Correlation of parasite load by quantitative PCR and clinical outcome in a heart transplant patient with disseminated toxoplasmosis

Running title: PCR monitoring of disseminated toxoplasmosis

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Abstract

Disseminated toxoplasmosis is a life-threatening infection in transplant recipients, and results from either reactivation of latent infection or from organ-transmitted primo-infection. Preventive measures and diagnostic screening methods differ between countries and are related to the seroprevalence of *Toxoplasma* in the general population. Here, we report a case of disseminated toxoplasmosis in a heart transplant recipient with previous immunity which occurred after cotrimoxazole prophylaxis for the prevention of *Pneumocystis jiroveci* pneumonia was stopped. Quantitative PCR proved useful for the diagnosis and monitoring of *Toxoplasma* infection. A decreasing parasitic burden in sequential samples of cerebrospinal fluid, blood and bronchoalveolar lavage correlated with a favorable outcome and allowed modulation of the immunosuppressive drug regimen. The duration of anti-*Toxoplasma* treatment and need for maintenance prophylaxis are discussed, as well as prophylaxis in solid-organ transplant recipients. Although a rare event in heart transplant recipients, *Toxoplasma* reactivation must be investigated promptly since early treatment improves the prognosis.

Key words: disseminated toxoplasmosis, immunocompromised, heart transplant recipient, PCR, diagnosis, prophylaxis
Introduction

Toxoplasmosis is a worldwide parasitic disease caused by the intracellular protozoan *Toxoplasma gondii*. After infection, mostly acquired through contaminated vegetables or undercooked meat, the parasite can persist for life encysted in different sites such as the muscles, heart, brain, eye, and, more rarely, in other organs. Whereas clinical symptoms are usually absent or mild in primo-infected immunocompetent individuals, the infection is life-threatening in immunocompromised patients (17). In transplant patients, severe or disseminated toxoplasmosis can result from either reactivation of latent infection in the recipient or from organ-transmitted infection from a seropositive donor to a seronegative recipient (6, 29), a situation where heart transplants carry the highest risk (16, 19, 22, 32).

Reactivation of a chronic infection may occur in the recipient irrespective of the type of graft, but the risk is closely related to the duration and degree of immunosuppression. The risk also varies according to the immunosuppression protocol and therefore to the graft, with hematopoietic stem cell transplantation (HSCT) carrying the highest risk (10). Furthermore, the incidence of *Toxoplasma* reactivation is greater in countries with a higher seroprevalence.

The diagnosis of acute toxoplasmosis in immunocompromised patients relies on PCR detection of parasite DNA in blood, cerebrospinal fluid (CSF), bronchoalveolar lavage (BAL) samples or in biopsy specimens. Serology has a poor performance for the diagnosis of reactivation infection due to a lack of sensitivity (in HSCT patients) or poor correlation with clinical reactivation (solid-organ transplantation (SOT)).

Here, we report a case of disseminated toxoplasmosis in a previously seropositive heart transplant recipient, who underwent several severe infectious complications leading to interruption of cotrimoxazole prophylaxis and subsequently to *Toxoplasma* reactivation. After initial diagnosis, the infection was monitored by quantitative PCR on blood, CSF and pulmonary samples. A decrease in parasite load correlated with a favorable clinical outcome.
on treatment. Quantitative PCR is considered to be a valuable tool for the diagnosis and monitoring of acute toxoplasmosis in SOT recipients. Our results re-emphasize the need to monitor *Toxoplasma* reactivation in seropositive recipients, particularly in countries with a high seroprevalence. Potential drug regimens for anti-*Toxoplasma* chemoprophylaxis in heart transplant patients are discussed.

**Case report**

A 57-year-old man underwent a heart transplant following ischemic cardiomyopathy with severe heart failure. The patient was seropositive for anti-*Toxoplasma* antibodies before transplant (Table 1), as was the donor (IgG = 43 IU/mL, no IgM detected). Both serological results were indicative of past infection. Primary preventive treatment with cotrimoxazole (40/200 mg/day) was started on the first postoperative day (day 1 (D1)) until D27 for *Pneumocystis jirovecii* pneumonia prophylaxis and was then stopped because of intolerance (aggravation of thrombocytopenia from 62 g/L to 39 g/L, leucopenia from 6 g/L to 3 g/L, and acute renal failure). Immunosuppressive therapy was started on D1 to D3 with 3 g/day mofetil mycophenolate (MFM), methylprednisolone hemisuccinate (440 mg/day on D1, 410 mg/day on D2, 240 mg/day on D3), and thymoglobulin. Methylprednisolone hemisuccinate was then reduced progressively to 30 mg/day on D30, without any change in the MFM regimen, and cyclosporin was introduced on D3 with residual rates ranging from 100–200 ng/mL.

The postoperative evolution was marked by several infectious and cardiovascular complications. During the early postoperative days (D3), he presented with mixed cardiogenic and septic shock due to *Escherichia coli* which required circulatory assistance and antibiotherapy with imipenem and gentamicin. On D28, he developed invasive pulmonary aspergillosis, diagnosed by positive BAL cultures and the presence of circulating galactomannan antigen, which was successfully treated with voriconazole. In the following
days, he developed catheter-related bacteremia due to *Enterococcus faecalis* and *Staphylococcus epidermidis* which was treated with vancomycin. He was readmitted to the intensive care unit (ICU) from D45 to D87 for severe successive infectious complications, including ESBL *E. coli* bacteremia and a scarpal abscess treated with imipenem, and pneumopathy due to *Enterobacter aerogenes* probably acquired through mechanical ventilation, which had a favorable outcome on cefotaxim antibiotherapy. Everolimus was introduced on D46 to optimize the immunosuppressive regimen and to reduce the cyclosporin dose. However, due to the lack of improvement of renal function, everolimus was stopped on D70. Cyclosporin was replaced by tacrolimus on D78, in combination with methylprednisolone hemisuccinate and MFM, because of the onset of thrombotic microangiopathy. On D104, he finally left the ICU with stable hemodynamics, despite renal failure requiring dialysis three times a week and encephalopathy attributed to anticalcineurins.

On D113, he presented with febrile respiratory distress, hypoxemia and persistent encephalopathy, leading to orotracheal intubation for invasive mechanical ventilation. Pulmonary radiography revealed bilateral pulmonary infiltrates. Direct microbiological examination of BAL was positive for *T. gondii* trophozoites and negative for bacteria and viruses by culture and/or PCR. Cerebral tomodensitometry was normal and lumbar puncture yielded a clear CSF sample with moderate hyperproteinorachia (0.56 g/L), normoglycorachia without hypercellularity (<1 leukocyte/µL), and negative direct examination for bacteria and parasites. PCR allowed the detection of high levels of *Toxoplasma* DNA in blood, BAL, and CSF. An endomyocardial biopsy sample taken on D124 did not reveal the presence of cysts or signs of acute rejection. *Toxoplasma* serology was checked concomitantly and showed strong serological reactivation with high titers of both IgG and IgM antibodies. Anti-*Toxoplasma* treatment was introduced immediately (D113) with pyrimethamine 50 mg/day and
sulfadiazine 3 g/day, replaced on D118 by clindamycin (2400 mg/day) because of premature
hematotoxicity. As *Toxoplasma* DNA could still be detected 2 weeks after the onset of
therapy, the immunosuppressive regimen was reduced until D131 by stopping MFM and
decreasing methylprednisolone hemisuccinate to 10 mg/day and tacrolimus to 3 ng/mL
residual rate (versus 15–20 ng/mL before). At the same time, there was an increase in
lymphocyte count from 0.02 g/L on D113 to 0.4 g/L on D138. This decrease in
immunosuppression was accompanied by a decrease in parasitic burden. Definitive clearance
of parasites from the different fluids was checked on D164. His fever started to decrease from
D142 and he was completely apyrexial on D149 (i.e. 36 days after beginning specific therapy
and 18 days after a change in immunosuppressive regimen). Extubation was delayed on D159
because of critical neuromyopathy.

After 48 days of antiparasitic treatment with a curative dose regimen, secondary prophylaxis
was continued with pyrimethamine 25 mg/day and clindamycin 1200 mg/day. On D180,
echocardiography showed a strong alteration of systolic function leading to an
endomyocardial biopsy which confirmed the diagnosis of acute rejection. Immunosuppressive
therapy was optimized with an increase in prednisone to 20 mg/day and the progressive
reintroduction of MFM in addition to tacrolimus. *Toxoplasma* secondary prophylaxis was
modified with pyrimethamine (25 mg/day) and cotrimoxazole (160/800 mg at the end of each
dialysis, three times a week).

The patient left the cardiology unit 206 days after transplantation with terminal chronic renal
failure, major malnutrition, and reduced mobility. His immunosuppressive regimen was
maintained with MFM 1000 mg/day reduced to 750 mg/day on D214, prednisone 20 mg/day
and tacrolimus with a residual rate of 5.9–7.5 mg/L. Secondary antiparasite prophylaxis was
continued. The patient is still in a rehabilitation center 1 year after transplantation.
Materials and methods

Clinical samples
Sequential serum, whole-blood, CSF, and pulmonary samples (BAL) were collected for diagnostic purposes (i.e. serology, direct examination (BAL), and quantitative PCR (whole blood, CSF, BAL)). After centrifugation, serum was removed and stored at -20°C until serological testing.Slides were prepared from BAL after cytocentrifugation at 91 x g for 5 min and stained using Giemsa coloration. All samples for DNA extraction were frozen at -80°C.

Serology
Specific anti-Toxoplasma IgG and IgM antibodies were detected using Platelia Toxo IgG® and Platelia Toxo IgM® ELISA tests (Biorad, Marnes-la-Coquette, France), and an immunosorbent agglutination assay (ISAGA IgM®; BioMérieux, Marcy l’Etoile, France).

Parasite DNA detection
DNA was extracted using QIAMP® DNA mini-kit tissue extraction columns (Qiagen, Courtaboeuf, France), after an initial step of overnight proteinase K digestion at 56°C for whole blood. Real-time PCR was performed using Taqman® probes on a Step One plus system (Applera, Villebon, France). The PCR assay targeted the highly repetitive sequence REP-529 (Genbank AF487550) (primers: 270F: 5’-AgAgACACCggAATgCgATCT; and 318R: 5’-TTCgTCCAAgCCTCCgACT) and a labeled probe (310T 6-FAM/TAMRA: 5’-TCgTggTgATggCggAgAGAATgA). Amplification was performed as described previously (28) after a decontamination step with uracil-DNA glycosylase. All DNA samples were tested in duplicate, with and without an internal control consisting of a small amount (5 copies/mL) of parasite genomic DNA from strain RH added to the well. A standard curve was established using serial dilutions of Toxoplasma RH strain recovered and purified from peritoneal lavage from an infected mouse. The sensitivity of the PCR assay has been evaluated previously; it
can detect 2–5 parasites/mL (data not shown) and is evaluated annually in the framework of the quality control program of the Centre National de Référence de la Toxoplasmose (www.chu-reims.fr). The parasitic load of each sample was evaluated from the standard curve.

**Mouse inoculation (in vivo culture)**

With the aim of isolating parasites for genotyping, two Swiss IOPS female mice were inoculated intraperitoneally with 0.5 mL of BAL. Mice were subjected to serological examination 5 weeks later using an hemagglutination assay (Toxoscreen®; BioMérieux), according to the manufacturer’s instructions. As expected, serological testing was positive, and infection was confirmed in mice by the presence of cerebral cysts in brain tissue on microscopic examination. The remaining brain tissue was sent to the Centre National de Référence de la Toxoplasmose (Biological Resource Center, Reims – Limoges, France) for genotyping.

**Results**

The serological and parasitological results for the sequential samples are shown in Table 1. Parasite DNA was concomitantly detected in BAL, CSF, and whole blood samples on D112 post-transplant. As tachyzoites were visible on Giemsa-stained smears of BAL, two Swiss IOPS mice were immediately inoculated with the remaining fluid. The isolated *Toxoplasma* strain was typed as genotype II. After initiating specific treatment, the parasite load was carefully monitored in whole blood by quantitative PCR. On D12 post initiation of therapy, parasites could still be detected at decreasing but nevertheless significant numbers in whole blood and BAL (Table 1), leading clinicians to perform another lumbar puncture that was still positive at a low level on D14 post-therapy. A new blood sample obtained after 21 days of treatment had a residual DNA parasite load of 6 tachyzoites/mL and was associated with
clinical improvement and a decrease in fever. Total clearance of parasite DNA from blood was demonstrated 1 month later (Table 1). *Toxoplasma* serology showed stable IgG and IgM antibody titers until 3 weeks before dissemination of the parasite. The patient had persistent IgM detection, probably related to chronic residual IgM from a past infection, as demonstrated by a previous serological result obtained 1 year before transplant (Table 1). IgG and IgM antibody titers began to rise dramatically from the day of parasite isolation, reaching a peak 2 months after the episode and decreasing slowly over the next 4 months.

**Discussion**

Disseminated toxoplasmosis is a well-known life-threatening infectious complication in transplant recipients, although it is a rare event in SOT patients. It usually results from a mismatch between donor and recipient *Toxoplasma* serological status (seropositive donor to seronegative recipient) and transplantation of a graft containing encysted *Toxoplasma* (16, 19). Heart and heart-lung transplants carry the highest risk of *Toxoplasma* graft transmission to a seronegative recipient (32), which can be prevented by cotrimoxazole prophylaxis (25). In high-risk mismatched heart recipients, the efficacy and safety of cotrimoxazole has been established (2, 18) at the same dose regimen commonly used to prevent *P. jiroveci* pneumonia, but trimethoprim-sulfamethoxazole (160/800 mg) three times a week is also effective (2, 24). Conversely, a combination of pyrimethamine-sulfadoxine (75 mg/1500 mg) every 2 weeks has been reported to be ineffective at preventing heart-transmitted infection (13). In cases of intolerance, pyrimethamine alone (25 mg/day) can be a valuable alternative (24, 33). Prevention also relies on donor and recipient serological screening and counseling of seronegative patients to avoid exposure through food (31). In France, where seroprevalence is high, serological screening is mandatory for every organ donor and screening of recipients is strongly recommended, reducing the risk of graft-transmitted toxoplasmosis by both
prolonged chemoprophylaxis and careful follow-up of patients in cases of mismatch.

However, in countries with low *Toxoplasma* seroprevalence, guidelines may differ and serological screening is not routinely recommended for all patients (18, 25, 34).

Reactivation of past infection is more frequently observed in HSCT patients than in SOT patients and is associated with a high mortality rate (10, 11). Consequently, some HSCT centers monitor patients carefully for several months by repeated screening for *Toxoplasma* DNA using quantitative PCR on blood samples, since prompt initiation of therapy is crucial in patients with profound immunodeficiency (7, 14, 20). In heart transplant recipients, disseminated toxoplasmosis due to reactivation is rare and in most cases is favored by enhancement of immunosuppressive regimens in cases of acute rejection. Moreover, the incidence of reactivation is related to the prevalence of toxoplasmosis in the general population, a fact that explains the lack of consensus concerning biological follow-up and anti-*Toxoplasma* chemoprophylaxis in heart transplant patients with infection acquired prior to transplantation. Prophylaxis in *Toxoplasma* seropositive heart transplant patients is usually ensured by cotrimoxazole in the framework of *P. jiroveci* pneumonia prevention. The very low incidence of *Toxoplasma* reactivation in such patients does not justify a specific prophylaxis regimen outside this context (3), but the potential risk of reactivation of latent infection should not be ignored in patients receiving pentamidine for *P. jiroveci* pneumonia prophylaxis or with interruption of cotrimoxazole prophylaxis (11), as was the case in our patient. In our case, multiple infectious events, although non viral, that combined to stop prophylaxis, could have heightened immunodeficiency and favored *Toxoplasma* reactivation. This case and others (8) raise the question of prolonging prophylaxis for up to 3 months in patients with latent toxoplasmosis before transplantation, particularly in patients with serious complications in the post-operative months. However, cotrimoxazole was reported to be ineffective at preventing *Toxoplasma* reactivation in a liver transplant recipient receiving
antilymphocytic immunoglobulins for the treatment of acute graft rejection (4), suggesting
that other prophylactic regimens could be used in severely immunocompromised patients. An
alternative using pyrimethamine-sulfadoxine was proposed as a twice-weekly regimen for *P. jiroveci* pneumonia and toxoplasmic encephalitis prevention in HIV-infected patients (30) or
once weekly in bone marrow transplant recipients (12).

It is widely agreed that early diagnosis improves the prognosis of patients with disseminated
toxoplasmosis, but diagnostic strategies differ from one country to another. In our hospital,
*Toxoplasma* serological follow-up is carried out two or three times during the first year and
once a year thereafter. Serological reactivation with significantly increasing IgG titers, even
associated with specific IgM detection, can be observed in the absence of any clinical
manifestations. Nevertheless, it should prompt the search for *Toxoplasma* infection in the
presence of clinical signs. In the present case, serology was unhelpful for diagnosis as the last
serum sample obtained 24 days prior to *Toxoplasma*-positive BAL did not show any sign of
serological reactivation. Quantitative PCR allowed confirmation of cerebral involvement and
was useful for monitoring the response to treatment. Immunosuppressive drugs (MFM,
methylprednisolone hemisuccinate, and tacrolimus) were temporarily reduced to prompt an
immune response against the parasite, and were then restored at the full dose to avoid acute
rejection when the infection was under control. The delay to obtaining negative blood PCR
results was quite long (more than 3 weeks), but was shorter than in other reports with lethal
outcomes (21), probably reflecting the extent of immunodeficiency. The level of parasitemia
was similar to other reports (6). Furthermore, the isolate obtained from our patient was typed
as genotype II, an avirulent genotype widely isolated from both congenitally-infected and
immunocompromised patients in France (1), a fact that could explain the favorable outcome
observed here, in contrast to the poor outcomes reported by Delhaes et al. in two allo-SCT
patients infected with virulent genotypes (9). Reactivation of the recipient’s own *Toxoplasma*
cysts is the most probable hypothesis, although it has also been suggested that disseminated toxoplasmosis could be due to re-infection with a new strain acquired from the transplanted organ (27). Here, no cysts were observed in the myocardial biopsy performed on D124. Despite the lack of sensitivity inherent in the examination of small biopsy samples, this result could suggest peripheral reactivation of the recipient’s cysts rather than reactivation of an organ-transmitted strain.

Anti-Toxoplasma curative therapy usually relies on a synergistic combination of antimicrobial agents including pyrimethamine and sulfadiazine or pyrimethamine and clindamycin (15), but cotrimoxazole has also been shown to be effective as first-line treatment in HIV-infected patients (5). A reduction in immunosuppressive therapy is essential wherever possible and could explain the short delay to a favorable evolution in our case. Curative therapy is generally given at high doses for 6 weeks (31), followed by maintenance therapy. However, data on the duration and drug regimen for secondary prophylaxis are scarce, particularly in transplant patients. Cotrimoxazole (5) or sulfadiazine-pyrimethamine (25 mg/2g daily or 50 mg/2 g three times a week) (26) have been proposed for maintenance therapy of toxoplasma encephalitis in HIV-infected patients. In the present case, secondary prophylaxis associating cotrimoxazole at the end of each dialysis and 25 mg/daily pyrimethamine was chosen, to ensure optimal serum levels of protective drugs. The optimal duration of secondary prophylaxis has not been clearly defined, but should be at least 6 months, depending on the ongoing risk factors for reactivation (31).

In conclusion, disseminated toxoplasmosis remains a life-threatening infection in heart transplant recipients with previous immunity, although it is a rare event that mostly depends on Toxoplasma seroprevalence in the general population. Despite its low incidence, Toxoplasma reactivation must be rapidly investigated in patients with neurological signs,
unexplained fever or pulmonary infiltrates, since the prognosis is highly dependent on early
treatment.

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REFERENCES


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Table 1: Results of *Toxoplasma* serological follow-up and *Toxoplasma* DNA detection by quantitative PCR

<table>
<thead>
<tr>
<th>Date of sample collection (from day of transplantation)</th>
<th>Anti-<em>Toxoplasma</em> antibody titers</th>
<th>Quantitative <em>T. gondii</em> PCR (no. of parasites/mL of sample)</th>
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<tr>
<td></td>
<td>IgG $^a$ (IU/mL)</td>
<td>IgM $^b$ (ELISA index)</td>
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<tr>
<td>Day - 358</td>
<td>55</td>
<td>1.24</td>
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<tr>
<td>Day 0</td>
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</tr>
</tbody>
</table>

$^a$Positivity threshold: ≥9 (Platelia Toxo IgG; Biorad)

$^b$Positivity threshold: ≥1 (Platelia Toxo IgM; Biorad)

$^c$Positivity threshold: 9–12 (ISAGA IgM; BioMérieux)

CSF: cerebrospinal fluid; BAL: bronchoalveolar lavage; -: not done; undet: undetectable.