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Serologic and Polymerase Chain Reaction Analysis of Intraocular Fluids in the Diagnosis of Infectious Uveitis

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• PURPOSE: Infectious uveitis entities are usually rapidly progressive blinding diseases that can be prevented by prompt administration of specific antimicrobial therapy. With the aim of improving early diagnosis in patients with infectious uveitis, intraocular fluid samples from patients with sight-threatening posterior uveitis were investigated to determine the causative agent.

• METHODS: Thirty-eight patients with acquired immunodeficiency syndrome (AIDS) and retinitis, eight immunosuppressed patients with retinitis, 16 immunocompetent patients with acute retinal necrosis, and 22 immunocompetent patients with toxoplasmic retinochoroiditis were analyzed by polymerase chain reaction for the presence of herpesviruses and Toxoplasma gondii DNA and for local antibody production against these microorganisms.

• RESULTS: In patients with AIDS and retinitis, polymerase chain reaction was positive for cytomegalovirus DNA in 21 (91%) of the 23 ocular fluid samples obtained during active cytomegalovirus retinitis, whereas local antibody production analysis was negative in all cases. In acute retinal necrosis, varicella-zoster virus or herpes simplex virus could be established as the inciting agent in 81% of the cases, using the combination of both techniques. Polymerase chain reaction was positive in all samples obtained within two weeks after the onset of disease. Toxoplasma gondii DNA was detected in 4 of 13 samples (31%) from immunocompetent patients with active toxoplasmic retinochoroiditis; in each case, local antibody production was also detected. In contrast, no local antibody production was observed in two of three samples from transplant recipients that were positive for T. gondii DNA. All the control samples tested were negative for the above-mentioned tests.

• CONCLUSIONS: In patients with AIDS, polymerase chain reaction analysis is preferable above local antibody production in detecting the inciting agent of retinitis. In other cases, the combination of both techniques can make a valuable contribution to the diagnosis.
A diagnosis of uveitis was based on clinical characteristics according to the criteria of the International Uveitis Study Group and the Research Committee of the American Uveitis Society (1994). Samples from thirty-eight patients with AIDS and retinitis, eight immunosuppressed patients without AIDS who had retinitis, 16 patients with acute retinal necrosis, and 22 patients with toxoplasmic retinochoroiditis were included.

Blood and aqueous humor or vitreous fluid samples were collected simultaneously. Aqueous humor samples were obtained by performing a paracentesis for diagnostic purposes, and vitreous fluid samples were collected during a therapeutic or diagnostic pars plana vitrectomy. In total, 58 aqueous humor samples and 26 vitreous fluid samples were collected and, depending on the amount of fluid, tested for the presence of different herpesviruses and T. gondii. In five patients with acute retinal necrosis, a second ocular fluid sample was obtained at a later stage during the disease.

Patients without intraocular inflammation included control subjects with 13 aqueous humor samples obtained during cataract or glaucoma surgery and vitreous fluid samples collected during a therapeutic vitrectomy in seven cases of proliferative vitreoretinopathy and six cases of diabetic retinopathy. The control patients were informed of these procedures and their consent was obtained. Twelve control vitreous samples were obtained from tissue donors. No information was available concerning seropositivity for herpesviruses or T. gondii.

Because it has been shown that aqueous humor and vitreous fluid contain polymerase chain reaction inhibitory factors, DNA was isolated from ocular fluid samples by using silica particles, according to the method of Boom and associates. Fifty μl of ocular fluid was used for DNA isolation, and afterward the template DNA was diluted in 50 μl of distilled water. The amplifiability of the DNA was tested by adding 12.5 fg of plasmid-containing modified human β-globin DNA, and no polymerase chain reaction inhibitory activity was detected. Primers for cytomegalovirus, varicella-zoster virus, herpes simplex virus, and T. gondii were selected from published sequences (Table 1). We performed a single polymerase chain reaction and chose the outer primers from publications describing a nested polymerase chain reaction. All primers were tested for the optimal annealing temperature and magnesium concentration.

**PATIENTS AND METHODS**

A diagnosis of uveitis was based on clinical characteristics according to the criteria of the International Uveitis Study Group and the Research Committee of the American Uveitis Society (1994). Samples from thirty-eight patients with AIDS and retinitis, eight immunosuppressed patients without AIDS who had retinitis, 16 patients with acute retinal necrosis, and 22 patients with toxoplasmic retinochoroiditis were included.
<table>
<thead>
<tr>
<th>POLYMERASE CHAIN REACTION</th>
<th>PRIMER SEQUENCES (5'→3')</th>
<th>POSITION</th>
<th>MAGNESIUM** (mmol)</th>
<th>ANNEALING TEMPERATURE (°C)</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytomegalovirus</td>
<td>AGCTGCATGATGTGACGCAAG</td>
<td>1767-1786</td>
<td>3.0</td>
<td>56</td>
<td>Cathomas and associates</td>
</tr>
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<td></td>
<td>GAAGGCTGAGTTCTTTGATAA</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Varicella zoster virus</td>
<td>TACGGGTCTTGCCGGAGCTGTTAT</td>
<td>51,066-51,090</td>
<td>2.0</td>
<td>60</td>
<td>Mahalingam and associates</td>
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<tr>
<td></td>
<td>AATGCCGTGACCAACAAATAT</td>
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<td></td>
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<tr>
<td>Herpes simplex virus 1</td>
<td>ATCACGGTGCCCGGCCGTGAC</td>
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<td>4.0</td>
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<td>Aurelius and associates</td>
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<td>Herpes simplex virus 2</td>
<td>AACTCGGGTGGCGTGTTGACA</td>
<td>1203</td>
<td>5.0</td>
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<td>CGGGAAACGCGGCGGGCCACAC</td>
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<td>Toxoplasma gondii</td>
<td>GCCATTTCTCGTGGAAGATT</td>
<td>Ribosomal DNA</td>
<td>2.0</td>
<td>58</td>
<td>Cazenave, Cheyrou, and Begueret</td>
</tr>
<tr>
<td></td>
<td>CTTGGGGGATAGGTCTAGG</td>
<td></td>
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</tr>
</tbody>
</table>

* R indicates A or G, S indicates G or C, Y indicates C or T.

The amplification mixture contained 0.4 pmol of 3' and 5' primer (Isogen Bioscience bv, Amsterdam, The Netherlands), 0.2 mmol of dUTP (Sphaero Q, Leiden, The Netherlands), dATP, dCTP, dGTP (Life Technologies, Breda, The Netherlands), 0.1 unit of Taq polymerase (Sphaero Q), 0.1 unit of uracil DNA glycosylase (Life Technologies), and 5 μl of isolated template DNA solution, diluted to a final volume of 50 μl with distilled water. The polymerase chain reaction was performed in a Biometra Trio-Thermoblock (Westburg, Leusden), as follows: the samples were incubated for ten minutes at 37°C and afterward at 95°C for five minutes, then 40 cycles were performed for one minute at 95°C denaturation, one minute at the optimal temperature for annealing, and one and a half minutes at 72°C for elongation. After the last cycle, the samples were incubated for seven minutes at 72°C for final elongation.

One fifth of the polymerase chain reaction product was subjected to electrophoresis on a 1.8% agarose gel, stained with ethidium bromide, and photographed. The gel was subjected to Southern blotting. The oligonucleotide probes (20 pmol) specific for the amplified fragments were labeled with 1.9 MBq of adenosine 5'-[γ-32P] triphosphate (ATP) by the method of Maniatis and associates. After hybridization, the nylon membranes were washed and exposed to x-ray film at −80°C for 24 hours.

For varicella-zoster virus and cytomegalovirus, the oligonucleotide probes were designed with the following sequences: varicella-zoster virus: CTC ACT ACC AGT CAT TTC TAT CCA; and cytomegalovirus: GGC TTT AGC CTG CAG TGC AC. For herpes simplex virus 1, the probe was used as previously described, with the following sequence: TAC GAG GAG GAG GGG TAT AAC AAA GTC TGT. For T. gondii, no Southern blotting was performed because of the short length of the polymerase chain reaction product, and for herpes simplex virus 2, no appropriate probe was available. In these cases, we identified the polymerase chain reaction product by ethidium bromide staining after electrophoresis on agarose gel and comparing the length of the product of experimental samples with a positive control.

Using known amounts of plasmid containing viral DNA, 20 target molecules were detected for cytomegalovirus DNA and varicella-zoster virus DNA, and 35 target molecules for herpes simplex virus 1 DNA. For herpes simplex virus 2 and T. gondii DNA, the detection limit of the polymerase chain reaction was...
RESULTS

The results of polymerase chain reaction analysis and local antibody production in the different diagnostic groups are provided in Table 2. None of the ocular fluid samples from the control subjects tested were positive for cytomegalovirus DNA (n = 38), varicella-zoster virus DNA (n = 35), herpes simplex virus DNA (n = 16), or T. gondii DNA (n = 25).

Thirty-one aqueous humor samples and seven vitreous fluid samples from 38 patients with AIDS and retinitis were analyzed. The clinical diagnoses included active cytomegalovirus retinitis (n = 23), inactive cytomegalovirus retinitis (n = 5), toxoplasmic retinochoroiditis (n = 4), progressive outer retinal necrosis (n = 3), retinitis of unknown origin (n = 2), and AIDS-related retinopathy (n = 1) (Table 2). Cytomegalovirus DNA was detectable in ocular fluids from 21 (91%) of 23 patients with active cytomegalovirus retinitis, while no local antibody production against cytomegalovirus was observed in these samples (Table 2). One of the samples that was negative for cytomegalovirus DNA was positive for varicella-zoster virus DNA, which indicated that the clinical diagnosis may need to be reevaluated. In nine of ten cases of active cytomegalovirus retinitis tested, all positive for cytomegalovirus DNA in ocular fluid, cytomegalovirus DNA was also detected in peripheral blood leukocyte samples by polymerase chain reaction. In two cases of cytomegalovirus retinitis, local antibody production against varicella-zoster virus was observed, but the polymerase chain reaction was positive for cytomegalovirus DNA and negative for varicella-zoster virus DNA (Table 2). One of the four vitreous fluid samples and one aqueous humor sample obtained during inactive cytomegalovirus retinitis were still positive for cytomegalovirus DNA. However, the number of patients is too small to allow concluding whether polymerase chain reaction results for cytomegalovirus are influenced by disease activity.

Results were not influenced by treatment with antiviral medication because polymerase chain reaction for cytomegalovirus was positive in nine of the ten patients with active cytomegalovirus retinitis without treatment and all five cases during treatment with ganciclovir or foscarnet. For patients with inactive cytomegalovirus retinitis, polymerase chain reaction was positive in one of the three patients treated with antiviral medication and one of two patients not treated. In the other eight cases, no information about treatment with antiviral medication was available at the time the samples were collected.

Varicella-zoster virus DNA was detected in ocular fluid of all patients with the clinical diagnosis of progressive outer retinal necrosis (Table 2). Local antibody production against varicella-zoster virus in these three patients was negative. T. gondii DNA was not detected in any of the patients with toxoplasmic retinochoroiditis (Table 2); two patients...
### TABLE 2

**LOCAL ANTIBODY PRODUCTION AND POLYMERASE CHAIN REACTION ANALYSIS OF OCULAR FLUIDS FOR HERPESVIRUSES AND TOXOPLASMA GONDII**

<table>
<thead>
<tr>
<th>OCULAR DIAGNOSIS</th>
<th>NO. OF PATIENTS</th>
<th>LOCAL ANTIBODY PRODUCTION</th>
<th>LOCAL ANTIBODY REACTION</th>
<th>POLYMERASE CHAIN REACTION</th>
<th>LOCAL ANTIBODY PRODUCTION</th>
<th>POLYMERASE CHAIN REACTION</th>
<th>LOCAL ANTIHERPES SIMPLEX VIRUS</th>
<th>POLYMERASE CHAIN REACTION</th>
<th>LOCAL ANTIHERPES SIMPLEX VIRUS</th>
<th>POLYMERASE CHAIN REACTION</th>
<th>LOCAL ANTI-TOXOPLASMA GONDII</th>
<th>POLYMERASE CHAIN REACTION</th>
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<tr>
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<tr>
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<tr>
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<td>10/16</td>
<td>7/16</td>
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<td>0/12</td>
<td>0/20</td>
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<td>15/22</td>
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<tr>
<td>Control subjects</td>
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<tr>
<td>No uveitis</td>
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<td>—</td>
<td>0/25</td>
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</tr>
</tbody>
</table>

| Varicella-Zoster Virus            |                 |                           |                         |                           |                           |                           |                             |                           |                             |                           |                             |                           |

| Herpes Simplex Virus 1            |                 |                           |                         |                           |                           |                           |                             |                           |                             |                           |                             |                           |
| Herpes Simplex Virus 2            |                 |                           |                         |                           |                           |                           |                             |                           |                             |                           |                             |                           |

had local antibody production for *T. gondii*. In one patient with retinitis of unknown origin, there was local antibody production for varicella-zoster virus, but no varicella-zoster virus DNA was detected.

Five aqueous humor samples and three vitreous fluid samples from eight immunosuppressed patients with retinitis were investigated; the patients' diagnoses were non-Hodgkin's lymphoma (n = 3), heart transplant recipients (n = 2), renal transplant recipients (n = 2), and systemic lupus erythematosus (n = 1). *Toxoplasma gondii* DNA was detected in samples from three transplant recipients; in one of these samples, local antibody production against both *T. gondii* and herpes simplex virus was positive (Table 2). Local antibody production against cytomegalovirus was observed in samples from two patients suffering from non-Hodgkin's lymphoma and systemic lupus erythematosus, respectively, and cytomegalovirus DNA was detected in one of these cases.

Nine aqueous humor samples and seven vitreous fluid samples from 16 patients with acute retinal necrosis were analyzed. Local antibody production against varicella-zoster virus was positive in ten cases (63%). The polymerase chain reaction for varicella-zoster virus was positive in six (aqueous humor, n = 4; vitreous fluid, n = 2) of these ten samples positive for local antibody production against varicella-zoster virus (Table 2). Two of the samples negative for local antibody production were positive for varicella-zoster virus or herpes simplex virus 1 DNA, respectively. Samples from three patients were positive for local antibody production against both varicella-zoster virus and herpes simplex virus, and one sample solely for herpes simplex virus but without herpes simplex virus 1 or 2 DNA. The samples positive for varicella-zoster virus or herpes simplex virus 1 DNA were all obtained during the active stage of the disease, and the majority of these samples were collected within three weeks after the disease onset (Figure). In five cases, a second ocular fluid sample was obtained. One of these patients had a positive Goldmann-Witmer coefficient and polymerase chain reaction for varicella-zoster virus in the first sample, but the polymerase chain reaction became negative for varicella-zoster virus in the second sample four weeks later, whereas local antibody production against varicella-zoster virus was still readily detectable. In the other four cases, there were no changes in the results of analysis of the first and second samples. Finally, in 12 cases (81%) of acute retinal necrosis, the inciting agent could be detected with both local antibody production and polymerase chain reaction (37.5%), local antibody production alone (31%), or polymerase chain reaction alone (12.5%).

In total, 22 samples (aqueous humor, n = 13; vitreous fluid, n = 9) from patients with toxoplasmic retinochoroiditis were analyzed. Local antibody production for *T. gondii* was observed in 15 of the 22 samples (68%) (Table 2). Local antibody production was positive in eight of the 13 samples (62%) obtained during active toxoplasmic retinochoroiditis and in seven of nine samples (77%) obtained during the convalescent stage of the disease. *Toxoplasma gondii* DNA could be detected by polymerase chain reaction in four of the 13 samples (31%) (aqueous humor, n = 2; vitreous fluid, n = 2) collected during active disease; these were all positive for local antibody production.

**DISCUSSION**

IN THIS STUDY, WE PERFORMED ANTIBODY AND POLYMERASE CHAIN REACTION ANALYSIS FOR HERPESVIRUSES AND *T. gondii* ON A RELATIVELY SMALL AMOUNT (150 µL) OF OCULAR FLUID. LOCAL ANTIBODY PRODUCTION AGAINST CYTOMEGALOVIRUS WAS NEGATIVE IN ALL PATIENTS WITH AIDS AND ACTIVE CYTOMEGALOVIRUS RETINITIS, WHILE IN 21 CASES (91%), CYTOMEGALOVIRUS DNA COULD BE DETECTED BY POLYMERASE CHAIN REACTION. LOCAL ANTIBODY PRODUCTION AGAINST VARICELLA-ZOSTER VIRUS WAS ALSO NEGATIVE IN SAMPLES FROM PATIENTS WITH AIDS AND PROGRESSIVE OUTER RETINAL NECROSIS IN WHICH VARICELLA-ZOSTER VIRUS DNA COULD BE DETECTED WITH POLYMERASE CHAIN REACTION. CURRENTLY, THERE IS NO EXPLANATION FOR THE CONSISTEN TLY NEGATIVE GOLDMANN-WITMER COEFFICIENTS IN PATIENTS WITH AIDS AND RETINITIS, BUT IN SOME CASES THE RELATIVELY HIGH SERUM ANTIBODY TITERS MIGHT HAVE MASKED THE LOCAL ANTIBODY PRODUCTION. HOWEVER, IN IMMUNOCOMPETENT PATIENTS WITH ACUTE RETINAL NECROSIS OR TOXOPLASMIC RETINOCHOROIDITIS, LOCAL ANTIBODY PRODUCTION WAS MORE FREQUENTLY POSITIVE THAN POLYMERASE CHAIN REACTION. FURTHERMORE, IN TWO PATIENTS WITH AIDS AND THE CLINICAL CHARACTERISTICS OF CYTOMEGALOVIRUS RETINITIS, THE RESULTS OF THE LOCAL ANTIBODY PRODUCTION, POSITIVE FOR VARICELLA-ZOSTER VIRUS, WERE NOT
Figure (de Boer and associates). Relation between moment of sample collection after the onset of the acute retinal necrosis, and positive result for polymerase chain reaction or local antibody production. A total of 21 samples were obtained from 16 patients. The majority of the polymerase chain reaction-positive samples were obtained within three weeks after onset of the disease. GW indicates Goldmann-Witmer coefficient; PCR, polymerase chain reaction; M, months after the onset of symptoms; W, weeks after the onset of symptoms.

Consistent with polymerase chain reaction results because in both cases cytomegalovirus DNA was detected. The interpretation of the positive Goldmann-Witmer coefficients in these cases is not clear because these patients had no signs of previous retinal lesions caused by a varicella-zoster virus infection. Therefore, polymerase chain reaction seems to be a preferable method for the diagnosis of retinitis in patients with AIDS.

In immunosuppressed patients without AIDS, local antibody production against cytomegalovirus was positive; therefore, the combination of both polymerase chain reaction and local antibody production might be beneficial in the diagnosis of retinitis in these patients. Furthermore, this study discloses that toxoplasmic retinochoroiditis appears to be an important differential diagnosis for transplant recipients with retinitis. The negative polymerase chain reaction in all four patients with AIDS and toxoplastic retinochoroiditis might indicate the lack of sensitivity of the polymerase chain reaction for T. gondii. More sensitive polymerase chain reaction techniques, for example, by the use of nested primer pairs, might improve the detection of T. gondii in ocular fluids in the future.

In both acute retinal necrosis and toxoplasmic retinochoroiditis, only samples obtained from patients during active ocular inflammation were positive for varicella-zoster virus, herpes simplex virus, or T. gondii DNA with polymerase chain reaction. However, local antibody production against these agents was still positive in 80% of samples from patients with acute retinal necrosis and 77% of the samples from patients with toxoplasmic retinochoroiditis collected during the convalescent stage of the disease. Previously, we and others observed negative results for local antibody production in patients with acute retinal necrosis and in those with herpes encephalitis.
during the first weeks after the onset of disease.\textsuperscript{8,9,15} In
the current study, all the samples collected within the
first two weeks after the disease onset were positive for
varicella-zoster virus or herpes simplex virus 1 DNA,
including two cases that were negative for local
antibody production against herpesviruses. The im-
portance of the delay between sample collection and
disease initiation was shown by a case where
varicella-zoster virus DNA was detected in aqueous
humor obtained one week after the onset of disease
but not in vitreous fluid obtained three weeks later.
Usui and associates\textsuperscript{11} reported positive results with
polymerase chain reaction for varicella-zoster virus
and herpes simplex virus DNA in samples from
patients with acute retinal necrosis that had been
obtained within one month after the onset of disease.
Samples that had been obtained later were all nega-
tive. Therefore, in cases of acute retinal necrosis,
the time of sample collection seems to be a crucial
factor for serologic and polymerase chain reaction
analysis of ocular fluids. In cases of acute retinal necrosis,
polymerase chain reaction analysis can make a valu-
able contribution to early diagnosis in this sight-
threatening disease.

In patients with posterior uveitis, positive polymer-
ase chain reaction results for herpesviruses and T.
gondii were obtained in aqueous humor as well as
vitreous fluid. This is an important finding because
aqueous humor is obtained by paracentesis, a safer
and less invasive procedure than taking vitreous or
choriotreital biopsies. In immunocompetent patients,
 systemic treatment with corticosteroids is common in
severe idiopathic uveitis but might be deleterious in
cases of infectious origin. The ophthalmologist
should consider performing a paracentesis before
beginning treatment with systemic corticosteroids
and while infectious cause is still being considered.

In this study, the diagnosis and therapy of several
patients were changed because of laboratory analysis
of intraocular fluids. In patients with AIDS, polymer-
ase chain reaction was preferred to detection of local
antibody production. In other cases, the combination
of both serologic and polymerase chain reaction
analysis of ocular fluids makes a valuable contribution
to early diagnosis of infectious uveitis. However,
polymerase chain reaction is a sensitive technique
that needs to be used with strict precautions to avoid
false-positive results by contamination of the samples.

Furthermore, the high sensitivity of this technique
might result in the detection of latent viruses. There-
fore, additional work in this area will be required
before the true value of polymerase chain reaction
analysis of ocular fluids in cases of infectious uveitis
can be established.

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