Detection of Intraocular Antibody Production to Herpesviruses in Acute Retinal Necrosis Syndrome

Joke H. de Boer, M.D., Leny Luyendijk, Aniki Rothova, M.D.,
G. Seerp Baarsma, M.D., Paulus T. V. M. de Jong, M.D., Jan-Geert Bollemeijer, M.D.,
Adrianus J. J. M. Rademakers, M.D., Allegonda Van der Lelij, M.D.,
Michel J. W. Zaal, M.D., and Aize Kijlstra, Ph.D.

In order to improve the determination of the causative agent in acute retinal necrosis syndrome, we evaluated the detection of intraocular antibody production to herpesviruses in 28 patients with this disease. Intraocular antibody production was determined by calculation of the Goldmann-Witmer coefficient whereby specific antibody titers in the inflamed eye and circulation are related to the total IgG content in ocular fluid and serum. Specific antibody titers to herpesviruses and *Toxoplasma* were determined by the indirect immunofluorescence technique. Thirty-five patients with ocular toxoplasmosis, cataract, or proliferative vitreoretinal disorders were tested as controls. By this technique, intraocular antibody production to varicella zoster virus or herpes simplex virus could be established in 16 (57%) of the patients with the typical clinical features of acute retinal necrosis, compared to none of the controls. Of the 33 affected eyes, 21 (64%) had a visual outcome of less than 20/200. We concluded that detection of intraocular antibody production to herpesviruses may be a useful diagnostic tool in

establishing the causative agents in acute retinal necrosis.

THE ACUTE RETINAL NECROSIS syndrome is a severe inflammatory eye disease that often leads to serious impairment of vision within days or weeks after onset. The clinical features, as first described by Urayama and associates¹ in 1971, are characterized by acute peripheral necrotizing retinitis, retinal arteritis, and panuveitis.^{1,2} Acute retinal necrosis is frequently complicated by retinal detachment with further visual deterioration.³ The acute retinal necrosis syndrome occurs predominantly in otherwise healthy persons but immunocompromised patients with this syndrome have also been described.⁴ In 1982, Culbertson and associates⁵ first demonstrated the presence of herpesviruses in the retina of eyes enucleated during the active stage of this disease. Several studies have confirmed this observation, and it is now generally accepted that most of the cases of acute retinal necrosis are caused by a varicella zoster virus or herpes simplex virus infection.⁶ It has been suggested that other herpesviruses may also be involved in the pathogenesis of acute retinal necrosis, but until now only two cases of acute retinal necrosis have been reported whereby cytomegalovirus was isolated from the retina.^{7,8} Identification of the causative agent can have important therapeutic consequences, and early diagnosis may influence the clinical outcome. Especially when severe opacities of the vitreous interfere with examination of the fundus, a discrimination between acute retinal necrosis and syndromes that share similarities may be difficult.

Detection of a viral causative agent in acute retinal necrosis can be established by different diagnostic techniques. A fourfold increase in serum antibody titers in serial measurements can indicate an active systemic infection, but

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From The Netherlands Ophthalmic Research Institute, Amsterdam (Dr. de Boer, Mrs. Luyendijk, and Dr. Kijlstra); the Department of Ophthalmology, University of Amsterdam, Amsterdam (Dr. Rothova); the Eye Hospital of Rotterdam, Rotterdam (Dr. Baarsma); the Department of Ophthalmology, Erasmus University, Rotterdam (Dr. de Jong); the Department of Ophthalmology, University of Leiden, Leiden (Dr. Bollemeijer); the Department of Ophthalmology, Catholic University, Nijmegen (Dr. Rademakers); the Department of Ophthalmology, University of Utrecht, Utrecht (Dr. Van der Lelij); and the Department of Ophthalmology, Free University of Amsterdam, Amsterdam (Dr. Zaal).

Reprint requests to J. H. de Boer, M.D., The Netherlands Ophthalmic Research Institute, P.O. Box 12141, 1100 AC Amsterdam, The Netherlands.

will take at least two weeks, and does not always occur in ocular disease.^{5,9} Local detection of herpesviruses in the affected eye has been performed by several techniques. Varicella zoster virus has, for instance, been cultured from vitreous fluid of a patient with acute retinal necrosis.¹⁰ However, viral cultures are time-consuming and are often negative, even if electron microscopic examination shows virus particles.¹¹ Recently, varicella zoster virus DNA or herpes simplex virus DNA was detected by the polymerase chain reaction¹² in the aqueous humor of six patients or the vitreous fluid of one patient, all of whom had acute retinal necrosis.

An indirect method to indicate a viral infection in the eye is the detection of intraocular antibody production in aqueous humor or vitreous fluid.¹³⁻¹⁵ Although this technique is gradually becoming an accepted method in Japan and Europe, ophthalmologists elsewhere are not yet convinced of the benefit of this procedure in establishing the causative agent in acute retinal necrosis patients. To address this issue, we have evaluated the laboratory methods in acute retinal necrosis and correlated laboratory findings with clinical prognosis.

Patients and Methods

Patients who fulfilled the clinical diagnostic criteria of acute retinal necrosis, as proposed by the education and research committee of the American Uveitis Society in 1992, and seen between 1983 and January 1993 by members of the Dutch uveitis study group, were selected for this study. Briefly, the clinical criteria for acute retinal necrosis included retinal necrosis located in the peripheral retina, circumferential spread, rapid progression, occlusive vasculopathy, and prominent inflammatory reaction in the vitreous and anterior chamber. Twenty-eight consecutive patients with the clinical features of acute retinal necrosis were included in this study.

Eleven patients with ocular toxoplasmosis were tested as a uveitis control group. The diagnosis of ocular toxoplasmosis was based on clinical features and supported by a positive Goldmann-Witmer coefficient for *Toxoplasma gondii*.¹⁶ Ten patients with cataract and 14 patients with proliferative vitreoretinal disorders (proliferative vitreoretinopathy) (five) and proliferative diabetic retinopathy (nine) served as nonuveitis controls.

Paired serum and aqueous humor or vitreous fluid samples were collected from 28 patients with acute retinal necrosis. The aqueous humor samples were obtained from 12 patients by performing a paracentesis for diagnostic purposes. We observed no complications after sampling. Vitreous fluid samples were collected undiluted during a therapeutic or diagnostic pars plana vitrectomy from nine patients. In seven patients a diagnostic paracentesis as well as a therapeutic vitrectomy were performed at different moments during the disease. In two patients a second paracentesis was performed for diagnostic purposes, and in one patient a third aqueous humor sample was collected during ocular surgery. A total of 22 aqueous humor samples and 16 vitreous fluid samples were collected, and all samples were analyzed. Most of the samples were collected within three months after the disease onset (range, one week to four years). The control aqueous humor samples were obtained during cataract surgery and the control vitreous fluid samples were collected during a therapeutic vitrectomy. All patients were informed about these investigations and their consent was obtained. Blood samples were obtained on the same day as the paracentesis or vitrectomy, left to clot for one hour, and centrifuged for ten minutes at 1,500g to obtain the serum. The samples were investigated directly after collection or stored at -20 C until use. For nine patients longitudinal serologic follow-up was available.

Intraocular antibody production to herpesviruses and *T. gondii* was performed exactly as previously described.^{12,17} The fixed cell immunofluorescence technique for antiviral antibodies, available as a ready-to-use kit (Gull Laboratories, Salt Lake City, Utah), served as a quantitative detection method for antibodies to varicella zoster virus, herpes simplex virus, cytomegalovirus, and Epstein Barr virus. *Toxoplasma* antibodies were measured by using a commercially available immunofluorescence test kit (*Toxoplasma* suspension OTGY 10/11; fluorescein isothiocyanate-labeled antihuman immunoglobulin OUHT 04/05 [Behringwerke AG, Marburg, Germany]) according to the manufacturer's instructions. Paired serum and aqueous humor or vitreous fluid were tested at the same time. Total IgG and albumin levels in serum and ocular fluids were determined by radial immunodiffusion according to a previously described technique.¹⁸ Results of specific antibody titers and IgG concentrations were obtained 48 hours after the samples arrived in the laboratory. All the mentioned tests together did not

require more than 50 to 100 μ l of ocular fluid.

Intraocular antibody production was determined by calculating the Goldmann-Witmer coefficient^{19,20}:

$$\frac{\text{antibody titer AH or VF}}{\text{antibody titer serum}} : \frac{\text{total IgG AH or VF}}{\text{total IgG serum}}$$

The ratio of antiherpes viral antibody levels in serum and aqueous humor or vitreous fluid was compared to the ratio of total IgG in serum and aqueous humor or vitreous fluid. Theoretically, a Goldmann-Witmer coefficient greater than 1 should indicate intraocular antibody production. In view of the variability in the results of various measurements, according to other authors, a coefficient greater than 3 was considered to be positive.^{19,21} Similar calculations were performed whereby antibody titers were related to the albumin content in serum and ocular fluid.

Eleven sera of patients with a positive Goldmann-Witmer coefficient for varicella zoster virus or herpes simplex virus were tested for IgM antibodies to varicella zoster virus as well as herpes simplex virus. Ten sera from patients with *Toxoplasma* chorioretinitis with positive serum antibody titers to varicella zoster virus and herpes simplex virus served as controls. Sera were first absorbed with IgG Sepharose to eliminate IgM rheumatoid factor and subsequently tested on virus-infected cells with an immunofluorescence technique as described.

Results

The acute retinal necrosis group included 11 women and 17 men with a mean age of 49 years (range, 23 to 90 years). In eight patients the disease was bilateral, and one additional patient was suspected of having bilateral disease because he had lost vision in one eye five years previously. Four patients were immunocompromised at the time they developed acute retinal necrosis. Two patients were renal transplant recipients and receiving immunosuppressive medication; one of them also had a non-Hodgkin's lymphoma. Another patient was treated with a low dose of corticosteroids. One patient had acquired immunodeficiency syndrome (AIDS).

Three of the immunocompromised patients suffered from zoster dermatitis just before or at the time they developed acute retinal necrosis. Two patients had herpes simplex dermatitis at

the time they developed acute retinal necrosis. Another patient had had herpes simplex encephalitis five years before the onset of acute retinal necrosis.

To investigate if serum antibody levels to herpesviruses are predictive for establishing the diagnosis of acute retinal necrosis, the serum levels to varicella zoster virus, herpes simplex virus, Epstein Barr virus and cytomegalovirus were compared with that of the three control groups. Six of the 28 patients with acute retinal necrosis (21%) and none of the control patients had antibody levels in serum to varicella zoster virus exceeding 1:1,024 (χ^2 test, $P = .004$). Seven of the 28 acute retinal necrosis patients (25%) and seven of the control patients (20%; all of the cataract group) had serum antibody titers to herpes simplex virus higher than 1:1,024 (χ^2 test, $P = .64$). Two acute retinal necrosis patients, one of them being a renal transplant recipient, and five patients with cataract had serum titers to Epstein Barr virus exceeding 1:1,024. The renal transplant recipient also had anticytomegalovirus antibody titers higher than 1:1,024; he was the only patient with high anticytomegalovirus antibody titers. Serum antibody titers to *Toxoplasma* higher than 1:1,024 were observed in three patients with acute retinal necrosis, one of the patients with ocular toxoplasmosis, and none of the patients with proliferative vitreoretinal disorders.

Table 1 summarizes the Goldmann-Witmer coefficients of the first collected ocular fluid samples.

Intraocular antibody production to varicella zoster virus was detected in aqueous humor or vitreous fluid samples obtained from 13 patients (46%). In ocular samples of nine patients, the antivariella zoster virus antibody level exceeded the serum level. Two patients had a positive Goldmann-Witmer coefficient for both varicella zoster virus and for herpes simplex virus in the first collected sample. Another patient was positive for varicella zoster virus in the first sample and for varicella zoster virus and herpes simplex virus in a second sample. Finally, three patients had a positive Goldmann-Witmer coefficient for varicella zoster virus and herpes simplex virus. In samples of two of these patients the varicella zoster virus antibody levels exceeded the herpes simplex virus antibody levels but in samples of the third patient the herpes simplex virus antibody levels were higher.

A positive Goldmann-Witmer coefficient to herpes simplex virus was detected in the first

TABLE 1
DETERMINATION OF INTRAOCULAR ANTIBODY PRODUCTION IN THE FIRST COLLECTED OCULAR
FLUID SAMPLES OF PATIENTS WITH ACUTE RETINAL NECROSIS

PATIENT NO., AGE (YRS), GENDER	SYSTEMIC DISEASES	SAMPLE	GOLDMANN-WITMER COEFFICIENT*				
			VARICELLA ZOSTER VIRUS	HERPES SIMPLEX VIRUS	EPSTEIN-BARR VIRUS	CYTOMEGALOVIRUS	TOXOPLASMA SP.
1, 48, M		Aqueous humor	>126.0	Not detectable	<1.0	Not detectable	Not detectable
2, 39, M		Aqueous humor	18.0	<1.0	2.3	<1.4	—
3, 30, M		Vitreous fluid	18.4	2.3	<1.0	1.1	<1.0
4, 45, F		Vitreous fluid	15.0	15.0	<1.0	<1.0	—
5, 60, M		Aqueous humor	14.0	<1.0	<1.0	Not detectable	1.7
6, 41, M		Aqueous humor	11.4	1.4	Not detectable	<1.0	—
7, 86, M		Aqueous humor	10.9	2.7	1.4	Not detectable	<1.7
8, 56, M		Vitreous fluid	9.4	1.2	<1.0	<1.0	—
9, 58, M	Polymyalgia rheu- matica, diabetes mellitus, trigeminal zoster dermatitis	Aqueous humor	6.7	1.7	—	—	—
10, 42, F		Vitreous fluid	4.2	Not detectable	Not detectable	Not detectable	<1.0
11, 30, F		Aqueous humor†	3.7	Not detectable	<1.0	Not detectable	Not detectable
12, 76, F		Aqueous humor	3.4	<1.0	<1.0	<1.0	<2.2
13, 62, M		Vitreous fluid	<1.0	144.0	<1.0	<1.0	Not detectable
14, 29, F		Aqueous humor	<1.0	14.6	Not detectable	<1.0	Not detectable
15, 45, F	Herpes simplex dermatitis	Vitreous fluid	3.5	7.0	<1.0	<1.0	Not detectable
16, 31, M	Herpes simplex dermatitis	Aqueous humor	<3.0	4.5	4.5	<1.5	Not detectable
17, 67, F		Aqueous humor‡	2.3	<1.0	<1.0	<1.0	<1.0
18, 27, M	Nasopharyngitis	Aqueous humor	<1.0	<1.0	Not detectable	<1.0	—
19, 41, M	Renal transplant recipient	Aqueous humor	<1.0	1.6	1.6	1.6	<1.0
20, 63, F		Vitreous fluid	<1.0	<1.0	<1.0	Not detectable	2.2
21, 43, M	Herpes simplex encephalitis	Vitreous fluid	<1.0	1.7	<1.0	<1.0	Not detectable
22, 57, F	Viral meningitis	Vitreous fluid	<2.0	<1.0	1.3	<3.0	<1.5
23, 23, M	Nasopharyngitis	Aqueous humor	<1.0	Not detectable	Not detectable	Not detectable	Not detectable
24, 60, M	Renal transplant recipient, non-Hodgkin lymphoma, zoster dermatitis	Aqueous humor	<1.0	2.1	—	1.0	1.6
25, 30, M	AIDS, trigeminal zoster dermatitis	Aqueous humor	Not detectable	<3.0	Not detectable	Not detectable	Not detectable
26, 29, M		Vitreous fluid	<1.0	<1.0	<1.0	—	Not detectable
27, 69, F		Vitreous fluid	<1.0	<1.0	<1.0	<1.0	Not detectable
28, 90, F		Vitreous fluid	Not detectable	Not detectable	Not detectable	Not detectable	Not detectable

*Goldmann-Witmer coefficient >3 is considered to be positive.

†A second sample (vitreous fluid) was positive for varicella zoster virus (Goldmann-Witmer coefficient, 23.2) and herpes simplex virus (Goldmann-Witmer coefficient, 6.0).

‡In a second (aqueous humor) and third sample (vitreous fluid) a positive Goldmann-Witmer coefficient for *Toxoplasma* (8.7 and 4.4, respectively) was observed.

collected samples from five patients (18%). The intraocular antibody titer to herpes simplex virus exceeded the serum titer in three patients. One patient had a Goldmann-Witmer coefficient of 4.5 for herpes simplex virus as well as for Epstein Barr virus. The aqueous humor and serum titer of antibodies to herpes simplex virus (serum: 1:1,024; aqueous humor: 1:32) were four times higher than the antibody titer to Epstein Barr virus (serum: 1:256; aqueous humor: 1:8).

Except for the patient with the multiple positive Goldmann-Witmer coefficients described above, there were no patients with intraocular antibody production to Epstein Barr virus or cytomegalovirus.

One patient had a positive Goldmann-Witmer coefficient for *Toxoplasma*; an infection with toxoplasmosis was confirmed by specific IgM antibodies in serum and a fourfold increase in serum antibodies to *Toxoplasma* after six weeks.

In all, 16 of the 28 cases (57%) of acute retinal necrosis were presumed to be caused by varicella zoster virus or herpes simplex virus, ten cases by varicella zoster virus (36%), three cases by herpes simplex virus (11%), and three cases by varicella zoster virus and herpes simplex virus (11%). The positive Goldmann-Witmer coefficient was observed in aqueous humor samples in eight of 12 cases (66%), in vitreous fluid in five of nine cases (55%), and in aqueous humor as well as vitreous fluid in three of seven cases (43%). Because no differences were observed in the percentage of positive samples by analyzing aqueous humor or vitreous fluid samples, the kind of ocular specimen collected does not seem to be important for this diagnostic method.

No positive Goldmann-Witmer coefficients for herpesviruses were observed in the three control groups and no positive Goldmann-Witmer coefficients for *Toxoplasma* were observed in the patient group with proliferative vitreoretinal disorders.

To investigate whether the Goldmann-Witmer coefficient is influenced by high antiviral serum antibody titers, both factors were compared. Four of the six patients with a serum titer to varicella zoster virus exceeding 1:1,024 had a positive Goldmann-Witmer coefficient to varicella zoster virus and one of the seven patients with a serum titer higher than 1:1,024 to herpes simplex virus had a positive Goldmann-Witmer coefficient to herpes simplex virus. Two of three other patients with high serum antibody titers

to herpes simplex virus had a positive Goldmann-Witmer coefficient for varicella zoster virus, and one of the three for *Toxoplasma*.

To investigate whether intraocular antibody production can be masked by protein diffusion from the circulation, the albumin levels in aqueous humor and vitreous fluid were determined as a measure for an increased permeability of the blood retinal barrier. Sixteen patients had albumin levels in their ocular fluids exceeding 10% of the serum albumin level. Despite increased permeability of the blood-retinal barrier, ten of these patients (63%) had a positive Goldmann-Witmer coefficient.

The ratio of antiviral antibodies in ocular fluids and serum was also compared with the albumin ratio in ocular fluids and serum. By this calculation method, 14 of the 16 patients with positive Goldmann-Witmer coefficients calculated by IgG ratios were positive for varicella zoster virus or herpes simplex virus, or both. One patient, positive for varicella zoster virus by comparison with IgG ratios, was also positive for herpes simplex virus by calculating the Goldmann-Witmer coefficient with albumin ratios.

Since timing between onset of disease and ocular fluid sampling might play an important role in the degree of intraocular antiviral antibody production, the data were analyzed to address this question (Figure). Eleven of the 16 negative samples (69%) were collected in the first two weeks or after six months of the disease onset. Two of the negative samples collected within two weeks were from immunocompromised patients with a systemic varicella zoster virus infection. From two patients with a negative sample, collected within two weeks after the onset of disease, a second sample was obtained in the second and fourth months, respectively. One of these second samples was positive for *Toxoplasma*; the test result of the other second sample remained negative. Another patient had positive samples for varicella zoster virus in the first and second months of the disease, but after seven months the Goldmann-Witmer coefficient was negative. From seven other patients two samples were obtained at different times, but both samples were collected later than two weeks after the onset of disease. The Goldmann-Witmer coefficient of the first and second samples was positive for three patients and negative for four patients.

In case of a systemic infection, a diagnostic change in antibody titers is defined as a fourfold increase or decrease in serial measure-

Figure (de Boer and associates). Relationship between time of sample collection after the disease onset and a positive (shaded bars) or negative (open bars) Goldmann-Witmer coefficient. W indicates weeks after the onset of complaints; M, months after the onset of complaints.

ments. For nine patients, longitudinal follow-up of serologic antiviral antibody titers was performed. A fourfold increase in antibody titer was observed to varicella zoster virus in the serum of two patients, and to both varicella zoster virus and herpes simplex virus in the serum of one patient. All three patients had a positive Goldmann-Witmer coefficient to varicella zoster virus. The serum titers of these patients are shown in Table 2. Four Goldmann-Witmer negative patients and two patients with a Goldmann-Witmer coefficient for varicella zoster virus and herpes simplex virus had no diagnostic increase to herpesviruses in serial serum samples.

One of the patients with a positive Goldmann-Witmer coefficient for varicella zoster virus had a weak IgM response to varicella zoster virus in serum. Ten other patients with acute retinal necrosis who had a Goldmann-Witmer coefficient positive for varicella zoster virus or herpes simplex virus, as well as ten control patients, did not have detectable circulating IgM antibodies to varicella zoster virus or herpes simplex virus.

Twenty-one of 33 affected eyes had a visual outcome of less than 20/200 (mean follow-up, 20 months; range, one month to seven years), ten of 18 eyes with a positive Goldmann-Witmer coefficient for varicella zoster virus or her-

TABLE 2
DIAGNOSTIC INCREASE OR DECREASE IN LONGITUDINAL FOLLOW-UP OF SERUM ANTIBODY TITERS TO VARICELLA ZOSTER VIRUS OR HERPES VIRUS

PATIENT NO.*	ANTI-VARICELLA ZOSTER VIRUS SERUM TITER				ANTI-HERPES SIMPLEX SERUM TITER				GOLDMANN-WITMER COEFFICIENT > 3 FOR
	SAMPLE†				SAMPLE†				
	FIRST	SECOND	THIRD	RISE	FIRST	SECOND	THIRD	RISE	
1	80	40	2,000	25	0	—	—	0	Varicella zoster virus
8	128	2,096	—	16	256	1,024	—	4	Varicella zoster virus
6	256	2,048	—	8	4,096	2,048	—	−2	Varicella zoster virus

*Patient numbering of Table 1.

†Time between collecting the first, second, and third samples of Patient 1 was two weeks and six months, respectively; the time between collecting the first and second samples of Patient 8 and Patient 6 were two weeks and five weeks, respectively.

pes simplex virus, and 11 of 15 eyes with a negative Goldmann-Witmer coefficient ($\chi^2 P = .29$). The visual outcome in relation to presumed causative agent is shown in Table 3.

Discussion

This study contains the results of the analysis of intraocular antibody production to herpesviruses and *Toxoplasma* in a group of patients with the clinical features of acute retinal necrosis. With this technique, varicella zoster virus or herpes simplex virus, or both, could be detected as a presumed causative agent in 16 (57%) of the patients. Unfortunately, no immunohistologic data are available to demonstrate that there is indeed a herpes infection in these eyes. In our series, we found no indication for an intraocular infection with solely cytomegalovirus or Epstein Barr virus in acute retinal necrosis. One patient with the clinical features of acute retinal necrosis had a Goldmann-Witmer coefficient positive for toxoplasmosis, and this infection was confirmed by specific IgM antibodies and a fourfold rise in serial measurement of specific antibodies in the serum. This observation had important therapeutic consequences; the acyclovir treatment was replaced by clindamycin. Whether more patients with ocular manifestation resembling that in acute retinal necrosis have signs of an infection with toxoplasmosis needs further investigation. No positive Goldmann-Witmer coefficients for viruses were observed in the three control groups

(ocular toxoplasmosis, cataract, and vitreoretinal disorders). Therefore, the value three, chosen according to previous authors,^{19,21} seems to be a valid cutoff point for the Goldmann-Witmer coefficient.

Recently, Pepose and associates⁹ reviewed a total of 33 patients with acute retinal necrosis from the literature and concluded that routine serology alone is not useful in establishing an etiologic diagnosis in acute retinal necrosis. Of the patients, 39% had a diagnostic change in herpes antibody levels in serum in serial measurements. IgM antibodies are often not detectable in sera of patients with acute retinal necrosis. The presence of antibodies precipitating the p32 major varicella zoster virus antigen in the serum of two patients with acute retinal necrosis was reported, indicating an active systemic varicella zoster virus infection.⁹ To investigate the predictive value of serum antibody level to herpesviruses in acute retinal necrosis, we compared serum antibody titers of acute retinal necrosis patients with those of the controls. High serum titers to varicella zoster virus were observed in six (21%) of the samples of acute retinal necrosis patients in contrast to none of the controls. Additionally, high serum titers to varicella zoster virus were observed only in two cases of an adult nonimmunocompromised uveitis population of 225 patients (data not shown). Therefore, high serum antibody levels to varicella zoster virus might be an indication of a varicella zoster virus infection, but not necessarily an intraocular infection. Normal serum antibody levels to varicella zoster virus certainly do not exclude an intraocular infec-

TABLE 3
RELATIONSHIP BETWEEN VISUAL OUTCOME OF 33 EYES WITH ACUTE RETINAL NECROSIS
AND PRESUMED VIRAL CAUSATIVE AGENT

VISUAL ACUITY	NO. OF EYES				TOTAL
	POSITIVE GOLDMANN-WITMER COEFFICIENT			NEGATIVE GOLDMANN- WITMER COEFFICIENT	
	VARICELLA ZOSTER VIRUS	HERPES SIMPLEX VIRUS	VARICELLA ZOSTER AND HERPES SIMPLEX VIRUS		
≥20/20	1	1		2	4
20/40 to 20/20	1	1			2
20/200 to 20/40	3	1		2	6
Light perception to 20/200	5*	1	2	5	13
0	2			6	8
Total	12	4	2	15	33

*One eye was amblyopic before the development of acute retinal necrosis.

tion because most of the patients with a positive Goldmann-Witmer coefficient for varicella zoster virus had normal antiviral zoster virus antibody titers. The number of acute retinal necrosis patients and control patients with high serum titer to herpes simplex virus was similar.

In 11 (39%) of the patients, we found no indication for a causative agent of acute retinal necrosis. The question arises whether causative agents other than those tested by us are involved, but our data do not allow conclusions with regard to this issue. However, no prognostic differences were observed between patients with a positive or negative Goldmann-Witmer coefficient. Another explanation is that our test results in these patients were false-negative. Under certain circumstances the Goldmann-Witmer coefficient might be negative, despite an intraocular infection. Several studies have shown that early in the disease the local antibody production may not have reached a detectable level, despite the presence of viral DNA in the eye or cerebrospinal fluid.^{12,22} We observed that most of the negative samples were collected early in the disease course, or later than six months after the disease onset. Longitudinal follow-up of two patients showed a reversion in Goldmann-Witmer coefficient. Therefore, the moment of collecting the samples seems to be important. At an early stage in the disease, detection of viral DNA with the polymerase chain reaction technique in ocular samples might supplement the detection of local antibody production. Intraocular antibody production can also be masked by increased permeability of the blood retinal barrier. However, the observations that most of the patients with an increased permeability of blood retinal barrier had a positive Goldmann-Witmer coefficient, and that most of the patients with a positive Goldmann-Witmer coefficient had a higher antibody titer in the eye than in serum, indicates the presence of a marked humoral immune response in the eye. Another possible explanation for a negative test result is that in case of a strong systemic response to the virus, the ratio between antiviral antibodies in ocular fluids and serum becomes small, but most of the patients with high serum titers to varicella zoster virus had a positive Goldmann-Witmer coefficient to varicella zoster virus. Three severely immunocompromised patients all had a negative Goldmann-Witmer coefficient, despite that two of them had a systemic varicella zoster virus infection. Although most

episodes of herpes zoster dermatitis in renal transplant recipients are associated with a positive antibody response,²³ no local antibody production was observed in two of these patients, one of them with zoster dermatitis before acute retinal necrosis and treated with acyclovir intravenously at the time of the onset of acute retinal necrosis. An early start with antiviral medication may cause a rapid elimination of the virus, which could influence the antibody response. The patient with AIDS in our study had a trigeminal herpes zoster infection, and therefore strong evidence that the acute retinal necrosis was caused by varicella zoster virus, but no intraocular antibody production to varicella zoster virus could be detected. Abnormalities in B-cell function are described in patients with AIDS; one study reported that 22% of patients with AIDS who had pathologically proven toxoplasmosis of the central nervous system had undetectable antitoxoplasma antibodies in serum.^{24,25}

In ocular samples of four patients, a positive Goldmann-Witmer coefficient for two herpesviruses was observed; one patient had a diagnostic increase in serum antibody level to varicella zoster virus, as well as herpes simplex virus. Pepose and associates⁹ described a patient with acute retinal necrosis and a simultaneous rise in serum antibodies to varicella zoster virus as well as cytomegalovirus. Several other studies²⁶⁻²⁹ have demonstrated a simultaneous rise in antibodies to herpesviruses during systemic infections, especially to varicella zoster virus and herpes simplex virus in varicella and herpes zoster. Earlier, an antigenic relationship between varicella zoster virus and herpes simplex virus was suggested,²⁶⁻²⁸ but others have postulated that a simultaneous rise in antiviral antibody titers to different viruses may be caused by simultaneous reactivation of multiple latent viruses.²⁹ The reactivation of a latent herpesvirus might occur during a state of depressed cellular immunity caused by an infection with another herpesvirus. Reactivation may also be the result of the interaction between two herpesviruses during DNA replication, as herpesviruses can transactivate viral and cellular genes.³⁰ However, the possibility of cross reactivity between herpesviruses or infections with multiple herpesviruses in acute retinal necrosis needs further investigation.

The visual outcome in acute retinal necrosis of less than 20/200 in 21 (64%) of the affected eyes underlines the seriousness of this disease. Other studies reported similar percentages of

64% to 72% of legal blindness.²⁻¹⁰ For some of the patients in our population, the ocular disease was recognized late as acute retinal necrosis. These patients had been treated with high doses of systemic corticosteroids without antiviral medication. Systemic corticosteroid therapy without antiviral medication may have exacerbated the ocular inflammation and may therefore have deteriorated the visual prognosis. Acute retinal necrosis can be a rapidly progressing disease. Therefore, early start with appropriate medical treatment and prevention of retinal detachment might improve the visual outcome. We established an intraocular infection with varicella zoster virus and herpes simplex virus in 16 (57%) in a series of 28 acute retinal necrosis patients by detection of intraocular antibody production. Determination of the Goldmann-Witmer coefficient was a quick diagnostic method. Particularly in patients in whom examination of the fundus is hindered by vitreous opacities, a positive Goldmann-Witmer coefficient may be decisive for the diagnosis. In the future, detection of intraocular antiviral antibody production in combination with the polymerase chain reaction technique to detect viral DNA may improve the establishment of the causative agents in acute retinal necrosis.

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OPHTHALMIC MINIATURE

What it came down to, Call concluded, was this: on the morning when he was injured, his eyesight had failed him. He hadn't once suspected that the buckskin horse was hobbled. He ought to have been alert to that possibility, but all he had seen were two horses grazing. His eyes had simply failed him. Horses moved differently, when they were hobbled. Earlier in his life, his eyes would have detected the difference. As it was, they hadn't. He should have had spectacles, but it had never occurred to him that his vision had fallen off so.

Larry McMurtry, *Streets of Laredo*
New York, Simon & Schuster, 1993, p. 544