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Correlation of Parasite Load Determined by Quantitative PCR to Clinical Outcome in a Heart Transplant Patient with Disseminated Toxoplasmosis▽

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

Toxoplasmosis is a worldwide parasitic disease caused by the intracellular protozoan *Toxoplasma gondii*. After infection, acquired mostly 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, other organs. Whereas clinical symptoms are usually absent or mild in primarily infected immunocompetent individuals, the infection is life-threatening for immunocompromised patients (17). In transplant patients, severe or disseminated toxoplasmosis can result either from 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 differs according to the immunosuppression protocol and therefore according 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 higher seroprevalences. 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 biopsy specimens. Serology performs poorly in the diagnosis of reactivation of infection, due to a lack of sensitivity (in HSCT patients) or poor correlation with clinical reactivation (for 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 the 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 upon treatment. Quantitative PCR is considered to be a valuable tool for the diagnosis and monitoring of acute toxoplasmosis in SOT recipients. Our results reemphasize the need to monitor *Toxoplasma* reactivation in seropositive recipients, particularly in countries with high seroprevalences. 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 transplantation (Table 1), as was the donor (IgG level, 43 IU/ml; no IgM detected). Both serological results were indicative of past infection. Primary preventive treatment with cotrimoxazole (40 and 200 mg/day) for *Pneumocystis jirovecii* pneumonia prophylaxis was started on the first postoperative day (day 1 [D1]), continued until D27, and was then stopped because of intolerance (aggravation of thrombocytopenia from 62 g/liter to 39 g/liter, aggravation of leucopenia from 6 g/liter to 3 g/liter, and acute renal failure). Immunosuppressive therapy was started

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TABLE 1. Results of *Toxoplasma* serological follow-up and *Toxoplasma* DNA detection by quantitative PCR

Date of sample collection[a]	Titer of anti-*Toxoplasma* antibody[b]			*T. gondii* burden (no. of parasites/ml of sample) by quantitative PCR[c]		
	IgG (IU/ml)	IgM (ELISA index)	IgM (ISAGA index)	Whole blood	CSF	BAL fluid
Day −358	55	1.24	—	—	—	—
Day 0	54	1.49	11	—	—	—
Day +86	50	1.54	—	—	—	—
Day +112	106	5.51	—	1,800	12	6,000
Day +119	—	—	—	1,000	—	—
Day +124	—	—	—	170	—	400
Day +126	—	—	—	—	3	—
Day +129	2,302	7.4	—	50	—	—
Day +133	—	—	—	6	—	—
Day +164	—	—	—	Undet.	—	—
Day +185	>2,400	6.4	—	—	—	—
Day +285	996	2.62	—	—	—	—

[a] From the day of transplantation.
[b] Positivity thresholds were ≥9 for IgG (Platelia Toxo IgG ELISA; Bio-Rad), ≥1 for IgM by the Platelia Toxo IgM ELISA (Bio-Rad), and 9 to 12 for IgM by the IgM ISAGA (bioMérieux). —, not done.
[c] Undet., undetectable.

on D1 to D3 with mofetil mycophenolate (MFM) at 3 g/day, methylprednisolone hemisuccinate (440 mg/day on D1, 410 mg/day on D2, and 240 mg/day on D3), and thymoglobulin. The dose of methylprednisolone hemisuccinate was then reduced progressively to 30 mg/day on D30, without any change in the MFM regimen, and cyclosporine was introduced on D3 with residual rates ranging from 100 to 200 ng/ml.

The postoperative evolution was marked by several infectious and cardiovascular complications. During the early postoperative days (D3), the patient 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 fluid 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 extended-spectrum β-lactamase (ESBL) *E. coli* bacteremia and a Scarpa's triangle abscess treated with imipenem, and pneumopathy due to *Enterobacter aerogenes*, probably acquired through mechanical ventilation, which had a favorable outcome upon cefotaxime antibiotherapy. Everolimus was introduced on D46 in order to optimize the immunosuppressive regimen and reduce the cyclosporine dose. However, due to the lack of improvement of renal function, everolimus was stopped on D70. Cyclosporine was replaced by tacrolimus on D78, in combination with methylprednisolone hemisuccinate and MFM, because of the onset of thrombotic microangiopathy. On D104, the patient finally left the ICU with stable hemodynamics, despite renal failure requiring dialysis three times a week and encephalopathy attributed to anti-calcineurins.

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. BAL fluid was positive for *T. gondii* trophozoites by direct microbiological examination and negative for bacteria and viruses by culture and/or PCR. Cerebral tomodensitometry gave a normal result, and lumbar puncture yielded a clear CSF sample, with moderate hyperproteinorachia (0.56 g/liter) and normoglycorachia without hypercellularity (<1 leukocyte/μl), which was negative for bacteria and parasites by direct examination. PCR allowed the detection of high levels of *Toxoplasma* DNA in blood, BAL fluid, 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 at 50 mg/day and sulfadiazine at 3 g/day; the latter was replaced on D118 by clindamycin (2,400 mg/day) because of premature hematotoxicity. Because *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 a 3-ng/ml residual rate (versus 15 to 20 ng/ml before). At the same time, the lymphocyte count increased from 0.02 g/liter on D113 to 0.4 g/liter on D138. This decrease in immunosuppression was accompanied by a decrease in the parasitic burden. Definitive clearance of parasites from the different fluids was checked on D164. The patient's 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 the 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 at 25 mg/day and clindamycin at 1,200 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 the dose of 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 and 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 at 1,000 mg/day (reduced to 750 mg/day on D214), prednisone at 20 mg/day, and tacrolimus with a residual rate of 5.9 to 7.5 mg/liter. 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 fluid) were collected for diagnostic purposes (i.e., serology, direct examination [BAL fluid], and quantitative PCR [whole blood, CSF, BAL fluid]). After centrifugation, serum was removed and stored at −20°C until serological

testing. Slides were prepared from BAL fluid after cytocentrifugation at 91 × *g* for 5 min and were stained using Giemsa stain. All samples for DNA extraction were frozen at −80°C.

Serology. Specific anti-*Toxoplasma* IgG and IgM antibodies were detected using the Platelia Toxo IgG and Platelia Toxo IgM enzyme-linked immunosorbent assays (ELISAs) (Bio-Rad, Marnes-la-Coquette, France) and an immunosorbent agglutination assay (ISAGA) (IgM ISAGA; bioMérieux, Marcy l'Etoile, France).

Parasite DNA detection. DNA was extracted using QIAamp DNA minikit 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 accession no. AF487550) with primers 270F (5′-AGAGACACCG GAATGCGATCT) and 318R (5′-TTCGTCCAAGCCTCCGACT) and with probe 310T, labeled with 6-carboxyfluorescein (6-FAM) and 6-carboxytetramethylrhodamine (TAMRA) (5′-TCGTGGTGATGGCGGAGAGAATT GA). 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* strain RH recovered and purified from peritoneal lavage fluid from an infected mouse. The sensitivity of the PCR assay has been evaluated previously; it can detect 2 to 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 fluid. Mice were subjected to serological examination 5 weeks later by using an hemagglutination assay (Toxoscreen; bioMérieux), according to the manufacturer's instructions. As expected, serological testing gave positive results, 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 fluid, CSF, and whole-blood samples on D112 posttransplantation. Because tachyzoites were visible on Giemsa-stained smears of BAL fluid, two Swiss IOPS mice were immediately inoculated with the remaining fluid. The isolated *Toxoplasma* strain was typed as genotype II. After the initiation of specific treatment, the parasite load in whole blood was carefully monitored 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 fluid (Table 1), leading clinicians to perform another lumbar puncture that still gave a low-level positive result on D14 posttherapy. A new blood sample obtained after 21 days of treatment had a residual parasite DNA 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 the 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 transplantation (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 the serological *Toxoplasma* statuses of the donor and the recipient (seropositive donor to seronegative recipient) and the 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. jirovecii* pneumonia, but trimethoprim-sulfamethoxazole (160 and 800 mg) three times a week is also effective (2, 24). In contrast, a combination of pyrimethamine and sulfadoxine (75 mg and 1,500 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 serological screening of the donor and the recipient, as well as counseling of seronegative patients to avoid exposure through food (31). In France, where seroprevalence is high, serological screening is mandatory for every organ donor; screening of recipients is strongly recommended; and the risk of graft-transmitted toxoplasmosis is reduced both by prolonged chemoprophylaxis and by 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 for 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 for heart transplant patients with infections acquired prior to transplantation (23). Prophylaxis in *Toxoplasma*-seropositive heart transplant patients is usually ensured by cotrimoxazole in the framework of *P. jirovecii* 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 for patients receiving pentamidine for *P. jirovecii* pneumonia prophylaxis or with interruption of cotrimoxazole prophylaxis (11), as was the case for our patient. In our case, multiple infectious events, although nonviral, 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 as long as 3 months for patients with latent toxoplasmosis before transplantation, particularly patients with serious complications in the postoperative months. However, cotrimoxazole has been 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 for severely immunocompromised patients. An alternative using pyrimethamine-sulfadoxine was proposed as a twice-weekly regimen for the prevention of *P. jirovecii* pneumonia and toxoplasmic encephalitis in HIV-infected patients (30) or as a once-weekly regimen for 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 present case, serology was unhelpful for diagnosis, since the last serum sample obtained 24 days prior to the finding of *Toxoplasma*-positive BAL fluid showed no sign of serological reactivation. Quantitative PCR allowed confirmation of cerebral involvement and was useful for monitoring the response to treatment. The doses of immunosuppressive drugs (MFM, methylprednisolone hemisuccinate, and tacrolimus) were temporarily reduced to prompt an immune response against the parasite, and these drugs were then restored at the full doses in order to avoid acute rejection when the infection was under control. The delay before PCR-negative blood results were obtained was quite long (more than 3 weeks), but shorter than that in reports with lethal outcomes (21), probably reflecting the extent of immunodeficiency. The level of parasitemia was similar to that in 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. for 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 reinfection 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 the first-line treatment for 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 for transplant patients. Cotrimoxazole (5) or sulfadiazine-pyrimethamine (25 mg and 2 g daily or 50 mg and 2 g three times a week) (26) has been proposed for maintenance therapy of toxoplasma encephalitis in HIV-infected patients. In the present case, secondary prophylaxis with cotrimoxazole at the end of each dialysis and 25 mg of pyrimethamine daily was chosen in order to ensure optimal levels of protective drugs in the serum. The optimal duration of secondary prophylaxis has not been clearly defined, but it 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 depends mostly on the seroprevalence of *Toxoplasma* in the general population. Despite its low incidence, *Toxoplasma* reactivation must be investigated rapidly for patients with neurological signs, unexplained fever, or pulmonary infiltrates, since the prognosis is highly dependent on early treatment.

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