




# Comparison of clinical characteristics in patients with acute zonal occult outer retinopathy according to anti-retinal antibody status

Yuki Hashimoto<sup>1,2</sup> · Wataru Saito<sup>1,3</sup>  · Saho Kanaizumi<sup>1</sup> · Michiyuki Saito<sup>1</sup> · Kousuke Noda<sup>1</sup> · Atsuhiko Kanda<sup>1</sup> · Susumu Ishida<sup>1</sup>

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## Abstract

**Purpose** To evaluate the clinical characteristics of patients with acute zonal occult outer retinopathy (AZOOR), according to the presence or absence of anti-retinal antibodies (ARAs) that are frequently detected in autoimmune retinopathy.

**Methods** Retrospective observational case series. This study included 33 patients with acute-stage AZOOR who had been followed up for more than 6 months after the initial visit. The median follow-up period was 26 months. Immunoblot analyses were used to detect autoantibodies for recoverin, carbonic anhydrase II, and  $\alpha$ -enolase in serum from these patients. Main outcome measures comprised clinical factors at the initial and final visits, including best-corrected visual acuity, mean deviation on Humphrey perimetry, and retinal morphology, which were statistically compared between patients with AZOOR who exhibited ARAs and those who did not.

**Results** At least one serum ARA was detected in 42% of patients with AZOOR. There were no significant differences in clinical factors between the two groups, including follow-up period, best-corrected visual acuity and mean deviation at the initial and final visits, a-wave amplitude on single-flash electroretinography at the initial visit, and frequencies of improvement of the macular ellipsoid zone and AZOOR recurrence.

**Conclusions** Our findings suggest that the presence of ARAs did not influence visual outcomes or outer retinal morphology in patients with AZOOR.

## Key messages

- The involvement of anti-retinal antibodies (ARAs) in the pathogenesis of acute zonal occult outer retinopathy (AZOOR) remains controversial at present.
- There were no significant differences in clinical factors between patients with AZOOR who exhibited ARAs and those who did not, including best-corrected visual acuity and mean deviation at the initial and final visits, and frequencies of improvement of the macular ellipsoid zone and AZOOR recurrence.
- These results suggest that ARAs have no pathogenicity for the retina of AZOOR.

**Keywords** Acute zonal occult outer retinopathy ·  $\alpha$ -enolase · Anti-retinal antibodies · Carbonic anhydrase II · Macular ellipsoid zone · Recoverin

## Introduction

Acute zonal occult outer retinopathy (AZOOR) is an idiopathic disease characterized by the development of a zonal area of acute outer retinal impairment, despite normal funduscopic retinal appearance at the initial stage [1, 2]. The outer retinal impairment is demonstrated by diminished responses

✉ Wataru Saito  
wsaito@med.hokudai.ac.jp

Extended author information available on the last page of the article

on full-field or multifocal electroretinography [3] and disrupted ellipsoid zone (EZ) detected with spectral domain optical coherence tomography (OCT) [4].

In Japanese patients with AZOOR, spontaneous improvement of visual function with good visual prognosis is more frequent, and the development of late-onset retinal degeneration corresponding to the zone of visual loss is less frequent than in Caucasian ones [2, 5–7]. These observations suggest that clinical features of AZOOR differ according to ethnicity and the Japanese patients have better visual prognosis. Strategies for the management of AZOOR are not clearly established thus far. However, several reports have suggested that systemic corticosteroid therapy is effective for patients with progressive visual impairment [5, 8, 9].

Autoimmune retinopathy (AIR) is a syndrome caused by the development of anti-retinal antibodies (ARAs), which result in retinal degeneration. Autoantibodies against retinal antigens, especially  $\alpha$ -enolase, carbonic anhydrase II, and recoverin, are present in the majority of patients with AIR who exhibit ARAs [10]. These antibodies are pathogenic for retinal tissues including photoreceptors [11–14]. Visual function generally worsens in a progressive manner over a period of weeks to months in patients with AIR who exhibit anti-recoverin antibody [15, 16], whereas it takes over a period of years to worsen in patients with anti- $\alpha$ -enolase antibody [17, 18]. Patients with AIR tend to have poor visual prognosis, despite administration of systemic immunosuppressive agents [15, 18]. AIR is diagnosed on the basis of the clinical features described above, as well as the presence of ARAs on immunoblot analysis; therefore, an important consideration for each patient is whether antibodies against recoverin and/or  $\alpha$ -enolase are present [19].

Thus far, two major hypotheses have been proposed regarding the mechanism underlying outer retinal impairment in patients with AZOOR [20]. The first hypothesis comprises the choroidal impairment theory [8, 21], on the basis of the prerequisite that the choriocapillaris supplies oxygen and nutrition to the outer retinal layers. Indocyanine green angiography has revealed hypofluorescence in areas related or unrelated to AZOOR lesions [5, 8, 22, 23]. Notably, subfoveal choroidal thickness significantly decreases and choroidal blood flow velocity increases, in combination with improvements in visual function and outer retinal morphology [8, 24]. Moreover, changes in choroidal thickness are negatively correlated with changes in sensitivity on perimetry [24]. These observations suggest a relationship between choroidal circulatory impairment and AZOOR pathogenesis. However, the choroidal impairment in patients with AZOOR might result from primary damage to photoreceptors [4, 21].

The second hypothesis comprises the ARA theory, on the basis of the observation that the features with normal fundus appearance, along with photoreceptor

impairment, in the early stages of AZOOR are similar to features present in patients with AIR. Immunoblot analyses have revealed non-specific ARAs in serum samples of all patients with AZOOR [25, 26]. In addition, anti- $\alpha$ -enolase antibody was detected in the serum of 26% of patients with AZOOR; anti-carbonic anhydrase II antibody was detected in the serum of 17% of these patients [27]. Based on these results, some clinicians have proposed that ARAs are involved in AZOOR pathogenesis [25, 26]. However, to the best of our knowledge, there remains no clear evidence of the relationship between ARAs and clinical characteristics of AZOOR [25–28]. Therefore, ARAs may be non-pathogenic for the retina if they are secondarily produced following exposure of retinal antigens at the onset of AZOOR [29]. Here, we hypothesized that visual prognosis in patients with AZOOR is worse in those with ARAs than in those without ARAs. To examine the association of ARAs with the pathogenesis of AZOOR, the aims of the present study were to determine the rates of detection regarding the three representative ARAs frequently associated with AIR (i.e., autoantibodies for recoverin, carbonic anhydrase II, and  $\alpha$ -enolase) in patients with AZOOR, and to evaluate clinical features of patients with AZOOR according to ARA status.

## Methods

### Patients

This retrospective observational case series included 33 patients (45 eyes) with AZOOR who visited Hokkaido University Hospital from October 2004 to March 2015. Patients were included if they had acute-stage AZOOR and had been followed up for more than 6 months after the initial visit. Patients were excluded if they had convalescent AZOOR at the initial visit or if they had AZOOR in combination with any disease of AZOOR complex such as multiple evanescent white dot syndrome. The presence of serum autoantibodies against recoverin, carbonic anhydrase II, and  $\alpha$ -enolase was examined in patients with AZOOR by using immunoblot analyses (Supplemental Fig. 1). Patients with AZOOR were divided into the ARA and non-ARA groups according to the presence or absence of  $\geq 1$  ARA, respectively. Characteristics including visual function at the initial and final visits were compared between the two groups. This study was approved by the ethics committee of Hokkaido University Hospital (approval number 020–0236) and was performed in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from each patient after the nature and potential consequences of the study had been explained.

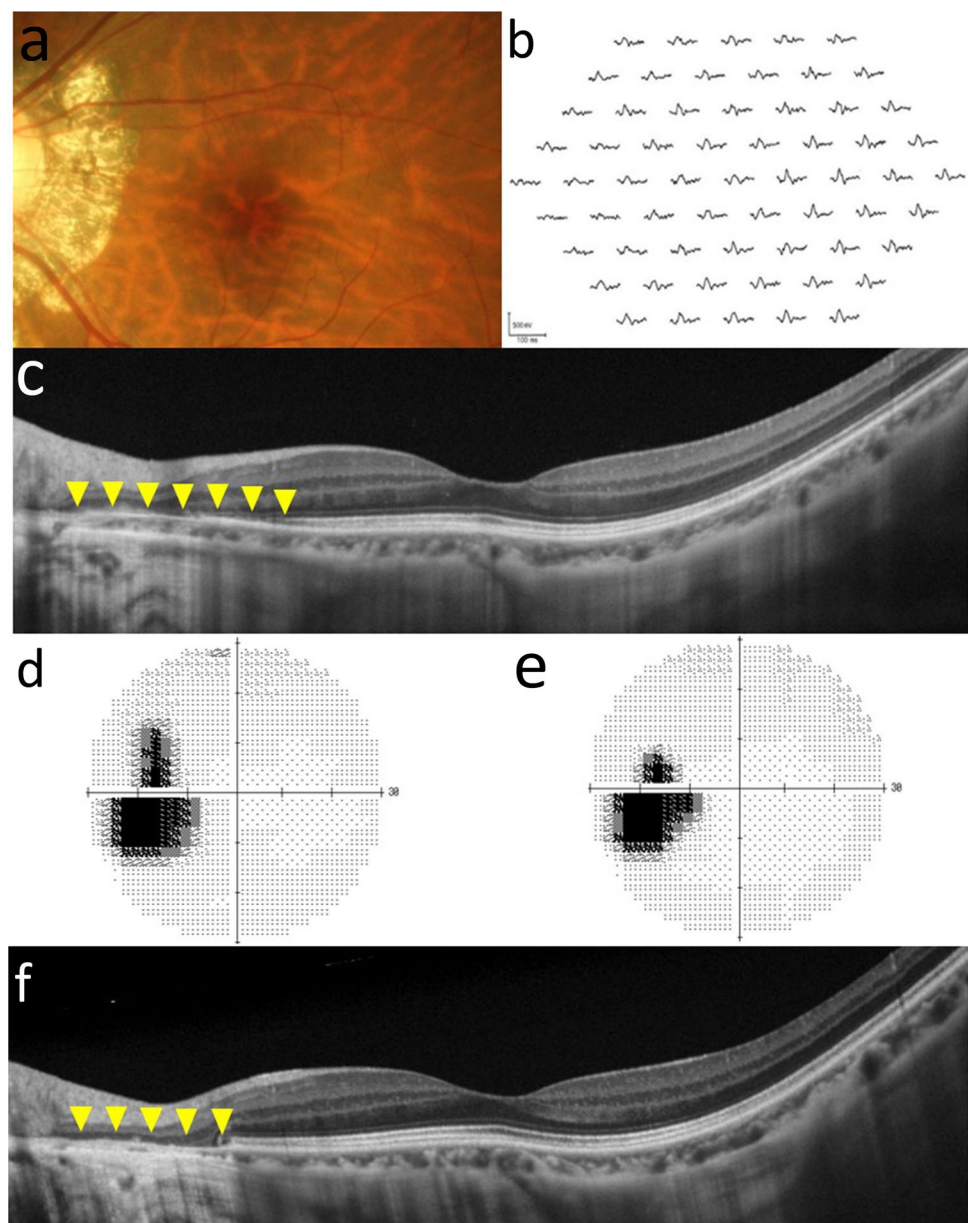
## Diagnosis

AZOOR was diagnosed for patients who fulfilled all of the following criteria [2, 5]: (i) acute visual field or vision loss, typically with concurrent photopsia; (ii)  $\geq 1$  visual field defect regions that could not be explained by the results of fundusoscopic examination or fluorescein angiography; (iii) reduced multifocal electroretinography responses corresponding to retinal sites with visual field defects; (iv) negative screening results for infection, including syphilis and tuberculosis; in addition to (i)–(iv), (v) beginning in 2007, outer retinal morphologic abnormalities, including absence or discontinuity of the EZ and/or interdigitation zone on OCT (available in 30 of 33 patients). A representative case is shown in Fig. 1.

## Ophthalmologic examinations

At their initial visit, patients underwent comprehensive ophthalmic examinations, including decimal best-corrected visual acuity (BCVA) measurement, indirect ophthalmoscopy, color fundus photography, fluorescein angiography, spectral domain and/or enhanced depth imaging OCT (OCT Ophthalmoscope C7, RS-3000, or RS-3000 Advance; both from Nidek, Gamagori, Japan), and 20 J scotopic single-flash electroretinography (LE-3000, Tomey, Nagoya, Japan), followed several days later by visual field testing (Goldmann perimetry and/or the Humphrey 30–2 Swedish interactive threshold algorithm standard test) and multifocal electroretinography (VERIS; EDI, San Mateo, CA, USA). BCVA assessment, Humphrey

**Fig. 1** Images of the left eye in a 43-year-old patient with acute zonal occult outer retinopathy (AZOOR) with anti- $\alpha$ -enolase antibody (case 14). **a** Fundus photograph at the initial visit shows no abnormal findings, except peripapillary atrophy. **b** Multifocal electroretinography shows reduced amplitudes corresponding to the retinal site with a visual field defect (**d**). **c** Horizontal image through the fovea on enhanced depth imaging optical coherence tomography (EDI-OCT) at the initial visit shows loss of the ellipsoid zone (EZ) (arrowheads), corresponding to the AZOOR lesion site. **d, e** On Humphrey perimetry, a blind spot enlargement observed at the initial visit (**d**) shrunk at the final visit (**e**). **f** On EDI-OCT, the area of EZ loss at the final visit was shortened, compared with the corresponding area at the initial visit



perimetry, fundus photography, and OCT were performed during follow-up. Two investigators (Y.H. and W.S.) routinely evaluated the development of late-onset zonal retinal atrophy by comparing fundus photographs taken at the initial visit and more than 6 months later.

## Treatment

Patients were not treated if they exhibited no central visual acuity loss at the initial visit with no clinical progression thereafter. Patients with progressive central visual acuity loss received systemic corticosteroid therapy including corticosteroid pulse therapy, as previously described [8].

## Blood samples

Blood samples were obtained at the initial visit in patients with AZOOR and were centrifuged at 3000 rpm for 10 min at 4°C to collect the serum. The serum was then carefully transferred into a polypropylene tube and stored at -80°C. Between November and December 2015, all the samples were thawed and immediately processed for immunoblot analyses.

## Immunoblot analyses for recoverin, carbonic anhydrase II, and $\alpha$ -enolase

The methods used for production and purification of glutathione S-transferase (GST) fusion recoverin protein and immunoblot analyses were described previously [30]. Recombinant human carbonic anhydrase II and  $\alpha$ -enolase proteins were purchased from Biovision (Milpitas, CA, USA) and ATGen (Gyeonggi-do, South Korea), respectively. Antibodies for protein detection were patients' and normal sera (1/2000 dilution), anti-recoverin antibody (1:20,000 dilution, Merck Millipore, Billerica, MA, USA), anti- $\alpha$ -enolase antibody (1:2000 dilution, Santa Cruz Biotechnology, Dallas, TX, USA), and anti-carbonic anhydrase II antibody (1:2000 dilution, Abcam, Cambridge, MA, USA). The normal sera used as negative serum controls were previously proven to be seronegative for ARAs [18, 30, 31], and GST was electrophoresed as a negative antigen control followed by each antibody or serum application [30, 31]. Preliminary results regarding the rates of detection (14% in total) for antibodies against recoverin (5%), carbonic anhydrase II (0%), and  $\alpha$ -enolase (10%) were determined in a study of 21 patients with idiopathic epiretinal membrane [18]. Given the non-autoimmune etiology of idiopathic epiretinal membrane, these results were similar to the previously reported detection rate (9% for  $\alpha$ -enolase) in normal healthy individuals [12], confirming the reliability of our detection system.

## Statistical analyses

Values were expressed as the mean  $\pm$  standard deviation. Decimal BCVA was converted to the logarithm of the minimum angle of resolution (logMAR). The Mann–Whitney *U* test or Fisher's exact test was used to compare patient demographics and clinical characteristics (e.g., logMAR values of BCVA and mean deviation [MD] values on Humphrey perimetry) at the initial and final visits between ARA and non-ARA groups. For all tests,  $P < 0.05$  was considered statistically significant. Significant improvement and deterioration in visual functions during follow-up were defined as logMAR BCVA change of  $\geq 0.3$  and MD value change of  $\geq 30\%$ .

## Results

### Rates of ARA detection in patients with AZOOR

At least one serum ARA was detected in 14 (42%) of 33 patients with AZOOR with 3 (9%), 4 (12%), and 10 patients (30%) having antibodies against recoverin, carbonic anhydrase II, and  $\alpha$ -enolase, respectively (Supplemental Fig. 1).

### Baseline clinical parameters

Clinical characteristics of patients in the ARA and non-ARA groups are summarized in Tables 1 and 2. Regarding baseline parameters, no statistically significant differences were detected between the two groups in any of the following parameters: age, follow-up period, refractive error, bilaterality, photopsia, a-wave amplitude of single-flash electroretinography, logMAR BCVA, and MD value at the initial visit; however, sex and blind spot enlargement significantly differed between groups (Table 3). Moreover, when patients with anti- $\alpha$ -enolase antibody alone were compared with patients who had no ARAs, only spherical equivalent significantly differed between the two groups (Supplemental Table 1).

### Clinical parameters at the final visit

Parameters at the final visit did not significantly differ between the two groups with respect to logMAR BCVA or MD values; the frequencies of patients who received systemic corticosteroid therapy, who exhibited improvement of macular EZ, who exhibited development of zonal retinal atrophy, and who exhibited AZOOR recurrence were also similar between groups. Moreover, when patients with anti- $\alpha$ -enolase antibody alone were compared with patients who had no ARAs, there were no significant differences between the two groups in any of the parameters at the final visit (Supplemental Table 1).

**Table 1** Clinical characteristics of patients with acute zonal occult outer retinopathy who exhibited anti-retinal antibodies

Case	Age/sex	Serum ARAs	Follow-up period (months)	Bilaterality	Photopsia (R/L)	Spherical equivalent (R/L)	BSE (R/L)	Baseline logMAR BCVA (R/L)	Final logMAR BCVA (R/L)	Baseline MD value (dB) (R/L)	Final MD value (dB) (R/L)	a-wave amplitude of ERG ( $\mu$ V)	Improvement of EZ (R/L)	Recurrence of AZOOR (R/L)	Development of retinal atrophy (R/L)
1	23/F	$\alpha$ -enolase	40	-	-/-	-4.50/	+/	-0.2/	-0.2/	-7.40/	-0.16/	268/	+/	-/-	-/-
2	39/F	$\alpha$ -enolase	38	-	+/	-5.00/	-/-	0.1/	-0.2/	-6.48/-	0.32/	390/	+/	-/-	-/-
3	29/F	$\alpha$ -enolase	38	-	+/	-1.00/	-/-	-0.2/	-0.2/	-9.23/	0.35/	223/	+/	-/-	-/-
4	28/F	$\alpha$ -enolase	34	-	/-	/-1.00	-/-	/-0.2	/-0.1	/-2.50	/-0.29	425	ND	/-	/-
5	29/F	Recoverin	27	-	+/	/-6.00	/-	/0.0	/0.1	ND	ND	7316	/-	/-	/-
6	36/F	$\alpha$ -enolase	19	+	+/+	-4.00/-12.00	-/-	-0.1/1.15	-0.1/0.0	-1.29/-5.74	-0.24/-5.10	388/265	-/+	-/-	-/-
7	35/F	CAII	16	+	-/-	-13.50/-13.50	-/-	-0.2/-0.2	-0.2/-0.2	-4.85/-3.31	-5.02/-3.52	366/330	-/-	-/-	-/-
8	21/F	Recoverin/ $\alpha$ -enolase	16	-	-/-	-8.00/	+/	0.5/	0.0/	-10.32/	-8.98/	270/	+/	-/-	-/-
9	34/F	Recoverin/CAII/ $\alpha$ -enolase	6	-	+/	/-2.00	/-	/0.0	/-0.1	/-5.26	/-0.20	7369	+/	/-	/-
10	46/F	CAII	10	+	+/+	2.50/-2.75	-/-	-0.2/-0.2	-0.1/0.0	0.13/-0.53	0.83/1.63	431/429	-/-	-/-	-/-
11	61/F	$\alpha$ -enolase	9	-	+/	0.00/	-/-	-0.2/	-0.1/	ND	ND	455/	+/	-/-	-/-
12	39/F	$\alpha$ -enolase	11	-	+/	-2.00/	+/	0.4/	0.5/	-1.78/	-0.44/	449/	-/-	-/-	-/-
13	45/F	CAII	28	-	/-	0.00/	/-	-0.1/	-0.2/	0.55	1.20	ND	/-	/-	/-
14	43/F	$\alpha$ -enolase	54	+	+/+	-4.75/-5.00	+/+	-0.1/0.0	-0.1/0.0	-4.03/-4.00	-7.28/-1.88	296/ND	-/+	-/-	-/+

F female, ARAs anti-retinal antibodies, CAII carbonic anhydrase II, R right eye, L left eye, BSE Blind spot enlargement, ND not done, BCVA best-corrected visual acuity, logMAR logarithm of the minimum angle of resolution, MD mean deviation, ERG electroretinography, EZ ellipsoid zone, AZOOR acute zonal occult outer retinopathy

**Table 2** Clinical characteristics of patients with acute zonal occult outer retinopathy who did not exhibit anti-retinal antibodies

Case	Age/sex	Follow-up period (months)	Bilaterality	Photopsia (R/L)	Spherical equivalent (diopters) (R/L)	BSE (R/L)	Baseline logMAR BCVA (R/L)	Final logMAR BCVA (R/L)	Baseline MD value (dB) (R/L)	Final MD value (dB) (R/L)	a-wave amplitude of ERG ( $\mu$ V)	Improvement of EZ (R/L)	Recurrence of AZOOR (R/L)	Development of retinal atrophy (R/L)
1	37/F	39	+	-/-	-1.00/-0.75	-/-	-0.1/0.3	-0.2/-0.2	-25.16/-27.89	-17.83/-21.48	384/343	+/+	-/-	+/-
2	24/F	86	+	+/-	-8.75/-8.75	+/+	-0.1/-0.1	-0.1/0.0	ND	ND	342/325	-/-	-/-	+/-
3	36/M	92	+	+/+	0.00/-0.25	-/-	-0.1/0.4	-0.2/-0.2	ND	ND	90/75	+/+	+/+	-/-
4	35/M	13	-	+/+	/-8.00	+/+	/-0.2	/-0.2	ND	ND	366	ND	/-	-/-
5	30/M	25	-	-/-	-5.50/	+/+	0.0/	-0.2/	ND	ND	447/	ND	-/-	-/-
6	39/F	44	-	-/-	-13.50/	ND	0.4/	-0.1/	-22.60/	-5.51/	267/	+/	+/	-/-
7	32/F	9	-	+/+	0.00	+/+	/-0.2	/-0.2	/-13.88	0.17	425	+/	/-	/-
8	24/F	39	-	-/-	-17.00/	+/+	0.4/	-0.1/	-3.48/	-3.64/	285/	+/	-/-	-/-
9	43/M	42	-	+/+	-9.50/	+/+	0.0/	-0.2/	ND	-1.83/	184/	+/	-/-	-/-
10	23/M	26	+	+/+	-7.00/-9.50	-/+	-0.2/0.1	-0.1/-0.2	ND	-1.38/-2.17	367/382	+/+	-/-	-/-
11	47/F	18	-	/-	/-8.50	/-	/-0.1	/-0.1	/-10.19	/-1.80	224	+/	/-	-/-
12	45/F	9	-	+/+	/-16.50	+/+	0.3	/-0.2	/-8.37	/-2.39	229	+/	/-	+/
13	41/M	14	-	+/	-4.50/	ND	-0.2/	-0.2/	-5.82/	-5.54/	417/	-/-	-/-	-/-
14	39/F	8	-	+/	-1.00/	+/	0.0/	-0.2/	-10.77/	-9.34/	563/	-/-	-/-	-/-
15	42/F	89	-	+/	/-14.50	+/	0.2	0.1	/-19.26	/-10.83	164	+/	+/	+/
16	64/M	30	+	-/-	-5.75/-5.25	ND	0.0/-0.1	-0.1/0.0	-0.79/0.11	0.44/-1.08	ND	+/+	-/-	-/-
17	56/M	44	+	+/+	0.75/0.25	+/+	-0.2/0.0	0.0/0.0	-0.80/-1.19	-0.60/0.03	ND	+/+	-/-	-/-
18	17/F	17	+	-/-	-6.50/-6.25	+/+	-0.2/0.05	-0.1/-0.1	-1.24/-5.90	0.31/-0.49	327/323	-/+	-/-	-/-
19	20/F	6	+	+/+	-4.75/-6.75	-/+	-0.1/0.0	-0.1/0.0	-2.65/-4.28	-2.80/-4.26	239/254	-/-	-/-	-/-

F female, M male, R right eye, L left eye, BSE Blind spot enlargement, ND not done, BCVA best-corrected visual acuity, logMAR logarithm of the minimum angle of resolution, MD mean deviation, ERG electroretinography, EZ ellipsoid zone, AZOOR acute zonal occult outer retinopathy



**Table 3** Comparison of clinical parameters in patients with acute zonal occult outer retinopathy according to anti-retinal antibody status

	ARA group (14 patients, 18 eyes)	Non-ARA group (19 patients, 27 eyes)	<i>P</i> value
Age at the onset (years)	36.2 ± 10.1	36.5 ± 11.7	\$0.869
Male: female	0:14	8:11	#0.010
Follow-up period (months)	24.7 ± 13.9	34.2 ± 26.8	\$0.477
Spherical equivalent (diopters)	−4.8 ± 4.1	−6.2 ± 5.0	\$0.302
Bilaterality (patients)	4	8	#0.470
Photopsia (eyes)	12	16	#0.756
Initial logMAR BCVA	0.01 ± 0.34	0.07 ± 0.42	\$0.279
Initial MD value (dB)	−4.12 ± 3.11	−9.15 ± 8.52	\$0.121
a-wave amplitude on ERG (μV)	354.3 ± 72.2	305.3 ± 112.7	\$0.10
Blind spots enlargement (eyes)	5	16	#0.012
Use of systemic corticosteroids (patients)	5	8	#1.000
Final logMAR BCVA	−0.06 ± 0.16	−0.11 ± 0.08	\$0.332
Final MD value (dB)	−1.79 ± 3.10	−4.38 ± 5.78	\$0.070
Improvement of ellipsoid zone (eyes)	8	17	#0.194
Development of retinal atrophy (eyes)	1	4	#0.633
Recurrence of AZOOR (eyes)	0	4	#0.138

ARA anti-retinal antibody, *logMAR* logarithm of the minimum angle of resolution, *BCVA* best-corrected visual acuity, *ERG* electroretinography, *MD* mean deviation, *AZOOR* acute zonal occult outer retinopathy

# Fisher's exact test

§ Mann–Whitney *U* test

## Visual outcomes

Compared with initial values, the final logMAR BCVA improved in 3 eyes (17%) and 7 eyes (26%), remained unchanged in 15 eyes (83%) and 20 eyes (74%), and worsened in 0 eyes and 0 eyes in the ARA ( $N=18$ ) and non-ARA ( $N=27$ ) groups, respectively. The frequency of BCVA improvement did not significantly differ between the two groups ( $P=0.716$ ).

The mean MD values were significantly higher at the final visit than at the initial visit in both groups (Table 3, ARA group;  $P=0.006$ , non-ARA group;  $P=0.0003$ ). Compared with initial values, the final MD values improved in 11 eyes (69%) and 12 eyes (57%), remained unchanged in 4 eyes (25%) and 8 eyes (38%), and worsened in 1 eye (6%) and 1 eye (5%) in the ARA ( $N=16$ ) and non-ARA ( $N=21$ ) groups, respectively. The frequencies of improvement or deterioration of the MD value did not significantly differ between the two groups ( $P=0.515$  and  $P=1.000$ , respectively).

## Discussion

The present study showed that the detection rate of  $\geq 1$  specific ARAs, which are frequently detected in patients with AIR, was 42% in the serum from patients with AZOOR. However, there were no significant differences in clinical parameters including photoreceptor function at the initial

visit, visual functions (i.e., BCVA and visual field) at the initial and final visits, and the frequencies of improvement of macular EZ and AZOOR recurrence between patients with ARAs and those without ARAs.

We recently reported similar results in Vogt-Koyanagi-Harada (VKH) disease [31], the mechanism of which is presumed to differ from that of AIR. In VKH disease, choroiditis caused by activation of CD4 + T lymphocyte-mediated cellular immunity against melanocyte-associated proteins leads to outer retinal disorder with serous retinal detachment [32]. The detection rate of  $\geq 1$  of the three serum ARAs examined was 50% in patients with VKH disease. However, there were no significant differences in clinical parameters (e.g., visual outcomes, recovery of the macular EZ, and uveitis recurrences) after systemic corticosteroid therapy between patients with and without ARAs [31]. These results suggest that the ARAs produced did not influence visual prognosis, outer retinal morphology, and the chronicity of uveitis in patients with VKH disease and that these ARAs would reasonably be non-pathogenic for the retina. Although VKH disease is associated with activated cellular immunity, it may have such a high rate of ARA detection because autoimmunity for both T cells and B cells is activated during its acute stage.

In the present study, final logMAR BCVA was  $\leq 0.0$  in nearly all eyes of patients with AZOOR. Perimetric sensitivity at the final visit improved or remained unchanged in  $> 90\%$  of eyes in both groups, compared with sensitivity at the initial visit. These results suggest that Japanese

patients with AZOOR have good visual prognosis, regardless of the presence or absence of ARAs. Moreover, there were no significant differences in clinical parameters including visual outcomes, improvement of macular morphology, and AZOOR recurrence between patients with AZOOR who exhibited ARAs and those who did not. Therefore, the current results suggest that the ARAs detected did not influence visual outcomes or AZOOR disease activity and were thus non-pathogenic for the retina in patients with AZOOR, similar to the findings in patients with VKH disease. This is presumably because differences in the ARA epitopes cause the diverse range of pathogenicity (often little or no) depending on the corresponding ARAs [33]. Therefore, our results could not validate the causative relationship of ARAs with AZOOR pathogenesis in terms of clinical outcomes. Notably, our conclusions are supported by evident differences in many clinical parameters, such as age, sex ratio, refraction, binocularity, and BCVA at the initial and final visits between patients with AZOOR and those with AIR, both of whom exhibited anti- $\alpha$ -enolase antibodies (Table 4).

Approximately 30% of patients with AZOOR have a history of systemic autoimmune diseases, such as Hashimoto disease [2]. The results of several studies have suggested the effectiveness of systemic corticosteroids and adalimumab [5, 8, 9, 22, 34]. Based on these observations, the previously reported high rates of ARA seropositivity [25–28], and our present results, we presume that an autoimmune mechanism other than ARAs (e.g., activation of T cells) might be involved in AZOOR pathogenesis. Further studies are needed to investigate the more detailed mechanisms involved in AZOOR.

ARAs reportedly have been detected in patients with various retinal diseases such as retinitis pigmentosa, age-related macular degeneration, uveitis, and diabetic maculopathy [35–39]. However, the previous studies revealed

no relationships between clinical parameters and the presence of ARAs in patients with various types of uveitis [37, 38] or in our patients with VKH disease [31], consistent with the present findings. Therefore, the association of ARAs with disease pathogenesis could not be determined simply by assessing the presence of ARAs.

This study has some limitations. First, it only examined the prevalence of three specific ARAs. It would be difficult to compare the rate of ARA detection in patients with AZOOR between the present study and previous studies because of differences regarding immunoblot methods [25, 26]. Second, in the present study, the major anti-retinal antibody detected was anti- $\alpha$ -enolase antibody; however, cases with other antibodies showed no distinct features (Table 1) and there were still no significant differences between patients with anti- $\alpha$ -enolase antibody alone and none of the three ARAs in parameters at the baseline and final visits (Supplemental Table 1). Third, the interval between serum collection and ARA detection varied depending on patients. The variation of interval among samples, even though stored at  $-80^{\circ}\text{C}$ , may have caused the detection rate of ARAs to be underestimated. Finally, the number of female patients was higher in the ARA group than in the non-ARA group. This is presumably because, compared with men, women exhibit higher prevalence for systemic autoimmune diseases [40].

In conclusion, autoantibodies against three major retinal antigens frequently associated with the onset of AIR were detected in 42% of patients with AZOOR. There were no significant differences in clinical parameters, including visual outcomes and improvement of macular morphology, between patients with AZOOR who exhibited ARAs and those who did not. These results suggest that the ARAs detected did not influence visual prognosis or disease activity in patients with AZOOR. ARAs produced in patients

**Table 4** Comparison of clinical features between patients with acute zonal occult outer retinopathy who exhibited anti- $\alpha$ -enolase antibody and anti- $\alpha$ -enolase antibody-positive autoimmune retinopathy

	AZOOR (present study) (8 patients, 10 eyes)	AIR (reference 18) (25 patients, 49 eyes)	<i>P</i> value
Male:female	0:8	9:16	#0.073
Follow-up period (months)	37.2 ± 10.9	22.2 ± 21.9	\$0.096
Mean age at first visit (years)	30.3 ± 14.7 (23–61)	60.8 ± 16.7 (16–83)	\$ < 0.01
Spherical equivalent (diopters)	− 2.6 ± 1.8	− 0.9 ± 2.9	\$0.033
Bilaterality (patients)	2/8 (25.0%)	24/25 (96.0%)	# < 0.001
Blind spot enlargement (eyes)	4/10	4/49	#0.022
Initial logMAR BCVA	0.06 ± 0.40	0.16 ± 0.33	\$0.045
Final logMAR BCVA	− 0.12 ± 0.07	0.33 ± 0.54	\$ < 0.0001
Association of systemic tumor	0/8 (0.0%)	7/23 (30.4%)	#0.146

AZOOR acute zonal occult outer retinopathy, AIR autoimmune retinopathy, logMAR logarithm of the minimum angle of resolution, BCVA best-corrected visual acuity

# Fisher's exact test

\$ Mann–Whitney *U* test



with AZOOR are suggested to be non-pathogenic for the retina, and may be secondarily produced following immunologic exposure to retinal antigens caused by destruction of retinal tissues.

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

## Declarations

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The current study was approved by the ethics committee of Hokkaido University Hospital (#020–0236).

**Consent to participate** Informed consent was obtained from all individual participants included in the study.

**Consent for publication** Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

**Competing interests** The authors declare no competing interests.


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## Authors and Affiliations

Yuki Hashimoto<sup>1,2</sup> · Wataru Saito<sup>1,3</sup>  · Saho Kanaizumi<sup>1</sup> · Michiyuki Saito<sup>1</sup> · Kousuke Noda<sup>1</sup> · Atsuhiko Kanda<sup>1</sup> · Susumu Ishida<sup>1</sup>

<sup>1</sup> Department of Ophthalmology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Nishi 7, Kita 15, Kita-ku, Sapporo, 060-8638, Japan

<sup>2</sup> Department of Orthoptics, Faculty of Medicine, Fukuoka International University of Health and Welfare, Fukuoka, Japan

<sup>3</sup> Kaimeido Eye and Dental Clinic, Sapporo, Japan