

# Biomarkers of inflammation and fibrosis in young adults with history of Kawasaki disease <sup>☆</sup>

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## ABSTRACT

**Background:** Myocardial histology from autopsies of young adults with giant coronary artery aneurysms following Kawasaki disease (KD) shows bridging fibrosis beyond the territories supplied by the aneurysmal arteries. The etiology of this fibrosis is unknown, but persistent, low-level myocardial inflammation and microcirculatory ischemia are both possible contributing factors. To investigate the possibility of subclinical myocardial inflammation or fibrosis, we measured validated biomarkers in young adults with a remote history of KD.

**Methods:** We measured plasma calprotectin, galectin-3 (Gal-3), growth differentiation factor-15 (GDF-15), soluble ST2 (sST2), and serum procollagen type 1C-terminal propeptide (P1CP) in 91 otherwise healthy young adults with a remote history of KD and in 88 age-similar, healthy controls. KD subjects were stratified by coronary artery aneurysm (CAA) status and history of remote myocardial infarction (MI).

**Results:** After correction for multiple testing, calprotectin, Gal-3, and GDF-15 levels were significantly higher in subjects with persistent CAA (n = 26) compared with KD subjects with remodeled CAA (n = 20, p = 0.005, 0.001, 0.0036, respectively). In a multivariable regression model with CA status as the main predictor and adjusting for sex, MI history, and interval from KD onset, CA status was a significant predictor (Persistent CAA vs KD Normal CA) of calprotectin, Gal-3, GDF-15 and sST2 levels (p = 0.004, <0.001, 0.007, and 0.049, respectively).

**Conclusions:** These results suggest that ongoing inflammation and fibrosis may be occurring in individuals with persistent CAA. Longitudinal follow-up is needed to clarify the clinical significance of these elevated biomarker levels in this patient population that requires life-long monitoring.

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## 1. Introduction

The most significant complications of acute Kawasaki disease (KD) are coronary artery aneurysms (CAA) that occur in 15–25% of untreated patients and 3–5% treated with intravenous immunoglobulin (IVIG) [1]. Once aneurysms have formed, they can either remodel, persist, or enlarge over time [2]. Little is known about the long-term outcomes of patients who follow these three

different paths. In addition to the coronary vasculitis, myocarditis is a universal feature of the acute illness that is rarely clinically apparent but has been documented by endomyocardial biopsy [3]. We recently reported that active inflammation in adult patients with a history of KD with giant CAA was detected by molecular profiling using shotgun proteomics, transcriptomics, and glycomics [4]. These individuals showed elevated levels of calprotectin, a marker of inflammation secreted by neutrophils and monocytes, while adults with a history of KD but no CAA had no signature of inflammation. Concerns regarding persistent myocardial inflammation have been raised by reports of myocardial fibrosis in explanted hearts from KD patients requiring transplant and from autopsy cases of young adults following KD in childhood

<sup>☆</sup> These authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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[5–6]. Histology showed diffuse, bridging fibrosis that was not confined to territories supplied by the affected coronary arteries [6]. In addition, murine models of KD have also shown accelerated myocardial fibrosis following adrenergic stimulation [7]. Detection of myocardial fibrosis or persistent inflammation in living patients would likely alter patient management.

To evaluate the extent of global cardiac fibrosis by non-invasive methods, several studies have focused on cardiac magnetic resonance imaging (CMRI), but the application of CMRI techniques including T1 mapping has largely failed to demonstrate abnormalities in KD patients in the absence of known ischemic events [8–10].

There is controversy regarding how well plasma biomarkers predict the histologic finding of myocardial fibrosis [11]. In the adult heart failure literature, the carboxy-terminal propeptide of procollagen type I (PIPC) has been used as a biomarker of myocardial fibrosis [12]. Increased serum PIPC levels correlate with adverse outcomes in heart failure and myocardial infarction [13–14]. Other candidate biomarkers implicated in both myocardial fibrosis and inflammation include soluble suppressor of tumorigenicity 2 (sST2), galectin-3 (Gal-3), growth differentiation factor-15 (GDF-15), and calprotectin [4,12]. We postulated that the subset of the KD patients with persistent giant CAA in adulthood would have elevated levels of biomarkers of myocardial fibrosis with or without evidence of inflammation.

## 2. Methods

### 2.1. Study population

**KD subjects:** We enrolled 91 subjects  $\geq 15$  years of age into the San Diego Adult KD Collaborative Study over the 11-year period from 2008 through 2018. Of these 91 subjects, 71 (78%) had been followed since the time of diagnosis by the pediatric KD team at Rady Children's Hospital San Diego while 21 (22%) subjects were diagnosed in childhood at other institutions and traveled to UCSD to join the San Diego Adult KD Collaborative study. Demographic data, medical history, and laboratory data were obtained from the medical record. Early family history of coronary artery disease (CAD) was defined as CAD in a first-degree relative before 60 years of age. Hyperlipidemia was defined as low-density lipoprotein cholesterol  $> 160$  mg/dL or physician-documented history of hyperlipidemia. Hypertension was defined as a physician-documented history of high blood pressure. The worst CA Z-score was defined as the largest internal diameter during the first year after KD onset of the right coronary artery (RCA) and left anterior descending coronary artery (LAD) normalized for body surface area and expressed as standard deviation units from the mean [15]. Giant CAA was defined as a Z-score  $\geq 10$  or an absolute dimension of  $> 8$  mm and an aneurysm was defined as a Z-score  $\geq 2.5$  and  $< 10$  or a segment with a lumen dimension that was 1.5 times the adjacent segment [16]. A remodeled CAA was defined as normalization of the arterial lumen by echocardiography (CA Z-score  $< 2.5$ ), CT angiography, or invasive angiography after prior evidence of CAA.

**Healthy controls:** We obtained blood samples from 88 young adult healthy volunteers who were medical students with no significant past medical history. (Table 1) The study was reviewed and approved by the Institutional Review Board at the University of California San Diego and parents and subjects signed informed consent or assent documents as appropriate.

### 2.2. Sample collection and assays

Blood samples from KD subjects were collected at convalescent time points (median: 14.5 years; range: 0.9–55.0 years post-KD

onset). Blood was collected and separated immediately by centrifugation and stored at  $-80$  °C. We measured EDTA plasma levels of calprotectin, Gal-3, sST2, GDF-15, and serum levels of PIPC by ELISA according to the manufacturer's instructions: calprotectin, Gal-3, GDF-15: R & D Systems, Minneapolis, MN, USA, sST2: Critical Diagnostics, San Diego, CA, USA and PIPC: Quidel, San Diego, CA, USA.

### 2.3. Statistical analysis

For each biomarker, a descriptive analysis was conducted with mean (standard error of the mean (SEM)) reported for continuous variables and a frequency table for categorical variables. Kruskal-Wallis test was performed for continuous variables and Fisher's exact test was performed for categorical variables for the comparison among the four CA status groups. For each biomarker outcome, a Kruskal-Wallis test was performed for the comparison among the four CA status groups. Wilcoxon Rank Sum tests were performed for the six pairwise comparisons. The Bonferroni critical p-value for each pairwise comparison was  $0.05/6 = 0.0083$ . Multi-variable regression analyses were performed for each biomarker. Each biomarker was log-transformed due to a non-Gaussian distribution. KD CA status was the main predictor (a variable with three categories: Normal CA, Persistent, and Remodeled; Normal CA was the reference group) in the models, adjusting for sex, MI history, and interval from KD onset. A p-value  $< 0.05$  was considered statistically significant unless otherwise specified except for the pairwise comparison for which the critical p value was 0.0083. Data were analyzed using R software version 3.5.2 (<http://www.r-project.org>).

## 3. Results

Among the four patient groups, the subjects with persistent giant CAA were older and had a longer interval between KD onset and study participation ( $p < 0.001$  and  $p = 0.01$ , respectively) (Table 1). There was no significant difference across the groups with respect to the frequency of traditional risk factors for atherosclerosis. Only 8/30 (26.7%) subjects with current ( $n = 25$ ) or remodeled ( $n = 5$ ) giant CAA were taking a statin medication at the time of phlebotomy for the study (Table 1). These subjects were treated with a statin not because of hyperlipidemia, but for the known anti-inflammatory effects of statins and the putative beneficial effect seen in *ex vivo* experiments with KD sera and human umbilical vein endothelial cells [17].

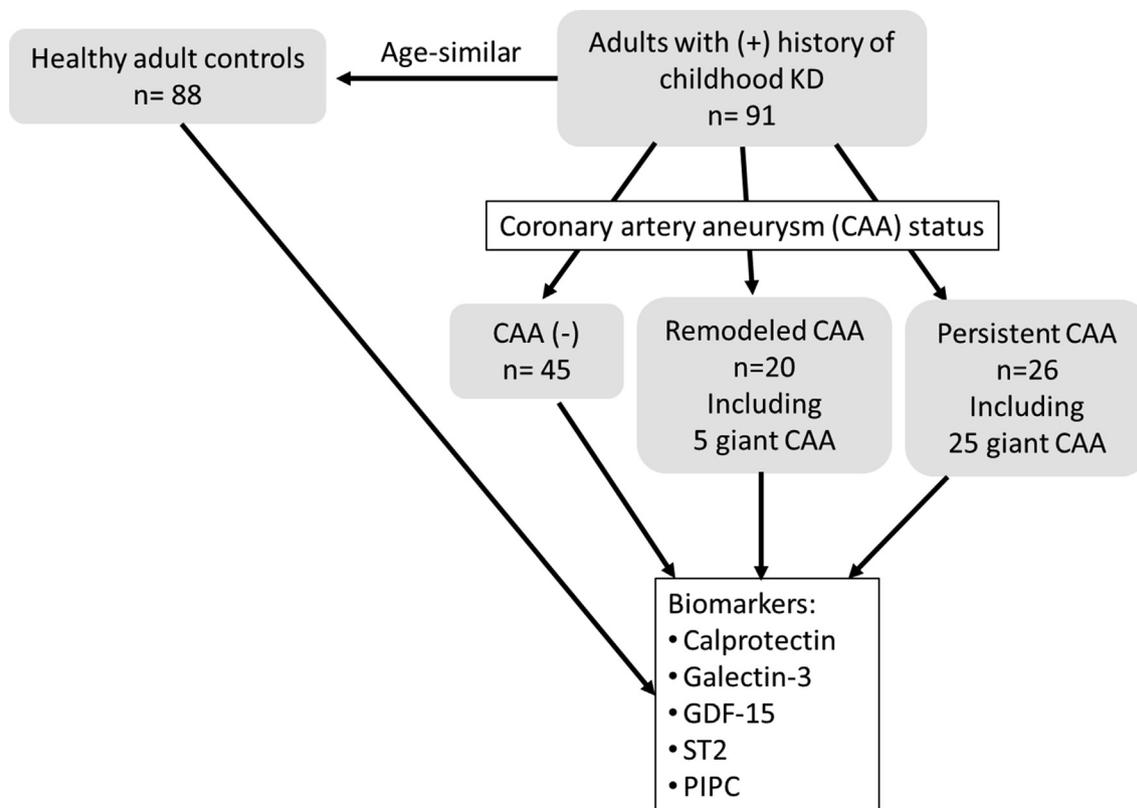
Of the 91 adults with a history of KD in childhood, 45 (49.5%) never had aneurysms (Z score  $< 2.5$ ), 16 (17.5%) had CAA (Z score 2.5–10), and 30 (33.0%) had giant CAA. (Fig. 1) Of the subjects with CAA, 20 (15 with CAA, 5 with giant CAA) had remodeled their CAA to a Z-scored internal diameter  $< 2.5$  as determined by echocardiography or computed tomography angiography by the time of their participation in this study. (Table 2) This interesting group of remodeled patients was characterized in many cases by rapid resolution of their CAA: 13 of the 14 evaluable patients normalized their internal dimension within 6 months after KD onset. The exact timing of remodeling could not be determined in six subjects because of gaps in the serial echocardiograms due to patients being transiently lost to follow up. As a group, these remodeled subjects did not differ with respect to age at onset, sex, or ethnicity compared to the subjects with persistent CAA.

In the univariate analysis, for the comparison across the four CA status groups, calprotectin, Gal-3, GDF-15 and PIPC showed a significant difference among the groups (Table 3). From the pairwise comparisons (with a critical p value threshold of 0.0083 after adjusting for multiple comparisons), calprotectin, Gal-3, and

**Table 1**  
Demographic and clinical characteristics in adult KD and healthy adult control cohorts stratified by coronary artery status.

Characteristic	HC (n = 88)	Normal CA (n = 45)	Remodeled (n = 20)	Persistent (n = 26)	P value
Age at KD onset, years	–	4.8 (2.2–7.3)	3.4 (0.9–5.7)	5.0 (1.5–13.4)	NS
Age at sample collection, years	22.2 (20.8–24.3)	17.9 (16.5–20.2)	17.5 (16.5–19.3)	25.4 (19.6–35.8)	<0.001
Male, %	47.7	53.3	70.0	65.4	NS
Interval from KD onset, years	–	13.6 (8.8–17.4)	15.5 (8.8–17.9)	17.3 (8.9–30.8)	0.01
BMI	NA	22.7 (19.0–26.5)	21.8 (19.4–22.7)	24.0 (21.1–26.0)	NS
Statin treatment	NA	0	0	8 (30.8)	
Race/ethnicity, n (%)					
Non-Hispanic white	29 (33.0)	26 (57.8)	10 (50.0)	14 (53.8)	0.01
Asian	40 (45.5)	5 (11.1)	6 (30.0)	5 (19.2)	
Hispanic	6 (6.8)	4 (8.9)	3 (15.0)	2 (7.7)	
Others	13 (14.8)	10 (22.2)	1 (5.0)	5 (19.2)	
Cardiovascular risk factors, n (%)					
Current smoking	9 (14.3)	1 (2.4)	2 (10.5)	1 (3.8)	NS
Family history of early CAD	6 (10.0)	10 (24.4)	0	2 (7.7)	NS
Hypertension	no data	2 (4.9)	0	2 (7.7)	NS
Hyperlipidemia	6 (10.0)	3 (7.3)	2 (11.8)	6 (23.1)	NS

Values are median (IQR) or n (%). Kruskal-Wallis test for continuous variables; Fisher’s exact test for categorical variables. HC: healthy adult control; CA: coronary artery.



**Fig. 1.** Flow chart of adult KD biomarker study with description of cohorts. CAA- subjects were KD subjects with normal CA dimensions on all echocardiograms. Remodeled CAA subjects had aneurysms that subsequently remodeled to an internal dimension of the CA < 2.5 S.D. units below the mean normalized for body surface area.

GDF-15 levels were significantly higher in subjects with persistent CAA (n = 26) compared with healthy controls (n = 88, p < 0.001, p < 0.001, p = 0.0027, respectively) and subjects with normal coronary arteries (CA) following KD (n = 45, p ≤ 0.001 for all three measures). Subjects with persistent CAA (n = 26) also had significantly higher levels of calprotectin, Gal-3, and GDF-15 compared with subjects with remodeled CAA (n = 20, p = 0.005, 0.001, 0.0036, respectively). PIPC was elevated in all CAA subjects regardless of remodeling compared with KD with normal CA and healthy controls. (Table 3 and Fig. 2). When the distribution of biomarkers was examined in subjects with persistent CAA, the majority of the values for calprotectin, Gal-3, and GDF-15 were in the highest quartile. (Fig. 3)

In multivariable regression analyses with CA status as the main predictor (with three categories: Normal CA, Persistent and Remodeled; Normal CA as the reference group), adjusting for sex, MI history, and interval from KD onset, in which each biomarker outcome (calprotectin, Gal-3, GDF-15, PIPC, and sST2) was analyzed separately, CA status was a significant predictor (Persistent CAA vs Normal CA) of calprotectin, Gal-3, GDF-15 and sST2 levels (p = 0.004, <0.001, 0.007, and 0.049, respectively)(Table 4). For the adjusting predictors, interval from KD onset was a significant predictor of GDF-15 and PIPC levels (p < 0.001 for both measures) and sex was a significant predictor of PIPC and sST2 (p = 0.008, 0.032, respectively) (Table 4). Because male sex and younger age are associated with higher levels of PIPC due to linear growth,

**Table 2**

Characteristics of the 20 KD subjects who remodeled their coronary artery aneurysms based on echocardiographic assessment of coronary arteries yielding a Z score < 2.5 S. D. units. Subjects are in order of descending worst Z score.

Age at onset (yrs)	Acute treatment	Worst Z Score*	Days until Z worst < 2.5
0.1	IVIG, ASA, warfarin	26.5	Gap
0.9	IVIGx3, steroid pulse x2, cyclophosphamide	20.9	Gap
2.4	Diagnosed 1 month after onset	18.4	Gap
1.6	ASA, persantine (no IVIG)	17.3	Gap
0.9	IVIG, ASA, persantine, PTX, warfarin	10.3	Gap
3.7	ASA, IVIG	9.0	286
0.5	ASA, IVIG	6.6	60
4.4	ASA, IVIG	5.4	160
13	ASA, IVIG	5.0	63
5.3	ASA, IVIG	5.0	83
0.7	ASA, IVIG	4.8	82
1.1	ASA, IVIG	4.6	12
15	ASA, IVIG, IFX	4.2	28
0.3	IVIG, ASA	4.2	17
11.3	ASA, IVIG	3.9	12
5.7	ASA, IVIG	3.3	24
3.1	ASA, IVIG	3.1	23
5.7	ASA, IVIG	3.0	57
2	ASA, IVIG	3.0	Gap
4.4	ASA, IVIG	2.6	24

\*Worst Z score: largest internal dimension of the coronary arteries expressed as standard deviation units from the mean normalized for body surface area. ASA: aspirin, IVIG: intravenous immune globulin, IFX: infliximab, PTX: pentoxifylline. Gap: consecutive echocardiographic follow-up with a gap of more than 1 year.

we tested the correlation between age at phlebotomy, sex, and PIPC levels and found that indeed sex and age at phlebotomy were significantly correlated with PIPC ( $p < 0.001$  and  $p = 0.0002$ , respectively).

#### 4. Discussion

This study explored biomarkers of inflammation and fibrosis in subsets of KD subjects years after disease onset and found elevated levels of calprotectin, Gal-3, GDF-15, and PIPC, almost exclusively in KD subjects with persistent CAA. PIPC levels were also elevated in KD subjects with remodeled CAA. Surprisingly, subjects who remodeled their CAA, including five subjects with documented giant CAA in childhood, had favorable biomarker profiles with the exception of PIPC, which was elevated compared to KD subjects with normal CA and healthy controls. Statin use was documented at the time of phlebotomy in only eight subjects with CAA, many of whom had elevated biomarkers of inflammation despite statin treatment (Fig. 2). In univariate analyses, calprotectin, Gal-3, and GDF-15 were significantly higher in the persistent CAA subjects compared to healthy controls and KD subjects with normal CA or remodeled CAA. Persistent CAA status was an independent predictor of elevated levels of calprotectin, Gal-3, sST2, and GDF-15.

**Table 3**

Biomarker concentrations in adult KD and healthy adult control cohorts stratified by coronary artery status.

	HC (n = 88)	Normal CA (n = 45)	Remodeled (n = 20)	Persistent (n = 26)	P value
Biomarkers					
Calprotectin, ng/ml	536.5 (80.7)	569.6 (87.2)	563.9 (120.3)	2256.2 (821.0)	<0.001
Galectin-3, ng/ml	5.7 (0.2)	5.7 (0.3)	6.5 (0.7)	10.7 (1.3)	<0.001
GDF-15, pg/ml	394.4 (15.4)	343.3 (13.5)	361.2 (22.0)	506.8 (38.5)	<0.001
ST2, ng/ml	32.3 (1.6)	31.5 (1.9)	34.6 (4.1)	27.4 (1.3)	NS
PIPC, ng/ml	147.4 (6.7)	175.2 (13.0)	216.7 (20.2)	217.1 (22.5)	<0.001

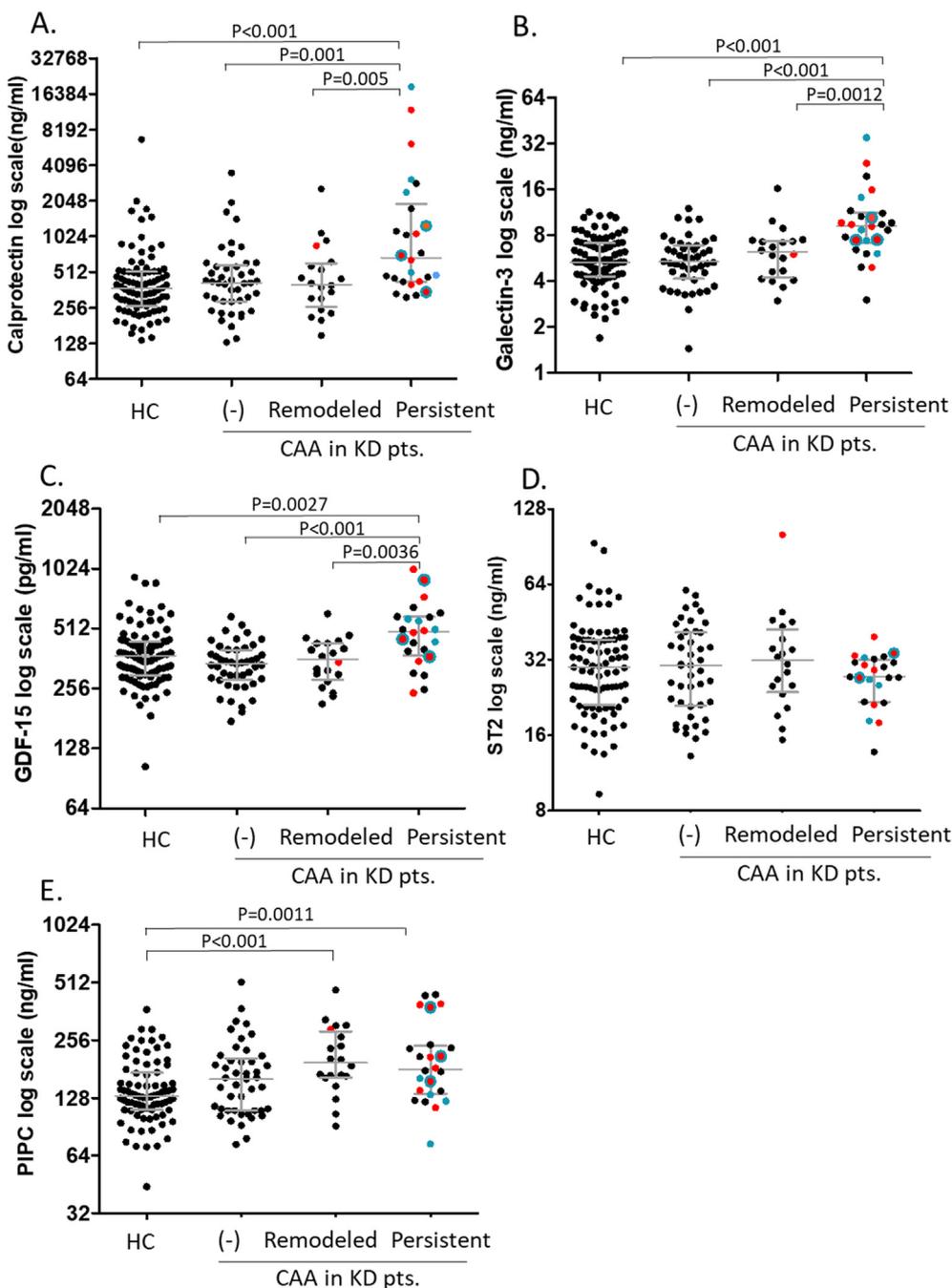
Values are mean (Standard error measurement). P values are from Kruskal-Wallis test.

Calprotectin, the heterodimer of the calcium-binding proteins S100A8 and S100A9, is not only a biomarker of inflammation, but also a mediator of inflammation that has been proposed as a therapeutic target. In a mouse model of ischemic/reperfusion injury, S100a8/a9 mediated cardiomyocyte death via suppression of mitochondrial function [18]. In humans, levels of calprotectin are elevated post-MI and are independently associated with poor outcomes [19]. Calprotectin binds directly to human microvascular endothelial cells and induces an inflammatory response with upregulated expression of chemokines and adhesion molecules [20]. In KD, neutrophils and monocytes, the cellular sources of calprotectin, infiltrate the arterial wall and mediate damage through pro-inflammatory signaling pathways [21]. Calprotectin was identified as a biomarker of persistent inflammation using a proteomic approach in adults and children with giant aneurysms after KD [4]. The finding of elevated levels of calprotectin in otherwise healthy KD subjects with persistent aneurysms suggests that a sub-clinical, smoldering inflammatory response is continuing in these young adults.

Gal-3, a member of the B-galactoside-binding lectin family, is a mediator of both inflammation and fibrosis. It is secreted by activated macrophages and has been used alone or in combination with natriuretic peptides as a biomarker for heart failure prognosis [22]. We previously showed that Gal-3 is expressed in the myocardium and coronary arterial wall of autopsy tissues from KD patients who developed giant coronary artery aneurysms [5]. In these tissues, Gal-3 was expressed in both infiltrating mononuclear cells and spindle-shaped myofibroblast-like cells, suggesting a dual role in both inflammation and fibrosis. The elevated levels in our subjects with persistent CAA suggest that both chronic inflammation and fibrosis may be present.

GDF-15 is a member of the TGF $\beta$  family whose expression is upregulated by oxidative stress. Increased levels of TGF $\beta$  were present in myofibroblast-like cells in the coronary arterial wall from autopsies of KD patients [23]. Elevated levels of GDF-15 after acute coronary syndrome predict negative outcomes including death and are independent risk factors for subclinical atherosclerosis [24–25]. The elevation of GDF-15 in KD patients with persistent CAA has not been previously reported and may be associated with on-going inflammation in the myocardium or arterial wall. Soluble ST2 is induced in response to mechanical stretch and acts as a decoy receptor for IL-33, which inhibits myocardial fibrosis. As such, it has been used as a prognostic indicator in chronic heart failure [26]. In the multivariable analyses, levels of sST2 had a borderline negative association with CAA persistence. Most of the subjects in this group had preserved left ventricular ejection fraction. Healthy females are known to have lower sST2 levels than males, which may explain the association with male sex seen in the multivariable analysis.

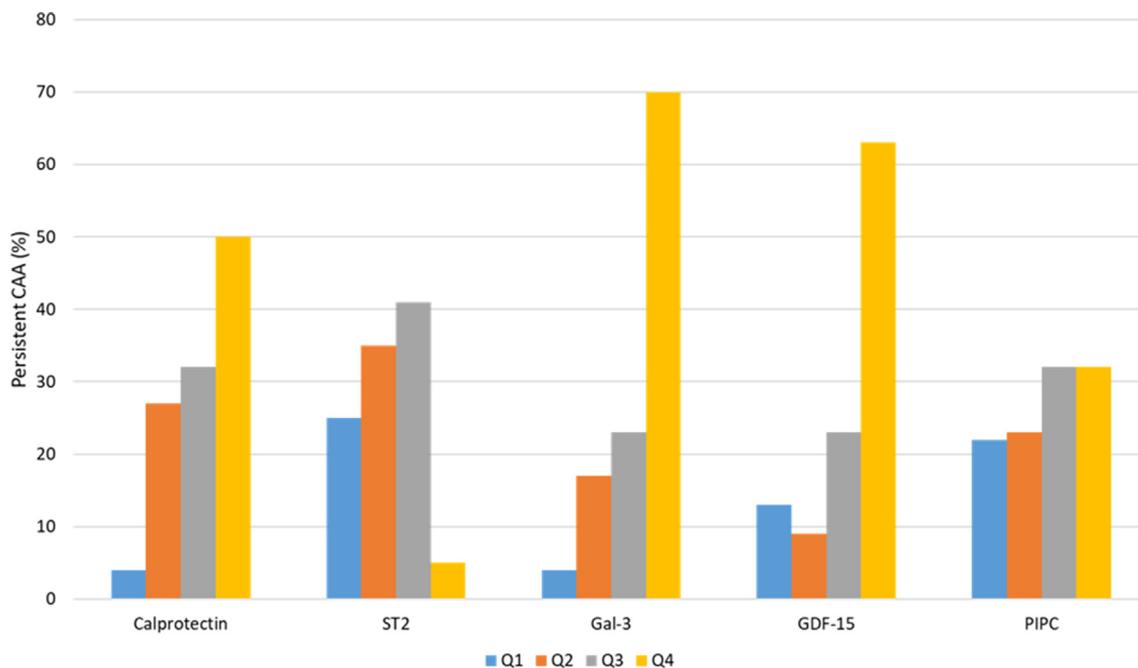
Myocardial and arterial wall fibrosis is associated with excessive crosslinking and deposition of Type 1 collagen and serum PIPC is a validated biomarker of this process [27]. Myocardial fibrosis has been documented by autopsy or histology of explanted hearts in KD patients with giant aneurysms requiring heart transplant



**Fig. 2.** Biomarker levels stratified by coronary artery status. A. Calprotectin, B. Galectin-3 (Gal-3), C. Growth differentiation factor-15 (GDF-15), D. Soluble ST2 (sST2), and E. procollagen I carboxy-terminal propeptide (PIPC). Red closed circle: subjects with history of MI prior to blood collection. Blue closed circle: subjects treated with a statin at the time of phlebotomy. Red circle with blue rim: subjects with a prior history of MI and taking a statin at time of phlebotomy. The critical p value for each pairwise comparison is  $0.05/6 = 0.0083$  using Bonferroni's correction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

[6,28]. Limited studies have shown late gadolinium enhancement by MRA, but the majority of the positive studies were in patients with previous MI [29]. The interval from KD onset was a significant predictor of both GDF-15 and PIPC levels. In the univariate analysis (Table 3 and Fig. 2), all KD patient groups had higher median levels of PIPC regardless of CA status. However, when the model included CA status, sex, MI history, and interval from KD onset, only interval from KD onset and sex were statistically significant for PIPC. Longitudinal studies of biomarkers and imaging in this patient population will help to clarify the meaning of these elevated biomarker levels.

Based on these data and previously published studies of calprotectin and Gal-3 in the acute phase of KD, we propose an evolution of plasma calprotectin and Gal-3 related to the stages of KD [4-5] (Fig. 4). Calprotectin and Gal-3 are initially expressed by inflammatory cells in the CA and myocardium during the acute phase. Subsequently, plasma levels of these biomarkers fall in patients with normal CA or remodeled CAA but remain elevated in patients with persistent CAA and reflect ongoing inflammation and fibrosis. The activation of fibroblasts to increase collagen synthesis and cross-linking results in elevated PIPC levels.



**Fig. 3.** Distribution of biomarker concentrations across quartiles in subjects with persistent coronary artery aneurysms. Quartiles were based on the distribution of values from healthy controls.

**Table 4**

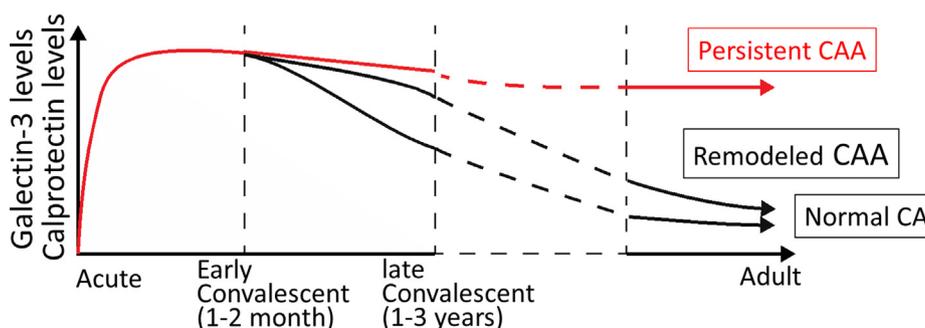
Multivariable regression analysis of subject characteristics predicting biomarker levels. For coronary artery (CA) status (remodeled or persistent), KD subjects with normal CA status were the reference group. For sex, male was the reference group. Each biomarker was log transformed to account for the non-normal distribution.

	Log_Calprotectin				Log_Galectin-3				Log_GDF-15			
	Est	SEM	95% CI	p	Est.	SEM	95% CI	p	Est.	SEM	95% CI	p
Remodeled CAA	-0.022	0.229	(-0.47,0.43)	0.92	0.116	0.120	(-0.12,0.35)	0.33	0.014	0.069	(-0.12,0.15)	0.84
Persistent CAA	0.724	0.245	(0.24,1.2)	<b>0.004</b>	0.537	0.129	(0.28,0.79)	<b>&lt;0.001</b>	0.217	0.078	(0.06,0.37)	<b>0.007</b>
Sex	-0.059	0.186	(-0.42,0.31)	0.75	-0.019	0.098	(-0.21,0.17)	0.85	-0.108	0.057	(-0.22,0.004)	0.06
MI.history	0.305	0.342	(-0.37,0.98)	0.38	0.101	0.180	(-0.25,0.45)	0.58	-0.014	0.106	(-0.22,0.19)	0.90
Interval from KD onset	0.0003	0.001	(-0.0017,0.0023)	0.97	0.0005	0.005	(-0.009,0.01)	0.92	0.016	0.003	(0.01,0.02)	<b>&lt;0.001</b>

	Log_ST2				Log_PIPC			
	Est.	SEM	95% CI	p	Est.	SEM	95% CI	p
Remodeled CAA	0.030	0.099	(-0.16,0.22)	0.76	0.194	0.107	(-0.02,0.04)	0.07
Persistent CAA	-0.222	0.111	(-0.44,-0.004)	<b>0.049</b>	0.213	0.119	(-0.02,0.45)	0.08
Sex	-0.178	0.082	(-0.34,-0.02)	<b>0.032</b>	-0.241	0.089	(-0.42,-0.07)	<b>0.008</b>
MI.history	0.133	0.158	(-0.18,0.44)	0.40	0.272	0.163	(-0.05,0.59)	0.10
Interval from KD onset	0.0076	0.0044	(-0.001,0.016)	0.09	-0.019	0.005	(-0.03,-0.01)	<b>&lt;0.001</b>

Est.: estimate; SEM: standard error of the mean.



**Fig. 4.** Proposed evolution of plasma calprotectin and galectin-3 relative to the stages of KD. Calprotectin and galectin-3 are initially expressed by inflammatory cells in the CA and myocardium during the acute phase. Plasma levels of calprotectin and galectin-3 remain elevated in patients with persistent CAA and reflect ongoing inflammation, which activates fibroblasts to increase collagen synthesis and cross-linking.

We recognize strengths and limitations to our study. This is the first study to compare biomarkers of inflammation and fibrosis among convalescent KD subjects with different CA outcomes. The results have potential implications for the monitoring and treatment of this patient population. An important limitation of the study is the absence of CMRI or tissue level validation of inflammation and fibrosis as suggested by the elevated biomarker levels in subjects with persistent CAA. To date, CMRI with T1 weighting or late gadolinium enhancement has not proven to be sufficiently sensitive to detect fibrosis in the absence of myocardial infarction in convalescent KD patients [10]. Longitudinal analysis of Gal-3 and PIPC would help clarify if there is biomarker evidence of progression of fibrosis over time. Our study did not include biomarker levels before and after initiation of statin therapy, so the anti-inflammatory impact of statins on these biomarker levels is unknown.

## 5. Conclusion

These results suggest that ongoing inflammation and fibrosis may be occurring in KD patients with persistent CAA. Ideally, these biomarker findings should be validated at the tissue level by either biopsy, autopsy, or imaging. Longitudinal follow-up will also clarify the clinical significance of these biomarker levels in this patient population that requires lifelong monitoring. Whether patients with persistent CAA would benefit from therapies designed to reduce cardiovascular inflammation should be addressed in future studies.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## References

- [1] J.W. Newburger, M. Takahashi, J.C. Burns, Kawasaki Disease, *J. Am. Coll. Cardiol.* 67 (14) (2016) 1738–1749.
- [2] M. Miura, T. Kobayashi, T. Kaneko, M. Ayusawa, R. Fukazawa, N. Fukushima, et al. Association of Severity of Coronary Artery Aneurysms in Patients With Kawasaki Disease and Risk of Later Coronary Events. *JAMA Pediatr.* 2018; e180030.
- [3] S. Yonesaka, T. Takahashi, T. Matubara, T. Nakada, H. Furukawa, K. Tomimoto, et al., Histopathological study on Kawasaki disease with special reference to the relation between the myocardial sequelae and regional wall motion abnormalities of the left ventricle. *Jpn. Circ. J.* 56 (4) (1992) 352–358.
- [4] M. Lech, J. Guess, J. Duffner, J. Oyamada, C. Shimizu, S. Hoshino, et al., Circulating Markers of Inflammation Persist in Children and Adults With Giant Aneurysms After Kawasaki Disease, *Circ. Genom. Precis. Med.* 12 (4) (2019) e002433.
- [5] F. Numano, C. Shimizu, S. Jimenez-Fernandez, M. Vejar, T. Oharaseki, K. Takahashi, et al., Galectin-3 is a marker of myocardial and vascular fibrosis in Kawasaki disease patients with giant aneurysms, *Int. J. Cardiol.* 201 (2015) 429–437.
- [6] C. Shimizu, A. Sood, H.D. Lau, T. Oharaseki, K. Takahashi, H.F. Krous, et al., Cardiovascular pathology in 2 young adults with sudden, unexpected death due to coronary aneurysms from Kawasaki disease in childhood, *Cardiovasc. Pathol.* 24 (5) (2015) 310–316.
- [7] H.H. Matundan, J. Sin, M.N. Rivas, M.C. Fishbein, T.J. Lehman, S. Chen, et al., Myocardial fibrosis after adrenergic stimulation as a long-term sequela in a mouse model of Kawasaki disease vasculitis, *JCI Insight.* 4 (3) (2019).
- [8] K. Bratis, P. Hachmann, N. Child, T. Krasemann, T. Hussain, S. Mavrogeni, et al., Cardiac magnetic resonance feature tracking in Kawasaki disease convalescence, *Ann. Pediatr. Cardiol.* 10 (1) (2017) 18–25.
- [9] C.E. Tacke, S. Romeih, I.M. Kuipers, A.M. Spijkerboer, M. Groenink, T.W. Kuijpers, Evaluation of cardiac function by magnetic resonance imaging during the follow-up of patients with Kawasaki disease, *Circ. Cardiovasc. Imaging.* 6 (1) (2013) 67–73.
- [10] S. Mavrogeni, K. Bratis, E. Karanasios, D. Georgakopoulos, S. Kaklis, G. Varlamis, et al., CMR evaluation of cardiac involvement during the convalescence of Kawasaki disease, *JACC Cardiovasc. Imaging.* 4 (10) (2011) 1140–1141.
- [11] J.L. Januzzi Jr., R.R. van Kimmenade, Importance of rigorous evaluation in comparative biomarker studies, *J. Am. Coll. Cardiol.* 63 (2) (2014) 167–169.
- [12] B. Lopez, A. Gonzalez, S. Ravassa, J. Beaumont, M.U. Moreno, G. San Jose, et al., Circulating Biomarkers of Myocardial Fibrosis: The Need for a Reappraisal, *J. Am. Coll. Cardiol.* 65 (22) (2015) 2449–2456.
- [13] S.H. Poulsen, N.B. Host, K. Egstrup, Long-term changes in collagen formation expressed by serum carboxyterminal propeptide of type-I procollagen and relation to left ventricular function after acute myocardial infarction, *Cardiology.* 96 (1) (2001) 45–50.
- [14] F. Zannad, F. Alla, B. Dousset, A. Perez, B. Pitt, Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insights from the randomized aldactone evaluation study (RALES), *Rales Investigators. Circulation.* 102 (22) (2000) 2700–2706.
- [15] F. Dallaire, A. Fournier, J. Breton, T.D. Nguyen, L. Spiegelblatt, N. Dahdah, Marked variations in serial coronary artery diameter measures in Kawasaki disease: a new indicator of coronary involvement, *J. Am. Soc. Echocardiogr.* 25 (8) (2012) 859–865.
- [16] B.W. McCrindle, A.H. Rowley, J.W. Newburger, J.C. Burns, A.F. Bolger, M. Gewitz, et al., Diagnosis, Treatment, and Long-Term Management of Kawasaki Disease: A Scientific Statement for Health Professionals From the American Heart Association, *Circulation* 135 (17) (2017) e927–e999.
- [17] M. He, Z. Chen, M. Martin, J. Zhang, P. Sangwung, B. Woo, et al., miR-483 Targeting of CTGF Suppresses Endothelial-to-Mesenchymal Transition: Therapeutic Implications in Kawasaki Disease, *Circ. Res.* 120 (2) (2017) 354–365.
- [18] Y. Li, B. Chen, X. Yang, C. Zhang, Y. Jiao, P. Li, et al., S100a8/a9 Signaling Causes Mitochondrial Dysfunction and Cardiomyocyte Death in Response to Ischemic/Reperfusion Injury, *Circulation* 140 (9) (2019) 751–764.
- [19] L.A. Altwegg, M. Neidhart, M. Hersberger, S. Muller, F.R. Eberli, R. Corti, et al., Myeloid-related protein 8/14 complex is released by monocytes and granulocytes at the site of coronary occlusion: a novel, early, and sensitive marker of acute coronary syndromes, *Eur. Heart J.* 28 (8) (2007) 941–948.
- [20] D. Viemann, A. Strey, A. Janning, K. Jurk, K. Klimmek, T. Vogl, et al., Myeloid-related proteins 8 and 14 induce a specific inflammatory response in human microvascular endothelial cells, *Blood* 105 (7) (2005) 2955–2962.
- [21] K. Takahashi, T. Oharaseki, S. Naoe, M. Wakayama, Y. Yokouchi, Neutrophilic involvement in the damage to coronary arteries in acute stage of Kawasaki disease, *Pediatr. Int.* 47 (3) (2005) 305–310.
- [22] R.R. van Kimmenade, J.L. Januzzi Jr., P.T. Ellinor, U.C. Sharma, J.A. Bakker, A.F. Low, et al., Utility of amino-terminal pro-brain natriuretic peptide, galectin-3, and apelin for the evaluation of patients with acute heart failure, *J. Am. Coll. Cardiol.* 48 (6) (2006) 1217–1224.
- [23] C. Shimizu, T. Oharaseki, K. Takahashi, A. Kottek, A. Franco, J.C. Burns, The role of TGF-beta and myofibroblasts in the arteritis of Kawasaki disease, *Hum. Pathol.* 44 (2) (2013) 189–198.
- [24] D. Lindholm, S.K. James, K. Gabrysich, R.F. Storey, A. Himmelmann, C.P. Cannon, et al., Association of Multiple Biomarkers With Risk of All-Cause and Cause-Specific Mortality After Acute Coronary Syndromes: A Secondary Analysis of the PLATO Biomarker Study, *JAMACardiol.* 3 (12) (2018) 1160–1166.
- [25] A. Rohatgi, P. Patel, S.R. Das, C.R. Ayers, A. Khera, A. Martinez-Rumayor, et al., Association of growth differentiation factor-15 with coronary atherosclerosis and mortality in a young, multiethnic population: observations from the Dallas Heart Study, *Clin. Chem.* 58 (1) (2012) 172–182.
- [26] A. Aimo, J.L. Januzzi Jr., A. Bayes-Genis, G. Vergaro, P. Sciarra, C. Passino, et al., Clinical and Prognostic Significance of sST2 in Heart Failure: JACC Review Topic of the Week, *J. Am. Coll. Cardiol.* 74 (17) (2019) 2193–2203.
- [27] R. Querejeta, B. Lopez, A. Gonzalez, E. Sanchez, M. Larman, J.L. Martinez Ubago, et al., Increased collagen type I synthesis in patients with heart failure of hypertensive origin: relation to myocardial fibrosis, *Circulation* 110 (10) (2004) 1263–1268.
- [28] J.B. Gordon, L.B. Daniels, A.M. Kahn, S. Jimenez-Fernandez, M. Vejar, F. Numano, et al., The Spectrum of Cardiovascular Lesions Requiring Intervention in Adults After Kawasaki Disease, *JACC Cardiovasc. Interv.* 9 (7) (2016) 687–696.
- [29] T.T. Doan, J.C. Wilkinson, R.W. Loar, A.S. Pednekar, P.M. Masand, C.V. Noel, Regadenoson Stress Perfusion Cardiac Magnetic Resonance Imaging in Children With Kawasaki Disease and Coronary Artery Disease, *Am. J. Cardiol.* 124 (7) (2019) 1125–1132.