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ARTICLE

Observation of varicella zoster virus-induced acute retinal necrosis: viral load detection and visual outcome

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Abstract

Objectives To observe the changes of viral load in aqueous humour samples and visual outcomes in varicella zoster virus (VZV)-induced acute retinal necrosis (ARN).

Methods Observational retrospective study. Medical records and viral load measured by real-time quantitative polymerase chain reaction (qPCR) of 20 eyes with VZV-induced ARN were reviewed.

Results The mean viral load at presentation was $5.7 \times 10^7 \pm 9.7 \times 10^7$ copies/mL. An initial plateau phase for viral load lasting up to 2 weeks occurred in most eyes (18 eyes, 90%). In the following logarithmic reduction phase, the mean slope of the decline in viral load was -0.103 ± 0.029 log/day, and the expected time for half reduction of the initial viral load was 3.2 ± 1.0 days. At the end of the first 8-week's antiviral treatment, the viral load was below detection threshold in all 20 eyes (100.0%). The mean logarithm of the minimum angle of resolution (logMAR) best-corrected visual acuity (BCVA) improved from 1.1 ± 0.7 (Snellen equivalent 20/250) to 0.7 ± 0.6 (Snellen equivalent 20/100) after a follow-up of 8.6 ± 2.0 months. Thirteen of the 20 eyes (65.0%) suffered retinal detachment and underwent vitrectomy. The initial viral load was the independent predictive factor of logMAR BCVA at the last follow-up ($\beta = 0.745$, $P < 0.001$).

Conclusions The observation of viral load changes by qPCR was useful for better monitoring of therapeutic efficacy and deciding needed antiviral duration in VZV-induced ARN patients.

Introduction

Acute retinal necrosis (ARN) is a panuveitis entity caused by herpes virus family infection, with varicella zoster virus (VZV) the most common causative pathogen [1]. ARN is

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characterised by acute necrotising retinitis, which may be complicated by retinal detachment (RD) [2, 3]. ARN is a rapidly destructive form of infectious uveitis, timely antiviral treatment and monitoring the extent of disease are vital. The extent of necrotising retinitis is routinely monitored under clinical observation, the assessment is usually subjective, and it is difficult to observe the posterior segment in some eyes with vitreous opacities [2, 4].

Real-time quantitative polymerase chain reaction (qPCR) is a technique which can quantify the genome copies of pathogen in samples, thus provides us a perspective to monitor the extent of infectious diseases beyond clinical observation [5]. Real-time qPCR has been used to diagnose viral infections and assess response to antiviral therapy in a series of studies, including ARN [6, 7]. But most of the studies were single case reports or small case series. The changes of viral deoxyribonucleic acid (DNA) load after antiviral treatment have not been well described in ARN patients in a substantial series.

In the present study, we assessed the changes of viral DNA load in aqueous humour samples by real-time qPCR in VZV-induced ARN patients, in order to evaluate the

contribution of real-time qPCR for monitoring and treatment of ARN.

Methods

Diagnosis criteria and data compilation

In this observational retrospective study, we examined the clinical records of all subjects with a diagnosis of ARN who visited our clinic between December 2018 and November 2019. All eyes were clinically diagnosed according to the diagnostic criteria for ARN defined by the American Uveitis Society [3], and eyes with VZV infection identified by PCR were included in the study.

Demographic and clinical data, including age, sex, laterality, duration between symptoms and presentation, and previous medical history were collected. Ophthalmologic characteristics were also collected, including best-corrected visual acuity (BCVA) at presentation and last follow-up, initial retinal quadrants and zones involved referring to the previous definition for cytomegalovirus (CMV) retinitis [8], type of treatment, and occurrence of RD. Visual acuity was recorded by Snellen visual acuity ratios, while logarithms of the minimum angle of resolution (logMAR) was applied for statistical analysis [9].

The study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the Eye and ENT Hospital of Fudan University.

Treatment protocol

All subjects received a combined antiviral treatment. Systemic treatment included intravenous antiviral treatment (acyclovir, 10 mg/kg, q8h) for the first 14 days, and a following oral administration (valaciclovir 2000 mg tid for 2 weeks, followed by a maintenance dose of 1000 mg tid). Intravitreal injections of ganciclovir, 4 mg/0.1 mL, twice a week were administered once ARN was suspected, and until the disappearance of active retinitis was observed by two senior doctors (R.J. and M.Z.), or the occurrence of RD requiring vitrectomy. Intravitreal antiviral injections could be administered for a maximal dose of nine injections (4 weeks' duration). An intravitreal or oral corticosteroid was given depending on the severity of intraocular inflammation. Patients with RD underwent vitrectomy with silicon oil tamponade.

Sample collection and virological analysis

Aqueous humour samples (~100 μL) were collected from each participant before intravitreal injections, and were used for a qualitative multiplex PCR assay to measure the genomic DNA levels of herpes simplex virus type 1, type 2,

VZV, Epstein–Barr virus, and CMV at the first visit, as previously described [10]. If VZV was detected, a real-time qPCR assay was performed. For eyes with VZV-induced ARN, the scheduled time point for aqueous humour samples collection and real-time qPCR analysis was the time of the first visit (day 1), day 4, week 1, 2, 3, 4 in the first 4-week's treatment, and was stopped at any time when intravitreal injections discontinued. During the following treatment, aqueous humour samples collection and real-time qPCR analysis was made once a month until the viral load was below detection threshold. The real-time qPCR assay was performed with a TaqMan PCR kit (Shanghai ZJ Bio-Tech Co., Ltd., Shanghai, China) and a real-time PCR system (ABI 7500, Thermo Fisher Scientific, Waltham, MA), as previously described [11]. The viral DNA load is expressed as the number of copies per millilitre of aqueous humour. The threshold of detection was 1×10^3 copies/mL.

Statistical analysis

Continuous data are described as means ± standard deviations. Categorical data are described as the absolute and relative frequencies in each category. Nonparametric statistical tests including the Wilcoxon test and Spearman test were used, for the data were positively skewed on histograms. A multiple regression analysis was used to identify the virological parameters that were independently associated with the visual outcome. The viral DNA load was modelled using a logarithmic decay curve; the exponential model was analysed using linear regression analysis.

P Values of <0.05 were considered as statistically significant. SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

## Results

Baseline characteristics

During the study period, 32 eyes in 28 patients were diagnosed as ARN. VZV was detected in the aqueous humour samples in 27 eyes of 25 patients. Continuous aqueous humour samples were collected in 20 eyes (13 left eyes and 7 right eyes) of 20 patients (13 men and 7 women), which were included in this study. No primary (congenital) or secondary immune deficiencies were found in any of the patients. The patients' demographic and baseline clinical data are listed in Table 1.

Treatment and outcomes

The logMAR BCVA was 1.1 ± 0.7 (Snellen equivalent 20/250; range, hand motion (HM) to 20/66) at first visit, and

Table 1 Baseline characteristics of the patients.

Gender	13 males (65%); 7 females (35%)
Age, years	49.5 ± 11.3 (range, 38–73)
Duration between symptoms and presentation, days	10.4 ± 5.1 (range, 3–20)
Retinal quadrants involved (n, %)	Two quadrants (6/20, 30%), Three quadrants (6/20, 30%), Four quadrants (7/20, 35%), Inaccessible (1/20, 5%)
Retinal zones involved (n, %)	Zone 3 (5/20, 25%), Zone 3 + 2 (10/20, 50%), Zone 3 + 2 + 1 (4/20, 20%), Inaccessible (1/20, 5%)
Duration of follow-up	8.6 ± 2.0 months (range, 6–12)
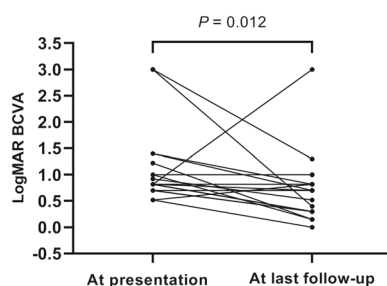
Fig. 1 LogMAR BCVA at presentation and last follow-up of the affected eyes. BCVA best-corrected visual acuity, logMAR logarithm of the minimum angle of resolution.

0.7 ± 0.6 (Snellen equivalent 20/100; range, HM to 20/20) at the last follow-up, at a mean of 8.6 ± 2.0 months (range, 6–12 months) ($P = 0.012$). Visual acuity improved in 13 eyes (65.0%), remained the same in five eyes (25.0%), and decreased in two eyes (10.0%). Visual acuity was 20/200 or less in four eyes (20.0%) at the last follow-up (Fig. 1).

RD occurred in 13 of 20 eyes (65.0%) and was treated with vitrectomy. The mean duration between symptoms and occurrence of RD was 8.0 ± 5.3 weeks (range, 1–18 weeks). The mean duration between occurrence of RD and vitrectomy was 4.8 ± 3.0 days (range, 1–9 days). After vitrectomy, all retinas were still attached at the last follow-up visit.

Virological analysis

The mean viral DNA load at presentation was $5.7 \times 10^7 \pm 9.7 \times 10^7$ copies/mL (range, 8.6×10^5–3.7×10^8/mL). The following changes of viral DNA load were described in the DNA curves (Fig. 2). The viral DNA profile in the aqueous humour displayed a kinetic pattern of decline after starting antiviral treatment, including a plateau phase and a logarithmic reduction phase [12, 13].

The plateau phase was defined as the period before a biologically significant decline of viral DNA load was observed (a decrease in viral DNA load of 0.5 log from baseline) [13], and was found to be within 1 week in six eyes (30.0%; Patient 3,6,9,10,17,19), between 1 and

2 weeks in 12 eyes (60.0%; Patient 1,2,4,5,7,8,11–15,18), and between 2 and 3 weeks in one eye (5.0%; Patient 16). We could not assess the plateau phase in one eye (Patient 20), which had limited measurements because of early RD.

After the viral load achieved a biologically significant decline, the viral kinetics of reduction were evaluated in eight patients (Patient 4,6–9,11,17,19) with a minimum of three samples collected after the time point of 0.5 log decrease in viral DNA load from baseline. There was an exponential decrease in the viral DNA load, and in 6 of the 8 eyes (Patient 4,6–9,11), the correlation was significant using linear regression analysis ($P < 0.05$). The mean slope of the decline of the base 10 logarithm of viral DNA load was −0.103 ± 0.029 log/day (range, −0.150 to −0.060 log/day). The expected time for half reduction in the initial viral DNA load was 3.2 ± 1.0 days (range, 2.0–5.0 days).

At the end of the first 4-week's antiviral treatment, the mean viral DNA load decreased to $3.1 \times 10^4 \pm 1.8 \times 10^4$ copies/mL (range, 1.6×10^4–6.0×10^4 copies/mL; $P = 0.012$). At the end of the first 8-week's antiviral treatment, the viral DNA load was below detection threshold in all 20 eyes (100.0%).

Relationship between visual outcome and virological parameters determined by real-time qPCR

The relationships between visual outcome and virological parameters determined by real-time qPCR were shown in Table 2. The logMAR BCVA at the last follow-up was assessed in a stepwise multivariable regression model (including initial retinal quadrants involved, initial retinal zones involved, occurrence of RD and initial viral DNA load). The parameter that remained independently associated with logMAR BCVA at the last follow-up was the initial viral DNA load ($\beta = 0.745$, $P < 0.001$).

Discussion

In the present study, we measured changes in the viral DNA load of aqueous humour samples in VZV-induced ARN patients and investigated the visual outcomes. As far as we know, this is the largest series of VZV-induced ARN patients treated under uniform antiviral therapy in a short time span.

The quantitative measurement of viral DNA load using real-time qPCR has recently been described for ocular infections by VZV, including patients with ARN [6, 14], which has become a reliable tool to monitor the extent of disease, especially in eyes with media opacity [5]. The observation by D. Bernheim et al. first depicted the kinetics of viral DNA load in aqueous humour samples during treatment in six patients of VZV-induced ARN [13]. All
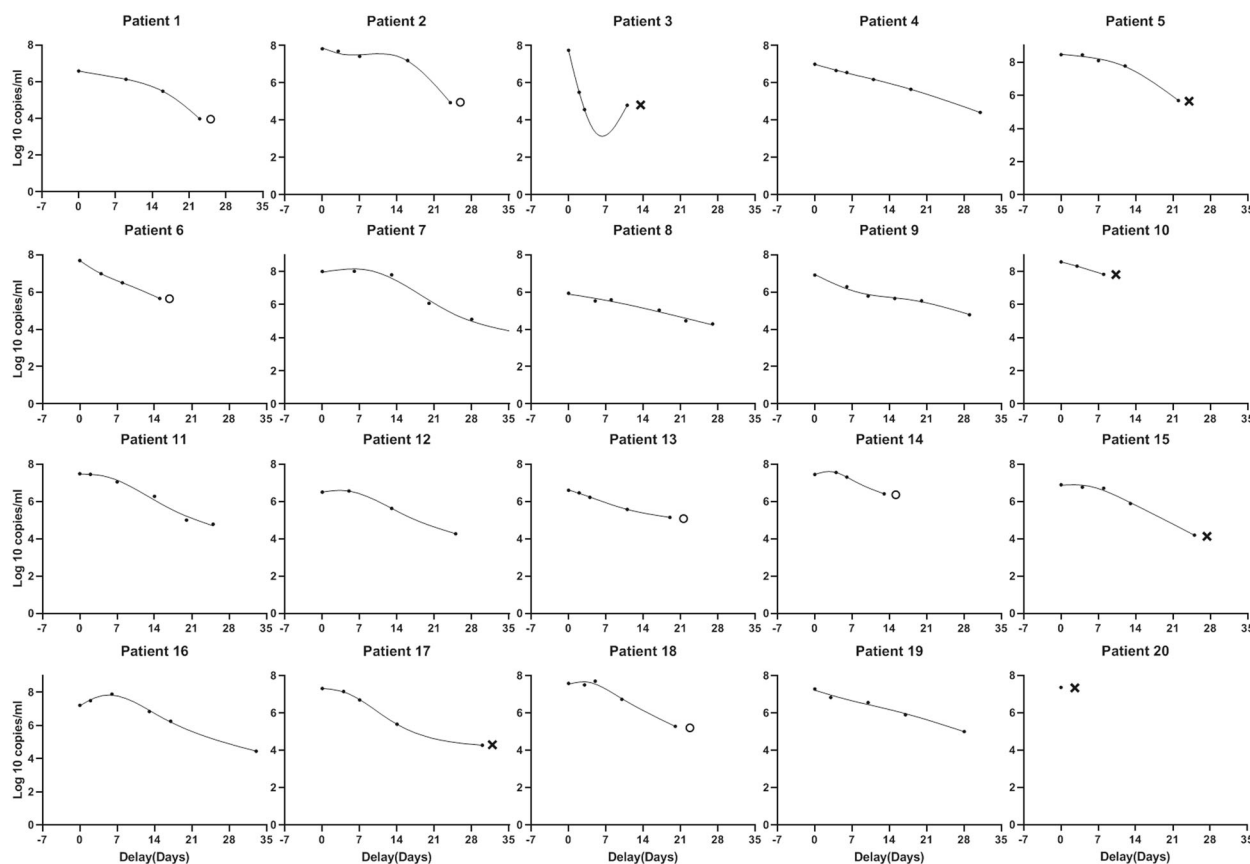
Fig. 2 Changes in the viral DNA load over time detected by real-time qPCR. Circles: time at which active retinitis disappearance was first found. Crosses: time at which retinal detachment occurred. Both the circles and crosses mark the early termination of intravitreal injection and real-time qPCR analysis in the first 4-week's antiviral treatment.

these publications were performed with small samples, which need to be validated in a substantial series.

In the present study, the initial viral DNA load covered a large dynamic range from 8.6×10^5 to 3.7×10^8 copies /mL, comparable to the studies published before [6, 13, 14]. In the study by Charles et al., initial viral DNA load of $\geq 5.0 \times 10^6$ copies/mL was associated with worse visual acuity, which was similar to what was found in the present study [14]. In the present study, the initial viral DNA load was the independent predictive factor of logMAR BCVA at the last follow-up ($\beta = 0.745$, $P < 0.001$).

Just as what has reported before, once antiviral treatment began, the profile of viral DNA load in the aqueous humour follows a well-defined kinetic pattern of virus clearance with a plateau phase and a following logarithmic reduction phase [12, 13]. The plateau phase was found to be within 2 weeks in most of the eyes (18 eyes, 90.0%) in the present study. This seems to be shorter than what was reported in Bernheim's study (mean, 27.8 days; range, 10–77 days) [13]. The length of plateau phase is reported to be related to a series of factors including the host immune responses and the choice of antiviral therapy [15, 16]. The antiviral susceptibility of the causal virus is also thought to be related to

the length of plateau phase [13], and in Hafidi's study, the patients with antiviral resistance shared a longer plateau phase of viral DNA load [17].

The kinetics of the viral DNA load might be a useful tool for evaluating the clinical course of ARN and help determining the treatment schedule. The most established treatment regimen of ARN included an induction therapy of intravenous acyclovir for 7–10 days followed by a maintenance treatment of oral antivirals for a long period of time [2, 18]. The duration of the currently used intravenous induction dose was consistent with the mean length of the plateau phase of the viral DNA load shown by qPCR. A prolonged plateau phase monitored by real-time qPCR might suggest the antiviral resistance, and the need for a more prolonged high-dose antivirals or change for another antiviral medication in clinical practice [17, 19]. Interestingly, the plateau phase was not correlated with the initial viral DNA load ($r = -0.269$, $P = 0.266$), which suggests that there could be far more other issues involved, as the retinal permeability, drug pharmacokinetics, vascular infraction or occlusion, and so on.

Although observation of the length of plateau phase can help us speculate on the possible antiviral resistance, a VZV

Table 2 Relationship between visual outcome and virological parameters determined by real-time qPCR.

	LogMAR BCVA at last follow-up		Initial viral DNA load		Time to achieve a biologically significant decline of viral DNA load		Slope of the decline of viral DNA load	
	r	P value[a]	r	P value[a]	r	P value[a]	r	P value[a]
Clinical characteristics								
Duration between symptoms and presentation	0.332	0.153	0.246	0.296	−0.353	0.138	−0.624	0.098
Initial retinal quadrants involved	0.685	0.001	0.514	0.024	−0.152	0.547	−0.620	0.138
Initial retinal zones involved	−0.560	0.013	−0.567	0.011	0.403	0.097	0.337	0.460
Occurrence of RD	0.514	0.02	0.700	0.001	−0.284	0.238	−0.850	0.007
Virological parameters determined by real-time qPCR								
Initial viral DNA load	0.450	0.046	–	–	−0.269	0.266	−0.731	0.04
Time to achieve a biologically significant decline of viral DNA load	−0.418	0.075	−0.269	0.266	–	–	0.055	0.897
Slope of the decline of viral DNA load	−0.491	0.217	−0.731	0.04	0.055	0.897	–	–

BCVA best corrected visual acuity, DNA deoxyribonucleic acid, logMAR logarithms of the minimum angle of resolution, qPCR quantitative polymerise chain reaction, RD retinal detachment.

[a]Spearman test.

genotypic antiviral testing is important to validate this speculation. Currently antiviral drugs to treat VZV-associated infections mainly target the viral DNA polymerase (Pol) and viral thymidine kinase (TK); thus mutations in Pol and TK encoding genes confer resistance to antiviral drugs [20]. As a relatively new sequencing technology, ultra-deep sequencing has an enhanced sensitivity to detect minor variants and allows quantitative evaluation of the viral drug resistance mutations [21, 22]. Ultra-deep sequencing might provide us with the antiviral resistance more quickly and accurately, compared to observation of the plateau phase, and is worthy further exploration, which is not investigated in the present study.

Once the viral DNA load starts to decrease (after a viral DNA load decline of 0.5 log), most of the DNA curves behaved similarly, with mean slopes of decline of $−0.103 \pm 0.029$ log/day, and a mean expected time for half reduction of the initial viral DNA load of 3.2 ± 1.0 days. This is consistent with the series reported by Bernheim et al. (3.0 ± 0.7 days) and Hafidi et al. (4.2 days) [13, 17]. Previous studies that measured the time-profile of viral DNA load of ARN patients with real-time qPCR are summarised in Table 3.

In the present study, at the end of the first 8-week's antiviral treatment, the viral DNA load was below detection threshold in all 20 eyes (100.0%), which might provide some guidance on duration of antiviral maintenance treatment for ARN. Systemic antiviral treatment stopped at 8 weeks in the present series when viral DNA load was not detected, the duration was shorter than most other reports [18, 23, 24]. Long-term oral antiviral treatment is commonly used as maintenance treatment for ARN to stabilise the retina and avoid bilateral involvement. The duration varies greatly, typically many months in the previous published studies [2, 4], and the doctors were perhaps treating ARN for too long. An intensive antiviral treatment conducted in the present series might be one reason for this shorter antiviral duration, with cautious inference. This shorter duration of antiviral treatment can reduce the financial burden and the risk of liver or renal dysfunction. Whether this shorter course of treatment would increase the risk of contralateral eye involvement needs further observation. Firstly, bilateral ARN often results from delays in the initial diagnosis and treatment of the first involved eye [25], while in the present series all eyes were treated with a timely antiviral therapy. Secondly, as Palay et al. investigated, the risk of contralateral eye involvement was greatest in the first 14 weeks after diagnosis [26]; in the present series except for one patient suffering from bilateral ARN at initial presentation, the remaining 19 patients did not suffer from fellow eye involvement after a follow-up of more than 6 months. The incidence of contralateral eye involvement was not higher than that of 11.10–22.70% in prior studies

Table 3 Summary of previous studies of the time-profile of viral DNA load determined by real-time qPCR in ARN patients.

Studies		Bernheim, 2013	Hafidi, 2019	The present series
Study period		2005–2009	2010.5–2016.12	2018.12–2019.11
Cases		6 eyes/6 patients	25 eyes/24 patients	20 eyes/20 patients
Average age, years (range)		52.0 ± 13.0 (35–68)	58.0 (20–86)	49.5 ± 11.3 (38–73)
Sex		1 female, 5 males	13 females,11 males	7 females, 13 males
Average duration from symptoms to presentation, days(range)		–	7.0 (1–45)	10.4 ± 5.1 (3–20)
Causal virus (n, %)		VZV: 6/6 (100%)	VZV: 15/24 (63%); HSV1: 4/24 (17%); HSV2: 3/24 (13%); CMV: 2/24 (8%)	VZV: 20/20 (100%)
Immune dysfunction (n, %)		2/6 (33%)	8/24 (33%)	None
Systemic antiviral therapy		Acyclovir intravenously and valaciclovir orally	Personalised, acyclovir/foscarnet/ganciclovir intravenously and valaciclovir/valganciclovir orally	Acyclovir intravenously and valaciclovir orally
Intravitreal therapy	Regimen	2 mg/0.1 ml ganciclovir; Or 2.4 mg/0.1 ml foscarnet	2 mg/0.05 ml ganciclovir; Or 2.4 mg/0.1 ml foscarnet	4 mg/0.1 ml ganciclovir
	Frequency	Weekly	Twice-weekly for the duration of intravenous treatment; followed by weekly injections	Twice-weekly
	Numbers	Mean:8.8 ± 5.3 Median: 8.0 Range:4–18	Mean: not provided Median: 9.0 Range: 0–28	Mean:7.1 ± 2.2 Median: 7.5 Range:1–9
Viral load kinetics	Mean initial load, copies/ml (range)	$3.4 \times 10^7 \pm 4.5 \times 10^7$ $(6 \times 10^6 – 1.2 \times 10^8)$	8.1×10^5 for VZV group	$5.7 \times 10^7 \pm 9.7 \times 10^7$ $(8.6 \times 10^5 – 3.7 \times 10^8)$
	Plateau phase, days (range)	27.8 ± 24.9 (10–77)	15.5 (7–28)	0–1 week in 6 eyes (30.0%), 1–2 weeks in 12 eyes (60.0%) and 2–3 weeks in 1 eye (5.0%)
	Mean time to achieve a 50% reduction in viral load, days	3.0 ± 0.7	4.2 for VZV group	3.2 ± 1.0
	Time of undetectable viral load, days	Mean:104.2 ± 45.9 Range:64–178	Mean: 56.1	Up to 56 days in all 20 eyes (100%)
Retinal detachment rate		83%	16%	65%
Mean LogMAR BCVA, Snellen equivalent	Initial	0.9 ± 1.1, 20/160	0.8, 20/125	1.1 ± 0.7, 20/250
	Final	2.0 ± 1.4, CF	0.6, 20/80	0.7 ± 0.6, 20/100
Average follow-up, months (range)		17.6 ± 16.4	17.0 (2–24)	8.6 ± 2.0 (6–12)

ARN acute retinal necrosis, *BCVA* best corrected visual acuity, *CF* counting fingers, *CMV* Cytomegalovirus, *DNA* deoxyribonucleic acid, *HSV* Herpes simplex virus, *logMAR* logarithms of the minimum angle of resolution, *qPCR* quantitative polymerise chain reaction, *VZV* Varicella zoster virus.

[23, 27, 28]. After the first eye was actively infected by virus, it becomes latent in sensory neurons. The balance of the host's immune responses and viral status determined whether and when the contralateral eye will be involved. In some patients, contralateral eye involvement may occur even several years later [25]. There is no evidence on the length of antiviral treatment required to prevent contralateral eye involvement after the viral load turns negative in the affected eye. Thus, we administered antiviral treatment until the viral load turned negative in the affected eye, and carefully observed the contralateral eye.

The strengths of this study include its larger sample size of VZV-induced ARN patients compared with other similar studies, a consistent pathogenic virus in all cases, and the same antiviral therapy used in all patients. However, the study also had several limitations. Firstly, the study was retrospective and the patient data were reviewed from medical records. Secondly, for some patients with mild disease or early RD, the number of aqueous humour samples acquired was limited, resulting in the disability of some statistical analyses. Thirdly, antiviral resistance was not investigated in the series, which is valuable for developing

personalised antiviral treatment. Finally, although continuous aqueous sampling and qPCR measurements are useful in the treatment for ARN, the procedure must be conducted with caution considering its invasive nature; explorations for other noninvasive indicators to guide treatment for ARN are needed in the following studies.

In conclusion, real-time qPCR is useful in monitoring the changes of viral DNA load in aqueous humour samples in VZV-induced ARN patients, which followed a clearance kinetics including a plateau phase and a logarithmic reduction phase. Compared with a single simple PCR test, the viral DNA load kinetics measured by continuous real-time qPCR might be useful to monitor the therapeutic efficacy and decide the needed antiviral duration in VZV-induced ARN patients.

Summary

What was known before

- ARN is caused by herpes virus family infection, with VZV the most common causative pathogen.
- The viral DNA load reflects the extent of disease and response to antiviral treatment.

What this study adds

- The viral load displayed a kinetic pattern of decline after starting antiviral treatment, including a plateau phase and a logarithmic reduction phase.
- The viral load was below detection threshold after 8-weeks' antiviral treatment.
- The observation of viral load changes by qPCR was useful for better monitoring of therapeutic efficacy and deciding needed antiviral duration in VZV-induced ARN patients.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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