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Atherosclerosis in Patients With Cyanotic Congenital Heart Disease

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Background: Cyanotic patients with congenital heart disease (CHD) might be protected against atherosclerosis.

Methods and Results: Atherosclerotic risk factors and carotid intima–media thickness (IMT) were investigated in adults with cyanotic CHD and in unaffected age- and sex-matched controls. Fifty-four cyanotic patients (30 men, mean age 38, range 19–60 years) and 54 controls were included. Mean transcutaneous saturation of the cyanotic patients was 81±6%. Mean carotid IMT adjusted for age was significantly decreased in cyanotic patients compared to controls (0.55±0.1 mm vs 0.58±0.08 mm; ∆IMT=0.04 mm [SE 0.015], P=0.01). In cyanotic patients lower total cholesterol levels were observed (4.4±1 mmol/L vs 4.9±1 mmol/L; P=0.02), as well as lower thrombocyte levels (173±81×10⁹/L vs 255±54×10⁹/L; P<0.01), higher bilirubin levels (18.6±11 μmol/L vs 12.7±6 μmol/L; P<0.01), and lower diastolic and systolic blood pressure (71±9 mmHg vs 76±9 mmHg, P<0.01; 113±14 mmHg vs 124±12 mmHg, P<0.01, respectively).

Conclusions: In patients with cyanotic CHD carotid IMT, and hence atherosclerosis disease risk, was decreased. This might be due to a combination of reduced atherosclerotic risk factors such as lower blood pressure, lower total cholesterol levels, higher bilirubin levels and lower thrombocyte levels. (Circ J 2010; 74: 1436–1441)

Key Words: Atherosclerosis; Carotid intima–media thickness; Cyanotic congenital heart disease; Eisenmenger syndrome
between adult patients with cyanotic CHD and unaffected age- and sex-matched controls.

### Methods

**Patients**

For the present study, patients were included from the following 6 Dutch hospitals: Academic Medical Center Amsterdam, University Medical Center Groningen, Radboud University Nijmegen Medical Center, Leiden University Medical Center, University Medical Center Utrecht, and St Antonius Hospital Nieuwegein, and one Italian hospital: University of Padua. Patients with a transcutaneous oxygen saturation <90% at rest were defined as cyanotic. Cyanotic CHD patients from the Netherlands were identified using the CONCOR database, a national registry and DNA bank of adult patients with CHD in the Netherlands.14 Italian cyanotic patients were identified at the outpatient clinic. Patients were requested to participate by their cardiologist. Patients with one of the following defects were asked to participate: Eisenmenger syndrome, univentricular heart, pulmonary atresia with ventricular septal defect (VSD), pulmonary atresia without VSD, or double outlet right ventricle. Age- and sex-matched controls were recruited among relatives and acquaintances by the participating patients. Patients who had previously undergone a Fontan procedure and patients with a liver or kidney disease, myeloproliferative disease, current or recent malignancy and patients using lipid-lowering drugs were excluded from the study.

**Study Protocol**

This was an observational, case–control study. Data were collected during a single visit to the outpatient clinic between March 2007 and May 2008. Data on coronary risk factors such as body mass index, diastolic and systolic blood pressure, current smoking status, positive family history such as parent or sibling with a cardiovascular event aged <55 years, medical history and use of medication such as thiazide diuretics, β-blockers, and lipid lowering drugs were collected both in cyanotic patients and in controls. Hypertension was defined as diastolic blood pressure >90 mmHg, systolic blood pressure >140 mmHg, or the use of anti-hypertensive agents. Furthermore, a fasting venous blood sample was collected.

Hematologic measurements included hemoglobin concentration, hematocrit, thrombocyte, and erythrocyte count. Hematocrit was based on automated electronic particle counts. Serum tests included glucose, uric acid, total bilirubin, folate acid and measurement of lipids. Transcutaneous saturation was measured in both patients and controls after 5 min of rest using a standard transcutaneous pulse oximeter at the finger.

Atherosclerotic changes were assessed in both cyanotic patients and controls by measuring carotid IMT using B-mode ultrasound. IMT images of the arterial far wall segments of the right and left common carotid artery, carotid bulb and, internal carotid artery were acquired according to a standardized protocol.11 Carotid ultrasound scans of the patients from the Academic Medical Center Amsterdam, Leiden University Medical Center, University Medical Center Utrecht, Radboud University Nijmegen Medical Center and the St Antonius Hospital Nieuwegein, The Netherlands, were performed in the academic Medical Center Amsterdam by a single well-trained and experienced sonographer. IMT measurements of subjects of the University Medical Center Groningen (n=13) and the University of Padua (n=6) were performed in the respective centers. In all centers, including the Italian site, scan protocols were standardized. The centers in the Netherlands used Acuson Aspen or Sequoia equipment, using L7 transducers and the magnification settings on both machine types (Siemens, Erlangen, Germany). In the Padua center images were measured online using an Acuson Sequoia, C512, echocardiography system, 4V1C transthoracic Sector Array Transducer and 6L3 IMT Sector Array Transducer (Siemens). Dynamic and high resolution still images were saved as DICOM files. Measurements were done on the 2×2-cm still frames; the clips were used as a dynamic reference to identify the lumen—intima and media—adventitia interfaces of the arterial far walls. All images were analyzed by the same image analyst blinded to clinical information. IMT was defined as the average of the IMTs of the right and left common carotid artery, carotid bulb and internal carotid artery segments. The institutional review committee approved the protocol and written informed consent was obtained from all participants prior to participation in the study. For patients with Down syndrome, parental consent was obtained.
Statistical Analysis
The descriptive data are presented as mean±SD if normally distributed or as median (range) as appropriate. Comparisons of continuous variables between groups were made using unpaired Student t-tests. In the case of a skewed distribution, the Mann−Whitney U-test was used. Correlation coefficients were used to assess the relationship between IMT and age and total cholesterol. Multivariate analysis of mean IMT, total cholesterol levels and HDL-cholesterol levels was used to assess contributing parameters. P<0.05 was considered to be significant.

Results
Patients
Between March 2007 and May 2008, 54 cyanotic patients (30 men and 24 women, mean age 38 years, range 19–60 years) and 54 age- and sex-matched controls were included in the study. Mean transcutaneous saturation of the cyanotic patients was 81±6%. Eisenmenger syndrome was present in 89% of the cyanotic patients (n=48), with VSD being the most frequent underlying diagnosis. Of the cyanotic patients 35% (n=19) had Down syndrome, of whom 68% (n=13) had hypothyroidism. All patients with hypothyroidism were adequately treated. Cyanotic patients had significantly lower diastolic and systolic blood pressure compared to controls (71±9 mmHg vs 76±9 mmHg, P=0.02; 113±14 mmHg vs 124±12 mmHg, P<0.01, respectively). Table 1 lists the population characteristics. One cyanotic patient had a previous cerebrovascular accident and 6 cyanotic patients had a previous thrombotic event.

IMT
The results of the B-mode ultrasound IMT measurements showed IMT increase with advancing age (r=0.4, P<0.01). Moreover, mean carotid IMT, adjusted for age using linear regression analysis, was significantly decreased in cyanotic patients compared to controls (0.55±0.1 mm vs 0.58±0.08 mm), with a mean difference of 0.04 mm (SE 0.015 mm, P=0.01), as shown in Figure 1. Sub-segmental analysis, after adjustment for age, showed these differences to be most explicit in the common carotid artery (P=0.02) and carotid bulb (P=0.004). There was no age × gender interaction in IMT (P=0.9). On multivariate linear regression analysis the influence of the following parameters was not statistically significant: Down syndrome (β=0.070, P=0.6); hypothyroidism (β=0.215, P=0.1); current smoking (β=−0.049, P=0.6); use of thiazide diuretics (β=−0.005, P=1); use of β-blockers (β=0.001, P=1); and uric acid levels (β=−0.052, P=0.6). In addition, after adjustment for diastolic and systolic blood pressure, mean IMT was comparable in both groups with a mean difference of 0.026 mm (SE 0.017 mm, P=0.1). Moreover, a positive association was found between IMT and diastolic and systolic blood pressure (r=0.3, P<0.01 and r=0.3, P<0.01, respectively). No correlations were observed between the decreased blood pressure and the use of β-blockers or diuretics.

Blood Analysis
To clarify the cause of the difference in IMT between cyanotic patients and controls, we analyzed risk factors for atherosclerosis through blood analysis (Table 2). Total cholesterol levels were significantly lower in cyanotic patients compared to controls (Figure 2), and reduced total cholesterol levels (<3.9 mmol/L) were more often seen in these patients (37% vs 17%, P=0.02). Total cholesterol was posi-
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tively associated with mean IMT (r=0.4, P<0.01) and with oxygen saturation (r=0.2, P=0.04). On multivariate analysis the presence of Down syndrome, hypothyroidism, smoking or the use of thiazide diuretics or β-blockers had no contributing effect on differences in total cholesterol between cyanotic patients and controls. Table 2 shows the equal distribution of LDL-cholesterol in both groups. Strikingly, HDL-cholesterol was significantly lower in cyanotic patients. Multivariate analysis indicated that 32% of the variation in HDL was due to the cyanotic state and the use of β-blockers (r=0.6, P<0.001). In addition, there were no gender differences in total, LDL- and HDL-cholesterol.

Additional blood analyses showed, as expected, significantly increased hemoglobin, hematocrit and erythrocyte levels in cyanotic patients, as well as bilirubin and uric acid levels (Table 2). No correlation was found between either hemoglobin, hematocrit or erythrocyte levels and total cholesterol. Total cholesterol was neither related with bilirubin nor with uric acid levels. Furthermore, 46% of the cyanotic patients (n=25) had thrombocytopenia, defined as platelet counts <150×10^9/L. Thrombocyte levels were associated with the severity of cyanosis (r=0.5, P<0.01).

### Discussion

This is the first study showing a reduced IMT in cyanotic CHD patients by means of non-invasive techniques. The reduction in IMT might be due to a combination of decreased atherosclerotic risk factors in cyanotic patients.

The present results are in accordance with the findings of Perloff, who found minimal or absent signs of atherosclerosis on coronary angiography in 25 cyanotic women, mean age 43±4 years and 24 cyanotic men, mean age 41±6 years.1 We confirmed these findings using non-invasive and quantitative carotid IMT measurements.

It is known that reduced atherosclerotic and thrombotic factors, for example hypcholesterolemia, hyperbilirubinemia, elevated nitric oxide levels and thrombocytopenia are associated with cyanosis.12,15 To further elucidate the mechanism underlying the cause of the reduced mean IMT in cyanotic patients, we analyzed these risk factors. Elaborating on the contribution of nitric oxide to IMT was beyond the scope of the present study.

Total cholesterol levels were significantly reduced in the present patients, and correlated positively with decreased oxygen saturation. Decreased total cholesterol is related to low IMT, as has been demonstrated in the general population.16-18 Hyperbilirubinemia is frequently seen in cyanotic patients due to secondary erythrocytosis, as confirmed in the present study.19 Serum bilirubin is considered anti-atherosclerotic, because it is an endogenous antioxidant that inhibits LDL oxidation.20 We were unable, however, to demonstrate a reduction in serum LDL levels because the inhibitive properties of hyperbilirubinemia, which promotes LDL oxidation, were counteracted by elevated uric acid levels.21 Unexpectedly, we found low—normal HDL levels, cut-off 1.1 mmol/L, in the cyanotic population compared to the control group. The cyanotic state and the use of β-blockers were found to be independent predictors for reduced HDL. Beta-blocker usage

### Table 2. Blood Analysis

<table>
<thead>
<tr>
<th></th>
<th>Cyanotic CHD patients (n=54)</th>
<th>Controls (n=54)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum lipids (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>4.4±1.2</td>
<td>4.9±1.0</td>
<td>0.02</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>2.8±1</td>
<td>2.9±0.9</td>
<td>0.5</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.2±0.3</td>
<td>1.6±0.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1±0.5</td>
<td>0.9±0.4</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Bilirubin total (μmol/L)</strong></td>
<td>18.6±11</td>
<td>12.7±6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Uric acid (mmol/L)</td>
<td>0.5±0.7</td>
<td>0.3±0.08</td>
<td>0.03</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.6±0.9</td>
<td>4.8±0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Folate acid (mmol/L)</td>
<td>21.5±13</td>
<td>24.8±6</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Hematology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (mmol/L)</td>
<td>11.9±2</td>
<td>8.9±0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hematocrit (L/L)</td>
<td>0.59±0.09</td>
<td>0.42±0.04</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Erythrocytes (10^12/L)</td>
<td>6.7±1.2</td>
<td>4.7±0.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Thrombocytes (10^9/L)</td>
<td>173±81</td>
<td>255±54</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

CHD, congenital heart disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

![Figure 2. Total fasting cholesterol of cyanotic patients and controls. Total cholesterol was significantly reduced in cyanotic patients compared to controls: 4.4±1 mmol/L vs 4.9±1 mmol/L, respectively (P=0.02).](image-url)
has previously been shown to negatively influence HDL-cholesterol levels. Only 13% of the cyanotic CHD patients, however, used a β-blocker, which could not completely explain the decreased HDL levels. Fyfe et al also found unexplained reduced HDL levels in their cyanotic patient group, whereas HDL levels were increased in the hypoxemic erythrocytotic of high altitudes. A possible hypothesis is the existence of hypoalphalipoproteinemia, because apo A-I plays an important role in HDL function. Furthermore, a number of rare genetic disorders are associated with low HDL-cholesterol levels. The genes that account for the variation in serum HDL-cholesterol in the general population, however, have not been identified as yet. In addition, it was beyond the scope of the present study to clarify the unexpected reduction of HDL levels in cyanotic CHD patients.

Blood pressure was significantly reduced in the present patients and an association was found between diastolic and systolic blood pressure and IMT. On multivariate analysis the reduced blood pressure could not explain the difference in IMT between patients and healthy controls.

Although not a risk factor for atherosclerosis, thrombocytopenia is frequently seen in cyanotic patients. We found low thrombocyte levels, which were negatively associated with the severity of pulmonary-to-systemic shunting and therefore with the level of cyanosis. Moreover, Kajimoto et al recently found elevated platelet activation in cyanotic patients, especially in patients after the Fontan procedure. These findings could well be applicable to the present patient population, and could partly explain the appearance of thromboembolic events in these patients. Contrary to the increased bleeding tendency caused by this thrombocytopenia, hyperviscosity, which is a frequent complication in cyanotic patients, increases thrombotic risk. We confirmed this increased hyperviscosity by demonstrating significantly elevated hemoglobin, hematocrit and erythrocyte levels in cyanotic patients. The literature is ambiguous, however, on the thrombotic risk of hyperviscosity, indicating the necessity of further investigation.

A limitation of the present study was the small difference in IMT between two small and relatively young groups. The IMT was within the normal range and both groups would be expected to be at low risk for cardiovascular event. Additionally, long-term outcome is unknown for the present patient group and regularly follow up is necessary to determine whether a reduced IMT in cyanotic patients is associated with a decreased risk for cardiovascular events. Moreover, a separate comparison of IMT and anti-atherosclerotic effects between patients with the Eisenmenger syndrome and cyanotic CHD patients without PH seemed inappropriate, because subgroups were small and heterogeneous. Another limitation was the lack of angiographic information of the coronary tree at the time of carotid ultrasound.

Conclusion

The present study showed lower carotid IMT in cyanotic CHD patients compared to unaffected controls, as measured on non-invasive ultrasound. The reduction in IMT in cyanotic patients might be due to a combination of reduced atherosclerotic risk factors such as lower blood pressure, higher bilirubin levels and lower thrombocyte levels. Therefore, in contrast to acyanotic CHD patients, it might be unlikely that atherosclerosis will pose an additional health problem to cyanotic CHD patients when they grow older and reach the age at which atherosclerosis becomes clinically relevant.

References

and ventricular function in young adults with right ventricular dysfunc-