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ORIGINAL PAPER

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Serum hepatocyte growth factor combined with vascular endothelial growth factor as a predictive indicator for the occurrence of coronary artery lesions in Kawasaki disease

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Abstract We investigated the possible use of serum hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) levels as a predictive indicator for the occurrence of coronary artery lesions (CAL) in Kawasaki disease (KD). Serum HGF and VEGF levels were measured by enzyme-linked immunosorbent assay in 41 patients with KD and 25 afebrile controls. Serum HGF levels of patients in the acute phase of KD were significantly higher than those of afebrile controls ($Pc < 0.05$) and decreased to lower levels during recovery ($P < 0.0001$). Univariate analysis showed significant correlations between occurrence of CAL and five variables: duration of fever ($P = 0.018$), serum C-reactive protein concentration ($P = 0.024$), albumin concentration ($P = 0.009$), serum VEGF level ($P = 0.009$) and serum HGF level ($P = 0.035$). Furthermore, multivariate analysis revealed that serum HGF and VEGF levels and presence of oedema were major risk factors for the occurrence of CAL. For prediction of the development of CAL, we established a new risk classification system with these three variables, which showed a sensitivity of 100% and a specificity of 94.4%. *Conclusion:* these data show that hepatocyte growth factor, together with vascular endothelial growth factor, might play an important role in the pathophysiology of Kawasaki disease and their serum levels could be a powerful predictor for the development of coronary artery lesions.

Keywords Coronary artery lesion · Kawasaki disease · Serum hepatocyte growth factor · Serum vascular endothelial growth factor

Abbreviations *CAL* coronary artery lesion · *CRP* C-reactive protein · *Pc* corrected *P* · HGF hepatocyte growth factor · *IL* interleukin · *IVGG* intravenous gammaglobulin · *KD* Kawasaki disease · *VEGF* vascular endothelial growth factor

Introduction

Kawasaki disease (KD) is an acute systemic vasculitis of unknown aetiology in infancy and early childhood, characterised by prolonged high fever, polymorphous skin rash, conjunctivitis, inflammation of mucous membranes, oedema of the peripheral extremities and cervical lymphadenopathy [12]. Activation of the immune system including neutrophils, monocytes and lymphocytes occurs in the acute stage of the disease [7, 8, 17, 20, 31]. Cytokines such as tumour necrosis factor-α, interferon-γ, interleukin (IL)-1 [7, 19, 20, 21], IL-6 and IL-8 [21, 36] appear to play important roles in the pathophysiology of the disease.

To identify predictive indicators for the development of coronary artery lesions (CAL) in KD, we have been focusing on cytokines/growth factors which exert direct effects on vascular tissues. Actually, there was an observation that sera from patients with acute phase of KD contained some humoural factors to enhance proliferation or migration of endothelial cells [34]. Vascular endothelial growth factor (VEGF) is a cytokine produced by smooth muscle cells, immune cells and other cells and works as a vascular permeability factor as well as an endothelial cell mitogen [3, 6, 16]. Recently, we reported that serum VEGF levels in patients with KD

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were associated with the development of CAL [32]. Hepatocyte growth factor (HGF) is a pleiotropic growth factor produced by smooth muscle cells, fibroblasts and other cells. In addition to the function as a stimulator for a broad spectrum of cell types [13, 33, 35, 40], HGF promotes the growth of endothelial cells without vascular smooth muscle cell replication and acts as a survival factor for endothelial cells. Since HGF exerts its direct effects on the endothelial cells and is produced by vascular smooth muscle cells, a local HGF system, consisting of HGF production and the presence of its receptor, c-*met* proto-oncogene product [4, 26], appears to exist in vascular tissues in vivo as well as in vitro [25]. Recently, it has been reported that HGF upregulated VEGF expression in smooth muscle cells and VEGF receptor flk-1 in human endothelial cells [37, 38]. Moreover, a recent report has shown that HGF served as an early marker of arterial thrombus formation, although this role of HGF has not been clarified [22]. Therefore, we measured serum HGF and VEGF levels in patients with KD to investigate their possible usefulness as a predictive indicator for the occurrence of CAL.

Subjects and methods

Subjects

A total of 41 Japanese children (27 boys and 14 girls, aged between 2 and 106 months, median: 22.0 months) who met the diagnostic guidelines for KD were studied. Sera were obtained in both acute (febrile) and recovery (afebrile) phases. They were treated at Kyushu University and Kyushu Kouseinenkin Hospitals between January 1994 and June 1999, and 5 (12.2%) of the 41 developed CAL. All patients received intravenous gammaglobulin (IVGG) treatment, initial administration on day 4.8 ± 1.8 (mean ± SD) of illness, with acetylsalicylic acid (30 mg/kg per day) except in two cases who received only acetylsalicylic acid because of mild symptoms. Seven patients received IVGG (400 mg/kg per day) for 3 to 6 consecutive days (until October 1994) and 32 patients had IVGG (1 g/kg per day, over 10 h) for 1 to 4 consecutive days (since November 1994). Of the 39 patients, 17 (43.6%) received at least 1.6 g/kg of IVGG.

In patients with KD, sera were obtained on day 4.7 ± 1.8 (mean ± SD) of illness in the acute phase (presence of fever) before IVGG administration as well as on day 12.9 ± 5.3 (mean ± SD) of illness in the recovery phase (absence of fever). Normal controls included 17 male and eight female children without infections, aged between 6.0 and 13.0 years (median 9.0 years) and disease controls comprised nine male and 12 female children with active infections (ten with pneumonia, five bronchitis, two infectious mononucleosis, two lymphadenitis, one with measles and one with upper airway infection), aged between 1 and 132 months (median 18.0 months). Informed consent was obtained from their parents before study. These sera were collected and immediately frozen at –80°C.

The following variables at the time of admission were considered as potential CAL risk factors: age (month), sex, major symptoms except fever, duration of fever (days), WBC, Hb concentration, platelet count, C-reactive protein (CRP) level, serum sodium and potassium concentrations, total protein and albumin concentrations, AST and ALT levels. Patients were considered to have fever when the body temperature rose above 38°C.

Evaluation of CAL by echocardiography

Two-dimensional echocardiography was performed in all patients at the time of diagnosis and repeated at least twice a week until discharge (admission period 19.8 ± 10.5 days, mean ± SD). CAL was defined as follows: 1) lumen diameter at least 3 mm in children ≥5 years of age; 2) internal diameter of a segment at least 1.5 times as large as that of an adjacent segment; or 3) clearly irregular lumen.

Hepatic growth factor assay

Serum HGF levels were determined by a sandwich enzyme-linked immunosorbent assay kit (R&D Systems Inc. Minneapolis, Minn., USA). Sera or recombinant human HGF standards (125–8000 pg/ml diluted in RD6X) (50 µl) and 150 µl assay diluent RD1W were added to the wells precoated with murine monoclonal antibody against human HGF and incubated for 2 h at 37°C. After washing, 200 µl of polyclonal antibody against HGF conjugated to horseradish peroxidase was added and incubated for 2 h at 37°C. Substrate solution (200 µl/well) was added and incubated for 30 min at 37°C under in the dark and the reaction was terminated with 200 µl of 2 N H_2SO_4. Absorbance was recorded at 450 nm. The detection limit of this assay was 40.0 pg/ml.

Vascular endothelial growth factor assay

Serum VEGF levels were determined by a sandwich enzyme-linked immunosorbent assay kit (Immuno-Biological Laboratories Co., Japan). Sera or recombinant human VEGF standards (R&D Systems, Minneapolis, Minn., USA, 15.6–1000 pg/ml) (100 µl) was added to the wells precoated with mouse immunoglobin G against human VEGF and incubated for 1 h at 37°C. After washing, 100 µl of rabbit immunoglobin G against human VEGF (0.5 mg/ml in 0.05% phosphate-buffered saline with 1% bovine serum albumin) conjugated to horseradish peroxidase was added and incubated for 30 min at 37°C. Substrate solution (100 µl/well) was added and incubated for 30 min at 37°C in the dark and the reaction was terminated with 100 µl of 1 N H_2SO_4. Absorbance was recorded at 450 nm. The detection limit of this assay was 15.6 pg/ml. Serum VEGF levels in 41 patients in the acute and recovery phases of KD included those of 30 patients of our previous report [31].

Statistical analyses

Medians and ranges (median, minimum, maximum) were used as descriptive statistics. Distributions of serum HGF levels of patients with KD, acute (febrile) and recovery (afebrile) phases, normal controls and disease controls were compared using the Mann-Whitney U test and the multiple comparison test (Bonferroni's method). The corrected P values (Pc) were obtained by multiplying the P values by the numbers of the tests. Statistical significance was defined as $Pc < 0.05$. Correlations between serum HGF levels and laboratory data including serum VEGF levels were studied by Spearman's correlation coefficients. Association between CAL development and all variables in patients with KD was studied by multivariate analysis (stepwise logistic regression analysis). Significance was defined as $P < 0.05$.

Results

Serial determination of serum hepatocyte growth factor and vascular endothelial growth factor levels

Serial serum HGF levels of patients with KD in acute (febrile) and recovery (afebrile) phases are shown in Fig. 1. Serum HGF levels in the acute phase (range 628.8–8920.9 pg/ml, median 1653.4 pg/ml) were significantly higher than those in the recovery phase (range 409.5–2804.3 pg/ml, median 409.5 pg/ml; $Pc < 0.05$), and

showed significant differences to those of normal controls (range 394.8–1360.0 pg/ml, median 817.8 pg/ml; $Pc < 0.05$) and those of disease controls (range 550.5–2820.8 pg/ml, median 762.2 pg/ml; $Pc < 0.05$). Serum VEGF levels in the acute phase (range 0.0–2003.6 pg/ml, median 68.0 pg/ml) were also higher than those in the recovery phase (range 0.0–328.7 pg/ml, median 58.4 pg/ml; $P = 0.0013$, Wilcoxon signed rank test). In 32 (78.0%) of 41 patients with KD, serum HGF levels in the acute phase were greater than the 90th percentile (1084.4 pg/ml) of those in normal controls. No significant differences were seen between serum HGF levels in the recovery phase of KD patients and those of normal controls or disease controls ($Pc > 0.05$, respectively). Serum HGF levels (acute phase) in five patients with CAL were 1489.1, 3095.4, 3194.7, 3710.5 and 6677.8 pg/ml, which decreased in recovery phase.

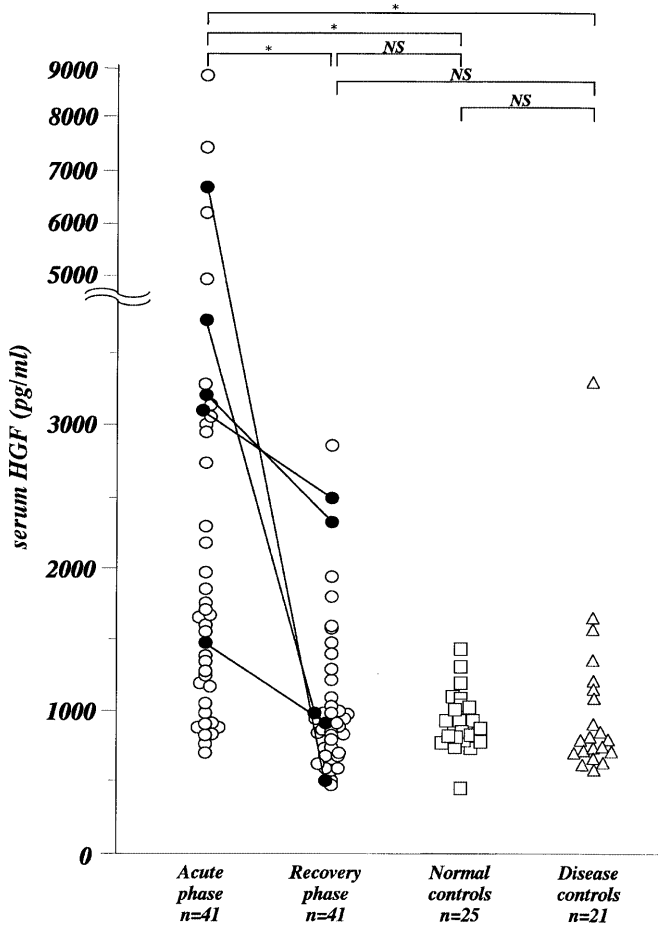

Fig. 1 Serum HGF levels in patients with KD in both acute and recovery phases. Absence of CAL (*open circles*), presence of CAL (*filled circles*), normal controls (*open squares*) and disease controls (*open triangles*). *NS* not significant. *$Pc < 0.05$ (Mann-Whitney U test and multiple comparison test)

Relationships between serum hepatocyte growth factor level and other laboratory data in the acute phase

Relationships between serum HGF level and other variables including serum VEGF level in patients with KD were studied by Spearman's correlation coefficients (Table 1). Serum HGF levels in the acute phase of KD showed significant positive correlations with duration of fever ($r_s = 0.352$, $P = 0.0262$), WBC count ($r_s = 0.426$, $P = 0.0071$), neutrophil count ($r_s = 0.496$, $P = 0.0020$), serum CRP concentration ($r_s = 0.643$, $P < 0.0001$) and serum VEGF level ($r_s = 0.526$, $P = 0.0009$), and a negative correlation with albumin concentration ($r_s = -0.415$, $P = 0.0095$). There were no significant correlations between serum HGF levels and liver enzymes (ALT and AST) in the acute phase of KD. Five patients developed CAL among nine patients with serum VEGF levels > 750 pg/ml or serum HGF levels > 6500 pg/ml, while none had CAL among 32 with serum VEGF levels ≤ 750 pg/ml and serum HGF levels ≤ 6500 pg/ml (Fig. 2). These criteria for selection of development of CAL had a sensitivity of 100% and a specificity of 88.9%. In the nine patients described above, oedema was observed in seven (77.8%) patients including five with CAL and two without CAL. On the other hand, 18

Table 1 Relationships between serum HGF level and other laboratory data in the acute phase of KD

Variable	Number (n)	Spearman rank correlation coefficient (r)	P
Duration of fever (days)	41	0.352	0.0262*
WBC count (/μl)	41	0.426	0.0071**
Neutrophil count (/μl)	40	0.496	0.002**
Hb (g/dl)	41	–0.229	0.1473
Platelets ($\times 10^4$/μl)	41	0.159	0.3136
Haematocrit (%)	41	–0.226	0.1522
CRP (mg/dl)	41	0.643	< 0.0001**
Na (mEq/l)	41	–0.302	0.056
K (mEq/l)	41	–0.223	0.8236
AST (IU/l)	41	0.114	0.4706
ALT (IU/l)	41	0.233	0.1399
Total protein (g/dl)	41	0.067	0.6733
Albumin (g/dl)	40	–0.415	0.0095**
VEGF (pg/ml)	41	0.526	0.0009**

*$P < 0.05$
**$P < 0.01$

(56.3%) of the 32 patients with low HGF and VEGF levels showed oedema of the hands or feet.

Determination of risk factors for coronary artery lesion development by univariate and multivariate analyses

Firstly, correlation between CAL development and continuous variables in patients with KD was studied by univariate analysis (Mann-Whitney U test). Table 2 shows significant correlations between occurrence of CAL and five variables: duration of fever ($P=0.018$), serum CRP concentration ($P=0.024$), albumin concentration ($P=0.009$), serum VEGF level ($P=0.009$) and serum HGF level ($P=0.035$). With respect to categorical variables such as gender and presence or absence of conjunctival injection, lymphadenopathy, rash, oedema of hands or feet and erythematous lip, there were no significant correlations as to the development of CAL in Chi-squared test with Yates correction (data not shown).

Secondly, stepwise logistic regression analysis revealed that significant correlations existed between occurrence of CAL and three variables: elevations of serum HGF and VEGF levels with every 100 pg/ml ($P=0.0398$, odds ratio: 1.09, 95% confidence interval: 0.97–1.22 and $P=0.0002$, odds ratio: 1.87, 95% confidence interval: 0.99–3.53, respectively) and the presence of oedema of the hands or feet ($P=0.0013$, odds ratio was not able to be estimated for monotone likelihood), as shown in Table 3.

For prediction of the development of CAL, we established a formula to determine risk score as follows: risk score = 0.08683 × serum HGF level (pg/ml)/100 + 0.626 × serum VEGF level (pg/ml)/100 + 13.14 × oedema of hands or feet (presence = 1, absence = 0) - 19.27. The risk score > 0 was considered as a high risk for the development of CAL. With this score, the sensitivity and specificity were 100% and 94.4%, respectively (Fig. 3).

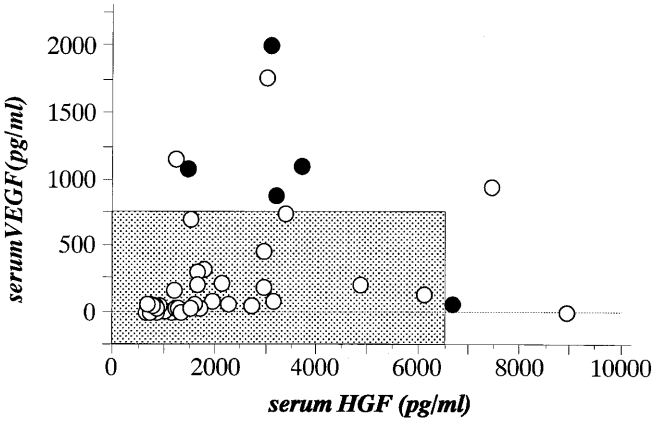

Fig. 2 Correlation between serum HGF and VEGF levels in 41 patients with KD in the acute phase. Absence of CAL (*open circles*), presence of CAL (*filled circles*)

Discussion

Coronary artery aneurysm or dilatation occurs in approximately 5%–16% of patients with KD despite the introduction of high-dose IVGG treatment [9, 24, 27, 28]. As coronary aneurysm is responsible for most of the mortality of the disease [5, 11], it is essential to determine risk factors for the development of coronary aneurysm in advance to establish a better treatment strategy.

Among various cytokines or growth factors that have direct effects on vascular tissues, we investigated serum HGF levels in patients with KD in the present study. Elevation of serum HGF levels in patients with KD was

Table 2 Univariate analysis of continuous variables related to CAL development

Variable	Number (*n*)		Median (range)		Mann-Whitney U test
	Total	CAL	CAL		*P*
		Positive/negative	Positive	Negative	
Age (months)	41	5/36	22.0 (4–55)	23.5 (2–106)	0.591
Duration of fever (days)	41	5/36	9 (7–13)	6 (2–15)	0.018**
WBC count (/μl)	41	5/36	16510 (9200–22350)	11290 (5300–22050)	0.087
Neutrophil count (/μl)	40	5/35	12292 (5612–14715)	5712 (550–18240)	0.082
Haemoglobin (g/dl)	41	5/36	11.6 (8.5–12.6)	11.5 (8.2–13.3)	0.765
Platelets ($\times 10^4$/μl)	41	5/36	29.5 (28.8–98.0)	31.0 (16.2–64.0)	0.55
CRP (mg/dl)	41	5/36	13.6 (6.7–17.0)	7.2 (0.3–34.5)	0.024*
Na (mEq/l)	41	5/36	136 (134–142)	137 (129–143)	0.841
K (mEq/l)	41	5/36	4.5 (3.8–4.9)	4.4 (3.2–5.2)	0.484
AST (IU/l)		5/36	44 (20–117)	33 (19–590)	0.921
ALT (IU/l)	41	5/36	43 (11–219)	23 (5–435)	0.604
Total protein (g/dl)	41	5/36	6.8 (6.4–7.1)	6.8 (5.4–8.2)	0.704
Albumin (g/dl)	40	5/35	3.5 (2.9–4.2)	4.2 (3.3–4.7)	0.009**
VEGF (pg/ml)	41	5/36	1088.8 (62.5–2003.6)	59.6 (0.0–1766.9)	0.009**
HGF (pg/ml)	41	5/36	3194.7(1489.1–6677.8)	1577.1 (628.8–8920.9)	0.035*

*$P < 0.05$
**$P < 0.01$

Table 3 Identification of risk factors for CAL by stepwise logistic regression analysis

Variable	*P*	Odds ratio (95% CI)
Oedema of hands or feet		
Positive[a]	0.0013	–[c]
Negative[b]		
Serum VEGF elevation with every 100 pg/ml	0.0002	1.87 (0.99–3.53)
Serum HGF elevation with every 100 pg/ml	0.0398	1.09 (0.97–1.22)

[a]Oedema of hands or feet; positive: five patients with CAL and 20 patients without CAL were included
[b]Oedema of hands or feet; negative: 16 patients without CAL were included
[c]Odds ratio was not able to be estimated for monotone likelihood

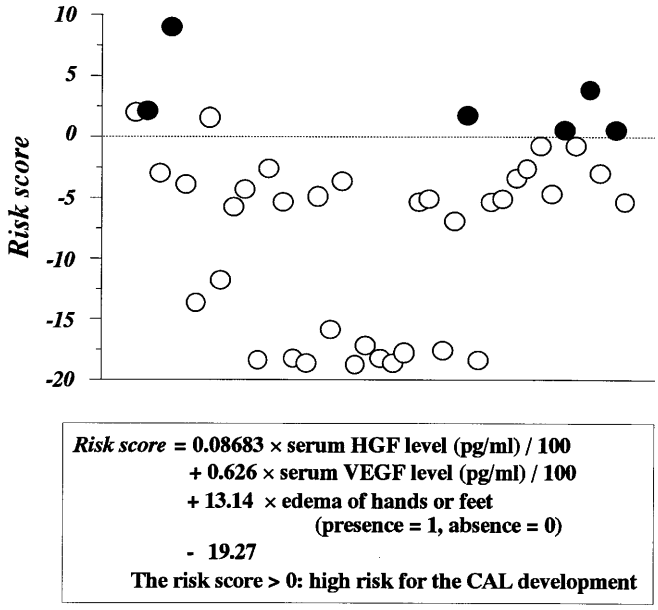

Fig. 3 Risk scores for prediction of the development of CAL in 41 patients with KD. Absence of CAL (*open circles*), presence of CAL (*filled circles*)

found to be a major risk factor for the occurrence of CAL. Although there is no definite information on topical HGF kinetics of damaged vessels, it is possible that HGF was produced locally and therefore correlated with severity of inflammation in vascular tissue because HGF is synthesised by smooth muscle cells and fibroblasts within vascular tissues [25]. In Henoch-Schönlein purpura [29], elevation of serum HGF levels was also probably due to the production of HGF by vascular tissue with inflammation. An alternative explanation includes that to repair or to regenerate injured vascular tissues, serum HGF was produced by intact tissues or cells in accordance with the degree of vascular damage. In fact, the HGF endocrine-related mechanism for tissue repair or regeneration of injured organs was suggested from animal studies. In partially hepatectomised or unilaterally nephrectomised rats, HGF mRNA increased rapidly in intact organs [39], and HGF receptor was markedly down-regulated in injured organs but not in intact organs [10, 23].

In the present study, serum HGF level showed positive correlations with duration of fever, WBC count, neutrophil count, serum CRP concentration and serum VEGF level, and a negative correlation with albumin. A positive correlation between serum HGF and VEGF levels was consistent with a recent observation that HGF upregulated VEGF expression in smooth muscle cells [37]. In addition, it is possible that to a certain extent, serum HGF level reflects a degree of systemic vascular inflammation because of its positive correlations with duration of fever, WBC count, neutrophil count and serum CRP concentration, and of a negative correlation with albumin. Furthermore, the correlations between serum HGF level and WBC count or neutrophil count might be ascribed in part to the direct effects of HGF on the proliferation of haematopoietic progenitor cells and the colony formation of granulocyte-erythroid-megakaryocyte lineages [14, 30].

Various risk factors for the development of CAL have been reported. Duration of fever has been one of the major risk factors to predict coronary aneurysms found in studies in Japan and the United States [1, 15]. Beiser et al. [2] reported a sequential risk classification instrument using neutrophil and band counts, haemoglobin concentration, platelet count, and fever on the following day after IVGG infusion to predict the development of coronary aneurysms. We have recently reported that elevated serum VEGF level served as a new risk factor for the development of CAL [32]. In the present study, we have identified three variables including the presence of oedema of the hands or feet, high serum VEGF level and high serum HGF level as major risk factors for CAL development by multivariate analysis. After the introduction of high-dose IVGG treatment, presence of oedema in the extremities was first identified as a risk factor in this study. Oedema appears to reflect the severity of vasculitis in extremities, which might be correlated with that in coronary arteries. Using the three variables, we have established a new classification system for prediction of the development of CAL. With respect to oedema of the hands or feet, since its quantitative evaluation was not performed in this study, we utilised oedema as a categorical variable in the formula. Although both HGF and VEGF, showing direct effects on the endothelial cells, were closely associated with each other in patients with KD, multivariative analysis showed that they appeared to work independently as risk factors for the development of CAL. This new risk classification showing higher specificity (94.4%) than previous ones (60%–65%), can be done at the time of diagnosis in contrast to the previous

classifications, which enables us to modulate the treatment strategy in advance [2, 15]. Furthermore, one other criterion using two growth factors (serum HGF and VEGF) only, also had a high sensitivity (100%) and specificity (88.9%), may be more informative for the clinician. Conversely, this system has a disadvantage that serum HGF and VEGF levels are not routine laboratory assays in hospitals.

Since there is a significant association between serum HGF and VEGF levels and CAL development, HGF and VEGF should play important roles in the pathophysiology of KD and their serum levels might serve as a predictor for the development of coronary aneurysms.

References

1. Asai T (1983) Evaluation method for the degree of seriousness in Kawasaki disease. Acta Paediatr Jpn 25: 170
2. Beiser AS, Takahashi M, Baker AL, Sundel RP, Newburger JW (1998) A predictive instrument for coronary artery aneurysms in Kawasaki disease. US Multicenter Kawasaki Disease Study Group. Am J Cardiol 81: 1116–1120
3. Berse B, Brown LF, Van de Water L, Dvorak HF, Senger DR (1992) Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors. Mol Biol Cell 3: 211–220
4. Bottaro DP, Rubin JS, Faletto DL, Chan AM, Kmiecik TE, Vande Woude GF, Aaronson SA (1991) Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. Science 251: 802–804
5. Dhillon R, Newton L, Rudd PT, Hall SM (1993) Management of Kawasaki disease in the British Isles. Arch Dis Child 69: 631–636
6. Ferrara N, Henzel WJ (1989) Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Biophys Res Commun 161: 851–858
7. Furukawa S, Matsubara T, Jujoh K, Yone K, Sugawara T, Sasai K, Kato H, Yabuta K (1988) Peripheral blood monocyte/macrophages and serum tumor necrosis factor in Kawasaki disease. Clin Immunol Immunopathol 48: 247–251
8. Furukawa S, Matsubara T, Motohashi T, Nakachi S, Sasai K, Yabuta K (1990) Expression of Fc epsilon R2/CD23 on peripheral blood macrophages/monocytes in Kawasaki disease. Clin Immunol Immunopathol 56: 280–286
9. Furusho K, Kamiya T, Nakano H, Kiyosawa N, Shinomiya K, Hayashidera T, Tamura T, Hirose O, Manabe Y, Yokoyama T, Kawarano M, Baba K, Baba K, Mori C (1984) High-dose intravenous gammaglobulin for Kawasaki disease. Lancet 2: 1055–1058
10. Higuchi O, Nakamura T (1991) Identification and change in the receptor for hepatocyte growth factor in rat liver after partial hepatectomy or induced hepatitis. Biochem Biophys Res Commun 176: 599–607
11. Kato H, Ichinose E, Kawasaki T (1986) Myocardial infarction in Kawasaki disease: clinical analyses in 195 cases. J Pediatr 108: 923–927
12. Kawasaki T, Kosaki F, Okawa S, Shigematsu I, Yanagawa H (1974) A new infantile acute febrile mucocutaneous lymph node syndrome (MLNS) prevailing in Japan. Pediatrics 54: 271–276
13. Kinoshita T, Tashiro K, Nakamura T (1989) Marked increase of HGF mRNA in non-parenchymal liver cells of rats treated with hepatotoxins. Biochem Biophys Res Commun 165: 1229–1234
14. Kmiecik TE, Keller JR, Rosen E, Van de Woude GF (1992) Hepatocyte growth factor is a synergistic factor for the growth of hematopoietic progenitor cells. Blood 80: 2454–2457
15. Koren G, Lavi S, Rose V, Rowe R (1986) Kawasaki disease: review of risk factors for coronary aneurysms. J Pediatr 108: 388–392
16. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N (1989) Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 246:1306–1309.17.
    Leung DY, Siegel RL, Grady S, Krensky A, Meade R, Reinherz EL, Geha RS (1982) Immunoregulatory abnormalities in mucocutaneous lymph node syndrome. Clin Immunol Immunopathol 23: 100–112
18. Leung DY, Chu ET, Wood N, Grady S, Meade R, Geha RS (1983) Immunoregulatory T cell abnormalities in mucocutaneous lymph node syndrome. J Immunol 130: 2002–2004
19. Leung DY, Collins T, Lapierre LA, Geha RS, Pober JS (1986) Immunoglobulin M antibodies present in the acute phase of Kawasaki syndrome lyse cultured vascular endothelial cells stimulated by gamma interferon. J Clin Invest 77: 1428–1435
20. Leung DY, Geha RS, Newburger JW, Burns JC, Fiers W, Lapierre LA, Pober JS (1986) Two monokines, interleukin 1 and tumor necrosis factor, render cultured vascular endothelial cells susceptible to lysis by antibodies circulating during Kawasaki syndrome. J Exp Med 164: 1958–1972
21. Lin CY, Lin CC, Hwang B, Chiang B (1992) Serial changes of serum interleukin-6, interleukin-8, and tumor necrosis factor alpha among patients with Kawasaki disease. J Pediatr 121: 924–926
22. Matsumori A, Ono K, Furukawa Y, Okada M, Sasayama S (1998) Circulating hepatocyte growth factor as an early marker of arterial thrombus formation. Jpn Circ J 62: 311–313
23. Nagaike M, Hirao S, Tajima H, Noji S, Taniguchi S, Matsumoto K, Nakamura T (1991) Renotropic functions of hepatocyte growth factor in renal regeneration after unilateral nephrectomy. J Biol Chem 266: 22781–22784
24. Nagashima M, Matsushima M, Matsuoka H, Ogawa A, Okumura N (1987) High-dose gammaglobulin therapy for Kawasaki disease. J Pediatr 110: 710–712
25. Nakamura Y, Morishita R, Higaki J, Kida I, Aoki M, Moriguchi A, Yamada K, Hayashi S, Yo Y, Matsumoto K, Nakamura T, Ogihara T (1995) Expression of local hepatocyte growth factor system in vascular tissues. Biochem Biophys Res Commun 215: 483–488
26. Naldini L, Vigna E, Narsimhan RP, Gaudino G, Zarnegar R, Michalopoulos GK, Comoglio PM (1991) Hepatocyte growth factor (HGF) stimulates the tyrosine kinase activity of the receptor encoded by the proto-oncogene c-MET. Oncogene 6: 501–504
27. Newburger JW, Takahashi M, Burns JC, Beiser AS, Chung KJ, Duffy CE, Glode MP, Mason WH, Reddy V, Sanders SP, Shulman ST, Wiggins JW, Hicks RV, Fulton DR, Lewis AB, Leung DYM, Golton T, Rosen FS, Melish ME (1986) The treatment of Kawasaki syndrome with intravenous gamma globulin. N Engl J Med 315: 341–347
28. Newburger JW, Takahashi M, Beiser AS, Burns JC, Bastian J, Chung KJ, Colan SD, Duffy CE, Fulton DR, Glode MP (1991) A single intravenous infusion of gamma globulin as compared with four infusions in the treatment of acute Kawasaki syndrome. N Engl J Med 324: 1633–1639
29. Nishida M, Kawakatsu H, Ishiwari K, Tamai M, Sawada T, Nishimura M, Yoshimura M (1999) Serum hepatocyte growth factor levels in Henoch-Schonlein purpura. Pediatr Int 41: 474–476
30. Nishino T, Hisha H, Nishino N, Adachi M, Ikehara S (1995) Hepatocyte growth factor as a hematopoietic regulator. Blood 85: 3093–3100
31. Niwa Y, Sohmiya K (1984) Enhanced neutrophilic functions in mucocutaneous lymph node syndrome, with special reference to the possible role of increased oxygen intermediate generation in the pathogenesis of coronary thromboarteritis. J Pediatr 104: 56–60
32. Ohno T, Igarashi H, Inoue K, Akazawa K, Joh-o K, Hara T (2000) Serum vascular endothelial growth factor: a new pre-

dictive indicator for the occurrence of coronary artery lesions in Kawasaki disease. Eur J Pediatr 159: 424–429
33. Rubin JS, Chan AM, Bottaro DP, Burgess WH, Taylor WG, Cech AC, Hirschfield DW, Wong J, Miki T, Finch PW, Aaronson SA (1991) A broad-spectrum human lung fibroblast-derived mitogen is a variant of hepatocyte growth factor. Proc Natl Acad Sci U S A 88: 415–419
34. Sakata K, Kita M, Imanishi J, Onouchi Z, Liu Y, Mitsui Y (1995) Effect of Kawasaki disease on migration of human umbilical vein endothelial cells. Pediatr Res 38: 501–505
35. Tashiro K, Hagiya M, Nishizawa T, Seki T, Shimonishi M, Shimizu S, Nakamura T (1990) Deduced primary structure of rat hepatocyte growth factor and expression of the mRNA in rat tissues. Proc Natl Acad Sci U S A 87: 3200–3204
36. Ueno Y, Takano N, Kanegane H, Yokoi T, Yachie A, Miyawaki T, Taniguchi N (1989) The acute phase nature of interleukin 6: studies in Kawasaki disease and other febrile illnesses. Clin Exp Immunol 76: 337–342
37. Van Belle E, Witzenbichler B, Chen D, Silver M, Chang L, Schwall R, Isner JM (1998) Potentiated angiogenic effect of scatter factor/hepatocyte growth factor via induction of vascular endothelial growth factor: the case for paracrine amplification of angiogenesis. Circulation 97: 381–390
38. Wojta J, Kaun C, Breuss JM, Koshelnick Y, Beckmann R, Hattey E, Mildner M, Weninger W, Nakamura T, Tschachler E, Binder BR (1999) Hepatocyte growth factor increases expression of vascular endothelial growth factor and plasminogen activator inhibitor-1 in human keratinocytes and the vascular endothelial growth factor receptor flk-1 in human endothelial cells. Lab Invest 79: 427–438
39. Yanagita K, Nagaike M, Ishibashi H, Niho Y, Matsumoto K, Nakamura T (1992) Lung may have an endocrine function producing hepatocyte growth factor in response to injury of distal organs. Biochem Biophys Res Commun 182: 802–809
40. Zarnegar R, Muga S, Rahija R, Michalopoulos G (1990) Tissue distribution of hepatopoietin-A: a heparin-binding polypeptide growth factor for hepatocytes. Proc Natl Acad Sci U S A 87: 1252–1256