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Subclinical atherosclerosis in patients with cyanotic congenital heart disease☆

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ABSTRACT

Introduction: Survival in patients with cyanotic congenital heart disease (CCHD) has improved dramatically. The result is an ageing population with risk of acquired heart disease. Previous small uncontrolled studies suggested that these patients are protected against the development of atherosclerosis. To test this hypothesis, we sought to determine the prevalence of subclinical atherosclerosis in a larger population of patients with CCHD.
Method: We compared the prevalence of subclinical atherosclerosis in adult CCHD patients from Denmark, Sweden, Norway and Australia, with that in age-, sex-, smoking status-, and body mass index matched controls. Coronary artery atherosclerosis was assessed on computed tomography with coronary artery calcification (CAC) score. Subclinical atherosclerosis was defined by CAC-score > 0. Carotid artery atherosclerosis was evaluated using ultrasound by measuring carotid plaque thickness (cPT-max) and carotid intima media thickness (CIMT). Lipid status was evaluated as an important atherosclerotic risk factor.
Results: Seventy-four patients with CCHD (57% women, median age 49.5 years) and 74 matched controls (57% women, median age 50.0 years) were included. There were no differences between the groups in: CAC-score > 0 (21% vs. 19%, respectively; $p = 0.8$), carotid plaques (19% vs. 9%, respectively; $p = 0.1$), cPT-max (2.3 mm vs. 2.8 mm, respectively; $p = 0.1$) or CIMT (0.61 mm vs. 0.61 mm, respectively; $p = 0.98$). And further no significant differences in lipoprotein concentrations measured by ultracentrifugation.
Conclusion: Young adults with CCHD have similar cardiovascular risk factor profiles and measures of subclinical atherosclerosis, compared with controls. Given their increasing life expectancies, athero-preventive strategies should be an important part of their clinical management.

1. Introduction

During the past decades the survival of patients with cyanotic congenital heart disease (CCHD) has improved due to better care and treatment options [1,2]. This has resulted in an ageing population of patients with CCHD in whom acquired heart diseases should be taken into account. Previous studies have suggested that patients with CCHD might be protected against developing atherosclerosis [3–7]. Atherosclerosis is a systemic disease initiated during adolescence, with accumulation of fat and inflammatory cells in the walls of arteries. Atherosclerosis remains in an asymptomatic subclinical state before progressing to clinically relevant stages causing coronary artery disease (CAD) and/or cerebrovascular disease in adulthood [8]. The aetiology is multifactorial and progression is influenced by risk factors, such as elevated plasma lipids, hypertension, diabetes mellitus, and smoking.

Although sparsely reported in the literature, the decreased prevalence of atherosclerosis in CCHD has been associated with low plasma lipoprotein levels and upregulated nitric oxide. Hyperbilirubinaemia

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and thrombocytopenia are thought to be important anti-atherosclerotic factors [6,9,10]. We sought to determine whether patients with CCHD are truly protected against atherosclerosis by examining a larger patient group for subclinical atherosclerosis using well-validated imaging modalities in the two major sites of atherosclerosis.

2. Method

2.1. Study participants

From August 2014 to February 2018, clinically stable adult patients with CCHD followed at below listed institutions were invited to participate in this multicentre prospective study: The University Hospital Rigshospitalet, Copenhagen and The University Hospital Aarhus, Denmark; The University Hospitals in Lund, Stockholm and Gothenburg, Sweden; The University Hospital Rikshospitalet, Oslo, Norway; and The Royal Prince Alfred Hospital, Sydney, Australia.

CCHD was defined as the presence of a congenital heart defect with a right-to-left or bidirectional shunt, with resting systemic oxygen saturation < 92% and/or <87% during exercise [11].

Patients from Australia and Gothenburg in Sweden were examined locally whereas all others were examined at Rigshospitalet in Denmark.

Healthy controls were recruited from Denmark by advertisements in local newspapers and the Internet on the homepages www.sundhed.dk and www.forsøgspersoner.dk. Matching was performed in a one-to-one ratio to reduce the atherosclerotic effect of the following variables; age (±five years), sex (women/men), smoking status (current smoker, former smoker, or never smoker) and body mass index (BMI) (<25 m^2, 25–29 m^2, ≥30 m^2). We included control persons with no history of cardiovascular disease (CVD) or diabetes mellitus. Exclusion criteria were pregnancy or breastfeeding.

2.2. Risk factors

Clinical examination was performed at rest including heart rate, transcutaneous oxygen saturation, waist circumference, as well as body weight and height measurements. BMI was calculated [12]. Blood pressures (BP) were measured via 24-hour readings on the upper arm every half hour during the daytime and every hour during the night.

Data on risk factors for CVD were collected, including hypertension, hypercholesterolemia, diabetes mellitus, previous myocardial infarction (MI), and cerebrovascular event (CVE) for participants and their parents and siblings. The participants were classified as current smokers, former smokers, or non-smokers. The number of pack-years was calculated by the number of packs per day multiplied by the number of years. Weekly alcohol consumption was recorded as less or >14 units per week. The participants' medications were registered.

Daily activity levels were measured using a seven-day pedometer, and the participants were asked about their weekly exercise activity.

The Framingham Risk score was calculated for all participants, and they were divided according to their risk of developing CVD within 10 years, into low risk (<10%), moderate risk (10–19%) and high risk (>20%) groups [13].

2.3. Assessment of subclinical atherosclerosis

2.3.1. Coronary artery calcification visualized on CT

An electrocardiography gated non-contrast multi-detector CT scan of the heart was performed. Participants were scanned on a 320 detector scanner (Acquilion ONE, ViSION edition, Toshiba Medical Systems, Japan) in Denmark, a 64 detector scanner (Discovery HD 750, GE Healthcare, Milwaukee, WI, USA) in Australia, and a 128 detector dual source stellar-detector scanner (Siemens Definition, Germany) in Gothenburg, Sweden. Since the purpose was to assess the total burden of coronary atherosclerosis, only non-contrast scans evaluating the CAC-score were obtained. Images were reconstructed with a slice thickness and increment of 3 mm. All CT scans were interpreted at the CT core laboratory at Rigshospitalet in Copenhagen, Denmark. The readers of the scans were blinded to the participants' status.

The CAC-score was the primary endpoint and was measured and quantified using the Agatston scoring method [14]. The Agatston score was used to categorize the patients into 4 ordered groups: 0, 1–100, 101–400, and >400, in concordance with previous studies [15,16]. Subclinical atherosclerosis was defined by an Agatston score > 0.

2.3.2. Carotid ultrasound scanning with plaque thickness and intima media thickness

The B-mode ultrasound scanning protocol included standard imaging of the carotid artery and its branches [17,18]. The CIMT was defined as the mean CIMT on the far wall of the vessel in 1-cm-lengths of the common carotid artery just proximal to the bifurcation, or where measured thickest. If a carotid plaque was identified, an experienced vascular surgeon, blinded to type of participant, reevaluated the cPT-max. Carotid plaques were defined as focal structures encroaching into the arterial lumen by: 1) at least 0.5 mm, 2) 50% of the surrounding IMT value, or 3) demonstrating a thickness > 1.5 mm, as measured from the media–adventitia interface to the intima–lumen interface [19,20].

2.3.3. Plaques in the coronary arteries and/or carotid arteries

We defined a categorical variable if the participants had a CAC-score > 0 and/or any presence of plaque in the carotid arteries, termed "coronary-carotid-plaque".

2.4. Blood measurements

Non-fasting blood samples were taken to determine haematologic and inflammatory markers. Standard hospital assays were used to measure lipoproteins (VITROS® 5600 Integrated System, US), including measurements of total-cholesterol, low-density-lipoprotein (LDL)-cholesterol, high-density-lipoprotein (HDL)-cholesterol and triglycerides.

2.4.1. Ultracentrifugation

Ultracentrifugation was used as the gold standard for measuring lipoproteins. Ultracentrifugation was performed at 50.000 rpm at 4 °C for 16 h using a Beckman Ti 50.3 rotor and a Beckman Optima LE-80 K ultracentrifuge (Beckman Coulter, Inc., Fullerton, CA). Cholesterol was measured using enzymatic kits (CHOD-PAP, Roche Applied Sciences, Denmark).

2.4.2. Fast protein liquid chromatography (FPLC) analysis

To determine if there were any size differences in the distribution of lipoprotein classes a fast protein liquid chromatography (FPLC) was performed. The patient and control groups were each separated into two groups according to the median total-cholesterol measured from ultracentrifugation. Pools of plasma were formed from each group and subjected to a supserose 6 column (Amersham) with a flow of 0.4 mL/min using phosphate buffered saline and ethylenediaminetetraacetic acid as buffer. Cholesterol measurements were performed as described above, and triglycerides were measured using an enzymatic kit (Serum Triglyceride Determination Kit, GPO-Trinder, Sigma, Denmark).

2.5. Statistics

All statistical analyses were conducted using SAS statistical software version 7.1 (SAS Institute, Cary, NC, USA). Continuous variables were expressed as means ± standard deviations (SD) if normally distributed and as medians and percentiles [25 and 75 percentiles] if non-normally distributed. Categorical variables were presented as percentages. For continuous variables, unpaired comparisons between the patients and controls were performed using the Student's *t*-test if normally distributed, and the Wilcoxon rank sum test if non-normally distributed. To compare categorical data, the Chi-square or Fisher's exact test was used. Paired comparison of the main endpoints ("CAC > 0", "coronary-carotid-plaque", "carotid plaque", "CIMT" and "cholesterol levels") in the two groups was performed as a sensitivity analysis with the use of conditional logistic regression if binary-outcome and by paired *t*-test in continuous outcomes. Throughout, a nominal level of 5% statistical significance (two-tailed) was assumed. To test for differences in the coronary and carotid-plaques between patients with CCHD and controls we performed a logistic regression analysis adjusted for the matching parameters: age, sex, BMI, and smoking status. In accordance with previous studies regarding anti-atherosclerotic factors, each of the following variables were added to the analysis: total-cholesterol, oxygen saturation, bilirubin, and platelets [4,6,7].

2.6. Ethics

The study was conducted in adherence with good clinical practice guidelines and in accordance with the Helsinki Declaration. The Danish (protocol number: H-1-2014-035), Norwegian (protocol number: 2014/2243/REK sør-øst A) and Swedish Ethic (protocol number: 2015/424-31) Australian (protocol number: HREC/16/RPAH/135, 16-0109), committees approved the study protocol. Written informed consent was obtained from all participants before inclusion.

3. Results

Seventy-four clinically stable patients (57% women, median age 49.5 years, range: 23–78 years) and 74 age-, BMI-, sex- and smoking status-matched controls (57% women, median age 50.0 years, range: 24–78 years) were included in the study. The demographics are shown in Table 1.

Ventricular septal defect (VSD) was the most frequent cause of CCHD (54%). There was no significant difference in BMI or waist circumference between the two groups. The mean oxygen saturation was 84 ± 5% in the patients with CCHD compared to 98 ± 1% in the controls ($p < 0.0001$). The patients with CCHD had significantly higher heart rates (75 bpm (±15) vs. 62 bpm (±11), $p < 0.0001$), and significantly lower blood pressure (systolic: 113 (±14) mm Hg vs. 119 (±15) mm Hg, $p = 0.02$; diastolic: 69 (±9) mm Hg vs. 73 (±9), $p = 0.01$).

The seven-day pedometer assessment showed that the daily activity level of the patients with CCHD was half the level of the controls (4015 (±2401) steps per day vs 8248 (±3414) steps per day, $p < 0.0001$). A significantly smaller number of patients reported regular exercise compared to the controls (38 (51%) vs. 59 (80%), respectively, $p = 0.002$).

Table 1
Demographics.

	Patients with CCHD (n = 74)	Controls (n = 74)	*p*-Value
Clinical characteristic			
Age, years (median, range)	49.5 [23.0–78.0]	50.0 [24.0–78.0]	0.9
Female sex, n (%)	42 (57)	42(57)	1.0
Cyanotic congenital heart disease diagnoses			
Ventricular septal defect, n (%)	40 (54)	0	
Atrial septal defect, n (%)	11 (15)	0	
Atrioventricular septal defect, n (%)	3 (4.0)	0	
Tetralogy of Fallot, n (%)	7 (9.5)	0	
Univentricular heart, n (%)	6 (8)	0	
Double Outlet Right Ventricle, n (%)	3 (4.0)	0	
Ebstein anomaly with atrial septal defect, n (%)	2 (2.7)	0	
Hemitruncus arteriosus	1 (1.4)	0	
Pulmonary arteriovenous malformation	1 (1.4)	0	
Other diagnoses			
Diabetes mellitus, n (%)	2 (3)	0 (0)	0.5
Myocardial infarction, n (%)	0 (0)	0 (0)	–
Stroke, n (%)	14 (19)	0 (0)	<0.0001
Transient ischemic attacks, n (%)	3 (4)	0 (0)	0.08
Family history of myocardial infarction, stroke, hypertension, hypercholesterolemia, diabetes, n (%)	42 (67)	54 (74)	0.26
Physical examination			
Oxygen saturation at rest %, mean (±SD)	84 (±5)	98 (±1)	<0.0001
Systolic blood pressure, average 24 h, (mm Hg), mean (±SD)	113 (±14)	119 (±15)	0.02
Diastolic blood pressure, average 24 h, (mm Hg), mean (±SD)	69 (±9)	73 (±9)	0.01
Heart rate at rest, (bpm) mean (±SD)	75 (±15)	62 (±11)	<0.0001
Body mass index (kg/m^2), mean (±SD)	23.2 (±4.6)	24.2 (±3.8)	0.2
Waist circumference (cm), mean (±SD)	90.0 (±14.7)	89.5 (±10.0)	0.8
Seven-day pedometer, average number of steps, mean (±SD)	4015 (±2401)	8248 (±3414)	<0.0001
Medication			
Lipid lowering medication, n (%)	5 (7)	1 (1)	0.2
Antithrombotic medication, n (%)	13 (18)	0	0.0002
Anticoagulation medication, n (%)	22 (30)	0	<0.0001
New oral anticoagulants, n (%)	1 (1)	0	0.3
Advanced therapy for pulmonary arterial hypertension, n (%)	53 (72)	0	<0.0001
Endothelin receptor antagonist, n (%)	47 (64)	0	<0.0001
Phosphodiesterase 5 inhibitor, n (%)	26 (35)	0	<0.0001
Framingham 10-year CVD risk score			0.8
Low (<10%), n (%)	61 (87)	65 (88)	
Moderate (10–19%), n (%)	7 (10)	8 (11)	
High (≥20%), n (%)	2 (3)	1 (1)	
Lifestyle			
Current smoker, n (%)	7 (6.5)	6 (6.5)	0.8
Number of pack years for smokers, mean (±SD)	16.7 (±15.5)	21.5 (±14.3)	0.58
Former smoker, n (%)	15 (20)	13 (18)	0.5
Number of pack years for former smokers, mean (±SD)	8.2 (±8.7)	12.6 (±9.0)	0.3
High alcohol consumption (>14 units per week), n (%)	2 (2.7)	4 (5.4)	0.6
Regular exercise, n (%)	38 (51)	59 (80)	0.002

CCHD = cyanotic congenital heart disease, n = number, SD = standard deviation, CAD = coronary artery disease, CVD = cardiovascular disease.

3.1. Clinical manifestations of atherosclerosis

None of the patients or controls reported any angina pectoris. Five patients and one control received lipid-lowering agents. Three patients with CCHD (4%) reported previous transient ischemic attacks (TIA) and 15 (19%) reported previous strokes. However, two of the patients experienced their cerebrovascular event (CVE) in association with cardiac procedures - defibrillation or cardiac catheterization - and other two were <25 years of age at the time of the CVE. No patients had any history of MI, and none of the controls had any history of either CVE or MI.

3.2. Subclinical atherosclerosis

3.2.1. Coronary artery calcification

Sixty-seven patients (median age: 50.0 years, range 23–78) and 74 controls (median age: 50.0 years, range 24–78 years) had a cardiac CT-scan performed. The prevalence of subclinical atherosclerosis in the coronary arteries (CAC-score > 0) was 21% (14/67) in the patients with CCHD and 19% (14/74) in the controls ($p = 0.8$) (Fig. 1).

3.2.2. Carotid B-mode ultrasound, cPT-max, and CIMT

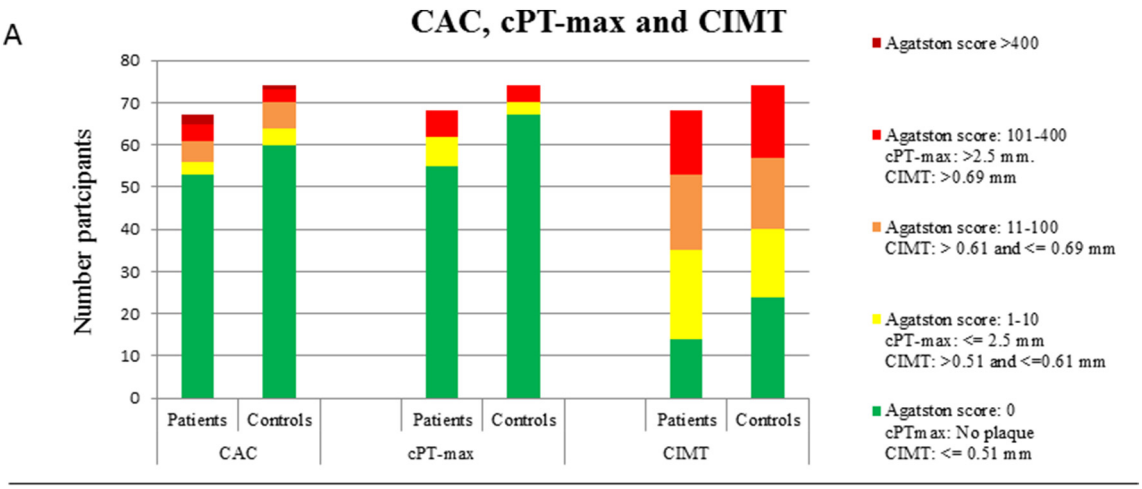
Sixty-eight patients (56% women, median age: 50.0 years, range 23–78 years) and 74 controls (57% women, median age 50.0 years, range 24–78 years) had B-mode carotid ultrasound scanning performed. There was no difference in CIMT between the two groups (0.61 ± 0.12 vs. 0.61 ± 0.13, $p = 0.98$). Plaque in the carotid arteries was found in 19% (13/68) of the patients and in 9% (7/74) of the controls ($p = 0.1$). The median cPT-max was 2.3 mm [1.3–2.9 mm] in the patients and 2.8 mm [1.8–6.7 mm] in the controls ($p = 0.1$) (Fig. 1).

In a model were subclinical atherosclerosis was defined by a CAC-score > 0 in the coronary arteries, and/or the presence of plaque in the carotid arteries (coronary-carotid-plaque), there was no difference between the two groups (31% in patients vs. 25% in controls, $p = 0.4$) (Fig. 1).

Paired comparison confirmed above listed results with no significant difference in the proportion of patients with CCHD compared to controls in CAC > 0, plaque in the carotid arteries, coronary-carotid-plaque or in CIMT.

The effect of the matching parameters - sex, age, smoking, BMI, total-cholesterol, oxygen saturation, bilirubin, and platelets - on the

A



B

Parameters of subclinical atherosclerosis	Patients with CCHD	Controls	*p*-value
CAC-score >0, % (n)	21 (14/67)	19 (14/74)	0.8
CIMT(mm), mean (±SD)	0.61 (0.12)	0.61 (0.13)	0.98
Plaque in carotid arteries, % (n)	19 (13/68)	9 (7/74)	0.1
cPT-max, median [p25-p75]	2.3 [1.3-2.9]	2.8 [1.8-6.7]	0.1
Plaque in the coronary or the carotid arteries, % (n)	31 (21/67)	25 (18/74)	0.4

Fig. 1. Measurements of subclinical atherosclerosis. A) Illustrates the differences in; -CAC-score, -maximum carotid plaque thickness (cPT-max) and carotid intima media thickness (CIMT) between patients with cyanotic congenital heart disease (CCHD) and matched controls. - CAC-score divided in subgroups according to Agatston score; 1) 0 2) 1–10 3) 11–100 4) 101–400 5) >400. - cPT - max divided according to; 1) No plaque; 2) Plaque thickness ≤ 2.5 mm; 3) Plaque thickness > 2.5 mm. - CIMT divided in quartiles; 1) ≤0.51; 2) >0.51 and ≤0.61; 3) >0.61 and ≤ 0.69; 4) >0.69 B) Listed differences in CAC > 0, CIMT, cPT-max, plaque in the carotid arteries or in either the carotid or coronary arteries between the patients with CCHD and the controls.

presence of coronary-carotid-plaque was examined in a logistic regression adjusted for the above-mentioned variables, and only age ($p < 0.0001$) was associated with coronary-carotid-plaque, data not shown.

Seven (33%) of the 21 patients with coronary-carotid-plaque had a history of previous CVE not related to a cardiac procedure and were aged above 25 years.

3.3. Blood analysis

The patients with CCHD had higher level of haemoglobin and haematocrit, and lower value of platelets than the controls (Table 2). The patients had higher leukocytes, neutrophils, CRP, and Hb1AC levels than the controls, although these differences were not considered clinically significant.

3.4. Lipoprotein characterization

3.4.1. Standard lipoprotein measurements

Standard plasma measurements did not reveal any differences between patients and controls regarding total-cholesterol or LDL-cholesterol. However, the patients had lower HDL-cholesterol and higher triglyceride levels compared to the controls (Table 2). The paired sensitivity analysis confirmed the results.

3.4.2. Ultracentrifugation of lipoproteins

Measurements of lipoproteins by ultracentrifugation on 42 of the patients (57% women, median age: 50.0 years, range: 25–78 years) and 42 matched controls (57% women, median age: 52.0 years, range: 26–76 years) revealed no difference in plasma total-cholesterol or plasma LDL-cholesterol. In contrast to the standard plasma measurements, ultracentrifugation did not show any significant difference in plasma HDL-cholesterol, though a small tendency to a low level of HDL-cholesterol in the patients group as in the standard measurements above mentioned (Table 2). The paired sensitivity analysis confirmed the results.

3.4.3. FPLC lipoprotein analysis

The cholesterol and triglyceride elution profiles representing the FPLC separation of lipoproteins were evaluated in 42 of the patients (57% women, median age: 50.0 years, range 25–78 years) and 42 matched controls (57% women, median age: 52.0 years, range 26–76 years). Each group was subdivided into two groups according to the median total-cholesterol (patients: 4.7 mmol/L; controls: 5.2 mmol/L) by ultracentrifugation measurements. There was a tendency toward less LDL and HDL in patients with high total cholesterol, but no difference in type or size of cholesterol particles in the patients and controls (Fig. 2). The patients presented with a higher ratio of LDL/HDL-cholesterol compared to the controls (In the group of high total-cholesterol: 2.0 vs.1.9; and in the group with low total-cholesterol: 2.0 vs. 1.67) (Fig. 2a+b).

The FPLC analysis of the triglycerides suggests that patients with high total-cholesterol have more VLDL and chylomicrons than the corresponding control group, and patients with low total-cholesterol have less VLDL and chylomicrons than matching controls (Fig. 2c+d).

4. Discussion

We found that in adult patients with CCHD, the lipoprotein profile, cardiovascular risk score, and prevalence of carotid and coronary subclinical atherosclerosis were similar to a matched group of controls.

Previous studies suggested that patients with CCHD have a lower atherosclerotic burden than the general population. Those studies assessed the prevalence of atherosclerosis using coronary angiography,

Table 2
Blood samples.

	Reference intervals	Patients with CCHD	Controls	*p*-Value
Haemoglobin (mmol/L), mean (±SD)	7.3–10.5	12 (±1.5)	8.72 (±0.7)	<0.0001
Haematocrit,(%) mean (±SD)	0.35–0.50	0.56 (±0.08)	0.41 (±0.03)	<0.0001
Thrombocyte, (×10E9/L), mean (±SD)	145–390	165 (±55)	241 (±48)	<0.0001
Leukocytes, (×10E9/L), mean (±SD)	3.5–8.8	6.71(±1.80)	5.9 (±1.9)	0.01
Neutrophils, (×10E9/L), mean (±SD)	1.6–5.9	4.3 (±1.5)	3.5 (±1.5)	0.001
CRP, (mg/L), median [p25–p75]	<10	3 [1.0–5.0]	1.0 [1.0–2.0]	0.002
Creatinine, (μmol/L), mean (±SD)	50–105	80.4 (±24.7)	77.6 (±15.7)	0.4
ALAT, (U/L), mean (±SD)	36–45	23.8 (±10.0)	22.7 (±9.2)	0.5
ASAT, (U/L), mean (±SD)	15–35	26.4 (±6.50)	25.8 (±7.7)	0.7
Bilirubin, (μmol/L), median [p25–p75]	5–25	12 [9.0–17.0]	8.0 [6.0–9.0]	<0.0001
Pro BNP, (pmol/L), median [p25–p75]		41.6 [18–120.5]	4.6 [3.0–11.3]	<0.0001
Hb1Ac, (mmol/mol), mean (±SD)	<48.0	37.8 (±3.6)	34.0 (±3.5)	<0.0001
Lipoproteins				
Plasma total-cholesterol, (mmol/L), mean (±SD)	<5.0	4.91 (±1.12)	5.18 (±0.96)	0.1
Plasma LDL-cholesterol, (mmol/L), mean (±SD)	<3.0	2.95 (±0.99)	3.09 (±0.78)	0.3
Plasma HDL-cholesterol, (mmol/L), mean (±SD)	>1.0	1.42 (±0.47)	1.78 (±0.46)	<0.0001
Plasma triglyceride, (mmol/L), median [p25–p75]	<2.0	1.38 [0.88–1.93]	0.96 [0.7–1.53]	0.002
Ultracentrifugation		Patients with CCHD (n = 42)	Controls (n = 42)	
Plasma total-cholesterol, (mmol/L), mean (±SD)	<5.0	4.76 (±0.97)	5.14 (±0.95)	0.08
Plasma LDL-cholesterol, (mmol/L), mean (±SD)	<3.0	2.59 (±0.64)	2.85 (±0.84)	0.1
Plasma HDL-cholesterol, (mmol/L), mean (±SD)	>1.0	1.69 (±0.66)	1.86 (±0.55)	0.2
Plasma VLDL-cholesterol, (mmol/L), mean (±SD)	<0.9	0.49 (±0.34)	0.43(±0.30)	0.4

CCHD = cyanotic congenital heart disease, SD = standard deviation, CRP = C-reactive protein, ALAT = alanine-aminotransferase, ASAT = aspartate-aminotransferase Pro-BNP = pro brain natriuretic peptide, Hb1Ac = haemoglobin A1C, LDL = low density lipoprotein, HDL = High density lipoprotein, VLDL = Very low-density lipoprotein,

CIMT measurements, lipid-profiles, and endothelial function in small cohorts of patients with mean ages of 11–44 years [3–7]. However, this study is the first to provide a comprehensive investigation of the two major sites of atherosclerosis in a larger group of patients with CCHD.

Previous studies have shown absence of coronary artery disease based on the results of coronary angiograms; however, coronary arteries in patients with CCHD are tortuous and dilated, thus complicating the detection of plaques on coronary angiograms [3,6,7]. Moreover, visual assessments of coronary angiograms cannot truly quantify the atherosclerotic burden.

CAC-score determined by CT, and CIMT and cPT-max assessed by ultrasound, are non-invasive tests known to be able to detect subclinical atherosclerosis and they correlate with future cardiovascular outcomes [21–23]. We found no significant difference in the prevalence of calcifications (CAC-score > 0) in the coronary arteries between the two groups. These findings were supported by a similar prevalence of carotid plaques, cPT-max, and CIMT on carotid ultrasound assessments.

Previous studies examining the prevalence of subclinical atherosclerosis in healthy subjects found a higher proportion of subjects with CAC-score > 0 (44–68% of the participants) than identified in this study. Moreover, the mean age in those studies was greater than in this study (50–68 years vs. 48 years (median 49.5)) [18,24,25]. Since atherosclerosis is a progressive disease and only a subset of the participants in our study were >60 years of age, progressions in CAC-score, cPT-max, and CIMT in patients and controls would be expected with increasing age.

The patients reported a relatively high number of previous CVEs, which might raise the suspicion of a higher prevalence of atherosclerotic disease at baseline. However, in structural heart disease the pathogenesis is more likely small vessel ischemia due to chronic hypoxia, erythrocytosis, or embolism from the heart, rather than true, in-situ atherosclerosis from carotid plaque thrombi or emboli [26,27]. Though, one-third of the patients with subclinical atherosclerosis in either the coronary or carotid arteries actually had a history of previous CVE although these could have been paradoxical thromboembolic events due to the right-to-left shunt.

It has been suggested that the low incidence of atherosclerosis in patients with CCHD is due to hypoxemia, upregulated nitric oxide, hyperbilirubinaemia, and thrombocytopaenia, which are all believed to be anti-atherogenic factors [3,4,6,7]. However, in a logistic regression analysis adjusted for total-cholesterol, oxygen saturation, bilirubin, and platelet levels, we did not find any correlation with coronary-carotid-plaque, which calls the above hypothesis into question.

4.1. Risk factors

It has been reported that patients with CCHD have lower lipoprotein levels than the general population, which might be correlated with hypoxaemia and secondary erythrocytosis [4,5,7,28]. There was no difference in plasma total-cholesterol and plasma LDL-cholesterol between the patients and the controls upon measurement of lipoproteins using standard techniques and ultracentrifugation. The standard measurements showed a significantly decreased plasma HDL-cholesterol in the patients compared to the controls, which is in accordance with results of previous studies [4,7]. This was further supported by ultracentrifugation and FPLC which showed a tendency toward reduced plasma HDL-cholesterol. The patients had higher triglyceride levels, which had not previously been described in patients with CCHD [6].

Our FPLC analysis did not support a previous, much smaller study suggesting that patients with CCHD have qualitative changes in lipoprotein distribution [7]. The patients with CCHD seemed to have a similar lipoprotein distribution and size as the general population, although an increased ratio of LDL/HDL-cholesterol was found. Thus, despite the lack of significant differences in total-cholesterol and LDL-cholesterol, the patients may have a more atherosclerotic-like lipid-profile with an increased ratio of LDL/HDL-cholesterol.

A previous study has suggested a need to correct for haematocrit when comparing lipid-profiles in cohorts with expected differences in haematocrit values [29]. It could be speculated that high haematocrit values in patients with CCHD would result in a high plasma lipid-profiles due to a reduced plasma volume. In contrast, the lipid-profile in whole blood may be significantly lower [29]. However, to our knowledge, no previous studies of patients with CCHD have measured whole blood lipoprotein concentrations after correcting for haematocrit values

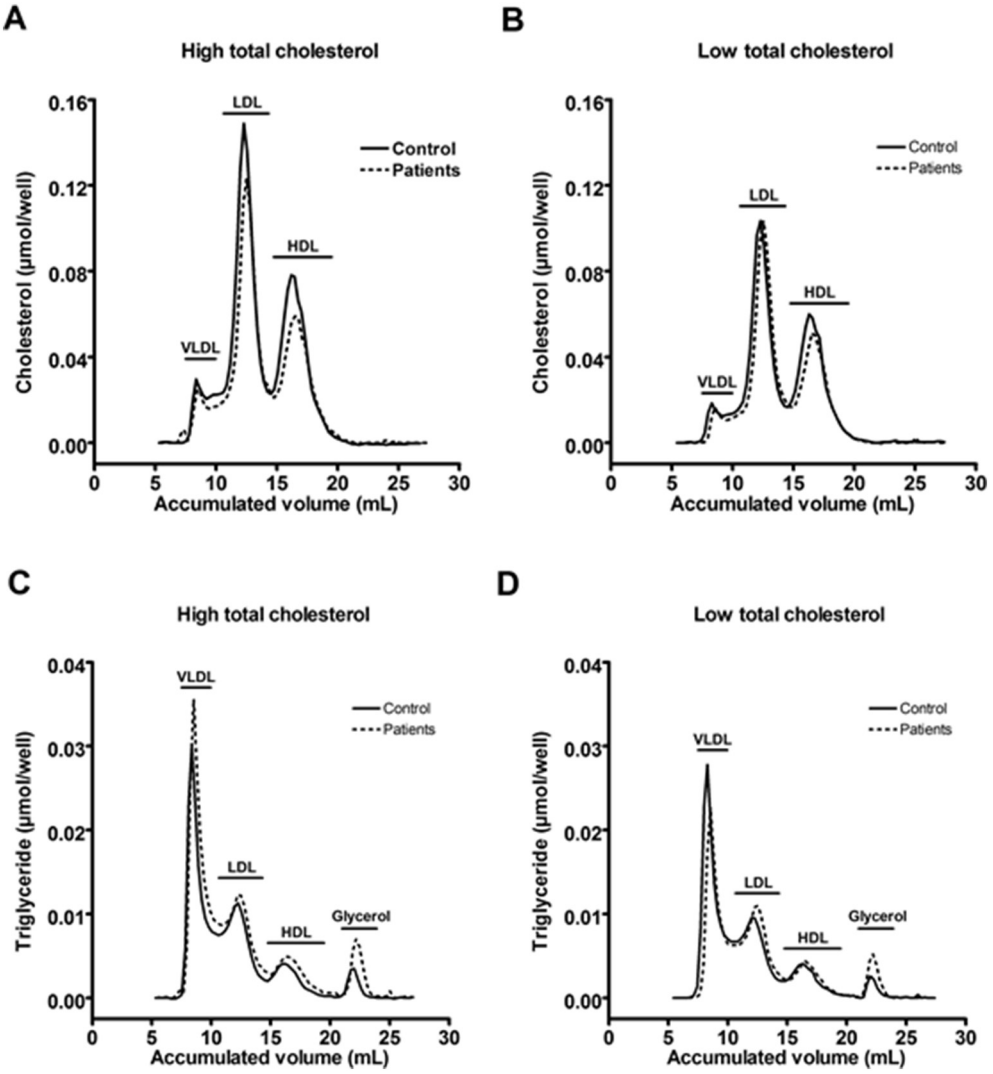


Fig. 2. Fast protein liquid chromatography (FPLC) analysis of 42 patients with CCHD and 42 matched controls subdivided according to the median of total-cholesterol measured by ultracentrifugation.

[4,5,7]. Further, previous studies and guidelines regarding lipoproteins and risk of CVD have not described the need for correction of haematocrit. Therefore, it is uncertain how important the whole blood lipoprotein concentration is in relation to the pathogenesis of atherosclerosis [30].

Inflammatory cells play a critical role in the pathogenesis of atherosclerosis and high levels can predict an increased risk of coronary heart disease [31]. Therefore, due to the higher level of inflammatory markers, the patients with CCHD may have an increased risk of atherosclerotic disease.

Atherosclerosis is a multifactorial disease, and the risk factors can be modulated through a healthy lifestyle. Patients with CCHD have severely impaired exercise capacity as reflected in the seven-day pedometer assessment [32]. Although there were no differences in waist circumferences or BMI, a significantly higher proportion of controls participated in regular sports compared to the patients. A previous study showed that patients with CCHD had an increased risk of developing diabetes mellitus type 2, which is in accordance with the observation of a slightly higher Hb1AC in our patients with CCHD [33].

In summary, our results did not show a decreased prevalence of subclinical atherosclerosis in CCHD patients but, may actually indicate a tendency toward patients with CCHD being at increased risk of atherosclerosis. Although we did not find significant differences in CAC-score > 0, cPT-max, or CIMT, there were twice as many patients with plaques in the carotid arteries and a minor tendency toward higher Agatston scores in the patient group compared to the controls. We found that the lipid-profiles showed higher LDL/HDL ratios and a tendency toward lower HDL-cholesterol levels in the CCHD groups. When taken together with an increased prevalence of CVE and an elevated inflammatory state, the patients with CCHD might have an increased risk of developing clinical atherosclerosis later in life.

4.2. Limitations

Due to the participants' ages, the a priori risk of atherosclerosis was low, making it more difficult to detect differences between the groups. The controls were recruited by advertisement, which often attracts more healthy subjects, and thus they may not represent the general population.

The patients and controls had different haematocrit values, possibly leading to bias in the measurements of the lipoproteins. An ideal background population for lipid measurements would have been one living at a high altitude with erythrocytosis. For logistical reasons, not all patients with CCHD had all assessments performed.

We did not report the patients NYHA classification or measure the six minutes walking distance which may have been useful in regard to the seven day pedometer assessment.

5. Conclusion

We did not find a decreased atherosclerotic burden or beneficial plasma lipoprotein-profiles in patients with CCHD compared to healthy controls. The patients with CCHD may actually have an increased risk of atherosclerosis due to higher LDL/HDL-ratios in combination with elevated levels of inflammatory markers.

Our results indicate that these patients should apply the same precautions and life-style modifications as recommended to the general population to avoid atherosclerotic diseases. As patients with CCHD live longer, healthcare in the Grown Up with Congenital Heart Disease outpatient clinics should be aware that their medical issues may further be complicated by atherosclerotic disease.

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Conflicts of interest

Dr. Sørgaard has received a PhD grant from the research foundation at Rigshospitalet and has received lecturing fees from Toshiba Medical Corporation, Japan.

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