Cardiovascular status after Kawasaki disease in the UK

V Shah, G Christov, T Mukasa, K S Brogan, A Wade, D Eleftheriou, M Levin, RM Tulloch, B Almeida, MJ Dillon, J Marek, N Klein, PA Brogan

ABSTRACT

Objective Kawasaki disease (KD) is an acute vasculitis that causes coronary artery aneurysms (CAA) in young children. Previous studies have emphasised poor long-term outcomes for those with severe CAA. Little is known about the fate of those without CAA or patients with regressed CAA. We aimed to study long-term cardiovascular status after KD by examining the relationship between coronary artery (CA) status, endothelial injury, systemic inflammatory markers, cardiocvascular risk factors (CRF), pulse-wave velocity (PWV) and carotid intima media thickness (cIMT) after KD.

Methods Circulating endothelial cells (CECs), endothelial microparticles (EMPs), soluble cell-adhesion molecules (sICAM-1, sVCAM-1), inflammatory markers, cardiovasculature cell adhesion molecules (VCAM-1, ICAM-1), and markers of systemic inflammation were measured in patients with KD and healthy controls (HC). CA status of the patients with KD was classified as CAA present (CAA+), absent (CAA−), or regressed (CAA−) according to their worst-ever CA status. Data are median (range).

Results Ninety-two KD subjects were studied, aged 11.9 years (4.3–32.2), 8.3 years (1.0–30.7) from KD diagnosis. 54 (59%) were CAA−, and 38 (41%) were CAA+. There were 51 demographically similar HC. Patients with KD had higher CECs than HC (p=0.00003), most evident in the CAA+ group (p=0.00009), but also higher in the CAA− group than HC (p=0.0010). Patients with persistent KD had the highest CECs, but even those with regressed KD had higher CECs than HC (p=0.011). C105 EMPs were also higher in the KD group versus HC (p=0.04), particularly in the CAA+ group (p=0.02), with similar findings for soluble vascular cell adhesion molecule 1 and soluble intercellular adhesion molecule 1. There was no difference in PWV, cIMT, CRF, or markers of systemic inflammation in the patients with KD (CAA+ or CAA−) compared with HC.

Conclusions Markers of endothelial injury persist for years after KD, including in a subset of patients without CAA.

INTRODUCTION

Kawasaki disease (KD) is a self-limiting medium vessel vasculitis of unknown aetiology affecting 8.39/100 000 children under the age of five per year in the UK;2 22.5/100 000 in white Californians, with higher incidence in Asian/Pacific Islanders (50.4/100 000) and 29.8/100 000 in the black population.2 The highest incidence is in Japan: 243.1/100 000 aged 0–4 years in 2011 and 264.8 in 2012.3 Coronary artery aneurysms (CAA) occur in 15%–25% of untreated patients; 2%–3% of untreated cases die as a result of coronary vasculitis.4 Despite the effectiveness of intravenous immunoglobulin (IVIG),4 20% of cases are IVIG resistant, and are at even higher risk of coronary complications. As more children with KD are advancing into adulthood, further studies are needed to improve our understanding of long-term cardiovascular sequelae.4–7 KD vasculopathy differs from atherosclerosis in several key characteristics.5 Historically, the vasculopathy of KD is characterised by an acute, predominantly neutrophilic necrotising vasculitis targeting the luminal coronary endothelium. Subsequently, a later subacute/chronic vasculitis ensues, targeting the adventitia, with myofibroblastic proliferation causing progressive arterial stenoses over many years. Importantly, histological features of true atherosclerosis such as lipid-laden macrophages are usually absent.8–10

The prognosis for patients with giant (>8 mm) CAA is extremely worrying, with 88% 30-year survival, 16% myocardial infarction rate and 59% requiring revascularisation within a 25-year follow-up.11 Common sense dictates that patients with persistent CAA require lifelong follow-up. It remains unclear, however, what long-term management is required for patients who never had CAA or in whom CAA have regressed. Although many patients with CAA undergo regression of aneurysmal dilatation, the coronaries remain abnormally thickened, and vessel-wall calcification is often detected.6 The long-term consequences of these changes remain largely unknown. The American Heart Association (AHA) have issued clinical guidelines for the long-term follow-up and management of patients with KD,10 recently adopted in the UK.4 These recommendations are not based on robust scientific data. To date, there has only been a single small study of late KD vascular outcome in the UK.11 Long-term studies to inform follow-up strategies for patients with KD in the UK are, therefore, required. We conducted the current study to address this unmet clinical need.

We hypothesised that chronic vasculitis persists years after the acute KD presentation, and is detectable using novel biomarkers of endothelial injury. We conducted a study to examine the relationship between coronary artery (CA) status, endothelial injury, systemic inflammatory markers, cardiovasculature cell adhesion molecules (CRF), pulse-wave velocity (PWV) and carotid intima media thickness (cIMT) years after KD.

METHODS

This was an observational comparative study, with ethical approval (08/H0713/80). All participants provided fully informed written consent; assent,
where appropriate, was also obtained from children under the age of 16 years. After an overnight fast, subjects attended the Somers Clinical Research Facility at Great Ormond St Hospital NHS Foundation Trust (GOSH) between January 2009 and May 2013 for a single research assessment. Deidentified clinical data collated included age, sex, ethnicity, age at KD diagnosis, features of KD at presentation, treatments past and present, CA status, body mass index, blood pressure and smoking status.

Patients with KD
Inclusion criteria were complete KD (≥12 months previously) as defined by AHA criteria, fever lasting at least 5 days plus four of five principal clinical criteria: (1) rash, (2) bilateral conjunctivitis without exudate, (3) inflammation of oral mucosa, (4) cervical lymphadenopathy and (5) extremity changes. Patients with atypical KD with fewer than four of the clinical features, but in the presence of CAAs, were also eligible for inclusion. Exclusions were presence of any significant acute or chronic comorbidity, including intercurrent infection. Patients with KD were recruited from two main sources: (1) GOSH and (2) via advertisement by the Kawasaki Syndrome Support Group (http://www.kssg.org.uk). CA status from the original clinical presentation for each patient was ascertained from independent direct scrutiny of patients’ medical records by a senior vasculitis expert (PAB) and a senior paediatric cardiologist (GC); any discrepant cases were discussed to achieve consensus. All clinical and scientific studies were performed blind to the subject status, however.

Definition of CA status and IVIG resistance
The primary data analysis was performed according to the worst-ever CAA status of the patients with KD, defined at any stage over the disease course using recommended AHA criteria. Thus, CAA+ patients were those where any of the following three conditions were met: (1) z score of left anterior descending (LAD) or right CA (RCA) ≥2.5, (2) abnormal CAs as per Japanese Ministry of Health criteria (internal diameter >3 mm in children under the age of 5 years, >4 mm for children 5 years and above, internal diameter greater than 1.5 times the size of an adjacent segment or obvious irregularity of the CA lumen) or (3) z score for LAD or RCA 2–2.5 in the presence of at least two other suggestive features (perivascular brightness, lack of arterial tapering, decreased IV function, mitral regurgitation or pericardial effusion). CAA– patients did not have any of the above features at any stage of their disease.

CAA on the day of study were defined as CA internal diameter ≥2 SD above the mean for age-adjusted body surface area, calculated using the web tool http://www.paramaterz.com, and included those with true CAA (internal diameter Z score >3, giant aneurysms as internal diameter >8 mm) and those with CA ectasia (internal vessel diameter Z score ≥2, but <3), or other obvious significant luminal abnormality.

For the purposes of a subgroup analysis of the CAA+ patients, subjects who still had echocardiographic evidence of CAA on the day of study were defined as persistent CAA; CAA+ patients who no longer had evidence of CAA were defined as regressed CAA.

IVIG resistance was defined as those who received more than one dose of IVIG or one dose of IVIG plus second-line or third-line treatment (other than aspirin or other non-steroidal anti-inflammatory drugs).

Controls
Age-similar (at least within 2 years) and sex-matched controls were recruited from healthy unaffected siblings of patients with KD. A pilot study comparing the preliminary data obtained from the first 19 such controls found no difference in any biomarker compared with other matched paediatric controls from previous studies from our group.

Conventional cardiovascular risk factor assessments
Echocardiography was performed and interpreted in all subjects by trained senior paediatric cardiologists (GC, TM and JM) who were blinded to the subject’s status using a predefined protocol that assessed CA and ventricular dimensions, conventional parameters of systolic and diastolic function (fractional shortening, EF, mitral inflow, pulsed wave tissue Doppler imaging annular velocities) and ventricular myocardial deformation using speckle-tracking imaging; 12-lead resting ECG and fasting lipids (total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL) and triglycerides (TG)) were also assessed using routine methodologies (see online supplementary methods). Resting (minimum of 15 min) blood pressure was measured using an oscillometric manual sphygmomanometer (Greenlight 300; ACCOSON, Essex, UK).

Assessment of inflammatory indices
High-sensitivity C reactive protein (hs-CRP), serum amyloid A (SAA), tumour necrosis factor α, interleukin-1β, 6, 8 and 10, monocyte chemotactant protein-1, vascular endothelial growth factor (VEGF), angiotropin 1 and 2, soluble E-selectin, soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble P-selectin and thrombomodulin were assessed using a validated commercial multiarray detection system based on electrochemiluminescence technology (SECTOR Imager 2400; MesoScale Discovery) (see online supplementary methods).

Assessment of endothelial injury
Circulating endothelial cells
Circulating endothelial cells (CECs) were identified with CD146-immunomagnetic bead extraction based on an international consensus standardised protocol, as described in the online supplementary methods.

Endothelial microparticles
Annexin V-positive endothelial microparticles (EMPs) expressing CD105, E-selectin, ICAM-1, VCAM-1, CD144, CD31, but negative for the platelet marker CD42a, were quantified from platelet poor plasma using a BD FACSArray flow cytometer as previously described by our group, (see online supplementary methods).

Vascular stiffness and cIMT
Carotid-femoral and carotid-radial PWVs were assessed by a trained investigator (VS) using the Vicorder device (Skidmore Medical) as per manufacturer’s instructions, and in accordance with AHA recommendations (see online supplementary methods). Measurement of cIMT was assessed by experienced vascular technicians using a standardised imaging protocol (see online supplementary methods).

Statistical analysis
Sample size calculations were based on CECs (the primary outcome measure). Pilot data from the first 30 patients with KD suggested that CECs were not normally distributed. Natural logarithmic transformation to normality provided an SD of 0.9 for the healthy controls (n=19) and 1.2 for patients with KD, and suggested that 40 subjects in each group were required to
detect a doubling of average CECs in each KD subgroup versus controls with 90% power, significance 0.05. Making adjustment for non-normality and the need to either use non-parametric tests or to transform prior to analysis, this number increased to 47 per group. Thus, we aimed to recruit 80 tests or to transform prior to analysis, this number increased to for non-normality and the need to either use non-parametric

w Values of <0.05 were considered significant for CECs; analysis of all the other indices was considered exploratory, and therefore, p values were not adjusted for multiple comparisons. Analysis of covariance was used to compare the slope of PWV versus age between the groups using linear regression. Multivariable linear regression was used to assess the relationship between CECs (dependent variable) and predictor variables: healthy control or KD, presence of CAA, male sex, age at diagnosis and at study assessment, IVIG resistance, length of follow-up from KD diagnosis and inflammatory status (hs-CRP or SAA). CECs were log-transformed, and hence, model coefficients were exponentiated to represent fold-increase in median CEC between groups. Statistical analyses were performed using GraphPad Prism V.4.0, SPSS V.22 and R V.3.1.2.

RESULTS

Demographics

Of 150 invites sent out, 92 positive responses and agreement to participate in the study were returned, yielding 92 patients with KD: 54 CAA– and 38 CAA+, and 51 healthy controls. The reason why some patients did not respond to the study invite was not formally ascertained; however, there was no obvious bias in CAA status in those who failed to respond. There was no difference in any demographic parameter, body mass index, blood pressure or smoking status between the KD group and controls (table 1). While the CAA+ group was slightly younger, this did not reach statistical significance. The CAA+ group was also younger at KD onset than the CAA– group. There were no other significant demographic differences between the controls and the KD subgroups. Subjects in both groups were predominantly Caucasian. Sixteen per cent in the control group were non-Caucasian; their ethnicities were Asian (n=4) or Afro-Caribbean (n=3); and 17% of the KD group were non-Caucasian; their ethnicities were Asian (n=8), Afro-Caribbean (n=6) and Brazilian (n=1).

KD clinical features

Eighty-three out of 92 (90%) had complete KD; the remaining nine cases (10%) had atypical KD. Seventy-five received IVIG (82%); eight (9%) did not receive IVIG; in seven cases, the KD treatment status was unknown. Forty-eight cases (64%) were IVIG resistant; 63% of the IVIG-resistant group developed CAA, and 15% of the non-IVIG-resistant group developed CAA (difference 48%, 95% CI 25% to 63%, p<0.0001). Table 2 summarises the CA status of the 92 patients with KD. Twenty-two of the KD group were currently receiving medication. For the CAA+ group, 12 were on low-dose aspirin alone; three on aspirin and warfarin; one on aspirin and clopidogrel; one on aspirin, carvedilol and lisinopril and two on clopidogrel alone. For the CAA– group, one patient was on warfarin (for deep vein thrombosis (DVT) that complicated the initial KD episode), and one was treated with verapamil (for Wolff–Parkinson–White syndrome). None of the healthy controls were receiving medication.

Routine laboratory indices

Table 3 summarises the routine laboratory indices between the KD and control groups. There was no significant difference in hs-CRP, SAA, fasting total cholesterol, LDL cholesterol, HDL cholesterol or TG between healthy controls and patients with KD, with or without CAA.

Circulating inflammatory indices

The results are summarised in table 4. VEGF was higher in the CAA+ group than controls. There was no significant difference between healthy controls and patients with KD, with or without CAA, in any other inflammatory parameter studied.

Assessment of endothelial injury

Circulating endothelial cells

Patients with KD had higher CECs than the healthy controls (table 5 and figure 1A). While the CAA+ group had the highest CECs, CAA– patients also had higher CECs compared with the controls. Subgroup analysis of the CAA+ group revealed patients with persistent CAA had the highest CECs, but those with regressed CAA also had significantly higher CECs than healthy controls (figure 1B). There was no significant correlation between CECs and time from the acute KD episode (figure 1C).

Table 1 Demographics of patients with KD and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th>KD N=54</th>
<th>KD vs controls p Value</th>
<th>CAA– N=38</th>
<th>CAA– vs controls p Value</th>
<th>CAA+ N=38</th>
<th>CAA+ vs controls p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at study (years, range)</td>
<td>13.5 (4.9, 30.3)</td>
<td>11.9 (4.3, 32.2)</td>
<td>0.51</td>
<td>13.4 (5.3, 32.2)</td>
<td>0.61</td>
<td>10.4 (4.3, 24.4)</td>
<td>0.051</td>
</tr>
<tr>
<td>Males (%)</td>
<td>53%</td>
<td>51%</td>
<td>0.86</td>
<td>53/11</td>
<td>0.56</td>
<td>23/35</td>
<td>0.67</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>20.2 (13.5, 35.5)</td>
<td>19.2 (11.2, 33.6)</td>
<td>0.97</td>
<td>21.1 (14.0, 33.6)</td>
<td>0.21</td>
<td>17.3 (11.2, 29.7)</td>
<td>0.13</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>110 (90, 133)</td>
<td>110 (78, 140)</td>
<td>0.72</td>
<td>110 (81, 137)</td>
<td>0.50</td>
<td>100 (78, 140)</td>
<td>0.12</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>60 (47, 80)</td>
<td>60 (40, 95)</td>
<td>0.99</td>
<td>60 (43, 95)</td>
<td>0.90</td>
<td>60 (40, 80)</td>
<td>1.0</td>
</tr>
<tr>
<td>Caucasian</td>
<td>82%</td>
<td>79%</td>
<td>0.82</td>
<td>80/7</td>
<td>0.78</td>
<td>74/7</td>
<td>0.46</td>
</tr>
<tr>
<td>Non-Caucasian</td>
<td>16%</td>
<td>17%</td>
<td>11%</td>
<td>13/11</td>
<td>24%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnicity unknown</td>
<td>2%</td>
<td>5%</td>
<td>2%</td>
<td>1/1</td>
<td>5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>0%</td>
<td>3%</td>
<td>0.55</td>
<td>0/1</td>
<td>4%</td>
<td>0/1</td>
<td>3%</td>
</tr>
<tr>
<td>Age at KD diagnosis (years)</td>
<td>–</td>
<td>4.9 (0.18, 11.3)</td>
<td>–</td>
<td>3.0 (0.3, 11.3)</td>
<td>–</td>
<td>0.9 (0.2, 8.8)</td>
<td>–</td>
</tr>
<tr>
<td>Time of study (years) after KD</td>
<td>–</td>
<td>8.3 (1.0, 30.7)</td>
<td>–</td>
<td>8.5 (2.0, 30.7)</td>
<td>–</td>
<td>7.8 (1.0, 23.5)</td>
<td>–</td>
</tr>
</tbody>
</table>

All data are median (range), unless otherwise specified.
BP, blood pressure; CAA, coronary artery aneurysms; KD, Kawasaki disease.
EMPs and soluble adhesion molecules

CD105 EMPs were higher in the KD group versus controls (table 5), particularly in the CAA+ group (figure 2A). Patients with persistent CAA had the highest CD105 EMPs, although patients with regressed CAA also had higher CD105 EMP than controls (figure 2B). sVCAM-1 was higher in the KD group versus controls (table 5), and even higher in the CAA+ group (table 5 and figure 2C). Patients with regressed CAA had the highest sVCAM-1 (figure 2D). sICAM-1 was higher in the CAA+ group versus the controls and the CAA− group (table 5 and figure 2E); patients with KD with persistent CAA had the highest sICAM-1 (figure 2F).

Predictors of CEC counts

There was no significant association between CEC and current age, male sex, age at diagnosis, IVIG resistance, length of follow-up from KD episode, hs-CRP or SAA in univariable models (see online supplementary table S1). Both subject status (KD or healthy control) and presence of CAA were univariably significantly associated with CEC count. In the multivariable model, KD was associated with a 64% increase on average in CEC count (95% CI 12% to 140%, p=0.0001), and presence of CAA remained independently associated with a further 59% increase (95% CI 6% to 140%, p=0.026). After accounting for these two variables, none of the other factors were independently predictive of CEC count.

Structural peripheral arterial parameters

There was a strong positive association between age and carotid-femoral PWV for all subject groups: \( r^2=0.65 \) for controls, \( r^2=0.62 \) for CAA− and \( r^2=0.73 \) for CAA+; \( p<0.0001 \) for all (figure 3). There was, however, no difference in the PWV (table 5 and figure 3) or cIMT (table 5) between the controls and the patients with KD, with or without CAA.

**DISCUSSION**

The long-term cardiovascular outcome for paediatric survivors of KD is an important concern. It is known that the prognosis for patients with persistent CAA remains guarded, particularly for those with giant CAA since myocardial infarction occurs in 16%–31%.20 Much less is known about the fate of those with regressed CAA or those who never developed CAA. We conducted a long-term follow-up study of KD in a UK-based

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**Table 2** Coronary artery status of the KD group (n=92)

<table>
<thead>
<tr>
<th>CAA status of the KD group (n=92)</th>
<th>n (% of all KD patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAA+</td>
<td>38 (41)</td>
</tr>
<tr>
<td>CAA &gt;8 mm</td>
<td>7 (8)</td>
</tr>
<tr>
<td>Persistent CAA</td>
<td>7 (8)</td>
</tr>
<tr>
<td>Regressed CAA</td>
<td>0 (0)</td>
</tr>
<tr>
<td>CAA &lt;8 mm</td>
<td>31 (34)</td>
</tr>
<tr>
<td>Persistent CAA</td>
<td>10 (11)</td>
</tr>
<tr>
<td>Regressed CAA</td>
<td>21 (23)</td>
</tr>
</tbody>
</table>

CAA+: CAA defined at any stage after the initial KD episode (see Methods). CAA−: no evidence of coronary aneurysm at any stage. Persistent CAA (n=17, 19%): any CAA+ subject who still had echocardiographic evidence of CAA on the day of study (see Methods). Regressed CAA (n=21, 23%): CAA+ subjects whose aneurysms had regressed on echocardiography on the day of study. 6/38 of the CAA+ patients developed coronary stenosis; these included 4/7 of the patients with giant (>8 mm) CAA, three of whom required a recanalisation procedure.

CAA, coronary artery aneurysm; KD, Kawasaki disease.

**Table 3** HS-CRP, SAA and fasting lipids

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Healthy controls (n=27)</th>
<th>All patients with KD Median (range)</th>
<th>KD CAA− vs control p Value (95% CI of diff)</th>
<th>KD CAA+ vs control p Value (95% CI of diff)</th>
<th>CAA− vs CAA+ p Value (95% CI of diff)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL (mmol/L)</td>
<td>1.40 (0.40, 2.40)</td>
<td>1.39 (0.40, 2.40)</td>
<td>0.16 [−0.2 to 0.12]</td>
<td>0.35 [0.12 to 0.58]</td>
<td>0.77 [−0.12 to 0.18]</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.27 (0.93, 4.90)</td>
<td>2.28 (0.93, 4.90)</td>
<td>0.38 [−0.2 to 0.10]</td>
<td>0.44 [0.01, 0.87]</td>
<td>0.35 [0.01, 0.70]</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.77 (0.38, 3.34)</td>
<td>0.78 (0.38, 3.34)</td>
<td>0.25 [−0.2 to 0.1]</td>
<td>0.44 [0.01, 0.87]</td>
<td>0.35 [0.01, 0.70]</td>
</tr>
<tr>
<td>SAA (mg/L)</td>
<td>0.80 (0.08, 14.71)</td>
<td>0.80 (0.08, 14.71)</td>
<td>0.84 [0.01, 8.49]</td>
<td>0.84 [0.01, 8.49]</td>
<td>0.84 [0.01, 8.49]</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>0.25 (0.00, 69.40)</td>
<td>0.26 (0.00, 69.40)</td>
<td>0.72 [−0.2 to 0.16]</td>
<td>0.73 [−0.2 to 0.16]</td>
<td>0.73 [−0.2 to 0.16]</td>
</tr>
</tbody>
</table>

All values are median (range) unless otherwise specified. Number (n) of subjects in each group as specified per test due to missing data points. *p Values from Kruskal-Wallis test comparing three groups: controls, CAA− and CAA+. 95% CI of diff, 95% CI of the difference of median. HDL, high-density lipoprotein; SAA, serum amyloid A; TG, triglycerides.

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population. Using a multimodal approach to the assessment of cardiovascular health, we observed elevation of markers of endothelial injury, a median of 8.3 (1.0–30.7) years after KD. CECs were significantly higher in KD than controls, and were highest in the CAA+ group, but also elevated in patients with regressed CAA and in the CAA− group (figure 1A, B). This observation was corroborated by further evidence of persistent markers of endothelial injury in KD versus controls from three other circulating indices: CD105 EMP sVCAM-1 and sICAM-1, particularly in the CAA+ group (figure 2) and by the observation that VEGF was higher in the CAA+ group than controls. Patients with regressed CAA also had significantly higher circulating CD105 EMPs and sVCAM-1 (figures 2B, D). Despite this, we did not detect any evidence of altered peripheral arterial health (PWV and cIMT) in the patients with KD or any perturbation in conventional cardiovascular risk factors. These novel observations could have implications for long-term monitoring, follow-up and secondary prevention of cardiovascular events in survivors of KD.

CECs are mature cells that have become detached from the vessel wall, and are associated with vascular injury, particularly in diseases where endothelial injury is central to the pathogenesis, such as the systemic vasculitides.16 21 Nakatani et al22 demonstrated high CECs in acute KD complicated by CAA, but also in patients without CAA. Yu et al23 demonstrated that these CECs expressed higher levels of inducible-nitric oxide synthase, mirroring findings in lesional coronary vasculitis observed at postmortem. These CECs carry surface-bound S100 and related proteins, including MRP-8/MRP-14,24 S100A12 25 and its receptor, RAGE (receptor for advanced glycation endproducts),25 26 and their levels correlate with the severity of coronary vasculitis. These studies suggest that CECs detach from medium-sized arteries as a result of neutrophil-endothelial interactions mediated by S100 proteins. CECs appear highest in those with CAA, but are also elevated in patients with acute KD without CAA. Yu et al23 demonstrated that these CECs remain elevated for years after KD, including in some patients who never had any evidence of CAA. This finding aligns well with the observation that coronary endothelial dysfunction assessed using paradoxical vasoconstriction response to intracoronary acetylcholine challenge is known to persist for many years in patients with KD and aneurysms.27 28 It is still unclear, however, how CECs relate to earlier studies that used flow-mediated dilatation (FMD) in the brachial artery. Dhillon et al11 observed that FMD of the brachial artery was markedly reduced in patients with KD compared with control subjects many years after the illness, even in patients without detectable early CA involvement. In contrast, McCrindle et al10 did not demonstrate any differences in brachial artery reactivity following KD. Brachial artery FMD, cIMT and PWV are unlikely to be reliable surrogates for assessing coronary vascular health after KD since these techniques were developed for the assessment of vascular health in atherosclerosis. The peripheral arteries are not typically affected in KD, and hence, these indices are not good surrogates for the study of late-KD coronary vasculopathy.

Our study now suggests that CECs remain elevated for years after KD, including in some patients who never had any evidence of CAA. This finding aligns well with the observation that coronary endothelial dysfunction assessed using paradoxical vasoconstriction response to intracoronary acetylcholine challenge is known to persist for many years in patients with KD and aneurysms.27 28 It is still unclear, however, how CECs relate to earlier studies that used flow-mediated dilatation (FMD) in the brachial artery. Dhillon et al11 observed that FMD of the brachial artery was markedly reduced in patients with KD compared with control subjects many years after the illness, even in patients without detectable early CA involvement. In contrast, McCrindle et al10 did not demonstrate any differences in brachial artery reactivity following KD. Brachial artery FMD, cIMT and PWV are unlikely to be reliable surrogates for assessing coronary vascular health after KD since these techniques were developed for the assessment of vascular health in atherosclerosis. The peripheral arteries are not typically affected in KD, and hence, these indices are not good surrogates for the study of late-KD coronary vasculopathy.

We did not demonstrate any evidence of systemic inflammation (table 4) or cardiovascular risk factors to account for the elevated CECs we observed. We suggest that our observations are consistent with an ongoing active subclinical vasculitis because there was no significant correlation between CECs and time from acute KD diagnosis (figure 1C), and we also...
Table 5  Endothelial injury markers, platelet microparticles, arterial stiffness and carotid IMT in the KD group and controls

<table>
<thead>
<tr>
<th>Marker</th>
<th>Healthy controls (Median (range))</th>
<th>All patients with KD (Median (range))</th>
<th>KD vs controls p Value (95% CI of diff)</th>
<th>KD CAA− vs controls p Value (95% CI of diff)</th>
<th>KD CAA+ vs controls p Value (95% CI of diff)</th>
<th>p Value Kruskal–Wallis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CECs (cells/mL)</td>
<td>12 (0, 32) (n=49)</td>
<td>24 (0, 224) (n=89)</td>
<td>p=0.00003 (4 to 16)</td>
<td>p=0.00010 (4 to 12)</td>
<td>p=0.00009 (8 to 44)</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Microparticles (×10^3/mL) plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total annexin V</td>
<td>990 (160, 6160)</td>
<td>970 (1190, 5410)</td>
<td>p=0.83 (−250 to 220)</td>
<td>p=0.42 (−360 to 160)</td>
<td>990 (230, 4360)</td>
<td>p=0.54 (−200 to 400)</td>
</tr>
<tr>
<td>CD54 (ICAM-1)</td>
<td>0.97 (0, 27.00)</td>
<td>0.87 (0, 18.00)</td>
<td>p=0.82 (−0.40 to 0.34)</td>
<td>p=0.86 (−0.50 to 0.43)</td>
<td>1.10 (0, 9.11)</td>
<td>p=0.83 (−0.62 to 0.46)</td>
</tr>
<tr>
<td>CD62E (E-selectin)</td>
<td>3.92 (0, 49.34)</td>
<td>2.87 (0, 22.37)</td>
<td>p=0.40 (−1.89 to 0.53)</td>
<td>p=0.84 (1.75 to 0.96)</td>
<td>1.87 (0, 16.98)</td>
<td>p=0.16 (−2.97 to 0.19)</td>
</tr>
<tr>
<td>CD105</td>
<td>0 (0, 206.50)</td>
<td>1.60 (0, 186.80)</td>
<td>p=0.04 (0.03 to 1.95)</td>
<td>p=0.14 (−0.02 to 1.67)</td>
<td>2.97 (0, 186.80)</td>
<td>p=0.02 (0.01 to 6.00)</td>
</tr>
<tr>
<td>CD62P (P-selectin)</td>
<td>0 (0, 12.90)</td>
<td>0 (0, 15.17)</td>
<td>p=0.59 (−0.01 to 0.01)</td>
<td>p=0.94 (−0.03 to 0.08)</td>
<td>0 (0, 8.62)</td>
<td>p=0.24 (−0.03 to 0.24)</td>
</tr>
<tr>
<td>CD1A4</td>
<td>0.20 (0, 12.36)</td>
<td>0.32 (0, 155.20)</td>
<td>p=0.42 (−0.02 to 0.29)</td>
<td>p=0.24 (−0.004 to 0.71)</td>
<td>0 (0, 155.20)</td>
<td>p=1.0 (−0.02 to 0.02)</td>
</tr>
<tr>
<td>CD31</td>
<td>20.59 (0, 556.50)</td>
<td>14.18 (0, 981.00)</td>
<td>p=0.98 (−8.37 to 6.74)</td>
<td>p=0.89 (−8.57 to 9.13)</td>
<td>17.63 (0, 258.30)</td>
<td>p=0.83 (−13.7 to 6.48)</td>
</tr>
<tr>
<td>CD42a (platelet MP)</td>
<td>24.93 (0, 930.70) (n=47)</td>
<td>14.04 (0, 586.50) (n=87)</td>
<td>p=0.26 (−15.88 to 2.02)</td>
<td>p=0.16 (−20.10 to 1.04)</td>
<td>13.61 (0, 509.90)</td>
<td>p=0.66 (−16.25 to 6.74)</td>
</tr>
<tr>
<td>Soluble adhesion molecules (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sICAM-1</td>
<td>251 (186, 504)</td>
<td>260 (131, 452)</td>
<td>p=0.58 (−16 to 30)</td>
<td>p=0.46 (−31 to 16)</td>
<td>300 (131, 452)</td>
<td>p=0.04 (1 to 59)</td>
</tr>
<tr>
<td>sVCAM-1</td>
<td>408 (261, 1066)</td>
<td>465 (253, 880)</td>
<td>p=0.007 (15 to 79)</td>
<td>p=0.08 (−4 to 66)</td>
<td>484 (274, 880)</td>
<td>p=0.0022 (30 to 113)</td>
</tr>
<tr>
<td>sE-Sel</td>
<td>13 (5, 62) (n=48)</td>
<td>16 (5, 59)</td>
<td>p=0.31 (−1 to 4)</td>
<td>p=0.49 (−2 to 4)</td>
<td>17 (5, 42)</td>
<td>p=0.27 (−2 to 5)</td>
</tr>
<tr>
<td>sP-Sel</td>
<td>70 (19, 262) (n=48)</td>
<td>82 (22, 204) (n=89)</td>
<td>p=0.10 (−2 to 23)</td>
<td>p=0.09 (−2 to 28)</td>
<td>77 (22, 139)</td>
<td>p=0.28 (−7 to 22)</td>
</tr>
<tr>
<td>Carotid-radial PWV (m/s)</td>
<td>7.30 (4.70, 9.60) (n=51)</td>
<td>7.40 (4.60, 11.20) (n=91)</td>
<td>p=0.40 (−0.30 to 0.50)</td>
<td>p=0.25 (−0.20 to 0.70)</td>
<td>7.40 (4.90, 11.20)</td>
<td>p=0.86 (−0.50 to 0.50)</td>
</tr>
<tr>
<td>Carotid-femoral PWV (m/s)</td>
<td>5.40 (4.00, 8.40) (n=51)</td>
<td>5.40 (3.80, 9.00) (n=91)</td>
<td>p=0.69 (−0.40 to 0.30)</td>
<td>p=0.58 (−0.30 to 0.60)</td>
<td>5.10 (3.80, 7.00)</td>
<td>p=0.13 (−0.80 to 0.10)</td>
</tr>
<tr>
<td>Right CCA IMT (mm)</td>
<td>0.47 (0.40, 0.60) (n=42)</td>
<td>0.47 (0.39, 0.58) (n=78)</td>
<td>p=0.77 (−0.02 to 0.01)</td>
<td>p=0.59 (−0.02 to 0.01)</td>
<td>0.47 (0.39, 0.57)</td>
<td>p=0.92 (−0.2 to 0.2)</td>
</tr>
<tr>
<td>Left CCA IMT (mm)</td>
<td>0.46 (0.41, 0.60) (n=40)</td>
<td>0.46 (0.37, 0.58) (n=76)</td>
<td>p=0.97 (−0.01 to 0.01)</td>
<td>p=0.99 (−0.02 to 0.02)</td>
<td>0.46 (0.39, 0.58)</td>
<td>p=0.95 (−0.02 to 0.02)</td>
</tr>
</tbody>
</table>

All values are median (range) unless otherwise specified. Number (n) of subjects in each group as specified per test.

*p Values from Kruskal–Wallis test comparing three groups: controls, CAA− and CAA+ (CA status at initial disease presentation). 95% CI of diff: 95% CI of the difference of median. Statistically significant results are in bold.

CAA, coronary artery aneurysms; CCA, common carotid artery; CECs, circulating endothelial cells; E-Sel, E-selectin; ICAM, intercellular adhesion molecule; IMT, intima media thickness; KD, Kawasaki disease; P-Sel, P-selectin; PWV, pulse-wave velocity; s, soluble; VCAM, vascular cell adhesion molecule.
consistently observed elevated soluble adhesion molecules (sVCAM-1 and sICAM-1) and CD105 EMP in the KD CAA+ group. We also observed significantly higher levels of VEGF in the CAA+ group. This latter observation could be explained by an attempt at ongoing endothelial repair/remodelling in patients with active subclinical vasculitis, as suggested by our previous studies of systemic vasculitis in children. It could also indicate an ongoing, and possibly permanent, disturbance in endothelial cell homoeostasis. Future studies examining the mechanisms driving this chronic endothelial injury and the prognostic relevance of this finding are now required. While we did not measure circulating S100 proteins or their expression on CECs, it is possible that this mechanism could account for the persistently high CEC levels.

Although we did (as expected) observe a strong positive relationship between PWV and age, there was no difference in patients with KD (with or without CAA) and healthy controls (Figure 3). Similarly, we did not detect any difference in cIMT.

These findings are in agreement with a recently published large study of cardiovascular health in 203 predominantly Caucasian North American patients with KD, a median of 11.6 years (1.2–26 years) after acute KD; the authors concluded that patients with KD without CAA or with CAA ectasia could be reassured, and may not need specialised surveillance. Moreover, we did not observe any difference in lipid profile between patients with KD and controls again, in agreement with the study conducted by Selamet Tierney et al. While we cannot yet conclude that patients with high CECs after KD are at higher risk of cardiovascular events, our study now provides a scientific rationale for the AHA recommendation for follow-up even for those without CAA, since persistence of subclinical vasculitis in an important subset of patients is likely to act in concert with traditional cardiovascular risk factors to adversely influence long-term coronary prognosis. Since it is currently not possible to clinically distinguish those with elevated CECs from patients with KD as a whole, we suggest that all patients with KD (irrespective of
EMP and soluble adhesion molecules. (A) KD CAA+ patients had higher CD105 EMP than HC (95% CI of difference 0.01 to 6.00×10³/mL), but there was no significant difference when the CAA+ group was compared with CAA− patients. CAA− patients did not significantly differ from HC. (B) Patients with KD with persistent CAA had the highest CD105 EMP, although this did not reach statistical significance compared with controls (95% CI of difference in median −0.00005 to 6.04×10³/mL). Patients with KD with regressed CAA had significantly higher CD105 EMP (median 2.59×10³/mL) than HC (0.00×10³/mL) (95% CI of difference in median −0.00006 to 10.49), but not compared with the persistent CAA group (95% CI of difference in median −5.68 to 10.40) or the CAA− group. (C) sVCAM-1 was significantly higher in the CAA+ group versus HC, but this was not significantly higher when compared with the CAA− group. (D) sVCAM-1 was particularly high in those with regressed CAA (Regr CAA: vs HC (95% CI of difference in median 32.3 to 144.6 ng/mL) and vs CAA−), but was not different from those with persistent CAA (95% CI of difference in median −44.4 to 111.1 ng/mL). (E) sICAM-1 was higher in the KD CAA+ group (vs HC (95% CI of difference in median 1 to 59 ng/mL) and vs CAA−). (F) sICAM-1 was highest in those with persistent CAA (Pers CAA: vs HC (95% CI of difference in median 2 to 86 ng/mL) and higher than the CAA− group). There was no difference in sICAM-1 levels between the persistent and regressed CAA groups (95% CI of difference in median −76 to 31 ng/mL), and no significant difference between Regr CAA and controls (95% CI of difference in median −11 to 52 ng/mL).

Horizontal lines represent median and IQR. CAA, coronary artery aneurysms; EMP, endothelial microparticles; HC, healthy control; KD, Kawasaki disease; Pers, persistent; Regr, regressed; sICAM, soluble intercellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule.

30 years after KD.

After KD, r²=0.62 for KD CAA− and r²=0.73 for KD CAA+;
p<0.0001 for all. Analysis of covariance of the carotid-femoral PWV
slope did not show any statistically significant difference from controls
(dashed line) for KD CAA− patients (solid black line, p=0.87) or KD
CAA+ patients (red line, p=0.66). CAA, coronary artery aneurysms; HC,
healthy control; KD, Kawasaki disease; PWV, pulse-wave velocity.

Figure 3  PWV versus age. There was a strong positive association
between age and carotid-femoral PWV for all subject groups: r²=0.65
for controls, r²=0.62 for KD CAA− and r²=0.73 for KD CAA+;
p<0.0001 for all. Analysis of covariance of the carotid-femoral PWV
slope did not show any statistically significant difference from controls
(dashed line) for KD CAA− patients (solid black line, p=0.87) or KD
CAA+ patients (red line, p=0.66). CAA, coronary artery aneurysms; HC,
healthy control; KD, Kawasaki disease; PWV, pulse-wave velocity.

Acknowledgements  Dr S Masi and Devina Bhowruth for assistance with
measurement of carotid IMT. Mrs Sue Davidson of the Kawasaki Support Group.
Dr E Menson and Dr T Krasemann (Evelina Children’s Hospital, London) for help
with recruitment of subjects. Mr M Mayes for help with study co-ordination. Staff at
the Somers Clinical Research Facility, Great Ormond Street Hospital NHS Foundation
Trust and the children, young adults and families who participated in the study. We
also acknowledge support from researchers at the National Institute for Health
Research Biomedical Research Centre at Great Ormond Street Hospital for Children
NHS Foundation Trust, and University College London.

Limitations
Since our study assessed medical records from multiple centres
going back over 30 years, it is possible that some patients with
minor degrees of coronary vasculitis could have been misclassified,
although medical records were assessed independently by
two experienced clinicians, thus limiting any potential bias.
Second, since our study was not a single-centre study, sequential
detailed echocardiographic data were not available to us to
study CAA regression rates and the potential influence of that
on CEC counts, an area worthy of a further prospective study.
Third, for practical reasons, we used healthy siblings of index
KD cases as controls. As yet unde

Caring for Kids Fund.

CONCLUSIONS
In conclusion, we have conducted a follow-up study of KD and
found: (1) evidence of persistent endothelial injury years after
KD in a subset of patients both with and without CAA and (2)
no evidence of structural peripheral arterial disease. Future
studies now need to validate the relevance of CECs as a poten-
tially important prognostic biomarker of late-KD vasculopathy.

What is already known on this subject?
Kawasaki disease (KD) is a self-limiting medium vessel vasculitis
of unknown aetiology, affecting predominantly children under
the age of 5 years, resulting in coronary artery aneurysms (CAA)
in approximately 25% of untreated patients. There is a need to
identify patients at risk of late KD vasculopathy to inform
clinical strategies for surveillance and prevention of late
cardiovascular events.

What might this study add?
This study examined circulating endothelial cells (CECs) years
after KD. CECs were increased in patients with KD, were highest
in those with CAA, but were also elevated in some patients
without CAA, compatible with a state of persistent subclinical
vasculitis years after the acute disease. In contrast, arterial
stiffness, carotid intima media thickness and conventional
cardiovascular risk factors were no different from controls.

How might this impact on clinical practice?
The significance of patients having high CECs is unknown. Our
study does, however, provide a scientific rationale for
recommending lifelong follow-up of all patients with KD.

REFERENCES
disease in Japan, 2011–2012: from the results of the 22nd nationwide survey.

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Coronary artery disease


Supplementary Methods and Results

Supplementary methods

Fasting lipid assay

Fasting lipids were measured using the VITROS Chemistry System (Ortho-Clinical Diagnostics, Raritan, NJ) from serum samples by multilayer film dry-slide chemistry with colorimetric detection.

Assessment of inflammatory indices

Circulating soluble markers of systemic and vascular inflammation were studied using a multi-parametric approach to explore potential relevant inflammatory pathways. High sensitivity C reactive protein (hs-CRP), serum amyloid A (SAA), tumour necrosis factor alpha (TNF-α), interleukin (IL)- 1β, 6, 8 and 10, monocyte chemoattractant protein-1 (MCP-1), vascular endothelial growth factor (VEGF), angiopoietin 1 and 2, soluble E-selectin (sE-sel), soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble P selectin (sP-Sel), and thrombomodulin (TM) were assessed using a multi-array detection system based on electro-chemiluminescence technology (SECTOR Imager 2400, MesoScale Discovery; see supplementary methods). In brief, this system uses multi-array plates fitted with multi-electrodes per well with each electrode being coated with a different catching antibody. The assay procedure then follows that of a classic sandwich ELISA with the analytes of interest captured on the relevant electrode. These captured analytes were then in turn detected by a secondary analyte-specific ruthenium-conjugated antibody, which is capable of emitting light after electrochemical stimulation. A particular advantage of this system is the ability to simultaneously measure different biomarkers in small (25ul or 50uL) serum or plasma samples. Tissue Factor (TF) was measured by sandwich enzyme immunoassay using a commercially available kit from R & D Systems, Europe Ltd, (Abingdon, UK).
Circulating endothelial cells

Venous blood (1 ml) collected into tubes containing EDTA was mixed with buffer (1 ml of phosphate buffered saline containing 0.1% bovine serum albumin and 0.6% sodium citrate) and 20 μl of Fc receptor–blocking reagent (Miltenyi Biotec) and incubated for 5 minutes at room temperature. Fifty microliters of a preparation of anti-CD146-coated immunomagnetic beads (clone S-endo-1; BioCytex and Dynal Biotech) was added, and the sample was incubated at 4°C for 30 minutes, with rotation. Bead-bound cells were separated using a magnet (MPC-L; Dynal Biotech) and washed 3 times with buffer. Cells were then resuspended in 100 μl of buffer containing 10 μl of a 2-mg/ml preparation of FITC-labeled Ulex europaeus lectin (Sigma-Aldrich) and incubated for 1 hour at room temperature in the dark. CECs in the sample were counted using a Nageotte chamber on a fluorescence microscope by an experienced scientist (VS) blinded to the study subject status. CECs were defined as Ulex bright cells that were >10 μm in size, with >5 magnetic beads attached.

Endothelial microparticles (EMPs)

Blood was collected in 3.2% buffered citrate and centrifuged at 5000 g for 5 min twice to obtain platelet-poor plasma (PPP). MPs were sedimented from 200μl of PPP after centrifugation at 17000g for 60 min and re-suspended in An V binding buffer (BD PharMingen, Oxford, United Kingdom) prior to incubating with conjugated fluorescent monoclonal proteins or antibodies labelled with fluorescein isothiocyanate [FITC], phycoerythrin [PE], or Allophycocyanin (APC): An V FITC (BD Oxford, United Kingdom), mouse (PE)-labeled anti-human CD62e (Clone 68-SH11, BD PharMingen Oxford, United Kingdom), mouse (PE)-labeled anti-human CD31 (clone WM59, BD Oxford, United Kingdom), mouse (PE)-labeled anti human 62P (clone AK-4 BD PharMingen Oxford, United Kingdom), mouse (PE)-labeled anti human CD105 (clone SN6 E-Bioscience Hatfield United Kingdom), mouse (PE)-labeled anti human CD54 (clone HA58 BD PharMingen Oxford, United Kingdom), mouse
(PE)-labeled anti human CD106 (clone BD 51-10C9 PharMingen Oxford, United Kingdom). Additional labeling with mouse anti-human CD42a-APC (clone GR-P Immunostep.com Salamanca Spain) to exclude MP of platelet-origin was conducted. Samples were analyzed with a FACS Array flow cytometer (BD).

**Vascular stiffness and carotid intima media thickness**

Carotid-femoral and carotid-radial pulse wave velocity (PWV) was assessed by a trained investigator (VS) using the Vicorder device (Skidmore Medical Limited) as per manufacturer instructions, and in accordance with American heart Association recommendations (23).

The technique most widely used to measure arterial stiffness is the determination of arterial pulse wave velocity (PWV). PWV is the speed of travel of the pulse along an arterial segment. Carotid-femoral PWV is a direct measurement, and it corresponds to the widely accepted propagative model of the arterial system. However, carotid-radial PWV may also provide other relevant information of arterial stiffness. For this study, PWV measurements were obtained using the Vicorder device (Skidmore Medical Devices) by placing a 100 mm wide blood pressure cuff around the upper thigh to measure the femoral pulse, and a 30mm partial cuff around the neck at the level of the carotid artery. High quality waveforms were recorded simultaneously for 3 seconds with the subject in the supine position, and the foot-to-foot transit time was determined using an in-built cross-correlation algorithm centered around the peak of the second derivative of pressure. For carotid to femoral PWV, path length was defined as the distance from the suprasternal notch to the middle of the thigh cuff as indicated by the manufacturer (mm). The measurement from the suprasternal notch to the umbilicus and then to the middle of the cuff was recorded, in addition. The distance from the suprasternal notch diagonally to the middle of the neck cuff was also recorded as a separate
measurement. For carotid to radial PWV, path length was defined from the distance from the suprasternal notch to the middle of the radial cuff.

Measurement of far wall carotid intima-media thickness (cIMT) with B-mode ultrasound is a non-invasive and reproducible technique for identifying and quantifying vascular disease and for evaluating cardiovascular risk. For this study, experienced vascular technicians carried out all cIMT measurements following a standardised imaging protocol (see acknowledgments). The Zonare ultrasound scanner (Zonare Medical System) with a high resolution probe, was used to image both the right (RCCA) and left (LCCA) common carotid arteries longitudinally 1cm proximal to the carotid bifurcation. Images were focussed on the posterior (far) wall of the artery and the zoom function was used to magnify the area. Ten second cineloops were recorded in DICOM format and downloaded for offline analysis. Three end-diastolic frames were selected and analysed for mean cIMT, defined as the interface between lumen-intima and media-adventitia, for both right and left carotid arteries using an automated carotid analyser (Carotid Analyser, M.I.A). The images were analysed by accredited readers and the mean of both the left and right-sided readings was used for the analysis.
Supplementary Results

**Supplemental Table 1:** Predictors of CEC counts

<table>
<thead>
<tr>
<th>Variables</th>
<th>Unadjusted fold increase in median CEC (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of CAA</td>
<td>1.37 (1.40,2.92)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Subject status (healthy control/KD)</td>
<td>1.38(1.40,2.74)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Current age (years)</td>
<td>0.99 (0.70,1.21)</td>
<td>0.559</td>
</tr>
<tr>
<td>Males</td>
<td>0.87 (0.55,1.07)</td>
<td>0.119</td>
</tr>
<tr>
<td>Serum amyloid A</td>
<td>1.01 (0.97,1.14)</td>
<td>0.244</td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>1.01 (0.99,1.07)</td>
<td>0.777</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>0.96 (0.87,1.05)</td>
<td>0.364</td>
</tr>
<tr>
<td>Years of follow up post KD</td>
<td>1.01 (0.97,1.0)</td>
<td>0.959</td>
</tr>
<tr>
<td>IVIG resistance</td>
<td>1.15 (1.76,1.53)</td>
<td>0.333</td>
</tr>
</tbody>
</table>

**Supplemental Table 1 legend:** Unadjusted univariable analysis for fold increase in median circulating endothelial cell (CEC) count in association with presence of CAA, subject status, current age, sex (male), serum amyloid A, hs-CRP, age at diagnosis of KD, years of follow up post KD, and IVIG resistance.