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# False negative result of polymerase chain reaction in very early stages of acute retinal necrosis

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## Abstract

**Background** Viral nucleic acid testing of intraocular fluid using polymerase chain reaction (PCR) is a major laboratory examination in the diagnosis of acute retinal necrosis (ARN). Importantly, false negative PCR results may occur in several special situations. We reported a case of ARN with a negative PCR result in the aqueous humour in the very early stages of disease.

**Case presentation** A female patient presented to the ophthalmologist with complaints of blurred vision and redness in her left eye. Her medical history included ARN in her right eye 10 years prior. Although the result of the aqueous viral analysis by PCR in her left eye was negative the first time (one day after the appearance of ocular symptoms), ARN in her left eye was presumed based on the clinical signs. With timely antiviral and anti-inflammatory treatments, the retinal lesions diminished. The viral load of herpes simplex virus (HSV) turned positive ( $7.25 \times 10^3$  copies/mL) one week later, increased to  $2.49 \times 10^5$  copies/mL after three weeks, and finally turned negative about five weeks after the onset of disease. The initial HSV-IgG level in the aqueous humour was 0.01 U/mL and increased to 222.64 U/mL in the final sampling.

**Conclusions** The results of PCR analysis can be negative in the very early stages of ARN. Diagnosis of ARN should be made based on the clinical features, and antiviral treatments should not be delayed. Repeated PCR analysis of the aqueous humour is necessary to confirm the diagnosis and monitor the disease process.

**Keywords** Acute retinal necrosis, Polymerase chain reaction, Aqueous humour, False negative result, Case report

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## Background

Acute retinal necrosis (ARN) is a severe sight-threatening ocular disease characterized by iritis, vitreitis, occlusive retinal vasculitis and necrotizing retinitis. Varicella zoster virus (VZV) and herpes simplex virus (HSV) are two leading causes of ARN. Bilateral involvement of ARN occurs in approximately one-third of patients [1], and the onset of intraocular inflammation in both eyes usually occurs at intervals of several days or weeks [2]. Delayed recurrence of ARN in the second eye, although rare, has been reported to occur up to 46 years later [3]. For a long time, the diagnosis of ARN depended on the clinical findings [4]. With the development of biological technology,

analysis of polymerase chain reaction (PCR) and the Goldmann-Witmer coefficient (GWC) were added to further confirm the diagnosis of ARN and to identify the causative virus [5]. These testing methods, usually PCR, are widely used in the diagnosis and treatment of ARN to monitor the activity of the disease [6] and estimate prognosis [7].

In this study, we present a case of ARN in which PCR analysis showed false negative results in the very early stages of disease. Bilateral occurrence of ARN in both eyes with an interval of 10 years occurred in this case, which was uncommon in the clinic.

### Case presentation

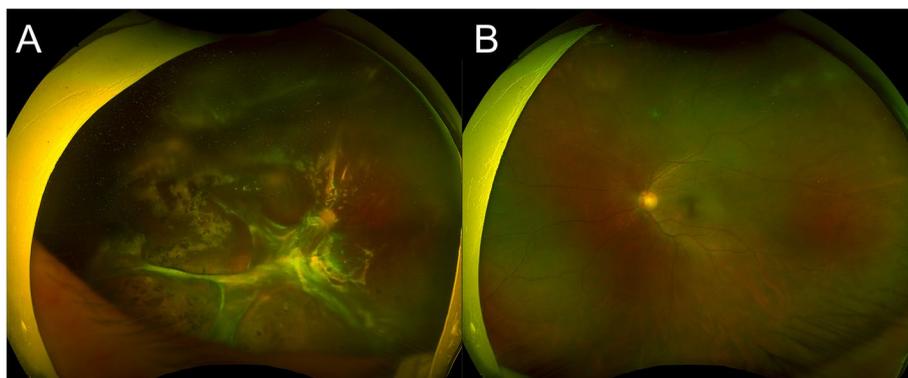
A 38-year-old female patient presented to the ophthalmologist on 16 January 2022 with complaints of blurred vision and redness in her left eye for one day. She had suffered sudden visual loss in her right eye 10 years prior, and was diagnosed with ARN in her right eye according to the standard diagnostic criteria for acute retinal necrosis syndrome [4]. No molecular, biological or immunological detection method was applied due to technical limitations at that time. Vitrectomy and silicone oil injections were performed due to rhegmatogenous retinal detachment.

On initial examination, best-corrected visual acuity (BCVA) was 20/70 (Snellen visual acuity chart) in the left eye and light perception (LP) in the right eye. The intraocular pressure (IOP) was 38 mmHg in the left eye and 17 mmHg in the right eye. Slit-lamp examination revealed conjunctival hyperaemia, mutton-fat keratic precipitates in the middle and below the corneal endothelium, and 3+ flare in the anterior chamber in the left eye. The anterior segment of the right eye was quiet. Dilated fundus examination of the left eye showed mild vitritis, peripheral retinal whitening and mild retinal vasculitis.

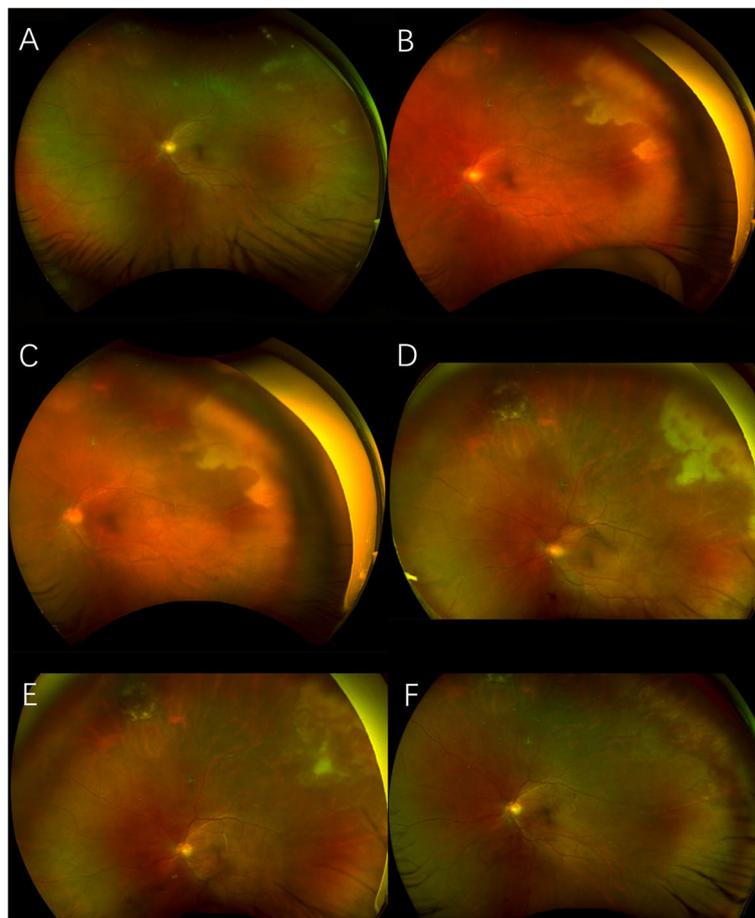
An exam of the right eye showed silicone tamponade in the vitreous cavity and extensive pre-retinal proliferation (Fig. 1). ARN in the left eye was presumed, and treatments including intravenous acyclovir (1500 mg/day), intravitreal injections of ganciclovir (3 mg) every four days and oral prednisolone were given.

To confirm the diagnosis of ARN and determine the causative virus, about 100 $\mu$ L aqueous humour was obtained through an aqueous tap. Real-time quantitative PCR (qPCR) of four common human herpes viruses (HSV, VZV, CMV, EBV) were performed. For qPCR, a mixture of about 20  $\mu$ L intraocular fluid and 20  $\mu$ L nucleic acid extraction liquid (NaCl+EDTA-Na<sub>2</sub>) was incubated at 99 °C for 10 min, then centrifuged at 13,000 rpm for 10 min. The recovered supernatant was used for qPCR assay using HSV (Z-SD-0017-02), VZV (Z-OD-0024-02), CMV (Z-OD-0022-02), and EBV (Z-OD-0023-02-25) real-time PCR kits (Liferiver, Shanghai, China), according to the manufacturer's instructions. Immunoglobulin G (IgG) measurements of these four viruses were also conducted via enzyme-linked immunosorbent assay (ELISA). Four antibody detection kits, including ESR-105G (HSV1&2-IgG), ESR-104G (VZV-IgG), ESR-109G (CMV-IgG) and ESR-1361G (EB-VCA-IgG) (Institute Virion/Serion GmbH, Würzburg, Germany) were used according to the manufacturer's instructions.

It was surprising that the results of qPCR for all four viruses were negative. We also did not find any increase in aqueous viral antibody levels. Fundus examination showed an apparent enlargement and integration of retinal lesions towards the posterior pole (Fig. 2). Taking the typical clinical characteristics into consideration, we insisted on the diagnosis of ARN in the left eye and postulated a false negative result from the qPCR. Antiviral treatment was continued. Another aqueous tap was performed on 24 January 2022, about one



**Fig. 1** Bilateral ultra-wide field fundus photography taken at the onset of ARN in the left eye. **A** Silicone tamponade in the vitreous cavity, extensive pre-retinal proliferation and retinal scarring could be observed in the right eye. **B** Mild vitritis and peripheral retinal whitening could be observed in the left eye



**Fig. 2** Changes in retinal lesions in the process of ARN. First, the peripheral yellowish-white lesions enlarged quickly towards the posterior pole and then diminished gradually with intensive antiviral and anti-inflammation treatments. **A** Three days after the onset of ARN in the left eye. **B** Ten days after the onset of ARN in the left eye. HSV DNA load in the aqueous humour was  $7.25 \times 10^3$  copies/mL at this time point. **C** Thirteen days after the onset of ARN in the left eye. **D** About 3 weeks after the onset of ARN in the left eye. HSV DNA load in the aqueous humour was  $2.49 \times 10^5$  copies/mL at this time point. **E** About 5 weeks after the onset of ARN in the left eye. HSV DNA load in the aqueous humour turned negative at this time point. **F** Thirty-seven days after the onset of ARN in the left eye, which showed nearly complete disappearance of retinal lesions in fundus photography

week after the first sampling, which showed positive HSV DNA load in the aqueous humour ( $7.25 \times 10^3$  copies/mL). Meanwhile, aqueous HSV-IgG was 1.93 U/mL. Despite initial enlargement, the necrotic retinal lesions diminished after intensive antiviral and anti-inflammation treatments (Fig. 2). The HSV DNA load in the aqueous humour was  $2.49 \times 10^5$  copies/mL on 6 February 2022 and finally turned negative on 17 February 2022, about five weeks after the onset of disease. The level of HSV-IgG in the aqueous humour increased to 276.08 U/mL and 222.64 U/mL at these two time points. BCVA in the left eye was maintained at 20/30. The changes in BCVA, the HSV DNA load and the level of HSV-IgG in the aqueous humour are listed in Table 1.

**Table 1** The variation of BCVA and the viral load of HSV in the aqueous humour

Date	BCVA (Snellen visual acuity chart)	Aqueous HSV load (copies/mL)	Aqueous HSV-IgG (U/mL)
16.Jan.2022	20/70	(-)	0.01
24.Jan. 2022	20/100	$7.25 \times 10^3$	1.93
6.Feb.2022	20/70	$2.49 \times 10^5$	276.08
17.Feb.2022	20/30	(-)	222.64

BCVA Best corrected visual acuity, HSV Herpes simplex virus

### Discussion and conclusions

PCR analysis of intraocular fluid samples is a useful tool in the diagnosis and monitoring of subsequent treatments

for viral posterior uveitis [8–10]. Its high specificity has been verified in previous studies, which have shown that PCR results are negative in most eyes without herpetic uveitis, even in the presence of positive serum antibodies [11]. However, the sensitivity of PCR varies among different studies. Schoenberger et al. reviewed studies with relevance to PCR testing of intraocular fluid samples in the setting of ARN and reported a positive rate of HSV or VZV DNA between 70 and 100% [12]. Several factors can cause a PCR false negative result, including limited amounts of DNA that are lower than required for detection, nucleolysis due to improper sampling or transportation, existence of nucleic acid amplification inhibitor in the reaction system and mutation of the primer binding site [13]. In this case, the most likely reason was the low aqueous HSV DNA load in the very early stages of ARN, which could not be identified by qPCR. The patient had almost no vision in her right eye due to ARN occurring 10 years prior, and used only her left eye in daily life. It was reasonable to deduce that she could easily perceive the loss of vision in her left eye and saw the ophthalmologist earlier than patients with unilateral ARN.

The change in HSV-IgG in the aqueous humour in this case reflected the host response to the viral infection during disease progression. Errera et al. studied a series of intraocular fluid samples from patients with ARN and proposed that positive PCR results were obtained from 24 h to 10 weeks, but GWC was positive after one week to three months after the onset of signs [14]. In our study, GWC was not calculated because no serum sample was collected. However, it was obvious that the viral antibody in the aqueous humour was maintained at a low level until three weeks after the onset of disease, which indicated that antibody tests in the early stages of ARN should be interpreted with prudence.

Another interesting point in this case was the delayed occurrence of ARN in the second eye after 10 years. The pathogenetic mechanism of bilateral ARN is not fully understood, but the virus is thought to spread via the optic nerve, optic chiasm, parasympathetic pathways and suprachiasmatic nucleus [15–17]. Okunuki et al. [18] reported four cases of delayed occurrence of ARN with latency periods ranging from 3 to 19 years and found that the causative virus species were the same in both eyes. They suggested that the reactivated latent virus that affected the first eye was the causative virus of the disease in the other eye, rather than the second eye being affected by a different virus. It is regretful that the patient in our case did not receive PCR analysis of her intraocular fluid sample 10 years ago, so it was unknown if the causative virus in her two eyes was the same. A previous study also reported less severe inflammation and a lower chance of receiving

surgical treatment in the second eye compared to the first affected eye [18], which corresponded to the visual outcome (LP in the right eye and 20/30 in the left eye) and treatment outcome (vitrectomy and silicone oil injections performed in the right eye but not in the left eye) in our case. We propose that the earlier diagnosis and antiviral treatment might contribute to a better prognosis of the eye with later onset.

In conclusion, we reported a case of bilateral ARN with a 10-year interval after the initial onset. The PCR analysis of the aqueous humour did not identify any causative virus the first time, which indicated the false negative possibility of PCR analysis at the onset of ARN. Antibody test results were also low in the early stages of disease. Diagnosis of ARN should be made based on the clinical features, and antiviral treatments should not be delayed. Repeated PCR analysis of the aqueous humour is necessary to further confirm the diagnosis and monitor the disease process.

#### Abbreviations

ARN	Acute retinal necrosis
PCR	Polymerase chain reaction
BCVA	Best-corrected visual acuity
IOP	Intraocular pressure
LP	Light perception
HSV	Herpes simplex virus
IgG	Immunoglobulin G

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Not applicable.

#### Authors' contributions

HBW and LC collected and analyzed the data; ZYQ and BCL drafted the manuscript; JXZ, LW and YT participated in the laboratory examination and case analysis; LJZ and LJ revised the manuscript and gave final approval of the version to be published. All authors read and approved the final manuscript.

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#### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

#### Declarations

##### Ethics approval and consent to participate

The patient provided written informed consent for the sample acquisition and publication of this case report. The requirement for review board approval was waived because of the nature of this study (case report).

##### Consent for publication

The work has not been published before and is not under consideration for publication elsewhere. Written informed consent to publish this information was obtained from study participant.

##### Competing interests

The authors declare no competing interests.

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## References

- Lee JH, Agarwal A, Mahendradas P, Lee CS, Gupta V, Pavesio CE, Agrawal R (2017) Viral posterior uveitis. *Surv Ophthalmol* 62(4):404–445
- Lei B, Jiang R, Wang Z, Xu G, Wu X, Zhou M (2020) Bilateral acute retinal necrosis: a case series. *Retina* 40(1):145–153
- La Cava M, Abbouda A, Restivo L, Zito R (2015) Delayed onset of bilateral acute retinal necrosis syndrome: a 46-year interval. *Semin Ophthalmol* 30(2):146–149
- Holland GN (1994) Standard diagnostic criteria for the acute retinal necrosis syndrome. Executive Committee of the American Uveitis Society. *Am J Ophthalmol* 117(5):663–667
- Takase H, Okada AA, Goto H, Mizuki N, Namba K, Ohguro N, Sonoda KH, Tomita M, Keino H, Kezuka T, Kubono R, Mizuuchi K, Shibuya E, Takahashi H, Yanai R, Mochizuki M (2015) Development and validation of new diagnostic criteria for acute retinal necrosis. *Jpn J Ophthalmol* 59(1):14–20
- Lei B, Wang Z, Shu Q, Gu R, Zhang Y, Jiang R, Chang Q, Zhou M, Xu G (2022) Observation of varicella zoster virus-induced acute retinal necrosis: viral load detection and visual outcome. *Eye (Lond)* 36(6):1209–1216
- Calvo CM, Khan MA, Mehta S, Garg SJ, Dunn JP (2017) Correlation of clinical outcomes with quantitative polymerase chain reaction DNA copy number in patients with acute retinal necrosis. *Ocul Immunol Inflamm* 25(2):246–252
- Sugita S, Shimizu N, Watanabe K, Mizukami M, Morio T, Sugamoto Y, Mochizuki M (2008) Use of multiplex PCR and real-time PCR to detect human herpes virus genome in ocular fluids of patients with uveitis. *Br J Ophthalmol* 92(7):928–932
- Tran TH, Rozenberg F, Cassoux N, Rao NA, LeHoang P, Bodaghi B (2003) Polymerase chain reaction analysis of aqueous humour samples in necrotising retinitis. *Br J Ophthalmol* 87(1):79–83
- Rothova A, de Boer JH, Ten Dam-van Loon NH, Postma G, de Visser L, Zuurveen SJ, Schuller M, Weersink AJ, van Loon AM, de Groot-Mijnes JD (2008) Usefulness of aqueous humor analysis for the diagnosis of posterior uveitis. *Ophthalmology* 115(2):306–311
- Pendergast SD, Werner J, Drevon A, Wiedbrauk DL (2000) Absence of herpesvirus DNA by polymerase chain reaction in ocular fluids obtained from immunocompetent patients. *Retina* 20(4):389–393
- Schoenberger SD, Kim SJ, Thorne JE, Mruthyunjaya P, Yeh S, Bakri SJ, Ehlers JP (2017) Diagnosis and treatment of acute retinal necrosis: a report by the American Academy of Ophthalmology. *Ophthalmology* 124(3):382–392
- Li JM (2016) *The technology of the real-time fluorescent PCR[M]*, 2nd edn. Science Press, Beijing
- Errera MH, Goldschmidt P, Batellier L, Degorge S, Héron E, Laroche L, Sahel JA, Westcott M, Chaumeil C (2011) Real-time polymerase chain reaction and intraocular antibody production for the diagnosis of viral versus toxoplasmic infectious posterior uveitis. *Graefes Arch Clin Exp Ophthalmol* 249(12):1837–1846
- Vann VR, Atherton SS (1991) Neural spread of herpes simplex virus after anterior chamber inoculation. *Invest Ophthalmol Vis Sci* 32(9):2462–2472
- Labetoulle M, Kucera P, Ugolini G, Lafay F, Frau E, Offret H, Flamand A (2000) Neuronal pathways for the propagation of herpes simplex virus type 1 from one retina to the other in a murine model. *J Gen Virol* 81(Pt 5):1201–1210
- Atherton SS (2001) Acute retinal necrosis: insights into pathogenesis from the mouse model. *Herpes* 8(3):69–73
- Okunuki Y, Usui Y, Kezuka T, Takeuchi M, Goto H (2011) Four cases of bilateral acute retinal necrosis with a long interval after the initial onset. *Br J Ophthalmol* 95(9):1251–1254

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