

Persistent monocytosis after intravenous immunoglobulin therapy correlated with the development of coronary artery lesions in patients with Kawasaki disease

Ho-Chang Kuo¹, Chih-Lu Wang², Chi-Di Liang³, Hong-Ren Yu¹, Hsin-Hsu Chen¹, Lin Wang¹, Kuender D. Yang¹

¹Division of Allergy, Immunology, and Rheumatology, Department of Pediatrics, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Graduate Institute of Clinical Medical Sciences, Chang Gung University College of Medicine, Kaohsiung; ²Department of Pediatrics, Po-Jen Hospital, Kaohsiung; and ³Division of Pediatric Cardiology, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Kaohsiung, Taiwan

Received: June 29, 2006 Revised: August 1, 2006 Accepted: August 30, 2006

Background and Purpose: This study was conducted to investigate whether changes in the complete blood count (CBC)/differential count (DC) and C-reactive protein (CRP) were correlated to Kawasaki disease (KD) with coronary artery lesions (CALs).

Methods: A retrospective analysis was performed of all children with KD at Chang Gung Memorial Hospital at Kaohsiung from 2001 to 2006. KD patients were divided into those with and without CALs for testing of correlations with changes in CBC/DC and CRP levels.

Results: A total of 147 patients were enrolled for this analysis. Serial CBC/DC and CRP measurements and echocardiographic data for determination of CAL formation were obtained before and after intravenous immunoglobulin (IVIG) treatment. There were 44 (29%) KD patients having CAL formation (>3 mm in diameter of internal lumen). There was no significant difference in terms of age distribution and major diagnostic criteria between KD patients with and without CALs. Male KD patients, however, had a significantly higher rate of CAL formation ($p=0.009$). In multivariate logistical regression analysis, persistent monocytosis after IVIG treatment was the only factor significantly correlated to CAL formation ($p=0.003$).

Conclusions: Of the febrile routine measurements of CBC/DC and CRP in KD, persistent monocytosis after IVIG treatment was correlated to CAL formation. Further studies to clarify the mechanism of monocytosis may help prevent the CALs of KD.

Key words: Coronary arteriosclerosis; C-reactive protein; Mucocutaneous lymph node syndrome

Introduction

Kawasaki disease (KD) is an acute febrile vasculitis of unknown etiology first described by Tomisaki Kawasaki [1]. KD occurs worldwide and primarily affects young children (<5 years of age). The incidence of KD appears to be the highest among children in Japan and the Japanese-American children living in Hawaii, followed

by the Hawaiian, Chinese-American, Korean-American, and Filipino-American children living in Hawaii, and the Taiwanese and Chinese children living in Asia [2-6]. The clinical characteristics of KD are prolonged fever, conjunctivitis, diffuse mucosal inflammation, polymorphous skin rashes, indurative edema of the hands and feet associated with subsequent peeling of finger tips, and non-suppurative cervical lymphadenopathy [1,7]. The most serious complication of KD is acute coronary syndrome showing myocardial infarction, coronary artery dilatation, or coronary artery aneurysms, which is pathognomonic when identified in the setting of a compatible febrile illness [7]. In addition

Corresponding author: Dr. Kuender D. Yang, Department of Pediatrics, Chang Gung Memorial Hospital-Kaohsiung Medical Center, 123 Ta-Pei Road, Niasung Hsiang, Kaohsiung, Taiwan.
E-mail: yangkd@adm.cgmh.org.tw

to the diagnostic criteria, a broad range of nonspecific clinical features can be found in KD, including irritability, uveitis, aseptic meningitis, cough, vomiting, diarrhea, abdominal pain, gallbladder hydrops, urethritis, arthralgia, arthritis, hypoalbuminemia, liver function impairment, and heart failure [8]. In some countries where newborn babies receive bacillus Calmette-Guérin (BCG) vaccination, KD can be associated with erythematous induration or even ulceration of the BCG scars in one-third of KD patients [9]. In developed countries, KD is currently the leading cause of acquired heart diseases in children [7-9].

Sequelae of coronary artery aneurysm developed in 20% of untreated KD children [2]. A multicenter study in the USA established that a single high-dose (2 g/kg) of intravenous immunoglobulin (IVIG) plus aspirin could lower the incidence of aneurysm from 20% to 3-5% [10]. The IVIG treatment could also shorten the duration of fever when it was given within 10 days of the fever [10,11]. Since coronary artery lesions (CALs) occurred at a mean of 10 days after the onset of KD, it is important to treat and prevent the progression of coronary artery injury within 9 days of the KD [7,12].

The aim of this study was to investigate whether common febrile routine measurements of complete blood counts (CBC), differential count (DC), and C-reactive protein (CRP) in KD before and after IVIG treatment could be correlated to CAL formation and to find early risk factors for the prediction and possible prevention of CALs in KD.

Methods

Patients

All subjects studied were children who fulfilled the criteria for KD [8] and were admitted for IVIG treatment at Chang Gung Memorial Hospital at Kaohsiung from 2001 to February 2006. Patients were initially treated with a single dose of IVIG (2 g/kg) during a 12-h period. Aspirin was also given until all signs of inflammation resolved and the CAL regressed as detected by two-dimensional (2D) echocardiography. We retrospectively analyzed the CBC/DC and CRP in KD patients with and without CALs.

Collection of clinical and laboratory data

Major clinical features of KD that occur in the acute stage of KD were recorded and coded for analyses. Laboratory data including CBC, DC, and CRP were collected for this analysis. Laboratory data were obtained

before IVIG treatment, and 2 to 3 days after the initial IVIG treatment. The absolute DCs were calculated from the total white blood cell (WBC) counts together with the percentage of DCs from peripheral blood samples. All patients with KD underwent 2D-echocardiography of their coronary artery before IVIG treatment, and 2 echocardiographies subsequently within 4 weeks of the IVIG treatment. CAL was defined as the internal diameter of the lumen >3 mm [13]. Patients were classified into 2 groups: group 1 (KD without CAL formation) included patients who did not develop CALs in any of the 2D-echocardiography examinations; and group 2 (KD with CAL formation) included KD patients with CALs in any of the 2D-echocardiography examinations.

Statistical analysis

Changes of CBC/DC and CRP levels before and after IVIG treatment were tested by the paired *t* test. The gender distribution and presentation of major clinical criteria of KD patients with and without CALs were tested by the chi-squared test. CBC/DC and CRP levels before and after IVIG treatment were tested by the Student's *t* test. Multivariate analysis with logistical regression was used to assess parameters between the 2 groups. A *p* value <0.05 was considered statistically significant. All statistical tests were performed using the Statistical Package for the Social Sciences for Windows (Version 12.0; SPSS Chicago, IL, USA).

Results

Clinical features of KD patients

The study enrolled 147 patients with KD, 101 boys (68%) and 46 girls (32%). 103 patients (71%) had no evidence of CALs and 44 patients (29%) had CALs. The most common signs and symptoms were conjunctivitis (97.9%), fissured lips (93%), strawberry tongue (88.6%), induration over extremities (88.1%), and skin rashes (89.6%). In contrast, only 46.2% had cervical lymphadenopathy in this series. A higher rate of BCG scar reactions (42.1%, 62/147) was found in this series. The IVIG retreatment rate was 14% (21/147) in this study. The IVIG retreatment rate in patients with CALs was significantly higher than that in patients without CALs (27.3% vs 8.7%, *p*=0.003).

Features of KD with and without CALs

There were no statistical differences in the major diagnostic criteria of KD between KD patients with and without CALs (Table 1). Age distribution of conjunctivitis, cervical lymphadenopathy, polymorphous skin rash,

Table 1. Demographic data of Kawasaki disease patients with and without coronary artery lesions (CALs)^a

	Without CALs No. of patients (%) [n = 103]	With CALs No. of patients (%) [n = 44]	<i>p</i> ^b
Age (months) ^c	18.81 ± 1.27	22.45 ± 3.12	0.19 ^d
Male gender	64 (62.1)	37 (84.1)	0.009
Conjunctivitis	100 (97.0)	44 (100)	0.25
Cervical lymphadenopathy	52 (50.5)	16 (36.4)	0.11
Polymorphous skin rash	93 (90.3)	38 (86.3)	0.36
Strawberry tongue	88 (85.4)	38 (86.3)	0.48
Induration of extremities	84 (81.5)	35 (79.5)	0.50
BCG scar reactions	45 (43.2)	16 (36.3)	0.53

Abbreviation: BCG = bacillus Calmette-Guérin

^aInternal diameter of lumen >3 mm.

^b*p* Values were tested by chi-squared test, except for age distribution.

^cMean ± standard error of the mean.

^dAge distribution was tested by independent *t* test.

strawberry tongue, induration over limbs, and BCG scar reactions was not significantly different between the 2 groups (*p*>0.05). Male children had a significantly higher frequency of CALs than females (37/101 and 7/46, respectively, *p*=0.009). The time to resolution of fever after IVIG treatment had no significant difference between KD with and without CALs (27.8 ± 4.2 vs 21.5 ± 2.4 h, *p*=0.4; Mann-Whitney *U* test).

Changes of CBC/DC and CRP after IVIG treatment

Total WBC (13,940 ± 374 vs 9293 ± 275/mm³, *p*<0.001), neutrophils (9009.1 ± 290.3 vs 3772.2 ± 234.8/mm³, *p*<0.001), monocytes (897.1 ± 97.1 vs 628.4 ± 26.1/mm³, *p*=0.004), hemoglobin (10.84 ± 0.10 vs 10.32 ± 0.09 g/dL, *p*<0.001), and CRP levels (96.6 ± 5.6 vs 55.9 ± 4.6 mg/L, *p*<0.001) were elevated before IVIG treatment and significantly decreased after IVIG treatment. In contrast,

platelets (34.5 ± 0.9 vs 44.8 ± 1.4 × 10⁴/mm³, *p*<0.001), eosinophils (345.8 ± 28.5 vs 432.4 ± 28.7/mm³, *p*=0.005), and lymphocytes (3318.0 ± 165.9 vs 4045.7 ± 158.4/mm³, *p*<0.001) were not elevated before IVIG treatment, but significantly increased after IVIG treatment. Basophils also increased after IVIG treatment, but this increase was not significant.

According to univariate analysis, CBC/DC and CRP data before and after IVIG treatment were not correlated to CALs, but total WBC and monocytes after IVIG treatment were significantly correlated to CAL formation (*p*=0.014 and *p*=0.01, respectively) [Table 2 and Table 3]. According to multivariate analysis with logistical regression, only monocytosis after IVIG treatment was significantly associated with CALs in KD (*p*=0.003) [Table 3]. As shown in Fig. 1, a decline of WBC after IVIG treatment was found in both KD patients with and without CALs, but total WBC after

Table 2. Comparison of complete blood count and C-reactive protein levels between Kawasaki disease patients with and without coronary artery lesions (CALs)^a before intravenous immunoglobulin treatment

Variable ^b	Without CALs	With CALs	Univariate analysis <i>p</i>	Multivariate analysis <i>p</i>
White blood cell/mm ³	13,682 ± 433	14,545 ± 730	0.29	0.14
Hemoglobin (g/dL)	10.6 ± 0.1	10.8 ± 0.2	0.37	0.36
Platelet (× 10 ⁴ /mm ³)	33.8 ± 0.9	37.6 ± 2.1	0.11	0.67
Neutrophil/mm ³	8997 ± 337	9668 ± 576	0.29	0.07
Lymphocyte/mm ³	3259 ± 183	3454 ± 353	0.59	0.43
Monocyte/mm ³	858 ± 51	844 ± 79	0.88	0.44
Eosinophil/mm ³	344 ± 33	349 ± 53	0.93	0.60
Basophil/mm ³	19.8 ± 3.5	30.4 ± 8.5	0.25	0.15
C-reactive protein (mg/L)	90.7 ± 5.9	107.3 ± 11.2	0.12	0.54

^aInternal diameter of lumen >3 mm.

^bMean ± standard error of the mean.

Table 3. Comparison of complete blood count and C-reactive protein levels between Kawasaki disease patients with and without coronary artery lesions (CALs)^a after intravenous immunoglobulin (IVIG) treatment^b

Variable ^c	Without CALs	With CALs	Univariate analysis <i>p</i>	Multivariate analysis <i>p</i>
White blood cell/mm ³	8691 ± 292	10,475 ± 606	0.014 ^d	0.210
Hemoglobin (g/dL)	10.0 ± 0.2	10.5 ± 0.4	0.630	0.860
Platelet (x 10 ⁴ /mm ³)	41.0 ± 2.9	52.1 ± 7.1	0.290	0.530
Neutrophil/mm ³	3671 ± 539	4374 ± 855	0.070	0.220
Lymphocyte/mm ³	3900 ± 202	4224 ± 230	0.460	0.900
Monocyte/mm ³	663 ± 29	850 ± 94	0.010 ^d	0.003 ^d
Eosinophil/mm ³	411 ± 27	478 ± 71	0.390	0.230
Basophil/mm ³	28.8 ± 4.8	24.5 ± 6.2	0.480	0.900
C-reactive protein (mg/L)	64.7 ± 13.5	33.7 ± 11.0	0.810	0.480

^aInternal diameter of lumen >3 mm.

^bUnivariate analysis showed total white blood cells and monocytes to have significant correlations with CALs after IVIG treatment; multivariate analysis with logistical regression showed higher monocytes to be the only factor significantly associated with CALs.

^cMean ± standard error of the mean.

^d*p*<0.05.

IVIG treatment remained higher in KD patients with CALs than in those without CALs. In contrast, persistent monocytosis after IVIG treatment was found in patients with CALs (*p*=0.01, Fig. 2).

Discussion

Detailed analyses of CBC/DC and CRP levels in KD have not been reported in the literature. In this study,

we found that monocytosis, neutrophilia, and lymphopenia were prominent in the acute phase of KD, suggesting that augmented innate immunity associated with lower adaptive immunity may be involved in the pathogenesis of KD. Supporting this possibility, we also found that a persistent monocytosis after IVIG treatment was significantly associated with CALs. Further studies to identify whether monocytosis is the cause or effect of the pathogenesis of KD are needed.

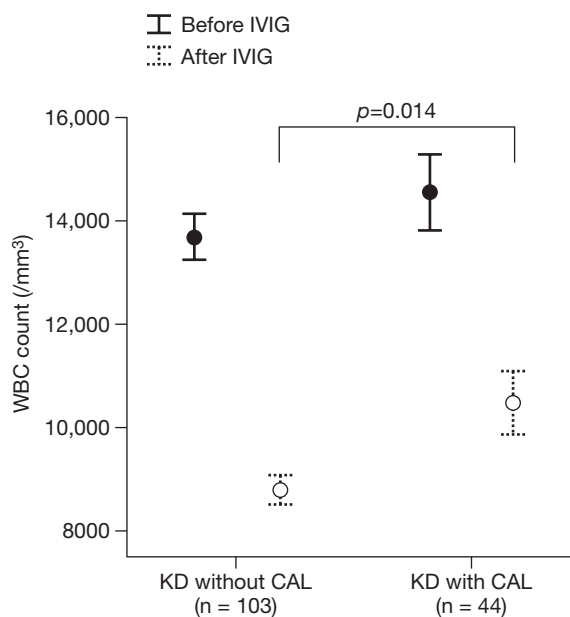


Fig. 1. Total white blood cell (WBC) levels before and after intravenous immunoglobulin (IVIG) treatment in Kawasaki disease (KD) patients with and without coronary artery lesions (CALs). Data are presented as mean ± standard error of the mean.

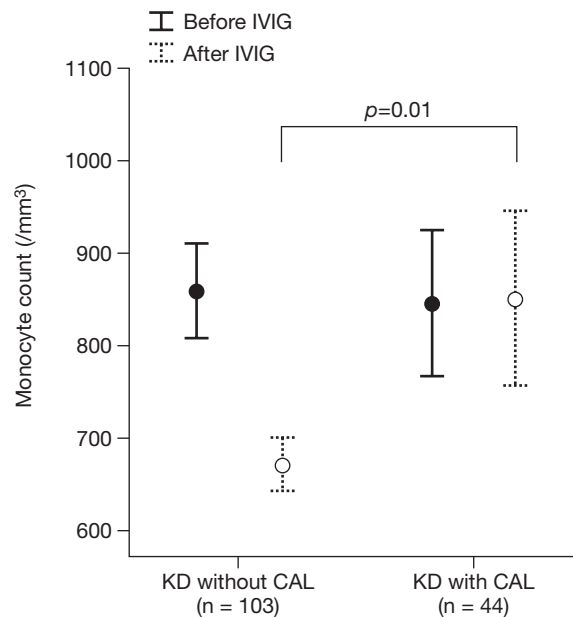


Fig. 2. Monocyte levels before and after intravenous immunoglobulin (IVIG) treatment in Kawasaki disease (KD) patients with and without coronary artery lesions (CALs). Data are presented as mean ± standard error of the mean.

Furukawa et al [14] have reported that KD patients with CALs showed an increase in CD14+ macrophages/monocytes when compared to KD patients without CALs in the acute phase. They also found a greater decrease in CD14+ macrophage/monocyte counts after IVIG treatment in the former group [15]. In our study, monocytes were significantly elevated in the acute stage before IVIG treatment, but not significantly different between KD patients with and without CALs. The average absolute blood monocyte counts vary with age — being high in newborns and children and then declining to 400/mm³ during adulthood [16]. The up-limited value of absolute blood monocyte counts in the age group of 6 months to 6 years is 750/mm³ [17]. We were, however, the first to find that a persistent monocytosis after IVIG treatment was significantly correlated to the presence of CALs in KD patients. This suggests that KD patients may progress to CAL formation if the initial IVIG treatment is unable to suppress the activation of monocytes. We plan to investigate the monocyte growth factors and monokines in KD patients with and without CALs next.

A lower hemoglobin level has been shown to be one of the risk factors of coronary artery aneurysms following IVIG treatment [18]. In this study, we did not find any correlation between hemoglobin levels and the presence of CALs in KD. Previous reports, however, observed that the occurrence of coronary artery aneurysms was higher among KD patients with more severe vasculitis and inflammation responses, such as elevated CRP and erythrocyte sedimentation rate. The risk of CALs increased in KD patients with prolonged fever, young age, male gender, high initial CRP, high neutrophils and band form counts [18-21]. In this study, we did not find any difference of age, CRP, neutrophilia, or lymphopenia between KD patients with and without CALs. We did, however, find male gender as the most significant risk factor for CAL formation. This suggests that sex-related genes may be involved in the remodeling of CALs in KD. In summary, we found that CALs were found more frequently in male KD patients than in females, and that persistent monocytosis after IVIG treatment was an important indicator for CAL formation in KD.

References

1. Kawasaki T, Kosaki F, Okawa S, Shigematsu I, Yanagawa H. A new infantile acute febrile mucocutaneous lymph node syndrome (MLNS) prevailing in Japan. *Pediatrics*. 1974;54:271-6.
2. Holman RC, Curns AT, Belay ED, Steiner CA, Effler PV, Yorita KL, et al. Kawasaki syndrome in Hawaii. *Pediatr Infect Dis J*. 2005;24:429-33.
3. Yanagawa H, Nakamura Y, Yashiro M, Oki I, Hirata S, Zhang T, et al. Incidence survey of Kawasaki disease in 1997 and 1998 in Japan. *Pediatrics*. 2001;107:E33.
4. Du ZD, Zhang T, Liang L, Meng X, Li T, Kawasaki T, et al. Epidemiologic picture of Kawasaki disease in Beijing from 1995 through 1999. *Pediatr Infect Dis J*. 2002;21:103-7.
5. Newburger JW, Taubert KA, Shulman ST, Rowley AH, Gewitz MH, Takahashi M, et al. Summary and abstracts of the Seventh International Kawasaki Disease Symposium: December 4-7, 2001, Hakone, Japan. *Pediatr Res*. 2003;53:153-7.
6. Chang LY, Chang IS, Lu CY, Chiang BL, Lee CY, Chen PJ, et al. Epidemiologic features of Kawasaki disease in Taiwan, 1996-2002. *Pediatrics*. 2004;114:e678-82.
7. Burns JC, Glodé MP. Kawasaki syndrome. *Lancet*. 2004;364:533-44.
8. Newburger JW, Takahashi M, Gerber MA, Gewitz MH, Tani LY, Burns JC, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. *Circulation*. 2004;110:2747-71.
9. Hsu YH, Wang YH, Hsu WY, Lee YP. Kawasaki disease characterized by erythema and induration at the Bacillus Calmette-Guérin and purified protein derivative inoculation sites. *Pediatr Infect Dis J*. 1987;6:576-8.
10. Newburger JW, Takahashi M, Beiser AS, Burns JC, Bastian J, Chung KJ, et al. A single intravenous infusion of gamma globulin as compared with four infusions in the treatment of acute Kawasaki syndrome. *N Engl J Med*. 1991;324:1633-9.
11. Newburger JW, Takahashi M, Burns JC, Beiser AS, Chung KJ, Duffy CE, et al. The treatment of Kawasaki syndrome with intravenous gamma globulin. *N Engl J Med*. 1986;315:341-7.
12. Wang CL, Wu YT, Liu CA, Kuo HC, Yang KD. Kawasaki disease: infection, immunity and genetics. *Pediatr Infect Dis J*. 2005;24:998-1004.
13. Wang CL, Wu YT, Lee CJ, Liu HC, Huang LT, Yang KD. Decreased nitric oxide production after intravenous immunoglobulin treatment in patients with Kawasaki disease. *J Pediatr*. 2002;141:560-5.
14. Furukawa S, Matsubara T, Yabuta K. Mononuclear cell subsets and coronary artery lesions in Kawasaki disease. *Arch Dis Child*. 1992;67:706-8.
15. Furukawa S, Matsubara T, Jujoh K, Sasai K, Nakachi S, Sugawara T, et al. Reduction of peripheral blood macrophages/

- monocytes in Kawasaki disease by intravenous gammaglobulin. *Eur J Pediatr.* 1990;150:43-7.
16. Nathan DG, Orkin SH, Look AT, Ginsburg D, eds. *Nathan and Oski's hematology of infancy and childhood.* 6th ed. Philadelphia: WB Saunders; 2003:963.
 17. Behrman RE, Kliegman RM, Jenson HB, eds. *Nelson textbook of pediatrics.* 17th ed. Philadelphia: WB Saunders; 2004: 1605.
 18. Morikawa Y, Ohashi Y, Harada K, Asai T, Okawa S, Nagashima M, et al. Coronary risks after high-dose gamma-globulin in children with Kawasaki disease. *Pediatr Int.* 2000;42:464-9.
 19. Burns JC, Capparelli EV, Brown JA, Newburger JW, Glode MP. Intravenous gamma-globulin treatment and retreatment in Kawasaki disease. *US/Canadian Kawasaki Syndrome Study Group. Pediatr Infect Dis J.* 1998;17:1144-8.
 20. Mori M, Imagawa T, Yasui K, Kanaya A, Yokota S. Predictors of coronary artery lesions after intravenous gamma-globulin treatment in Kawasaki disease. *J Pediatr.* 2000;137:177-80.
 21. Beiser AS, Takahashi M, Baker AL, Sundel RP, Newburger JW. A predictive instrument for coronary artery aneurysms in Kawasaki disease. *US Multicenter Kawasaki Disease Study Group. Am J Cardiol.* 1998;81:1116-20.