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Galectin-3 is a marker of myocardial and vascular fibrosis in Kawasaki disease patients with giant aneurysms☆

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ABSTRACT

Backgrounds: Galectin-3 (Gal-3) is a multifunctional matricellular protein associated with heart failure and cardiovascular events. Gal-3 is required for transforming growth factor-β pathway-mediated myofibroblast activation that is a key process in coronary artery aneurysm formation in Kawasaki Disease (KD). Autopsies from young adults late after KD onset (AKD) have demonstrated bridging fibrosis throughout the myocardium and arteries. In this study, we postulated that Gal-3 may participate in the pathogenesis of myocardial and vascular fibrosis and the remodeling of coronary artery aneurysms following acute KD.

Methods and results: We measured plasma Gal-3 levels in 63 pediatric KD (PKD) and 81 AKD subjects. AKD subjects with giant aneurysms had significantly higher Gal-3 levels compared to the other adult groups (all $p < 0.05$). All PKD groups had significantly higher Gal-3 levels than pediatric healthy controls (HC) (all $p < 0.05$). Histological and immunohistochemical staining was performed on tissues from 10 KD autopsies and one explanted heart. Gal-3 positive staining was detected associated with acute inflammation and in spindle-shaped cells in the myocardium and arterial wall in KD subjects with giant aneurysms.

Conclusions: AKD subjects with giant aneurysms and PKD subjects had significantly higher plasma Gal-3 levels than HC and Gal-3 expression was increased in the myocardium of KD subjects who died with either acute inflammation or marked myocardial fibrosis. Gal-3 may be a clinically useful biomarker that identifies a subset of KD patients at highest risk of myocardial and vascular fibrosis, and may be an attractive therapeutic target to prevent myocardial dysfunction in this subset.

1. Introduction

Kawasaki disease (KD) is a self-limited, acute vasculitis of young children whose etiology remains unknown. Coronary artery aneurysms (CAA) are the most significant complication, and are found in 15–25% of untreated patients and 3–5% treated with intravenous immunoglobulin (IVIG) [1,2]. Thrombosis of these aneurysms or stenosis due to luminal myofibroblastic proliferation can lead to myocardial infarction, ischemic heart disease, or sudden death. Myocarditis is associated with coronary artery vasculitis in the majority of KD cases based on histologic studies of autopsies and endomyocardial biopsies [3–5]. Emerging recognition of the long-term significance of myocardial fibrosis in young adults following KD is based largely on case reports from cardiac transplantation and small series of autopsy cases [6].

Galectin-3 (Gal-3) is a β-galactoside-binding lectin and a matricellular protein that plays a multifunctional role in inflammation, fibrosis, and cell differentiation [7] and is required for transforming growth factor (TGF)-β pathway-mediated myofibroblast activation [8] that is a key process in CAA formation in KD [9]. Gal-3 is expressed by fibroblasts [10] and inflammatory cells including monocytes/macrophages [11–13]. Gal-3 is recognized as a marker of heart failure and cardiovascular events [14–16], and autopsies in young adults late after KD onset have demonstrated bridging fibrosis throughout the myocardium and arteries [6]. We postulated that Gal-3 may participate in the pathogenesis of myocardial and vascular fibrosis and the remodeling of CAAs following acute KD. To

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test this hypothesis, we measured plasma Gal-3 levels in acute and convalescent KD patient plasma samples and stained for Gal-3 in the myocardium and coronary arterial walls from KD autopsy and cardiac transplant cases.

2. Methods

2.1. Subjects

We enrolled 63 pediatric subjects who met American Heart Association clinical criteria for complete KD [17] at Rady Children's Hospital San Diego (Table 1 and supplemental table A.2) and 81 adult subjects with history of KD who participated in the San Diego Adult KD Collaborative Study. The investigation was performed according to the Declaration of Helsinki, and the Institutional Review Board of the University of California San Diego reviewed and approved this study. All subjects or their parents gave written informed consent for study participation. For the adult KD subjects, giant aneurysm was defined as a CAA with an internal diameter of at least 8 mm as measured by computed tomography (CT) or standard coronary angiography. The pediatric subjects were defined as having normal coronary arteries if the internal diameter of the right coronary artery and left anterior descending coronary artery (RCA and LAD) normalized for body surface area and expressed as standard deviation units from the mean (Z-score) was less than 2.5 as determined by transthoracic echocardiography. Giant aneurysm was defined as a Z-score greater than 10 and an aneurysm was defined as a Z-score greater than 4.0 and less than 10. For comparison, we studied 68 normotensive, age-similar and sex-matched healthy adult control subjects (median age 23.4 years, IQR 21.8–27.0 years; 41% male) who were recruited from our university campus and seven normotensive pediatric healthy controls who were age-similar patients undergoing minor orthopedic surgery procedures.

2.2. Sample collection

Blood samples from pediatric KD patients were collected at the following time points: acute (pre-treatment), early convalescent (illness day 21–82), and late convalescent (illness day 263–4396). Illness Day 1 was considered to be the first day of fever. Clinical laboratory data were also collected for pediatric KD subjects from the same phlebotomy sample used for Gal-3 measurements. Blood was collected in tubes containing sodium EDTA and plasma was separated immediately by centrifugation and stored at −80 °C.

Galectin-3 assay: Gal-3 levels were measured by enzyme-linked immunosorbent assay (ELISA) (BG Medicine, Waltham, MA, USA). The intra-assay and inter-assay coefficients of variation were 0.8% to 6.1% and 1.0% to 4.9%, respectively.

2.3. Tissue samples

We obtained formalin fixed, paraffin-embedded tissues from 11 KD patients (Table 2 and Supplemental Table A.1). Seven of these patients had giant aneurysms (Cases 1–7). The four remaining patients did not have aneurysms but died of other causes after recovery from KD (Cases N-1 to N-4). Tissues were obtained either at the time of autopsy (Cases 1, 2, 4, and 5, Cases N-1–N-4) or surgery (Cases 3, 6, and 7) following written informed consent from the subjects or their parents. The tissue sampling protocol was approved by the Investigational Review Board of the University of California San Diego and Toho University Ohashi Medical Center. The clinical details of the cases are summarized in Table 2 and Supplemental Table A.1. We also obtained control tissue samples from children who died from complications of congenital diaphragmatic hernia and from adults who died of Hodgkin lymphoma. All tissues (5 μm sections) were stained with hematoxylin and eosin (H&E) and Masson's trichrome stain according to standard protocols.

Immunohistochemistry (IHC): All tissues were fixed in formalin and embedded in paraffin. Tissue sections were deparaffinized and rehydrated. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in methanol. Antigen retrieval was performed either in citrate buffer in a microwave oven for 10 min. Slides were incubated with 1.5% normal swine serum blocking solution (Vector Laboratories) at room temperature for 60 min then incubated in PBS with 1.5% goat or horse serum overnight at 4 °C with anti-Galectin-3 antibody (rabbit polyclonal H-160: sc-20157, Santa Cruz Biotechnology). Specificity of this Gal-3 antibody was shown by Western blotting with images available at the company website (http://www.scbt.com/datasheet-20157-galectin-3-h-160-antibody.html). Antibodies were detected using biotin–avidin, LSAB2 System-HRP kit (DAKO K0675), and followed by coloring with AEC peroxidase substrate kit (Vector SK4200) according to the manufacturer's instructions. Negative staining controls included normal rabbit immunoglobulin G (IgG) (Dako, Cat.X0936) as the first antibody. Digital microscopic images were captured by Nanozoomer 2.0HT using NanoZoomer Digital Pathology (NDP) v2.1 (Hamamatsu Photonics K.K., Japan).

Immunofluorescent double-staining for α-SMA and Gal-3 were performed as previously described [9]. Binding of antibodies was detected following incubation with donkey anti-mouse IgG Alexa Fluor 488 (Jackson Immunoresearch laboratory, Cat. 715-545-150) (1:100) for α-SMA and donkey anti-rabbit IgG Alexa Fluor 594 (Jackson Immunoresearch laboratory, Cat. 711-585-152) (1:100) for the Gal-3.

2.4. Statistical methods

Data were analyzed by the Kruskal–Wallis test and Mann–Whitney test using Prism software (Graphpad software, La Jolla, CA, USA), and two-tailed $p < 0.05$ was considered to be statistically significant.

Table 1
Demographic and clinical characteristics of the study population.

Characteristic	Pediatric KD			
	Pediatric healthy control (n = 7)	Normal coronary (n = 42)	Aneurysm (n = 14)	Giant aneurysm (n = 7)
Coronary artery Z score[a]	–	<2.5	10 > Z score ≥ 4.0	≥10
Age, months (median, range)[b]	35.4 (10–69)	34.0 (2.0–130)	24.0 (3.0–85)	5.0 (2.0–49)
% Male	57	53	60	100
Coronary Z score worst (median, IQR)	–	1.2 (0.7–1.7)	4.4 (4.1–5.5)	14.8 (11.7–25.7)
Illness Day at diagnosis, (range)	–	6 (2–14)	6.5 (3–11)	10 (4–19)
Early convalescent time point, (days, range)	–	47 (25–71)	39 (21–55)	37 (28–82)
Late convalescent time point, (years, range)	–	1.36 (0.99–12.04)	1.82 (0.72–11.84)	1.99 (0.94–2.25)

IQR: interquartile range.
[a] Coronary artery Z score: internal diameter normalized for body surface area and expressed as standard deviation units from the mean.
[b] Age at sample collection for pediatric healthy controls, age at onset of KD for pediatric KD subjects.

Table 2
Demographic and clinical characteristics of Kawasaki disease subjects for histologic studies.

Case	Age/sex[a]	Race/ethnicity	Age of onset	Interval between KD onset and surgery or death	Procedure	Clinical course	Tissues obtained
1	3y7m/M	Hispanic	3y7m	7d	Autopsy	Died on illness day 7 of complications of KD shock without coronary artery thrombosis.	Myocardium/coronary artery
2	15 m/M	Caucasian	5 m	10 m	Autopsy	GA at 5 months, cardiac arrest from thrombotic occlusion of GA 10 months.	Myocardium/coronary artery
3	29y/M	Caucasian	3y	26y	Cardiac transplantation	KD with moderate CAA at age 3 yrs. Cardiac transplantation for end-stage CHF at age 29 yrs.	Myocardium/coronary artery
4	22y/M	Asian	2y9m	19y	Autopsy	KD with GA at 2y9m. Cardiac arrest due to thrombotic occlusion of GA age 22 yrs.	Myocardium/coronary artery
5	30y/M	Hispanic	6y	24y	Autopsy	Diagnosis of "Scarlet fever" age 6 yrs. Cardiac arrest due to thrombotic occlusion of GA age 30 yrs.	Myocardium/coronary artery
6	31y/M	Caucasian	7w	30y	Bilateral iliac artery endarterectomy	KD with GA and bilateral common femoral artery aneurysms at age 7 wks. Onset of lower extremity claudication age 22 yrs relieved by endarterectomy age 30 yrs.	Intima & media of bilateral iliac artery
7	23y/F	Caucasian	1 m 18 m(recurrent)	20y	Resection of right axillary artery aneurysm	KD with GA and bilateral axillary aneurysms. Upper extremity claudication treated with bilateral axillary artery aneurysm resection and angioplasty age 23 yrs.	Right axillary artery

Abbreviations: KD; Kawasaki disease, CAA; Coronary artery aneurysm, GA; Giant aneurysm.
[a] Age at tissue harvest.

3. Results

3.1. Plasma Gal-3 levels in adult KD subjects

Plasma Gal-3 levels were measured in 81 young adults with a history of KD (AKD) who were classified into three groups based on the coronary artery internal diameter measured by CT or invasive coronary angiography. Only the AKD subjects with giant aneurysms (>8 mm) had significantly higher Gal-3 levels compared to the other groups (all $p < 0.05$) (Fig. 1-A). There was no correlation between Gal-3 levels and sex or age (data not shown).

3.2. Plasma Gal-3 levels in pediatric KD subjects

Plasma Gal-3 levels were measured in 63 acute (pre-treatment) pediatric KD subjects (PKD) who were classified into three groups based on Z scores determined by echocardiography: normal coronary arteries, aneurysm, and giant aneurysm (Fig. 1-B). All of the PKD groups had significantly higher levels of Gal-3 than pediatric HC (all $p < 0.05$). However, there were no significant differences among the three acute PKD groups. To test whether the elevation of the PKD Gal-3 levels was associated with level of inflammation, we tested the correlation between values of Gal-3 and white blood cell count, absolute neutrophil

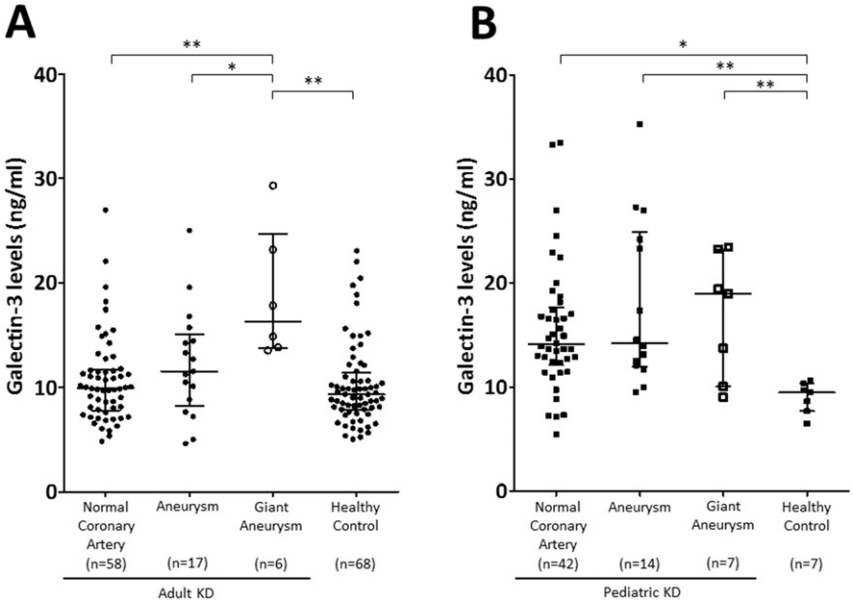

Fig. 1. Plasma Gal-3 levels in adult and acute pediatric KD subjects. (A) Comparison of plasma Gal-3 levels in adult KD subjects. Only AKD subjects with giant aneurysms ($n = 6$) had significantly higher Gal-3 levels than the other subjects with smaller aneurysms ($n = 17$), normal coronary arteries ($n = 58$), and adult healthy controls ($n = 68$). (B) Comparison of plasma Gal-3 levels in acute pediatric KD subjects. PKD subjects with giant aneurysms ($n = 7$), smaller aneurysms ($n = 14$), or normal coronary arteries ($n = 42$) had significantly higher levels of plasma Gal-3 than pediatric healthy controls ($n = 7$). Results bar shown as medians and interquartile range (IQR). Open circle and box: subject of giant aneurysm. $^{*}p \le 0.05$, $^{**}p \le 0.01$, p value by Mann–Whitney test.

count, erythrocyte sedimentation rate, and CRP, but no significant relationship emerged (Supplemental Figure A.1).

For our PKD groups we measured Gal-3 levels over time and found that only in the late convalescent phase did CAA subjects have higher Gal-3 levels than non-CAA subjects (Fig. 2). Gal-3 levels remained elevated in all PKD groups compared to pediatric HC even after more than one year from the onset of KD and regardless of coronary artery status (data not shown).

3.3. Gal-3 expression in tissue from autopsies with coronary artery aneurysms

To understand the relationship between fibrosis and Gal-3 expression, we performed immunohistochemical staining for Gal-3 in KD autopsy cases with CAAs. In the myocardium from an acute KD subject (illness days 7, Case 1), round-shaped inflammatory cells were seen infiltrating the myocardium in a perivascular distribution and positive staining for Gal-3 was noted in these same regions (Fig. 3A–D). Myocardial tissue from Cases 2 and 3, which were obtained 10 months and 27 years after the onset of KD, showed two patterns of fibrosis: a) widespread cardiomyocyte degeneration and necrosis with extensive bridging fibrosis and b) collagen deposition between cells with diffuse, interstitial lacy fibrosis (Fig. 3E, F, I, J). Infiltration of inflammatory cells was not observed. Spindle-shaped cells that expressed Gal-3 in the cytoplasm were observed between cardiomyocytes. Gal-3 expression was not observed in the cytoplasm of cardiomyocytes (Fig. 3E–L). Two other cases (Cases 4 and 5) whose tissues were obtained 19 and 24 years after the onset of KD also showed focal or interstitial fibrosis and Gal-3 expression in spindle-shaped cells (Supplemental Figure A.2). In the coronary artery, infiltrating mononuclear cells that expressed Gal-3 in the cytoplasm were observed in the thickened intima, the media, and the adventitia from an autopsy performed on illness day 7 (Case 1, Fig. 4a–c). In addition to infiltrating inflammatory cells, positively stained spindle-shaped cells were noted in the media (Fig. 4b). In Case 2, whose autopsy was performed 10 months after the onset of KD, there were infiltrating mononuclear cells expressing Gal-3 (Fig. 4e). Both the giant aneurysm with thrombotic occlusion of Case 2 and the recanalized right axillary artery of Case 7 showed destruction of the internal elastic lamina and intimal thickening with Gal-3 positive spindle-shaped cells (Fig. 4d–f, j–l). Similar spindle-shaped cells expressing Gal-3 were also observed in the surgically resected axillary aneurysm and endarterectomy specimen from the two AKD subjects with axillary and femoral artery aneurysms as a complication of acute KD in infancy (Fig. 4g–i).

3.4. Gal-3 expression in tissues from subjects with a history of KD who died from non-cardiovascular causes

To understand whether KD subjects with normal coronary arteries may develop fibrosis following the vasculitis and myocarditis associated with acute KD, we performed Trichrome and Gal-3 staining on myocardium from the autopsies of subjects with a history of KD who died from unrelated, non-cardiovascular causes. Myocardial fibrosis was not observed in any section of myocardium from these four subjects who died 2.5 months to 14 years after KD onset. Gal-3 expression was observed only in infiltrating inflammatory cells from Case N-1 who died 14 years after KD of sepsis. In the other cases, neither Gal-3 expression nor infiltrating mononuclear cells were observed (Supplemental Figure A.3).

3.5. Co-expression of α-SMA and Gal-3 in spindle-shaped cells in the myocardium

We previously demonstrated α-SMA-expressing spindle-shaped cells with staining characteristics of myofibroblasts in the aneurysmal arterial wall of a KD patient [9]. To determine whether Gal-3 expressing cells also express α-SMA in the myocardium, we performed double staining for Gal-3 and α-SMA on the myocardium from the explanted heart of Case 3. Spindle-shaped cells co-expressed both Gal-3 (red) and α-SMA (green) suggesting a myofibroblast phenotype (Fig. 5).

4. Discussion

We examined Gal-3 plasma levels and tissue expression in KD subjects from the time of the acute disease to decades after KD onset. Plasma levels were elevated acutely in all pediatric KD patients and elevated levels persisted until at least one year post-onset. In the adult KD population, Gal-3 levels were only elevated in patients with giant aneurysms. Examination of autopsy or explanted heart tissues revealed two distinct patterns of Gal-3 expression. In the acute phase, Gal-3 was

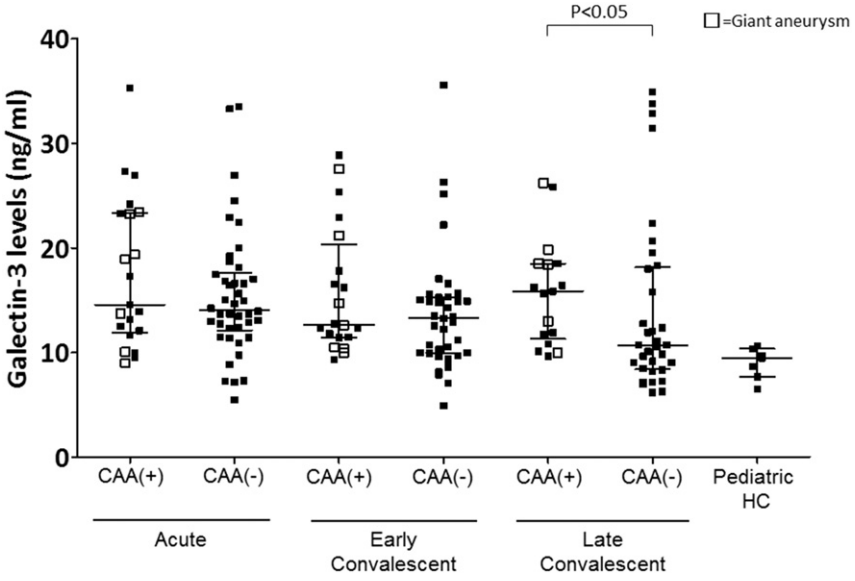

Fig. 2. Comparison of plasma Galectin-3 levels between pediatric KD subjects with and without coronary artery aneurysms (CAA). Plasma Gal-3 levels in pediatric KD subjects with CAA at three phases (acute: n = 21, early convalescent: n = 20, and late convalescent: n = 17) and without CAA (n = 42, n = 37, and n = 33, respectively) and pediatric healthy controls (n = 7). Gal-3 levels of pediatric subjects who had no coronary artery aneurysms were significantly lower than subjects who had aneurysms, only after the late convalescent phase. Results bar shown as medians and IQR. Open box: subject of giant aneurysm. p value by Mann–Whitney test.

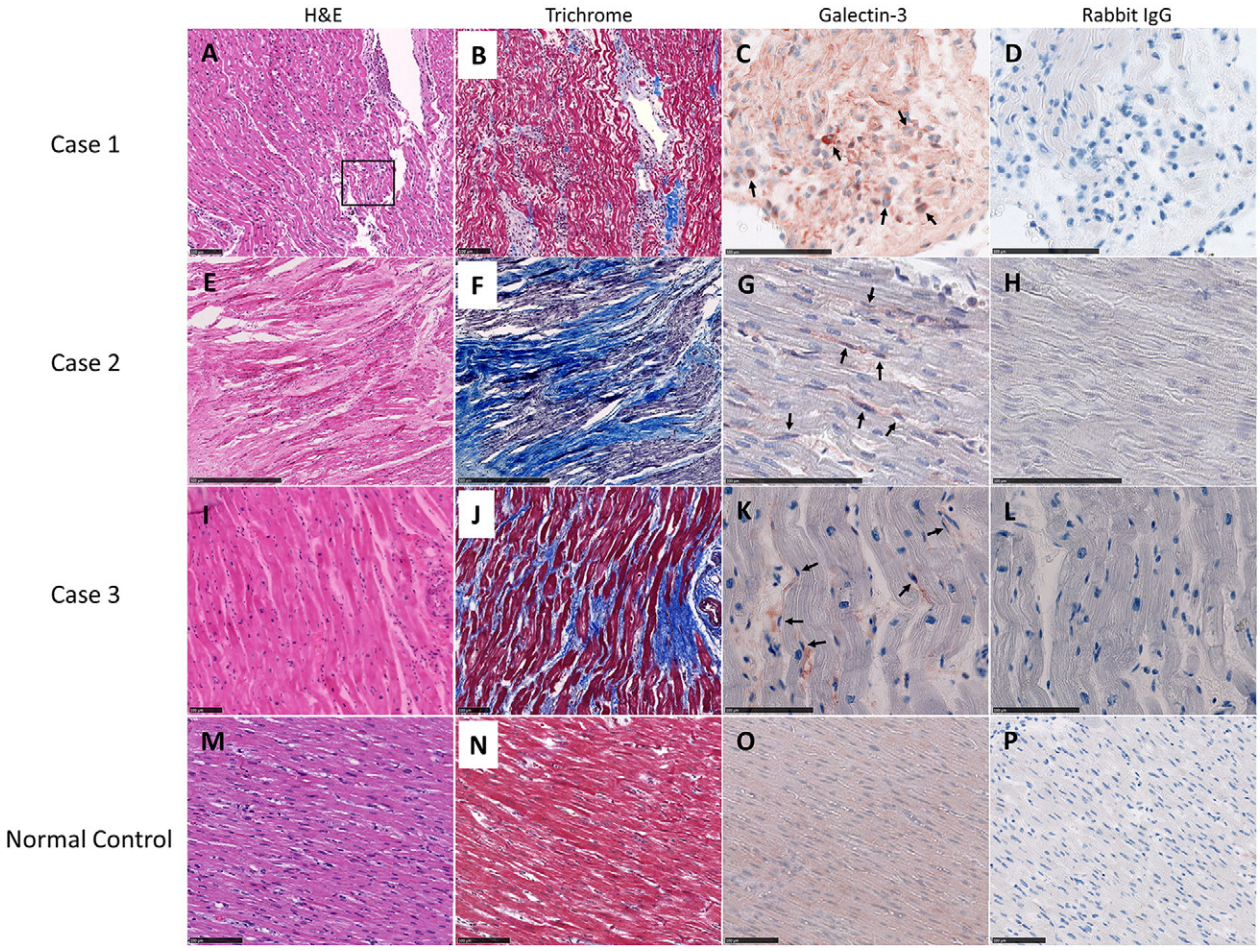

Fig. 3. Histological and immunohistochemical (IHC) analysis of KD and normal control myocardium. (A–D) Case 1, death from complications of KD shock on Illness Day 7. (A) Hemotoxylin and eosin staining showed acute inflammation with neutrophil and mononuclear cell infiltration into myocardium. Boxed area indicates location of section shown in (C) and (D). (B) Masson's trichrome staining (red indicates myocardial bundles, blue indicates collagen) showed rare deposits of collagen. (C, D) IHC showing marked expression of Gal-3 in acutely inflamed myocardium. Gal-3 expressed in cytoplasm of infiltrating mononuclear cells (arrowhead). (E–H) Case 2, cardiac arrest at 10 months post-KD. (E, F) Cardiomyocytes replaced by marked degenerative fibrosis. (G) Cytoplasmic Gal-3 expression in spindle-shaped cells (arrows). (I–L) Case 3, myocardium from explanted heart 26 years post-KD. (I, J) Diffuse interstitial lacy fibrosis with focal cardiomyocyte necrosis. (K) Cytoplasmic Gal-3 expression in spindle-shaped cells (arrows). (M–P) Control myocardium from 4 y.o. boy with congenital diaphragmatic hernia showing no inflammation, fibrosis, or Gal-3 expression. Scale bar: 100 μm.

expressed by infiltrating inflammatory cells. In the late convalescent phase in patients with giant aneurysms, Gal-3 was expressed by spindle-shaped cells in the densely fibrotic regions of the myocardium and arterial media. Our data suggest that plasma levels of Gal-3 may be a biomarker for myocardial and vascular fibrosis that occurs in a subset of convalescent KD patients with giant aneurysms. There is currently no accepted method to detect myocardial fibrosis after KD ante-mortem although research groups are testing late gadolinium contrast enhancement by magnetic resonance imaging [18]. A reliable biomarker for fibrosis would allow assessment of potential therapeutic interventions to prevent progression of fibrous scar in the myocardium and arterial wall.

Gal-3, a β-galactoside-binding lectin, is a pleiotropic molecule mediating inflammation, fibrosis, and cell differentiation [7]. Gal-3 is expressed by many different cells including fibroblasts [10], endothelial cells [19], inflammatory cells including neutrophils [20], monocyte/macrophages [11–13], and dendritic cells [21]. Gal-3 participates in many aspects of inflammation including neutrophil activation and adhesion, chemoattraction of monocytes/macrophages, opsonization of apoptotic neutrophils, and activation of mast cells [12].

The expression of Gal-3 is low in normal human myocardium, but is significantly up-regulated in certain the pathologic states [22]. Gal-3 promotes macrophage migration into the myocardium in hypertrophied hearts and active myocarditis [13,23]. In the hypertrophied rat heart, activated myocardial macrophages expressed Gal-3, a potent mitogen for fibroblasts, and Gal-3 binding sites were detected on fibroblasts and extracellular matrix [13], suggesting that Gal-3 plays an important role in tissue fibrogenesis. Gal-3 also stimulates differentiation of cardiac fibroblasts into myofibroblasts with attendant increase in collagen production [24]. In this study, we showed that plasma Gal-3 levels were elevated in most of pediatric KD subjects for more than a year. On the one hand, histologic analysis showed that Gal-3 co-localized with infiltrating inflammatory cells, suggesting that increased plasma Gal-3 levels in acute KD might be related to inflammation. On the other hand, late convalescent tissues showed no active inflammation, thus suggesting that the elevated plasma Gal-3 levels were due to vascular and myocardial fibrosis (Fig. 6). Plasma Gal-3 levels in pediatric KD subjects without giant aneurysms were still elevated at one year post-onset of disease at a time when all systemic markers of inflammation, such as CRP, had normalized. This observation raises the question of a potential role of persistent myofibroblast activation months to years after acute KD. Further longitudinal studies will be necessary to determine when levels normalize in children with normal coronary arteries years after the acute KD. Our adult KD data suggest that these levels do, in fact, normalize in patients without giant aneurysms at some time point beyond one year.

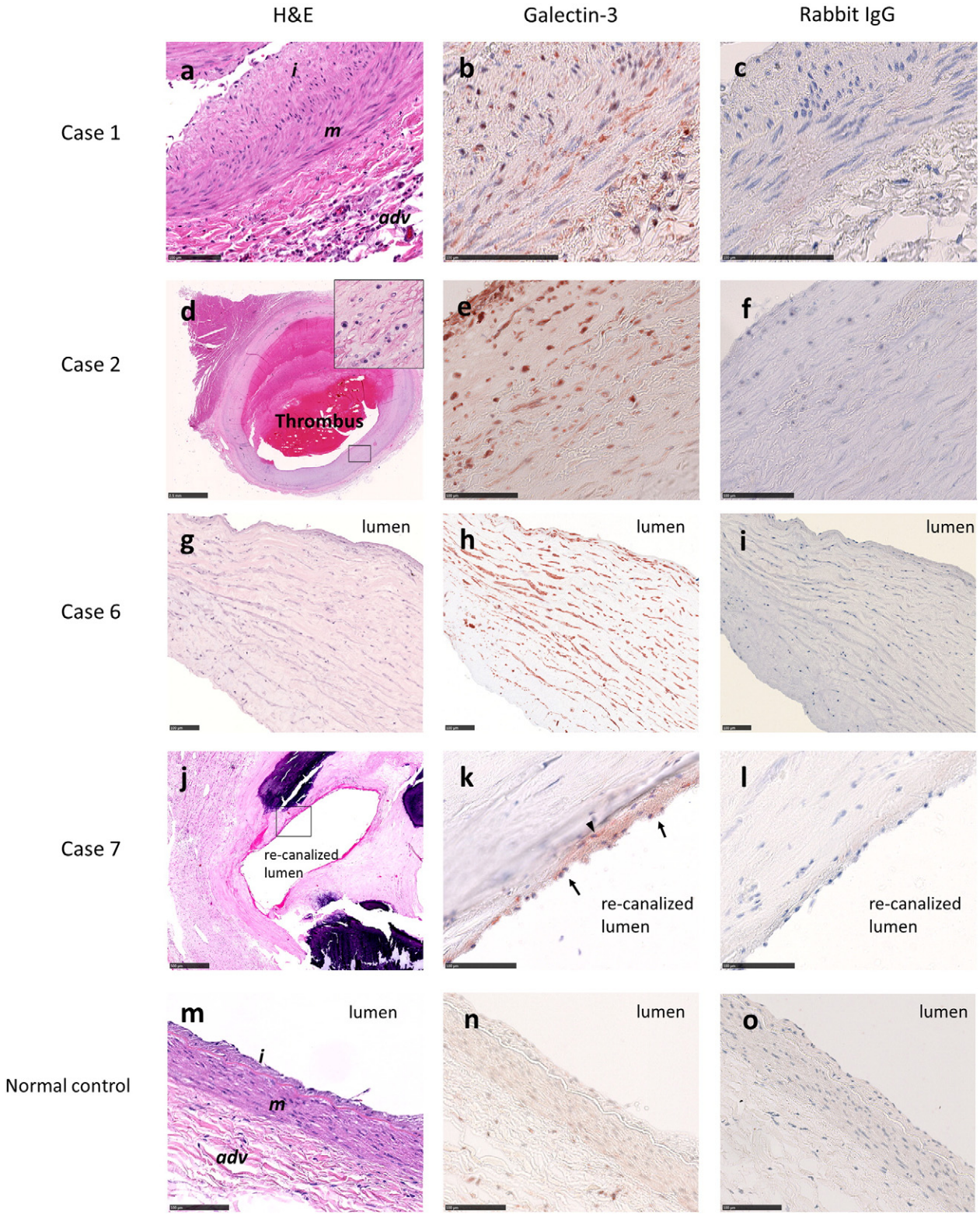

Fig. 4. Histological and immunohistochemical (IHC) analysis of KD coronary arteries (Cases 1 and 2), systemic arteries (Cases 6 and 7) and normal control. Boxed areas indicate locations of sections used for IHC in (e), (f), (k) and (l). (a–c) Case 1: Coronary artery wall of severe acute KD patient (case1; 7 illness days) (a) Infiltration of inflammatory cells in all vessel layers. (b) Marked Gal-3 expression in infiltrating inflammatory cells in the intima and adventitia and spindle-shaped cells in the media. (c) Negative control for IHC. (d–f) Case 2: Giant An of LAD 10 months post-KD). (d) Multiple calcification, intimal hyperplasia, and destruction of both internal and external elastic membrane were seen, intimal lumen was occluded by thrombus. (Box) Persistent inflammation with mononuclear infiltration at luminal side of hyperplasic intima. (e) Gal-3 expression in infiltrating mononuclear cells and spindle-shaped cells. (g–i) Case 6: Endarterectomy specimen with hyperplastic intima from common femoral artery (h) Gal-3 expression by abundant spindle-shaped cells. (j–l) Case 7: surgically resected axillary artery aneurysm with re-canalization. (k) Gal-3 expression in endothelial cells (arrows) and spindle-shaped cells (arrowhead). (m–o) Coronary artery wall from a 4 y.o. male with diaphragmatic hernia. Scale bar: 500 μm (j), 100 μm (others), i: intima, m: media, adv: adventitia.

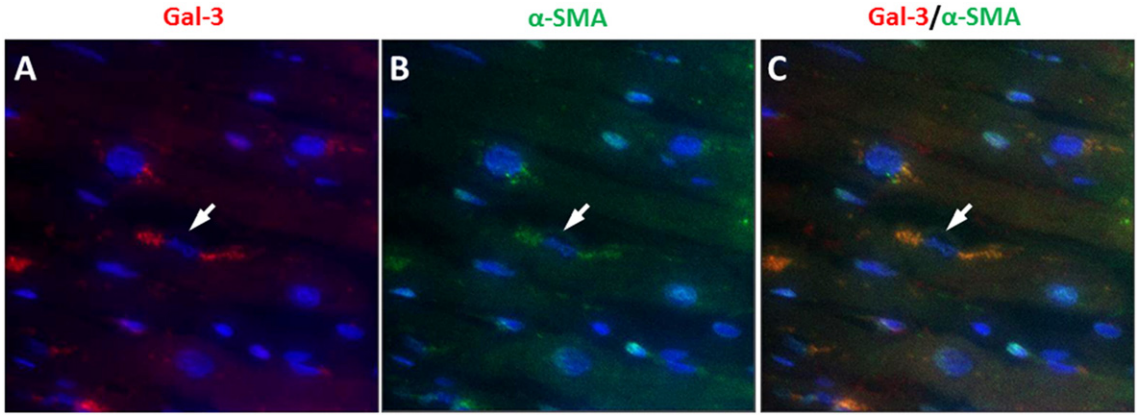

Fig. 5. Case 3. Gal-3 + (red, panel A) and α-SMA + (green, panel B) spindle-shaped cells in explanted heart 26 years post-KD. Fluorescent double-staining for Gal-3 and α-SMA (orange, panel C) suggests a myofibroblast phenotype for cells expressing Gal-3.

Multiple studies of endomyocardial biopsies have documented that myocardial inflammation is a universal feature of acute KD [4,5]. Of the more than 200 subjects studied by different investigators, approximately one-third also had changes of myocardial fibrosis. Harada et al. conducted histological studies of myocardial tissues from 29 KD patients who died within 40 days from KD onset and included eight cases with no evidence of CAA [3], inflammatory cell infiltration in the myocardium was seen in all cases. From our histologic study of the myocardium from patients who had a history of KD but who died from non-cardiac causes, we observed neither myocardial fibrosis nor Gal-3 expression with the exception of Case N-1 who died of sepsis and had infiltrating inflammatory cells expressing Gal-3. In contrast, late convalescent KD subjects with giant aneurysms (Cases 2–5) had evidence of myocardial fibrosis and high levels of Gal-3 tissue expression.

Cardiac magnetic resonance imaging (CMRI) can detect myocardial scarring and has excellent agreement with histology in animal and human studies [25]. However, in a KD patients who initially had left ventricular dysfunction during the acute phase by echocardiography, only 2/60 (3.3%) had abnormalities of the myocardium seen by CMRI late gadolinium contrast enhancement (CMRI-LGE) during the late convalescent phase [18]. Thus, using currently available techniques, CMRI-LGE may miss diffuse fibrosis and a biomarker for these histologic changes is needed.

In this study, Gal-3 expression was demonstrated in the walls of both coronary and systemic artery aneurysms and the histologic details of fibrosis in these tissues have been previously reported by our group for Cases 4–7 [6,26]. Thus, these late convalescent KD subjects with giant aneurysms had both myocardial and arterial wall fibrosis, and it is likely that both these tissue sources contributed to the elevated Gal-3 levels reported here.

Experiments in hypertensive rats have demonstrated that the naturally occurring tetra-peptide, N-acetyl-ser-asp-lys-pro (AcSDKP) reduces myocardial inflammation and prevents fibrosis mediated by Gal-3 [27,28]. In vitro experiments have also demonstrated inhibition of epithelial-to-mesenchymal transition in tissue culture [29]. Mineralocorticoid receptor antagonists have been demonstrated to modulate Gal-3 expression after acute myocardial infarction [30] and to prevent aldosterone-salt induced cardiac hypertrophy, dysfunction, and fibrosis [31]. Fibrosis in these settings is mediated through the TGFβ-SMAD3 signaling pathway, which is implicated in CAA formation in KD. Genetic variants in TGF-β influence KD susceptibility and CAA formation [32]. TGF-β may contribute to coronary arteritis and aneurysm formation by promoting the generation of myofibroblasts from different cell lineages including endothelial cells and smooth muscle cells in media, fibroblasts in adventitia, and fibrocytes in the peripheral circulation [9], and expression of TGF-β pathway molecules in aneurysmal arterial walls

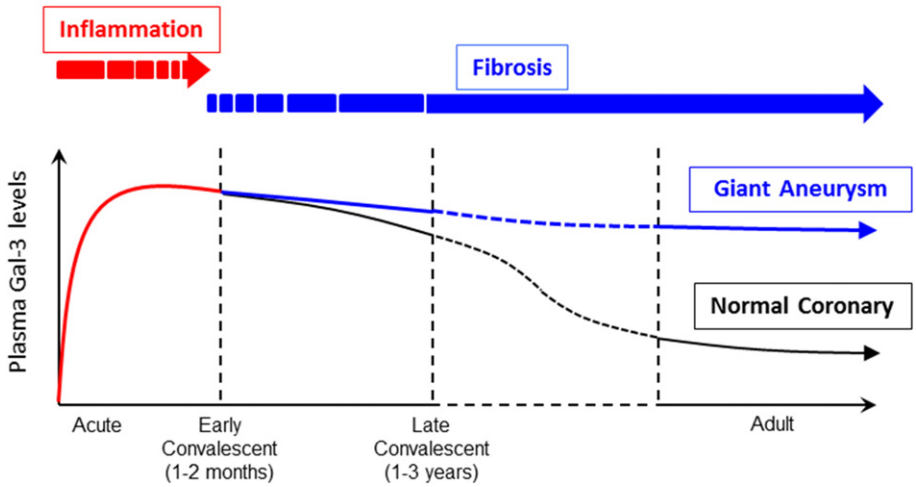

Fig. 6. Proposed evolution of plasma Galectin-3 levels related to stages of Kawasaki Disease. Gal-3 is initially expressed by inflammatory cells infiltrating the coronary arterial wall and myocardium during the acute phase. Plasma levels then wane over time in patients with normal coronary arteries or persist at elevated levels in KD patients with giant aneurysms. Levels remain elevated due to fibroblasts and myofibroblasts activation associated with progressive fibrosis. The dotted lines represent uncertainty about this time period for which we have no data.

from autopsies of young adult KD patients has been demonstrated by immunohistochemistry [6]. Myofibroblasts in KD patients may remain activated and cause progressive luminal narrowing of coronary artery to the point of total occlusion [33]. In the present study, we demonstrated expression of Gal-3 in SMA-expressing spindle-shaped cells in myocardium. Inhibition of Gal-3 with consequent reduction of TGF-β signaling may be an appealing therapeutic strategy for KD patients with severe coronary artery involvement and elevated serum levels of Gal-3.

We recognize several strengths and limitations of our study. This is the first report to study Gal-3 longitudinally in KD patients with a variety of different clinical outcomes and to demonstrate Gal-3 expression in KD tissues. The link between Gal-3 and TGF-β signaling provides a plausible mechanism for how Gal-3 may mediate myocardial and vascular fibrosis in KD. Data from large adult cohorts show that circulating Gal-3 levels gradually increase with age, and are higher in females [34]. We therefore chose age-similar, sex-matched subjects for healthy controls [14,34]. As a limitation, our study was restricted by the types of clinical tissue samples available. Since plasma Gal-3 levels were from living patients and tissues were available only from autopsies with no available plasma, we were unable to study myocardial fibrosis in tissue and plasma Gal-3 levels in the same patients. Caution must therefore be exercised in assuming that plasma Gal-3 levels and myocardial fibrosis are mechanistically linked. In an investigation of myocardial and blood Gal-3 levels in hypertensive patients with heart failure, there was no correlation between myocardial or plasma Gal-3 levels and collagen degradation products [35]. MRI extra-cellular volume fraction studies may help to resolve the relationship of plasma Gal-3 levels and myocardial fibrosis in the adult KD patients.

5. Conclusion

Adult KD subjects with giant coronary aneurysms had significantly higher plasma Gal-3 levels compared to other adult KD and HC subjects. Gal-3 is recognized as a novel marker associated with heart failure and cardiovascular events in different clinical settings. With the development of more sensitive imaging techniques and the measurement of circulating procollagen fragments to detect fibrosis in KD patients antemortem, correlation studies can be performed to test the hypothesis that plasma Gal-3 is a clinically useful biomarker for myocardial and vascular fibrosis and that inhibition of Gal-3 may lead to improved myocardial function in the subset of KD patients.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2015.07.063.

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