

Investigation of varicella-zoster virus immunoglobulin G levels in general health checkup subjects and patients with the Ramsay Hunt syndrome for a diagnosis of zoster sine herpette

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Objectives: The objectives were: to investigate the distribution of and age-related changes in the varicella-zoster virus (VZV) immunoglobulin G (IgG) levels in subjects attending a health checkup, and to investigate the validity of diagnosis based on the IgG levels in patients with peripheral facial nerve paralysis without other symptoms of herpes zoster reactivation (zoster sine herpette [ZSH]).

Methods: The serum VZV IgG levels were examined in 2,372 subjects during a general health checkup conducted. Among 25 patients with Ramsay Hunt syndrome, the VZV IgG levels measured within 3 days and after 3 weeks of onset were investigated. In addition, the sensitivity and specificity of VZV IgG levels for diagnosing ZSH were evaluated.

Results: During health checkup, there was a significant increase in the VZV IgG levels in subjects who were in their 20s–30s, 20s–40s, and 20s–50s. In patients with Ramsay Hunt syndrome, the VZV IgG levels measured within 3 days and after 3 weeks of onset did not differ from those in the health checkup subjects. ZSH can be diagnosed only by the change in VZV IgG levels, and the sensitivity was low (16%).

Conclusion: It was difficult to diagnose ZSH using VZV IgG levels.

Key words: facial nerve paralysis, Ramsay Hunt syndrome, herpes zoster, zoster sine herpette, varicella-zoster virus immunoglobulin G

Introduction

In Japan, publicly supported routine vaccination for varicella-zoster virus (VZV) was not administered until 2014; therefore, chickenpox was common and was almost exclusively acquired during childhood. Hence, most adults have VZV IgG antibodies in their serum. Herpes zoster (HZ) and Ramsay Hunt syndrome, also known as, herpes zoster oticus, are caused by VZV reactivation. Risk factors for HZ were largely unknown, older age and diminished cell-mediated immunity were well-documented risk factors but cannot fully explain the epidemiology of HZ. Detection of serum IgM and PCR are of limited value for the confirmation of HZ.¹ However, the relationship between the levels of serum

VZV IgG and the occurrence of VZV reactivation remains unknown. There is no consensus regarding the age-related change in the serum VZV IgG levels. A previous study reported no age-related change.²

In contrast, it has been reported that certain cases of peripheral facial palsy without rash, sensorineural hearing loss, or vertigo can be caused by VZV reactivation, known as zoster sine herpette (ZSH). ZSH is associated with poor prognosis.³ Therefore, a strong treatment regimen should be considered. It is clinically important to diagnose ZSH in the early stage of the disease. However, as mentioned above, a majority of the adults have VZV IgG antibodies in their serum, and the levels of these antibodies may change with age. Therefore, validity of using VZV IgG levels for ZSH diagnosis requires verification by

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comparing the serum levels of VZV IgG in the general population with those in patients with Ramsay Hunt syndrome.

In the present study, the distribution of and age-related changes in the serum VZV IgG levels were investigated in subjects attending a health checkup on the basis of the results of a general health checkup conducted at our university. Subsequently, the validity of using serum VZV IgG levels for ZSH diagnosis was investigated by comparing the results of the health checkup subjects with those of patients with Ramsay Hunt syndrome.

Materials

Subjects undergoing the annual employees' health checkup

We investigated the results of 2,372 subjects attending the annual employee's general health checkup conducted at the Kitasato University and the Kitasato University Hospital in 2014. Subjects for whom data regarding age, sex, and serum VZV IgG levels were unavailable and those who did not provide written informed consent were excluded from the study.

Patients with Ramsay Hunt syndrome

We investigated the clinical data of 25 patients with Ramsay Hunt syndrome who, on an outpatient basis, visited the Department of Otorhinolaryngology of the Kitasato University Hospital between January 1, 2011 and December 31, 2014. Ramsay Hunt syndrome was diagnosed on the basis of the presence of peripheral facial palsy and a rash with blisters of the mucocutaneous division of the VII cranial nerve innervation and/or the VIII cranial nerve symptoms, including sensorineural hearing loss, and/or vestibular symptoms (with nystagmus). Consequently, peripheral facial palsy patients with ZSH were not included in this study. Patients for whom data regarding age, sex, serum VZV IgG levels, and follow-ups were unavailable, and those who did not provide written informed consent were excluded from the study.

This study was approved by the ethics committee of the Kitasato University (B14-171).

Methods

A total of 2,372 subjects (age range, 20–77 years) who underwent the general health checkup were divided into 6 age groups: those in their 20s, 30s, 40s, 50s, 60s, and 70s. The patients with Ramsay Hunt syndrome (age range, 24–77 years) were divided into the same 6 age

groups. Complete recovery of facial palsy in patients with Ramsay Hunt syndrome was defined as a Yanagihara score of $\geq 36/40$ a year after the onset of paralysis.⁴

The serum VZV IgG levels were measured using an enzyme immunoassay (Denka Seiken Co., Ltd., Tokyo) in both groups. In patients with Ramsay Hunt syndrome, the serum VZV IgG levels were measured once within 3 days and again 3 weeks following the onset of facial palsy. When the VZV IgG levels were >128 , we considered these levels as 128.

For the diagnosis of ZSH, we investigated whether the diagnosis of Ramsay Hunt syndrome could be made by the values of VZV IgG alone. We evaluated the sensitivity and specificity for the diagnosis of Ramsay Hunt syndrome using the VZV IgG levels calculated from the results of the health checkups of subjects and the patients with Ramsay Hunt syndrome using receiver operating characteristic (ROC) curves. A threshold of 50 for VZV IgG levels, which is generally used in Japan,⁵ was used in this study. In patients with Ramsay Hunt syndrome, we investigated the relationship between the treatment results and the change in the VZV IgG levels from those measured within 3 days to those measured after 3 weeks of onset.

Statistical analyses

All statistical analyses were performed using the software package JMP (ver 11.2 for Windows). The Mann-Whitney or chi-square test was used for between-group comparisons, the Kruskal-Wallis test for age group comparisons, and followed up by the Wilcoxon multiple test. For the first three tests, $P < 0.05$ was considered statistically significant. In the Wilcoxon multiple test for the 6 age groups, $P < 0.003333$ (0.05/15 pairs) was considered statistically significant.

Results

VZV IgG levels in health checkup subjects during the general health checkup

The distribution of age and gender of subjects during the general health checkup is shown in Figure 1A. A total of 794 men and 1,578 women were included in the study. The median age was 37 and 34 years (top 25th percentile [first quartile], 29 and 27, bottom 25th percentile [third quartile] 46 and 45) for men and women, respectively. The proportion of women was larger in the health checkup subjects; however, there was no significant difference in the VZV IgG levels between gender ($P = 0.1719$), therefore, eliminating gender difference.

The distribution of the VZV IgG levels in the total

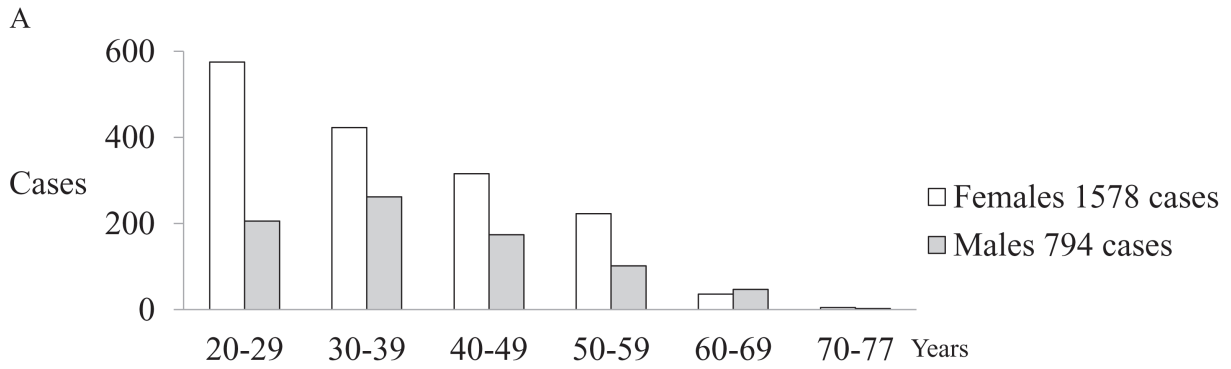


Figure 1A. Age groups and gender in the general health check

The median age was 37 and 34 years (top 25th percentile [first quartile], 29 and 27, bottom 25th percentile [third quartile] 46 and 45) for men and women, respectively.

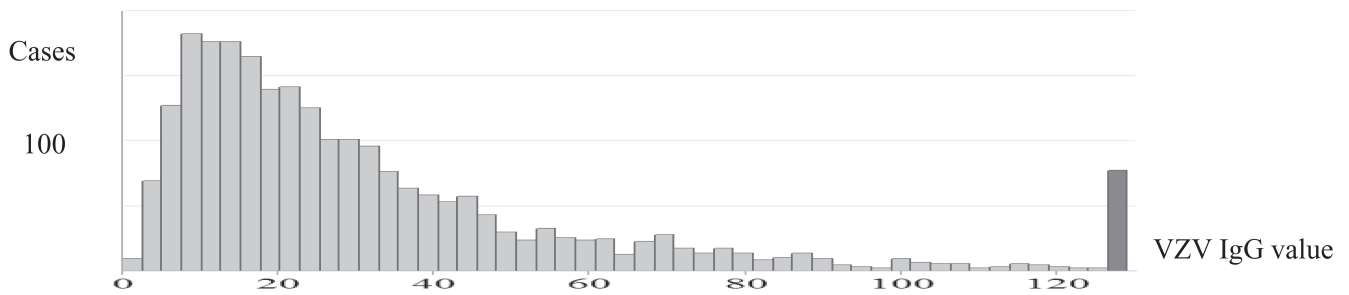


Figure 1B. VZV IgG in general health check (all ages)

The median VZV IgG level was 23.1 and the geometric average was 32.4 (SD, 28.2). The percentage of subjects with a VZV IgG level ≥ 128 was 3.2% (n = 76/2,372).

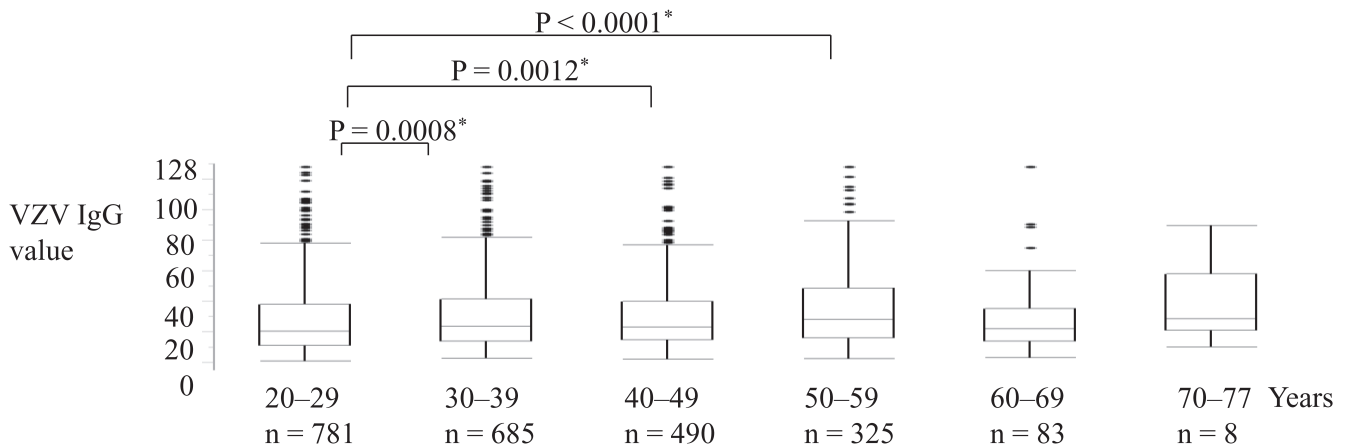


Figure 1C. VZV IgG from the general health check by age group

A statistically significant increase in the VZV IgG levels was observed among the subjects in their 20s, 30s, 40s, and 50s.

population who underwent the general health checkup is shown in Figure 1B. The median VZV IgG level was 23.1 and the geometric average was 32.4 (SD, 28.2). The percentage of subjects with a VZV IgG level ≥ 128 was 3.2% (76 of 2,372). The significance value using the Kruskal-Wallis test was $P < 0.0001$. Subsequently, we performed the Wilcoxon multiple test, and a statistically significant increase in the VZV IgG levels was observed in their 20s, 30s, 40s, and 50s (Figure 1C). The P values from the Wilcoxon multiple test are shown in Table 1. In each age group, the percentage of subjects with a VZV IgG level ≥ 128 was nearly constant at 2.4% and 4.6% for subjects in their 20s, 30s, 40s, and 50s, respectively (Figure 1C).

VZV IgG levels in patients with Ramsay Hunt syndrome
 The distributions of age and sex in patients with Ramsay Hunt syndrome are shown in Figure 2. A total of 15 men and 10 women were included in the study. The average age was 48.8 and 60.4 (SD, 16.7 and 14.3) years for men and women, respectively. The distribution of the VZV IgG levels in patients with Ramsay Hunt syndrome is shown in Figure 3. The median VZV IgG level measured within 3 days of onset was 27.0 and the geometric average

was 26.9 (SD, 33.7). The median VZV IgG level measured after 3 weeks of onset was 29.7 and the geometric average was 36.6 (SD, 42.6).

In all patients who were in their 20s – 50s, the VZV IgG levels measured within 3 days of onset were < 50 , with no age group differences observed (Figure 3). In patients aged > 60 years, the VZV IgG levels measured within 3 days of onset were relatively high and scattered. However, there was no significant difference in the values of patients aged < 59 and > 60 years ($P = 0.2888$).

Only 4 patients (16%) exhibited > 2 -fold increase in the VZV IgG levels measured after 3 weeks of onset compared with those measured within 3 days. Among these 4 patients, 2 were in their 20s, and 2 were in their 30s (Figure 3). Three of the patients with levels > 128 measured after 3 weeks showed a favorable outcome (complete recovery), whereas the other patients, whose levels changed from 0.3 to 5.9 in their 20s (Figure 3), showed a poor outcome (poor recovery). The average VZV IgG level measured after 3 weeks was 50.2 and 33.7 in patients with complete ($n = 15$) and poor outcome ($n = 10$), respectively. These values were not significantly different ($P = 0.5045$).

In several patients with Ramsay Hunt syndrome, the

Table 1. VZV IgG in general health check by age groups

Age groups	20–29	30–39	40–49	50–59	60–69	70–77
20–29		0.0008*	0.0012*	< 0.0001 *	0.4515	0.1432
30–39			0.8798	0.0120	0.3937	0.2841
40–49				0.0184	0.3506	0.2752
50–59					0.0250	0.6542
60–69						0.1853
70–77						

The Kruskal-Wallis test for more than two groups shows a P value < 0.0001 . Significant evaluation was conducted among the 20s, 30s, 40s, and 50s by the Wilcoxon multiple test. P values are according to the Wilcoxon multiple test.

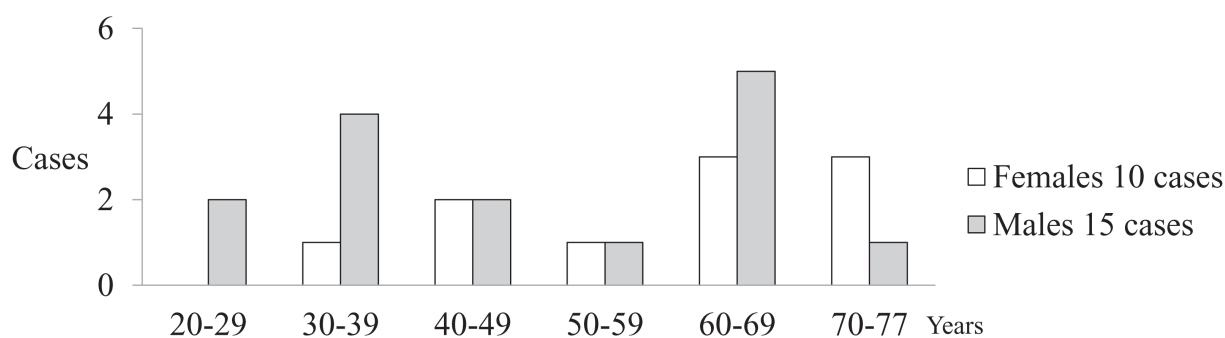


Figure 2. Age groups and gender in patients with Ramsay Hunt syndrome

The average age (\pm SD) of men was 48.7 ± 16.7 years, and that of women was 60.4 ± 14.3 years.

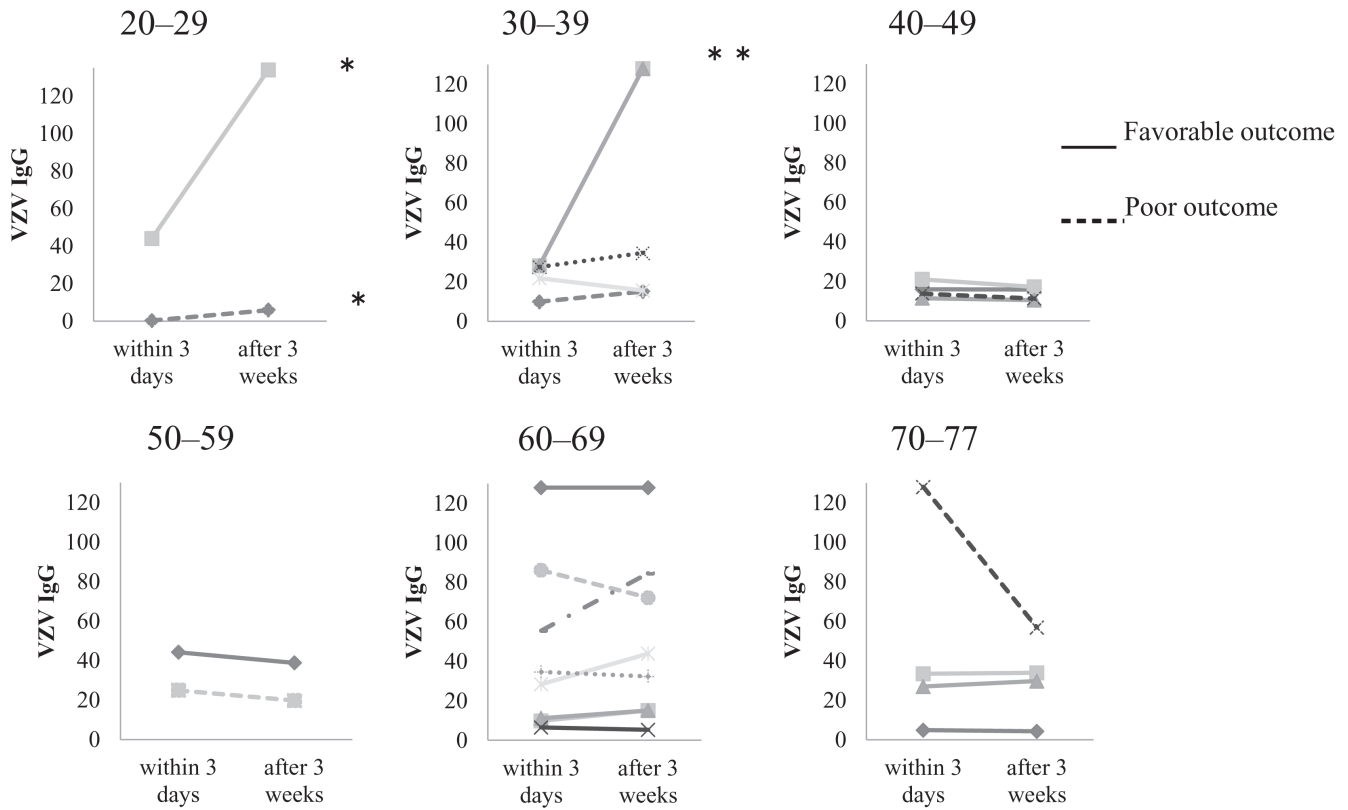
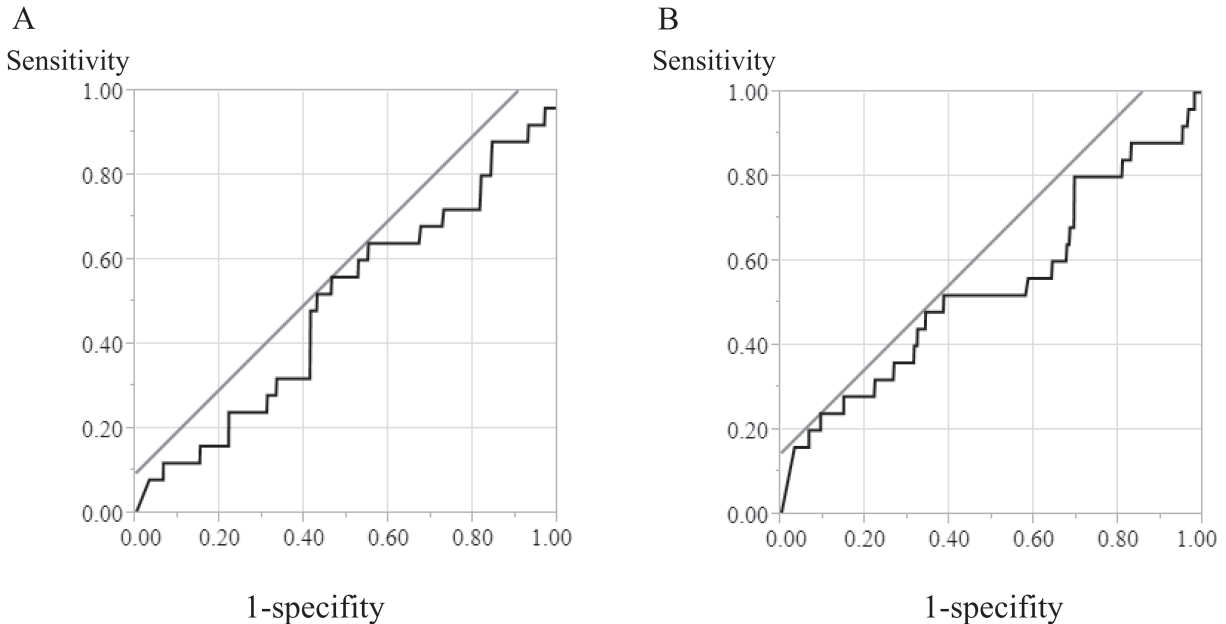


Figure 3. Change of VZV IgG in the Ramsay Hunt syndrome by age groups

The solid line indicates a favorable outcome (Yanagihara score of $\geq 36/40$), and the dashed line indicates a poor outcome (Yanagihara score of $\leq 34/40$). * >2 -fold increase in the VZV IgG levels measured after 3 weeks of onset compared with those measured within 3 days. **Patients in their 30s, 1 was 28.1/128, and 1 was 28.3/128.



A. ROC curve for Ramsay Hunt syndrome by VZV IgG within 3 days

The ROC curve was almost a straight line with a slope of 1 and the AUC of the ROC was 0.4972.

B. ROC curve for Ramsay Hunt syndrome by VZV IgG after 3 weeks

The ROC curve was almost a straight line with a slope of 1 and the AUC of the ROC was 0.5432.

Figure 4

VZV IgG levels remained unchanged after 3 weeks of onset compared with those measured within 3 days (Figure 3). There were no significant differences in the VZV IgG levels measured within 3 days and after 3 weeks among the age groups.

Comparison of VZV IgG levels between the health checkup subjects and patients with Ramsay Hunt syndrome

ROC analysis was used to assess the threshold of the VZV IgG levels for a ZSH diagnosis in the health checkup subjects and patients with Ramsay Hunt syndrome. In those patients, when the VZV IgG levels measured within 3 days of onset were considered, the ROC curve was almost a straight line with a slope of 1 and the area under the curve (AUC) of the ROC was 0.4972 (Figure 4A). If a threshold of 50 for VZV IgG levels, as is occasionally used in Japan, was considered, the sensitivity was 0.1600, and the specificity was 0.8179.

Only 16% (4/25) of the patients with Ramsay Hunt syndrome had VZV IgG levels >50 measured within 3 days, and all of these patients were aged >60 years. By contrast, the percentage of health checkup subjects with a VZV IgG level >50 was 18% (432/2,372). In the patients with Ramsay Hunt syndrome, when the VZV IgG levels measured after 3 weeks of onset were considered, the ROC curve showed a shape similar to that obtained when the levels within 3 days were considered. The AUC of the ROC was 0.5432, the sensitivity was 0.2800, and the specificity was 0.8179 at a threshold of 50 (Figure 4B). A total of 16.0% (4/25) of patients with Ramsay Hunt syndrome had VZV IgG levels \geq 128 measured after 3 weeks, and this percentage was significantly different compared with that in the health checkup subjects (3.2%, 76/2,372 subjects; $P < 0.001$).

Discussion

Distribution of VZV IgG levels by age in the health checkup subjects

In the present study, the subjects who underwent the general health checkup were comprised of university hospital and university staffs, including those from the Department of Liberal Science, the School of Medicine, the School of Nursing, the School of Allied Health Sciences, and the School of Sciences. We hypothesized that the health checkup subjects would have a similar distribution to the general population. However, because subjects who were working in the hospital were included in the study, the percentage of subjects who had come into contact with VZV-infected patients was considered

to be higher than that in the general population. Ogunjimi et al.⁶ reported that the incidence of HZ was 5.95% in pediatricians, 9.27% in dermatologists, and 10.82% in psychiatrists.⁶ Ogunjimi et al. concluded that exogenous boosting exists, although not for all persons, nor in all situations.⁶ Its magnitude is yet to be determined adequately in any study field.⁶ Ogunjimi et al.'s report, did not reveal large differences in VZV-specific antibody titers.⁶ Their results suggested that a booster effect accompanied with the elevation of VZV IgG levels cannot be induced without frequent and close contact with VZV-infected patients, as with pediatricians. Because the proportion of pediatricians and pediatric nurses who underwent the general health checkup in the present study was about 2%, it was assumed that there was not a significant difference between the health checkup subjects in the present study and the general population. An increase in the VZV IgG levels was observed for subjects in their 30s compared with those in their 20s. This may have occurred as the unaffected subjects had been vaccinated, either the subjects had their first infection after contact with chicken pox-infected patients, or the booster effect was induced accompanied with antibody production after contact with VZV-infected patients. It has been reported that the rate of normal subjects with VZV IgG positivity in their serum increases until the age of 25 years,⁶ and the data in the present study were consistent with this.

Although not statistically significant, there was a tendency for the VZV IgG levels to be lower in subjects in their 60s compared with those <60 years old. We considered the following possibilities for that result: 1. a decrease in antibody production due to age-related decline in immunity, 2. an attenuation due to the time effect of the antigen-antibody reaction with the HZ virus, and 3. multiple individual variations with circumstantial changes due to retirement and an increase of life style-related diseases. Ihara⁷ reported that the half-life of antibody production following VZV infection was 50 years. The ability of the body to produce antibodies against VZV infection induced during childhood most likely decreases with age.

The percentage of health checkup subjects with VZV IgG levels >128 was nearly constant across the age groups (2.4% and 4.6% among those in their 20s, 30s, 40s, 50s and 60s, respectively). The incidence rate of HZ ranged from 3–5/1,000 person-years in North America, Europe, and Asia-Pacific.⁸ The age-specific incidence rates of HZ were similar across countries, with a steep rise after 50 years of age.⁸ The incidence rate was about 6–8/1,000 person-years at 60 years of age and 8–12/1,000

person-years at 80 years of age.⁸ We observed that the percentage of the health checkup subjects with VZV IgG levels >128 in the present study appeared to be marginally higher than that of the accumulative rate⁸ of patients with HZ.

Investigation of VZV IgG levels in health checkup subjects and patients with Ramsay Hunt syndrome

Because Ramsay Hunt syndrome sample number was ineffectively small, it was not possible to perform multiple comparisons among age groups or gender. We statistically investigated the subjects with Ramsay Hunt syndrome and conducted ROC analysis. In patients with Ramsay Hunt syndrome, the VZV IgG levels measured within 3 days and after 3 weeks of onset did not differ significantly from those in the health checkup subjects. The ROC curve based on the values measured within 3 days was almost a straight line with a slope of 1. Therefore, it was not considered to be possible to diagnose ZSH on the basis of VZV IgG levels measured within 3 days. The ROC curve based on the values measured after 3 weeks was similar to that obtained for those measured within 3 days making it difficult to diagnose ZSH on the basis of VZV IgG levels. Therefore, making such diagnoses is inadequate resulting in the revelation that the VZV IgG levels are not useful for the diagnosis of ZSH.

Among patients with Ramsay Hunt syndrome, only 4 of 25 patients showed an increase of >2-fold in the VZV IgG levels measured within 3 days of onset compared with those measured after 3 weeks. Therefore, it can be inferred that the induction of VZV IgG production was infrequent in these patients. The reasons for these results are: 1. serum VZV IgG levels reflect the viral load in blisters, and 2. patients with Ramsay Hunt syndrome have a smaller area of blisters than do patients with trunk HZ.

Because with ZSH there is lack of blisters, the changes of serum VZV IgG levels in ZSH patients may be smaller than those in patients with Ramsay Hunt syndrome. Therefore, it must be more difficult to diagnose ZSH on the basis of VZV IgG levels.

Three of the 4 patients who had an increase of >2-fold in the serum VZV IgG levels exhibited favorable outcome and an increase of >128 in the level. Therefore, a good prognosis can be predicted when VZV IgG levels markedly increase after 3 weeks of onset. The remaining patients with a poor outcome had a low VZV IgG level

(0.3) within 3 days of onset, which may be due to insufficient vaccination.

In conclusion, ZSH can be diagnosed on the basis of VZV IgG levels only if, after 3 weeks of onset, the levels increase by ≥ 2 -fold compared with those measured within 3 days of onset. However, the sensitivity is estimated to be low; 4 of 25 cases (16%) in all age groups, 4 of 7 cases (57%) in those <40 years old, and no cases in those >40 years old. It was not considered possible to diagnose ZSH using a single VZV IgG level. We concluded that it is difficult to diagnose ZSH in patients with peripheral facial nerve paralysis using VZV IgG levels.

This study was presented at the 25th Annual Meeting of the Japanese Otological Society, on October 9, 2015.

Conflicts of Interest: None

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