not show the correlation with all parameters in their variation from immediately after IVIG to 2 weeks after IVIG ($P<0.05$).

ROM and BAP Levels in the Control Group

In the control group, the ROM level was 312 (327/300) (median (upper/lower quartile U.CARR) and the BAP level was 2,674 (2,745/2,572) (mol/L) (**Table 3**). These values are slightly higher than those that have previously been reported as adult standard levels (ROM: 200–300 U.CARR; BAP: >2,200 mol/L).[24]

Dynamics of ROM During the Acute Stage of KD

Immediately before IVIG, the ROM level were significantly higher in Group A than in the control group (633 (687/555) U.CARR; $P<0.0001$) (**Table 3**; **Figure 1**). However, ROM rapidly declined to significantly lower levels both immediately after (555 (646/478) U.CARR; $P<0.05$ and 2 weeks after (466 (506/381) U.CARR; $P<0.01$) the IVIG treatment.

Immediately before the IVIG treatment, the ROM level was also significantly higher in Group B than in the control group (564 (611/531) U.CARR; $P<0.01$). The IVIG treatment did not facilitate any immediate declines in ROM levels among Group B patients (557 (610/483) U.CARR, NS). After 2 weeks, however, ROM was significantly lower than it had been immediately before the treatment (381 (423/304) U. CARR; $P<0.01$).

It is important to note that ROM level was statistically similar in Groups A and B immediately before the treatment. Thus, the different responses to the IVIG treatment were not caused by differences in levels of ROS.

Dynamics of BAP During the Acute Stage of KD (Figure 2)

In Group A, BAP did not change significantly from immediately before the IVIG treatment (2,705 (2,883/2,510) mol/L) to immediately afterward (2,714 (2,940/2,462) mol/L). However, BAP was obviously higher 2 weeks after the treatment (2,778 (3,015/2,705) mol/L). This was significantly higher than both the baseline value ($P<0.01$) and that recorded immediately after the treatment ($P<0.05$).

Immediately before the IVIG treatment, BAP was significantly lower in Group B than in both the control group ($P<0.01$) and in Group A ($P<0.05$). Unlike the pattern observed in Group A, BAP levels in Group B did not change significantly from immediately before the treatment (2,474 (2,557/ 2,413) mol/L), to immediately after the treatment (2,438 (2,626/2,313) mol/L), to 2 weeks after the treatment (2,622 (2,703/2,380) mol/L).

We did not perform multiple regression analysis because we could not find any significant variable excluding BAP in univariate analysis. Furthermore, only BAP was still selected although the model for the multivariate analysis. The area under the receiver operating curve is 0.80 (95% confidence interval 0.59–0.99).

Discussion

Here, we found that the reduction of ROM from immediately after the IVIG to 2 weeks after IVIG correlated to the movement of hs-CRP, which is a general inflammatory marker. The fact might suggest their close relationship between the oxidative stress and inflammation. Furthermore, we found that patients with acute stage KD have abnormally high levels of ROM in their blood, indicating an increased production of ROS. When given an IVIG treatment, some patients experienced ROM reductions, while others did not, suggesting that this treatment will not always be effective in suppressing ROS production.

Baseline BAP levels were either similar to (Group A) or lower than (Group B) those in control individuals. Either way, this indicates that the KD patients had not launched an endogenous antioxidant response. However, patients in Group A experienced clear increases in BAP 2 weeks after receiving IVIG, demonstrating the effectiveness of this treatment in stimulating antioxidant activity. The pattern of change in BAP level was similar among patients in Group B, but their absolute BAP values were significantly lower than those in both control individuals and IVIG-treated patients in Group A. Again, this indicates that while the IVIG treatment is effective against acute KD symptoms, it cannot be relied upon to work equally well in all cases.

In cases of KD-associated vasculitis, cytokines such as TNF-α and IL-6 increase during the acute stage in response to the release of various prophlogistic substances from the infiltrated monocyte/macrophage.[11–15] The released TNF-α induces vascular endothelial cells to express adhesive factors that prime neutrophils and monocytes. Furthermore, it also acts on endothelial cells and fibroblasts, induces various chemokines, facilitates migration of inflammatory cells to the inflammatory site, and increases production of cytokines such as IL-6, thus increasing inflammation. NAD(P)H oxidase is then activated in the inflammatory cells (eg, neutrophils and macrophages) that were primed by the inflammatory cytokines; this leads to the rapid production of a large amount of ROS.[25] Inducible nitric oxide (NO) synthase in the inflammatory cells produces NO, an unstable radical that changes to peroxynitrite (ONOO-) when exposed to ROS. Peroxynitrite is extremely responsive, and its strong oxidation activity is capable of directly disabling vascular tissues.[26] We suspect that this might be an important element driving the progression of vascular disorders associated with KD. NAD(P)H oxidase[27] on the endothelial cell membrane also reacts with the xanthine oxidase system in vascular endothelial cells[28] and TNF-α in the blood, and causes the activation of the arachidonate cascade, which generates prophlogistic substances such as leukotriene[29] and produces ROS as a by-product of metabolism.

The ROM measured in this study is a generic name for organic molecules that have been oxidized by ROS such as hydroperoxide (R-OOH). One important ROM is hydroxyperoxide, which is produced by the oxidization of physiologically vital organic molecules such as lipids, proteins, and nucleic acids. The presence of hydroxyperoxide is considered an excellent marker of oxidative damage,[30–33] and can easily be measured by the FREE® system used in this study. Results from this method correlate highly with those produced using ESR methodologies.[34,35] The patterns of ROM decrease shown in both treatment groups here are not surprising, given what is currently known about KD and the inflammation pathway. The more novel result of this work is the discovery that ROS increased so rapidly. Additionally, our techniques here have shown that the ROM measurement is useful in clinical situations for assessing inflammatory dynamics in lesions, as well as investigating the efficacy of inflammation alleviation treatments. Further, our results clearly demonstrate that the IVIG inflammation alleviation treatment cannot be relied upon to significantly reduce inflammation in all patients, although it does reduce ROS production more often than not (eg, in 13 of 19 patients).

There are also homeostasis mechanisms that combat the presence of ROS by eliminating these elements in order to maintain the balance between oxidization and antioxidization.[36,37] Antioxidants have both endogenous (eg, albumin,

transferrin, ceruloplasmin, bilirubin, ureic acid, reduced glutathione, etc) and exogenous (eg, tocopherol, carotin, ubiquinone, ascorbic acid, methionine, flavonoid, polyphenol, etc) origins. The FREE® system, used here to measure the comprehensive antioxidative potency in patients' blood, is based on the same principle as the ferric reducing ability of plasma assay method,[38] which has widely been recognized as an effective measurement technique.

Here, we found that changes in BAP in acute KD patients did not mirror changes in ROS. Furthermore, BAP activity was clearly lower in some patients (Group B) than in others (Group A), even after the IVIG treatment. Oxidative stress alleviation was delayed in Group B, and we hypothesize that this led to a build-up of abnormal ROS, leading to further increases in oxidative stress and additional inflammation, via a positive feedback loop. This phenomenon might be one factor leading to the higher rate of coronary arterial disorders in IVIG-unresponsive cases.[39,40]

The results of our work also suggest that baseline BAP levels can be used to predict whether patients will respond well to IVIG treatment and to assess the clinical conditions of vasculitis. Previously, it has been shown that prolonged inflammation, such as that likely present in Group B, increases the rate at which patients suffer coronary arterial complications.[41] Therefore, it is important to select an effective initial treatment in order to decrease inflammation as early and as rapidly as possible. This can be facilitated via the use of an appropriate biomarker that can be used as an index of the severity of inflammation. Our work indicates that BAP levels, either before or during treatment, can be used for this purpose. Moreover, BAP measurements can be performed within a few minutes following sample collection, which further enhances the clinical usefulness of this modality.

Although we have not directly shown a link between the clinical conditions of KD and activity of the oxidation/reduction control mechanism, we believe there is good evidence suggesting that the two are closely related: In patients with KD-associated coronary arteritis, inflammatory cells such as macrophages remain in place for approximately 2–3 months after infiltration. Since these macrophages are producing ROS, the coronary arteries are exposed to excessive oxidative stress for long periods of time. Thus, in patients who respond to IVIG treatment by upregulating their antioxidant activity, vascular disorders should be shorter-lived and vascular remodeling should proceed at a higher rate, than among patients who do not respond to the IVIG treatment. In order to investigate this hypothesis, it will be important to plot ROM and BAP values, as well as document clinical conditions, of a large study group over a long study period.

Further study is needed to make this research results more significant because the number of cases is still little in this study. The relationship between the antioxidative potency and the IVIG reactiveness obtained this time is a very intriguing result. We think that further examination leads to the elucidation of the cause of KD and the proposal of new treatment methods.

Conclusion

We have shown that patients with acute stage KD suffer from increased levels of oxidative stress, which, in most cases, can be reduced via the anti-inflammatory activities of IVIG. We also found that antioxidant activities increase slowly, relative to changes in ROS levels. Nearly a third of patients examined here responded poorly to the IVIG treatment, and these individuals had lower initial BAP levels than either controls or IVIG-responsive patients. This suggests that BAP can be used to predict the likelihood that anti-inflammatory treatments will be effective in KD patients. The ROM measurement, also, might be useful in a clinical setting, for evaluating inflammatory dynamics in lesions and tracking the effectiveness of anti-inflammatory treatments.

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Disclosures

None.

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ORIGINAL ARTICLE

Serum adipokine profiles in Kawasaki disease

Yasushi Kemmotsu · Tsutomu Saji · Natsuko Kusunoki · Nahoko Tanaka · Chiaki Nishimura · Akira Ishiguro · Shinichi Kawai

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Abstract Adipokines are cytokines derived from adipose tissue. Recently it has been established that adipokines are closely linked to the pathophysiology of not only metabolic diseases, such as diabetes mellitus, obesity, and atherosclerosis, but also to inflammation and immune diseases. In this study we measured serum levels of adipokines in patients with acute Kawasaki disease to investigate the role of adipokines in the pathophysiology of Kawasaki disease. Serum resistin, high-molecular-weight (HMW) adiponectin, leptin, and visfatin levels were measured by enzyme-linked immunosorbent assay in a total of 117 subjects: 56 patients with acute Kawasaki disease, 30 healthy children, and 31 patients with acute infectious diseases. Serum resistin levels in patients with Kawasaki disease were significantly higher than those of healthy children and patients with acute infectious diseases. In contrast, mean serum HMW adiponectin, leptin, and visfatin levels in patients with Kawasaki disease exhibited no statistically significant differences compared with those in healthy children and patients with infectious diseases. Serum resistin levels decreased significantly after administration of intravenous immune globulin. Serum resistin levels on admission were significantly higher in nonresponders compared with responders to intravenous immune globulin therapy. A multivariate model revealed that C-reactive protein was a factor that was significantly related to elevated serum resistin level in patients with Kawasaki disease. In patients with Kawasaki disease, serum resistin levels were elevated, but decreased to nearly normal after intravenous administration of immune globulin. In contrast, serum HMW adiponectin, leptin, and visfatin levels showed no statistically significant changes. These findings suggest that resistin plays an important role, while other adipokines do not play a major role, in the pathogenesis of Kawasaki disease.

Keywords Adipokines · Resistin · C-reactive protein · Kawasaki disease

Y. Kemmotsu · T. Saji
Department of Pediatrics, School of Medicine, Faculty of Medicine, Toho University, Tokyo 143-8541, Japan

N. Kusunoki · N. Tanaka · S. Kawai (✉)
Division of Rheumatology, Department of Internal Medicine (Omori), School of Medicine, Faculty of Medicine, Toho University, 6-11-1 Omori-Nishi, Ota-ku, Tokyo 143-8541, Japan
e-mail: skawai@med.toho-u.ac.jp

C. Nishimura
Department of Medical Informatics, School of Medicine, Faculty of Medicine, Toho University, Tokyo 143-8540, Japan

A. Ishiguro
National Center for Child Health and Development, Tokyo 157-8535, Japan

Introduction

Kawasaki disease is a systemic vasculitis of childhood that was first reported by Tomisaku Kawasaki in 1967 [1]. Patients manifest with fever, bulbar conjunctival injection, changes of the oropharyngeal mucosa, changes of the peripheral extremities, cervical lymphadenopathy, and polymorphous rash [2]. In Japan there are approximately 10,000 new patients annually [3]. The most important complication of this disease is development of coronary lesions that result in acute myocardial infarction. Intravenous immune globulin is a standard therapy that is effective in about 70% of patients, but the cause of the disease has been unclear.

Adipokines or adipocytokines including resistin, adiponectin, leptin, and visfatin are bioactive molecules that are produced and secreted by adipose tissue [4]. Adipokines have various actions in the human body that regulate metabolic conditions, and may have a central role in regulation of insulin resistance [5, 6].

Resistin is an amino acid peptide that belongs to a cysteine-rich secretory protein family [7]. Circulating resistin levels are elevated in humans by obesity and diabetes [8]. Resistin levels are also associated with increasing coronary artery calcification and are predictive of coronary atherosclerosis [9]. Adiponectin is a 244-amino-acid polypeptide that has three isoforms: low molecular weight, middle molecular weight, and high molecular weight (HMW). Decreased levels of HMW adiponectin are associated with coronary artery disease and type 2 diabetes [10, 11]. Leptin is a protein of 167 amino acids. Circulating leptin levels reflect adipose tissue mass, and hyperleptinemia is associated with obesity and other metabolic diseases [12, 13]. Visfatin is one of the adipokines identified in 2004, being predominantly produced and secreted in visceral fat; its expression level in plasma increases during development of obesity [14].

These adipokines show obviously links to metabolic diseases, however recent studies have also suggested that some adipokines might play a role in inflammation and immune diseases [15]; for instance, we have previously shown that serum levels of resistin, leptin, and adiponectin were all associated with C-reactive protein (CRP) level in patients with rheumatoid arthritis, suggesting that these adipokines may act as proinflammatory cytokines in this disease [16].

The object of this study is to clarify serum levels of resistin, HMW adiponectin, leptin, and visfatin in patients with Kawasaki disease during treatment with intravenous immune globulin, and to evaluate the relationships between serum adipokines and their clinical measures.

Methods

Patients

Fifty-six patients (36 males and 20 females, mean age 29.8 ± 1.7 months) with acute-phase Kawasaki disease who were admitted to our university hospital participated in this study. All patients met American Heart Association diagnostic criteria for Kawasaki disease [2]. These patients were treated with oral aspirin and 1 or 2 g/kg intravenous immune globulin after admission. As controls, we collected serum samples from 30 healthy children and 31 patients with acute infectious diseases (14 patients with pharyngitis, 9 with bronchitis, 5 with gastroenteritis, and 3 with exanthema subitum). The protocol for the study was approved by the Ethics Committee of Toho University Hospital. Informed consent was obtained from the parents of all patients.

Adipokine measurements

Blood samples were collected from the patients with acute-phase Kawasaki disease upon admission (before intravenous immune globulin) and at 24–48 h after intravenous immune globulin treatment. Serum resistin, HMW adiponectin, leptin, and visfatin were measured using enzyme-linked immunosorbent assay (ELISA) kits. Serum resistin and leptin levels were measured in all 56 patients, but HMW adiponectin and visfatin were measured in 38 patients because the volume of serum samples was too small to perform all four analyses. Resistin and leptin ELISA kits were both purchased from B-Bridge International, Inc. (Sunnyvale, CA, USA). ELISA kits for HMW adiponectin and visfatin were obtained from Fujirebio, Inc. (Tokyo, Japan) and Phoenix Pharmaceuticals, Inc. (Burlingame, CA, USA), respectively.

Biochemical measurements

All of the patients with Kawasaki disease were examined for complete blood cell counts and serum chemistry, including CRP and electrolytes, before immune globulin therapy. Latex nephelometry (Sekisui Medical Co., Tokyo, Japan) was used for CRP measurement.

Statistical analysis

Comparisons between the three groups were made using the Kruskal–Wallis test. Serum adipokine levels before and after intravenous immune globulin were compared by the Wilcoxon matched-pairs signed-rank test. Correlations between serum adipokines and laboratory data were analyzed by simple linear regression analysis. Multiple regression analysis was used for studying multivariable models. Statistical significance was determined at $p < 0.05$. Statistical analyses of the data were conducted using the StatMate III software program (ATMS, Tokyo, Japan).

Results

Characteristics of the study population

The characteristics of the 3 groups are shown in Table 1. There were no statistically significant differences in age, gender or body weight among the 3 groups of children. In patients with Kawasaki disease, mean ± SD age was

Table 1 Background characteristics of the three patient groups

	Age (months)	Gender (M/F)	Body weight (kg)
Patients with Kawasaki disease ($n = 56$)	29.8 ± 21.7	36/20	12.1 ± 3.6
Patients with acute infectious diseases ($n = 31$)	29.2 ± 17.5	20/11	12.1 ± 3.5
Healthy children ($n = 30$)	26.9 ± 13.0	19/11	11.7 ± 2.4

Values are mean ± SD

Table 2 Clinical characteristics of the patients with Kawasaki disease

	Age (months)	Days on IVIG	Serum CRP conc. (mg/dl)	WBC counts ($\times 10^3/\mu l$)	Sodium conc. (mEq/l)	IVIG responder*	CAL
Male 36	26 ± 18	4.5 ± 1.8	7.2 ± 5.2	14.2 ± 5.2	131.5 ± 2.8	21 (58.3%)	3 (8.3%)
Female 20	37 ± 25	4.9 ± 1.9	4.7 ± 4.6	7.5 ± 3.1	135.5 ± 2.3	17 (85.0%)	1 (5.0%)

Values are mean ± SD or cases (percentages)

IVIG intravenous immune globulin therapy, *CRP* C-reactive protein, *conc.* concentrations, *WBC* white blood cell, *CAL* coronary arterial lesion

* Patients who had cessation of fever (<37.5°C) after IVIG and needed no additional therapy

29.8 ± 21.7 months. The clinical profiles of patients with Kawasaki disease are presented in Table 2. Thirty-eight patients (67.9%, 21 males, 17 females) responded to intravenous immune globulin infusion. Four patients had coronary lesions detected by echocardiography at discharge, even after immune globulin therapy.

Serum adipokine levels in patients with Kawasaki disease

Serum adipokine levels are shown in Fig. 1. Serum resistin levels were significantly higher in patients with Kawasaki disease (mean 31.5 ± 20.0, median 27.5 ng/ml) compared with healthy controls (mean 5.0 ± 6.8, median 3.3 ng/ml, $p < 0.001$) and patients with acute infectious diseases (mean 10.6 ± 9.2, median 6.9 ng/ml, $p < 0.001$). However, serum HMW adiponectin, leptin, and visfatin levels in patients with Kawasaki disease (HMW adiponectin: mean 10.8 ± 5.1, median 10.1 μg/ml; leptin: mean 2.4 ± 4.0, median 1.6 ng/ml; visfatin: mean 11.1 ±5.5, median 9.5 ng/ml) showed no statistically significant differences compared with those in healthy controls (HMW adiponectin: mean 23.5 ±9.9, median 22.7 μg/ml; leptin: mean 2.0 ±0.7, median 1.9 ng/ml; visfatin: mean 14.9 ±15.7,

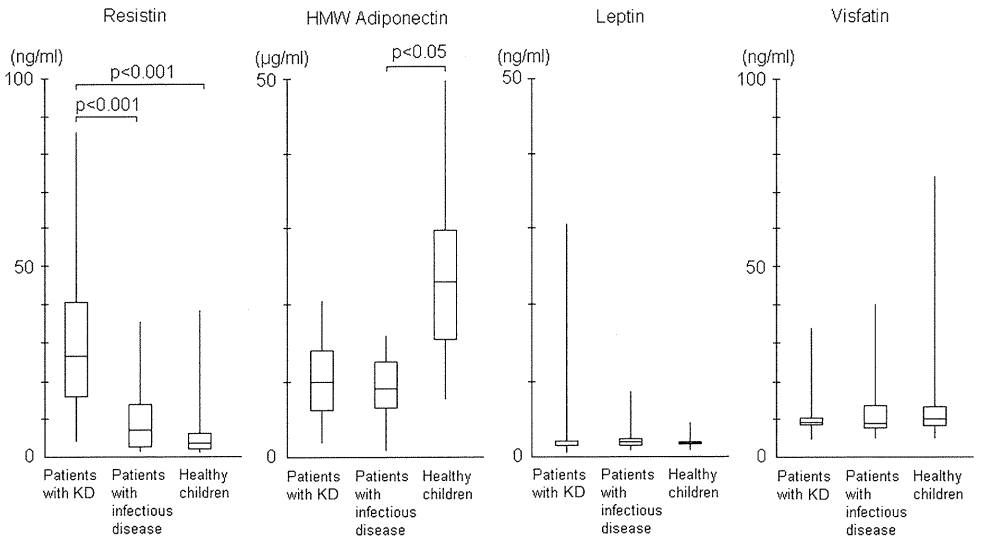

Fig. 1 Serum adipokine levels in the three groups. In the *box plots*, *horizontal lines* indicate median values, and the *lower* and *upper ends* of *boxes* represent the 25th and 75th percentiles. In patients with Kawasaki disease, serum resistin levels were significantly higher than in patients with infectious diseases and in healthy children ($p < 0.001$). Serum high-molecular-weight adiponectin, leptin, and visfatin levels in patients with Kawasaki disease exhibited no statistically significant differences compared with those in healthy controls and patients with acute infectious diseases. *KD* Kawasaki disease, *HMW* high molecular weight

median 11.0 ng/ml) and patients with infectious diseases (HMW adiponectin: mean 9.8 ±4.0, median 8.8 μg/ml; leptin: mean 2.2 ±1.3, median 1.9 ng/ml; visfatin: mean 11.7 ±8.0, median 9.1 ng/ml).

Adipokine levels before and after intravenous immune globulin therapy

Figure 2 shows the changes in serum adipokine levels after intravenous immune globulin treatment. Serum resistin levels decreased significantly after treatment (mean 28.7 ± 18.4 to 9.2 ± 8.3, median 24.5 to 6.7 ng/ml, $p < 0.001$). However, there were no significant changes in serum HMW adiponectin (mean 11.7 ± 5.5 to 11.3 ± 4.8, median 11.9 to 11.1 μg/ml), leptin (mean 3.0 ± 5.9 to 2.2 ± 2.4, median 1.7 to 1.7 ng/ml) or visfatin (mean 11.0 ± 5.3 to 10.9 ± 3.0, median 9.0 to 10.7 ng/ml) levels after intravenous immune globulin.

Comparison of serum adipokine levels in responders and nonresponders to intravenous immune globulin therapy

We compared serum adipokine levels in patients who responded to intravenous immune globulin infusion and in those who did not respond to the treatment, and found that serum resistin levels on admission were significantly ($p < 0.05$) higher in nonresponders (mean 37.2 ± 17.0, median 33.4 ng/ml) compared with responders (mean 28.9 ± 21.0, median 22.9 ng/ml). Serum HMW adiponectin, leptin, and visfatin levels were not significantly different between responders (HMW adiponectin: mean 11.1 ± 9.9, median 10.0 μg/ml; leptin: mean 1.7 ± 0.7, median 1.6 ng/ml; visfatin: mean 11.5 ±6.3, median 9.4 ng/ml) and nonresponders (HMW adiponectin: mean 10.0 ±5.8, median 10.1 μg/ml; leptin: mean 3.7 ± 1.7, median 1.7 ng/ml; visfatin: mean 10.2 ± 2.0, median 10.0 ng/ml).

Correlations between serum resistin levels and clinical data in patients with Kawasaki disease

Since significant elevation of only serum resistin levels was observed in Kawasaki disease, we further analyzed the relationship between serum resistin levels and clinical conditions. Table 3 presents the correlations between serum resistin levels and clinical data considered to be related to disease severity of Kawasaki disease before intravenous immune globulin therapy. Significant univariate predictors of severity included age, CRP, peripheral white blood cell count, and serum sodium level. Simultaneous inclusion of univariate predictors into a multivariate model revealed that high CRP level was a predictor of elevated serum resistin level.

Discussion

In this study, we found that serum resistin levels were elevated in patients with Kawasaki disease compared with in healthy children and in patients with acute infectious diseases. However, there were no significant differences in serum levels of HMW adiponectin, leptin, and visfatin between the various patient groups. Nozue et al. [17] recently reported that serum resistin levels in patients with Kawasaki disease were significantly higher than in healthy controls; however, they did not measure the levels of other adipokines.

Human resistin is produced and released mainly in mononuclear cells (monocytes/macrophages) rather than adipocytes [18, 19]. Mononuclear cells are also important in the pathogenesis of Kawasaki disease, and histopathological

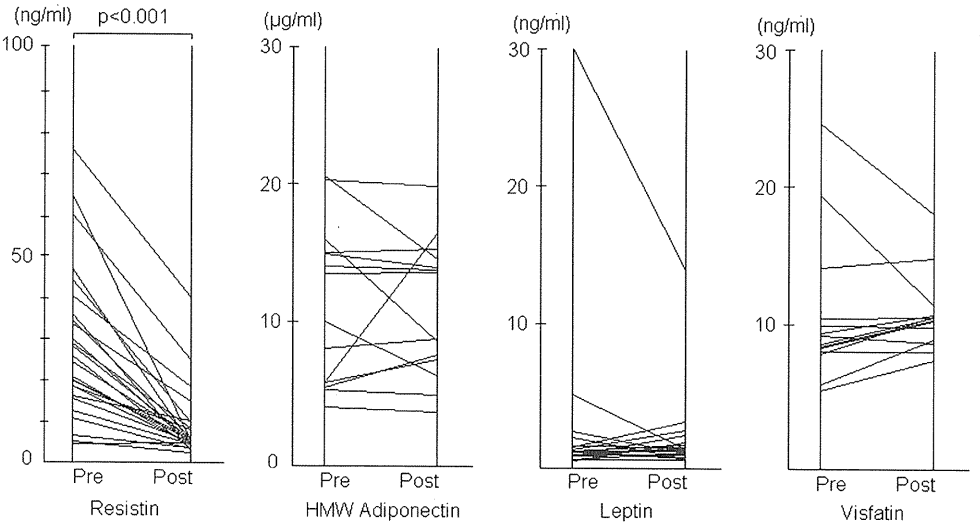

Fig. 2 Adipokine changes after treatment with intravenous immune globulin. Serum resistin levels decreased significantly after administration of intravenous immune globulin ($p < 0.001$). However, serum high-molecular-weight adiponectin, leptin, and visfatin levels did not exhibit any statistically significant changes after intravenous immune globulin treatment. *HMW* high molecular weight

Table 3 Correlations between serum resistin levels and clinical data in patients with Kawasaki disease

Characteristic	Univariate			Multivariate	
	β	p	R^2	β	p
Female	4.887	0.387	0.014	4.013	0.471
Age	0.266	*0.034*	0.081	0.146	0.677
Weight	1.334	0.077	0.057	−0.327	0.871
CRP	1.609	*0.001*	0.205	1.151	*0.022*
WBC	0.001	*0.041*	0.075	0.000	0.373
Sodium	−2.062	*0.019*	0.098	−1.163	0.199
				R^2 0.276	

β regression coefficient, *CRP* C-reactive protein, *WBC* white blood cell

Italics mean significant p values

findings in Kawasaki disease include panvasculitis with infiltration of mononuclear cells [20]. It has been reported that CD14+ monocytes/macrophages play an important role in cytokine production during acute Kawasaki disease [21]. The elevated serum resistin levels in Kawasaki disease shown in our study might have been caused by overproduction by monocytes/macrophages. It was recently shown that resistin competes with lipopolysaccharide for binding to toll-like receptor 4 (TLR4) on peripheral blood mononuclear cells. Torkowski [22] suggested that resistin may partly act as a proinflammatory cytokine via TLR4. It has also been reported that expression of TLR4 is upregulated during the acute phase of Kawasaki disease [23]. These reports and our clinical data of the present study suggest that resistin may be a key cytokine involved in the pathophysiology of Kawasaki disease, possibly as a ligand for TLR4.

After administration of intravenous immune globulin, which is a standard therapy for acute Kawasaki disease, serum resistin levels decreased significantly to nearly normal levels. This suggests that high resistin levels indicate high disease activity. We then examined the correlations between serum resistin level and laboratory parameters considered to be related to disease activity of Kawasaki disease [24, 25].

On simple regression analysis, inflammatory markers (CRP and peripheral white blood cell count) had significant positive correlations with serum resistin levels, while serum sodium levels had a negative correlation with serum resistin levels. Hyponatremia is a common finding in patients with severe Kawasaki disease [26]. Simultaneous inclusion of univariate predictors into a multivariate model resulted in a final parsimonious model with CRP in our study. In contrast, Nozue et al. [17] showed that the only variable significantly associated with resistin concentrations before intravenous immune globulin therapy was body mass index. There were no obvious differences in the background characteristics of patients, including age, gender, weight, and CRP levels on admission, between their study and our present study. We were not able to identify any reasons for the differences in the studies other than the different cohorts of patients.

In our present study, we also compared serum resistin levels in responders and nonresponders to intravenous immune globulin treatment. Serum resistin levels were significantly higher in patients who did not respond to intravenous immune globulin therapy. This result suggests that high serum resistin level may be a predictor of nonresponsiveness to intravenous immune globulin therapy. There were only four patients with coronary arterial lesions, and they had no trend for increased serum resistin levels compared with patients without coronary lesions [serum resistin levels in the four patients with coronary artery lesions: 19.8, 25.8, 29.2, 54.2 ng/ml, patients without coronary lesions ($n = 52$): mean 31.5 ± 20.3, median 28.8 ng/ml].

There has been one previous report dealing with adipokines other than resistin in Kawasaki disease. Takeshita et al. [27] reported that plasma total adiponectin levels in patients with acute Kawasaki disease were significantly lower than in those with convalescent Kawasaki disease or acute febrile disease or in healthy children. In our study, HMW adiponectin in patients with Kawasaki disease had a trend toward being lower than the serum levels in healthy children, but the difference was not statistically significant. Since the HMW fraction of adiponectin is associated more strongly with coronary artery disease than other fractions [10, 11], the trend toward a lower levels might not be highly attributable to the complications of coronary lesions in Kawasaki disease. This may be because of the differences in the pathogenesis of coronary lesions. Coronary lesions in Kawasaki disease are generally panarteritis with acute inflammatory cell infiltrations, in contrast to the progressive atherosclerotic changes associated with adult coronary lesions [28].

We have previously suggested that serum adiponectin level is elevated in adult patients with rheumatoid arthritis [16]. It was also shown that adiponectin stimulates the production of interleukin-8 [29] and prostaglandin E_2 [30] by rheumatoid synovial fibroblasts, suggesting that adiponectin might act as a proinflammatory cytokine in rheumatoid inflammation. Adiponectin is secreted by not only adipocytes, but also in synovial fibroblasts in patients with rheumatoid arthritis [31]. Therefore, the differences in serum adiponectin levels between patients with Kawasaki disease and rheumatoid arthritis may be related to differences in the major affected organs or cells. The different adiponectin levels between Kawasaki disease and rheumatoid arthritis may also be related to their acute and chronic inflammatory condition, respectively.

There have been no previous reports about leptin and visfatin in Kawasaki disease. We found that serum levels of these adipokines in patients with Kawasaki disease exhibited no statistically significant differences compared with in healthy children and patients with acute infectious diseases. In our previous study, significant elevation in serum leptin level was observed in patients with rheumatoid arthritis, and a significant correlation between serum leptin and CRP levels was shown by multivariate analysis in these patients [16]. It was reported that serum levels of visfatin are higher in patients with rheumatoid arthritis, but not in patients with systemic lupus erythematosus and systemic sclerosis [32]. The differences in serum levels of these adipokines in different inflammatory diseases remain to be studied.

In conclusion, we demonstrate herein that serum resistin levels were elevated in patients during the acute phase of Kawasaki disease and decreased to nearly normal after intravenous immune globulin treatment. In contrast, serum HMW adiponectin, leptin, and visfatin levels showed no significant changes. Although further investigations are needed to better understand the detailed roles of adipokines in Kawasaki disease, our data suggest that, among these four adipokines, only resistin participates in the pathogenesis of this disease.

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Conflict of interest None.

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ORIGINAL ARTICLE

Nationwide survey of severe respiratory syncytial virus infection in children who do not meet indications for palivizumab in Japan

Masaaki Mori · Hisashi Kawashima · Hidefumi Nakamura · Masao Nakagawa · Satoshi Kusuda · Tsutomu Saji · Hiroyuki Tsutsumi · Shumpei Yokota · Susumu Itoh · Surveillance Committee for Severe RSV Infection

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Abstract In Japan, palivizumab, a humanized monoclonal antibody specific for respiratory syncytial virus (RSV), has been available since 2002. However, its use is limited to children at risk of severe RSV infection, with specific criteria that have been validated in large-scale clinical studies. The Pharmaceutical Committee of the Japan Pediatric Society established a committee to conduct a nationwide questionnaire survey to determine which diseases place children at risk of severe RSV infection and require preventive measures. A questionnaire sent to 613 medical institutions, including major pediatric hospitals and general hospitals with pediatric services, received 272 responses (44.4%). In total, 1,115 children not meeting current indications for palivizumab therapy were hospitalized for severe RSV infection, 16 (1.4%) of whom died; this suggests that palivizumab therapy should be considered for children with severe immunodeficiency or those at risk of nosocomial RSV infection in whom prevention of RSV infection by standard control measures appears difficult.

Keywords Nationwide survey · Questionnaire · Respiratory syncytial virus · Child · Palivizumab off-label use

Introduction

Respiratory syncytial virus (RSV) is a common virus that is prevalent from fall through spring, and primary RSV infection occurs in almost 100% of children by 2 years of age [1]. RSV may cause lower respiratory tract infection in

M. Mori (✉)
Department of Pediatrics,
Yokohama City University Medical Center,
4-57 Urafune-cho, Minami-ku, Yokohama 232-0024, Japan
e-mail: mmori@med.yokohama-cu.ac.jp

H. Kawashima
Department of Pediatrics, Tokyo Medical University,
Tokyo, Japan

H. Nakamura
Clinical Trial Center, National Center for Child Health and Development, Tokyo, Japan

M. Nakagawa
Department of Pediatrics, Shiga University of Medical Science, Shiga, Japan

S. Kusuda
Department of Neonatology, Maternal and Perinatal Center, Tokyo Women's Medical University, Tokyo, Japan

T. Saji
Department of Pediatrics, Toho University Omori Medical Center, Tokyo, Japan

H. Tsutsumi
Department of Pediatrics, Sapporo Medical University Hospital, Sapporo, Japan

S. Yokota
Department of Pediatrics, Yokohama City University Hospital, Yokohama, Japan

S. Itoh
Department of Pediatrics, Kagawa University, Kagawa, Japan

children, and 30–40% of infants <12 months of age may develop severe illness, necessitating hospitalization in 2–3% of cases [2]. It has been estimated that 20,000–30,000 children are hospitalized due to severe RSV infection each year [3]. Immunocompromised children and children with underlying diseases often associated with cardiopulmonary disorders are especially susceptible to developing severe RSV infection. Since there is no specific treatment for RSV infection, management is difficult, and some children have a fatal outcome [4].

In Japan, palivizumab, an anti-RSV humanized monoclonal antibody, has been available since 2002 for the prophylaxis of severe RSV lower respiratory tract infection in high-risk infants. As of December 2009, palivizumab has been indicated only for children at risk of severe RSV infection, with specific criteria that have been validated in large-scale clinical studies. The effects of palivizumab prophylaxis in premature infants and children with chronic lung disease (bronchopulmonary dysplasia) were evaluated in an international Phase II multicenter placebo-controlled double-blind clinical study [5], which demonstrated a 55% reduction in RSV hospitalization for these children, as well as decreases in the duration of hospitalization, days of oxygen therapy, and the prevalence of intensive care unit (ICU) admission. In a Phase I/II bridging study conducted in Japan, it was considered legitimate to extrapolate the international efficacy data to the Japanese population. The Ministry of Health, Labor, and Welfare of Japan reviewed existing clinical trials and domestic data and approved palivizumab prophylaxis for: (1) infants ≤12 months of age born at ≤28 weeks of gestation, (2) infants ≤6 months of age born at 29–35 weeks of gestation, and (3) infants or young children ≤24 months of age who had been treated for bronchopulmonary dysplasia at any time during the previous 6 months. The use of palivizumab for children ≤24 months of age who have hemodynamically significant congenital heart disease (CHD) was additionally approved on the basis of the results of a Phase III placebo-controlled double-blind clinical study in children with CHD [6], which demonstrated a 45% decrease in RSV hospitalization and decreases in the duration of hospital stay and oxygen therapy, as well as the results of a Phase III clinical study in Japan which yielded profiles of serum palivizumab concentration in Japanese patients that were similar to those of participants in the international trial.

There are data from descriptive reports and case-control studies that support the use of palivizumab to prevent severe RSV infection in high-risk immunocompromised children and those with airway diseases, neuromuscular disorders, or chromosomal abnormalities/malformation syndromes, and children receiving home oxygen therapy, though no such findings have been reported in Japan. Although cases of serious complications of RSV infection such as sudden infant death syndrome (SIDS), encephalopathy/encephalitis, and cardiomyopathy have recently been documented, the epidemiology of such cases has not yet been clearly determined.

Accordingly, members of the Japan Pediatric Society established an RSV Survey Committee to investigate the types and risks of underlying diseases that could potentially be considered for prophylaxis against severe RSV infection.

Purpose

The present survey was conducted to: (1) identify the diseases and conditions in which prevention of RSV infection might be particularly important, and (2) determine the effects of severe RSV infection in high-risk children.

Patients and methods

Findings for children <4 years of age who did not qualify for palivizumab prophylaxis under existing guidelines and were hospitalized due to or died of laboratory-confirmed RSV infection during the period between August 2006 and July 2008 were retrospectively obtained from medical institutions throughout Japan, using two questionnaire forms.

In Survey A, a survey of RSV hospitalization in children with underlying diseases, physicians were asked to document information on age, sex, duration of hospitalization, treatment, and presence/absence of severe sequelae, defined as conditions requiring support/assistance with daily activities. In Survey B, a survey of severe RSV infection in children without underlying disease, physicians were asked to document data similar to the data involving the hospitalization of children with severe RSV infection, i.e., RSV infection associated with SIDS, apparent life-threatening events, encephalopathy/encephalitis, cardiomyopathy, severe bronchiolitis (defined as bronchiolitis with serious respiratory disorder such as expiratory wheezing, polypnea, chest-wall retraction, and cyanosis), or other diagnoses. The items investigated were the presence/absence and type of underlying disease, month of RSV infection, sex, age, duration of hospitalization, the presence/absence and duration (in days), if present, of oxygen therapy, and the presence/absence and duration (in days), if present, of ventilation (Table 1).

The study protocol was approved by the Ethics Committee of Yokohama City University.

Table 1 Underlying diseases and items of survey

I. Underlying diseases evaluated in the survey	
Respiratory diseases ($n = 414$)	
Asthma	348 (84.1%)
Bronchomalacia	10 (2.4%)
CLD in child over 2 years of age on HOT	10 (2.4%)
Pulmonary hypoplasia	9 (2.2%)
Others	37
Chromosomal abnormalities/malformation syndromes ($n = 130$)	
Trisomy 21 (no CHD)	57 (43.8%)
Congenital anomaly	35 (26.9%)
Other chromosomal abnormalities (no CHD)	30 (23.1%)
Others	8
Neuromuscular disorders ($n = 125$)	
Epilepsy	62 (49.6%)
Sequelae of cerebral hemorrhage/infarction	19 (15.2%)
Cerebral palsy	13 (10.4%)
Sequelae of meningitis/encephalitis	8 (6.4%)
Myasthenia gravis	0 (0%)
Others	23
Heart diseases (did not qualify for palivizumab) ($n = 27$)	
CHD in child over 2 years of age	18 (67%)
Arrhythmia	4 (15%)
Cardiomyopathy	3 (11%)
Others	2
Immunocompromised hosts ($n = 12$)	
Leukemia	3 (25%)
Solid tumor	3 (25%)
Primary immunodeficiency syndrome	3 (25%)
Other transplant recipient	1 (8.3%)
Kidney transplant recipient	0 (0%)
Liver transplant recipient	0 (0%)
Others	2
Congenital metabolic disorders ($n = 8$)	
Other diseases ($n = 40$)	
Kawasaki disease	26 (65.0%)
Rheumatic diseases	0 (0%)
Autoinflammatory syndrome	0 (0%)
Other diseases	14
II. Items of survey	
Underlying diseases[a]	
Severe RSV infection[b]	
Reasons for RSV hospitalization[a]	
Month of RSV infection	
Sex	
Age at RSV infection	
Duration of hospitalization (days)	
Use and duration (days) of oxygen therapy	
Use and duration (days) of ventilation	
Intensive care unit admission[a]	
Presence/absence of sequelae {of RSV infection}	

RSV respiratory syncytial virus, *CHD* congenital heart disease, *CLD* chronic lung disease, *HOT* home oxygen therapy

[a] Investigated only in Survey A

[b] Investigated only in Survey B

Data analysis

The findings for hospitalized patients were tested using univariate logistic regression analysis to evaluate the effect of each factor on the outcome of RSV infection, with p values and crude odds ratios obtained for each factor. Significance was examined using χ^2 tests for categorical variables such as the type of underlying disease, reason for hospitalization, and month of RSV hospitalization; Fisher's exact test for binary variables such as sex and the presence/absence of oxygen therapy; and Student's t-test for continuous variables.

Results

In June 2008, a questionnaire was sent to 613 medical institutions, including teaching hospitals with pediatric residency programs, as well as major pediatric hospitals and general hospitals with pediatric services equivalent to those provided in teaching hospitals, to report cases of RSV hospitalization in children not indicated for palivizumab prophylaxis during the period between August 2006 and July 2008, and 272 institutions (44.4%) responded (Table 2). After the data of Surveys A and B were reconciled to ensure

Table 2 Characteristics of patients evaluated in the nationwide survey

	Survey A ($n = 756$)	Survey B ($n = 359$)
Sex		
Male	447 (69.7%)	194 (30.3%)
Female	273 (63.9%)	155 (36.2%)
Age at RSV infection (months)[a]	20.4 ± 12.17 [0–47]	6.7 ± 8.55 [1–40]
Duration of hospitalization (days)[a]	10.5 ± 21.76 [1–540]	11.5 ± 13.08 [0–210]
≤2 weeks	650 (88.0%)	285 (79.4%)
>2 weeks	89 (12.0%)	74 (20.6%)
Use of oxygen therapy	458 (60.6%)	351 (98.6%) *1
Duration (days)[a]	7.7 ± 27.15 [1–540]	6.8 ± 4.79 [1–51]
Use of ventilation	48 (6.3%)	144 (40.4%) *2
Duration (days)[a]	22.8 ± 83.48 [1–540]	6.4 ± 3.87 [1–22]
Oxygen therapy alone	411 (54.4%)	206 (58.4%) *3
No oxygen/ventilation	297 (39.3%)	5 (1.4%) *3

Data during the period between August 2006 and July 2008 were collected through a questionnaire sent to 613 institutions, 272 (44.4%) of which responded

RSV respiratory syncytial virus, *SD* standard deviation

[a] Values are means ± standard deviation [range]; *1 No data in 3 cases; *2 no data in 3 cases; *3 no data in 6 cases. Cases with no data in *1 and *2 are separate patients

the absence of overlapping cases and to ensure that the data were sufficient, the numbers of patients reported in Surveys A and B were 756 and 359, respectively, and the total number reported was thus 1,115 patients.

The characteristics of the patients enrolled in the study are shown in Table 2. Figure 1 shows the age distribution of the reported patients. Of the children without underlying diseases reported in Survey B, 277 were less than 1 year of age, and the number of reported patients decreased as the age increased. In Survey A, of children with underlying diseases, there were substantial numbers of reported patients throughout the age range evaluated. The relative risk of having an underlying disease was about 1.3 times higher in males than in females, and this difference was statistically significant (1.000 vs. 0.7644, $p = 0.0421$, Fisher's exact test). The mean durations of hospitalization were 10.5 ± 21.76 and 11.5 ± 13.08 weeks in Surveys A and B, respectively. The most frequent duration of hospitalization was 0–2 weeks, and the longest duration of hospitalization was 77 weeks. The distribution of month of RSV infection was consistent with the fact that RSV infection is prevalent during winter months. However, reported cases were distributed through all months of the year, particularly in the patients with underlying diseases. Among the patients with underlying diseases, some were not treated with oxygen therapy or ventilation, while among the patients without underlying diseases, RSV infection was relatively severe, and the number of patients receiving neither oxygen therapy nor ventilation was small.

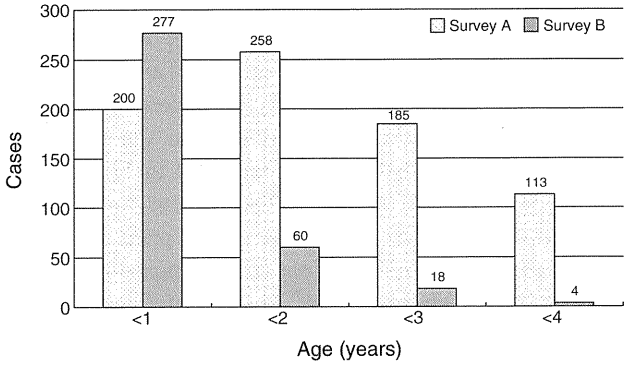

Fig. 1 Age distribution of reported patients

RSV hospitalization in patients with underlying diseases

Respiratory diseases were the most prevalent underlying conditions: 54.8% of reported patients had respiratory diseases, and asthma was the most prevalent respiratory disease (46.0% of the reported patients). Children with chromosomal abnormalities/malformation syndromes, neuromuscular diseases, cardiac diseases, immunocompromised status, and congenital metabolic disorders were also reported (Fig. 2a). Table 1 lists the numbers of patients by underlying disease.

Of the patients with underlying respiratory diseases ($n = 414$), 84.1% had asthma, and the remaining children had various conditions such as bronchomalacia, chronic lung disease (bronchopulmonary dysplasia) in patients over 2 years of age, and pulmonary hypoplasia. The second most prevalent category of underlying diseases was chromosomal abnormalities/malformation syndromes, 130 cases of which were reported, 57 (43.8%) involving children with trisomy 21 without CHD. RSV hospitalization in children with neuromuscular disorders was also common ($n = 125$). About half of these children had epilepsy, and cases of cerebral hemorrhage/infarction, meningitis, and encephalitis were also reported. Among children reported to have cardiac diseases not indicated for palivizumab prophylaxis ($n = 27$), 18 children (67%) had CHD and were over 2 years of age, and 3 children (11%) had arrhythmia or cardiomyopathy. Twelve children were immunocompromised due to treatment for leukemia or solid tumors, primary immunodeficiency syndrome, or post-transplant status. Of the children in the "other diseases" category ($n = 40$; see Table 1), 26 (65%) had a history of Kawasaki disease.

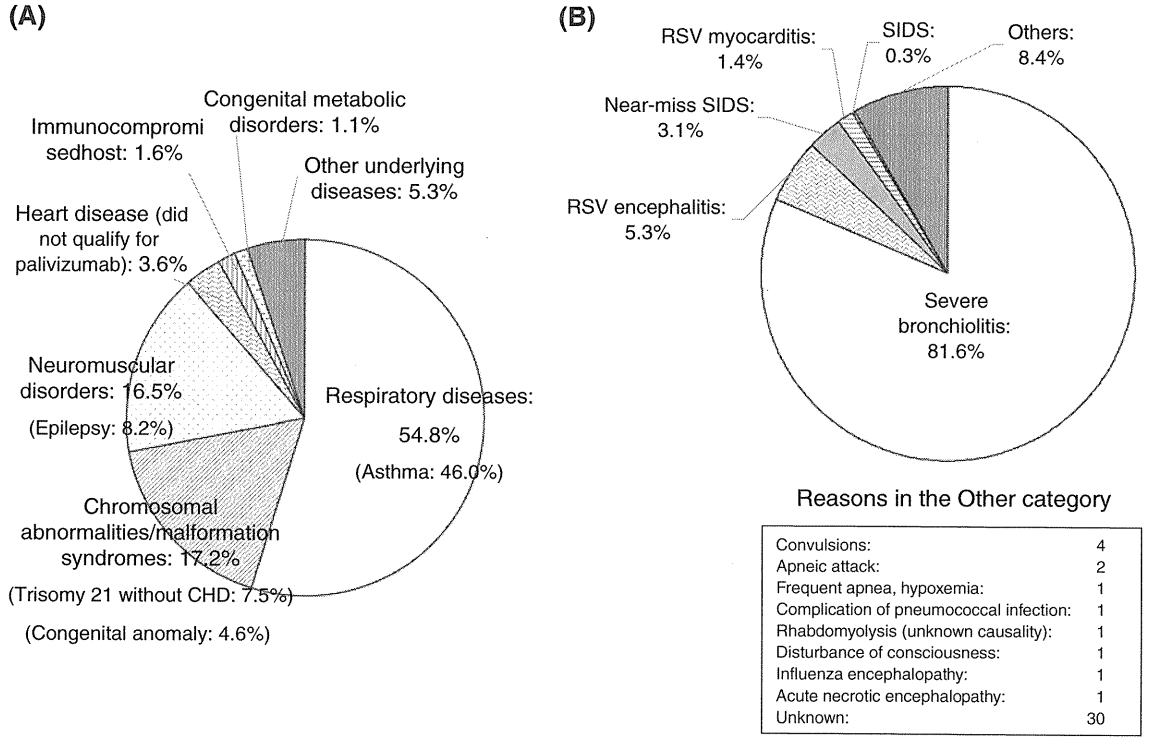

Fig. 2 **a** Types of underlying diseases in children hospitalized for respiratory syncytial virus (*RSV*) infection in Survey A ($n = 756$), and **b** reasons for RSV hospitalization in children without underlying diseases in Survey B ($n = 359$). *CHD* congenital heart disease, *SIDS* sudden infant death syndrome

Table 3 shows the outcome of RSV infection stratified by patient characteristics. When "severe sequelae" and "death" were considered to represent poor outcomes of RSV infection, the risk of poor outcome of RSV infection was about 1,000 times higher in immunocompromised children than in children with respiratory disorders and about 2.8–4.3 times higher than that in patients with other underlying diseases. These findings indicate that immunocompromised status is significantly associated with a poor outcome of RSV infection ($p < 0.0001$, χ^2 test). Oxygen therapy, ventilation, and ICU hospitalization were also significantly associated with poor outcomes of RSV infection ($p < 0.0023$, $p < 0.0001$, and $p < 0.0001$, Fisher's exact test), and the risks of poor outcome were about 11.4, 186.1, and 23.3 times higher in patients receiving oxygen therapy, those receiving ventilation, and those hospitalized in the ICU, respectively, than in patients without the corresponding treatment. The risk of poor outcome was about 1.2 higher in males than in females, but this difference was not statistically significant.

RSV hospitalization in patients without underlying diseases

Of patients with severe RSV infection without underlying diseases, 81.6% had severe bronchiolitis. Cases of RSV encephalopathy/encephalitis, apparent life-threatening events, myocarditis, and SIDS were also reported. Convulsions and apnea were also observed (Fig. 2b). Table 3 lists the results of analysis by patient characteristics. Although a crude odds ratio could not be obtained due to the distribution of cases, the month of RSV infection significantly affected the outcome of RSV infection ($p = 0.0015$, χ^2 test). The relative risk of poor outcome was about 6.5 times higher in patients requiring ventilation, and the use of ventilation was significantly associated with poor outcome of RSV infection ($p = 0.0162$, Fisher's exact test). The relative risk of poor outcome in male patients was about 0.5 times that in female patients, though this difference was not statistically significant.

A total of 16 deaths (Table 4) were reported in children with or without underlying diseases. Seven of the children who died (43.8%) wereunder 1 year of age, and 11 (68.7%) children had underlying diseases, i.e., leukemia and post-transplantation status in 2, neuromuscular disorder in 3, chromosomal abnormalities without CHD in 5, and CHD beyond 24 months of age in 1. The reasons for the RSV hospitalization of these 11 children were pneumonia in 4 patients and bronchiolitis in 1 patient, and nosocomial RSV infection during chemotherapy, unsuccessful resuscitation following cardiopulmonary arrest (CPA), shock, aspiration pneumonia, and deterioration of upper respiratory tract infection with CPA in one patient each.

Table 3 Severity of outcome of RSV infection by patient characteristics—survey in children with and without underlying diseases

Factors	With underlying diseases				Without underlying diseases			
	No/mild sequelae	Severe sequelae[a] or death	Odds ratio	*p* value	No/mild sequelae	Severe sequelae or death	Odds ratio	*p* value
Underlying diseases								
Immunocompromised hosts	9 (81.8%)	2 (18.2%)	1.000	<0.0001				
Respiratory diseases	411 (99.8%)	1 (0.2%)	0.001					
Neuromuscular disorders	116 (95.1%)	6 (4.9%)	0.233					
Chromosomal abnormalities/ malformation syndromes	120 (94.5%)	7 (5.5%)	0.262					
Heart diseases	25 (92.6%)	2 (7.4%)	0.360					
Congenital metabolic disorders	8 (100%)	0 (0%)	–					
Others	39 (100%)	0 (0%)	–					
Severe RSV infection								
SIDS					0 (0%)	1 (100%)	–	<0.0001
ALTE					11 (100%)	0 (0%)	–	
RSV encephalopathy					13 (68.4%)	6 (31.6%)	–	
RSV myocarditis					3 (75%)	1 (25%)	–	
Severe bronchiolitis					283 (99.6%)	1 (0.4%)	–	
Others					29 (96.7%)	1 (3.3%)	–	
Reasons for RSV hospitalization								
Bronchiolitis	374 (98.7%)	5 (1.3%)	1.000	<0.0001				
Pneumonia	171 (96.6%)	6 (3.4%)	–					
Encephalitis	0 (0%)	1 (100%)	–					
Bronchitis	46 (100%)	0 (0%)	–					
Atelectasis	3 (100%)	0 (0%)	–					
Asthma attack	25 (100%)	0 (0%)	–					
Others	55 (91.7%)	5 (8.3%)	6.800					
Sex								
Male	430 (97.3%)	12 (2.7%)	1.000	0.6790	183 (97.9%)	4 (2.1%)	1.000	0.3331
Female	265 (97.8%)	6 (2.2%)	0.811		147 (96.1%)	6 (3.9%)	1.867	
Month of RSV infection								
January	163 (96.4%)	6 (3.6%)	–	0.3994	94 (94.9%)	5 (5.1%)	–	0.0015
February	74 (94.9%)	4 (5.1%)	–		48 (98%)	1 (2%)	–	
March	39 (95.1%)	2 (4.9%)	–		17 (100%)	0 (0%)	–	
April	27 (96.4%)	1 (3.6%)	–		8 (88.9%)	1 (11.1%)	–	
May	12 (100%)	0 (0%)	–		3 (100%)	0 (0%)	–	
June	16 (100%)	0 (0%)	–		3 (75%)	1 (25%)	–	
July	12 (100%)	0 (0%)	–		5 (100%)	0 (0%)	–	
August	18 (94.7%)	1 (5.3%)	–		4 (100%)	0 (0%)	–	
September	23 (100%)	0 (0%)	–		5 (100%)	0 (0%)	–	
October	45 (100%)	0 (0%)	–		6 (75%)	2 (25%)	–	
November	87 (96.7%)	3 (3.3%)	–		25 (100%)	0 (0%)	–	
December	209 (99.5%)	1 (0.5%)	–		119 (100%)	0 (0%)	–	
Age at RSV infection (months) mean (±SD) [range]	20.51 ± 12.10 [0–47]	21.61 ± 16.13 [1–47]	–	0.0538	6.68 ± 8.65 [1–40]	11.2 ± 7.18 [1–21]	–	0.5606
Duration of RSV hospitalization (days) mean (±SD) [range]	9.48 ± 8.65 [1–124]	47.71 ± 129.93 [1–540]	–	0.2427	11.19 ± 12.25 [1–210]	27.9 ± 28.56 [0–94]	–	0.0976
Oxygen therapy								
Present	436	17	11.385	0.0023	332	9	0.1084	0.1371

Table 3 continued

Factors	With underlying diseases				Without underlying diseases			
	No/mild sequelae	Severe sequelae[a] or death	Odds ratio	*p* value	No/mild sequelae	Severe sequelae or death	Odds ratio	*p* value
Absent	292	1	1.000	–	4	1	1.0000	
Use of oxygen therapy	59.9%	94.4%	–	–	98.8%	90.0%	–	–
Duration of oxygen therapy (days) mean (±SD) [range]	6.19 ± 7.33 [1–124]	42.56 ± 132.98 [1–540]	–	0.2912	6.68 ± 4.62 [1–51]	9.88 ± 8.77 [1–23]	–	0.3391
Ventilation								
Present	30	16	186.116	<0.0001	128	8	6.500	0.0162
Absent	698	2	1.000	–	208	2	1.000	
Use of ventilation	4.1%	88.9%	–	–	37.9%	80.0%	–	–
Duration of ventilation (days) mean (±SD) [range]	10.65 ± 11.71 [2–60]	43.20 ± 137.74 [1–540]	–	0.3765	6.33 ± 3.35 [1–15]	9.13 ± 7.99 [1–22]	–	0.3574
ICU hospitalization								
Present	37	10	23.345	<0.0001				
Absent	691	8	1.000	–				
ICU hospitalization rate	5.1%	55.6%	–	–				

SD standard deviation, *SIDS* sudden infant death syndrome, *ALTE* apparent life-threatening events, *ICU* intensive care unit

[a] Severe sequelae were defined as conditions requiring support/assistance with daily activities

In the five children without underlying disease who died of RSV infection, the reason for hospitalization was SIDS in 1 case, severe bronchiolitis in 1, RSV encephalitis in 1, RSV myocarditis in 1, and 'other' (acute necrotic encephalopathy) in 1 case.

Discussion

The present nationwide questionnaire survey was conducted to identify the conditions for which prophylactic treatment may be considered to prevent severe RSV infection and the associated serious sequelae. During the 2-year period of the survey, a total of 1,115 children under 4 years of age were hospitalized due to RSV infection, 16 of whom died. Notably, 756 children had underlying diseases that did not meet the criteria for palivizumab prophylaxis in Japan. This suggests that the current coverage of palivizumab prophylaxis by the National Health Insurance in Japan is perhaps insufficient to protect against the wide spectrum of severe RSV infections in high-risk children.

In the present survey, the outcomes of severe RSV infection were more serious among immunocompromised children than among immunocompetent children with underlying diseases. It has been reported that the mortality rate of RSV infection is 80% when adult bone marrow transplant recipients receive no specific treatment for this infection [7, 8]. Pediatric immunocompromised organ transplant recipients are susceptible to severe RSV infection [9], and are at high risk of developing respiratory failure and death due to RSV infection [10, 11]. The high incidence of poor outcome of severe RSV infection among immunocompromised children in the present survey (2 of 11 children, 18.2%) is consistent with these findings, and indicates the importance of RSV prophylaxis in this population. A recent study using decision analysis modeling indicated that mortality in children receiving bone marrow transplantation was decreased by the addition of palivizumab to protect against RSV lung disease [12]. The American Society of Transplantation has recommended that infants (<1 year) who undergo solid organ transplantation during the RSV season receive immunoprophylaxis to prevent severe RSV infection [13]. Large-scale studies should be conducted in the future to develop guidelines for the prevention of RSV infection after transplantation.

In the present survey, the number of RSV hospitalizations was high among children with trisomy 21 (Down syndrome) without CHD: of the 57 children with trisomy 21 without CHD, two patients died and one had severe sequelae. This finding is supported by Bloemers et al. [14], who have reported that children with Down syndrome are at high risk of RSV hospitalization irrespective of CHD, indicating that patients with Down syndrome with or without CHD should also be considered for prophylaxis. Although the Japanese Society of Pediatric Cardiology and Cardiac Surgery recommends that children under 24 months of age with CHD

Table 4 Characteristics of patients who died due to RSV infection

		Survey A ($n = 11$)		Survey B ($n = 5$)	
Underlying diseases		Leukemia/transplant recipient	2	–	
		Neuromuscular disorders	3	–	
		Chromosomal abnormality without CHD (trisomy 21 in 2, trisomy 31 in 1, 4p-syndrome in 1, Aicardi syndrome in 1)	5	–	
		CHD in child over 24 months of age	1	–	
Reasons for RSV hospitalization		Pneumonia	4	SIDS	1
		Bronchiolitis	1	Severe bronchiolitis	1
		Other (nosocomial pneumonia during chemotherapy)	1	RSV encephalopathy	1
		Other (unsuccessful resuscitation following CPA)	1	RSV myocarditis	1
		Other (shock)		Other (acute necrotic encephalopathy)	1
		Other (aspiration pneumonia)	1	–	
		Other (URTI → CPA)	1	–	
		No data	1	–	
Sex	Male	6 (54.5%)		2 (40.0%)	
	Female	5 (45.5%)		3 (60.0%)	
Age at RSV infection (months)	Mean (±SD) [range]	22.9 ± 15.52 [1–47]		11.2 ± 8.06 [3–21]	
	<12	4 (36.4%)		3 (60%)	
	≥12	7 (63.6%)		2 (40%)	
Duration of hospitalization (days)	Mean (±SD) [range]	18.6 ± 33.34 [1–120]		7.0 ± 8.29 [0–22]	
	≤2 weeks	7 (63.6%)		4 (80%)	
	>2 weeks	4 (36.4%)		1 (20%)	
Oxygen therapy		11 (100%)		11 (100%)	
Duration (days)	Mean (±SD) [range]	8.5 ± 9.23 [1–27]		7.2 ± 8.13 [1–22]	
Ventilation		11 (100%)		11 (100%)	
Duration (days)	Mean (±SD) [range]	7.3 ± 9.17 [1–27]		7.2 ± 8.13 [1–22]	
Oxygen therapy alone		0 (0%)		0 (0%)	
No oxygen/ventilation		0 (0%)		0 (0%)	

CPA cardiopulmonary arrest, *URTI* upper respiratory tract infection

associated with chromosomal aberrations or genetic abnormalities, including trisomy 21, be treated prophylactically to prevent severe RSV infection even when they exhibit no significant signs/symptoms of CHD or have obtained complete cure of CHD [15], such children are not yet officially indicated for RSV prophylaxis in Japan.

The present survey found that severe RSV infections developed throughout the year. We were unable to analyze the relationship between month of RSV infection and corresponding serious outcomes, because of the unequal distribution of cases annually. Patients with underlying diseases should therefore be carefully observed for RSV infection throughout the year. Data in the present study were insufficient to permit analysis by district. In a nationwide epidemiological study, there were no differences in patterns of RSV epidemics among districts of Japan [16]. However, that survey did not include Okinawa Prefecture, which consists of islands in a subtropical zone in which it has been reported that RSV outbreaks also occur in spring and summer [17]. We therefore consider our findings representative of the pattern of RSV infection in most of Japan.

In the present survey, patients with neuromuscular disorders accounted for 16.5% of the children with underlying disease with poorer outcomes than other patient groups (poor outcome in 6 of 116 patients); they thus represent one of the important patient groups in which RSV prophylaxis would be potentially beneficial. In a prospective observational registry of children who received at least one dose of palivizumab injection during the RSV seasons from 2002 to 2004 in the United States, the incidence of RSV

hospitalization was found to be significantly higher in children with congenital airway abnormality or severe neuromuscular disorder than in children without such conditions [18]. Moreover, in a prospective multicenter study conducted in Germany between 1999 and 2005, patients hospitalized with RSV infection and neuromuscular impairment had a greater risk of requiring mechanical ventilation and developing seizures, with a statistically significant, higher attributable mortality compared to controls (5.5 vs. 0.2%) [19].

In the recommendations for the use of palivizumab as prophylaxis against RSV in infants with CHD, the Working Group of the British Paediatric Cardiac Association has included children with cardiomyopathy requiring treatment in the list of children likely to benefit from prophylaxis [20]. Although the number of patients with cardiomyopathy reported in the present survey was too small to examine effects on the outcome of severe RSV infection, this patient group should be carefully evaluated for the presence of congestive heart failure that requires prophylaxis in accordance with the CHD trial [6].

In a cohort study of all children with severe RSV infection in England from 1999 to 2007, all the children who died of RSV infection ($n = 35$) had preexisting diseases (relative risk 2.36), and multiple preexisting diseases (4.38), cardiac anomaly (2.98), and nosocomial RSV infection (2.89) were considered risk factors for death from severe RSV infection. An interaction among preexisting disease, nosocomial RSV infection, and mortality was also found [21].

Although the number of large-scale studies of palivizumab in patients with underlying diseases is limited, in a meta-analysis in 2007, treatment with palivizumab was found to increase the survival of patients undergoing bone marrow transplantation by about 10%, from 83 to 92% [12]; also, the use of palivizumab in children with certain specific diseases is reimbursed in some provinces of Canada and by some insurance companies in Western countries [22–26]. The results of the present survey indicate that palivizumab prophylaxis should be strongly considered in children with severe immunodeficiency and children with nosocomial RSV infection that is uncontrollable with conventional infection control measures.

In the present survey, in children without underlying disease, severe bronchiolitis was the most common reason for RSV hospitalization, though RSV encephalopathy/encephalitis accounted for 5.3% of RSV hospitalizations of otherwise healthy children. The mechanisms of development of severe RSV infection remain unclear in many respects, and it is also unclear which serotypes of RSV cause more severe RSV infection. Since encephalopathy/encephalitis may result in permanent neurological damage and significantly affect the quality of life of patients and their families, it is important to be aware of RSV infection as a cause of encephalopathy/encephalitis.

In summary, this first nationwide Japanese survey of RSV infection in high-risk children who did not receive prophylaxis based on current recommendations provides epidemiological data useful for the determination of additional indications for palivizumab. We hope that our results will help healthcare professionals to investigate the diverse presentations of RSV disease and target patients with RSV infection in pediatric emergency services. The survey also sets the stage for larger prospective studies of underlying medical disorders that place infants at greater risk of compromise from severe RSV infection, and it is such infants who may benefit substantially from prophylaxis.

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