Assessment of skin test with varicella-zoster virus antigen for predicting the risk of herpes zoster

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SUMMARY

The Shozu Herpes Zoster (SHEZ) Study was designed to clarify the incidence of and predictive and immunological factors for herpes zoster in a defined community-based Japanese population. As part of this series, a total of 5683 residents aged $\geqslant 50$ years received a varicella-zoster virus (VZV) skin test with VZV antigen, and 48 h later, the erythema and oedema were assessed by measuring the longest diameter. The diameters of both the erythema and oedema decreased with the increasing age of the subject. Sixty-three subjects contracted herpes zoster within a year after receiving the VZV skin test. Analysis of the herpes zoster incidence rate *vs.* the skin test reaction revealed that the shorter the diameter of erythema or oedema, the greater the likelihood of herpes zoster. These results demonstrated that the VZV skin test is an excellent surrogate marker for predicting the risk of herpes zoster.

Key words: Cell-mediated immunity, herpes zoster, skin test, surrogate marker, varicella-zoster virus.

INTRODUCTION

Herpes zoster is a painful disease characterized by papulovesicular skin lesions, mostly restricted to a single dermatome, and frequently followed by severe pain. Varicella-zoster virus (VZV) causes the disease by the reactivation of latently infected VZV in the dorsal root ganglia that had become established during the primary infection, namely varicella, in childhood. The incidence of herpes zoster and its complication, post-herpetic neuralgia (PHN), increases with age [1–3]. Herpes zoster is particularly frequent in patients with leukaemia, bone marrow and other organ transplants, and individuals with HIV infection [2]. These observations strongly suggest that an increased incidence of herpes zoster should be correlated with a decrease in VZV-specific cell-mediated immunity (VZV-CMI).

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Table 1. *General characteristics of subjects at baseline*

	Men		Women		Total	
	No. of subjects	(%)	No. of subjects	(%)	No. of subjects	(%)
Age (years)						
50–59	693	(26·9)	838	(26·9)	1531	(26·9)
60–69	942	(36·6)	1016	(32·7)	1958	(34·5)
70–79	675	(26·2)	902	(29·0)	1577	(27·7)
⩾80	263	(10·2)	354	(11·4)	617	(10·9)
Total	2573	(100)	3110	(100)	5683	(100)
Mean age (±S.D.)	66·4	(±9·2)	67·0	(±9·6)	66·7	(±9·4)
Past history of HZ						
Yes	384	(14·9)	663	(21·3)	1047	(18·4)
VZV skin test						
Erythema not measurable	76	(3·0)	80	(2·6)	156	(2·7)

HZ, Herpes zoster, VZV, varicella-zoster virus.

To determine the magnitude of VZV-CMI in an individual, several methods, including an interferon-γ enzyme-linked immunospot (ELISPOT) assay and VZV skin test have been developed. Sadaoka et al. [4] compared these two methods in healthy volunteers, and found a significant correlation between the skin test reaction and ELISPOT count. However, the skin test is more practical for assessing VZV-CMI in a large group of subjects, because it is simple and safe and requires no special skills or laboratory equipment. A skin test has been developed and improved in Japan [5–8], and its usefulness for assessing VZV-CMI in patients with varicella and herpes zoster has been reported [9–11]. Moreover, it was demonstrated using the skin test that the live varicella vaccine enhances CMI to VZV [12–14].

Recently, Oxman et al. [3] reported in a large-scale clinical trial that the live attenuated Oka/Merck VZV vaccine markedly reduced the morbidity from herpes zoster and PHN in adults aged ⩾60 years. They also showed in a substudy [15] that VZV-CMI was significantly increased in vaccine recipients. These findings prompted us to examine whether the original Oka VZV vaccine produced in Japan would be effective in preventing zoster in adults. As a first step toward this goal, we planned a large-scale prospective epidemiological study to clarify the actual features of herpes zoster in Japan. One of the features of this study was that many of the subjects received a VZV skin test to determine factors contributing to the risk of herpes zoster.

The study was conducted in Shozu County, Kagawa Prefecture; we therefore named it the Shozu Herpes Zoster (SHEZ) Study. We recruited 12 522 subjects, and had a 72·3% participation rate of the target population. The subjects were assigned to studies A, B, and C as described in our previous paper [16]. Here we report the results of study B, in which 5683 subjects received the VZV skin test. Although we are presenting the data from the first year of a 3-year project, these data provide meaningful information about the usefulness of the VZV skin test and immunological aspects of herpes zoster.

MATERIALS AND METHODS

Study design

This study was approved by the ethical committees of all the involved institutions. Between December 2008 and November 2009, a total of 5683 residents aged ⩾50 years in Shozu County, Kagawa Prefecture were enrolled in study B of the SHEZ Study, and gave informed consent to have a VZV skin test (Table 1). Of the groups classified by age, the 60–69 years age group was the largest ($n=1958$) and the ⩾80 years age group was the smallest ($n=617$). There were more women ($n=3110$) than men ($n=2573$); women comprised 54·7% of the total. After the subjects agreed to have the skin test, they completed questionnaires, which included a past history of herpes zoster. Of the 5683 subjects, 1047 (18·4%) had experienced herpes zoster in the past (Table 1). Women (21·3%) showed

a greater likelihood of developing herpes zoster than men (14·9%).

VZV skin test

The viral antigen used for the skin test was produced by Biken (Research Foundation for Microbial Diseases of Osaka University. Japan), and is commercially available in Japan. The antigen was prepared according to the method of Asano *et al.* [7]. Briefly, MRC-5 cells were inoculated with the Oka strain of VZV and incubated for 24 h at 37 °C. The infected cells were washed with Earle's solution, TCM-199 medium was added, and the cells were cultured for another 2 days. The cells and medium were then harvested together, and frozen at −60 °C until required. The frozen cells and medium were thawed, heated for 30 min at 56 °C, and centrifuged at 800 *g* for 10 min. The virus in the resultant supernatant was purified by concentration with a UF membrane, ultra-centrifugation at 45 000 *g* for 2 h, followed by filtration with a 0·22 μm filter.

To maintain technical consistency, the same three nurses performed the skin test throughout the study. They injected 0·1 ml of the skin test antigen intradermally into the forearm of each subject using disposable tuberculin syringes. In addition, certain Biken staff members received information and training from a dermatologist on how to evaluate the skin's reaction. In particular, the diameter of the oedema was precisely measured by touching the reaction site with the forefinger. Forty-eight hours after the injection, the longest and shortest diameters of both the erythema and oedema were measured using digital vernier calipers which indicate values to the second decimal place. Although oedema diameter could be measured for all subjects, some developed purpura at the injection site, which made it impossible to measure the diameter of the erythema. Therefore, we excluded 156 subjects from the erythema data (Table 1).

Survey of patients with herpes zoster

As reported in our previous paper [16], all subjects that received an initial clinical diagnosis of herpes zoster or possible herpes zoster were confirmed by PCR test. We collected samples from the patients' lesions, and transported them immediately to the surveillance centre at Kanonji Institute. In this study, 63 subjects were confirmed as having herpes zoster within a year after registration, and were analysed with respect to the results of the skin test.

DNA extraction and real-time PCR

The total DNA was extracted from the vesicular fluid or crust using a DNeasy Blood and Tissue kit (Qiagen, USA) in a volume of 200 μl, and a portion was used as a template for the real-time PCR assay.

To detect VZV DNA, the reaction mixtures contained distilled water (7·5 μl), 2*TaqMan Gene Expression Master Mix (25·0 μl), 10 μM forward primer (gene 62B-F; 5′-TCGGCCAGCGGGATTAC-3′; 108811-108827) (2·5 μl), 18 μM reverse primer (gene 62B-R; 5′-TTGGCGAAGAGCTAACACA AGA-3′; 108892-108871) (2·5 μl), 5 μM TaqMan MGB probe (gene 62B-T; 5′-FAM-TCCTCGCA TACGTAGTC-3′-MGB; 108853-108869), and 10 μl of template DNA. The real-time PCR reaction conditions were 1 cycle at 50 °C for 2 min, 1 cycle at 95 °C for 10 min, and 40 cycles at 95 °C for 15 s and at 60 °C for 1 min. In addition, we analysed the samples for human herpes virus type 1 (HSV-1) and type 2 (HSV-2): forward primer (HSV_gB-F; 5′-GGCATCG CGGTGGTCTT-3′; 980-996), reverse primer (HSV_ gB-R; 5′-TTGTAGTACATGGTGGCCTTGAA-3′; 1044-1022), TaqMan MGB probe (HSV_gB-T; 5′-FAM-AAGGAGAACATCGCCCCG-3′-MGB; 998-1015).

Statistical analysis

To adjust for confounding variables for sex, age group, and past history of herpes zoster, we applied ANCOVA to the statistical analysis of the skin test reaction.

RESULTS

Overall results of the VZV skin test

We enrolled 5683 subjects in study B of the SHEZ Study, the subjects received a VZV skin test at the time of registration (Table 1). Although the mean age of the men and women was almost the same, there were more women than men in all of the age groups. Forty-eight hours after intradermal injection with the VZV skin test antigen, the skin reaction at the injection site was inspected. We measured the diameter of the erythema and oedema in each individual, and analysed the distribution pattern of the longest
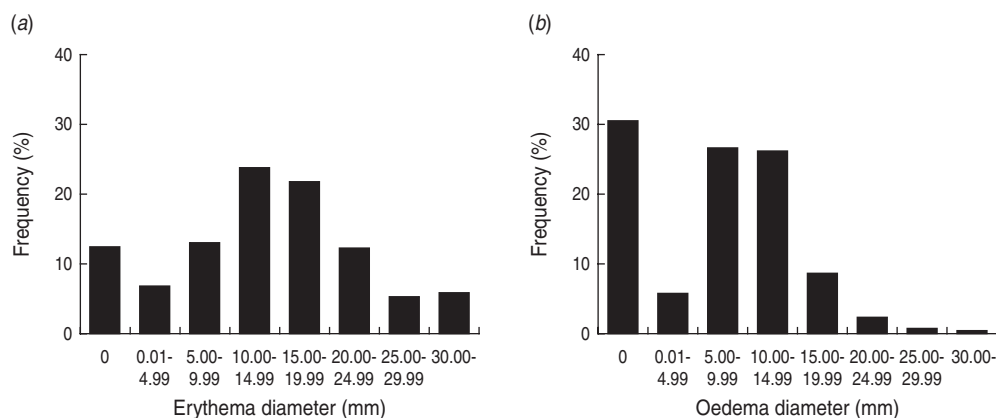
Fig. 1. Frequency distribution of diameters of (*a*) erythema and (*b*) oedema in subjects by the VZV skin test. Although all subjects (*n*=5683) were measured for oedema, some developed purpura at the injection site which resulted in slightly fewer subjects (*n*=5527) being measured for erythema.
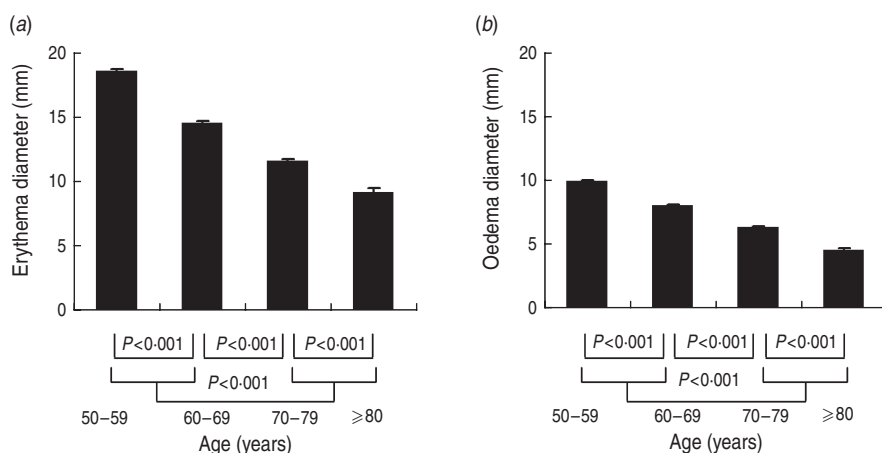

Fig. 2. Age-specific means and standard errors of diameters of (*a*) erythema and (*b*) oedema by the VZV skin test, after adjustment for sex and past history of herpes zoster by ANCOVA. All subjects were divided into four age groups and analysed statistically. *P* values for differences berween age groups are shown below the graphs.

diameter (Fig. 1). For erythema, the 10- to 14-mm group was the largest (23·6% of subjects), and 68·2% of subjects showed a diameter of >10 mm. For oedema, the 0-mm group was the largest (30·4% of subjects), and 37·6% of subjects showed a diameter of >10 mm.

VZV skin test reaction differentiated by sex and age

We examined the difference of the skin test reaction categorized by sex after adjusting for age and past history of herpes zoster. The mean of the longest diameter for erythema was 13·97 mm in men and 14·46 mm in women; statistical analysis revealed that the difference between them was small ($P=0.050$). A similar small difference between men and women was found for oedema (mean diameter 7·78 mm for men and 7·45 mm for women; $P=0.050$).

We then divided the subjects into four age groups and compared the skin reaction in these groups (Fig. 2). The longest diameter for both erythema and oedema clearly decreased with increasing age. Statistical analysis showed that this trend was highly significant. Table 2 shows the frequency distribution of the diameters of erythema and oedema in each age group. In the 50–59 years age group, the proportion of subjects with a diameter of >5 mm was 90·7% for erythema and 79·2% for oedema. These percentages decreased with increasing age, and in the oldest age group (≥80 years), the proportion was 62·6% and 40·7% for erythema and oedema, respectively.

Table 2. *Age-specific frequency distribution of of erythema and oedema diameters by the VZV skin test*

	Frequency (%)				
	0	0·01–4·99	5·00–9·99	⩾10·00	Total
Erythema diameter (mm)					
Age (years)					
50–59	5·4	3·9	8·2	82·5	100·0
60–69	10·2	5·7	13·3	70·8	100·0
70–79	17·1	8·7	16·3	57·9	100·0
⩾80	25·2	12·2	14·7	47·9	100·0
Oedema diameter (mm)					
Age (years)					
50–59	17·2	3·5	26·8	52·4	100·0
60–69	27·6	5·6	27·1	39·7	100·0
70–79	37·8	7·4	26·7	28·1	100·0
⩾80	53·0	6·3	22·4	18·3	100·0

VZV, Varicella-zoster virus.

VZV skin test reaction differentiated by a past history of herpes zoster

Of the subjects who received a VZV skin test, we determined that 1047 (18·4%) had developed herpes zoster in the past, based on their answers to the questionnaires (Table 1). The diameters of erythema and oedema were analysed according to whether the subject had a past history of herpes zoster, after adjusting for age and sex (Fig. 3). Although the mean diameter of the erythema was not greatly different between subjects with (15·76 mm) and without (13·90 mm) a past history of herpes zoster, the difference was significant. The mean diameter of oedema also showed a small but significant difference between subjects with (8·53 mm) and without (7·39 mm) a past history of herpes zoster. These results indicate that previous development of herpes zoster induces a prolonged VZV-CMI.

VZV skin test reaction differentiated by subjects with or without herpes zoster

By PCR and serological tests, 63 (1·09%) of the 5683 subjects were confirmed as having contracted herpes zoster within 1 year following registration. Of these individuals, 22 were men (22/2573, 0·86%) and 40 were women (40/3110, 1·29%). We then analysed their skin test reactions compared to those of the subjects without herpes zoster. The diameters of erythema and oedema in subjects with herpes zoster

(mean values 7·23 mm and 3·07 mm for erythema and oedema, respectively) were considerably shorter than those in subjects without herpes zoster (mean values 14·32 mm and 7·65 mm for erythema and oedema, respectively) (Fig. 4).

We next investigated the relationship between the individual skin test data and the development of herpes zoster in more detail (Table 3). When 5 mm was adopted as the cut-off value for the erythema diameter, 1047 of the total subjects with erythema diameter measuring < 5 mm were classified as negative, and 4480 with a diameter measuring ⩾ 5 mm were classified as positive. The incidence rate of herpes zoster in the negative and positive groups was 2·77% (29/1047) and 0·69% (31/4480), respectively. When these figures were adjusted for age, sex, and past history of herpes zoster, the relative risk (odds ratio) of herpes zoster was calculated as 0·23 (pattern A of erythema). The same analysis for other patterns is shown in Table 3. When we added a cut-off value of ⩾ 5 mm to < 10 mm as weakly positive, the odds ratio between the < 5-mm group and weakly positive group was not confident [pattern C of erythema, 95% confidence interval (CI) 0·32-1·22]. However, if the weakly positive group was adopted as a reference, the odds ratio indicated a higher reliability between the weakly positive and positive (⩾ 10 mm) groups (pattern D of erythema, 95% CI 0·12-0·50). The smallest odds ratios were found between < 5 mm and ⩾ 10 mm (0·15 for pattern C of erythema and 0·10 for pattern D of oedema). These results suggest that the skin test reaction caused by the VZV antigen is a promising indicator for predicting the development of herpes zoster.

DISCUSSION

We utilized the VZV skin test to investigate the magnitude of VZV-CMI in individuals, because the test has become established and is actively used in Japan, and because it enables the practical collection of large-scale epidemiological data for VZV-CMI. In fact, we were able to enrol a large number of subjects who agreed to have the skin test and the results from these individuals provided us with a great deal of information about VZV-CMI.

Although differences in the diameters of erythema and oedema between men and women and between subjects with and without a past history of herpes zoster were not so large, the differences between age groups were observed to be highly significant.
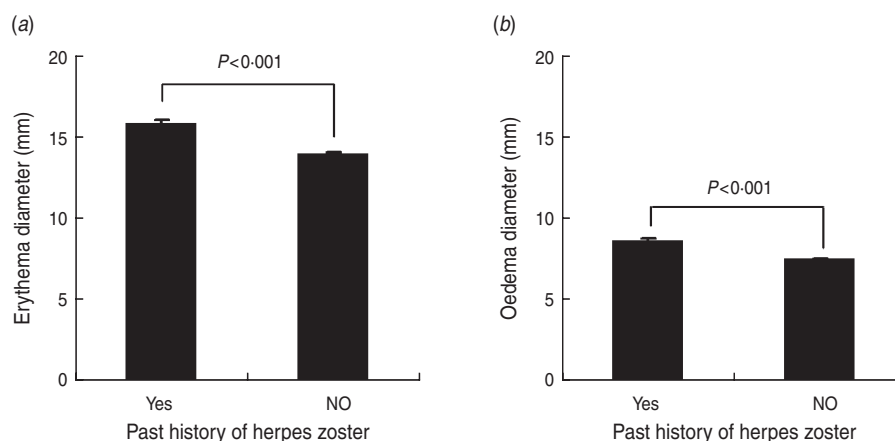
Fig. 3. Means and standard errors of diameters of (*a*) erythema and (*b*) oedema by the VZV skin test in subjects with and without a past history of herpes zoster, after adjustment for age and sex by ANCOVA. Subjects who experienced herpes zoster in the past are designated as 'Yes' and those that did not as 'No'.
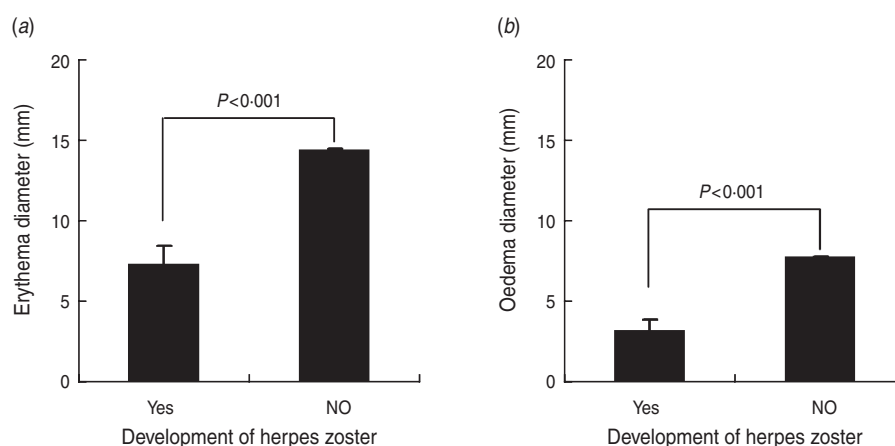

Fig. 4. Means and standard errors of diameters of (*a*) erythema and (*b*) oedema by the VZV skin test in subjects with and without a past history of herpes zoster within a year after registration. Subjects were adjusted for age, sex and past history of herpes zoster by ANCOVA. Subjects who developed herpes zoster within a year after registration are designated as 'Yes' those that did not as 'No'.

Previous studies have shown the incidence of herpes zoster increases with age, in connection with a decline in VZV-CMI [2, 15, 17–20], which is in agreement with the findings of the present study (Fig. 2, Table 2). Several reports have demonstrated an age-related decline in VZV-CMI using the VZV skin test [14, 18]. These results agree well with our data, suggesting that the skin test is reliable for assessing VZV-CMI in an individual of any age.

Our conclusions about the predictive usefulness of the VZV skin test are based on the data presented in Figure 4 and Table 3. Large differences in the diameters of erythema and oedema between subjects with and without later herpes zoster were found (Fig. 4). Considering the subjects developed herpes zoster within a year after the skin test, the weak skin test reaction appears to reflect the actual state of VZV-CMI in patients with herpes zoster. The above-mentioned results were elucidated by the incidence rates of herpes zoster (Table 3). The small odds ratios for positive diagnoses suggest that VZV-CMI plays an important role in preventing the development of herpes zoster. Moreover, the results indicate that the VZV skin test is an excellent surrogate marker for predicting the risk of herpes zoster.

The development of herpes zoster must stimulate the VZV-CMI of subjects and this stimulation appears to be demonstrated by a post-zoster skin test. However, our study design restricted us to applying the skin test after development of herpes zoster.

Table 3. *Risk of herpes zoster by VZV skin test diagnosis, after adjustment for age, sex, and past history of herpes zoster*

Cut-off value	Diagnosis	No. at risk	No. of cases	Incidence rate (%)	OR	95% CI
Erythema diameter						
Pattern A						
<5 mm	Negative	1047	29	2·77	1	(Reference)
≥5 mm	Positive	4480	31	0·69	0·23	0·14–0·39
Pattern B						
<10 mm	Negative	1757	42	2·39	1	(Reference)
≥10 mm	Positive	3770	18	0·48	0·18	0·10–0·32
Pattern C						
<5 mm	Negative	1047	29	2·77	1	(Reference)
≥5 mm to <10 mm	Weakly positive	710	13	1·83	0·63	0·32–1·22
≥10 mm	Positive	3770	18	0·48	0·15	0·08–0·28
Pattern D						
≥5 mm to <10 mm	Weakly positive	710	13	1·83	1	(Reference)
≥10 mm	Positive	3770	18	0·48	0·24	0·12–0·50
Oedema diameter						
Pattern A						
0 mm	Negative	1728	40	2·31	1	(Reference)
>0 mm	Positive	3955	23	0·58	0·24	0·14–0·42
Pattern B						
<5 mm	Negative	2048	45	2·20	1	(Reference)
≥5 mm	Positive	3635	18	0·50	0·22	0·12–0·38
Pattern C						
<10 mm	Negative	3548	58	1·63	1	(Reference)
≥10 mm	Positive	2135	5	0·23	0·14	0·06–0·36
Pattern D						
<5 mm	Negative	2048	45	2·20	1	(Reference)
≥5 mm to <10 mm	Weakly positive	1500	13	0·87	0·38	0·20–0·72
≥10 mm	Positive	2135	5	0·23	0·10	0·04–0·26

VZV, Varicella-zoster virus; OR, odds ratio; CI, confidence interval.

Therefore, to compensate for the shortcoming in this part in the SHEZ Study, we administered the skin test to a number of zoster patients who visited private clinics of dermatologists in another study. These analytical data together with those of the SHEZ Study provide useful knowledge about enhancement of VZV-CMI in patients with herpes zoster.

Several reports have shown that live varicella vaccine enhances VZV-CMI in the elderly [13–15, 20–23]. However, only a few reports have used the VZV skin test to evaluate the vaccine's ability to enhance an individual's immunity against VZV [13, 14, 24]. Takahashi *et al.* [14] demonstrated that in 42 subjects whose longest erythema diameter was <5 mm, 28 (66·7%) showed a diameter of >10 mm after immunization with live varicella vaccine (Oka strain, Biken). In connection with the lower frequency of herpes zoster in subjects who have a longer erythema diameter, as demonstrated in the SHEZ Study, Takahashi *et al.*'s report [14] might suggest that the varicella vaccine is effective for preventing herpes zoster. Currently, the only licensed zoster vaccine is Zostavax™ (Merck, USA) which was derived from Oka strain (Biken). Moreover, newly developed zoster vaccines are likely to be licensed in the near future. Under those circumstances, the skin test will be a helpful tool to estimate efficacy of the vaccines and to evaluate the duration of zoster vaccine-induced VZV-CMI.

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DECLARTION OF INTEREST

None.

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