Early Aqueous Humor Analysis in Patients with Human Ocular Toxoplasmosis

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To evaluate the diagnostic sensitivity of a panel of laboratory tests for ocular toxoplasmosis performed at the time of presentation, paired samples of aqueous humor and serum were collected from 49 consecutive episodes of ocular toxoplasmosis with a clinical course of less than 3 weeks. Total immunoglobulin G (IgG) and Toxoplasma gondii-specific IgG, IgM, and IgA were quantified by enzyme-linked immunosorbent assay. The avidity of T. gondii-specific IgG was determined, and DNA extracted from aqueous humor was amplified for detection of a glycoprotein B gene sequence of T. gondii. The diagnosis was confirmed for 73% (36 of 49) of the patients; this rate rose to 79.5% if data from a later analysis of aqueous humor derived from five of the negative patients were included. The analysis of serum (detection of T. gondii-specific IgM and analysis of consecutive serum samples) alone did not contribute to the diagnosis. Calculation of local antibody production lacked diagnostic sensitivity when it was determined less than 3 weeks after the manifestation of clinical symptoms (28 of 49 patients [57%]), but this rose to 70% after an analysis of a second aqueous humor sample. The antibody avidity index attained diagnostic significance in only 8 of 43 instances (19%), and T. gondii DNA was amplified from no more than 6 of 39 (16%) aqueous humor samples. However, T. gondii-specific IgA was found within the aqueous humors of 11 of 43 patients (26%); measurement of the T. gondii-specific IgA level thus contributed substantially to the diagnostic sensitivity of the laboratory tests.

Ocular toxoplasmosis is allegedly the most common cause of posterior uveitis in immunocompetent individuals (29, 42, 60). In most patients it is presumed to be a reactivated congenital condition (43, 53), but instances of acquired infection have also been reported (56). Clinical diagnosis is based on the manifestation of characteristic biomicroscopic features (13, 58, 61). A white, sharp-edged but irregular neuroretinal inflammatory focus (0.5 to 2 optic disc diameters in size) is usually seen, frequently in association with an old scar. When revealed by fluorescein angiography, this lesion may appear to be much larger than the same lesion viewed directly (2, 13, 46, 60). A largely cellular vitreal infiltration is regularly observed, with maximal density over the inflamed area, often in association with a moderate focal vasculitis. In recurrent ocular toxoplasmosis, acute inflammation may be restricted to a discrete zone at the margin of an old scar. The latter, as well as the small neuroretinal inflammatory satellites, are best detected by means of red light-free illumination (2, 61). The biomicroscopic indications of ocular toxoplasmosis are not, however, always so obvious, as signified here. Indeed, the clinical picture may often be far from typical, particularly in elderly patients (13, 26, 30).

Over the past three decades, many different serological tests have been introduced to confirm past infection with Toxoplasma gondii by the detection of T. gondii-specific immunoglobulin G (IgG) antibodies (27). According to current understanding, the presence of T. gondii-specific IgM is the hallmark of a recently acquired systemic or, possibly, ocular infection, although the rate of false-positive results due to persisting antibodies of this type is known to be fairly high (39). Given the absence or low levels of T. gondii-specific IgM in patients with reactivated ocular toxoplasmosis, it cannot therefore serve as a reliable marker of this disease (19, 34, 36, 56, 62).

Laboratory confirmation of ocular toxoplasmosis may be achieved in 50 to 80% of patients by analyzing paired samples of aqueous humor and serum for the accelerated local production of anti-Toxoplasma IgG (6, 12, 14, 32, 49) and, with late onset, of specific IgA also (28, 43, 59) but, possibly except in cases of acquired ocular disease, not of anti-Toxoplasma IgM in the aqueous humor (19, 34, 36, 56, 62).

On the basis of published data and our own observations, we have gained the impression that the rate of laboratory confirmation of ocular toxoplasmosis increases as a function of the time interval between the onset of symptoms and sample collection, which spans 4 to 52 weeks in the literature (11, 14, 49, 56). Since timely laboratory verification of the disease may be of therapeutic relevance, we wished to ascertain whether an early analysis (at least 3 weeks after the onset of symptoms) substantially reduced the rate of confirmation rate of ocular toxoplasmosis.

MATERIALS AND METHODS

Patients. Forty-nine consecutive episodes of ocular toxoplasmosis in 45 patients who manifested the typical clinical picture (as outlined above) were included in this study from the time of their first presentation. Twenty-four (53%) of the patients were female, and their ages spanned 12 to 83 years (mean age, 27.9 years). Each patient presented at the clinical activation stage of the disease, as revealed by the presence of vitreal floaters, with this state being followed by a drop in visual acuity, usually within 14 days but occasionally after a delay of up to 3 weeks (mean ± standard deviation, 9.7 ± 8.4 days; range, 1 to 42 days; median, 7 days). Patients with symptoms that were not obviously attributable to newly reactivated ocular toxoplasmosis, as well as those with underlying inflammatory diseases or immunodeficiency syndromes, were excluded from the study. Patients were subjected to a thorough ocular examination, which included binocular fundoscopy with pupillary dilation, on their first presentation and after 2 and 6 weeks. A 50° fundus photograph was taken to document the course of the disease, and blood was drawn for the quantification of specific antibodies and to determine whether the therapy was causing toxic side effects. A sample of aqueous humor was taken at the first presentation (prior to the onset of treatment) and thereafter at 6 weeks, on a voluntary basis, if the initial analysis had

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failed to confirm the clinical diagnosis or if an adequate scarification of the active zone had not occurred during the treatment period. All patients received a standard therapy; i.e., they were administered pyrithymine, sulfadiazine, and leucovorin (Table 1).

**Analyses of blood and aqueous humor samples.** Serum was obtained from centrifuged blood samples and was stored for a maximum of 24 h at 4°C prior to the analysis of immunoglobulins. Aliquots of aqueous humor of 150 to 250 μl were withdrawn after anterior chamber paracentesis. The sediments obtained after the centrifugation of these samples were dissolved in 50 μl of proteinases K buffer, and the solution was used for the amplification of *T. gondii* DNA (18); supernatants were used for the analysis of immunoglobulins.

**Immunooassay procedures.** The total IgG concentrations within the aqueous humor supernatants (dilution, 1/10) and serum samples (dilution, 1/100) were estimated by high-sensitivity nephelometry (detection limit, 4 mg/ml), the levels of *T. gondii*-specific IgG, IgM, and IgA were determined with a commercial available test system (Platelia Toxo; Sanofi-Diagnostics Pasteur, Marnes la Coquette, France; dilutions, 1/20 [aqueous humor supernatants] and 1/100 [serum samples]). The positive cutoff for the *T. gondii*-specific IgG test corresponds to 6 IU/ml on a scale set with World Health Organization-standardized samples of control sera containing defined concentrations of specific antibodies (devised according to the manufacturer's instructions).

The Goldmann-Witmer coefficient, also known as the antibody ratio (C), was calculated by using the Goldmann-Witmer formula, where $C = \frac{C_{\text{IgG}}}{C_{\text{IgA}}}$, and the antibody avidity index was calculated from the optical density quotient: optical density for the well with aqueous humor/serum for the standard solution. Each enzyme-linked immunosorbent assay procedure was performed according to the manufacturer's protocol (Platelia Toxo; Sanofi-Diagnostics Pasteur). Optical densities were measured at an emission wavelength of 492 nm (Microwell reader's manufacturer's instructions).

**RESULTS**

**Confirmation of diagnosis at the time of presentation.** Among the 49 consecutive episodes of ocular toxoplasmosis that satisfied the inclusion criteria, the clinical diagnosis was supported by indicative laboratory results for 9 episodes (18.4%) and was confirmed for 27 episodes (55.1%) at the time of presentation. Positive results for paired aqueous humor and serum samples by only one of the various laboratory tests supported the clinical diagnosis for 21 episodes (42.9%), whereas positive findings by two or more were indicative of or confirmatory for 15 episodes (30.6%). For the remaining 13 episodes (26.5%), specific antibodies were found in the serum, but neither these nor parasitic DNA was detected within the aqueous humor (Table 2). Neither the length of time between the onset of symptoms and presentation nor the absence or presence of scars influenced the laboratory confirmation rates ($P = 0.1$).

A threefold rise in the concentration of specific IgG in serum was found in only 1 of the 30 patients (3%) from whom blood had been withdrawn after 2 and 6 weeks. In this case, the antibody ratio for *T. gondii*-specific IgG was confirmatory, as was the level of *T. gondii*-specific IgA in the aqueous humor. *T. gondii*-specific IgM was detected in the sera of five patients; in two of these, it was present in the aqueous humor as well, with a higher index in the latter. Since, however, *T. gondii*-specific IgM may persist for long periods in an as yet undetermined proportion of patients, these results were not included in the rate-of-success analysis.

The antibody ratio was found to be indicative of the diagnosis.

**TABLE 2. Laboratory confirmation of ocular toxoplasmosis at time of presentation**

<table>
<thead>
<tr>
<th>No. of tests with indicative and confirmatory results</th>
<th>No. (%) of paired aqueous humor and serum samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ........................................................................... 13 (26.5)</td>
<td></td>
</tr>
<tr>
<td>1 ........................................................................... 21 (42.9)</td>
<td></td>
</tr>
<tr>
<td>More than 1 .................................................................. 15 (30.6)</td>
<td></td>
</tr>
<tr>
<td>Indicative .................................................................. 9 (18.4)</td>
<td></td>
</tr>
<tr>
<td>Confirmatory ................................................................ 27 (55.1)</td>
<td></td>
</tr>
</tbody>
</table>

*Indicative and confirmatory are defined in Materials and Methods section under “Criteria for laboratory support of the clinical diagnosis.”*
nosis for 8 patients and confirmatory for 20 patients, with the levels of specific IgA in the aqueous humor likewise being indicative of or confirmatory for the diagnosis for three and eight of these patients, respectively. The antibody avidity ratio was indicative of the diagnosis for five patients and confirmatory for three patients. It supported a suspicion of newly acquired toxoplasmosis with ocular involvement in two patients; one of these patients had suffered an episode of highly febrile systemic disease involving the liver and lungs 3 months prior to presentation for the ocular disease. Detection of parasitic DNA by PCR supported the diagnosis in six patients (16%); in three of these patients it constituted the only positive laboratory test (Table 3). Of these three patients, two consented to analysis of a second aqueous humor sample within 6 weeks. In one of these patients no further laboratory evidence for the diagnosis was revealed; in the other, a delayed onset of local antibody production was revealed, thus confirming the diagnosis.

**Confirmation of the diagnosis by analysis of a second aqueous humor sample.** In five patients, a second anterior chamber puncture was performed within 6 weeks of the first one to confirm the late onset of a humoral immune response. Four additional cases patients granted a request for a second puncture at 10, 21, 22, or 53 weeks (Table 1) owing to the persistence of inflammatory activity, even with standard therapy. In three of the five patients with no initial evidence of local antibody formation (including the two who were positive for parasitic DNA), the diagnosis was finally confirmed by a positive antibody ratio. In one of the patients in whom parasitic DNA was detected and in one patient with no laboratory evidence of active ocular toxoplasmosis, no laboratory confirmation was obtained, despite the manifestation of a typical clinical picture in each patient (Table 4).

In summary, the performances of all available tests with paired samples of aqueous humor and serum derived at the

### TABLE 3. Independent efficacy of each laboratory test performed with paired samples of aqueous humor and serum for confirming the diagnosis of ocular toxoplasmosis

<table>
<thead>
<tr>
<th>Laboratory test result</th>
<th>Increase in level of T. gondii-specific IgG</th>
<th>C</th>
<th>T. gondii-specific IgA in aqueous humor</th>
<th>T. gondii-specific IgG avidity</th>
<th>Detection of T. gondii DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>29 (97)</td>
<td>21 (43)</td>
<td>32 (74)</td>
<td>35 (78)</td>
<td>32 (84)</td>
</tr>
<tr>
<td>Indicative</td>
<td>1 (3)</td>
<td>8 (16)</td>
<td>3 (7)</td>
<td>5 (11)</td>
<td>—</td>
</tr>
<tr>
<td>Confirmatory</td>
<td>—</td>
<td>20 (41)</td>
<td>8 (19)</td>
<td>3 (7)</td>
<td>6 (16)</td>
</tr>
</tbody>
</table>

* The various results are defined in Materials and Methods under “Criteria for laboratory support of the clinical diagnosis.”

**Confirmation of the diagnosis by analysis of a second aqueous humor sample.** In five patients, a second anterior chamber puncture was performed within 6 weeks of the first one to confirm the late onset of a humoral immune response. Four additional cases patients granted a request for a second puncture at 10, 21, 22, or 53 weeks (Table 1) owing to the persistence of inflammatory activity, even with standard therapy. In three of the five patients with no initial evidence of local antibody formation (including the two who were positive for parasitic DNA), the diagnosis was finally confirmed by a positive antibody ratio. In one of the patients in whom parasitic DNA was detected and in one patient with no laboratory evidence of active ocular toxoplasmosis, no laboratory confirmation was obtained, despite the manifestation of a typical clinical picture in each patient (Table 4).

In summary, the performances of all available tests with paired samples of aqueous humor and serum derived at the
time of presentation and at 2 to 53 weeks for 9 patients yielded no laboratory support for the clinical diagnosis for 10 patients (20.4%); the results were indicative of the diagnosis for 9 patients (18.4%) and confirmatory for the diagnosis for 30 patients (61.1%) (Table 5).

**DISCUSSION**

Biomicroscopic signs of ocular toxoplasmosis are regarded as the “gold standard” for the clinical diagnosis of toxoplasmosis, with the proportion of false-positive and false-negative results depending largely upon the relative experience of the ophthalmologist (27). Any investigation that addresses the sensitivities and specificities of laboratory tests instigated to confirm a diagnosis of presumed ocular toxoplasmosis will inevitably draw on a group of patients defined according to the clinical picture. Although we included only those individuals who manifested a very typical clinical picture, it should nonetheless be borne in mind that any such study will carry the bias of being performed with a clinically preselected group of patients, and this bias will underlie the emergence of any clear-cut case definitions based on the evaluation of laboratory results (37). Ideally, then, the confirmatory value of any laboratory test should be judged according to an approved standard. The oldest and most firmly established of these is the Goldmann-Witmer coefficient (21), otherwise known as C. Even this, however, is not an objective one, since the generally accepted cutoff value for C has been set according to clinical experience, and for ocular toxoplasmosis, this has not been confirmed by prospective studies with appropriate controls (i.e., patients with systemic but not ocular toxoplasmosis and partner eyes from individuals with ocular toxoplasmosis [12, 14, 15]). Since no single analytical method currently at our disposal is, in itself, sufficiently sensitive to confirm the clinical diagnosis (57), the surest way of obtaining reliable information is to broaden the scope of the tests applied. With a view to ascertaining whether the rate of confirmation of ocular toxoplasmosis was substantially reduced by analyzing paired aqueous humor and serum samples at the earliest possible opportunity, i.e., at the time of presentation, rather than at a later date, we therefore instigated several of these tests.

As anticipated (12, 57), analysis of serum samples alone for the presence of *T. gondii*-specific IgM did not contribute to the diagnosis in any of our patients, and evidence for *Toxoplasma* infection activity based on analysis of consecutive serum samples was obtained for only one patient (3%). Laboratory tests performed with paired samples of aqueous humor and serum at the time of presentation supported the clinical diagnosis for 73% (18% indicative, 55% confirmatory) of our patients. However, when the results for local antibody production alone are considered, the rate drops to 57% (16% indicative, 41% confirmatory); this rises again to 76% or more upon the performance of an analysis of a second aqueous humor sample at a later date. Hence, discrepancies in the confirmation rates obtained by various investigators on the basis of the Goldmann-Witmer coefficient could partially be explained by differences in the time interval between the onset of symptoms and sample collection (14, 19, 20, 48, 49, 55), but this information is not specified in most publications (3, 6, 11, 12, 15, 31, 32, 43, 54, 62). Calculation of C alone at the time of presentation has not, indeed, proved to be a sufficiently reliable diagnostic index (6, 57). Likewise, determination of the avidity of *T. gondii*-specific IgG is not, in itself, a sufficiently sensitive test, although it contributed to the diagnosis for 18% of our patients, all of whom had an antibody ratio above 3. This method is now used as a means of differentiating between recently acquired and preexisting toxoplasmosis in pregnant women, independent of the persisting IgM status (25, 39). Vinhal et al. (63) was the first to apply this test to paired samples of aqueous humor and serum derived from patients with acute ocular toxoplasmosis, and they found a marked difference in avidity between the two. We defined the criteria for interpreting our own avidity data on the basis of the findings of Vinhal et al. (63) but failed to detect a difference between paired samples of aqueous humor and serum for all but eight patients (Table 3). In five of these patients avidity in the aqueous humor was, as expected, lower than that in the serum, but in the other three patients the reverse situation held true. A much larger body of data is required to interpret avidity findings on a pathological basis. Such information, however, could, perhaps, afford an insight into the patterns of antibody production and consumption at different sites.

**TABLE 5. Definitive laboratory support for clinical diagnosis**

<table>
<thead>
<tr>
<th>Laboratory contribution</th>
<th>No. (%) of patients</th>
</tr>
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<tbody>
<tr>
<td>Definitively negative$^{a,b}$</td>
<td>10 (20.4)</td>
</tr>
<tr>
<td>Indicative</td>
<td>9 (18.4)</td>
</tr>
<tr>
<td>Confirmatory</td>
<td>30 (61.2)</td>
</tr>
</tbody>
</table>

$^{a}$ The various results are defined in Materials and Methods under “Criteria for laboratory support of the clinical diagnosis.”

$^{b}$ Of the 15 patients for whom the clinical diagnosis was not confirmed by laboratory results at the time of presentation and the 9 patients for whom the results were only indicative, consent for a second anterior chamber puncture was obtained from only five patients; for three of these patients laboratory tests confirmed the diagnosis.

Quantification of *T. gondii*-specific IgA in the aqueous humor proved to be of diagnostic significance in only 11 of 45 patients (24.5%), but in five of these patients it represented the only confirmatory antibody test, which accords with data published by Ronday et al. (57). The relatively low success rate may again be explained by the short time interval between symptom onset and sample collection. We know, indeed, from experiments with animal models that *T. gondii*-specific IgA is not to be expected before the 4th to 8th week of infection, often in the absence of *T. gondii*-specific IgM or IgG (36). Our own clinical data, as well as those from a study on human systemic toxoplasmosis (59), correspond well to these experimental findings and lend credence to the inclusion of this test among those used for diagnostic purposes. It should be borne in mind, however, that *T. gondii*-specific IgA tends to persist for considerable periods of time in the serum (59), and this renders its quantification therein of no diagnostic value in oft encountered cases of recurrent systemic disease. Data pertaining to the persistence of *T. gondii*-specific IgA in the aqueous humors of patients with ocular toxoplasmosis are not available.

Amplification of *T. gondii* DNA is known to be a sensitive index of acute fetal infection when it is performed with samples of amniotic fluid (22) and to be helpful in the diagnosis of cerebral toxoplasmosis when it is conducted with aliquots of cerebrospinal fluid derived from immunocompromised patients (9, 50); it has also been claimed to be of value in the diagnosis of ocular toxoplasmosis when it is carried out with samples of aqueous humor (1, 8; A. P. Brezin, C. E. Eqwuagu, C. Silveira, P. Thulliez, M. C. Martins, R. M. Mahdi, R. Belfort, Jr., and R. B. Nussenblatt, Letter, N. Engl. J. Med. 324:699, 1991). However, even when precautionary measures are taken to prevent false-positive results generated by the incorporation of stray DNA (35) and to digest amplified target DNA fragments prior to amplification, which is now the routine practice in most laboratories (40), the amplification of *T. gondii* DNA from samples of aqueous humor is of little diag-
nostic value for immunocompetent patients with ocular toxoplasmosis (12, 19, 57). Only in immunodeficient patients, e.g., in patients with AIDS (4, 10, 51), and in individuals with what is presumed to be recently acquired ocular toxoplasmosis (41) is it a useful index of this disease. These findings are not perhaps surprising, since immunodeficient patients have markedly higher parasite DNA copy numbers and manifest a feebler immunological degradation of target DNA than immunocompetent patients. Furthermore, the ocular disease in immunodeficient individuals is an acute form of a generalized infection, which is not immunologically controlled. Consequently, the probability of detecting parasites or their DNA in any bodily compartment of immunodeficient patients is much higher than that for immunocompetent individuals with a previous or chronic infection (17, 23), such as reactivated ocular toxoplasmosis, a condition in which local reactivation of the chronic infection is confined almost exclusively to the region abutting a preexisting retinal scar and only rarely spreads into the anterior segment of the eye. Hence, the chances of detecting target DNA within the aqueous humors of immunocompetent individuals are fairly remote. Amplification of \( T. gondii \) DNA from samples of vitreous might possibly prove to be of diagnostic value, since the turnover, and thus clearance, of parasitic DNA in this medium is much slower than that in the aqueous humor (3, 12, 45). However, the potential complications associated with vitreal puncture are too severe to justify this undertaking on a routine basis.

In evaluating the data reported in the literature, it is also necessary to bear in mind that the detection sensitivity of DNA amplification varies between laboratories, since not all of these use standardized procedures or adopt those performed in certified reference establishments (24, 52). Our own low detection rates were not, however, attributable to this cause, since the PCR method that we used complied with the one-copy-per-sample standard set by reference laboratories (52) and included measures for the internal inhibition of amplification as well as for the destruction of stray DNA (44).

Relatively little is in fact known about the time dependence of antibody production in the eye (16, 26), and existing data pertaining thereto do not always fit into our current immunopathophysiological understanding of this phenomenon. In necrotizing retinopathy, for example, local antibody production is regularly observed at about the time of clinical presentation in immunocompetent individuals (11), whereas in ocular toxoplasmosis, a delayed response of up to 4 weeks can be expected (12, 14, 49, 56). This delay in local antibody production is not consistent with the more rapid development of clinical symptoms in the latter case than in the former (11), nor is it consistent with the circumstance that necrotizing viral retinopathy is believed to be a first-episode ocular disease, whereas ocular toxoplasmosis is a recurring one. These observations can be partially reconciled with our current understanding by allowing for differences in the rate of local antibody consumption or in the severity of the local inflammatory response (15), but not entirely. A severe inflammatory reaction, such as the one characteristic of necrotizing viral retinopathy, would be expected to give rise to a high proportion of false-negative results, which is not the case for this disease (33).

Host immune responses are subject to interindividual differences in the rate of activation of immunological mechanisms (and eventually also of immunogenetic mechanisms) (5, 26, 47). Until these mechanisms are more fully understood, a meaningful interpretation of laboratory findings would be greatly facilitated by devising scoring systems for the different diagnostic strategies, with the scoring systems based on the pooled data of nominated reference laboratories. Until such databases are established, we cannot judge whether the cases of disease in the 20% of patients with unconfirmed ocular toxoplasmosis were attributable to a mismatch between the clinical and laboratory diagnoses.

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33. Perkins, E. S.