Effectiveness of real-time PCR for diagnosis and prognosis of varicella-zoster virus keratitis

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Abstract

Purpose: To determine the efficacy of real-time PCR for the diagnosis and prognosis of varicella-zoster virus (VZV) keratitis.

Study design: Retrospective case series.

Methods: Patients: 545 consecutive patients with keratitis were examined to quantify copy numbers of VZV DNA by real-time PCR. Association of copy numbers of DNA of VZV to clinical signs and disease course was assessed using logistic regression analysis and Cox proportional hazard model.

Results: Of the 545 eyes, 38 eyes (6.9%) were diagnosed as VZV keratitis. The copy numbers of the DNA of VZV (median: $10^{4.19}$ copy) was significantly associated with diagnosis of VZV keratitis with the highest odds ratio (OR) of 3390 (for median copy) compared to clinical signs. Diagnostic accuracy of the VZV DNA copy indicated good diagnostic value of area under the curve (0.92) by receiver operating characteristic analysis, and detection of unrelated VZV DNA from the cornea was very rare (0.2%). When the VZV DNA copy and clinical signs were assessed for association with the disease course after herpes zoster ophthalmicus, the disease duration was significantly prolonged in VZV keratitis cases with higher numbers of VZV DNA copies, iritis, and history of recurrences. The amount of VZV DNA led to a continuous risk to prolong disease duration until the ocular inflammation subsides (hazard ratio (HR)

0.17, 95%CI: 0.07 - 0.42, for median copies).

Conclusions: Higher VZV DNA copy numbers are associated with the refractoriness of VZV keratitis, and its evaluation may be a clinically useful way to diagnose and manage VZV keratitis.

Keywords:

varicella-zoster virus keratitis, real-time PCR, herpes zoster ophthalmicus

Introduction

Herpes zoster contributes significantly to morbidity in elderly individuals and is mainly caused by a reactivation of the varicella-zoster virus (VZV). Almost one-third of individuals are estimated to be affected by herpes zoster, [1, 2] and up to 20% of herpes zoster infections are expressed as herpes zoster ophthalmicus (HZO).[2, 3] Patients with HZO have significantly higher risks of strokes and post-herpetic neuralgia. [4-6] Importantly, an ocular complication is observed in 35.1% to 65% of HZO patients.[7, 8]

However, the etiology of the ocular complication has not been determined. For example, the VZV DNA in the ocular lesions of VZV keratitis is occasionally not detected by conventional PCR. [9] For VZV keratitis cases positive for VZV DNA after HZO, VZV DNA was believed to disappear soon after the onset. Thus, it is still unclear whether active keratitis lesions are caused by VZV replication or an inflammatory response of the host.

The purpose of this study was to determine the relationship between the presence of VZV DNA in the eye and the diagnosis of HZO. To accomplish this, we quantified the

VZV DNA by quantitative real-time PCR (qPCR) and assessed the association of the copy numbers with the clinical signs with and without previous HZO. We then analyzed course of ocular complications after HZO. We shall show that VZV DNA was a significant risk factor for prolonged ocular complications after HZO.

Materials and Methods

Patients eligibility and diagnostic criteria of VZV keratitis

The medical records of 545 consecutive cases with clinically diagnosed keratitis were reviewed, and all were examined at the Tottori University Medical Hospital between November 2005 and September 2016. All of these cases had undergone qPCR for VZV. Of these 545 cases, 283 patients were men, and the mean age was 56.1 ± 23.1 years.

The diagnosis of HZO was based on the presence of primary skin rashes with erythema within the ophthalmic dermatome as described in detail.[9] Acute VZV keratitis was diagnosed when keratitis was present with preceding skin rashes <90 days from the onset of the skin lesions.[2] In cases without preceding skin rash with positive VZV by conventional PCR, a diagnosis of acute VZV keratitis was made by

the responsiveness to oral valaciclovir/acyclovir or topical acyclovir ointment, or the combination of these anti-herpetic drugs and steroid treatments. Chronic VZV keratitis was defined to be present when the activity required anti-viral drugs or steroids for ≥90 days from the onset of skin lesions.[2] Recurrent disease was defined to be present, when keratitis recurred ≥90 days after the resolution of the signs without the use of anti-viral drugs or steroids. DNA samples were collected at each outpatient visit until the clinical symptoms were resolved for the VZV keratitis cases.

A diagnosis of herpetic keratitis was made when herpes simplex virus (HSV) was detected by PCR. A diagnosis of other non-VZV keratitis, including bacterial keratitis, fungal keratitis, acanthamoeba keratitis, adenoviral keratitis, or autoimmune keratitis, was made by conventional culturing, smearing, and PCR as described.[10-13]

This study was approved by the Institutional Review Board of Tottori University, Faculty of Medicine, Tottori, Japan. An informed consent was obtained prior to the procedures from all of the participants after an explanation of the procedures to be used.

Quantitative real-time PCR

Samples were collected from the ocular surface and cornea by rinsing them with 400 μl of saline without touching the eyelids and skin lesions. DNA was extracted from the samples with the QIAamp DNA mini kit (Qiagen, Hilden, Germany) and were amplified with the LightCycler (Roche, Basel, Switzerland) using QuantiTect Probe PCR kit (Qiagen) and primers (Supplementary Table 1).[14, 15] To determine the copy numbers of the DNA of VZV, a standard curve was generated with serial dilutions of synthesized DNA fragments of the VZV polymerase gene.[14] The limit of detection at a 95% detection probability was 49 copies/assay. VZV copy number was adjusted by measurement of human GAPDH copy. [15]

Statistical analyses

Data are presented as the means ± standard deviations (SDs). For bilateral cases, the more severely affected eye was used for the statistical analyses. Cox proportional hazard model with shared frailty was used to calculate the hazard ratio (HR) during the course of the disease. Statistical analyses were conducted using Stata 14. A P <0.05 was considered significant.

Results

Diagnostic efficacy of qPCR for VZV keratitis

Patients with corneal ulcer or inflammatory keratitis which was suspected to be caused by VZV infection or required the exclusion of VZV for diagnosis were studied. Of the 545 eyes, 38 eyes (6.9%) were diagnosed with VZV keratitis. Thirty-seven eyes had HZO keratitis and 1 eye had varicella keratitis. The mean age of the HZO keratitis patients was 63.2 ± 20.0 years (Table 1).

qPCR for VZV was positive for 32 eyes (5.9%) of all the cases, and 30 eyes of PCR positive cases had HZO keratitis and 1 eye had varicella keratitis. All the VZV keratitis cases had prior periocular skin rashes, and 35 eyes (92.1%) were within 3 months of the initial visit. The percentage of eyes with ocular shedding of VZV DNA by non-VZV infection was very low in the diseased corneas (0.2%).

For the eyes diagnosed with VZV keratitis, the mean copy number was $10^{4.21} \pm 10^{2.61}$, and the median copy number was $10^{4.19}$. VZV DNA was significantly associated with VZV keratitis (Odds ratio (OR): 3390 for $10^{4.19}$ (median) copies, 6.9/log copy, after age and GAPDH adjustments, $P = 0.000$; Table 2). Periocular skin rashes (irrespective of dermatomal distribution and within the prior 3 months) also significantly associated with VZV keratitis, followed by ocular hypertension, iritis with mutton fat keratic precipitates, dendritic lesions, and scleritis (Table 2).

Next, we determined how these clinical signs and VZV DNA copy numbers are related to the diagnosis of VZV keratitis (Table 2). VZV DNA qPCR had a sensitivity of 81.6% and specificity of 99.8%. The likelihood ratio, which denotes the efficiency of the test, was the highest at 408 (Table 2). In contrast to the clinical signs, qPCR for VZV had higher positive predictive value and likelihood ratio, indicating that qPCR is accurate for both the negative and positive results for the diagnosis of VZV keratitis.

Of the 545 eyes, periocular skin rashes were observed in 49 eyes (9.0%), and 15 of these eyes (30.6%) were not related to VZV infection. The sensitivity and specificity of the skin rashes was 92.1% and 97.2%, and its likelihood ratio was lower at 32.9.

Other clinical signs, including ocular hypertension, iritis, corneal dendritic lesion, and scleritis, were also significantly associated with VZV keratitis, however, lower in positive predictive value and likelihood ratio.

Next, we assessed the diagnostic accuracy of these signs in comparison to that of qPCR using receiver operating characteristic analysis (ROC; Figure 1). The area under the curve (AUC) for periocular skin rashes calculated as reference was 0.95 (95%CI: 0.90 – 0.99). The AUC for VZV qPCR was 0.92 (95%CI: 0.86 – 0.98) after

GAPDH adjustments and was not different for the skin rashes. The AUC of dendritic lesions, ocular hypertension, and iritis was 0.66 (95%CI: 0.58 – 0.74), 0.62 (95%CI: 0.55 -0.70), and 0.60 (95%CI: 0.53 – 0.67) respectively, and their diagnostic accuracy was significantly lower than that of qPCR (*P* =0.000).

Association of copy numbers of DNA of VZV to clinical signs and disease course

We determined whether the copy numbers of the DNA of VZV was significantly associated with the clinical signs or outcomes using logistic regression analysis (Table 3). As expected, the copy number of DNA at the first visit was significantly associated with the presence of periocular skin rashes (OR: 100.6 for median copies, 95% CI: 22.9-441.9, *P* = 0.000, age and GAPDH adjusted). Notably, the copy number of DNA was significantly associated with iritis with OR of 6.0 indicating that high VZV copy number is especially associated with intraocular inflammation. This was followed by ocular hypertension (OR: 3.7) and dendritic lesion (OR: 3.5).

Therefore, we determined whether the amount of the DNA of VZV at the initial diagnosis of keratitis can predict the refractoriness and prognosis of HZO keratitis. A

higher copy number of VZV DNA at the first visit was significantly correlated with the duration of the disease (ρ = 0.53, *P* = 0.0007, Spearman correlation analysis). In refractory cases with iritis as the intraocular inflammation, the VZV genome was detected until all clinical signs of the keratitis were not detected (Figure 2).

When the history of recurrences was evaluated in HZO keratitis patients, 8 eyes (21.6%) had a history of recurrences. Of these 8 eyes, 2 eyes (25%) were from immune compromised patients. Immune compromised patients had significantly higher number of recurrences by a 50.6% increase of chance (*P* = 0.019 after VZV DNA copy adjustment).

The mean duration of the HZO keratitis was 119 days (95%CI: 82 – 155). Kaplan Meier survival analyses were performed to determine whether high copy numbers (more than the median) at the first visit, the clinical characteristics and previous recurrences were associated with the disease duration. The results showed that high VZV copy numbers (≥median; *P* = 0.008, log-rank test), iritis (*P* = 0.01), and history of recurrences were significantly associated with the duration of the disease (*P* = 0.006, Figure 3). Importantly, iritis with high VZV copy number had the most significant effect on the disease duration.

During the course of the disease process, we monitored the copy numbers of the DNA of VZV until HZO keratitis resolved. DNA of VZV was continuously detected at the outpatient visits with declining tendency, and become detectable when signs become absent. Then, we calculated the hazard ratio of VZV copy numbers during the course (including the first visit) on the disease duration. VZV copy numbers indicated highly significant HR, and was 0.17 for median of VZV copies (95% CI: 0.07- 0.42, *P* =0.000). Thus, detection of VZV copy number predicted a prolonged disease course, and the disease prolonging effect declines with its decrease and become negligible when it is not detected. HR indicated that the keratitis was six times more likely not to be resolved on a given date when the VZV copy numbers exceeded median. Presence of iritis were also significant risk factor associated with longer disease duration with comparable HR (HR, 0.14, 95% CI: 0.04- 0.49, *P* = 0.002). When the history of recurrences was assessed, the hazard ratio was calculated to be 0.15 (95%CI: 0.03 – 0.69, *P* = 0.01), indicating that recurrences were also associated significantly with a prolongation of the disease duration.

Discussion

Our results showed that qPCR is highly efficacious for diagnosing ocular VZV infection. Using qPCR, we here show two important findings. First, in case of high viral loads at ocular surface after HZO, the prolongation of keratitis was significantly associated with continuous viral production. Second, VZV keratitis was strictly associated with previous HZO, although VZV iritis without keratitis can often occur without noticeable history of HZO. These information is important for the management of refractory ocular complications after HZO because ocular inflammation was previously thought to be an anti-viral immune response without viral production and often treated without antiviral medications.

Zaal et al examined the inflammation of VZV keratitis after HZO by conventional PCR.[9] Because the inflammation often persisted after the VZV genome became undetectable, they suggested that inflammation was an important component of the pathology of VZV keratitis.

Considering the inflammatory aspect of ocular complications after HZO, a combination of antiviral drugs and steroids is generally used, however consensus has not been reached on how antiviral agents should be used. [16] The general belief was

that the period of VZV replication in the lesion is not prolonged beyond the acute phase of skin lesion stage. Currently, one-third of corneal specialists use antiviral drugs for 2 weeks and 18% do not use antivirals for HZO.[16]

In chronic and refractory cases, high amounts of VZV DNA were detected for a long time. If antivirals are discontinued after presumed clinical remission with significant viral replication, a recurrence in the form of delayed dendritic ulcer or iritis develops as ocular complications can be expected. Thus, VZV qPCR will be beneficial for clinicians to optimize drug dosage and duration during the disease course when the signs are resolving.

The presence of iritis was recently shown to be significantly associated with a risk of recurrences or chronicity in VZV keratitis after HZO.[2] We found that iritis with low copy numbers of the DNA of VZV was not a significant risk factor for refractoriness (Figure 2**a**). Instead, a combination of high amounts of VZV DNA copy and iritis were the most significant factors in the refractory cases. In the refractory or chronic cases, high VZV DNA copy numbers were maintained until the inflammation subsided. This suggests that prolonged viral replication and not the presence of iritis, determines the disease course in refractory cases.

The results of two studies have suggested that the detection of VZV may be caused by shedding and was independent of the refractoriness.[8, 17] In addition, VZV may be often reactivated in the saliva of healthy individuals when under extreme stress.[17] In bone marrow transplant patients, 19% have subclinical viremia without signs of HZO.[3, 18] This suggested detection of viral genome was frequent, and may not reflect diseases. In Japanese population, seropositivity reaches almost 100% in elderly subjects. [19] However, we found that the rate of unrelated VZV shedding from the eye was very low (0.2%) even in eyes stressed by non-VZV keratitis. This is in marked contrast to ocular HSV infection, in which spontaneous HSV shedding in tear is observed in one third of healthy subjects. [20]

Recurrences of ocular complication after HZO are frequent. The percentage of eyes with recurrences range between 5 to 25% depending on the duration of the observation period.[2, 21, 22] Consistent with previous reports, the recurrence percentage in our study was 21.6%. Tran et al reported that a recurrence of keratitis after HZO was associated with uveitis and ocular hypertension.[2] However, we could not confirm any significant associations of the recurrences with these clinical signs or the VZV DNA copy numbers. Instead, we found that the disease duration of single

episode was the only significant risk factor for recurrences. We suggest that the clinical signs are secondary to the long disease course which would directly explain the recurrences.

There are several limitations in our study. Our data were obtained at a tertiary referral institution, and selection or referral bias may have affected our outcomes. However, our data are based on 12 consecutive years of observation and should provide epidemiological evidence for Asians. In addition, the outcomes from a large series of VZV qPCR data from the eye have not been available.

In conclusion, VZV qPCR revealed an unexpectedly longer viral replication period and provided an effective measure to assess the viral load accurately during the course of the disease process. We propose that the management strategy of ocular complication after HZO would be significantly improved in the future with the use of VZV qPCR.

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References

1. Yawn BP, Saddier P, Wollan PC, St Sauver JL, Kurland MJ, Sy LS. A population-based study of the incidence and complication rates of herpes zoster before zoster vaccine introduction. Mayo Clin Proc. 2007;82(11):1341-9.

2. Tran KD, Falcone MM, Choi DS, Goldhardt R, Karp CL, Davis JL, et al. Epidemiology of Herpes Zoster Ophthalmicus: Recurrence and Chronicity. Ophthalmology. 2016;123(7):1469-75.

3. Liesegang TJ. Herpes zoster ophthalmicus natural history, risk factors, clinical presentation, and morbidity. Ophthalmology. 2008;115(2 Suppl):S3-12.

4. Kang JH, Ho JD, Chen YH, Lin HC. Increased risk of stroke after a herpes zoster attack: a population-based follow-up study. Stroke. 2009;40(11):3443-8.

5. Lin HC, Chien CW, Ho JD. Herpes zoster ophthalmicus and the risk of stroke: a population-based follow-up study. Neurology. 2010;74(10):792-7.

6. Bouhassira D, Chassany O, Gaillat J, Hanslik T, Launay O, Mann C, et al. Patient perspective on herpes zoster and its complications: an observational prospective study in patients aged over 50 years in general practice. Pain. 2012;153(2):342-9.

7. Kaufman SC. Anterior segment complications of herpes zoster ophthalmicus. Ophthalmology. 2008;115(2 Suppl):S24-32.

8. Cohen EJ, Kessler J. Persistent dilemmas in zoster eye disease. Br J Ophthalmol. 2016;100(1):56-61.

9. Zaal MJ, Volker-Dieben HJ, Wienesen M, D'Amaro J, Kijlstra A. Longitudinal analysis of varicella-zoster virus DNA on the ocular surface associated with herpes zoster ophthalmicus. Am J Ophthalmol. 2001;131(1):25-9.

10. Kakimaru-Hasegawa A, Kuo CH, Komatsu N, Komatsu K, Miyazaki D, Inoue Y. Clinical application of real-time polymerase chain reaction for diagnosis of herpetic diseases of the anterior segment of the eye. Jpn J Ophthalmol. 2008;52(1):24-31.

11. Kandori M, Miyazaki D, Yakura K, Komatsu N, Touge C, Ishikura R, et al. Relationship between the number of cytomegalovirus in anterior chamber and severity of anterior segment inflammation. Jpn J Ophthalmol. 2013;57(6):497-502.

12. Ikeda Y, Miyazaki D, Yakura K, Kawaguchi A, Ishikura R, Inoue Y, et al. Assessment of real-time polymerase chain reaction detection of Acanthamoeba and prognosis determinants of Acanthamoeba keratitis. Ophthalmology. 2012;119(6):1111-9.

13. Koizumi N, Miyazaki D, Inoue T, Ohtani F, Kandori-Inoue M, Inatomi T, et al. The effect of topical application of 0.15% ganciclovir gel on cytomegalovirus corneal endotheliitis. Br J Ophthalmol. 2016.

14. Weidmann M, Meyer-Konig U, Hufert FT. Rapid detection of herpes simplex

340 virus and varicella-zoster virus infections by real-time PCR. J Clin Microbiol.
341 2003;41(4):1565-8.
342 15. Miyazaki D, Uotani R, Inoue M, Haruki T, Shimizu Y, Yakura K, et al. Corneal
343 endothelial cells activate innate and acquired arm of anti-viral responses after
344 cytomegalovirus infection. Exp Eye Res. 2017;161:143-52.
345 16. Sy A, McLeod SD, Cohen EJ, Margolis TP, Mannis MJ, Lietman TM, et al.
346 Practice patterns and opinions in the management of recurrent or chronic herpes
347 zoster ophthalmicus. Cornea. 2012;31(7):786-90.
348 17. Cohrs RJ, Mehta SK, Schmid DS, Gilden DH, Pierson DL. Asymptomatic
349 reactivation and shed of infectious varicella zoster virus in astronauts. J Med Virol.
350 2008;80(6):1116-22.
351 18. Wilson A, Sharp M, Koropchak CM, Ting SF, Arvin AM. Subclinical
352 varicella-zoster virus viremia, herpes zoster, and T lymphocyte immunity to
353 varicella-zoster viral antigens after bone marrow transplantation. J Infect Dis.
354 1992;165(1):119-26.
355 19. The National Institute of Infectious Diseases.
356 https://www.niid.go.jp/niid/ja/y-graphs/7179-varicella-yosoku-serum2016.html.
357 Accessed 11 Oct 2017.
358 20. Kaufman HE, Azcuy AM, Varnell ED, Sloop GD, Thompson HW, Hill JM.

HSV-1 DNA in tears and saliva of normal adults. Invest Ophthalmol Vis Sci. 2005;46(1):241-7.

21. Miserocchi E, Fogliato G, Bianchi I, Bandello F, Modorati G. Clinical features of ocular herpetic infection in an italian referral center. Cornea. 2014;33(6):565-70.

22. Donahue JG, Choo PW, Manson JE, Platt R. The incidence of herpes zoster. Arch Intern Med. 1995;155(15):1605-9.

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Table 1 Demographic information of patients and VZV keratitis

Total 545 eyes	HZO keratitis N=37	Varicella keratitis N=1	Non-VZV-associated keratitis N=507
Age	63.2 ± 20.0	2	55.7 ± 23.1
Male	24 eyes (64.9%)	0 eyes	259 eyes (51.1%)
Asthma	3 eyes (8.1%)	0 eyes	11 eyes (2.2%)
Eczema	1 eyes (2.7%)	1 eyes	31 eyes (6.1%)

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Table 2 Association of VZV DNA copy numbers and clinical signs of VZV keratitis and evaluation of their diagnostic accuracy

Total 545 eyes	ratio of positive eyes	Odds ratio after age adjustment	positive predictive value	negative predictive value	Sensitivity	Specificity	likelihood ratio
VZV DNA copy	32* (5.9%)	3390* (135-85383) :median copy 6.9* (3.2-15.0) :log copy	96.9% (80.3-99.6%)	98.6% (97.2-99.3%)	81.6% (65.8-91.1%)	99.8% (98.6-100%)	408 (47-3037)
Periocular skin rashes	49 * (9.0%)	701* (162 - 3057)	71.4% (57.2-82.4%)	99.4% (98.1-99.8%)	92.1% (77.9-97.5%)	97.2% (95.3-98.3%)	32.9 (16.6-57.4)
Ocular hypertension	47* (8.6%)	5.8* (2.7 – 12.6)	25.5% (15.0-40.0%)	94.8% (92.4-96.4%)	31.6% (18.7-48.0%)	93.1% (90.5-95.0%)	4.6 (2.0-9.6)
Iritis with mutton fat keratic precipitates	39* (7.2%)	5.4* (2.4 - 12.5)	25.6% (14.3-41.7%)	94.5% (92.1-96.2%)	26.3 (14.6-42.6%)	94.3% (91.9-96.0%)	4.6 (1.8-10.7)
Corneal dendritic lesion	85* (15.6%)	5.0* (2.5 - 10.1)	20.0% (12.8-30.0%)	95.4% (93.1-97.0%)	44.7% (29.7-60.8%)	86.6% (83.3%-89.3%)	3.3 (1.8-5.7)
Scleritis	28** (5.1%)	3.9 *** (1.5 – 10.4)	21.4% (9.8-40.7%)	93.8% (91.4-95.6%)	15.8% (7.2-31.3%)	95.7% (93.5-97.1%)	3.7 (1.1-10.8)

*: statistically significant, *: P=0.000, **: P=0.009, ***: P=0.006, 95% confidence interval (95%CI)

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Table 3 Association of VZV copy numbers to clinical signs and characteristics

	Odds ratio (Median copy)	95% confidence interval	*P* value
Periocular skin rashes	100.6	22.9 – 441.9	0.000
Iritis (with mutton fat keratic precipitates)	6.0	2.4 – 15.2	0.000
Ocular hypertension	3.7	1.6 - 8.9	0.003
Corneal dendritic lesion	3.5	1.6 – 7.9	0.002

Logistic regression analysis after age and GAPDH adjustment

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Figure captions

Figure 1. Diagnostic accuracy of qPCR for VZV and clinical signs.

The sensitivity and specificity of VZV qPCR and clinical signs to diagnose VZV keratitis are plotted to determine the area under the curve (AUC) as diagnostic accuracy by receiver operating characteristic analysis. The VZV DNA copy number has a very high accuracy comparable to that for skin rashes calculated as reference and is significantly better than the other signs.

Figure 2. Case of VZV keratitis with duration of VZV disease. A 53-year-old man presented with herpes zoster ophthalmicus (HZO) with periocular skin rashes. One month later, he developed VZV keratitis with corneal edema and iritis, and the copy number of the DNA of VZV was 4.7×10^5. The elevated copy number was present for 4 months.

a VZV keratitis with stromal infiltration (arrow) and iritis with VZV copy of 4.5×10^5 at 2 months after the onset of HZO.

b Prolonged elevation of VZV DNA copy number during the course of the disease.

Figure 3. Association of duration of VZV keratitis to VZV DNA copy number and

clinical characteristics.

Disease duration was analyzed using Kaplan Meier survival analysis and plotted on non-healing rate. **a** High VZV DNA copy number (≥median ($10^{4.19}$ copies); $P = 0.008$, log-rank test), the presence of iritis ($P = 0.01$), and **b** history of recurrences ($P = 0.006$) were significantly associated with the disease duration.

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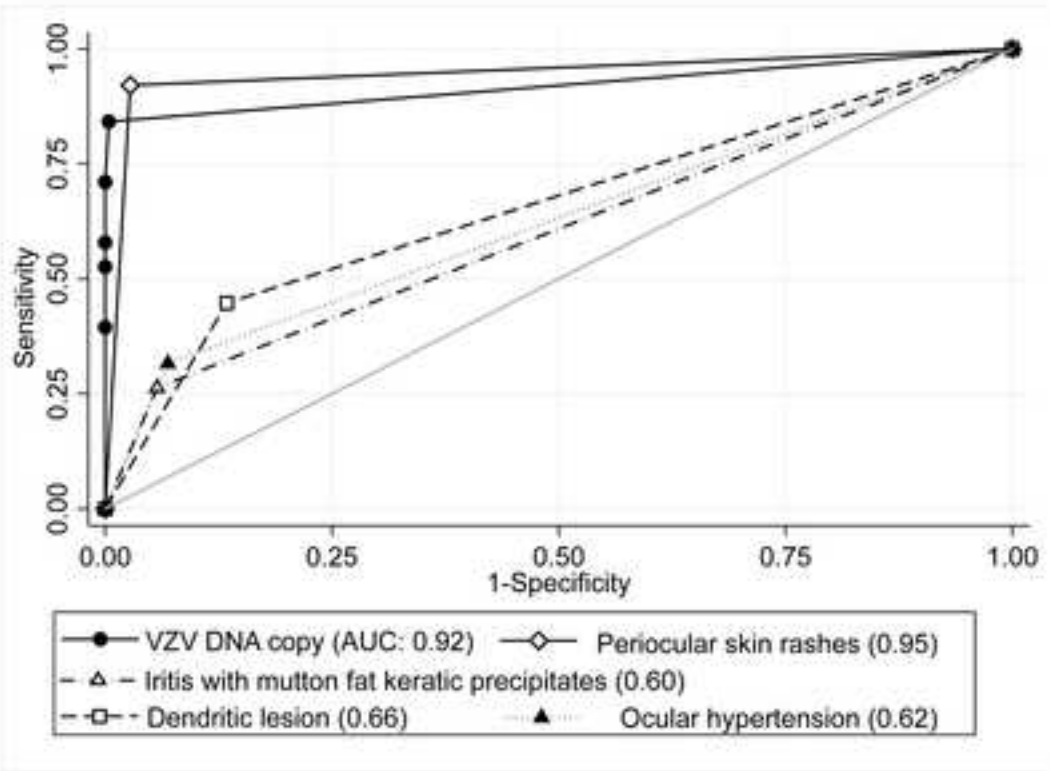

Figure 1

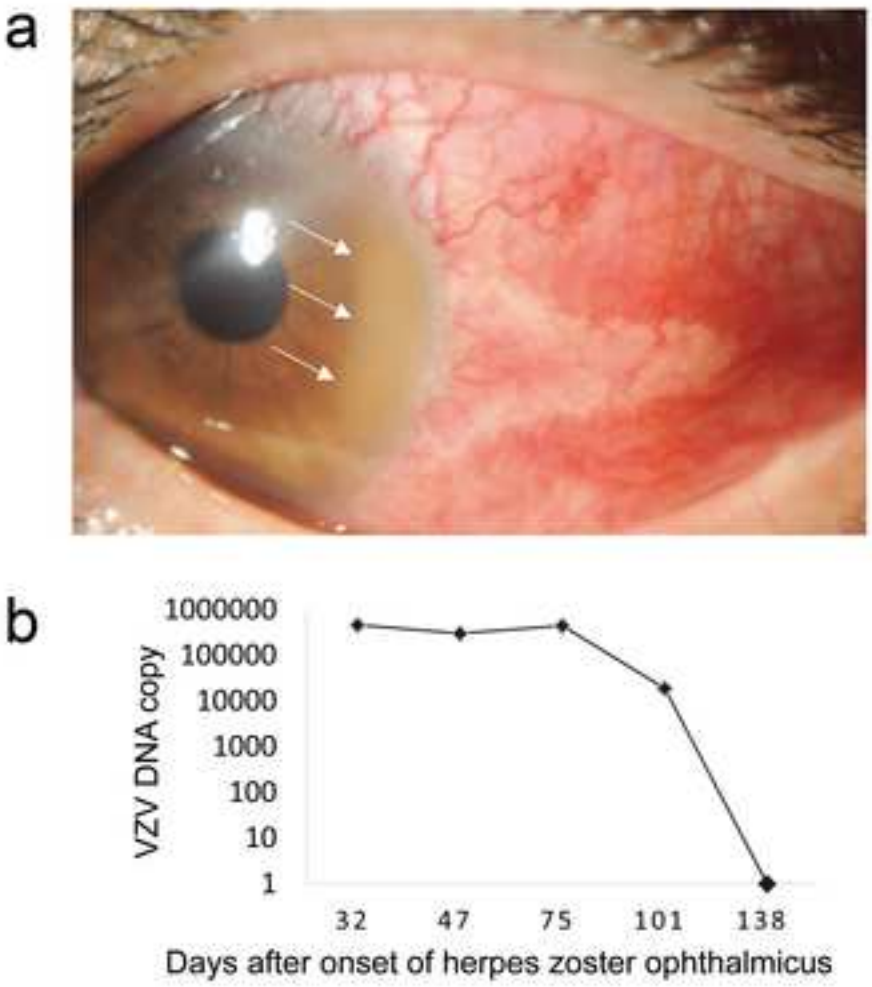

Figure 2

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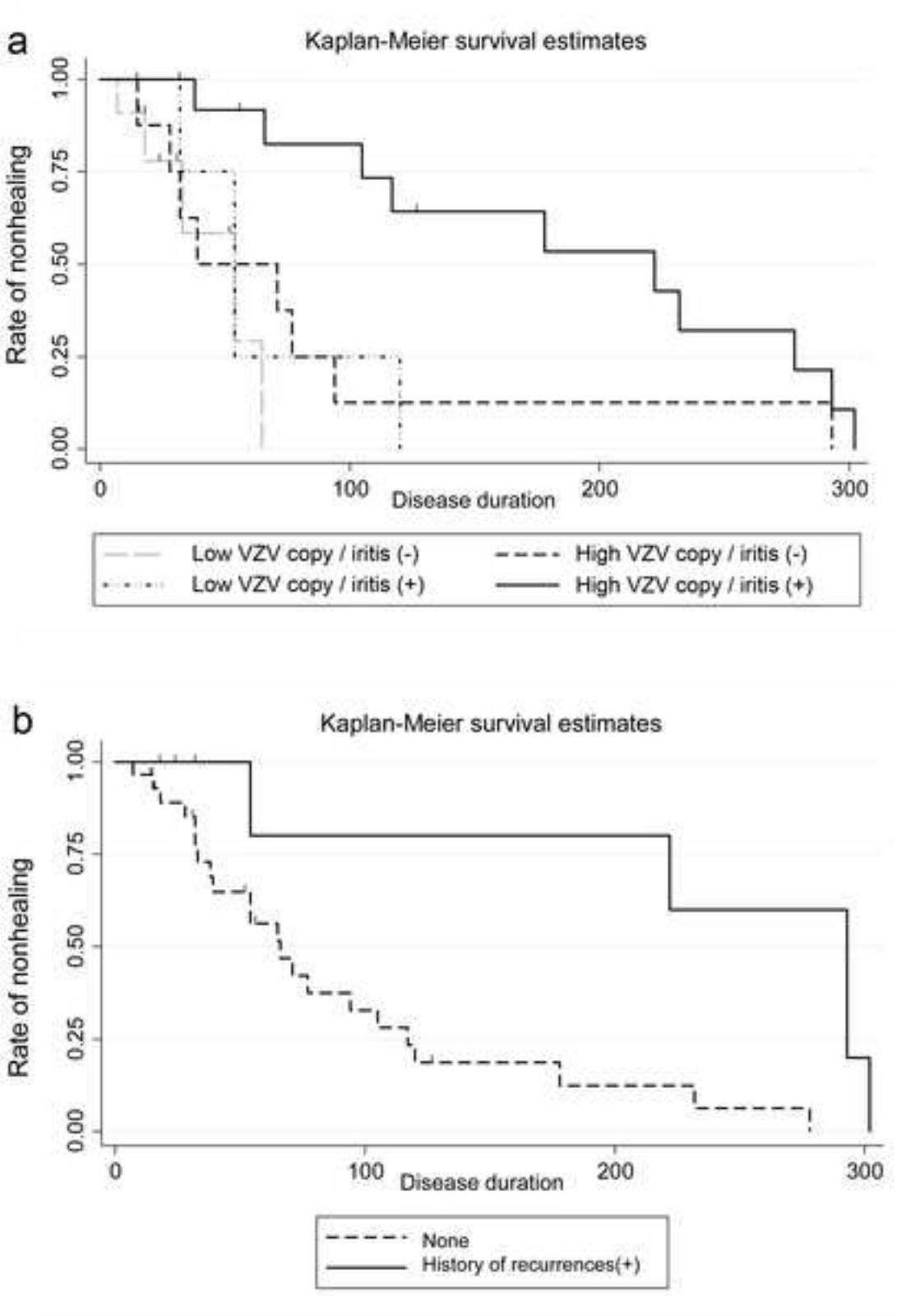

Figure 3