Molecular diagnosis of toxoplasmosis in immunocompromised patients

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Purpose of review
Toxoplasmosis in immunocompromised patients is associated with a high mortality rate. Molecular techniques are important tools to diagnose acute disease in immunocompromised patients, but there are various methods with variable efficiency. Some of them have been validated for the diagnosis of congenital toxoplasmosis, but the impact of their use has not been evaluated in immunocompromised patients.

Recent findings
Toxoplasmosis is of increasing importance in non-HIV immunocompromised patients. In addition, the picture of disease shows greater severity in South America, both in immunocompetent study participants and in congenitally infected infants. These epidemiological differences could influence the sensitivity of diagnostic methods. This review analyzes recent data on molecular diagnosis and compares them with older ones, in light of progress gained in molecular techniques and of recent epidemiological findings. Most recent studies were conducted in South America and used PCR targeting the B1 gene. PCR on blood could allow diagnosing a significant proportion of patients with ocular toxoplasmosis in Brazil.

Summary
Quantitative PCR methods with specific probes should be used to improve sensitivity and warrant specificity. Performance of quantitative PCR targeting the repeated 529 bp sequence for the diagnosis of toxoplasmosis in immunocompromised patients needs evaluation in field studies in South America and in western countries.

Keywords
blood, immunocompromised patients, molecular diagnosis, PCR, toxoplasmosis

INTRODUCTION

Toxoplasma gondii has been early recognized as a protozoon parasite responsible for severe opportunistic infection in immunocompromised patients [1]. First cases of toxoplasmosis were described in transplant patients in the early 1980s, and in HIV-infected patients a few years later, enlightening the concept of reactivation of past infection and the mechanisms of parasite control. It appeared that primary infection and parasite reactivation in patients with previous immunity were equally life-threatening in patients with low CD4\textsuperscript{+} T-cell counts. Initially, diagnosis was made by microscopic techniques, showing Toxoplasma tachyzoites in biopsy samples or fluids, such as broncho-alveolar lavage (BAL), cerebrospinal fluid (CSF), or blood. However, such observation is infrequent, and requires trained readers and high parasite loads. The few published cases were mainly pneumonias, as tachyzoites are more easily seen in BAL [2\textsuperscript{**},3].

However, immunocompromised patients soon benefited from molecular diagnosis, which was developed in the early 1990s to diagnose congenital toxoplasmosis [4,5]. Since the last 25 years, various gene targets have been described to detect Toxoplasma DNA, and the PCR techniques have greatly evolved to gain in sensitivity and to meet quality guidelines to avoid false positive results. These techniques have been mostly evaluated in the setting of congenital toxoplasmosis, a situation where it is easy to confirm the diagnosis by the serologic...
screening of the newborn or the examination of fetal biopsies in case of termination of pregnancy. By contrast, the assessment of PCR methods is far more difficult in immunocompromised patients, as deep sites of infection may be difficult to reach by biopsy, thus definite diagnosis usually relies on the association of imaging findings, molecular diagnosis, serology, and response to specific therapy. In this context, field evaluation of molecular methods is difficult, but this review will describe the state of the art of PCR methods and what may be expected from their use.

Published literature, restricted to human studies, was searched in the PubMed database, using the terms ‘pcr toxoplasmosis’ or ‘pcr toxoplasma’ combined or not with ‘diagnosis’, ‘toxoplasma b1’, ‘toxoplasma 529’, ‘toxoplasma rDNA’, ‘TGR1E’. The authors focused on the most recent articles but also included a few articles published several decades ago which were thought to be of technical or historical interest. Recommendations provided in this article are based upon critical analysis of the literature when available and upon the authors’ own experience in other cases. When needed, comparison of results is expressed as mean ± SEM.

THE EVOLUTION OF MOLECULAR DIAGNOSIS

The ability of PCR methods to detect low amounts of parasites in fluids or tissues is a key issue, as Toxoplasma can circulate at low concentrations, or inconstantly. In amniotic fluids from congenitally infected infants, concentrations as low as 10 tachyzoites/ml were observed in about 40% of cases in a French study [6]. Therefore, continuous efforts are undertaken to improve the sensitivity of molecular diagnosis, and immunocompromised patients benefit from advances in the diagnosis of congenital toxoplasmosis.

Gene targets

The p30 protein surface single-copy gene has been the first gene target described for the detection of T. gondii DNA by PCR [7]. Other sequence targets have been proposed thereafter which were given to be repeated from 30 to 300-fold in the parasite genome [8–13] (Table 1), but the true number of repetitions is still a matter of debate and could be lower than expected, or differ among parasite strains [14]. However, it has become clear that PCR methods targeting the repeated 529 bp (REP-529) sequence [11] have a better sensitivity, as compared with B1 PCR, with a mean gain of three amplification cycles [15,16–19]. In a multicenter study comparing the efficiency of B1 and REP-529 targets on spiked samples, it appeared that the difference in sensitivity was perceptible for parasite loads below 2 Toxoplasma per reaction tube [20]. Although some doubt has been raised on the conservatism of the REP-529 sequence, particularly in atypical genotype strains [21], those threats have not yet been confirmed [22], and this target is now used by all reference laboratories in France.

Table 1. Gene targets used in molecular diagnosis of toxoplasmosis

<table>
<thead>
<tr>
<th>Target</th>
<th>Number of repetitions</th>
<th>Main use</th>
<th>First description for each application (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P30</td>
<td>Single</td>
<td>Diagnosis</td>
<td>1988 [7]</td>
</tr>
<tr>
<td>B1</td>
<td>30–35</td>
<td>Diagnosis</td>
<td>1989 [8]</td>
</tr>
<tr>
<td>rDNA</td>
<td>≈ 110</td>
<td>Diagnosis, genotyping</td>
<td>1991 [9], 1987 [12]</td>
</tr>
<tr>
<td>TGR1E</td>
<td>≈ 100</td>
<td>Diagnosis, genotyping</td>
<td>1992 [13], 1991 [10]</td>
</tr>
</tbody>
</table>
Infections of the immunocompromised host

(www.cnr-toxoplasma.fr), as well as in the few commercial assays available on the market.

PCR methods

In the industrialized countries, real-time quantitative PCR (qPCR) was introduced in 2000 [23] and has gradually replaced conventional end point PCR. It has the advantage to minimize contaminations and to amplify and detect PCR products in a single step, by the use of specific fluorescent probes. In a multicenter evaluation, it has been shown that qPCR had a similar sensitivity than nested PCR, and a better specificity [24]. However, in developing countries, nested PCR and conventional PCR (cnPCR) methods are still widely used, whether associated to hybridization, digestion with restriction enzymes, or none of both. As qPCR is usually reputed to be more sensitive than cnPCR [24–26], this may introduce considerable bias in the comparison of data between series from various continents. On the other hand, nested PCR carries a higher risk of contamination [24,26], and thus has a lower specificity, which can also bias interpretation. The lack of sensitivity of cnPCR is perceptible for parasite concentrations below 20/ml [24]. However, the sole PCR method does not account for all gaps in sensitivity, and the optimization of the whole PCR method (extraction and amplification) is a key point [20].

As underlined in a French multicenter study, the extraction method can also influence the PCR sensitivity for low parasite concentrations [27]. Although qPCR easily offers the possibility to quantify parasite loads in fluid samples, no clear correlation has been made to date between parasite concentration and severity of infection. In addition, interlaboratory comparison of parasite quantification points to huge discrepancies between results, which motivated the validation and diffusion of a calibrated Toxoplasma suspension among centers of the National Reference Center for Toxoplasmosis in France [28].

Another alternative method to PCR, the loop-mediated isothermal amplification (LAMP) has been described in recent years. LAMP relies on autocycling strand displacement DNA synthesis by the Bst DNA polymerase, under isothermal conditions, usually at 63°C. High specificity of amplification results from the use of four to six different primers. Amplification proceeds by displacing and releasing a single-strand DNA. It is initiated by an inner primer, followed by strand displacement by an outer primer. LAMP products can be detected by direct fluorescence by the use of fluorescent dyes such as SYBR Green, and quantified by spectrophotometry. LAMP assays targeting the SAG1 gene, REP-529, B1 gene, or SAG2 gene of *T. gondii* were developed for human or animal diagnosis [29–31]. Although described as a robust and specific technique in veterinary studies, LAMP has been used in only one human study in Malaysia, where it showed a better sensitivity than nested PCR to detect *Toxoplasma* DNA in blood from immunocompromised patients [32]. Its place among other molecular tools still needs to be clarified, but this technique could be of interest in developing countries.

To date, several qPCR assays for molecular detection of *Toxoplasma* are commercialized, but few of them have been evaluated on clinical samples. The assay by Clonit (Milan, Italy) has been evaluated on spiked samples, but showed a higher detection threshold (i.e., lower sensitivity) than the in-house PCR method. This lack of sensitivity was confirmed on amniotic fluids, as only 50% of samples from congenitally infected fetuses were detected, whereas all tested positive using the in-house method [33]. Another qPCR assay (bio-evolution) was evaluated in a recent study which showed a good sensitivity and specificity on amniotic fluid samples [34*]. Although this assay is validated for parasite DNA detection in blood, BAL and CSF, no data are available on its use in field studies, thus its diagnostic performance in immunocompromised patients still needs evaluation. Other commercial PCR assays by Roche Diagnostics (Meylan, France), Elitech (Puteaux, France), as well as a LAMP commercial assay by Diasorin (Antony, France) are currently under evaluation by the French National Reference Center for Toxoplasmosis, and results should be released soon.

Another important point to be underlined is the diversity of the preanalytical treatments applied to samples prior to DNA extraction, which may influence the sensitivity of the PCR assay in clinical studies. The centrifugation of liquids (aqueous humor, CSF, and BAL) and DNA extraction from the pellet must be the rule to improve sensitivity. In addition, the results of PCR on blood may greatly vary according to procedures, that is, prior isolation of leukocyte cells from 5 or 10 ml of whole blood, or direct DNA extraction of whole blood. In a murine model of disseminated toxoplasmosis, Brenier-Pinchart et al. [35*] nicely showed that parasite loads detected in buffy coats obtained from 1200 μl of blood were higher than those detected from 200 μl. Additionally, buffy coat isolated from 1200 μl of blood yielded better results than 200 μl of whole blood, to detect circulating *Toxoplasma*. This observation can be explained by the fact that *T. gondii* replicates in leukocyte cells, and whole blood is rich in PCR inhibitors, thus can decrease the sensitivity of parasite DNA detection, as shown in a study comparing spiked samples of amniotic fluid.
and blood [36]. These interesting data pave the way for future recommendations regarding molecular diagnosis of toxoplasmosis in immunocompromised patients.

**VALUE OF MOLECULAR DIAGNOSIS ACCORDING TO THE CLINICAL SETTING**

The performance of molecular diagnosis has been mostly evaluated in cerebral toxoplasmosis and ocular toxoplasmosis, two clinical settings where final diagnosis can be confirmed by clinical response, at least partial, to specific therapy.

**Cerebral toxoplasmosis**

Most studies evaluating molecular diagnosis of toxoplasmosis were conducted in HIV-infected patients in Europe during the 1990s [37–46]. Consequently, all accumulated data are based on cnPCR techniques, and on small series of cerebral toxoplasmosis cases (mean 18 ± 2 patients). These studies reported a sensitivity of PCR in CSF and blood samples ranging from 17 to 65% (mean 42 ± 5.7%), and from 13 to 69% (mean 38.4 ± 8.8), respectively (Table 2). These techniques have been adopted by developing countries, which now provide data about diagnostic performances of molecular tools [47–53*]. Much attention is paid to these results, particularly from Southern America, where the pattern of infection is different [54,55], because of the predominance of atypical and type I parasite strains, displaying higher virulence [56]. In this context, it might be expected that virulent strains, which were shown to produce higher parasite loads than avirulent genotype II strains [57], could be detected more easily in circulating fluids. However, most recent data do not support this hypothesis, as they show similar or slightly higher levels of sensitivity, ranging from 35 to 72% (mean 55.6 ± 4.7), and from 15 to 80% (mean 41 ± 39), in CSF and blood, respectively, using comparable methods [47–53*] (Table 2). In fact, only two studies are available regarding blood samples; they reported very contrasting results which explain the huge SEM. Studies reporting data obtained with qPCR methods are still scarce, and do not provide significantly different results than those using nested PCR, excepting a better specificity (100%) [48,49]. As expected, qPCR targeting the REP-529 sequence yielded a better sensitivity (69%) [48] than that targeting the B1 gene (35%) [49].

The sensitivity of molecular diagnosis on CSF samples was reported to be higher when cellularity exceeds 4 cells/μl (P < 0.05) or for patients with at least four brain lesions seen on MRI (P < 0.05) [49]. A high specificity of a positive PCR in CSF suggests that the parasite circulates in this compartment only in case of reactivation, thus a positive PCR must be taken into account, even in case of low parasite counts (i.e., high cerebral toxoplasmosis value).

The time between CSF collection and beginning of specific treatment was pointed out to influence the sensitivity in several studies. For Anselmo et al. [53*] 13 of 15 and zero of 14 patients had a positive CSF, when puncture was performed less than 1 week or more than 1 week of treatment, respectively. Such a trend had been previously reported in a study, where 11 of 11 and seven of 24 patients sampled less than 1 week and more than 1 week of treatment, respectively, were diagnosed with a positive PCR [40]. Therefore, PCR can be useful to monitor treatment efficacy.

**Ocular toxoplasmosis**

Diagnosis of ocular toxoplasmosis mainly relies on ophthalmological examination. However, immunocompromised patients may present with atypical ocular fundus, which can mislead the ophthalmologist, as seen in clinical studies scoring the pretest diagnosis by physicians and the final diagnosis retained [58,59*]. Biological diagnosis of ocular toxoplasmosis mostly relies on immunologic and molecular methods on ocular fluids (aqueous humor or vitreous fluid), but *Toxoplasma* PCR on peripheral blood has also been proposed as a noninvasive diagnostic tool. Immunological diagnosis consists of the comparison of antibody synthesis in two compartments, that is, eye and blood, using quantitative or qualitative approaches. The quantitative one relies on the calculation of the Goldmann–Wittner coefficient, whereas the qualitative one relies on the use of western-blot to demonstrate the production of neosynthesized antibody (IgG, IgM, or IgA) in the eye compartment [60,61]. Contrasting with cerebral toxoplasmosis, a greater amount of recent data evaluating the molecular diagnosis is available in ocular toxoplasmosis, most of them using qPCR methods. However, most studies included both immunocompetent and immunocompromised patients, making it sometimes uneasy to analyze the performance of each technique according to the patient immune background.

Taken together, it appears that the sensitivity of molecular techniques depends on both the immunological status of the patients and the delay between onset of ocular symptoms and sampling. Indeed, in several studies, molecular diagnosis was the most sensitive method in the first weeks after the onset of symptoms [62,63], and it performed better than immunologic methods in immunocompromised patients [64–68**]. As seen in Table 3, the
Table 2. Main studies evaluating the sensitivity of molecular techniques for the diagnosis of cerebral toxoplasmosis

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Number of confirmed CT</th>
<th>Country</th>
<th>Samples (number)</th>
<th>PCR Target</th>
<th>PCR technique</th>
<th>Sensibility n/N (%)</th>
<th>Specificity (%)</th>
<th>HIV status</th>
<th>Reference</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>9</td>
<td>USA</td>
<td>CSF</td>
<td>B1</td>
<td>cnPCR + hyb</td>
<td>4/9 (44.5)</td>
<td>100</td>
<td>HIV+</td>
<td>[37]</td>
<td>1992</td>
</tr>
<tr>
<td>38</td>
<td>13</td>
<td>France</td>
<td>Blood (71)</td>
<td>P30</td>
<td>cnPCR + hyb</td>
<td>9/13 (69)</td>
<td>76.5</td>
<td>HIV+</td>
<td>[38]</td>
<td>1993</td>
</tr>
<tr>
<td>33</td>
<td>20</td>
<td>The Netherlands</td>
<td>CSF (49)</td>
<td>B1</td>
<td>cnPCR + hyb</td>
<td>13/20 (65)</td>
<td>100</td>
<td>HIV+</td>
<td>[39]</td>
<td>1993</td>
</tr>
<tr>
<td>82</td>
<td>35</td>
<td>Italy</td>
<td>CSF (82)</td>
<td>B1</td>
<td>Nested PCR</td>
<td>18/35 (51)</td>
<td>100</td>
<td>HIV+</td>
<td>[40]</td>
<td>1994</td>
</tr>
<tr>
<td>52</td>
<td>15</td>
<td>Germany</td>
<td>CSF (8) Blood (15)</td>
<td>B1</td>
<td>cnPCR + hyb</td>
<td>3/8 (37.5)</td>
<td>100</td>
<td>HIV+</td>
<td>[41]</td>
<td>1994</td>
</tr>
<tr>
<td>72</td>
<td>13</td>
<td>France</td>
<td>Blood (72)</td>
<td>18SrDNA</td>
<td>cnPCR + hyb</td>
<td>5/13 (38.5)</td>
<td>100</td>
<td>HIV+</td>
<td>[42]</td>
<td>1995</td>
</tr>
<tr>
<td>88</td>
<td>18</td>
<td>Italy</td>
<td>CSF</td>
<td>B1</td>
<td>Nested PCR</td>
<td>6/18 (33)</td>
<td>100</td>
<td>HIV+</td>
<td>[43]</td>
<td>1996</td>
</tr>
<tr>
<td>100</td>
<td>19</td>
<td>France</td>
<td>Blood (19)</td>
<td>TGR1E</td>
<td>cnPCR + hyb</td>
<td>5/19 (26)</td>
<td>95</td>
<td>HIV+</td>
<td>[44]</td>
<td>1996</td>
</tr>
<tr>
<td>83</td>
<td>18</td>
<td>France</td>
<td>CSF (33) Blood (93)</td>
<td>B1</td>
<td>TGR1E</td>
<td>1/6 (17)</td>
<td>86</td>
<td>HIV+</td>
<td>[45]</td>
<td>1996</td>
</tr>
<tr>
<td>192</td>
<td>64</td>
<td>Brazil</td>
<td>Blood (192)</td>
<td>B1</td>
<td>qPCR</td>
<td>51/64 (80)</td>
<td>98</td>
<td>HIV+</td>
<td>[47]</td>
<td>2005</td>
</tr>
<tr>
<td>51</td>
<td>16</td>
<td>Brazil</td>
<td>CSF</td>
<td>REP529</td>
<td>qPCR</td>
<td>11/16 (69)</td>
<td>100</td>
<td>39 HIV+12 HIV-</td>
<td>[48]</td>
<td>2009</td>
</tr>
<tr>
<td>135</td>
<td>85</td>
<td>Brazil</td>
<td>CSF (120) Blood (120)</td>
<td>B1</td>
<td>qPCR</td>
<td>30/85 (35)</td>
<td>100</td>
<td>HIV+</td>
<td>[49]</td>
<td>2010</td>
</tr>
<tr>
<td>207</td>
<td>59</td>
<td>Cuba</td>
<td>CSF (207)</td>
<td>T1-T4 (B1) S1-AS1 (B1) B22-B23 (B1)</td>
<td>Nested PCR Nested PCR cnPCR</td>
<td>30/59 (51) 40/58 (69) 42/58 (72.5)</td>
<td>98 97 95</td>
<td>147 HIV+60 HIV-</td>
<td>[50]</td>
<td>2013</td>
</tr>
<tr>
<td>28</td>
<td>16</td>
<td>Japan</td>
<td>CSF (16)</td>
<td>18SrDNA</td>
<td>Nested PCR LAMP LAMP</td>
<td>8/16 (50) ND (lower) ND (lower)</td>
<td>100 100</td>
<td>HIV+</td>
<td>[51]</td>
<td>2013</td>
</tr>
<tr>
<td>13</td>
<td>13</td>
<td>Japan</td>
<td>CSF (13)</td>
<td>18SrDNA</td>
<td>Nested PCR LAMP LAMP</td>
<td>3/7 (43) NA NA</td>
<td>NA 78 HIV+1 HIV-</td>
<td>[52]</td>
<td>2014</td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>27</td>
<td>Brazil</td>
<td>CSF (79)</td>
<td>B1</td>
<td>Nested PCR</td>
<td>15/27 (56)</td>
<td>100</td>
<td>HIV+</td>
<td>[53*]</td>
<td>2014</td>
</tr>
</tbody>
</table>

cPCR, conventional PCR; CSF, cerebrospinal fluid; CT, cerebral toxoplasmosis; hyb, hybridization; LAMP, loop-mediated isothermal amplification; ND, not determined; qPCR, real-time quantitative PCR.
<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Number of confirmed OT</th>
<th>Country</th>
<th>Samples</th>
<th>PCR target</th>
<th>PCR technique</th>
<th>Sensitivity n/N (%)</th>
<th>Specificity (%)</th>
<th>Immune background</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>7</td>
<td>USA</td>
<td>VF</td>
<td>B1</td>
<td>cnPCR</td>
<td>7/7 (100)</td>
<td>100</td>
<td>OT: 3 IC, 4 non IC</td>
<td>[69]</td>
</tr>
<tr>
<td>67</td>
<td>43</td>
<td>France</td>
<td>AH</td>
<td>B1</td>
<td>cnPCR</td>
<td>9/43 (21)</td>
<td>100</td>
<td>All: 14 IC</td>
<td>[64]</td>
</tr>
<tr>
<td>22</td>
<td>16</td>
<td>USA</td>
<td>5 AH, 13 VF, 3 retinal biopsies</td>
<td>B1</td>
<td>qPCR</td>
<td>All: 7/16 (44)</td>
<td>ND</td>
<td>All: 17 IC (6 HIV+), 5 non IC</td>
<td>[65]</td>
</tr>
<tr>
<td>56</td>
<td>11</td>
<td>The Netherlands</td>
<td>27 AH</td>
<td>B1</td>
<td>cnPCR</td>
<td>4/10 (40)</td>
<td>100</td>
<td>All: 56 IC (27 HIV+), OT: 11 IC (1 HIV+)</td>
<td>[66]</td>
</tr>
<tr>
<td>110</td>
<td>34</td>
<td>France</td>
<td>102 AH, 18 VF</td>
<td>B1</td>
<td>qPCR</td>
<td>9/34 (26.5)</td>
<td>100</td>
<td>OT: 4 HIV+, 30 non IC</td>
<td>[70]</td>
</tr>
<tr>
<td>54</td>
<td>40</td>
<td>France</td>
<td>51 AH, 3 VF</td>
<td>REP-529</td>
<td>qPCR</td>
<td>22/40 (55)</td>
<td>100</td>
<td>OT: 11 IC (6 HIV+), 29 non IC</td>
<td>[66]</td>
</tr>
<tr>
<td>133</td>
<td>18</td>
<td>USA</td>
<td>105 AH + 38 VF</td>
<td>B1</td>
<td>qPCR</td>
<td>12/18 (67)</td>
<td>100</td>
<td>79 IC (61 HIV+), 54 non IC</td>
<td>[58]</td>
</tr>
<tr>
<td>132</td>
<td>61</td>
<td>France</td>
<td>133 AH, 7 VF</td>
<td>?</td>
<td>qPCR</td>
<td>15/55 (27)</td>
<td>100</td>
<td>All: 16 IC</td>
<td>[63]</td>
</tr>
<tr>
<td>159</td>
<td>16</td>
<td>South Africa</td>
<td>150 AH + VF</td>
<td>B1</td>
<td>Nested PCR</td>
<td>11/16 (69)</td>
<td>100</td>
<td>All: 142 HIV+</td>
<td>[59*]</td>
</tr>
<tr>
<td>405</td>
<td>114</td>
<td>France</td>
<td>Blood (200 µl)</td>
<td>B1 and REP-529</td>
<td>qPCR</td>
<td>ND/ND (4)</td>
<td>99.6</td>
<td>All: 157 non HIV, 57 IC (31 HIV+)</td>
<td>[68**]</td>
</tr>
</tbody>
</table>

AH, aqueous humor; cnPCR, conventional PCR; IC, immunocompromised; ND, not determined; OT, ocular toxoplasmosis; qPCR, quantitative real-time PCR; VF, vitreous fluid.

*The number of immunocompromised patients in the group of confirmed OT is given, and when available, the number of immunocompromised patients in the whole population study ('all').
sensitivity in immunocompromised patients ranged from 61.5 to 100% in 8/10 studies, whereas it ranged from 21 to 100% in the whole study population, and from 25 to 53% in studies including only immunocompetent patients [62,69–73]. In addition, the size of the lesions was another factor which was reported to influence the positivity of PCR in aqueous humor, as lesions with disk area, more than three, were more likely associated with positive PCR results [64,67,74,75]. Finally, in spite of the limitation because of small series, it appears that PCR on vitreous fluid is more often positive than on aqueous humor [59*,69].

The performance of PCR on aqueous humor is usually better than on peripheral blood, whatever the immunological background of the patients. Bourdin et al. [68**] showed in a large series that molecular diagnosis on peripheral blood is less sensitive than in aqueous humor, especially in immunocompetent patients (1 vs. 24%, respectively). They also confirmed that molecular diagnosis can be improved by increasing the volume of whole blood extracted. Overall, combining the results of B1 and REP-529 qPCR allowed them to confirm the diagnosis of ocular toxoplasmosis in 61.5%, and only 24% of immunocompromised patients and of immunocompetent patients, respectively (P < 0.001). Interestingly, the sensitivity of Goldmann–Witmer coefficient (72%) was higher than that of western-blot (48%). Combining the three methods allowed them to reach a sensitivity of 89%, as seen by others [67].

As for congenital and neurotoxoplasmosis, the targeted sequence REP-529 is more sensitive than B1 [68**,70] and qPCR methods seem the most sensitive and specific PCR techniques.

However, it must be kept in mind that the best results are obtained by combining molecular and immunological methods on ocular samples. Despite the constraint regarding the small volume available, this is made possible by the completion of the PCR on the pellets and western-blot on the supernatant, as this latter technique is sample-sparing.

The role of the parasite genotype on the sensitivity of PCR in ocular toxoplasmosis is difficult to assess, as very few studies are available from South America. Some Brazilian authors recently reported the positivity of PCR result in blood samples from four of five patients with ocular toxoplasmosis [76]; all were immunocompetent, thus this rate seems unusually high. Mattos et al. [77] previously described a sensitivity of 41%, in Brazilian patients of unknown HIV status. If such results are confirmed, the diagnosis of ocular toxoplasmosis in Brazilian immunocompromised patients might rely at first on blood PCR, thus avoiding invasive procedures in a significant amount of cases.

**Pulmonary toxoplasmosis and disseminated toxoplasmosis**

The value of molecular diagnosis in these settings is difficult to establish, as the evolution of infection in immunocompromised patients may be rapidly lethal, and the diagnosis may remain undone, or unrecognized because of multiple opportunistic infections. Case reports are regularly published, but few series meet the required criteria to evaluate diagnostic efficiency, or even disease prevalence in immunocompromised patients. A recent study reported *Toxoplasma* screening by qPCR and direct examination in 336 BAL fluids from immunocompromised patients over a 2-year period, and found two patients with positive microscopic examination and PCR [2**]. Although the authors did not find a gain in sensitivity in favor of PCR, it must be underlined that microscopic diagnosis may be difficult, and requires specific skills, thus the diagnosis of pulmonary toxoplasmosis cannot rely only on direct examination. This incidence seems low, but is in agreement with a recent study recording the number of patients diagnosed with a positive PCR result in 15 French University Hospitals [78*].

### Interest of Molecular Tools to Monitor Treatment

qPCR can be of interest for monitoring treatment efficacy, by showing decreasing parasite loads in samples (blood, BAL, and CSF), as shown in transplant patients [79,80]. Circulating parasites should become undetectable within 3 weeks of treatment.

On the other hand, several surveys have reported the use of PCR screening to monitor allo-HSCT patients in the months following engraftment, by the completion of weekly PCR on blood samples [81–83]. Although they found circulating parasite DNA in patients without *Toxoplasma* disease in 6–13% of patients, the treatment of such patients probably allowed avoiding full-blown disease. In a recent study, the survival of allo-HSCT patients with circulating *Toxoplasma* DNA was compared among 11 French University Hospitals, and it appeared that better survival was observed in centers who had implemented a weekly or 2-monthly PCR screening, than in those who had not [78*]. Whether this screening is cost-effective and should be systematically included in the patient workup remains to be determined. The prevention of toxoplasmosis has...
been addressed in a recent review examining in detail recommendations for HSCT patients [84**].

CONCLUSION

Despite a long history of molecular diagnosis of toxoplasmosis and numerous evaluations, a new sheet remains to be written, as the picture of the disease in developing countries, in particular South America, has been shown to be different from Europe and Northern America. Therefore, it might have diagnostic implications and modify the use of diagnostic tools, in particular in ocular toxoplasmosis. Regarding cerebral toxoplasmosis, large series using qPCR methods are awaited, as most of our data of sensitivity are based on old methods which were subjected to technical pitfalls. New studies should precise the contribution of qPCR, and its use could bring new insights into the comprehension of the pathophysiology of the disease, which may differ among continents [85].

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest


The only prospective study evaluating the diagnostic methods and incidence of pulmonary toxoplasmosis in immunocompromised patients.


Infections of the immunocompromised host


45. The article describes a large series of ocular toxoplasmosis in immunocompromised and nonimmunocompromised patients, and compares different sample types and diagnostic methods.


49. The article describes a large series of ocular toxoplasmosis in immunocompromised and nonimmunocompromised patients, and compares different sample types and diagnostic methods.


53. The work includes a homogeneous series of immunocompromised patients, and points out the necessity of early samples of CSF after the treatment beginning for the diagnosis of cerebral toxoplasmosis.


68. Robert-Gangneux F, Sterkers Y, Yera H, et al. Molecular diagnosis of toxoplasmosis in immunocompromised patients: a 3-year multicenter retro-

69. Study report the practices of 15 French University Hospitals for the molecular diagnosis of toxoplasmosis. The crude mortality of all-HSCT patients diagnosed with a positive blood PCR was compared among centers according to systematic PCR screening practices.


