Carotid Intima-Media Thickness in Patients With a History of Kawasaki Disease

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Background: Kawasaki disease (KD) is an acute pediatric vasculitis with coronary artery aneurysms (CAA) as its main complication. Concerns have been raised regarding the possibility of a predisposition of KD to premature cardiovascular disease (CVD) risk later in life. Our aim was to assess carotid intima-media thickness (cIMT), as a surrogate marker of CVD risk, in patients with a history of KD compared with unaffected controls.

Methods and Results: B-mode ultrasound cIMT measurements were performed in 168 patients with a history of KD, and 82 controls; 7 patients were excluded because of incomplete cIMT assessments. Mean cIMT (±SD) was increased in patients with KD compared with controls (0.378±0.030 mm vs. 0.360±0.027 mm, respectively; P adjusted <0.0001). If the cIMTs of CAA-negative patients and controls were plotted against age, increased cIMT was only apparent at young age. In patients with CAA, increased cIMT was observed over the entire age range.

Conclusions: Our findings show that arterial wall thickening is more apparent in patients with a history of KD as compared with controls. In CAA-negative patients, cIMT is indistinguishable from controls at older age, whereas an increased cIMT is observed at any age in patients with CAA, suggesting a more general and severe effect of KD on the arterial wall.

Key Words: Cardiovascular disease risk; Carotid intima-media thickness; Coronary artery aneurysm; Kawasaki disease

Kawasaki disease (KD) is an acute systemic vasculitis that predominantly occurs in children less than 5 years of age. The disease is thought to be caused by an infectious agent in genetically predisposed children. Coronary artery aneurysms (CAA) develop in 15–25% of untreated patients and may lead to myocardial ischemia, infarction, and sudden death. Although treatment with high-dose intravenous immunoglobulins (IVIG) has reduced this risk to less than 10%, KD is the leading cause of acquired heart disease in developed countries. The disease is self-limiting and only rarely recurs, but there has been ongoing concern that patients both with and without coronary artery involvement may have a predisposition to endothelial damage and premature atherosclerotic disease in adulthood. Thickening of the coronary arterial wall has been shown in persisting and regressed dilatations, but also in always-normal coronary segments. Since the first case of KD was reported in 1967, patients who have recovered will now be middle-aged or younger, and therefore the follow-up of these patients has not been long enough to establish the natural history of the disease.

To determine if KD is a risk factor for the future development of cardiovascular disease (CVD), several studies have reported on the carotid intima-media thickness (cIMT) of patients with a history of KD. A thickened intima-media complex can be a result of atherosclerosis, but can also be caused by other processes involving injury and inflammation. As assessed by B-mode ultrasound, it is currently the best-validated non-invasive surrogate marker for cardiovascular risk available. In the past, an increased cIMT has been reported in former KD patients both with and without CAA, although these findings have not been confirmed by other studies.

We hypothesized that, because of their history of a systemic
vasculitis, patients with KD have an increased risk of CVD. Therefore, to determine the CVD risk in patients with a history of KD, B-mode ultrasound cIMT measurements (as a surrogate marker of CVD risk) in KD and unaffected control subjects were performed.

**Methods**

**Participants**
The study was conducted between January 2008 and September 2013 at the Emma Children’s Hospital, a tertiary referral center. Children aged 7–20 years with a history of KD were recruited consecutively during follow-up as outpatients. The diagnosis of KD was based on criteria from the American Heart Association. Patients diagnosed as having KD within 6 months of the study were excluded to minimize the potential confounding influence of (sub)acute inflammation. If multiple IMT measurements had been performed, the last measurement was included in the study.

Unaffected siblings of the children with KD and other unaffected subjects (family from the staff at the hospital) without a history of KD were eligible for the control cohort if they were in the same age range and did not take any cardiovascular medication. All subjects and/or their parents gave informed consent as approved by the institution’s Research Ethics Board.

**Study Protocol**
A medical (family) history was obtained from all participants, and body height, weight and blood pressure was measured. A non-invasive measurement of the cIMT was performed as described below. The mean arterial pressure (MAP) was calculated using the following formula: (systolic blood pressure+2 times diastolic blood pressure)/3. Using data of the fifth Dutch growth study performed in 2009 in 20,867 children in The Netherlands, standard deviation scores (SDS) for body mass index (BMI) were calculated based on the age and sex of each participant (http://groeiweb.pgdata.nl/calculator.asp).

The medical records of the patients with KD were reviewed retrospectively to collect the following clinical details: age at disease onset, time interval between disease onset and time of study, treatment with IVIG, aspirin and/or steroids, and the presence of CAA. The coronary arteries had been evaluated by 2D echocardiography. We defined CAA by worst-ever z-scores: CA dimensions as standard deviation units normalized for basal surface area. We choose to define the CAA by their worst-ever score because even when the lumen of a previously affected coronary artery has returned to its normal size, the artery can still be damaged and thus the initial systemic vasculitis was clearly more severe when compared with children with normal-sized arteries who had never had any enlargement at all. CAA was defined as a coronary z-score ≥2.5, a giant aneurysm was defined as a z-score ≥10 or a diameter ≥8 mm. In the patients with KD, a venous blood sample was taken after an overnight fast for measurement of total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, apolipoprotein A1, apolipoprotein B, lipoprotein(a) and, apolipoprotein E genotype. LDL-cholesterol was calculated using the Friedewald formula. All B-mode ultrasound scans were done according to a standardized protocol. For every subject the right and left common carotid arteries, carotid bulb and internal carotid arterial segments were visualized. One image of each segment was saved as a 2×2 cm high resolution DICON still. Image analysis was done off-line in a core lab, for which 20 images were analyzed twice to assess intra-rater reliability. The intraclass correlation coefficient was 0.92 (95% confidence interval [95% CI], 0.75–0.97) for the mean cIMT.

One image analyst performed all cIMT measurements blinded for the patient’s case status and risk factor levels. The per subject mean combined cIMT was calculated as follows: (mean of the left and right common carotid arteries+the mean of the left and right carotid bulb+the mean of the left and right internal carotid far wall segments)/3. For subjects in whom the scan of one of the segments had failed, the measurement of the same segment of the opposite carotid artery was taken as the mean of both carotid arteries. If both left- and right-side values were unavailable, the IMT was considered missing for that segment, and in that case the mean combined cIMT was also considered missing.

**Statistical Analysis**
We evaluated differences in demographics between patients with KD and controls by linear or logistic regression analysis. Differences in cIMT between patients with KD and controls were evaluated using linear regression analyses. We adjusted for the following potential confounders: age, sex, BMI SDS, MAP, family history. In addition, we performed stepwise backward elimination. Furthermore, in the group of KD patients we evaluated if IVIG treatment, IVIG resistance, total cholesterol, LDL-cholesterol and triglycerides were associated with cIMT by linear regression analysis.

An equation for difference in cIMT (ΔcIMT) was derived by subtracting the equation for patients with KD (if GROUP=1), that is, IMTKD=β1AGE+β2+β3AGE, from the equation for the unaffected controls (if GROUP=0), that is, IMTCO=β1AGE. This calculation resulted in ΔcIMT=β2+β3AGE. Betas and standard errors were derived from the output of a linear regression analysis for the whole group. Linear and logistic regression analyses were performed using the generalized estimating equation method in the SAS procedure GENMOD to account for correlations within families. The exchangeable correlation structure was used for these models. A P-value <0.05 was considered statistically significant. Statistical analyses were performed using SAS release version 9.2 (SAS Institute, Cary, NC, USA) and SPSS version 20.0 software (SPSS Inc, Chicago, IL, USA).

**Results**
In total, 168 former patients with KD and 82 controls subjects were enrolled. The control group consisted of 74 unaffected siblings and 8 family members recruited from among the hospital staff; 7 patients were excluded because of missing cIMT segments. Demographic characteristics of the remaining 161 patients with KD and the 82 controls were similar with respect to mean age, sex distribution, mean BMI SDS and MAP (Table 1). Clinical and laboratory data of patients with a history of KD are shown in Table 1. Median (interquartile range) onset of KD disease was 3.1 (1.2–5.3) years and 145 (90%) patients were treated with IVIG.

Based on their worst-ever coronary artery z-score, 119 (75%) had no CAA (z-score <2.5 during the (sub)acute phase)
The mean combined cIMT (±SD) was increased in patients with KD when compared with unaffected controls (0.378±0.030 mm vs. 0.360±0.027 mm; P<0.0001). After adjustment for age, sex, BMI, MAP, family history and family relationship, the difference remained statistically significant (P<0.0001).

and 42 patients had CAA (31 with a z-score of 2.5–10; and 11 with a z-score ≥10 or a diameter ≥8 mm). Of all patients, 145 (90%) had been treated with IVIG and of these, 27 (18.6%) were IVIG-resistant and had received another dose of IVIG.
For all subjects in the patient group, KD occurred around the age of 3 years. Because the age at cIMT measurement varied considerably (range: 7–20 years), we could explore the association between the difference in cIMT between KD patients and controls ($\Delta$IMT), and the time since KD onset. For this purpose, we plotted $\Delta$IMT against age at cIMT measurement (Figure A).

Based on their worst-ever z-score, plots were also created. This result did not change when BMI, MAP and family history were removed using stepwise backward elimination. Table 2 shows the mean cIMT, adjusted for age, sex and family relationship, for the controls and separate subgroups of patients with KD.

In the univariate analysis, IVIG treatment, IVIG resistance, total cholesterol, LDL-cholesterol and triglycerides were not significantly associated with mean cIMT.

Figure. Difference in mean combined carotid intima-media thickness (cIMT) and 95% confidence interval (CI) between patients with a history of Kawasaki disease (KD) and unaffected controls. The difference in mean combined cIMT and 95% CI between patients with a history of KD and unaffected subjects plotted against age, taking family relationship into account. (A) Difference between the whole group of patients and unaffected subjects ($n=243$), (B) Difference between the CAA-negative patients and unaffected subjects ($n=201$), (C) Difference between CAA-positive patients and unaffected subjects ($n=124$). Mean=thick line; 95% CI=dashed lines. CAA, coronary artery aneurysm.
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for subgroups without CAA (Figure B) and with CAA (Figure C). In CAA-negative patients, ∆IMT diminished with age at the time of cIMT measurement, and disappeared as age increased. In contrast, in patients with CAA a difference between patients and controls in cIMT remained present at all ages. When we excluded patients with giant aneurysms from the statistical analyses, these results on outcome remained unchanged.

Discussion

This study shows that children with a history of KD have an increased cIMT compared with unaffected controls. Plotting the difference in cIMT between patients and controls against age indicated that the observed difference in cIMT diminished with increasing age and disappeared in young adulthood in patients without CAA. In contrast, in children with CAA during acute KD (being either transient or persistent), the difference in cIMT was observed at all ages and remained significantly different from unaffected controls at all ages.

Studies evaluating the cIMT in patients with a history of KD have been performed mainly included small numbers of patients and have produced conflicting results. We recently published a systematic review and meta-analysis of CVD risk in patients with KD, including a total of 15 cIMT studies.18 Some of the studies reported no difference between KD patients and controls, whereas others found an increased cIMT in CAA-positive patients or also in CAA-negative patients.14 Quality assessment showed that all the studies had some significant methodological limitations. Mean cIMT in the whole KD group and the CAA-positive group did not differ significantly with controls on statistical meta-analysis, although there was a trend toward a thicker cIMT in patients (0.01 mm, 95% CI 0.00–0.02 and 0.01 mm, 95% CI 0.00–0.03 mm, respectively).

In contrast to those findings, the results of the present study showed that subjects with a history of KD had on average a significantly greater mean cIMT compared with unaffected controls. However, the difference in cIMT diminished when analyzed at different ages in the CAA-negative but not in CAA-positive patients. When analyzing the cIMT data in this way (ie, plotted against age at time of measurements), our findings may explain, at least in part, the conflicting data of others found an increased cIMT in CAA-positive patients or also in CAA-negative patients.14 Quality assessment showed that all the studies had some significant methodological limitations. Mean cIMT in the whole KD group and the CAA-positive group did not differ significantly with controls on statistical meta-analysis, although there was a trend toward a thicker cIMT in patients (0.01 mm, 95% CI 0.00–0.02 and 0.01 mm, 95% CI 0.00–0.03 mm, respectively).

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KD is a vasculitis that predominantly occurs in very young children.27 The median age at KD onset in our study cohort was 3.1 years. Therefore, the mean time interval from disease onset to participation in this study increases with age. Although it is unclear which pathophysiologic process results in the increased cIMT of CAA-positive patients, as well as the initially increased cIMT of CAA-negative patients, our findings suggested that this process represents a form of vasculopathy that may be different from premature atherosclerosis. If KD does result in premature atherosclerosis, ∆IMT would be expected to further increase with older age, whereas the cIMT of CAA-negative patients would become indistinguishable from controls in the older children instead.

The assumption that it may be a vasculopathy would be in line with an earlier study looking at the pathology of arteries in KD patients who died of myocardial infarction or heart failure 2–12 years after the onset of KD.28 A markedly thickened intima was found in the aneurysms that became stenotic and showed active remodeling of the arterial lesions many years after the disease. This process of remodeling was accompanied by the expression of multiple vascular growth factors, including platelet-derived growth factor and transforming growth factor (TGF)-β1, but no fatty streaks or accumulation of macrophages as is seen in (premature) atherosclerosis. Although we may not extrapolate these findings to other vascular structures, including the carotid artery, the finding that TGF-β is involved in arterial remodeling could explain the nonprogressive nature of the cIMT in CAA-negative patients up to 15 years after the disease. Such would not be in line with a process of premature atherosclerosis and supports the possibility of a different form of remodeling, provisionally designated as KD-vasculopathy.

In CAA-positive patients this pathway might continue to be active, resulting in a continuing increase of the cIMT. We have previously reported on the presence of vascular growth factors, TGF-β and their genetic association with KD.29,30 The precise nature of the vasculopathy that might result in a thickened cIMT in KD patients has not yet been determined.

The American Heart Association recommends life-long follow-up for children with and without CAA to assess CVD risk.29 Controversy exists on whether there is a need for this in children who never or only transiently experienced CAA. The uncertainty about a possible increased CVD risk based on a systemic vasculitis in the past feeds a big part of this discussion. Our study results indicate that life-long follow-up of children without CAA might not be necessary, although additional factors may be in play. For that reason, longitudinal studies have to be performed to define the real risk for CVD using the same methodology to decide about the follow-up of these children.

A major strength of the present study was the large study group and the consistent use of a standardized carotid imaging and image analysis protocol. Ultrasound measurements of all participants were obtained by 2 experienced sonographers, and 1 image analyst blinded for case or CAA status read all the images.

Some methodological aspects of our study merit discussion. First, we have only included 1 IMT measurement of each patient. The cIMT progression estimates were therefore based on cross-sectional data. Long-term follow-up studies are warranted to assess the ‘real’ course of cIMT change over time in the same KD patients. Second, patients were stratified based on their ‘worst-ever’ z-score. Because the study was performed in a tertiary referral center, pediatric cardiologists in centers other than ours generated many of the early echocardiograms. This might have caused misclassification of patients in the stratification for CAA subgroups. Third, although patients were included in a consecutive order at the outpatient clinic, the study population contains a high percentage of patients with CAA, as explained by referral bias with the more severe cases at our tertiary center. This may have overestimated the mean IMT of the total KD group.

Conclusions

Our study of cIMT in KD patients showed that the signs of early arterial wall changes were more apparent in patients with a history of KD, in particular in those with CAA. When plotting the difference in cIMT between patients and controls in
patients without CAA, cIMT became indistinguishable from controls with age. In CAA-positive patients an increased cIMT was observed at any age, the latter demonstrating a more severe effect of KD on the arterial wall. Although longitudinal data are missing, this result suggested that follow-up seems justified in CAA-positive patients, but may not be necessary in CAA-negative patients.

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