in line with previous studies showing arterial morphology to be congruent with body size in neonates following intrauterine growth restriction or excessive intrauterine growth,[23] as well as during childhood[24,25] and adolescence[14] in subjects with variable anthropometrics and body composition.

Severe pericardial constriction and mild myocardial restriction-related diastolic dysfunction, as well as mild systolic dysfunction, are relatively common findings in pediatric MUL disease.[11] Congestive heart failure is a major determinant of outcomes.[8] Most severe cases develop significant right heart failure and ascites with pericardiectomy indicated. Veins are commonly prominent in MUL disease due to elevated venous pressures, as observed in the present study. Left heart cardiac output is commonly in the lower range in MUL due to combined pericardial constriction and myocardial restriction, but pulmonary hypertension or systemic hypotension is not found in our representative pediatric MUL cohort.[11] We have previously shown that local blood pressure impacts arterial wall layer thickness in teenagers following aortic coarctation repair during infancy and early childhood.[13] The normal range arterial wall layer thickness in our pediatric MUL patients is then in agreement with normal systemic blood pressures when adjusted for the considerably smaller body size. Similarly, we have shown significant vascular remodeling, i.e., arterial wall thinning and venous wall thickening, among teenagers with inherently elevated systemic venous pressures and low systemic

arterial blood pressure after the Fontan procedure performed during childhood.[15] Our present findings of venous wall thickening in pediatric MUL with elevated systemic venous pressures are then similarly in line with our previous findings in pediatric Fontan patients.

Our study is limited by the relatively small sample size, precluding more detailed analyses. However, we studied a representative pediatric MUL cohort with age-ranged control patients. Exercise data were not included due to methodological limitations attributed to age and body size-related challenges in co-operation. Bone age was not measured, and body composition was only assessed using previously established formulas.[20] Puberty was not assessed. In addition, prospective longitudinal data collected pre and post-pericardiectomy was not included due to the rarity of interventions.

Conclusions

In conclusion, we report a comprehensive clinical and ultrasound assessment delineating both arterial and venous morphology, as well as arterial function, in a representative pediatric MUL cohort known to be challenged with significant growth restriction and variable pericardial and myocardial disease-related diastolic dysfunction leading to congestive heart failure. Our results show that the venous wall is mildly thickened in pediatric MUL, but arterial morphology and function are largely congruent with body anthropometrics and not different from age-matched healthy controls.

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Table 1. Characteristics, anthropometrics, and clinical findings of MUL patients and controls at study visit.

Variable	**MUL (N=23)**	**Controls (N=23)**	
	Mean (SD) or	**Mean (SD) or**	**p-value**
	Median (range) or N	**Median (range) or N**	
Male sex (N)	11	11	
Age (y)	10.3 (1.9-15.2	10.0 (1.2-15.8)	NS
Weight (kg)	14.8 (6.6-47.0)	33.0 (13.0-68.7)	<0.001
Weight-for-height (Z)	-2.11 (1.70)	0.08 (0.94)	<0.001
Height (cm)	110.5 (69.5-157.0)	146.4 (93.0-175.5)	<0.001
Height-for-age (Z)	-4.43 (1.76)	0.23 (1.10)	<0.001
BSA (m2)	0.77 (0.32)	1.16 (0.42)	<0.001
Lean Body Mass[a]	14.7 (6.8 - 36.1)	30.5 (11.3)	<0.001
Waist (cm)	52.3 (9.6)	61.4 (10.3)	<0.001
Hip (cm)	55.8 (13.2)	72.6 (16.8)	<0.001
Waist-Hip-ratio	0.96 (0.12)	0.87 (0.13)	0.018
Arm length (mm)	305 (91)	439 (101)	<0.001
Brachial length (mm)	194 (59)	281 (65)	<0.001
Brachial circumference (mm)	156 (38)	215 (40)	<0.001
Antebrachial circumference (mm)	150 (31)	196 (29)	<0.001
Leg length (mm)	543 (170)	714 (184)	<0.001
Thigh circumference (mm)	270 (78)	409 (99)	<0.001
Calf circumference (mm)	202 (51)	277 (56)	<0.001
Thorax circumference (mm)	538 (111)	645 (100)	<0.001
Head circumference (mm)	510 (28)	532 (20)	<0.001
Blood sample data			
Total cholesterol (mmol/L)	3.73 (0.61)	NA	

LDL cholesterol (mmol/L)	2.30 (0.49)		
HDL cholesterol (mmol/L)	1.30 (0.38)		
Plasma glucose (mmol/L)	4.94 (0.55)		
Serum insulin (mU/L)	10.9 (1.0-52.6)		
HbA1c (mmol/L)	33 (5)		
HbA1c (%)	5.2 (0.4)		
Alanine aminotransferase (U/lL)	35 (19)		
Plasma pro-BNP (ng/L)	181 (41-3264)		
Serum hs-CRP (mg/L)	0.32 (0.14-4.04)		
Estimated jugular venous pressure (mmHg)	7 (3)	Jugular veins not observable	
Liver (cm from costal margin)	2 (0-5)	Not palpable	
Modified Ross score	1 (0-13)	0	
Modified Ross class	1 (1-3)	1	
Systolic BP (mmHg)	95 (13)	104 (10)	0.009
SBP (Z)	-0.14 (1.32)	0.27 (0.67)	0,210
Diastolic BP (mmHg)	58 (7)	60 (7)	0.405
DBP (Z)	-0.16 (1.22)	0.13 (0.52)	0,310
Heart rate (bpm)	91 (20)	76 (14)	0.005

Data is presented as mean (SD), median (range), or as count (N). BP, blood pressure. NA, not assessed. [a]N=18 for lean body mass for MUL and individually matched controls.

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Table 2. Vascular dimensions and function at study visit in MUL patients and controls.

Variable	**MUL (N=23)**	**Control (N=23)**	**Adjusted for BSA**	**Adjusted for organ circumference**
	Mean (SD)	**Mean (SD)**	**Mean diff (CI95%)**	**Mean diff (CI95%)**
Carotid artery				
Intima-media thickness (mm)	0.35 (0.047)	0.40 (0.026)	-0.22 (-0.44-0.001)	-0.29 (-0.53 - -0.006)*
Lumen diameter (mm)	4.45 (0.49)	4.83 (0.60)	0.10 (-0.137-0.334)	-0.03 (-0.29-0.24)
Compliance (%/10mmHg)	4.53 (1.43)	4.46 (1.46)		
Stiffness (no unit)	3.33 (0.94)	3.14 (1.35)		
Radial artery (mm)				
Intima-media thickness	0.099 (0.029)	0.11 (0.03)	0.009 (-0.002-0.021)	0.017 (0.003-0.031)*
Adventitia thickness	0.066 (0.016)	0.061 (0.016)	0.009 (-0.002-0.019)	0.009 (-0.003-0.021)
Lumen diameter	1.32 (0.30)	1.51 (0.20)	-0.039 (-0.176-0.098)	0.132 (0.118-0.183)*
Ulnar artery (mm)				
Intima-media thickness	0.133 (0.038)	0.137 (0.038)	0.016 (-0.006-0.039	0.021 (-0.005-0.047)
Adventitia thickness	0.071 (0.014)	0.079 (0.032)	-0.004 (-0.021-0.014)	-0.001 (-0.021-0.018)
Lumen diameter	1.62 (0.30)	1.29 (0.26)	0.36 (0.16-0.56)**	0.36 (0.14-0.59)**
Brachial artery (mm)				
Intima-media thickness	0.092 (0.025)	0.110 (0.022)	-0.002 (-0.013-0.010)	0.005 (-0.008-0.018)
Adventitia thickness	0.090 (0.017)	0.10 (0.026)	-0.001 (-0.015-0.013)	0.002 (-0.014-0.018)
Lumen diameter	2.28 (0.62)	2.58 (0.51)	0.125 (-0.142-0.393)	0.266 (-0.053-0.585)
Femoral artery (mm)				
Intima-media thickness	0.191 (0.051)	0.206 (0.037)	0.029 (0.011-0.047)**	0.039 (0.015-0.064)**
Adventitia thickness	0.189 (0.060)	0.181 (0.038)	0.016 (-0.018-0.050)	0.019 (-0.019-0.058)
Lumen diameter	4.01 (0.91)	5.28 (1.16)	-0.403 (-0.821-0.017)	-0.093 (-0.645-0.459)
Dorsal tibial artery (mm)				
Intima-media thickness	0.181 (0.040)	0.210 (0.050)	-0.011 (-0.039-0.018)	-0.001 (-0.033-0.031)

Adventitia thickness	0.088 (0.024)	0.088 (0.030)	-0.001 (-0.017-0.015)	-0.001 (-0.018-0.017)
Lumen diameter	1.44 (0.27)	1.46 (0.21)	0.049 (-0.119-0.217)	0.085 (-0.100-0.271)
Ascending aorta				
Lumen diameter systole *(mm)*	16.3 (3.7)	20.8 (4.4)	-0.54 (-1.85-0.78)	-0.98 (-2.65- 0.69)
Abdominal aorta				
Lumen diameter systole(mm)	7.1 (2.4)	10.0 (2.9)	-0.92 (-2.22-0.37)	-1.18 (-2.59-0.23)
Compliance (%/10mmHg)	7.19 (3.48)*	5.37 (1.75)		
Stiffness (no unit)	2.22 (0.78)	2.64 (1.11)		
Central aortic pulse wave velocity (m/s)	5.0 (1.4)	4.7 (0.5)		
Veins (mm)				
Internal jugular vein intima-media-adventitia thickness	0.217 (0.052)	0.170 (0.034)	0.060 (0.031-0.090)***	0.054 (0.024-0.084)***
Cubital vein intima-media thickness	0.102 (0.027)	0.105 (0.032)	0.007 (-0.016-0.030)	0.012 (-0.013-0.037)
Cubital vein intima-media-adventitia thickness	0.175 (0.045)	0.174 (0.052)	0.031 (0.002-0.061)*	0.046 (0.013-0.078)**
Cubital vein lumen diameter	2.93 (0.89)	2.92 (0.94)	0.58 (-0.70-1.23)	0.66 (0.03-1.30)*
Dorsal tibial vein intima-media thickness	0.085 (0.021)	0.088 (0.030)	0.014 (-0.004-0.031)	0.017 (-0.003-0.037)
Dorsal tibial vein intima-media-adventitia thickness	0.149 (0.033)	0.164 (0.047)	0.015 (-0.008-0.037)	0.021 (-0.007-0.049)
Dorsal tibial vein lumen diameter	1.74 (0.41)	1.77 (0.65)	0.10 (-0.29-0.50)	0.14 (-0.30-0.58)

Data is presented as mean (SD). Groups compared with Students t-test. Adjusted mean difference analyzed with ANCOVA. Cubital vein IMT MUL N=19 and Controls N=15. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

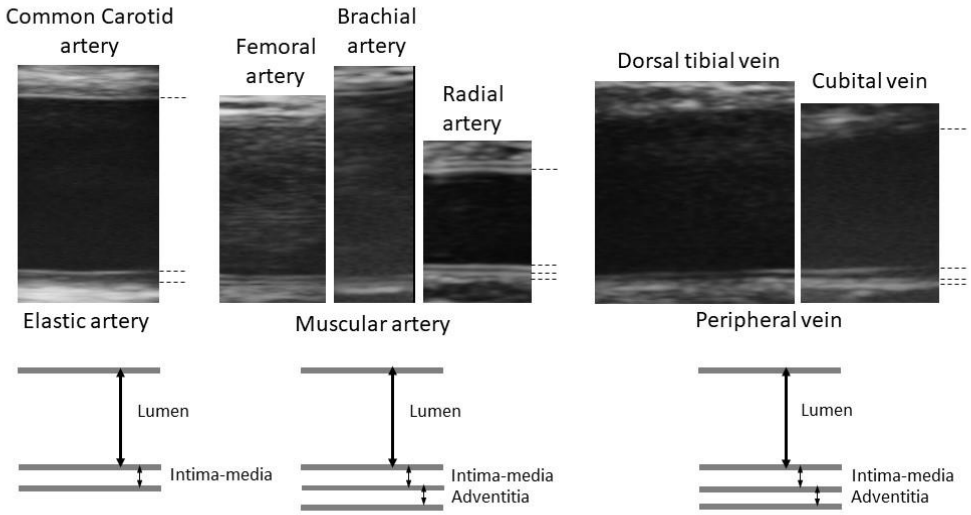

Figure 1. Sample images obtained with ultra-high frequency ultrasound showing common carotid, femoral, brachial, radial, as well as cubital and dorsal tibial veins in a 5-year-old MUL patient without pericardiectomy performed. Carotid and femoral arteries were imaged with 48 MHz and all the other vessels with 70 MHz.

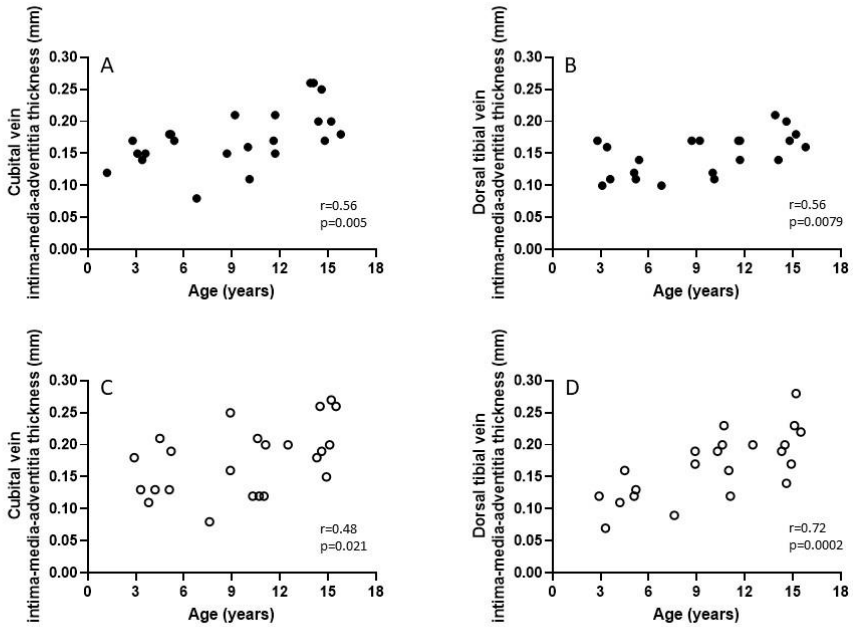

Figure 2. Statistically significant positive associations between age and cubital vein wall thickness (A and C) and dorsal tibial vein wall thickness (B and D) among MUL patients (A and B) and healthy controls (C and D).