

Limited Clinical Value of Anti-Retinal Antibody Titers and Numbers in Autoimmune Retinopathy

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Purpose: To assess the possible correlation of anti-retinal antibody titers and number of anti-retinal antibodies with outcome measurements including visual acuity, subjective vision loss, visual field, and electroretinography in patients with autoimmune retinopathy.

Design: Single-center, retrospective cross-sectional study.

Patients and Methods: Patients with autoimmune retinopathy who underwent anti-retinal antibody testing at least twice during their follow-up were enrolled. Anti-retinal antibody titers and numbers were grouped as improved, stable, or worsened. Outcomes included Snellen visual acuity, patient-reported vision loss, Humphrey visual field mean deviations, and electroretinography parameters.

Results: Thirty-one eyes among 16 patients with autoimmune retinopathy were included. Between-group analyses of visual acuity, subjective vision loss, visual field, and electroretinography outcomes did not reveal any significant differences by anti-retinal antibody titer or number group at a 95% confidence interval.

Conclusion: Changes in anti-retinal antibody titers or numbers were not associated with any vision outcome. Repeated anti-retinal antibody testing may be unnecessary after diagnosis of autoimmune retinopathy and detection of an anti-retinal antibody.

Keywords: autoimmune retinopathy, anti-retinal antibody, visual field, electroretinography, outcome measurements

Introduction

Autoimmune retinopathy (AIR) is a rare group of autoimmune diseases driven by auto-antibodies against the retina and typically results in progressive vision loss. Most instances primarily affect the peripheral retina and are characterized as either paraneoplastic (pAIR) or non-paraneoplastic (npAIR). Differences in disease course also exist between the two groups. The paraneoplastic group, including cancer-associated retinopathy (CAR) and melanoma-associated retinopathy (MAR), usually involves the pigment epithelial membrane while the non-paraneoplastic group does not.¹

Early stages of the disease often present with a normal fundus examination. However, pigment epithelium damage and vascular attenuation may be seen in later stages. Patients may report unilateral, bilateral, or asymmetric scotomas, photopsias, visual field defects, and rapid progressive visual loss. Objective central visual acuity may be decreased if the macula is affected. Perimetry may also reveal visual field deficits and scotomas, often correlating with retinal damage and inflammation seen on optical coherence tomography (OCT), fundus autofluorescence (FAF) and fluorescein angiography (FA). In addition, electrophysical activity of retinal rod and cone cells may be assessed by electroretinography (ERG) and the integrity of the retinal pigment epithelium may be measured with electro-oculograms (EOGs).^{1,2} Treatment involves the use of corticosteroids, immunomodulatory therapies (IMTs), and intravenous immunoglobulin (IVIG) with regular follow-up.

The rarity of the disease complicates efforts to draft a diagnostic criterion aside from the presence of serum antiretinal antibodies (ARAs), which are essential for diagnosis. Antiretinal antibodies may be classified by serum titers and number of binding targets. However, associations between serum ARAs and clinical outcomes have been questionable. The aim of this study is to examine correlations between ARA titers and numbers, and outcome measurements including visual acuity, subjective vision loss, visual fields, and ERG findings.

Materials and Methods

Patients

This was a single-center, retrospective study of patients with AIR who presented to the Massachusetts Eye Research and Surgery Institution (MERSI) between August 2011 and August 2021. Approval for this study was obtained through the New England Independent Review Board which issued a waiver of informed consent based on standard procedures for retrospective chart reviews. Study practices were performed in accordance with the Declaration of Helsinki and were compliant with the Health Insurance Portability and Accountability Act.

Diagnosis criteria for AIR included the presence of serum ARAs with at least one of the following findings not better explained by another etiology: 1. vision symptoms including vision loss, scotomas, visual field deficits, photopsias, nyctalopia, or photosensitivity; 2. abnormal ERG; 3. abnormal fundus examination with vascular attenuation, retinal pigment epithelial changes, retinal atrophy, or optic disc pallor. All patients were diagnosed with AIR by one of the three experienced uveitis specialists (Peter Y. Chang, MD, Stephen D. Anesi, MD or C. Stephen Foster, MD).

The electronic medical records of all AIR patients with ARA testing at least twice during follow-up were included. Data on age, sex, race, subjective visual loss, Snellen best-corrected visual acuity (BCVA) within one week of ARA testing, ocular comorbidities, systemic diseases, family history of cancers and autoimmune diseases, current medications, related laboratory examinations, and ocular findings on slit-lamp microscopy were analyzed. Both eyes were included except for monocular patients. Each eye was considered independent in statistical analysis. Data were recorded within 3 months of serum collection for ARA testing.

Ophthalmic Testing

Two ERG machines were used according to the standard protocol recommended by the International Society for Clinical Electrophysiology of Vision.^{3,4} Full-field ERG (ffERG) with SG-2002 Ganzfeld system (LKC Technologies, Gaithersburg, MD) parameters consisted of 30-Hz flicker implicit time.⁵ Diopsys® Fixed Luminance Flicker (FL-flicker) and Multi-Luminance Flicker (ML-flicker) ERG parameters included FL-flicker magnitude and phase, ML magnitude and phase area under the curve (AUC),⁶ but were later excluded due to small sample size (4 patients).

Visual field mean deviations (MDs) were obtained with the Humphrey Visual Field Analyzer (Carl Zeiss Meditec Inc., Dublin, California, USA) using the Swedish Interactive Threshold Algorithm (SITA) fast 24–2 test protocol. Short-wavelength automated perimetry (SITA-SWAP) was performed in some patients with previous suspicion for birdshot chorioretinopathy as it has been shown to be more sensitive.^{7,8}

ARA testing was performed at the Casey Eye Institute Ocular Immunology Laboratory, Oregon Health & Science University, currently the only Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory in the United States for ARA testing. Western blot (WB) was used to detect specific retinal proteins based on molecular weight in kilodaltons (kDa), and repeated titers were reported by the laboratory as increasing, decreasing, or no change according to standard protocols.⁹ ARA changes were considered improved if there was a decrease in ARA titers or number of WB bands, stable if there was no change in ARA titers and number of WB bands, and worsened if there was an increase in ARA titers or appearance of new WB bands.

Statistical Analysis

Data collection and management was performed on Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA). Appropriate parametric tests were performed, and assumptions assessed on IBM SPSS 28 (International Business Machines Corp., Armonk, NY, USA). A 95% confidence interval was used for statistical significance unless otherwise stated. Snellen visual acuities were converted to logarithm of the minimum angle of resolution (logMAR) for statistical analysis. All vision outcomes were averaged between both eyes at each time point.

Results

Sixteen patients with AIR had at least two serum tests for ARAs. Patient demographics are reported in Table 1. The mean age of patients was 52.5 years (SD 13.18) and most were female (81.25%). Fourteen of 16 patients were diagnosed with npAIR. The other two patients were diagnosed with MAR and CAR. One patient had a history of cutaneous malignant melanoma, and one patient had a history of squamous cell carcinoma of the mandible and basal cell carcinoma of the medial canthus. One patient had a history of monoclonal gammopathy. Seven patients had a family history of malignancy. One-third of patients had another autoimmune disease such as spondyloarthropathy, systemic lupus erythematosus, ocular cicatricial pemphigoid, or psoriasis. The most common presenting symptoms were decreased vision (93.75%), followed by visual field deficits (37.5%) and nyctalopia (31.25%). Ocular examination findings by slit-lamp microscopy and indirect fundoscopy are described in Table 2. The most common ARA targets (Table 3) in our patients were α -enolase (46 kDa), followed by carbonic anhydrase II (30 kDa).

Between-group analyses of change in visual field mean deviations (ANOVA, $p = 0.189$), 30-Hz flicker implicit times (ANOVA, $p = 0.679$), or logMAR visual acuities (ANOVA, $p = 0.820$) did not reveal any significant

Table 1 Autoimmune Retinopathy Patient Characteristics

Characteristic	Category	Value
Age (years)	Mean (SD)	52.3 (12.8)
Sex	Male (%)	3 (17.6)
	Female (%)	14 (82.4)
Race	White (%)	16 (94.1)
	Not Reported (%)	1 (5.9)
Comorbidity	Malignancy (%)	2 (12.5)
	Arthritis (%)	4 (25)
	Gout (%)	1 (6.25)
	Fibromyalgia (%)	3 (18.75)
	Systemic Lupus Erythematosus (%)	2 (12.5)
	Sjogren's syndrome (%)	1 (6.25)
	Pemphigoid (%)	1 (6.25)
	Irritable bowel syndrome (%)	1 (6.25)
	Autoimmune thyroid disease (%)	6 (37.5)
	Cardiovascular disease (%)	8 (50)
	Respiratory disorders (%)	3 (18.75)
Laterality	Right eye (%)	15 (93.8)
	Left eye (%)	16 (100)
Initial BCVA (logMAR)	Right eye (SD)	0.39 (0.45)
	Left eye (SD)	0.35 (0.7)

Note: N = 16 patients.

Abbreviations: SD, standard deviation; BCVA, best corrected visual acuity.

Table 2 Ocular Findings of Autoimmune Retinopathy on Initial Examination

Category	Ocular Finding	Frequency (%)
Anterior	Iritis	4 (12.9)
Intermediate	Vitritis	2 (6.5)
Posterior	Normal fundus	11 (35.5)
	Vascular attenuation	7 (22.6)
	Retinal pigmentary changes	10 (32.3)
	Retinal atrophy or degeneration	5 (16.1)
	Optic disc pallor	7 (22.6)

Note: N = 31 eyes.

differences by ARA status (Figure 1). Only four patients had repeated Diopsys measurements, and statistical power was insufficient to draw any reliable conclusion. Kruskal–Wallis H -test also did not reveal any association between ARA status and patient-reported vision symptom change ($H = 0.751$, $p = 0.687$).

Table 3 Anti-Retinal Antibodies at Diagnosis by Western Blot

Retinal Antigens	Molecular Weight (kDa)	Numbers (%)
Neuronal antigen	22	1 (6.25)
Recoverin	23	2 (12.50)
HSP27	27	1 (6.25)
–	28	2 (12.50)
Carbonic Anhydrase II	30	9 (56.25)
–	31	2 (12.50)
–	32	1 (6.25)
Myelin basic protein	33	1 (6.25)
–	34	1 (6.25)
Müller cell specific protein, transducin- β	35	1 (6.25)
Aldolase, transducin- α , rhodopsin	40	1 (6.25)
–	43	1 (6.25)
–	45	1 (6.25)
α -enolase	46	12 (75)
Arrestin	48	1 (6.25)
Pigment epithelium-derived factor	50	1 (6.25)
Tubulin	52	1 (6.25)
PKM2	58	1 (6.25)
CRMP 5	62	2 (12.50)
–	64	1 (6.25)
Bestrophin	68	1 (6.25)
–	72	1 (6.25)
–	76	1 (6.25)
–	96	1 (6.25)
–	97	1 (6.25)
–	112	2 (12.50)
–	136	1 (6.25)
–	139	1 (6.25)
–	180	1 (6.25)
–	187	1 (6.25)

Note: Unknown or uncharacterized binding targets are marked with a dash.

Abbreviations: CRMP 5, collapsing response mediator protein 5; GADPH, glyceraldehyde 3-phosphate dehydrogenase; HSP, heat shock protein; kDa, kilodalton; PKM2, pyruvate kinase M2.

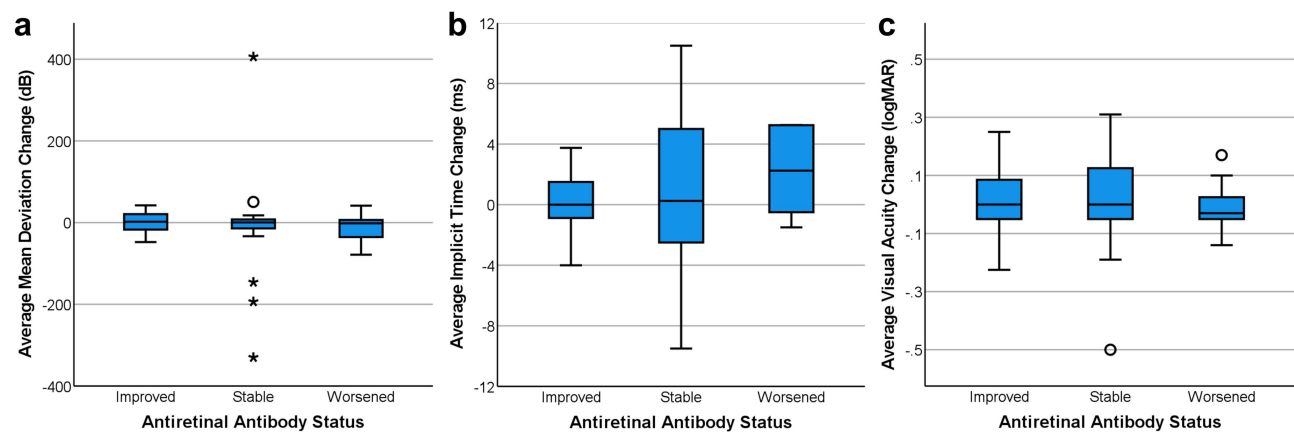


Figure 1 Boxplots of vision change compared by antibody status. Circles indicate outliers and asterisks indicate far outliers. (a) Average change in visual field mean deviations for both eyes in decibels, (b) average change in ERG implicit time for both eyes in milliseconds, and (c) average change in visual acuity for both eyes in logMAR.

Discussion

Diagnosis and management of AIR is complicated by its low incidence, lack of standard diagnostic criteria, lack of management guidelines, and limited availability of clinical biomarkers. Therefore, multimodal assessment is necessary for diagnosis and follow-up of treatment response. This practice pattern, however, may result in delayed diagnosis and treatment due to multiple tests and serologies needed to rule out more common and likely diagnoses. Within this delay, AIR may cause rapidly progressive vision loss.

The presence of ARAs is currently the most definitive modality for diagnosis. Some antibodies are specific for the retina, such as recoverin (23 kDa) and rhodopsin (40 kDa),¹⁰ while others such as α -enolase (46 kDa) are not.¹¹ Numerous antibodies have been reported to be associated with AIR such as carbonic anhydrase (30kDa), arrestin (48 kDa), Muller-cell-specific antigen (35 kDa), and transducing (35 or 40 kDa). The most commonly detected and relevant antibodies include recoverin, α -enolase (46 kDa), and transient receptor potential cation channel subfamily M member 1 (TRPM1), while recoverin and α -enolase are the most widely studied.^{2,11} ARAs can be found in other ocular diseases such as retinitis pigmentosa, Vogt-Koyanagi-Harada, sympathetic ophthalmia, toxoplasma retinochoroiditis, diabetic retinopathy, exudative age-related macular degeneration, and cystoid macular edema as well as in systemic diseases such as systemic lupus erythematosus, multiple sclerosis, Behcet's disease, and inflammatory bowel disease.¹² ARAs may also be absent in diagnosed patients and present in healthy patients^{2,13,14} or patients with AIR quiescence for years.¹⁵

Three laboratory techniques have been described for serum ARA detection, including immunohistochemistry (IHC), WB, and enzyme-linked immunosorbent assay (ELIZA), among which IHC and WB are more commonly used. However, none of them are definitive. Each technique has advantages and disadvantages.¹¹ For example, WB identifies antibodies based on the size of the protein and is both technically difficult and lacking in specificity. Therefore, two techniques should be performed concurrently to maximize sensitivity and specificity.¹⁶

The knowledge on correlation of ARAs with clinical presentation and treatment outcomes is limited. Saito et al reported a patient complained night blindness and central visual loss, but WB analysis did not detect antibodies. One year after presentation, an ARA was detected but systemic workup did not reveal malignancy. Two years after presentation, BCVA continued to decrease. On repeat examination for malignancy, the patient was diagnosed with colonic adenoma. ARA seropositivity may therefore be indicative of malignancy and demonstrates the value of repeat ARA testing in ARA seronegative patients with a strong clinical suspicion for AIR.¹⁷ We similarly report that one patient who presented with worsening vision was diagnosed with presumptive AIR and treated with IMTs. ERG testing disclosed further deterioration in the electrophysiologic parameters while on IMTs. Two repeated ARA tests were initially negative, but repeated testing 3 months later demonstrated ARAs. Ten Berge et al demonstrated that antibody staining between the inner nuclear layer and the outer plexiform layer and/or between the outer nuclear layer and the photoreceptors on indirect immunofluorescence was more common in patients with a visual acuity of <0.1 .² Sudden onset of vision loss was more often related to anti-recoverin, anti-P62, and anti-arrestin in CAR.¹⁸

Presentation of visual loss and severe deterioration have been found more often in ARA seropositive women with breast cancer, compared to seronegative women.¹⁹ Another case report showed decrease or absence of antibody titer corresponding to improvement in VA in CAR after a variety of treatments including plasmapheresis, chemotherapy, and prednisone.^{20–22} Conversely, antibody titers increased while BCVA worsened.²⁰ Therefore, some ophthalmologists hypothesized that ARA titers can be used as a biomarker of disease activity. However, there was a case where BCVA did not improve after treatment despite the appearance of antibody.²³ Another study by Stanwyck et al and Moussa et al did not show a correlation between ARA band decrease and VA, ERG parameters, or central retinal thickness on optical coherence tomography. They also concluded that ARAs were not a biomarker for response to treatment.^{24,25} In review, Fox et al developed consensus among uveitis specialists in the American Uveitis Society, concluding that there is not enough evidence to determine whether the number of positive ARAs or any specific subtypes are clinically relevant.¹⁶

Our study did not show significant correlation between ARA titers or numbers and VA, subjective vision, or investigations including ff-ERG parameter and MD in HVF. In summary, we found that ARA status is not associated with vision outcomes, with a few study limitations. AIR diagnosis in our cohort was limited to ARA testing by WB in a single laboratory and included both retina-specific and retina-nonspecific ARAs. The limited number of patients due to disease rarity also limits the statistical power of our methodology.

Conclusion

ARA testing is essential for diagnosis of AIR and should be repeated in seronegative patients if clinical suspicion is high, as seroconversion is sometimes observed. However, ARA titers and numbers are not significantly associated with vision outcomes or symptomatology. Therefore, after diagnosis of AIR with ARA seropositivity, repeat testing should not be used to monitor therapeutic response or indicate treatment modality. Also, the presence or absence of antibody should not be the primary driver for treatment decisions. Instead, multimodal investigation and clinical response should drive clinical decisions.

Disclosure

Dr C Stephen Foster reports personal fees from Bausch & Lomb, outside the submitted work. The authors report no other conflicts of interest in this work.

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