SENSITIVITY AND SPECIFICITY OF ANTI-VIRAL ANTIBODY DETERMINATION IN THE AQUEOUS OR VITREOUS OF UVEITIS PATIENTS


1Department of Ophthalmic-Immunology, The Netherlands Ophthalmic Research Institute; 2Department of Ophthalmology, University of Amsterdam; 3Eye Hospital, Rotterdam; 4Department of Ophthalmology, Erasmus University, Rotterdam; 5Department of Ophthalmology, Catholic University, Nijmegen; The Netherlands

Abstract

Intraocular synthesis of immunoglobulin G antibodies against micro-organisms is considered to be indirect proof of uveoretinal infection. To prove the validity of this hypothesis, the authors determined local anti-viral and anti-toxoplasma antibody production in a large group of uveitis patients and controls. Paired serum and aqueous or vitreous samples were tested for total IgG levels and antibodies to HSV, VZV, EBV, CMV and Toxoplasma, using commercially available immunofluorescence test kits. The sensitivity of VZV/HSV local antibody testing in patients with ARN was 50%, whereas the specificity was 96.5%. CMV antibody testing in AIDS patients with retinitis resulted in a sensitivity of 47.3% and a specificity of 98.0%. Local Toxoplasma antibody testing in Toxoplasma chorioretinitis had a sensitivity of 74.0% and a specificity of 100%. These data show that local antibody testing against infectious micro-organisms is a valuable tool in the laboratory work-up of patients with sight threatening intraocular inflammation.

Infectious disease still plays an important role in uveitis. Several retinal disorders have a viral etiology. Cytomegalovirus retinitis has, for instance, become a common clinical problem with the increase in the number of immuno-compromised patients due to the acquired immune deficiency syndrome.

Over the past few years, it has become accepted that the acute retinal necrosis syndrome is caused by reactivation of latent neurotropic viruses of the herpes group. Although these diseases embody a constellation of distinct clinical signs and symptoms, their appearance may suggest a broad differential diagnosis including Toxoplasma retinitis. A laboratory test would be most useful in identifying the causative agent.

There are a number of reasons for determining local antibody production in the ocular fluid. It may give an indirect proof of the micro-organism causing an intraocular infection and may confirm the clinically suspected diagnosis. Furthermore, it may provide reasonable arguments for starting therapy and can be helpful in monitoring the effects of therapy.

Address for correspondence: Prof. Dr. A. Kijlstra, Netherlands Ophthalmic Research Institute, P.O. Box 12141, 1100 AC Amsterdam, The Netherlands

Recent Advances in Uveitis, pp. 265-267
Proceedings of the Third International Symposium on Uveitis
Brussels, Belgium, May 24-27, 1992
edited by J.P. Demouchamps, C. Veroustraete, L. Caspers-Velu and M.J. Tassignon
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The aim of this study was to prove the validity of intraocular antibody testing by evaluating the sensitivity and specificity of the tests in different groups of uveitis patients.

Various techniques exist for the detection of these antibodies. We chose the commercially available indirect immunofluorescence method, whereby only small amounts of samples are needed\(^3\). Results of testing against the herpes simplex, Varicella zoster, Epstein-Barr and cytomegalovirus as well as against the Toxoplasma parasite will be presented. Various dilutions of serum and intraocular fluid were incubated for half an hour on a slide with virus-infected cells to determine the titer of anti-viral antibodies. Slides coated with formalin fixed Toxoplasma parasites were employed to detect Toxoplasma antibodies\(^4\). After a short washing procedure, a second incubation with fluorescein labelled anti-human IgG is performed.

The highest dilution still giving a positive result under the fluorescence microscope is defined as the titer of antibodies present in the serum, aqueous or vitreous.

Antibodies detected in the ocular fluid can originate from the peripheral blood or from within the eye. To discriminate between the two, it is necessary to relate the specific antibodies to the total amount of immunoglobulin G present in the blood and ocular fluid. By means of the radial immunodiffusion method, according to Mancini, both local and serum IgG levels were quantitated.

Intraocular synthesis of antibodies is considered to have taken place when the relative amount of specific antibodies compared to the total IgG level found in the ocular fluid exceeds that measured in a paired serum sample.

The quotient of the relative amount of antibodies is called the Goldmann-Witmer coefficient (C)\(^5\). Theoretically, a C larger than 1 would indicate a local production of antibodies within the eye. In view of the variability in the results of the various measurements, a C larger than 3 is considered significant.

We tested paired serum and intraocular fluid from:
- 24 patients with the acute retinal necrosis syndrome
- 43 AIDS patients with retinitis
- 46 Toxoplasma chorioretinitis patients

As controls, we used 32 aqueous samples obtained during senile cataract operations and 15 vitreous samples of patients with proliferative intraocular disorders who underwent vitrectomy. Eighty-four other uveitis patients in whom the exact diagnosis was not known at the time of writing formed a separate group. Transplantation patients were omitted.

Aqueous humor was taken for diagnostic reasons, whereas vitreous fluid came from patients who underwent vitrectomy for therapeutic reasons.

Table 1 shows the local antibody synthesis in the various groups of patients against the various micro-organisms tested. Due to the limited availability of samples, not all tests were performed for each individual sample.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>TOXO</th>
<th>VZV</th>
<th>HSV</th>
<th>CMV</th>
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<td>10/24</td>
<td>4/22</td>
<td>0/16</td>
<td>1/16</td>
</tr>
<tr>
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<td>1/29</td>
<td>11/43</td>
<td>1/23</td>
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<tr>
<td>TOXO</td>
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<td>0/15</td>
<td>0/10</td>
<td>0/9</td>
</tr>
<tr>
<td>CATAR</td>
<td>0/32</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>PID</td>
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<tr>
<td>Uveits e.c.i.</td>
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<td>2/58</td>
<td>1/58</td>
<td>1/50</td>
<td>2/52</td>
</tr>
</tbody>
</table>
- 10 of the 24 ARN patients showed intraocular production of antibodies against Varicella zoster and four against herpes simplex;
- 11 of the 43 AIDS patients with retinitis showed intraocular production of antibodies against cytomegalovirus. Only 19 of the 43 patients had definite clinical CMV retinitis. Nine of these 19 were positive for our CMV test;
- Two of the AIDS patients tested had Toxoplasma chorioretinitis;
- 34 of the 46 Toxoplasma retinitis patients showed intraocular production of antibodies against the Toxoplasma parasite.

None of the cataract or vitrectomy controls showed detectable intraocular synthesis of antibodies against the micro-organisms tested. In the other "unknown" uveitis patients group, we found a few coefficients above 3. Two intermediate uveitis patients had a positive test result for HSV and VZV and one case of lymphoma had a positive coefficient for CMV. Two cases were seen with a positive test result for EBV.

In what way are these data helpful, and what is their value regarding sensitivity and specificity? Table 2 shows the sensitivity and specificity of local antibody testing in uveitis patients. The sensitivity of Varicella zoster and herpes simplex virus antibodies testing in ARN was 50%. Compared to the Toxoplasma chorioretinitis and other uveitis patients, the specificity was 96%. The sensitivity of local CMV antibodies testing in AIDS patients with CMV retinitis was 47%. Compared to the other uveitis patients, the specificity was 98%. The sensitivity of local Toxoplasma antibody testing in the Toxoplasma chorioretinitis patients was 74%. Compared to the ARN and other uveitis patients, the specificity was 100% as no C > 3 was found.

| Table 2. Specificity and sensitivity of local antibody testing in uveitis |
|-----------------------------|-----------------------------|
|                             | Sensitivity | Specificity |
| VZV/HSV in ARN               | 50.0%        | 96.5%        |
| CMV in AIDS                 | 47.3%        | 98.0%        |
| Toxoplasma in Toxoplasma    | 74.0%        | 100.0%       |
|                         chorioretinitis |               |              |

The findings reported here show that the determination of local antibody production directed against infectious micro-organisms is a valuable tool in the management of uveitis.

References

2. Culbertson WW, Blumenkranz MS, Pepose JS, Stewart JA, Curtin VT: Varicella zoster virus is a cause of the acute retinal necrosis syndrome. Ophthalmology 93:559-569, 1986