Abnormal Matrix Remodeling in Adolescents and Young Adults with Kawasaki Disease Late after Onset

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BACKGROUND: Patients with a history of Kawasaki disease (KD), have been found to have pericoronary and myocardial fibrosis. Serum biomarkers of fibrosis may be sensitive indices for detection of these late cardiac complications in KD patients.

METHODS: We studied a cohort of 60 adolescents and young adults comprising 10 KD patients with persistent coronary artery lesions (CAL) occurring at a mean (SD) time of 14.5 (4.4) years after disease onset, 25 KD patients with no CAL after disease onset, and 25 healthy age-matched volunteers. We compared laboratory data from the patients and volunteers, including lipid profile, liver function, amino-terminal propeptide of type III procollagen (PIIINP), matrix metalloproteinase 9 (MMP-9), tissue inhibitor of metalloproteinase 1 (TIMP-1), and MMP-9:TIMP-1 ratios. Severity of CAL was determined on the basis of computed tomography determinations of the frequency of aneurysms and the extent of coronary stenosis/occlusion, thrombosis, and calcification.

RESULTS: Increased PIIINP and decreased MMP-9 and TIMP-1 concentrations and decreased MMP-9:TIMP-1 ratios were found not only in KD patients with persistent CAL but also in KD patients without CAL, although to a lesser extent in the latter group. In KD patients, the concentrations of PIIINP were positively associated with the severity of coronary stenosis/occlusion (r = 0.72, P = 0.011) and with the extent of coronary thrombus (r = 0.64, P = 0.014). The concentrations of high-sensitivity C-reactive protein, however, did not differ across groups.

CONCLUSIONS: Our results demonstrate alterations in extracellular matrix biomarkers in KD patients, suggesting enhanced collagen synthesis and ameliorated degradation in adolescents and young adults late after the onset of KD. We also observed an association between the concentrations of PIIINP and the extent of coronary stenosis/occlusion or thrombosis in KD patients, a finding that needs confirmation in further studies.

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Kawasaki disease (KD) is an acute systemic vasculitis of unknown etiology that may result in long-term sequelae of coronary arterial lesions (CAL). KD occurs predominantly in infants and young children, and its incidence appears to be increasing worldwide. KD is now the leading cause of acquired heart disease in children (1, 2). During the acute stage, CAL (i.e., coronary artery aneurysms or ectasia) develop in 15% to 25% of untreated KD children and may lead to myocardial ischemia/infarction or sudden death. Since the introduction of intravenous immunoglobulin therapy, the incidence of CAL has declined and the severity of CAL has decreased. Even with therapy, significant CAL still occurs in about 5%–10% of KD patients (2, 3). These coronary lesions may undergo vascular remodeling, such as recanalization and fibrosis, and progress to stenosis along with aneurysms later in adulthood (4, 5). Persistent CAL may be associated with impaired coronary endothelial function (6, 7) and low-grade inflammation (8, 9). Pathological data from the hearts of KD patients who suffered from sudden death showed thickening of coronary arteries and arterioles as well as perivascular and myocardial fibrosis (10–12). Alterations in the build-up and breakdown of arterial extracellular matrix are key features in vascular remodeling. Recently-developed assays permit assessment of matrix remodeling through measurement of circulating con-
Materials and Methods

STUDY POPULATION: PATIENTS AND CONTROL PARTICIPANTS

The institutional research committee of this institution approved the study protocol, which conformed to the principles of the Helsinki Declaration. Written informed consent was taken from all adult participants and from the parents of participants younger than 18 years.

The diagnosis of KD was made according to the clinical criteria of the American Heart Association, the Council on Cardiovascular Disease in the Young, and the Committee on Rheumatic Fever, Endocarditis and Kawasaki Disease (1). CAL were defined as having a lumen diameter of at least 3 mm (4 mm for patients ≥5 years of age) or an internal diameter of a segment at least 1.5 times as large as that of an adjacent segment.

KD patients who met the following study entry criteria were enrolled through the outpatient clinics of the National Taiwan University Hospital: (a) a diagnosis of KD, (b) an interval between the disease onset and time of study greater than or equal to 10 years, (c) an appointment for an echocardiographic evaluation of CAL during the acute phase of KD and a treatment protocol of regular follow-ups by use of echocardiography and/or other imaging modalities [computed tomography (CT) or coronary angiography], and (d) diagnosis of KD without CAL since onset of KD (KD-NCAL group) or with persistent CAL (KD-PCAL group). Patients were excluded from the study who had only irregular coronary arterial lumens with increased perivascular echogenicity or ectatic dilation (the size of the coronary artery, though enlarged, did not meet the criteria of CAL) during the acute phase. Control participants consisted of 25 age-matched healthy volunteers whose body mass indices were <25 kg/m² and who did not have a history of KD or other evident major diseases.

All patients received a detailed clinical evaluation for the assessment of general and cardiac conditions. Participants, including the controls, who had infectious disease or injury within 1 month before the study, abnormal liver function, chronic inflammatory disease, or clinical evidence of heart failure were excluded because abnormal collagen turnover has been reported in these disease entities (19).

CT EVALUATION

CT for coronary imaging has been shown to be reliable for visualizing the coronary arteries in adults (20) and children (21, 22) with or without coronary arterial anomalies. Application of CT to KD patients is a useful modality for demonstrating the presence of coronary aneurysm and stenosis (23, 24).

SCANNING PROTOCOLS AND IMAGE RECONSTRUCTION

Electrocardiography gated cardiac CT was performed by use of a 64 detector-row multidetector CT scanner (Lightspeed VCT, General Electric Medical Systems). Data acquisition and image reconstruction were performed as previously described (22). Briefly, the voltage and current of the x-ray tube in the scanner were 120 kV and 250–550 mA, respectively, and the matrix size in the X-Y plane was 512 × 512 pixels. The calculated total radiation dose was approximately 4–11 mSv (25, 26). Nonionic iodinated contrast medium (2 mL/kg Ultravist 370; Schering) was delivered via a power injector to all patients with 80% of the maximally allowable injection rate. Image reconstruction was performed with thin-slab reformatted images to identify the coronary artery segments. The left main coronary artery was identified first. After that, the left anterior descending coronary artery, left circumflex coronary artery, and right coronary artery were sequentially evaluated (27).

INTERPRETATION AND QUANTIFICATION OF CAL

Two image readers, with 12 years (the second author, S-J.C.) and 5 years (the eighth author, W.-J.L.) of experience in cardiac CT, assessed the CT images together to confirm identification of the coronary artery segments of interest and their measurements. In each patient, the severity of the CAL was evaluated using 4 parameters: (a) the number of coronary aneurysms, (b) the calcium burden of the coronary arteries (28),

Matrix Remodeling in Young Adults with Prior KD

(c) the extent of thrombus in the coronary aneurysms, and (d) the extent of stenosis of the coronary arteries. The calcium burden of the coronary artery was calculated by using the Agatston scoring algorithm, and the sum of the scores of all coronary arteries in a patient represented the total calcium burden of this patient (28). To define the extent of coronary thrombosis, we scored 1 point for the presence of any thrombus in each coronary aneurysm and 2 points for the presence of thrombosis occupying the whole aneurysm and summed these points over all coronary aneurysms to give a total score for coronary thrombosis in a patient. To define the extent of stenosis of the coronary arteries, we scored 1 point for the presence of any luminal narrowing >50% but <100% of the adjacent coronary segment, and 2 points for the presence of total occlusion of each coronary segment and summed all these scores to give a total score for coronary stenosis in a patient.

BIOCHEMICAL ANALYSIS
Venous blood samples were collected at the time of clinical examination and stored at −80 °C until analysis. Serum total cholesterol, HDL cholesterol (HDL-c), LDL-c, triglyceride, aspartate aminotransferase, and alanine aminotransferase were determined enzymatically by use of standard laboratory procedures. High-sensitivity C-reactive protein (hs-CRP) concentrations were measured in the plasma by use of a commercially available high-sensitivity method (Immulite®, Siemens). PIIINP was determined by a coated-tube RIA performed by use of commercial antisera specifically directed against the amino-terminal propeptide (Orion Diagnostica) as described previously (14). The intra- and interassay CVs of plasma PIIINP were <5%. On the basis of our protocol, the assay sensitivity of PIIINP was 0.3 μg/L, which was defined as twice the SD of the zero-binding value. The reference interval of serum PIIINP was 2.3–6.4 μg/L in adults. Plasma MMP-9 and TIMP-1 concentrations were measured by solid-phase sandwich ELISA (R&D). Duplicate measurements were performed and the intra- and interassay CVs were <7%. The assay sensitivity was 0.156 μg/L for MMP-9 and 0.08 μg/L for TIMP-1, as determined by adding 2 SDs to the mean absorbance value of 20 zero-standard replicates and calculating the corresponding concentration.

STATISTICAL ANALYSIS
We performed analysis using SPSS v11.5. Data are presented as mean (SD) or as median with interquartile range for characteristics of study participants, whereas frequencies and percentages (in parentheses) summarize categorical variables. Comparisons among 3 groups were conducted with nonparametric Kruskal–Wallis tests (α = 0.05). For 2-group comparisons, the Mann–Whitney U-test was used. Differences in proportion were tested by χ² analysis. In KD patients the associations between biomarkers of collagen turnover and image findings were assessed by use of Spearman correlation coefficients. Because this study was explorative and the comparisons were being made to discover any association between the biomarkers and image findings and to generate hypotheses that could be evaluated in further studies, adjustments (such as Bonferroni correction) to reduce the probability of type I errors due to multiple testing were not applied. Stepwise linear regression was run to assess the trends between various clinical and biomedical factors associated with quantitative CT-defined factors of CAL. Independent variables included in the multivariate model were age, sex, body mass index, systolic blood pressure, and concentrations of PIIINP and HDL-c.

Results

CLINICAL CHARACTERISTICS
There were 35 KD adolescents and young adults (25 in the KD-NCAL group and 10 in the KD-PCAL group) and 25 age-matched control participants enrolled in this study. Patients with persistent CAL received CT examinations (8 of 10 or 80% of sample) or angiography (7 of 10 or 70% of sample) to confirm the status of the CAL. The CAL defined by the imaging studies included coronary aneurysms (10 of 10), stenosis or occlusion (10 of 10), and calcifications (9 of 10). One 17-year-old patient in the KD-PCAL group reported angina. He received percutaneous transcatheter coronary angioplasty, which relieved the symptom. Medications administered to the patients included antiplatelet agents (n = 9; aspirin in 9 and dipyridamole in 3), warfarin (n = 3), β-blockers (n = 4), and angiotensin-converting enzyme inhibitors (n = 1). No statistically significant differences in body mass index, systolic blood pressure, liver function, or concentrations of triglyceride, HDL-c, and LDL-c were observed across groups (Table 1).

CONCENTRATIONS OF PIIINP, MMP-9, TIMP-1, AND hs-CRP
The concentrations of PIIINP (median, interquartile range) in the KD-PCAL group (11.22 μg/L, 6.73–13.09 μg/L) were significantly higher than those in the KD-NCAL group patients (5.96 μg/L, 5.38–8.74 μg/L) (P = 0.04). Concentrations of PIIINP in both the KD-PCAL and KD-NCAL groups were significantly higher than those in the control participants (5.00 μg/L, 3.75–6.53 μg/L) (P < 0.001, P = 0.002, respectively) (Fig. 1A). Because the quantitative balance between MMP and TIMP plays a central role in matrix breakdown, we
also calculated the MMP-9:TIMP-1 ratios. Although the variability in the data of MMP-9 and TIMP-1 was relatively large in the controls, we found the variability in the MMP-9:TIMP-1 ratio data to be similar in the 3 groups. Patients of both the KD-NCAL and KD-PCAL groups had much lower concentrations of MMP-9 and TIMP-1 and lower MMP-9:TIMP-1 ratios compared to the control participants ($P < 0.05$). Although patients in the KD-PCAL group tended to have even lower MMP-9 and TIMP-1 concentrations than patients in the KD-NCAL group, the differences were not significant (Fig. 1B, C, and D). The median (interquartile range) concentrations of plasma hs-CRP were 0.27 mg/L (0.18–1.05 mg/L) in the control group, 0.28 mg/L (0.21–1.13 mg/L) in the KD-NCAL group, and 0.24 mg/L (0.14–0.87 mg/L) in the KD-PCAL group. No significant differences in the hs-CRP concentrations were detected among the 3 groups ($P > 0.05$).

### The Concentrations of PIIINP and Their Relationship with CT-Imaging Findings

Fourteen patients (8 from the KD-PCAL group and 6 from the KD-NCAL group) underwent both CT examinations and evaluation of the plasma concentrations of MMP-9, TIMP-1, and PIIINP. Of 25 KD patients who never had coronary involvement, 6 patients (24%) received CT examination, 11.2 to 21 years after the KD onset, because they suffered from intermittent chest pain that could not be explained by results of other noninvasive evaluations. None of the 6 examinations showed abnormal findings with regard to size of coronary arteries, luminal stenosis, and intravascular thrombi. In these 6 patients, the median (interquartile range) diameters of the left main coronary artery and right coronary artery were 4.05 mm (3.5–4.1 mm) and 3.45 mm (3.0–3.5 mm), respectively.

The relationship between these biomarkers of matrix remodeling and parameters representing coronary abnormalities are shown in Table 2. We found that the concentrations of PIIINP best reflected coronary abnormalities and were highly correlated to the CT parameters for CAL. The CT imaging of CAL and the concentrations of PIIINP of the 14 KD patients are summarized in Table 3. The PIIINP concentrations correlated well with the coronary stenosis scores ($r = 0.72$, $P = 0.011$) and the coronary thrombus scores ($r = 0.64$, $P = 0.014$) (Fig. 2A, 2B). Further trend tests using stepwise linear regression analysis also showed that after adjustment for age, sex, body mass index, systolic blood pressure, and concentrations of HDL-c, increasing concentrations of PIIINP were strongly associated with the severity of CAL assessed by CT, including the coronary stenosis score ($P = 0.03$) and coronary thrombus score ($P = 0.05$). To elucidate whether the imaging parameters were related and would confound the analysis, correlation analysis between each parameter was performed and revealed a high correlation between coronary arterial stenosis and coronary thrombi ($R = 0.962$, $P < 0.001$).

### Table 1. Clinical characteristics of subgroups with KD and controls.\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 25)</th>
<th>KD-NCAL(^b) (n = 25)</th>
<th>KD-PCAL (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male, n</td>
<td>11 (44%)</td>
<td>16 (64%)</td>
<td>7 (70%)</td>
<td>NS</td>
</tr>
<tr>
<td>Age, years</td>
<td>17.4 (1.8)</td>
<td>18.4 (2.8)</td>
<td>18.6 (3.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Age at KD diagnosis, years</td>
<td>N/A</td>
<td>3.2 (3.2)</td>
<td>3.3 (3.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Interval after KD onset, years</td>
<td>N/A</td>
<td>14.7 (5.7)</td>
<td>14.5 (4.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.63 (0.07)</td>
<td>1.70 (0.09)</td>
<td>1.70 (0.08)</td>
<td>NS</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>54.9 (9.4)</td>
<td>61.1 (15.7)</td>
<td>65.3 (12.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>20.7 (2.4)</td>
<td>21.2 (3.7)</td>
<td>22.6 (3.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>99 (12)</td>
<td>105 (12)</td>
<td>110 (11)</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>60 (10)</td>
<td>65 (6)</td>
<td>64 (7)</td>
<td>NS</td>
</tr>
<tr>
<td>Aspartate aminotransferase, U/L</td>
<td>28 (4)</td>
<td>25 (4)</td>
<td>24 (3)</td>
<td>NS</td>
</tr>
<tr>
<td>Alanine aminotransferase, U/L</td>
<td>19 (3)</td>
<td>21 (4)</td>
<td>22 (4)</td>
<td>NS</td>
</tr>
<tr>
<td>Tchol, mmol/L</td>
<td>4.47 (0.81)</td>
<td>4.01 (1.21)</td>
<td>4.56 (0.92)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-c, mmol/L</td>
<td>1.67 (0.35)</td>
<td>1.34 (0.50)</td>
<td>1.22 (0.31)</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-c, mmol/L</td>
<td>2.48 (0.71)</td>
<td>2.35 (0.88)</td>
<td>2.65 (0.78)</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.63 (0.26)</td>
<td>0.71 (0.53)</td>
<td>0.85 (0.50)</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^a\) Results are expressed as n (%) or mean (SD).

\(^b\) NCAL, no CAL since the onset of KD; PCAL, persistent CAL since the onset of KD; NS, nonsignificant; N/A, not available; BP, blood pressure; Tchol, total cholesterol.
Discussion

This is the first study of the biomarker profile of the extracellular matrix in KD adult patients more than 10 years after disease onset. We report several novel findings. The index of collagen synthesis, PIIINP, was increased and the concentrations of MMP-9, TIMP, and the MMP-9:TIMP ratio were diminished in KD adolescents and young adults, especially in patients with persistent CAL. We also found that in patients with KD, the concentrations of PIIINP were closely associated with quantifiable characteristics of severity of CAL (coronary stenosis/occlusion and coronary thrombosis). In patients without gross coronary complications, collagen turnover was still abnormal. Together these findings suggest that late after the onset of KD, collagen turnover is still abnormal.

Table 2. Correlation analysis between makers of matrix remodeling and the 4 parameters obtained from CT imaging findings.¹

<table>
<thead>
<tr>
<th></th>
<th>MMP-9</th>
<th>TIMP-1</th>
<th>Ratio</th>
<th>PIIIINP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of aneurysms</td>
<td>$R = -0.323$  $P = 0.259$</td>
<td>$R = -0.591$  $P = 0.026$</td>
<td>$R = -0.08$  $P = 0.878$</td>
<td>$R = 0.590$  $P = 0.026$</td>
</tr>
<tr>
<td>Calcium A-J score</td>
<td>$R = -0.126$  $P = 0.668$</td>
<td>$R = -0.515$  $P = 0.059$</td>
<td>$R = 0.057$  $P = 0.846$</td>
<td>$R = 0.579$  $P = 0.03$</td>
</tr>
<tr>
<td>Stenosis score</td>
<td>$R = -0.552$  $P = 0.041$</td>
<td>$R = -0.610$  $P = 0.02$</td>
<td>$R = -0.392$  $P = 0.166$</td>
<td>$R = 0.720$  $P = 0.011$</td>
</tr>
<tr>
<td>Thrombosis score</td>
<td>$R = -0.535$  $P = 0.049$</td>
<td>$R = -0.641$  $P = 0.013$</td>
<td>$R = -0.359$  $P = 0.207$</td>
<td>$R = 0.640$  $P = 0.014$</td>
</tr>
</tbody>
</table>

¹ $R$, correlation coefficient.
turnover is still far from normal and may be related to the severity of CAL, microangiopathy, and myocardial fibrosis.

As described in the pathological reports of KD patients, active remodeling processes, including recanalization, narrowing, thrombosis, and calcification, continued in CAL many years after onset of KD (10–12). In stenotic areas distal to aneurysms, abundant vascular smooth muscle cells are associated with vascular thrombosis. These cells express growth factors such as transforming growth factor β1 and platelet-derived growth factor. In addition to being proinflammatory, transforming growth factor β1 is an important profibrotic cytokine that leads to an increased amount of extracellular matrix, including collagen III (29). In patients without gross CAL, the coronary vessels and myocardium also revealed some degree of intimal thickening, myocardial fibrosis, microvascular thrombosis, and microangiopathy, with abundant production of collagen and fibrin (12). These observations may account for pathological mechanism(s) leading to increased PIIINP concentrations in our patients, to a marked degree in those with persistent CAL and to a modest degree in those without.

The collagen turnover process involves a balance between collagen production and degradation. MMP and TIMP are major enzymes controlling collagen degradation. Previous studies on KD patients have demonstrated that increased concentrations of MMP-9 and increased MMP:TIMP ratio in the acute stage of KD are associated with CAL at the later convalescent stage (17, 18). Whether these extracellular matrix-

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Group</th>
<th>Number of aneurysms</th>
<th>Calcium A-J score</th>
<th>Stenosis score</th>
<th>Thrombus score</th>
<th>PIIINP concentration, µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PCAL</td>
<td>2</td>
<td>574</td>
<td>3</td>
<td>2</td>
<td>5.42</td>
</tr>
<tr>
<td>2</td>
<td>PCAL</td>
<td>1</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>6.43</td>
</tr>
<tr>
<td>3</td>
<td>PCAL</td>
<td>1</td>
<td>1784</td>
<td>3</td>
<td>1</td>
<td>6.82</td>
</tr>
<tr>
<td>4</td>
<td>PCAL</td>
<td>1</td>
<td>867</td>
<td>3</td>
<td>1</td>
<td>11.07</td>
</tr>
<tr>
<td>5</td>
<td>PCAL</td>
<td>1</td>
<td>140</td>
<td>4</td>
<td>2</td>
<td>11.37</td>
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<tr>
<td>6</td>
<td>PCAL</td>
<td>2</td>
<td>1469</td>
<td>3</td>
<td>1</td>
<td>12.96</td>
</tr>
<tr>
<td>7</td>
<td>PCAL</td>
<td>2</td>
<td>887</td>
<td>2</td>
<td>1</td>
<td>13.49</td>
</tr>
<tr>
<td>8</td>
<td>PCAL</td>
<td>2</td>
<td>352</td>
<td>5</td>
<td>5</td>
<td>16.07</td>
</tr>
<tr>
<td>9–14</td>
<td>NCAL</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.9–8.0</td>
</tr>
</tbody>
</table>

* PCAL, persistent coronary arterial lesion since the onset of KD; NCAL, no coronary arterial lesions since the onset of KD.

Fig. 2. Correlation between concentrations of PIIINP and score of (A) coronary stenosis and (B) score of coronary thrombus.
controling enzymes remain abnormal or not during late follow-up has never been investigated, however. In the present study, we demonstrated alterations in extracellular matrix indices in adult KD patients (i.e., increased PIIINP and decreased MMP-9 and TIMP concentrations and decreased MMP-9:TIMP-1 ratios). The profiles of MMP-9 and TIMP-1 years after diagnosis were distinct from those observed during the acute stage, indicating that the degradation of the extracellular matrix was ameliorated owing to decreased enzyme activities with damped tissue inhibitor. Collagen accmulation and fibrotic processes continued to progress in the KD adult patients late after disease onset, even in those without gross CAL initially. In those with persistent CAL, such alterations were augmented owing to the vascular remodeling in the coronary aneurysms. Although the PIIINP was significantly higher in the KD-PCAL group, however, there were no significant differences in data of MMP-9, TIMP-1, and MMP-9:TIMP-1 between the KD-NCAL and KD-PCAL groups. We suspect that coexisting general arterial problems outside the coronary arteries may also have contributed to the altered extracellular matrix, but this possibility needs to be investigated in further studies.

In a study of adult patients with coronary arterial disease but without prior myocardial infarction, concentrations of PIIINP were positively correlated with number of diseased vessels (30). In our 14 KD patients, this positive correlation was replicated between concentrations of PIIINP and severity of CAL, namely the scores of coronary stenosis/occlusion and the scores of coronary thrombosis. Because only 14 of 35 (40%) of all enrolled KD patients received CT examination, the data for correlation analysis may not be representative of the data of the whole KD population; nonetheless, this relationship is still likely to be informative for therapeautic intervention and may offer the potential of applying the PIIINP assay to evaluate the severity of CAL in patient follow-up. Because this study was a cross-sectional case-control study on a relatively small group of patients and therefore was exploratory, however, the calculated levels of significance for individual comparisons should be interpreted with caution. It is still early to conclude that PIIINP is an effective biomarker for assessing severity of CAL during long-term follow-up. The efficacy of this marker must be further validated in large-scale, longitudinal follow-up studies of changes of PIIINP in KD patients.

We also found a close correlation between the extent of coronary stenosis and thrombi. This finding may indicate that optimized anticoagulation was not achieved in the present study population. Whether increased PIIINP in patients with coronary thrombi was due to the presence of thrombi per se or was secondary to the presence of coronary stenosis also needs to be clarified.

The role of low-grade inflammation during late stages of KD is controversial. Interestingly, we did not observe any difference in the hs-CRP among the KD-PCAL, KD-NCAL, and control groups. This result was different from the results of 2 previous studies (8, 9), which demonstrated that increased concentrations of hs-CRP were associated with persistent CAL in KD patients. The mean intervals from diagnosis to time of study in these 2 studies were 10.8 years (8) and 7.8 years (9). In the present study, the age of the study cohort was a bit older (mean 18.5 years), and the intervals between the onset of KD and age at study were relatively longer (mean 14.6 years). If this discrepancy in observed hs-CRP results is related to age, it may be possible that the role played by inflammation during the remodeling process of CAL decreases with age and becomes less important during adulthood in KD patients. In adults with atherosclerosis in which inflammation is a key feature, the changes of MMP-9 usually parallel the changes of hs-CRP and other inflammation indices, such as interleukin 6, interleukin 18, and CD40 ligand (31). In our adult KD patients, concentrations of MMP-9 decreased and hs-CRP did not change significantly. These observations may indicate that the role of inflammation is less important in adult patients late after the onset of KD. Pathological studies of KD patients have also provided evidence suggestive of a relatively limited role of inflammation. The changes in the wall of coronary aneurysms consisted of abundant smooth muscle cells and thickened intima but only scanty macrophages, or fatty streaks (10–12).

In conclusion, we demonstrated alterations in extracellular matrix biomarkers (increased PIIINP and decreased MMP-9 and TIMP-1 concentrations and decreased MMP-9:TIMP-1 ratios) indicating enhanced collagen synthesis and ameliorated degradation in adult patients late after the onset of KD. In KD patients, there is a possible association between the concentrations of PIIINP and the most important coronary lesions, stenosis/occlusion and thrombosis. This finding must be confirmed in further studies. Abnormal collagen turnover was still noted in KD patients without a history of CAL.

Our study had several limitations. First, we did not perform endomyocardial biopsy to document whether myocardial fibrotic processes were present. Second, we adopted the criteria of CALs suggested by the Japanese Ministry of Health instead of the z-scores of the coronary arteries and may have underdiagnosed the patients with only mild coronary dilation. We therefore excluded patients with only mild coronary dilation from both the KD-NCAL and KD-PCAL groups in this study to minimize the possibility of missed mild coro-
nary dilation during the acute phase in the KD-NCAL group. Third, the number of cases studied was relatively small and the study therefore may have been susceptible to type I or type II errors in statistical analysis. Future larger-scale and longitudinal studies are needed to validate the findings of the present study.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Acknowledgments: This study was supported by a grant from the Children’s Cardiac Foundation of the Republic of China (CCF06-02) and a grant from Nation Science Council (NSC-96-2314-B-002-046-MY2).

Conflict of Interest: None declared.

Role of Sponsor: None declared.

References


