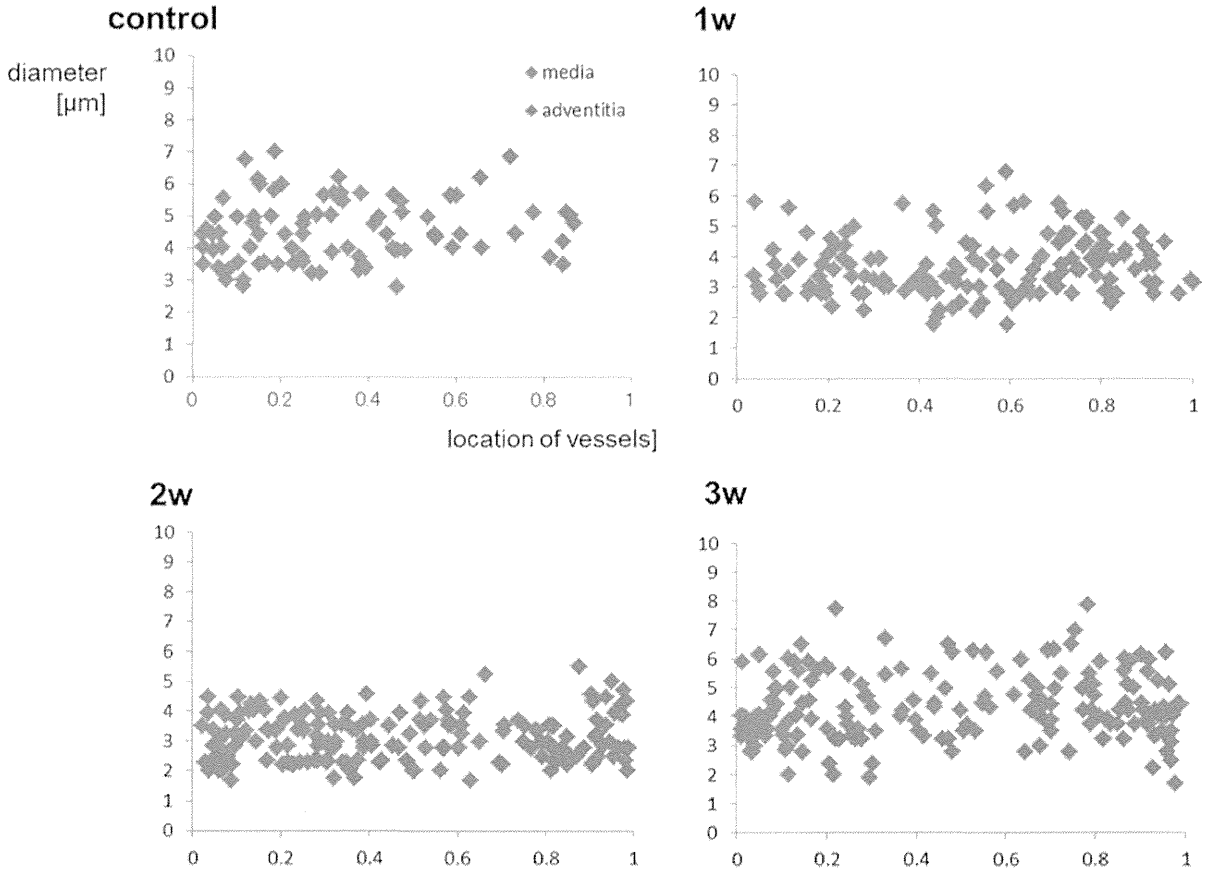

Figure 4 The size and distribution of small vessels, including the vasa vasorum. The X-axis shows the location of each vessel. "1" indicates external to the vessel, and "0" indicates the center of the lumen of that vessel.The Y-axis shows the size of vessels.The vessels proliferated at the adventitia along with the development of the inflammation.

SEM

There were many vessels surrounding the aorta and coronary arteries in a net-like appearance. (Figure 5). These were considered to be part of the vasa vasorum, because they were arising from or invading the host vessels, as reported previously [28,33-35].

In the group with inflammation, the number of vasa vasorum increased. Furthermore, these networks of vasa vasorum proliferated as the infiltration of the inflammation increased (Figure 6).

Micro CT

On scanning the aorta and coronary arteries, the left coronary artery was found to clearly bifurcate from the aorta in the control group and in the animal model one week after the injection of CAWS. However, at more than two weeks after the injection, the bifurcation of the left coronary artery was no longer detectable due to the leakage of contrast agent (Figure 7). This suggested that the blood vessel wall was fragile due to the development of inflammation.

Discussion

Kawasaki disease (KD) is characterized by systemic vasculitis with tissue edema at the initial phase of the disease. Although the inflammation is initially localized to the capillaries and microvessels, it eventually expands to medium-sized muscular arteries and veins. Our study found that the inflammation originated in the adventitia. The inflammation enhances the expression of growth factors and the resulting edema, leading to hypoxia due to the proliferation of the vasa vasorum (Figures 1, 2, 3). The proliferation of the vasa vasorum may act as a conduit for the entry of various growth factors [35], cytokines and blood cells. The spread of the proliferated vasa vasorum to the media thus broadens the inflammation.

In general, the inflammatory cells infiltrate from the adventitia toward the external elastic lamina around the Valsalva sinus and aortic valves by approximately one week after the administration of CAWS. During the second week, significant inflammatory changes develop from the adventitia and media toward the intima, as shown in Figures 1, 2 and 3, especially around the right

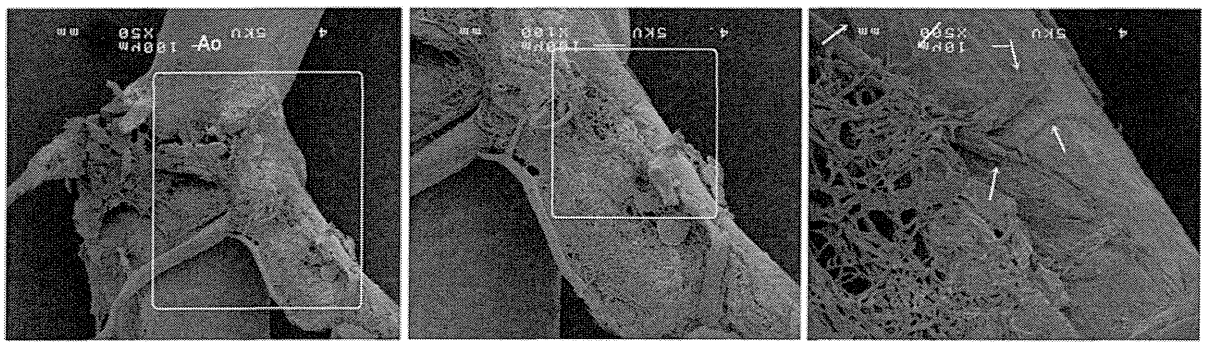

Figure 5 An example of the small vessels around the aorta as examined by a scanning electron microscope (SEM). These vessels surrounded the aorta and coronary arteries with a net-like appearance, and flowed into the host vessels (yellow arrows).

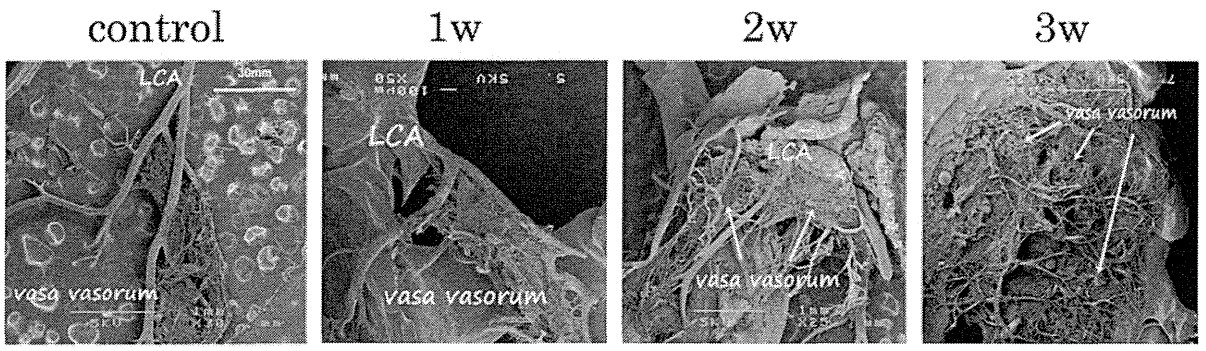

Figure 6 The four developmental stages of vasculitis in the murine model of Kawasaki disease as examined using a scanning electron microscope (SEM). The proliferation of the vasa vasorum was accompanied by an increase in the inflammation.

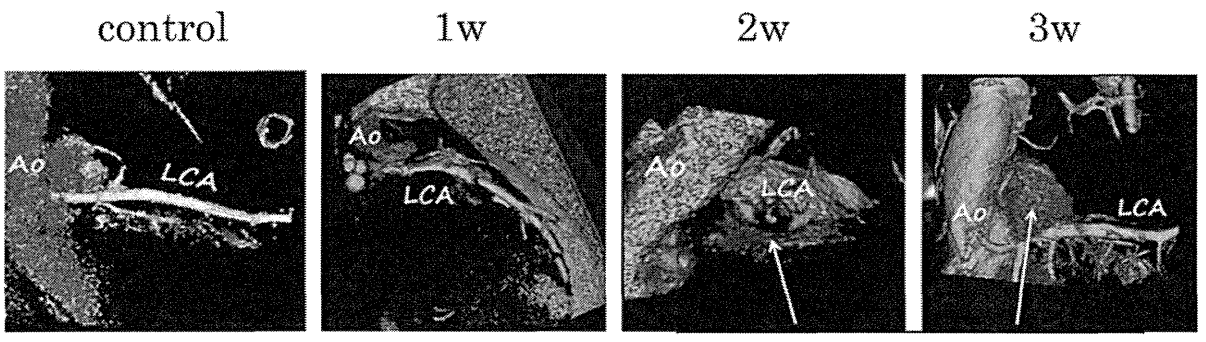

Figure 7 The four developmental stages of vasculitis in the murine model of Kawasaki disease as examined by micro computed tomography (CT). The aorta and the coronary arteries were detected. Beginning approximately two weeks after the injection of *Candida albicans* water-soluble fraction (CAWS), the bifurcation of the coronary arteries became unclear because of the leakage of the contrast agent.

and left coronary sinuses. The inflammatory cells, predominantly consisting of polymorphonuclear neutrophil leukocytes, are observed along the elastic lamina. More than three weeks later, the inflammation expands circumferentially to include the non-coronary sinus, and the beginning of an abscess can be observed. Fibrinoid necrosis is detected in the internal elastic lamina, and the intima and the adventitia markedly thicken. Therefore, the basic structure of blood vessels is destroyed, and they become fragile.

In the very early phase of inflammation, although the inflammatory cells accumulate at the adventitia, the detachment of endothelial cells or a thrombus indicative of the damaged intima are not detected. This suggests that the vasculitis is initiated from the side of the adventitia [36].

As the inflammatory cells infiltrate through the vasa vasorum, the neogenesis of more vasa vasorum occurs inward, and the inflammatory lesion expands along the elastic lamina.

While the arteries that form aneurysms are typically muscular, and occur along with the vasa vasorum (e.g., coronary artery, axillary artery, internal iliac artery), edema and cell infiltration are also seen at arterioles, venules, and microvessels. The pathophysiology of coronary aneurysms remains unclear, however, we hypothesize that both endothelial cell disorders and inflammation of the vasa vasorum are involved. If there is prolonged edema of the microvessels, the supply of oxygen to the coronary artery wall through the vasa vasorum will be compromised, and the coronary media may thus become ischemic. Hence, the coronary artery structure becomes very fragile [37].

As previously noted, we believe that after the development of inflammation around micro arteries in the adventitia, the neovascularization diverges from the vasa vasorum and proliferates toward the media and the intima to supply blood, inflammatory cells, cytokines and chemokines in order to quell the inflammation.

The vasa vasorum have been of considerable interest to scientists and physicians for more than a century [38,39]. They nurture the outer component of the vessel wall, including the adventitia and outer one-third of the media, and the intima is supplied with oxygen from the lumen [30]. Under normal conditions, the vasa vasorum run longitudinally along the long axis of the adventitia of the blood vessels (primary vasa vasorum), and in some places, flow into the adventitia and the media (second vasa vasorum).When any injury or inflammation of blood vessels occurs (e.g., arterial sclerosis), the vasa vasorum proliferate and penetrate into the adventitia [13] and the media, thus buttressing the capillary network [14].

Developments in diagnostic imaging have allowed the microvessels inside lesions to be detected if the neointima is thicker than 500 μm. Furthermore, any infiltration of microvessels from the adventitia into the vessels can now be visualized [15].In inflammatory lesions, the permeability of microvessels can be seen in the acute phase as these microvessels expand or progress. Hypoxia and endothelial reactions are the major driving forces behind the progression of the vasa vasorum [40]. Moreover, there is a disproportionate increase in growth factors for the endothelium of the vasa vasorum, and further development of the vasa vasorum causes the plasma to extravasate around the vasa vasorum.

Conclusions

In conclusion, we investigated the involvement of the vasa vasorum in the KD like vasculitis using a murine model induced by CAWS. We demonstrated that the vasa vasorum might serve as the initiator of vasculitis in this model. Initially, the inflammatory cells accumulated at the adventitia, and then diverged through neovascularization toward the media and the intima. Therefore, the vasa vasorum provides a critical route for the infiltration of inflammatory cells. The proliferation of the new vasa vasorum toward the media and the intima is an important criterion for both the expansion and the termination of the inflammation.

Abbreviations

KD: Kawasaki disease; SEM: Scanning electron microscope; CT: Computed tomography; CAWS: *Candida albicans* water-soluble fraction; IVIG: Intravenous immunoglobulin; CAL: Coronary arterial lesion; CADS: *Candida albicans*- derived substance; HE: Hematoxylin and eosin; EVG: Elastica van Gieson.

Competing interests

The authors declare no conflicts of interests.

Authors contributions

AHO: Preparation of the murine model and data analysis. CS: Preparation of the murine model and data analysis. TY: Preparation of the murine model and data analysis. KI: Preparation of the murine model and data analysis. NNM: Preparation of the CAWS. NO: Preparation of the CAWS. YA: Analysis using micro CT. HT: Histological evaluation of vasculitis. TT: Histological evaluation of vasculitis. KH: Preparation of the murine model and data analysis. All authors read and approved the final manuscript.

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A survey of the 3-decade outcome for patients with giant aneurysms caused by Kawasaki disease

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Background Our purpose was to determine the outcome in patients with a more-than-20-year history of giant coronary aneurysms (GAs) caused by Kawasaki disease (KD).

Methods Between 2010 and 2011, the incidence and outcome of cardiac events (CEs) in patients with GA was surveyed by questionnaire by the Kinki area Society of KD research. Death, acute myocardial infarction (AMI), coronary artery bypass grafting (CABG), percutaneous coronary catheter intervention, syncope, and ventricular tachycardia were considered as CEs. Survival rate and CE-free rate were analyzed by the Kaplan-Meier method.

Results We enrolled 245 patients (187 were male, 58 were female), 141 with bilateral GA and 104 with unilateral GA. The interval between the onset of acute KD to the time of survey ranged from 0.2 to 51 years, and the median was 20 years. Death, AMI, and CABG occurred in 15 (6%), 57 (23%), and 90 patients (37%), respectively. The CE-free rate and the survival rate at 30 years after KD were 36% (95% CI 28-45) and 90% (95% CI 84-94), respectively. The 30-year survival rate for bilateral GA was 87% (95% CI 78-93), and for unilateral GA, it was 96% (95% CI 85-96; hazard ratio 4.60, 95% CI 1.27-29.4, $P = .027$). The 30-year survival rate in patients with AMI was 49% (95% CI 27-71), and the 25-year survival rate in patients undergoing CABG was 92% (95% CI 81-98).

Conclusions The outcome differed significantly between bilateral GA and unilateral GA. The results focus attention on the need to preserve myocardial perfusion, especially in high-risk patients with bilateral GA. An understanding of the optimal CABG would be useful in bilateral GA. (Am Heart J 2014;167:249-58.)

Background

National surveys of Kawasaki disease (KD) in Japan have been performed every 2 years since 1976. Recently, about 10,000 patients with KD have been reported annually, and 1 month after the onset, giant coronary aneurysms (GAs) were present in less than 0.4% of patients. Until 2010, 272,749 patients had had acute KD, with 436 deaths. Although the mortality of KD exceeded 0.2% in the 1970 to 1984, it had decreased to less than 0.1% by the 1990s.[1] Most deaths occurred early after the acute illness and were attributable to the development of coronary aneurysms, especially giant lesions (GA).[2] Patients with GA often experienced cardiac events (CEs), such as acute myocardial infarction (AMI) or sudden death.[3-5] In contrast, the long-term outcome after coronary artery bypass grafting (CABG) was good,[6] and optimal coronary revascularization is believed to improve the prognosis for patients with GA. A previous report about patients with GA from a single institution suggested favorable results.[7] Because the goal is to prevent death and improve the quality of life, we surveyed the long-term outcome in patients with GA caused by KD believing that the knowledge gained would help improve the future management of patients with GA.

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Methods

Definitions

In this study, GA implies a coronary aneurysm due to KD with a diameter of more than or equal to 8 mm. In coronary artery lesions, localized stenosis (LS) means stenosis equal to or more than 25%, and occlusion means complete occlusion or segmental stenosis (SS) with new small vessels indicating

Table I. Characteristics of patients with GA

GA	RCA	LCA	Bilateral	Total
n	48 (20%)	56 (23%)	141 (57%)	245
M/F	40/8	34/22	113/28	187/58
Onset (mo), median (range)	27 (0.7-110)	26 (2-244)	21 (1-251)	24 (0.7-251)
Acute treatment				
Aspirin	10 (19%)	13 (25%)	29 (56%)	52
Aspirin + IVIG	15 (22%)	14 (21%)	38 (57%)	67
Including steroid	7 (17%)	8 (19%)	37 (94%)	52
Medicine*				
Antiplatelets	28 (19%)	35 (24%)	82 (57%)	145
Antiplatelets + warfarin	14 (18%)	19 (25%)	43 (57%)	76
Warfarin	1 (20%)	1 (20%)	3 (60%)	5
None	1 (10%)	1 (10%)	8 (80%)	10
Unknown	3 (33%)	0	6 (67%)	9
CEs (+)	11 (9%)	18 (14%)	98 (77%)	127
CEs (–)	37 (31%)	38 (32%)	43 (37%)	118
CEs				
Death	1 (7%)	1 (7%)	13 (86%)	15
AMI				
1	6 (12%)	5 (11%)	36 (77%)	47
≤2	0	0	10	10
ICT	3 (10%)	2 (7%)	24 (83%)	29
Successful	1 (7%)	2 (14%)	11 (79%)	14
Failed	2 (13%)	0	13 (87%)	15
CABG	3 (3%)	12 (13%)	75 (84%)	90
PTCRA	0	1 (13%)	7 (87%)	8
PCBA	2 (100%)	0	0	2
NSVT	1 (7%)	2 (13%)	12 (80%)	15
VT	1 (25%)	0	3 (75%)	4

Abbreviations: *IVIG*, Intravenous immunoglobulin; *ICT*, intracoronary thrombolysis; *PTCRA*, percutaneous transluminal coronary rotational ablation; *PCBA*, percutaneous transluminal coronary balloon angioplasty.
* Antithrombotic agents immediately after acute KD.

recanalization.[5] Stenotic lesions include LS, occlusion, and SS. Death, AMI, coronary revascularization procedures such as CABG and percutaneous coronary catheter intervention (PCI), syncope, and ventricular tachycardia (VT) were defined as CEs. Asymptomatic myocardial infarction was excluded from AMI. Patients who had not visited their hospital for 5 years were considered dropouts.

Our initial purpose was to define the clinical features, incidence of CE, and long-term outcome in patients with GA. About 2,000 cases of acute KD occur in the Kinki area each year, representing about one-fifth of the Japanese total. The Kinki area KD Research Society has a membership of about 250, and E. Tsuda recruited the members who participated in the study. Nine institutions including those of the authors participated. Between 2010 and 2011, the outcome for patients with GA in the 9 institutions was surveyed by a mail questionnaire.

The contents of the questionnaire are listed here: gender, date of birth, history of KD and its date of onset, acute-phase medication of KD (within 40 days of its onset) and medication immediately after the acute KD (>40 days after its onset), coronary arterial lesions at the initial selective coronary angiography (CAG) and its timing, aneurysm other than coronary; CE (AMI, CABG, PCI, syncope, VT, death), date of CE, coronary arterial lesions at the latest angiography and its timing, outcome, date of the latest clinic attendance, the latest clinical status (arrhythmia, New York Heart Association, delivery, left ventricular shortening fraction [LVSF], and left ventricular diastolic dimension [LVDd] by 2-dimensional echocardiogram, and its timing), and medication at the latest clinic attendance.

We analyzed the characteristic features of the patients with GA from the results of the questionnaire, and these are listed in Table I. We then divided them into 3 groups based on the GA location, namely, right coronary artery (RCA), left coronary artery (LCA), or bilateral involvement. We calculated the incidence of stenotic lesions in the respective groups based on the time after KD and compared the incidence of stenotic lesions in the RCA, left anterior descending artery (LAD), and left circumflex (LCX). Furthermore, we analyzed LVSF and LVDd, the incidence of VT or nonsustained VT (NSVT), and the incidence of death in the 3 groups divided by the number of AMIs.

Survival rates and free rates for CE, AMI, and CABG were calculated, and the same values were compared between the unilateral GA group and the bilateral GA group. The unilateral GA group consists of patients with GA in the RCA or LCA, and bilateral GA means the patients with GA in both coronary arteries. Survival rates were also calculated in the respective groups based on CE or medication as a subgroup analysis.

Statistical analysis

The mean values are shown as mean ± SD. χ^2 Test was used to compare differences in incidence between groups. Turkey-

Kramer test was used to test for differences in LVSF and LVDd between the 3 groups. Logistic regression model was used to compare the incidence of stenotic lesions by the time after KD. In addition, survival rates and CE-free rates were analyzed by the Kaplan-Meier method with 95% CIs, and differences were assessed by the log-rank test. We used the Cox proportional hazards model to assess the incidence of CE for a bilateral GA compared with unilateral GA. Differences were considered statistically significant at a P value less than .05. JMP7.0 (SAS institute Inc, Cary, NC) was used as statistical software.

No extramural funding was used to support this work. E. Tsuda was responsible for all aspects of this study.

Results

Number and background of patients with GA

A total of 261 patients with GA were reported by the 9 institutions; 16 were excluded because they failed to meet the definition of GA for the study. Data on 245 patients with GA were analyzed including 187 men (76%) and 58 women (24%). The outpatient department (OPD) records were available for all 245 patients. Age at the latest OPD visit ranged from 1 to 56 years (median 23 years), and the interval from the onset of KD to the latest OPD attendance ranged from 2 months to 51 years (median 20 years). Thirty-four patients (14%) were lost to follow-up.

The median interval from the onset of KD to the initial CAGs was 4 months (range 16 days-33 years). In the initial CAGs, 48 (20%) patients had GA confined to the RCA and 56 (23%) GA involving the LCA alone. Bilateral involvement was present in 141 (57%). By the year of the onset of KD, the numbers per decade were follows: 1960 to 1969, 3; 1970 to 1979, 45; 1980 to 1989, 102; 1990 to 1999, 54; and 2000 to 2009, 41. The age at the onset of KD ranged from 20 days to 20 years (median 24 months) and was less than 5 years in 194 patients (79%). Twelve patients (5%) had 2 or more attacks of KD. Aneurysms other than coronary were observed in 26 patients (11%). The treatment of acute KD was as follows: aspirin, 52 (21%); aspirin and intravenous immunoglobulin, 68 (28%); treatment including steroids, 43 (18%); others, 14 (6%); none, 11 (4%); and unknown, 57 (23%).

Medication

Medications received immediately after the acute phase are summarized in Table I. In the latest clinic attendance, 225 (92%) patients were receiving medication, 18 patients (7%) were not receiving medication, and medication was unrecorded in 2 patients (1%). One hundred sixty patients (65%) were taking antiplatelet agents, and 61 (25%) were taking antiplatelet agents plus warfarin. One patient had warfarin alone. Seventy-one (30%) of the patients were receiving other medications including the follows: β-blocker 33 (13%); angiotensin converting enzyme inhibitor 22 (9%); nitrate 9; statins 7; calcium antagonists 6; diuretics 5; antiarrhythmics 4; angiotensin 1 receptor blocker 2 and digoxin 1.

Coronary artery lesions in the late period

Two hundred thirty-eight patients (97%) underwent cardiac catheterization including CAG in the late period, and in the remaining 7 patients, coronary evaluation depended on multidetector computed tomography. The median interval from the onset of KD to the latest CAG was 15 years (range 73 days to 43 years). The incidence of stenotic lesions in the respective groups is shown in Figures 1 and 2 and significantly increased in the late period in all 3 groups ($P < .01$) (Figure 1). The incidence of stenotic lesions in the LCX was significantly lower than that in the RCA and LAD ($P < .01$) (Figure 2).

Myocardial infarction

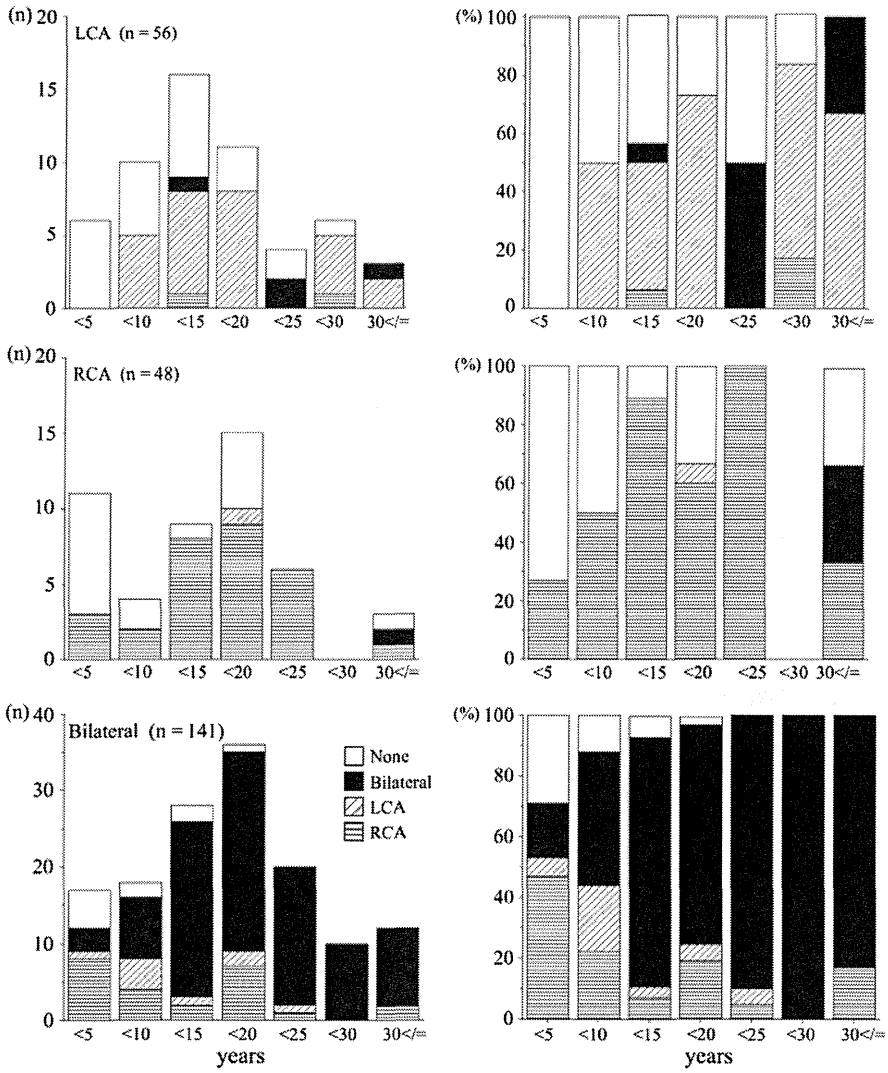
Acute MI occurred in 57 patients (23%). The median interval from the onset of KD to AMI was 8 months (range 18 days to 35 years). In 40 (70%) of the 57 patients, AMI occurred within 2 years of the onset of KD. Infarcts were inferoposterior in 28 (49%), anteroseptal in 27 (47%), and lateral in 2 (4%). Ten patients (4%) had more than 1 AMI. Thirteen deaths (23%) relating to myocardial infarction (MI) were as follows: sudden death due to AMI, 4; early death, 1; sudden death in the late period, 5; late death due to severe heart failure, 1; and death after heart transplantation, 2. Coronary revascularization for AMI was attempted in 29 patients (51%), thrombolytic therapy in 28 patients, and PCI in 1 patient. Fourteen attempts were successful and 15 failed. No major complications occurred. In the late period, 27 (47%) had CABG and 6 (11%) had PCI. Three deaths occurred among the 15 who failed thrombolytic therapy.

Coronary revascularization results

Ninety patients had undergone revascularization procedures, and 1 patient had open angioplasty. Six patients had a reoperation. In the 90 patients undergoing CABG, the numbers of vessels grafted were as follows: single graft, 43 (49%); 2 grafts, 32 (36%); and 3 grafts, 13 (14%). Four and 5 grafts were done in 1 patient, respectively. The mean number of grafts was 1.7 per patient. The target vessels were as follows: LAD, 85; RCA, 48; obtuse marginalis, posterolateral, or posterodescending, 17; and diagonal, 5. The grafts used the internal thoracic artery in 88 patients, saphenous vein in 8, radial artery in 8, and gastroepiploic artery in 2. The median age at operation was 11 years (range 1-44 years), and the interval from the onset of KD to operation ranged from 52 days to 43 years (median 7 years). Twenty-seven (30%) among 90 patients had a history of MI. The interval from the onset of AMI to operation ranged from 58 days to 26 years (median 12 years).

Elective PCI for a native artery was performed for 10 lesions in 10 patients (4%); their ages at PCI ranged from 6

Figure 1



Incidence of stenotic lesions in patients with GA. (upper) GA in the LCA; (middle) GA in the RCA; (lower) GA in bilateral GA.

to 27 years (median 13 years); and the onset of KD to PCI ranged from 5 to 25 years (median 12 years). Eight patients had percutaneous transluminal rotational angioplasty, and 2 had percutaneous transluminal balloon angioplasty.

Death

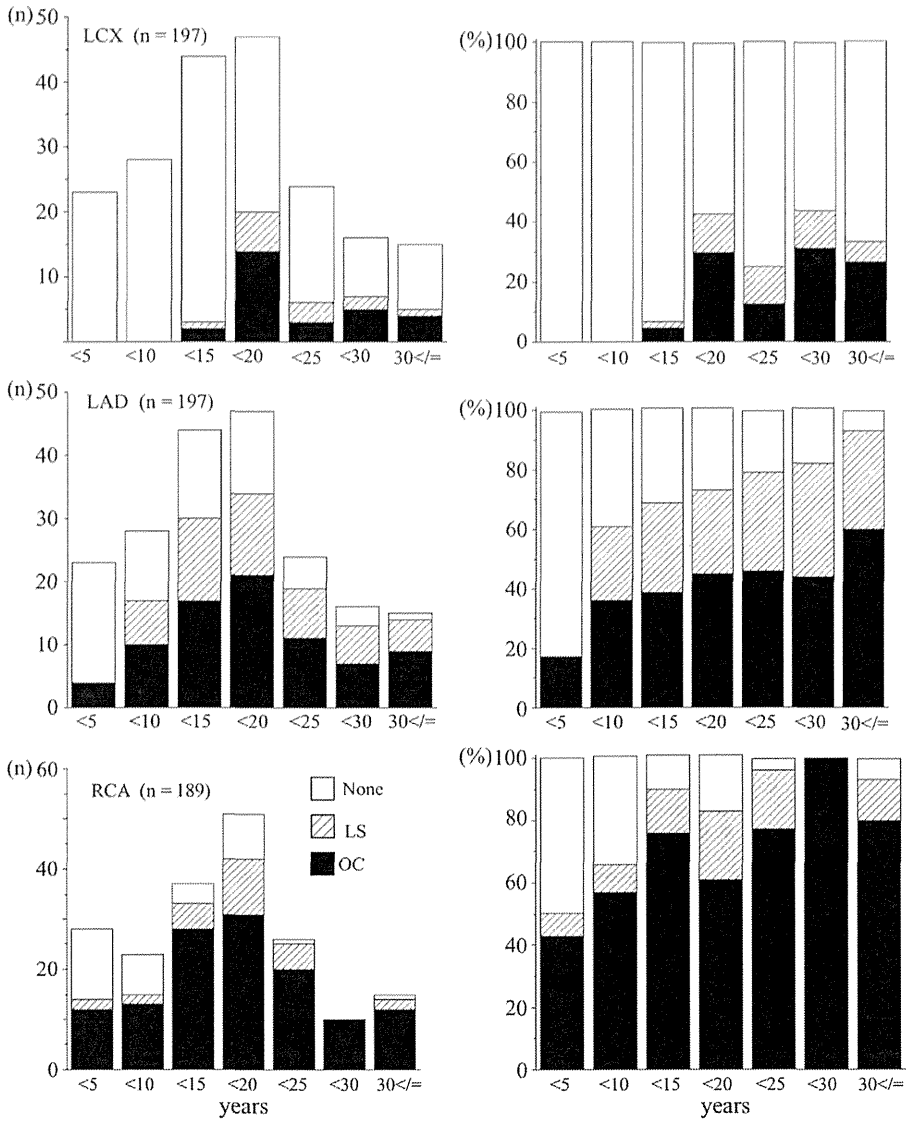
Fifteen (6%) of the 245 patients died (12 were male and 3 were female); 12 of 15 had had a previous MI; and 2 had had asymptomatic coronary occlusion. The age at death ranged from 1 to 28 years (median 16 years), and the interval from KD onset to death was from 2 months to 26 years (median 11 years). Eleven deaths were sudden (67%), and 4 were due to heart failure.

Clinical status

Excepting the 15 deaths, the remaining 230 patients were New York Heart Association I. Eleven women had delivered babies. Of patients with ventricular arrhythmia, VT was found in 4 (1%) and NSVT was detected in 15 patients (6%).

The LVSF and LVDd were recorded by 2-dimensional echocardiogram in 240 patients (98%). The mean LVSFs in the respective groups based on the number of AMI were as follows (in mean ± SD): none, 36% ± 6%; once, 28% ± 10%; and twice, 26% ± 12%. Left ventricular shortening fractions in the AMI groups were significantly lower than those in the non-AMI groups ($P < .001$) (Figure 3). The mean LVDd as a percent normal in the respective groups were as

Figure 2



Incidence of occlusion and LS in respective branches. OC, occlusion including SS.

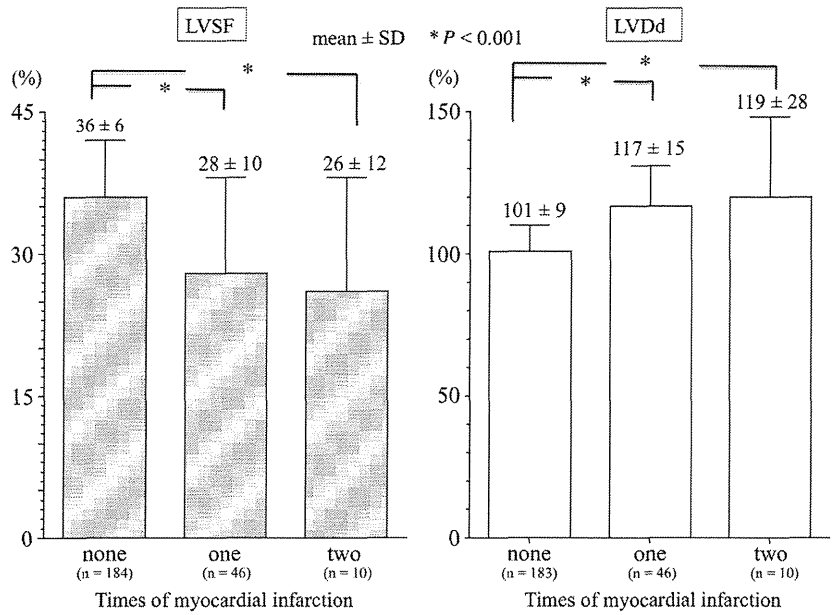
follows (in mean ± SD): none, 101% ± 9%; once, 117% ± 15%; and twice, 119% ± 28%. The LVDd in the groups with AMI were significantly larger than those in the groups without AMI ($P < .001$) (Figure 3). The incidence of VT or NSVT in the respective groups was as follows: none, 4 (2%) of 184; once, 12 (26%) of 46; and twice, 3 (30%) of 10 ($P < .001$). The incidence of VT or NSVT with AMI was significantly higher than that in the group without AMI. Furthermore, the incidence of death with AMI was significantly higher than that in those without AMI, being 2 (1%) of 184, once 9 (20%) of 46, and twice, 4 (40%) of 10 ($P < .001$).

Survival rate and CE-free rate

The overall survival rates in patients with GA at 10, 20, and 30 years were 97% (95% CI 94-99), 95% (95% CI 91-98), and 90% (95% CI 84-93), respectively (n = 245) (Table II; Figure 4, left upper). The 30-year survival rate in patients with a bilateral GA was 87% (95% CI 78-93, n = 141). The 30-year survival rate for a bilateral GA was significantly lower than the unilateral GA of 96% (95% CI 85-99, n = 104, $P = .027$) (Figure 4, right upper).

Overall CE-free rate in patients with GA at 30 years was 36% (95% CI 28-45, n = 245) (Table II; Figure 4, left lower). The 30-year CE-free rate in patients with a

Figure 3



The LVSF and the LVDd based on times of MI.

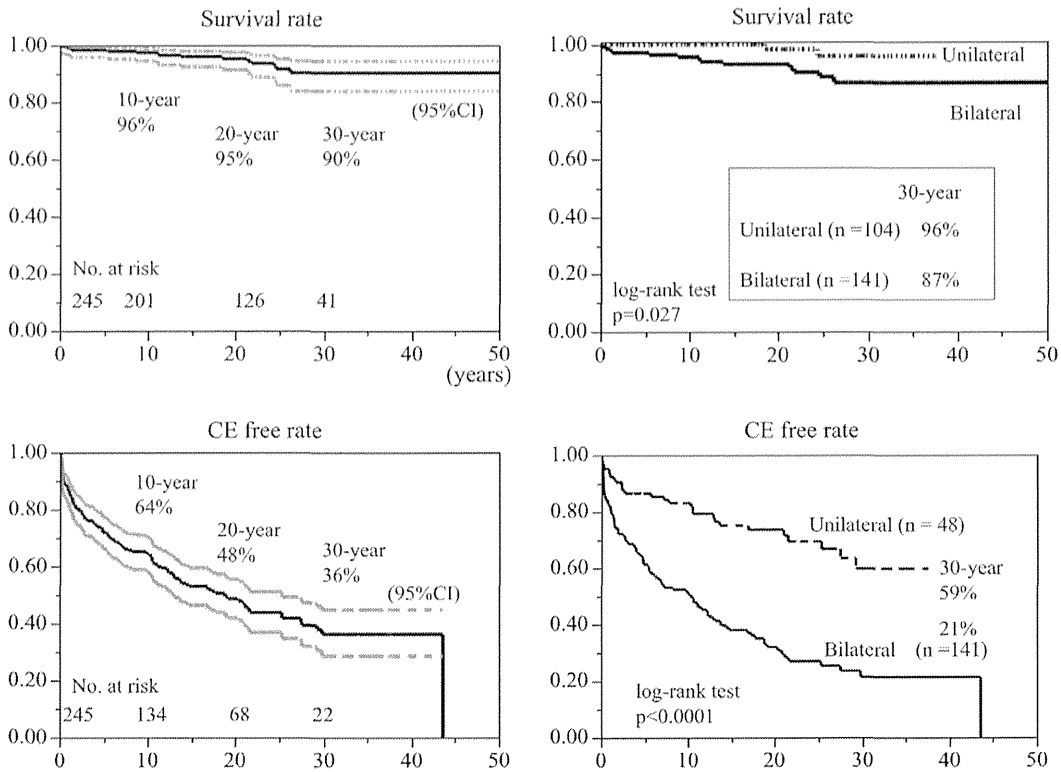
Table II. Survival rate and CE-free rate

	Unilateral			Bilateral			Total		
GA		**95% CI**	**n**		**95% CI**	**n**		**95% CI**	**n**
Survival rate			104			141			245
10-y	100		83	97	92-99	120	97	94-99	203
20-y	96	89-100	54	93	87-96	74	95	91-98	128
30-y	96	85-99	17	87	78-93	26	90	84-94	43
AMI-free rate			104			141			245
1-y	93	87-97	99	82	75-88	113	86	82-91	212
10-y	89	82-94	74	70	63-75	88	78	73-83	162
20-y	89	82-94	49	67	60-75	53	77	71-82	102
30-y	85	75-93	14	66	57-74	23	74	68-80	37
CABG-free rate			104			141			245
10-y	92	96-85	76	67	59-75	83	78	72-83	159
20-y	84	75-91	47	44	36-54	34	61	54-68	81
30-y	80	69-88	14	31	22-44	11	51	43-60	25
CE-free rate			104			141			245
10-y	83	74-89	68	51	43-60	68	64	58-71	134
20-y	73	63-82	42	31	24-41	28	48	42-55	70
30-y	59	46-72	13	21	14-31	11	36	28-45	24

bilateral GA at 30 years was significantly lower than that patients with a unilateral GA 59% (95% CI 46-72, n = 104) vs 21% (95% CI 14-30, n = 141, $P < .0001$) (Figure 5, right lower).

Overall AMI-free rate in patients with GA at 30 years was 74% (95% CI 68-80, n = 245) (Table II; Figure 5, left upper); for patients with bilateral GA, it was 66% (95% CI 57-74, n = 141), and for those with unilateral GA, it was 85% (95% CI 75-93, n = 104). The 30-year AMI-free rate in the bilateral GA was significantly lower than that in the unilateral GA ($P = .0003$) (Figure 5, right upper). Overall CABG-free rate in patients with GA at 30 years was 51% (95% CI 43-60, n = 245) (Table II; Figure 5, left lower). The 30-year CABG-free rates in patients with a bilateral GA was 31% (95% CI 22-44, n = 141), compared with 80% (95% CI 69-88) in the unilateral GA group (n = 104). The

Figure 4



Survival rate and CE-free rate. Left upper: overall survival rate. Right upper: survival rate in the patients with unilateral and bilateral GA. Left lower: overall CE-free rate. Right lower: CE-free rate in the patients with unilateral and bilateral GA.

30-year CABG-free rate in patients with bilateral GA was significantly lower than that in the patients with a unilateral GA ($P < .0001$) (Figure 5, right lower).

Hazard ratios for the incidence of the respective events in the bilateral GA compared with the unilateral GA groups are given in Table III.

Subgroup analysis

Survival rates from the onset of AMI at 10, 20, and 30 years were 87% (95% CI 74-93), 82% (95% CI 67-91), and 49% (95% CI 27-71), respectively (n = 57) (Figure 6, left upper), Without AMI, the 30-year survival was 98% (95% CI 95-100, n = 188). In patients with AMI, the 30-year survival rate after successful revascularization was 100% (n = 14), but after failed revascularization, it was 59% (95% CI 24-87, n = 15) ($P = .061$).

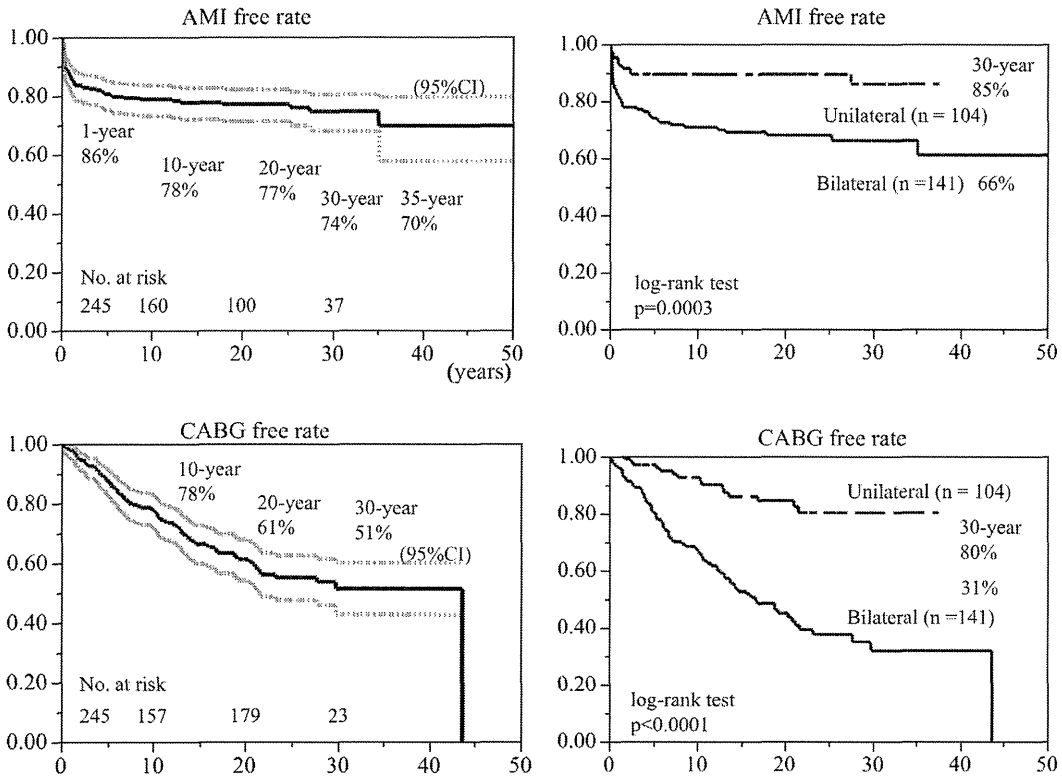
The overall survival rates after CABG at 10 and 25 years were 98% (95% CI 92-99) and 92% (95% CI 81-97), respectively (n = 90) (Figure 6, left lower). After CABG, patients with a history of AMI had a 25-year survival rate of 73 (95% CI 47-89, n = 27), and for those without AMI, survival was 100% (n = 63, $P = .003$) (Figure 6, left lower). The 25-year survival rate for those who had not undergone CABG after AMI was 40% (95% CI 12-76) (n = 30).

Antiplatelet agent therapy given immediately after acute KD resulted in a 30-year survival rate of 90% (95% CI 82-94, n = 145), and treatment with antiplatelet agents plus warfarin was 100% (n = 76, $P = .0466$). (Figure 6, right upper). The incidence of AMI in patients with antiplatelet agents plus warfarin was 22% (17/76), and that in patients with antiplatelet agents alone was 27% (39/145). There was no difference in AMI-free rate between these 2 groups.

Discussion

Acute MI is the most important determinant of the long-term outcome of patients with GA. Although most AMIs occurred within 2 years of the onset of KD, a decreased survival rate was found more than 20 years later.[8] Acute MI after KD in most patients is triggered by sudden coronary occlusion due to thrombosis in the GA predisposed to by endothelial dysfunction, turbulence or stasis within the GA, and hypercoagulability caused by accelerated coagulant and thrombolytic systems, which complicate the immediate post-acute period. Antithrombotic therapy is also beneficial in the critical period immediately after the acute KD.[9] Consequently, antithrombotic treatment, which combines antiplatelet agents

Figure 5



AMI-free rate and CABG-free rate. Left upper: overall AMI-free rate. Right upper: AMI-free rate in the patients with unilateral and bilateral GA. Left lower: overall CABG-free rate. Right lower: CABG-free rate in the patients with unilateral and bilateral GA.

with warfarin immediately after the onset of KD influenced the prognosis, although the incidence of AMI remained unchanged. Furthermore, because successful revascularization after AMI also increases the survival rate, the precise diagnosis and the optimal treatment of AMI are critically needed information.[8]

Characteristically coronary aneurysms caused by KD involve aneurysms at the bifurcation of the LCA, making it difficult to distinguish LAD aneurysms from those of the LCX. As a result, the incidence of stenotic lesions in the LCX was low.[10] Although the 30-year survival rate in patients with a unilateral GA was fairly good, the survival rate for patients with bilateral GA was low. If the number of GA branches increases, it is reasonable that the hazard ratio also increases. However, this study shows that the hazards ratio for bilateral GA compared with unilateral GA in the risk of death and CE was about 4-fold, not 2-fold. We believe that these new findings on the factors that influence the outcome for patients with GA should be the basis for treatment and management of future patients with GA. Episodes of AMI can result in widespread myocardial damage with reduced left ventricular ejection function. Such myocardial involvement can lead to fatal ventricular arrhythmia and other CE years later.[8] Possibly

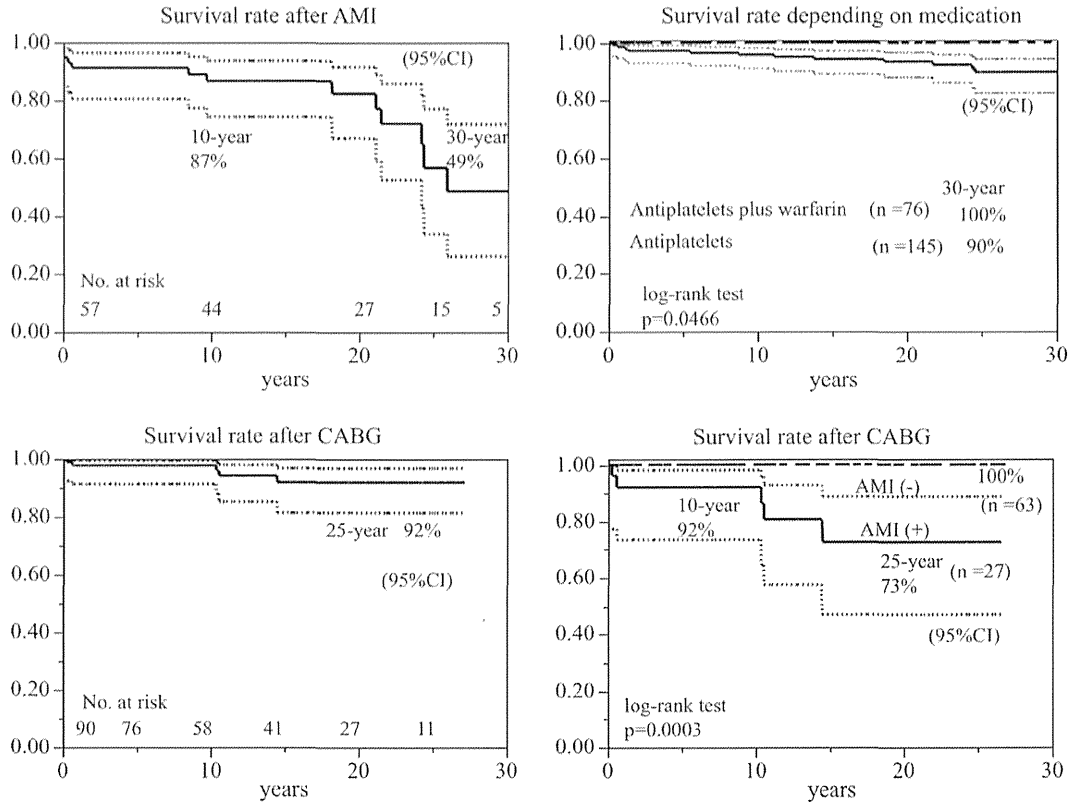
Table III. Hazard ratio for CEs in the patients with bilateral GA compared with the patients with unilateral GA

	Hazard ratio	95% CI	P
Death	4.6	1.27-29.4	.017
AMI	3.04	1.66-6.03	.0002
CABG	4.69	2.77-8.5	.0001
CEs	3.26	2.18-5.03	.001

good collateral arteries may develop more easily in the unilateral GA situation rather than with bilateral GA,[11,12] thereby preventing the decrease of coronary blood flow and preserving ventricular function. Our study showed a significantly better prognosis for unilateral GA.

Stenotic lesions were found in most of the working GA branches after 20 years, although their incidence was very low in vessels without GA.[13] In patients with bilateral GA, the incidence of stenotic lesions was high and the CE-free rate was low. In this situation with multivessel disease, CABG is likely to be more effective. Most patients with bilateral GA will need at least 1 good revascularization procedure over the long-term period after KD, and experience suggests that it increases survival rate.[6,14] Furthermore, CABG for

Figure 6



Survival rates in subgroups. Left upper: survival rate after AMI. Right upper: survival rate in patients given antiplatelet agents alone and in patients given antiplatelet agents plus warfarin. Left lower: survival rate after CABG. Right lower: Survival rate after CABG in patients with AMI and without AMI.

coronary revascularization would be better than PCI in some characteristics in this population.[15] On the other hand, PCI would be more useful for 1-vessel disease.[16] However, this study shows that prognosis may not necessarily be bad in patients with a unilateral GA, even if coronary revascularization is not done. Management that combines CABG and PCI might be adopted in children because of their long life from childhood to late adulthood.[17-19]

The appearance of GA due to acute KD does not always lead to immediate myocardial damage and ventricular dysfunction. In our study, the outcome in patients with GA in the absence of MI was good. The outcome is poor for patients with a decreased left ventricular ejection function. To prevent myocardial damage, the decision to advise coronary revascularization is important and difficult.[15] If ischemia on examination such as treadmill tests or radioisotope stressed myocardial perfusion imaging is detected, coronary revascularization should be considered based on benefit and risk.

Limitations of this study include its retrospective nature and its reliance on multi-institution data, with potential varying indications and selection criteria for interventions. Furthermore, the diagnosis and the treatment have changed and improved dramatically over the last 30 years. Detailed analyses about specific issues are limited because the data are based on a questionnaire. The determinants of survival rates and CEs should be further studied because of their potential to improve the care of future patients.

Conclusions

The long-term outcome was significantly different between bilateral GA and unilateral GA, and these differences are important in making treatment decisions in both the early and late stages. The results focus attention on the need to preserve myocardial perfusion, especially in high-risk patients with bilateral GA through the judicious use of revascularization procedures. The optimal CABG approach would be useful in bilateral GA. The study appears to confirm the value of appropriately used antithrombotic therapy.

Acknowledgements

We thank Professor Peter for his kind English language consultation.

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ORIGINAL ARTICLE
Pediatric Cardiology and Adult Congenital Heart Disease

Platelet Activation Dynamics Evaluated Using Platelet-Derived Microparticles in Kawasaki Disease

Tomoyo Yahata, MD; Chinatsu Suzuki; Ayako Yoshioka; Akiko Hamaoka, MD; Kazuyuki Ikeda, MD

Background: Little is known about the platelet dynamics and the effect of antiplatelet therapy in Kawasaki disease (KD). The aim of this study was to clarify platelet activation dynamics in acute-phase KD patients by assaying platelet-derived microparticles (PDMPs).

Methods and Results: The PDMP level in 18 patients with acute KD was measured on ELISA. Of the 18 patients, 14 were receiving oral aspirin and i.v. immunoglobulin (IVIG) and 4, oral aspirin alone. Blood samples were drawn before, immediately after, and 10–14 days after IVIG infusion; thereafter, at 1, 2, and 3 months after the onset of disease. PDMP level before aspirin treatment was significantly higher in acute-phase KD patients than in the control subjects with common febrile diseases ($P<0.01$). In the acute-phase KD patients, IVIG significantly decreased PDMP level; the PDMP level was not lower on the similar day of KD in the patients who did not receive IVIG. Eight patients' PDMP level rebounded after aspirin was discontinued.

Conclusions: Platelets are activated during acute-phase KD, which confirms the importance of antiplatelet therapy. In addition, platelet activation continues as long as 2 or 3 months after the acute phase, the time at which aspirin is commonly discontinued, and the timing of aspirin discontinuation should therefore be evaluated in each individual patient. (*Circ J* 2014; **78:** 188–193)

Key Words: Antiplatelet therapy; Kawasaki disease; Platelet-derived microparticle

Kawasaki disease (KD) is a form of systemic panvasculitis.[1–3] Typically, vasculitis damages vascular endothelial cells and causes loss of function, including antithrombotic action.[4] The acute phase of KD is therefore thought to involve platelet activation, and antiplatelet therapy is included in the treatment protocol for this phase.[5]

Based on this hypothesis, antiplatelet therapy is a common component of KD treatment strategies. The actual evidence for platelet activation, however, is limited to a few studies in which platelet aggregation tests showed increased aggregation capacity.[6–8] Therefore, antiplatelet agent treatment is only a so-called empiric therapy, being based mainly on the coronary artery thrombotic occlusions that are often confirmed on autopsy after acute phase deaths. In other words, we are routinely using antiplatelet therapy although we do not understand the dynamics of platelet activation.

In this study, we therefore measured the levels of platelet-derived microparticles (PDMPs), which have recently received attention as a marker of platelet activation, in order to elucidate the platelet activation dynamics in KD.

PDMPs have recently been reported as a platelet activation marker, and the PDMP level can therefore be used as an index of platelet activation.[9,10] PDMPs are endoplasmic reticulum-derived vesicles ranging from 0.02 to 0.1 μm in size that are discharged from platelets upon activation by some sort of stimulus. PDMPs have phospholipids with procoagulant activity on their surfaces and contain platelet granule components. PDMPs are not merely debris discharged from activated platelets but also possess intrinsic procoagulant activity and can activate platelets, white blood cells, and vascular endothelial cells to promote cell adhesion and contribute to blood coagulation. In addition, a vicious circle can develop as follows: cells activated by PDMPs produce inflammatory cytokines,[11,12] which induce monocytes, macrophages, neutrophils, and vascular endothelial cells to express tissue factors, which promote the generation of thrombin, which mediates further activation of platelets, and which generates more PDMPs. Consequently, PDMPs are considered as important functional particles involved in the pathogenesis of vascular inflammation; in other words, PDMPs are a marker of both platelet activation and vascular inflammation.

Accordingly, the measurement of PDMP level in patients with KD with panvasculitis should allow us to examine the dynam-

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ics of both platelet activation and the vasculitis itself. The aim of this study was to prove that a state of platelet activation exists in patients with KD; to determine the clinical significance of antiplatelet therapy; and to demonstrate the usefulness of PDMP level in evaluating the degree of vasculitis and the effect of antiplatelet therapy in KD.

Methods

Subjects

The subjects were 18 patients with KD in the acute phase. According to the treatment protocol for the acute phase, 14 patients (patients 1 14; mean age, 2 years 7 months ±2 years) received i.v. immunoglobulin (IVIG; 2 g/kg) and oral aspirin as antiplatelet agents, while 4 patients (patients 15–18; mean age, 1 year 7 months ±1 year) received oral aspirin alone. The dose of aspirin was 30–50 mg·kg^{-1}·day^{-1} during having fever, and we changed the dose (5 mg·kg^{-1}·day^{-1}) when the patients became afebrile. Aspirin was discontinued after 2 or 3 months. These subjects were compared with 33 age-matched patients with common febrile diseases as controls. All of the subjects were confirmed to have KD, with no questionable or incomplete diagnoses. Of the 14 subjects who received IVIG, none exhibited symptoms refractory to this treatment; the fever abated within 48 h after the start of the IVIG infusion in all subjects, and no subject developed any coronary artery disorder.

Written consent to participate in the study was obtained from all of the patients' guardians as their representatives. This study obtained Ethics Committee approval in University Hospital, Kyoto Prefectural University of Medicine.

Blood Sampling

Two milliliters of blood were obtained via a 21-G needle and mixed with a one-tenth volume of acid citrate dextrose/ethylene diamine tetraacetate (ACD/EDTA, Nipro Neotube; Nipro, Japan). The samples were centrifuged at 8,000–10,000×*g* to obtain platelet-poor plasma (PPP). All procedures were performed at room temperature. The samples were stored in deep-freeze (from −40 to −80°C) until analysis.

Blood samples were collected from acute-stage patients at the following times: (1) before IVIG; (2) 2 days after IVIG;

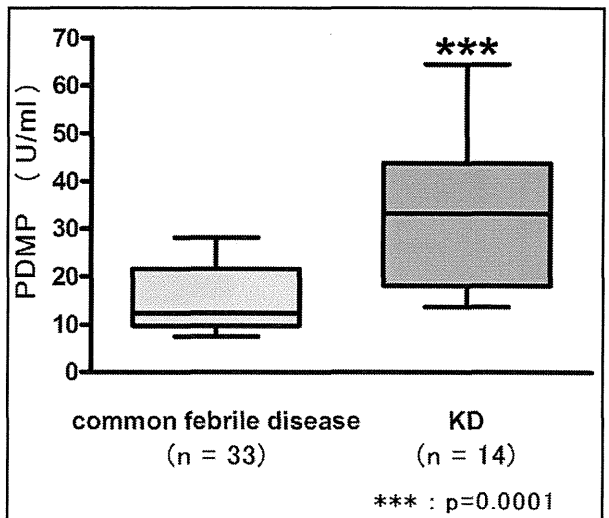

Figure 1. Comparison of platelet-derived microparticle (PDMP) level between controls (common febrile diseases) and Kawasaki disease (KD) patients. PDMP level was significantly higher in the KD patients (P=0.0001).

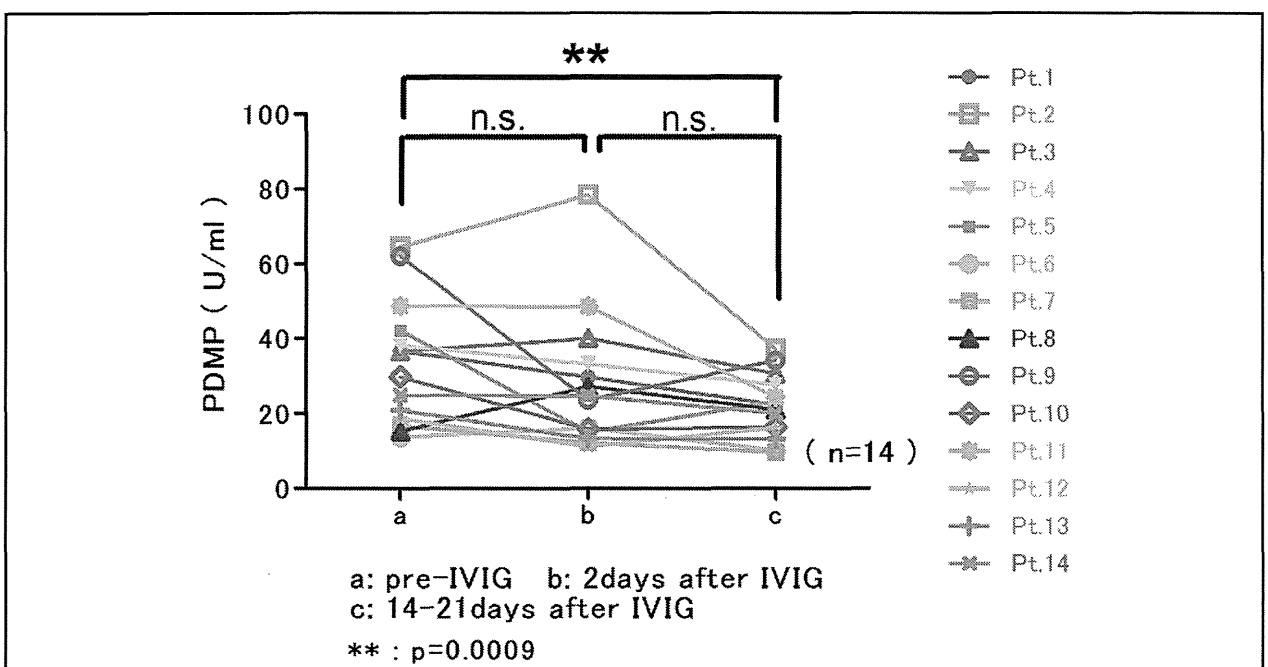

Figure 2. Dynamics of platelet-derived microparticle (PDMP) level in Kawasaki disease patients after i.v. immunoglobulin (IVIG). (a) before IVIG; (b) 2 days after IVIG; (c) 10–14 days after IVIG.

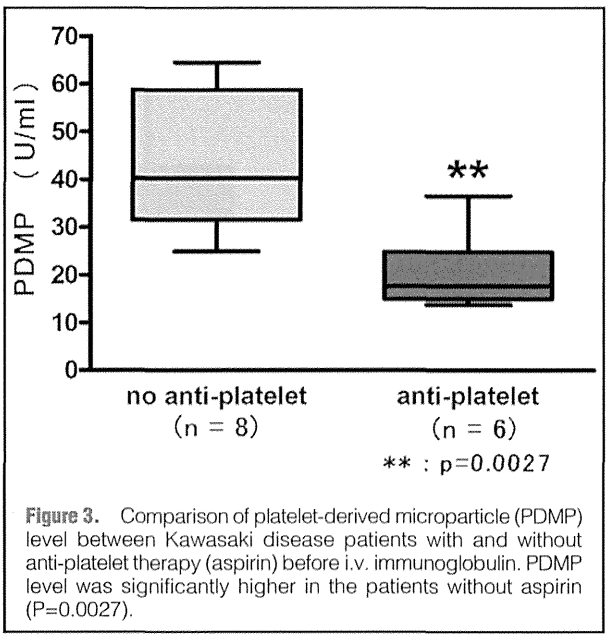

Figure 3. Comparison of platelet-derived microparticle (PDMP) level between Kawasaki disease patients with and without anti-platelet therapy (aspirin) before i.v. immunoglobulin. PDMP level was significantly higher in the patients without aspirin (P=0.0027).

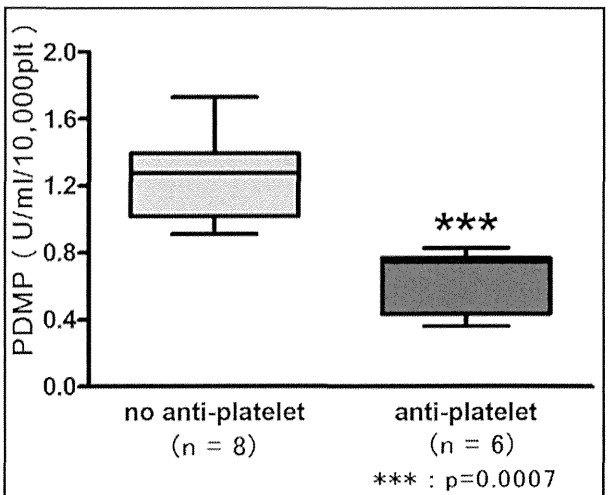

Figure 4. Comparison of platelet-derived microparticle (PDMP) level per 10,000 platelets (modified evaluation of **Figure 3**).

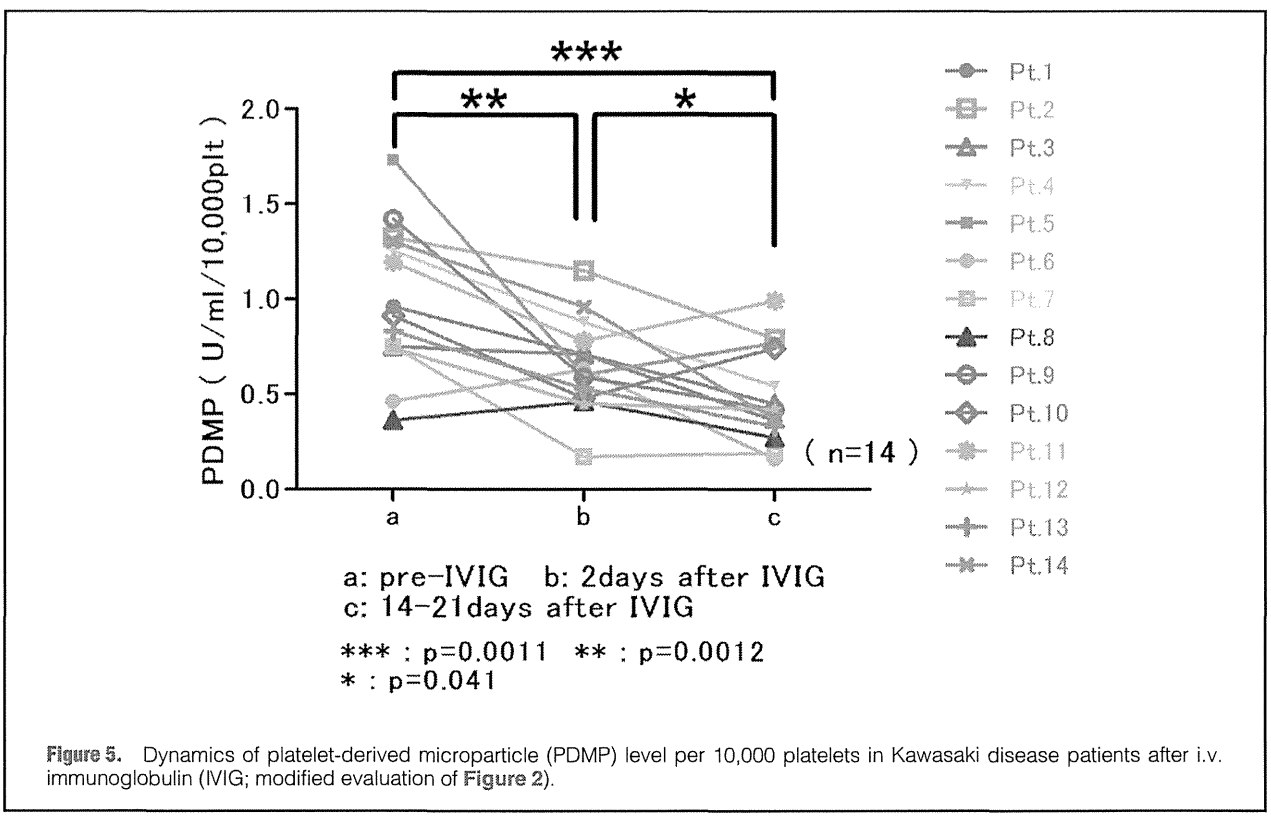

Figure 5. Dynamics of platelet-derived microparticle (PDMP) level per 10,000 platelets in Kawasaki disease patients after i.v. immunoglobulin (IVIG; modified evaluation of **Figure 2**).

(3) 10–14 days after IVIG; (4) 1 month; (5) 2 months; and (6) 3 months after the onset of disease. In patients treated only with aspirin, samples were collected at (1') day 5; (2') day 7; and (3') day 14–21 after onset. These time points (1'–3') were almost the same as time points (1–3).

Biochemistry

PDMP level was measured using an enzyme-linked immunosorbent assay (ELISA) system modified from the method reported by Osumi et al.[13] The ELISA used is now available as a kit from Ootsuka Pharmaceutical (Tokyo, Japan). Fifty microliters of pretreatment solution and 50μl of a PPP (PDMP sample) or standard were added to each well of a 96-well plate

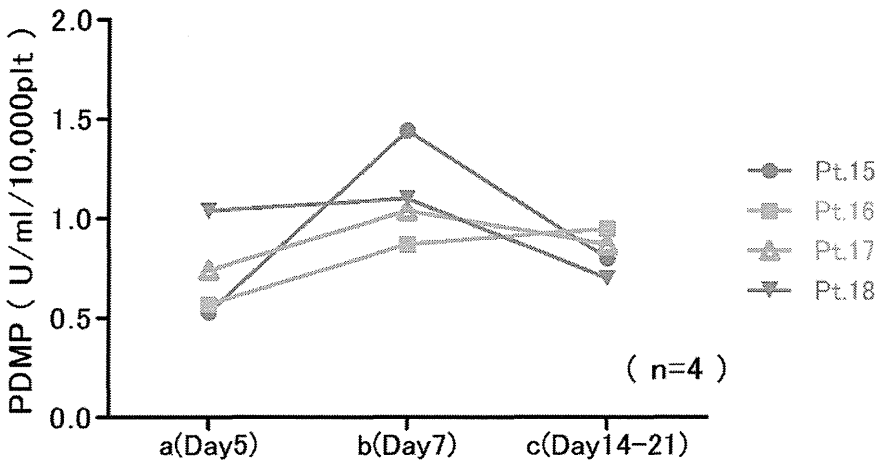

Figure 6. Dynamics of platelet-derived microparticle (PDMP) level per 10,000 platelets in Kawasaki disease patients without i.v. immunoglobulin. The 3 time points of blood collection in **Figure 5** and **Figure 6** are almost the same. The dynamics did not show a tendency to decrease.

and incubated for 3 h at 25°C on a plate shaker (200 rpm). The plates were washed 3 times with 350-μl/well of wash buffer (0.05% Tween 20 in PBS). One hundred microliters of peroxidase-conjugated GPIb antibody was added to each well and incubated for 1 h at 25°C on a plate shaker. Each well was washed 3 times with 350 μl of wash buffer and then incubated with 100 μl of peroxidase substrate solution for 20 min at room temperature. Finally, 100 μl of stop solution was added to each well, and the absorbance was measured with an microplate reader at 450 nm.

Statistical Analysis

Inter-group differences in continuous variables were evaluated using the Wilcoxon signed rank test, because the data were not normally distributed. The intra-group differences among the time points were analyzed using Mann-Whitney test. $P<0.05$ was considered statistically significant.

Results

We first compared PDMP level between the patients with acute-phase KD (43.9±13.5 U/ml) and those with common febrile diseases (15.4±6.8 U/ml). The PDMP level was significantly higher in the patients with acute-phase KD (P=0.0001; **Figure 1**). Second, we examined the PDMP dynamics during the acute phase of KD and found no statistically significant reduction in the PDMP level immediately after IVIG treatment (27.8±18.3 U/ml) compared with the pre-IVIG level (33.5±16.6 U/ml; **Figure 2**). Even before IVIG treatment, however, the PDMP level was significantly lower in the patients who had already received aspirin (20.3±8.3 U/ml) than in those who had received no anti-platelet therapy (43.4±14.3 U/ml; P=0.0027; **Figure 3**). The PDMP level 10–14 days after IVIG treatment (21.9±8.5 U/ml) was significantly lower than the pre-IVIG level (P=0.0009; **Figure 2**).

Third, in consideration of the fact that the platelet count changes greatly during the acute phase of KD, we evaluated the dynamics of the PDMP level per 10,000 platelets. The PDMP level per 10,000 platelets before IVIG infusion was 1.00±0.39 U/ml. The PDMP level of the patients who started

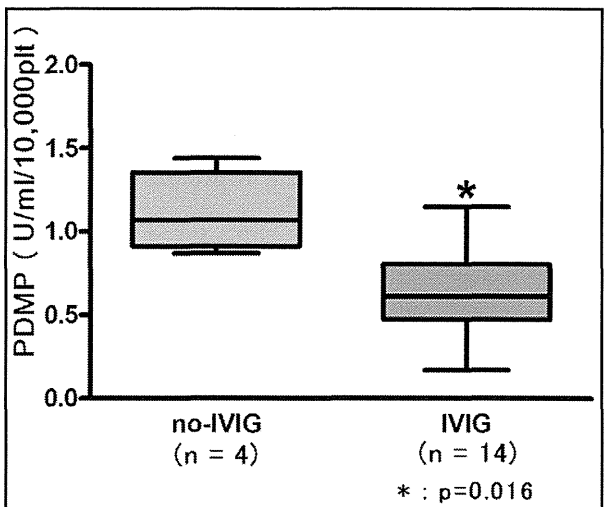

Figure 7. Comparison of platelet-derived microparticle (PDMP) level in Kawasaki disease patients without i.v. immunoglobulin (no IVIG) at day 7, and with IVIG at 2 days after IVIG. The PDMP level in patients after IVIG is significantly low (P=0.016).

receiving aspirin before IVIG (0.65±0.19 U/ml) was lower than that of the patients who received no aspirin before IVIG (1.26±0.26 U/ml; P=0.0007; **Figure 4**). The PDMP levels both immediately (0.65±0.24 U/ml) and 10–14 days after (0.49±0.25 U/ml) IVIG treatment were significantly lower than the pre-IVIG level (P=0.0012 and P=0.0011, respectively; **Figure 5**).

Fourth, comparison between the patients who did and did not receive IVIG showed that respective PDMP level increased in all the patients who did not receive IVIG (n=4; **Figure 6**), and this trend was opposite to the dynamics in the patients who received IVIG. Furthermore, the PDMP level of the patients who received IVIG was significantly lower than that in the patients

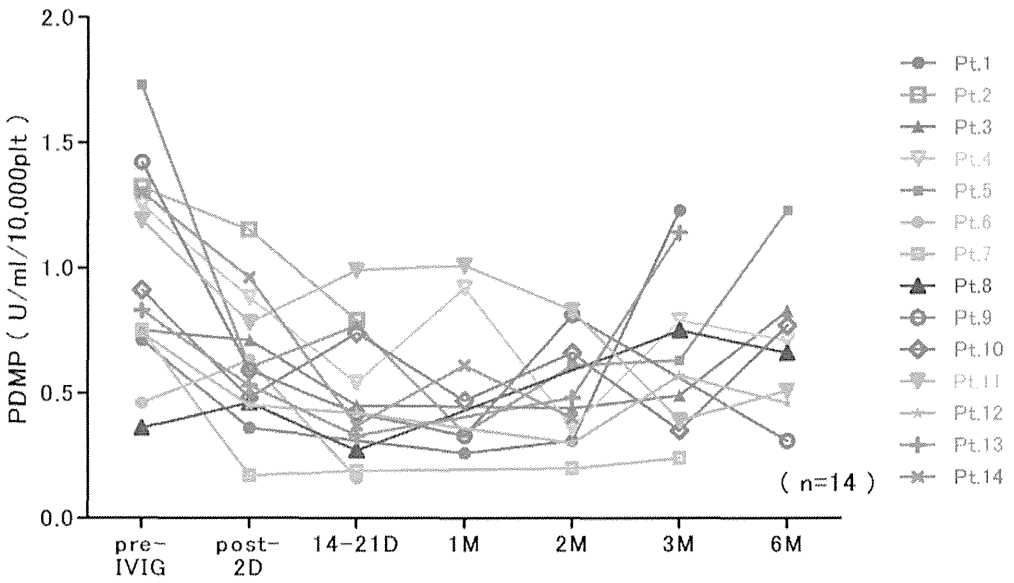

Figure 8. Dynamics of the platelet-derived microparticle (PDMP) level per 10,000 platelets in Kawasaki disease convalescence. The PDMP level of 8 patients increased after the discontinuation of aspirin.

who did not receive IVIG at the same time points (P=0.016; Figure 7). And even if PDMP level initially declined, 4 patients had recurrent rising PDMP level when aspirin was discontinued after 2 months, and so did another 4 when it was discontinued after 3 months (Figure 8).

Discussion

In the present study, PDMP level was elevated, indicating greater platelet activation in patients with acute-phase KD than in other febrile patients. Furthermore, the use of aspirin as an antiplatelet agent significantly changed PDMP level, which supports the value of antiplatelet therapy during the acute phase.

We used PDMP as an index of platelet activation to show that platelet activation is present during the acute phase of KD and that antiplatelet therapy is clinically significant. Although PDMP has recently received a great deal of attention as a marker of platelet activation in adults,[9,10] there are no data on PDMP level in pediatric patients. PDMP can be expected to be a clinically useful index of platelet activation in pediatric patients in the future.

In adults, PDMP has been featured increasingly often in reports covering such diverse clinical areas as oncology, in addition to thrombotic diseases,[14,15] and is a subject of great interest in the understanding of the pathogenesis of vascular disease. The mechanism by which PDMPs are generated has been previously described as follows: platelets are activated by some stimulus, causing phospholipids to be displayed on the platelet surface due to the flip-flop phenomenon; the catalytic activity of these phospholipids then increases the surface's procoagulant activity. A portion of the platelet surface membrane separates and is released as a PDMP that has phospholipids on its surface and contains platelet granule components. Therefore, PDMPs have been reported to have stronger procoagulant activity (50- to 100-fold higher) than platelets.[16]

Furthermore, discharged PDMPs have many other functions besides procoagulant activity. Among these is increasing the expression of adhesion molecules by white blood cells or vascular endothelial cells, which may contribute to vascular inflammation.[11] Conversely, increased expression of inflammatory cytokines has been reported to promote platelet aggregation and the generation of PDMPs. From this point of view, PDMP can be an index for evaluation of vasculitis.

In the present study, we used a sandwich ELISA based on antibodies against the platelet-specific markers CD42b (GPIb) and CD42a (GPIX), which was recently developed by Nomura et al,[13,17,18] and obtained stable reproducibility. PDMPs are expected to become an even more useful marker now that standardization, which was an issue of flow cytometry, has become easy to do, and the reliability of the measured results has increased.

The mechanism of platelet activation in KD was theorized to proceed as follows: the intense inflammation during the acute phase damages the vascular endothelial cells, leading to loss of the anti-thrombotic function of the vascular endothelium and causing platelet activation. But there has been no actual evidence for this mechanism. Only the observation of thrombi, which are the final result of platelet activation, during autopsies of deceased patients with acute-phase KD supported this theory. Previously, the representative report on platelet activation in the acute phase was the study by Taki et al that measured platelet aggregation capacity.[6] They reported that platelet coagulant activity is elevated during the acute phase and might continue to increase for several months. That method, however, measures the reactivity of retrieved platelets and does not necessarily reflect their status in vitro. The present PDMP measurement method evaluates platelet activation in vitro and is therefore an extremely significant advance.

In the present study, the PDMP level of several patients without obvious coronary artery disorders rebounded when aspirin was discontinued after 2–3 months, which indicates that platelet activation may persist long past previous expectations. Aspirin

as part of the treatment protocol for acute-phase KD is usually discontinued 2 or 3 months after onset if there are no coronary artery complications, because clinical experience has suggested that the platelet activation component of KD may resolve within that time frame. The present results suggest that the duration of platelet activation varies among individual patients and that the timing of discontinuation of antiplatelet therapy should be determined by the respective dynamics of platelet activation.

Conclusions

Platelets are activated during the acute phase of KD, which confirms the clinical importance of antiplatelet therapy in these patients. We also showed that IVIG, the standard treatment for acute-phase disease, may act secondarily to reduce platelet activation by suppressing inflammation. Furthermore, the timing of discontinuation of antiplatelet therapy should be determined on an individual basis.

Acknowledgments

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Disclosures

None.

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