

Disseminated Toxoplasmosis, Resulting from Infection of Allograft, after Orthotopic Liver Transplantation: Usefulness of Quantitative PCR

F. Botterel,^{1*} P. Ichai,² C. Feray,² P. Bouree,¹ F. Saliba,² R. Tur Raspa,³ D. Samuel,² and S. Romand^{1,4}

Service de Microbiologie, Département de Parasitologie-Mycologie, Hôpital de Bicêtre, Faculté de Médecine Paris-Sud, 94275 Kremlin-Bicêtre Cedex,¹ Centre Hépatobiliaire, Paul Brousse Hospital, 94804 Villejuif,² and Institut de Puericulture, F 75014 Paris,⁴ France, and Liver Unit, Rabin Medical Center, Tel Aviv, Israel³

Received 4 September 2001/Returned for modification 4 December 2001/Accepted 12 February 2002

Disseminated toxoplasmosis is a life-threatening disease in liver transplant recipients that can result from an organ-transmitted infection. We report here a case of fatal disseminated toxoplasmosis after orthotopic liver transplantation from a seropositive donor (immunoglobulin G [IgG]⁺ and IgM⁻) in a patient who was nonimmune for toxoplasmosis prior to transplantation. Quantitative PCR analyses of various clinical specimens, including serum samples, appeared retrospectively to be a valuable diagnostic tool that might guide therapeutic attitudes.

Toxoplasmosis is a worldwide infectious disease caused by the protozoan *Toxoplasma gondii*. Disseminated life-threatening infection is more common in immunocompromised individuals, such as patients with human immunodeficiency virus infection or transplant recipients. In these situations, early treatment significantly reduces the extent of the damage (5). However, the classical diagnosis of toxoplasmosis based on serological tests is inefficient and inadequate in these patients (5, 7). Therefore, the diagnosis is based on the direct demonstration of the parasite in tissues or biological fluids or detection of *Toxoplasma* DNA by PCR. We report here a case of disseminated fatal toxoplasmosis after orthotopic liver transplantation in a patient who had negative toxoplasmosis serology, and we also consider the contribution of quantitative PCR in various clinical specimens as a valuable diagnostic tool.

CASE REPORT

A 27-year-old French female, a native of Israel, underwent an orthotopic liver transplantation for end-stage liver cirrhosis (biliary cirrhosis due to autoimmune cholangitis) on 6 October 2000. The donor was a 29-year-old French female who died of aneurysm rupture. The immune serologic profile of the donor was consistent with a prior infection (i.e., the presence of immunoglobulin G [IgG] and the absence of IgM), whereas the recipient had a negative serology prior to transplantation (Table 1). The recipient maintenance immunosuppressive regimen included tacrolimus, mycophenolate mofetil, and methyl-prednisolone. At 12 days posttransplantation, fever and fatigue were noted. Thereafter, an explorative laparotomy was performed because of suspicion of peritonitis. Culture of the peri-

toneal fluid specimen indicated the presence of methicillin-resistant staphylococci. Antimicrobial therapy consisting of targocid, fosfomycin, and tazocillin was immediately started. Biological parameters showed pancytopenia and hypoxia. A bronchoalveolar lavage (BAL) was performed at day 26 after transplantation, and cytomegalovirus was diagnosed and treated with ganciclovir.

Despite this therapy, there was no improvement in the patient's condition. Specific screening tests, including blood cultures and urine cultures, were negative. At day 40 after transplantation, the patient developed acute respiratory failure with shock. Upon admission at the intensive care unit, the patient presented with severe dyspnea, unstable hemodynamics, and hyperthermia. Tazocillin was stopped and tienam, fluconazole, and filgrastim were started. A chest radiograph showed alveolar pneumopathy with pleural effusion. A second BAL was promptly performed. Direct microscopic examination of the BAL fluid specimen (by Giemsa staining) revealed several structures that were pathognomonic of tachyzoites of *T. gondii*, and therapy with pyrimethamine and sulfadiazine was initiated. Nevertheless, the patient died 5 days after receiving this specific anti-*Toxoplasma* treatment (day 50 after transplantation).

More than 9 months after transplantation, other recipients who received organs from the same donor (heart, lungs, kidneys, and cornea) remain in good health, with no evidence of infection. These recipients had positive serology for toxoplasmosis before transplantation.

MATERIALS AND METHODS

PCR analyses. Detection and quantitative evaluation of *Toxoplasma* DNA were attempted retrospectively by a PCR test on various clinical specimens from the patient. The newly developed, commercially available LightCycler system was used for the PCR analyses. This system allows real-time detection and quantification of specific PCR products by fluorescence resonance energy transfer (2).

Nucleic acid extraction. Nucleic acid was extracted from 0.2 ml of each specimen by using the High Pure PCR Template preparation kit (Roche Molecular Biochemicals, Meylan, France) according to the manufacturer's protocol. DNA

* Corresponding author. Mailing address: Parasitology and Mycology Department, Le Kremlin-Bicêtre Hospital, 78, rue du Général Leclerc, Paris XI University, F 94275 Le Kremlin-Bicêtre, France. Phone: (33) 1-45-21-33-21. Fax: (33) 1-45-21-33-19. E-mail: francoise.botterel@bct.ap-hop-paris.fr.

TABLE 1. Quantitative *T. gondii* real-time PCR test analysis of sequential samples from a patient with disseminated toxoplasmosis after orthotopic liver transplantation

Sample collection point (day) ^c	Anti- <i>T. gondii</i> antibodies in serum				Quantitative <i>T. gondii</i> PCR (no. of tachyzoites/ml of sample) in:		
	IgG (IU/ml) ^a		IgM (index) ^b		BAL fluid	Whole blood	Serum
	ELISA	Dye test	ELISA	ISAGA			
-28	<8	<1	0.07	0			Negative
0						Transplantation	
3	9	2	0.05	0			Negative
10	<8	1	0.05	0			Negative
13	<8	1	0.05	0			Negative
27					5 × 10 ²		
31	<8	1	0.1	0			7 × 10 ²
38	<8	1	0.09	0			
42					7.5 × 10 ³		6.5 × 10 ²
44	<8	1	0.16	0		3 × 10 ³	5 × 10 ²

^a IgG titers are expressed in international units (IU) per milliliter of serum. For the commercially available ELISA (Vidas), the cutoff value was 8 IU/ml. The cutoff value of the dye test was 1 IU/ml.

^b IgM values are expressed as an index. The cutoff value of the Vidas ELISA was 0.6; the cutoff value of the immunosorbent agglutination assay (ISAGA) was 6.

^c That is, the number of days relative to the day of transplantation.

was eluted from the column with 50 µl of PCR-grade H₂O and then amplified by LightCycler PCR (LC-PCR; Roche).

LC-PCR. LC-PCR was targeted at the *T. gondii* B1 gene, according to a protocol described in detail elsewhere (2). The primers for amplification were the 23-mer 5'-GGAGCACTGGCAACCTGGTGTGCG-3' (sense) and the 25-mer 5'-TTGTTTACCCCGGACCGTTTAGCAG-3' (antisense). The two hybridization probes (Tib MolBiol, Berlin, Germany) were the 25-mer 5'-ACGG GCGAGTAGCACCTGAGGAGAT-3', which was labeled at the 5' end with LC-red 640 and phosphorylated at the 3' end, and the 27-mer 5'-CGGAAATA GAAAGCCATGAGGCACTCC-3', which was labeled at the 3' end with fluorescein.

For quantification, one 10-fold serial dilution of *T. gondii* DNA (RH strain) was prepared that ranged from 0.1 to 10 parasites/µl. This series of 10-fold dilutions was included in each amplification run.

LC-PCRs were performed in a final volume of 20 µl by using the DNA Master Hybridization Probes Kit (Roche), with a 0.5 µM concentration of each primer, a 0.25 µM concentration of each probe, and 5 µl of extracted DNA sample. Carryover contamination was prevented by using heat-labile uracil-DNA-glycosylase (UNG; Roche Molecular Biochemicals). The amplification was carried out in a LightCycler Instrument (Roche) according to a previously described protocol (2). Quantitative results of the PCR were expressed by plotting the fluorescence signal of each positive sample against the standard curve obtained with known concentrations of the parasites, and the parasitic load was calculated by interpolation from this standard curve.

Clinical samples. Several serum, whole-blood, and BAL samples from the patients were consecutively collected for serology (serum), light microscopy (BAL), or culture (whole blood). All samples were frozen within 4 h of receipt in the laboratory and kept stored at -20°C until retrospective analyses by LC-PCR.

Serology antibody. Titers against *T. gondii* in transplant recipients were measured by an enzyme-linked immunosorbent assay (ELISA) for IgG and IgM (Vidas; Biomérieux, Marcy l'Étoile, France), with the addition of an immunosorbent agglutination assay for IgM (Biomérieux) and a direct agglutination assay (Toxoscreen; Biomérieux) for IgG. A dye test was retrospectively performed to detect IgG in selected serum samples to monitor the sensitivity of these reactions.

RESULTS

The PCR and serology results obtained in various specimens before and after transplantation are summarized in Table 1. IgG and IgM antibodies were not detected prior to transplantation (day -28). After transplantation, IgG antibodies were weakly positive but without detection of IgM.

The retrospective and simultaneous analysis of nine serum samples by LC-PCR showed that the first PCR-positive serum

sample was collected on day 31 after transplantation and on day 27 in BAL fluid.

DISCUSSION

Disseminated toxoplasmosis after liver transplantation is a rare event but in most cases results in a fatal outcome (1, 3, 5, 7, 10). The rarity of the disease and the nonspecificity of the symptoms have led to a general lack of awareness among clinicians and, hence, a high mortality rate among transplanted patients due to the delayed initiation of therapy.

In the case described here, toxoplasmosis was probably transmitted via an infected allograft since all serologic reactions, including the highly sensitive dye test, did not detect specific IgG antibodies in the recipient prior to transplantation. This assumption is also supported by the serologic status of the donor, who was seropositive for toxoplasmosis. In addition, the weak and delayed appearance of IgG antibodies after transplantation, detected by both ELISA and dye test, could be considered as a seroconversion, despite the absence of IgM, in a severely immunocompromised patient, as previously reported in otherwise immunosuppressed patients (5). Nevertheless, serologic diagnosis failed to demonstrate a severe *Toxoplasma* infection clearly and early in our patient. Thus, in immunocompromised patients diagnosis is usually based on the direct demonstration of the parasites in biological specimens by light microscopy or tissue culture (9). However, these techniques are often time-consuming, lack sensitivity and, as illustrated here, may delay the time of diagnosis and initiation of treatment. In contrast, the PCR technique allows a simple, rapid, and highly sensitive detection of *T. gondii* DNA in various specimens and represents a valuable diagnostic tool for assessing disseminated toxoplasmosis (2, 4, 6). Additionally, the LC-PCR system provides real-time detection and quantification of amplified products in less than 3 h, including the DNA extraction steps (11). Thus, postmortem PCR assays applied to serum samples could retrospectively assess a disseminated infection as early as day 31 after transplantation, i.e., 14 days before specific anti-*Toxoplasma* treatment had

been administered. As shown in Table 1, despite much lower parasitic loads in serum than in whole-blood samples, it is noteworthy that specific PCR products could be detected from unconcentrated serum samples. Subsequent samples showed increasing parasitic loads, a finding that is probably consistent with the progression of the disease. The high level of parasitic load in the first BAL sample also illustrates the sensitivity of PCR, although light microscopy failed to detect tachyzoites, even after a careful reexamination of BAL smears.

As suggested by others, this case raises the question of specific surveillance and prophylaxis of toxoplasmosis after liver transplantation (5, 8). Although it is recommended that human immunodeficiency virus-positive people who are also *Toxoplasma* antibody positive receive toxoplasmosis prophylaxis, there are currently no recommendations for the management of transplant recipients. Serologic screening of donor and recipient may not be relevant since disseminated toxoplasmosis can result either from reactivation of a latent infection or from a contaminated graft (5). Additionally, in otherwise seronegative but severely immunocompromised recipients, such as the patient described here, serologic methods lack sensitivity and may fail to clearly show a seroconversion. Since early initiation of specific anti-*Toxoplasma* therapy is a critical prognosis factor, highly sensitive PCR methods that can be applied directly to serum samples should be of great help in cases of unexplained fever in immunocompromised recipients. In addition, the real-time quantitative LC-PCR would be useful for monitoring treatment.

REFERENCES

1. Anthony, C. W. 1972. Disseminated toxoplasmosis in a liver transplant patient. *J. Am. Med. Womens Assoc.* **27**:601–603.
2. Costa, J. M., C. Pautas, P. Ernault, F. Foulet, C. Cordonnier, and S. Bretagne. 2000. Real-time PCR for diagnosis and follow-up of *Toxoplasma* reactivation after allogeneic stem cell transplantation by using fluorescence resonance energy transfer hybridization probes. *J. Clin. Microbiol.* **38**:2929–2932.
3. Hasegawa, S., K. Tanaka, H. Egawa, Y. Inomata, M. Murakawa, Y. Terada, T. Matsui, T. Arai, Y. Yamaoka, and K. Mori. 1996. Perioperative respiratory management with fiberoptic bronchoscopy in pediatric living-related liver transplantation. *Surgery* **119**:198–201.
4. Kupferschmidt, O., D. Kruger, T. K. Held, H. Ellerbrok, W. Siegert, and K. Janitschke. 2001. Quantitative detection of *Toxoplasma gondii* DNA in human body fluids by TaqMan polymerase chain reaction. *Clin. Microbiol. Infect.* **7**:120–124.
5. Lappalainen, M., T. S. Jokiranta, L. Halme, O. Tynneninen, I. Lautenschlager, K. Hedman, K. Hockerstedt, and S. Meri. 1998. Disseminated toxoplasmosis after liver transplantation: case report and review. *Clin. Infect. Dis.* **27**:1327–1328.
6. Lin, M. H., T. C. Chen, T. Kuo, C. C. Tseng, and C. P. Tseng. 2000. Real-time PCR for quantitative detection of *Toxoplasma gondii*. *J. Clin. Microbiol.* **38**:4121–4125.
7. Mayes, J. T., B. J. O'Connor, R. Avery, W. Castellani, and W. Carey. 1995. Transmission of *Toxoplasma gondii* infection by liver transplantation. *Clin. Infect. Dis.* **21**:511–515.
8. Patel, R. 1999. Disseminated toxoplasmosis after liver transplantation. *Clin. Infect. Dis.* **29**:705–706.
9. Renoult, E., E. Georges, M. F. Biava, C. Hulin, L. Frimat, D. Hestin, and M. Kessler. 1997. Toxoplasmosis in kidney transplant recipients: report of six cases and review. *Clin. Infect. Dis.* **24**:625–634.
10. Singh, N., T. Gayowski, and I. R. Marino. 1996. *Toxoplasma gondii* pneumonia in a liver transplant recipient: implications for diagnosis. *Liver Transpl. Surg.* **2**:299–300.
11. Wittwer, C. T., K. M. Ririe, R. V. Andrew, D. A. David, R. A. Gundry, and U. J. Balis. 1997. The LightCycler: a microvolume multisample fluorimeter with rapid temperature control. *BioTechniques* **22**:176–181.