

The positive duration of varicella zoster immunoglobulin M antibody test in herpes zoster

Seong-Won Min, MD^a, Yung Suk Kim, MD^b, Francis Sahngun Nahm, MD^{b,*}, Da Hye Yoo, MD^b, Eunjoo Choi, MD^b, Pyung-Bok Lee, MD^b, Hyunjung Choo, MD^c, Zee-Yong Park, PhD^d, Catherine Seonghee Yang, PharmD^e

Abstract

Laboratory tests for herpes zoster (HZ) are required to confirm varicella zoster virus (VZV) infection, especially when a skin lesion is not typical or apparent. The serological test for VZV IgM antibody is simple and cost-effective; however, the change in the VZV IgM-positive rate over the time course of the disease has not been investigated. Therefore, we conducted an observational study to evaluate the positive rate of VZV IgM results during the time course of HZ and estimate the VZV IgM-positive period.

After obtaining serum from patients with typical HZ, the VZV IgM titer was examined using enzyme-linked immunosorbent assay methods. After logarithmic transformation of the VZV IgM titer and the period after the onset of HZ, regression analysis was performed with the 2 transformed variables.

A total of 62 patients were included in this study, and VZV IgM antibody was positive only in 23 patients (37%). The estimated antibody-positive period after HZ onset was 3.5 weeks (95% confidence interval 2.8–4.6 weeks).

These findings suggest that the serological diagnosis of VZV IgM to confirm HZ is only useful within 3.5 weeks after the onset of symptoms.

Abbreviations: HZ = herpes zoster, IgG = immunoglobulin G, IgM = immunoglobulin M, PCR = polymerase chain reaction, VZV = varicella zoster virus, ZSH = zoster sine herpette.

Keywords: enzyme-linked immunosorbent assay, herpes zoster, immunoglobulin M, postherpetic neuralgia, serological test, zoster sine herpette

1. Introduction

Herpes zoster (HZ) is a viral disease that causes vesicular rash, pain, and/or paresthesia according to the dermatome that is affected. HZ is caused by reactivation of the varicella zoster virus (VZV), which can remain latent in the dorsal root ganglia and cranial nerve ganglia following the initial infection.^[1] Reactivation leads to proliferation of the virus in the sensory ganglia, followed by the infection spreading along the nerves to the skin,

resulting in a vesicular rash and pain. The incidence of HZ increases in old age, during human immunodeficiency virus infection, during steroid treatment, and after receiving immunosuppression treatment for an organ transplant or cancer.^[2] In rare cases, no lesions are formed on the skin, a condition known as zoster sine herpette (ZSH).^[3]

Diagnosis of HZ based on the characteristic rash and vesicles that appear before and after the manifestation of neuropathy symptoms is relatively accurate. However, when there are atypical skin lesions, especially those occurring in the mouth or genitals of immunosuppressed patients, differential diagnosis is required.^[4] In addition, there are cases of ZSH that need to be differentiated in order to determine whether the dermatomal pain is from VZV infection.^[5] Laboratory tests for differential diagnosis consist of serological testing and extracting the virus from the affected tissue through tissue culture or molecular biology.^[6]

After VZV infection, IgG and IgM antibodies appear 2 to 5 days after the rash and show the highest titers at 2 to 3 weeks. The VZV IgM antibody levels then rapidly decrease and cannot be detected at 1 year after infection, and the IgG antibody levels gradually decrease, showing positive test results for several years.^[7–9] However, these reports of titer changes in VZV antibodies are usually based on patients with a primary infection of VZV; consequently, there have been no reports that have specifically examined the titer changes in HZ patients. The serological testing of VZV-specific antibodies has been widely used in the diagnosis of both acute and latent infections of HZ.^[10] Detecting VZV IgM antibody by enzyme-linked immunosorbent assay (ELISA) is simple and has a high sensitivity and specificity compared to the traditional method of directly separating the

Editor: Kazuo Hanaoka.

The authors have no conflict of interest to declare.

^a Department of Anesthesiology and Pain Medicine, Seoul Metropolitan Government Seoul National University Boramae Hospital, Seoul National University College of Medicine, Seoul, Korea, ^b Department of Anesthesiology and Pain Medicine, Seoul National University Bundang Hospital, Seongnam, ^c Department of Anesthesiology and Pain Medicine, Seoul National University Hospital, Seoul, ^d School of Life Science, Gwangju Institute of Science and Technology, Gwangju, ^e Department of Bioinformatics and Statistics, Korea National Open University Graduate School, Seoul, Korea.

* Correspondence: Francis Sahngun Nahm, Associate Professor, Department of Anesthesiology and Pain Medicine, Seoul National University Bundang Hospital, Gumi-dong 300, Bundang-gu, Seongnam-si 463707, Korea (e-mail: hiitsme@hanmail.net).

Copyright © 2016 the Author(s). Published by Wolters Kluwer Health, Inc. All rights reserved.

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially.

Medicine (2016) 95:33(e4616)

Received: 6 July 2016 / Received in final form: 23 July 2016 / Accepted: 26 July 2016

http://dx.doi.org/10.1097/MD.0000000000004616

virus from the skin lesion. Furthermore, ELISA is known to be a more economical method with respect to cost and use of human resources in the laboratory.^[11] The ELISA method for detecting VZV IgM is especially useful during the diagnosis of a central nervous system infection, which requires the early administration of antiviral agents and is difficult to differentiate from other diseases when ZSH is suspected because it lacks the typical rashes usually found in HZ.^[11,11] However, there have been no clear reports of the positive rate of VZV IgM antibodies over the time course of HZ. Consequently, there are limits to the diagnosis of HZ through serological testing because it is difficult to differentiate whether negative results are because of a long time interval since the initial onset or to a non-HZ infection.

Therefore, we conducted an observational study to evaluate the positive rate of VZV IgM results over the time course after the onset of HZ and estimate the VZV IgM-positive period.

2. Methods

2.1. Study participants

This observational study was approved by the institutional review board of our hospital (IRB no. B-1311/228-107). Analysis was performed from August 2010 to July 2013 on the patients who visited the pain center of our hospital. The inclusion criteria were: diagnosis with HZ owing to the typical dermatomal pain after rash and vesicle formation and receiving antiviral medications. The disease period after the onset of skin lesions in each patient was investigated, and serum from the venous blood of the patients was collected for the viral serological test.

2.2. Detection of VZV IgM by ELISA

The Platelia VZV IgM kit (Bio-Rad Laboratories, Inc., Hercules, CA), which uses an ELISA principle, was used to measure the titer of the plasma VZV IgM according to the guidelines of the manufacturer. An aliquot of serum diluted to a ratio of 1:101 in a volume of 100 μ L was placed in a well coated with anti-human IgM monoclonal antibodies, then incubated for 45 minutes at 37°C. Afterward, it was washed 4 times with phosphate-buffered saline for 30 seconds. Then, 100 μ L of antigen-labeled monoclonal antibodies was added and the mixture was incubated again for 45 minutes at 37°C, followed by 4 washes with phosphate-buffered saline for 30 seconds each time. Finally, 100 μ L of the substrate was added to the well and then incubated for 15 minutes at room temperature (24°C \pm 1°C). Within 30 minutes of adding 100 μ L of stop solution, the absorbance was measured using an Evolis ELISA analysis instrument (Bio-Rad Laboratories, Inc., CA). For every test, negative and positive reference materials were compared with each sample and used to calculate the absorbance ratio. When the absorbance ratio was ≥ 1.0 compared to the positive reference, the result was determined to be positive. When the absorbance ratio was below the positive reference (i.e., the absorbance ratio was < 1.0), it was determined to be negative.

2.3. Statistical analysis

The free statistical software program “R” version 2.15.0 (The R Foundation for Statistical Computing, Vienna, Austria) was used for statistical analysis. Before regression analysis, the normality of the VZV IgM titer (X variable) and the period after the onset of HZ (Y variable) were tested using the Shapiro–Wilks test, and if

Table 1

Patient demographics.

Parameter	Data
Sex (M/F)	29/33 (total 62 patients)
Age, y	63 \pm 17.8 years
Period since rash, wk	Range 0–40 (median 9.2)
Affected PHN sites	
Trigeminal and other cranial, n (%)	15 (24.2)
Cervical, n (%)	6 (9.7)
Thoracic, n (%)	24 (38.7)
Lumbosacral, n (%)	17 (27.4)

Data are presented as the mean \pm standard deviation. PHN=postherpetic neuralgia.

the assumptions of normality were not satisfied, these variables were transformed logarithmically. After logarithmic transformation of the X and Y variables, regression analysis was performed between these variables. Then, the estimated antibody-positive period ($X \geq 1.0$) was calculated from the regression equation model. *P* values < 0.05 were considered statistically significant.

3. Results

The research was conducted on a group of 62 patients, and the time taken from a skin lesion outbreak to serological testing ranged from 2 days to 40 weeks. The sex, age, time from skin lesion outbreak to serological testing, and area of affected skin of the patients are shown in Table 1. Of the 62 patients, the VZV IgM antibody appeared positive in 23 patients (37%) and negative in 39 patients (63%). The VZV IgM titer started to increase after the skin lesions appeared, showing maximum levels at 6 to 10 days following the rash, and all patients showed negative results after 10 weeks (Fig. 1). The positive rate over the time course indicated that 83% of the patients were positive when tested within 1 week (5/6); 73% were positive when tested between 1 to 2 weeks (11/15); then the rate decreased to 36% when tested between 2 to 4 weeks (4/11), and the positive rate was 0% when tested after 10 weeks (Fig. 2).

The achieved regression equation model after logarithmic transformation was ($\text{Log [Y]} = 1.26652 - 48116 \times \text{Log [X]}$) ($R^2 = 0.382$, $P < 0.001$). The estimated positive duration calculated

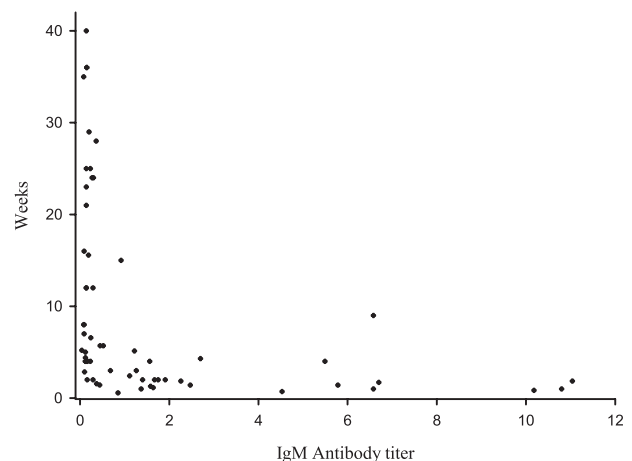


Figure 1. Scatter plot of VZV IgM antibody levels against the duration of rash according to serological test. VZV=Varicella zoster virus.

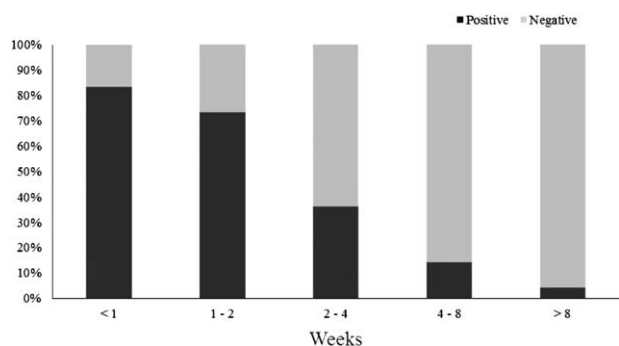


Figure 2. VZV IgM-positive ratio according to the duration of rash. VZV=Varicella zoster virus.

from this equation was 3.5 weeks (95% confidence interval 2.8–4.6 weeks, Fig. 3).

4. Discussion

HZ is relatively easy to diagnose clinically because of its characteristic painful skin blisters, rash, and dermatomal pattern. The period from the start of pain to the occurrence of lesions has been reported to range from 7 days to >100 days, and symptoms similar to the common cold, such as fatigue and headache, can appear a few days before the lesions occur.^[12] However, there may be a need for differential diagnosis of HZ from other skin diseases, such as zosteriform herpes simplex, eczema herpeticum, and vesicular enterovirus eruption. Furthermore, clinical diagnosis can be difficult when an atypical manifestation of HZ occurs, such as one with no skin lesions, no pain in the case of a previous vaccination, or a reduction in the diseased area of skin.^[3,13] Cases of HZ that do not show the characteristic skin lesions are called ZSH and have been estimated to have a prevalence of approximately 0.2%; however, there have been no attempts to thoroughly investigate the prevalence of ZSH.^[14]

There are various laboratory tests for the diagnosis of HZ, such as the Tzanck smear test, virus culture, serological methods, immunofluorescence methods, and polymerase chain reaction (PCR). These test methods have various strengths and weaknesses in terms of their sensitivity, specificity, convenience, reproducibility, required time, and cost.^[15] Morphological studies, such as the Tzanck smear test, are simple methods in which a diagnosis can be made by collecting a specimen from the blisters in the skin lesion, allowing fast verification of HZ for skilled examiners. However, such tests cannot be used when there are no skin lesions, and differential diagnosis from other viruses, such as herpes simplex virus, is impossible.^[6,16] PCR is a method that can be used to detect VZV DNA in blister fluid, blood, and cerebrospinal fluid; this method allows fast testing with very high sensitivity. It is especially helpful when there is a suspicion of ZSH or a central nervous system infection, which requires the early administration of antiviral agents.^[6] However, the accuracy of the PCR method for detecting ZSH remains controversial; it has drawbacks, for example, when analyzing blister fluid, it is difficult to manipulate the test material, and this method cannot be used when blisters are not present. PCR using blood is also limited by the fact that VZV DNA can only be discovered during the first few days of symptom manifestation. In addition, this method is difficult to apply generally to patients suspected of having HZ in terms of its cost and patient compliance.^[17]

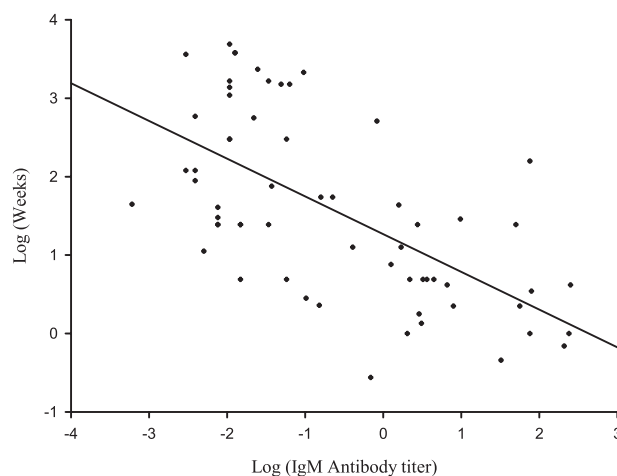


Figure 3. Regression analysis of the logarithmically transformed VZV IgM titer versus the duration of disease. The estimated duration during which patients would test positive for VZV IgM antibody was 3.5 weeks (95% confidence interval 2.8–4.6 weeks). VZV=Varicella zoster virus.

The antibody serological test for HZ virus consists of detecting the IgG and IgM antibodies for VZV, and it is used to differentially diagnose primary infection or recurrence of VZV, or to check the immune state.^[18] The IgM antibody test, which reveals acute VZV infection, has a low sensitivity, cross-reacts with other herpes viruses, and has a high false-negative error rate when the titer of IgG antibody is high.^[6,15] Although the IgM antibody test is accepted as an early diagnosis method for many other viral infections, the change in IgM titer in VZV infection does not currently offer significant diagnostic value in many cases. This is because the change in IgM titer is yet to be clearly defined, although there have been some reports that the titer appears 2 to 5 days after the lesions and shows the highest value after 2 to 3 weeks, rapidly decreasing to the point of being undetectable after 1 year.^[7,8,19] Despite this issue, it can be a “diagnostic test of choice” when there are no skin lesions, as it offers fast test results and is less invasive and has lower cost than other methods. For these reasons, it remains useful, compared to methods that separate the virus from blisters.^[8,11]

In our study, the positive rate of VZV IgM was lower than expected at 37%. There have been a few studies on the positive rate of serological tests in patients with HZ. According to research by Sundqvist et al^[7] that investigated the frequency and specificity of the VZV IgM response using ELISA, 21 of 25 patients were shown to be positive, which is equivalent to a positive rate of 84%. Dobec et al^[17] reported a positive rate of 47% for the detection of VZV IgM in HZ in a study to investigate the sensitivities of various laboratory approaches for the diagnosis of HZ in 53 patients with typical morphology and distribution of the skin lesions. The positive rate of VZV IgM antibody in HZ patients has been reported to range from 10% to 70% according to the test method, test period, and clinical symptoms of the patients.^[6,20,21]

Early diagnosis using the VZV IgM antibody test is not usually possible because it takes 2 to 5 days to form antibodies after the lesions appear.^[6] Even in our research, 1 of the 6 patients who received the test within 1 week of a skin lesion presented negative results. The patient who presented negative results was tested 4 days after the appearance of a skin lesion.

It is known that the disease period of postherpetic neuralgia before treatment is diverse, ranging from 0 to 312 months, and the mean disease period is known to be close to 1 month.^[14] Therefore, in patients exhibiting neural segmental pain without a clear etiology and no history of surgery or injury, along with suspected HZ and pain for approximately 1 month, the VZV IgM antibody test can be useful in identifying sources of unexplainable pain such as ZSH. The results of our study showed an estimated positive duration of 3.5 weeks for this test.

The limitation of this research is that HZ is caused by reactivation of VZV; thus, to make an accurate serological diagnosis, the IgG antibody should be measured twice during the acute period and the recovery period to observe the progress of the titer, but our study only measured the IgM antibody titer once. However, an IgM-positive result always signifies a recent infection; therefore, it may be considered that an IgM-positive result reflects a VZV infection. In addition, no comparison was performed between the VZV IgM titer and the clinical symptoms of the patients. Hence, it is thought that further research is necessary to evaluate the correlation between the degree of skin lesion and severity of pain with the titer. Our study included 1 patient who showed the characteristic skin lesion and was clearly presumed to be HZ, but was VZV IgM-negative in the serological test; therefore, there is need for a comparative study that includes other accurate test methods besides PCR.

In conclusion, when HZ is suspected, a VZV IgM test within 3.5 weeks of onset can be helpful; however, the diagnostic value of the test decreases after this period. Therefore, for a patient with suspected VZV infection who suffers nerve segmental pain for unknown reasons, a serological test for VZV IgM-specific antibody performed once within 3.5 weeks of pain onset would be easy, simple, and relatively cheap. This serological ELISA test can ultimately be clinically useful for making a differential diagnosis of VZV from other causes of nerve segmental pain.

References

- [1] Cohen JL. Clinical practice: Herpes zoster. *N Engl J Med* 2013;369:255–63.
- [2] Kim KH. Herpes zoster vaccination. *Korean J Pain* 2013;26:242–8.

- [3] Blumenthal DT, Shacham-Shmueli E, Bokstein F, et al. Zoster sine herpette: virologic verification by detection of anti-VZV IgG antibody in CSF. *Neurology* 2011;76:484–5.
- [4] Weinberg JM. Herpes zoster: epidemiology, natural history, and common complications. *J Am Acad Dermatol* 2007;57:S130–135.
- [5] Whitley RJ. A 70-year-old woman with shingles: review of herpes zoster. *JAMA* 2009;302:73–80.
- [6] Sauerbrei A, Eichhorn U, Schacke M, et al. Laboratory diagnosis of herpes zoster. *J Clin Virol* 1999;14:31–6.
- [7] Sundqvist VA. Frequency and specificity of varicella zoster virus IgM response. *J Virol Methods* 1982;5:219–27.
- [8] Kangro HO, Ward A, Argent S, et al. Detection of specific IgM in varicella and herpes zoster by antibody-capture radioimmunoassay. *Epidemiol Infect* 1988;101:187–95.
- [9] van Loon AM, van der Logt JT, Heessen FW, et al. Antibody-capture enzyme-linked immunosorbent assays that use enzyme-labelled antigen for detection of virus-specific immunoglobulin M, A and G in patients with varicella or herpes zoster. *Epidemiol Infect* 1992;108:165–74.
- [10] Sauerbrei A, Farber I, Brandstadt A, et al. Immunofluorescence test for sensitive detection of varicella-zoster virus-specific IgG: an alternative to fluorescent antibody to membrane antigen test. *J Virol Methods* 2004;119:25–30.
- [11] Oladepo DK, Klapper PE, Percival D, et al. Serological diagnosis of varicella-zoster virus in sera with antibody-capture enzyme-linked immunosorbent assay of IgM. *J Virol Methods* 2000;84:169–73.
- [12] Gildea DH, Dueland AN, Cohrs R, et al. Preherpetic neuralgia. *Neurology* 1991;41:1215–8.
- [13] Oxman MN, Levin MJ, Johnson GR, et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. *N Engl J Medicine* 2005;352:2271–84.
- [14] Nahm FS, Kim SH, Kim HS, et al. Survey on the treatment of postherpetic neuralgia in Korea; multicenter study of 1,414 patients. *Korean J Pain* 2013;26:21–6.
- [15] Cohen PR. Tests for detecting herpes simplex virus and varicella-zoster virus infections. *Dermatol Clin* 1994;12:51–68.
- [16] Kang JO, Yu HJ, Park IK. Comparison of direct immunofluorescent staining, shell vial culture and tube culture for the detection of varicella-zoster virus in skin lesions. *Korean J Clin Pathol* 1999;19:320–5.
- [17] Dobec M, Bossart W, Kaeppli F, et al. Serology and serum DNA detection in shingles. *Swiss Med Wkly* 2008;138:47–51.
- [18] Yeo JS, Sim WS, Kim YC. Herpes sine zoster: is the cause for the segmental intercostal neuralgia of unknown cause?: a case report. *Korean J Pain* 2005;18:226–8.
- [19] Heininger U, Seward JF. Varicella. *Lancet* 2006;368:1365–76.
- [20] Schmidt NJ, Arvin AM. Sensitivity of different assay systems for immunoglobulin M responses to varicella-zoster virus in reactivated infections (zoster). *J Clin Microbiol* 1986;23:978–9.
- [21] Gershon AA, Steinberg SP, Borkowsky W, et al. IgM to varicella-zoster virus: demonstration in patients with and without clinical zoster. *Pediatr Infect Dis* 1982;1:164–7.