 10.1111/j.1469-0691.2008.02091.x

Prevention of toxoplasmosis in transplant patients

F. Derouin[1] and H. Pelloux[2], on behalf of the ESCMID Study Group on Clinical Parasitology

[1]Laboratory of Parasitology and Mycology, University Paris and Hôpital Saint-Louis, Assistance Publique-Hôpitaux de Paris, and [2]Parasitology-Mycology laboratory, A Michallon teaching Hospital, and UMR CNRS-UJF 5163, Grenoble, France

ABSTRACT

Toxoplasmosis is a life-threatening opportunistic infection that affects haematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT) recipients. Its incidence in these patients is closely related to the prevalence of toxoplasmosis in the general population, which is high in Europe. In SOT recipients, toxoplasmosis results mainly from transmission of the parasite with the transplanted organ from a *Toxoplasma*-seropositive donor to a *Toxoplasma*-seronegative recipient. This risk is high in cases of transplantation of organs that are recognized sites of encystation of the parasite, e.g. the heart, and is markedly lower in other SOT recipients. Clinical symptoms usually occur within the first 3 months after transplantation, sometimes as early as 2 weeks post transplant, and involve febrile myocarditis, encephalitis or pneumonitis. In HSCT recipients, the major risk of toxoplasmosis results from the reactivation of a pre-transplant latent infection in seropositive recipients. The median point of disease onset is estimated at 2 months post transplant, with <10% of cases occurring before 30 days and 15–20% later than day 100. Toxoplasmosis usually manifests as encephalitis or pneumonitis, and frequently disseminates with multiple organ involvement. Diagnosis of toxoplasmosis is based on the demonstration of parasites or parasitic DNA in blood, bone marrow, cerebrospinal fluid, bronchoalveolar lavage fluid or biopsy specimens, and serological tests do not often contribute to the diagnosis. For prevention of toxoplasmosis, serological screening of donors and recipients before transplantation allows the identification of patients at higher risk of toxoplasmosis, i.e. seropositive HSCT recipients and mismatched (seropositive donor/seronegative recipients) SOT recipients. Preventing toxoplasmosis disease in those patients presently relies on prophylaxis via prescription of co-trimoxazole.

Keywords Prevention, *Toxoplasma gondii*, toxoplasmosis, transplantation

Accepted: 16 August 2008

Clin Microbiol Infect 2008; **14**: 1089–1101

INTRODUCTION

Toxoplasma gondii is a protozoan parasite with a worldwide prevalence in the general population that exceeds 50% in some countries. Human infection is acquired mainly by ingestion of undercooked infected meat containing *Toxoplasma* cysts or by ingestion of oocysts from faecally contaminated foods or via poor hand hygiene. In immunocompetent individuals, acute infection is usually self-limited and rarely symptomatic, although some cases of severe infection due to unusual *Toxoplasma* genotypes have been reported in South and Central America [1]. The acute phase of infection is followed by a latent chronic phase that is characterized by the life-long persistence of cysts in tissues. The containment of cysts by specific immunity is a determiming factor for this latency, as it has been shown experimentally that depleting cellular immunity or cytokine mediators of macrophage activation results in the reactivation of chronic toxoplasmosis [2]. In humans, the relationship between immunosuppression and occurrence of severe toxoplasmosis is well recognized. In human immunodeficiency virus (HIV)-infected patients, the incidence of toxoplasmic encephalitis is

Corresponding author and reprint requests: Francis Derouin, Laboratoire de Parasitologie-Mycologie, Hôpital Saint-Louis, 1 avenue Claude Vellefaux, 75475 Paris Cedex 10, France
E-mail: francis.derouin@sls.aphp.fr

closely related to the progression of immunodeficiency and a decrease in CD4 counts [3,4], whereas restoration of immunity following highly active antiretroviral therapy markedly decreases this incidence [5].

This relationship supports the rationale for preventing toxoplasmosis in HIV-infected patients via specific prophylaxis in patients with CD counts <100/mm^3 and by initiating highly active antiretroviral therapy to restore cellular immunity [6,7].

In non-AIDS immunocompromised patients, the risk of severe toxoplasmosis is also well recognized. In an extensive review of the literature, Israelki and Remington [8] identified 212 cases between 1953 and 1993, highlighting the high frequency of life-threatening toxoplasmosis in organ transplant patients. Since then, the practice of solid organ and bone marrow transplantation has markedly progressed, and the conditions of transplantation, as well as measures undertaken for prevention of infection, have improved.

Taking into consideration what has been learned from the management of toxoplasmosis in AIDS patients, and considering the new methods for diagnosis of toxoplasmosis, the objective of this review is to consider the specific features of toxoplasmosis in transplant patients, focusing on practical approaches to diagnosis and prevention.

PATHOGENESIS OF TOXOPLASMOSIS IN TRANSPLANT RECIPIENTS

In transplant recipients, toxoplasmosis can result either from the transmission of the parasite with the transplanted organ from a *Toxoplasma*-seropositive donor (D^+) to a *Toxoplasma*-seronegative recipient (R^-) or from the reactivation of a pretransplant latent infection in a seropositive recipient (R^+). Transmission of *T. gondii* via organ transplantation from a seropositive donor to a seropositive recipient seems to be possible. *Toxoplasma* transmission by blood transfusion is extremely rare.

Graft-transmitted toxoplasmosis

A clear differentiation should be made between solid organ transplantation (SOT), during which the risk of transmission is potentially high, and allogeneic haematopoietic stem cell transplantation (HSCT), during which this risk is more theoretical than real (Table 1).

Transmission of T. gondii from a recently infected donor to a seronegative recipient. This risk of transmission is related to the possible presence of *T. gondii* in the blood and tissue of a donor who had been infected by *T. gondii* several days or weeks before blood/tissue collection. In that case,

Table 1. Summary of risk factors for toxoplasmosis in transplant patients

	Type of risk		Clinical disease and prevention	
	Transmission	**Reactivation**	**Onset of symptoms**	**Efficient prophylaxis**
Haematologous stem cell transplantation				
Autologous	No risk	Very low in R^+	9–120 days	ND
Allogenic[a]	Possible but no case reported	High in R^+, regardless of donor's serology. Increased risk in cord blood SCT	Median: 62 days. 80% of cases in the second and third months post-HCST	CTX for pneumocystosis and toxoplasmosis. Alternatively, pyrimethamine–sulphadoxine. Given from day 30 to day 180 after SCT. Prolonged or restarted in cases of GVHD or sustained immunosuppression
Solid organ transplantation				
Heart/heart–lung[b]	High in mismatched D^+/R^-	Low in R^+	25–195 days	CTX given for pneumocystosis
Liver[c]	Low in mismatched D^+/R^-	Low in R^+	Median: 24 days in D^+/R^-; up to 6 years in R^+	CTX given for pneumocystosis
Kidney[c]	Low in mismatched D^+/R^-	Low in R^+	Median: 19 days in D^+/R^-, up to 2 months in R^+	ND
Intestine[c]	Low in mismatched D^+/R^-	ND	1 case, month 3	ND
Other (eye, bone, artery)	No risk	No risk	–	–

D, donor; R, recipient; +, positive pretransplant *Toxoplasma* serology; –, negative pretransplant *Toxoplasma* serology; ND, not documented; CTX, co-trimoxazole; SCT, stem cell transplant; HCST, haematopoietic stem cell transplant; GVHD, graft-versus-host disease.
[a]Adapted from references [12,42,45–47].
[b]Adapted from reference [25].
[c]Adapted from reference [32].

tachyzoites of *T. gondii* (i.e. the rapidly dividing stage of the parasite) may be present in the blood, bone marrow and in various organs, as the early phase of infection is characterized by a brief parasitaemia. Several cases of transmission of *T. gondii* to SOT patients who received organs from a single donor whose *Toxoplasma* serology was indicative of a recently acquired toxoplasmosis have been reported [9,10]. In HSCT, there is a potential risk of transmission if the donor is parasitaemic at the time of bone marrow collection, but, to our knowledge, it has never been confirmed, although it was suspected in several cases occurring in pre-transplant seronegative recipients [11,12].

The duration of the potential risk of transmission after an acquired infection of the donor is not known. In animal models of toxoplasmosis, parasitaemia can be detected up to 3 weeks following oral contamination by *T. gondii* cysts [13]. In humans, the precise duration of parasitaemia is not known. One study reported isolation of *T. gondii* by mouse inoculation 2 and 14 months after onset of toxoplasmosis [14]. In a more recent study using nested PCR, 53% of the patients with acute toxoplasmic lymphadenopathies had a detectable parasitaemia 5 weeks after the onset of symptoms [15].

Transmission from a chronically infected donor to a seronegative recipient. At the chronic stage of toxoplasmosis, tissue cysts are formed in the neural and muscular tissues, including the brain, eyes, skeletal muscles and cardiac muscles, but may also develop in visceral organs, including the lungs, liver and kidneys [16,17]. Their size range between 10–150 μm in diameter, and they contain hundreds to thousands of bradyzoites (i.e. the slowly dividing stage of the parasite). It is believed that tissue cysts can occasionally rupture without any clinical symptoms in immunocompetent hosts, the released bradyzoites being destroyed by the specific anti-*Toxoplasma* immunity [18].

Therefore, some organs taken from a donor who has been previously infected by *T. gondii*, even years before, probably contain cysts. Without specific immunity to *T. gondii* (seronegative recipient) and in the context of immunosuppressive therapy for transplant engraftment and tolerance, cysts present in the transplanted organ may be uncontrolled and may rupture, leading to an active infection. This risk is predominant in cases of transplantation of organs that are recognized sites of encystation, such as the heart (striated muscle), and is markedly lower when other organs are involved. It is estimated that there is no risk of cyst transmission with haematopoietic stem cells.

Heart and heart–lung transplantation

Since the initial reports in the 1970s [19,20], most cases of toxoplasmosis in heart and heart–lung transplantation have been reported in seronegative recipients receiving a heart transplant from a seropositive donor [8,21–27]. Clinical symptoms usually occur in the first 3 months after transplantation (sometimes as early as 2 weeks post transplant) and consist of febrile myocarditis, encephalitis or pneumonitis [8,22,25]. The true incidence of toxoplasmosis in cases of mismatch (D^+/R^-) is not precisely known but can reach 25–75% in the absence of prophylaxis (Table 2). Administration of co-trimoxazole for prophylaxis of pneumocystosis proved efficient in preventing toxoplasmosis in cases of mismatch patients [28,29].

Non-cardiac SOT

Cases of organ-transmitted toxoplasmosis are much less frequent with organs other than the heart [8,30,31]. In a recent review of the literature covering 40 years, Cambpell *et al.* [32] identified 52 cases of toxoplasmosis in non-cardiac SOT recipients (kidney, 34; liver, 12; pancreas, one; multivisceral, four; small bowel, one), i.e. a very low risk of toxoplasmosis in light of the number of such transplantations performed each year throughout the world. In that review, the serological status of donors and recipients was available for 20/52 patients, with a D^+/R^- mismatch in 16 cases. In these patients, toxoplasmosis manifested early after transplantation, with a median time from transplant to symptoms of 21 days (range 14–28 days). In most cases, serological data were insufficient to determine whether transmission from a recently infected donor could have occurred. However, it is estimated that the risk of transmission of *T. gondii* cysts with liver, kidney, pancreas or intestine from a chronically infected donor is very low, because *T. gondii* is not believed to form persistent cysts in these organs [32,33].

Table 2. Reported prevalence of organ-transmitted and reactivated toxoplasmosis in heart and heart–lung transplant recipients in various countries

	Transmission in seronegative recipients			Reactivation in seropositive recipients			
Country	Number of seronegative recipients	Number of D^+/R^- mismatches	Clinical toxoplasmosis (prevalence)	Number of seropositive recipients	Serological reactivations (prevalence)	Clinical toxoplasmosis	Reference
USA	509 (84%)	32	4 (12.5%; 25% in the 16 patients without prophylaxis)	98 (16%)	ND	0/98	Montoya *et al.* [27]
USA	31 (62%)	4	3 (75%)	19 (38%)	10/19 (52.6%)	0	Luft *et al.* [21]
Switzerland	52 (43%)	18	3 (16.7%)	69 (57%)	5/69 (7.2%)	2/5	Gallino *et al.* [24]
UK	175 (70%)	21	6 (28.5%; 57% in the four of seven patients without prophylaxis)	75 (30%)	3/75 (4%)	2/3	Wregitt *et al.* [22]
France	23 (36%)	ND	0	42 (64%)	10 (23.8%)	0	Lavarde *et al.* [59]

D^+, seropositive donor; R^-, seronegative recipient; ND, not documented.

Transmission of T. gondii from a seropositive donor to a seropositive recipient. Cases of severe toxoplasmosis have also been observed in seropositive recipients receiving a heart transplant from a seropositive donor [34], but this is far less frequent than in cases of mismatch (D^+/R^-), supporting the notion that recipient immunity is protective against the transmission of *T. gondii*. In this case, graft transmission is difficult to confirm and to differentiate from a reactivation of latent infection in the recipient. However, the hypothesis that a seropositive recipient could be re-infected by a transplant from a seropositive donor has been suggested by Robert-Gangneux *et al.* [35]. In this study, western blot analysis of post-transplant sera of seropositive recipients showed neosynthesized IgG that could be related to the recognition of a new parasite strain, possibly acquired via the transplanted organ from a *Toxoplasma*-seropositive donor. The only means of proving this re-infection would be to identify the infecting strain(s), by serotyping or genotyping [36,37].

Reactivation of a latent toxoplasmosis

The reactivation of a previously acquired and chronic infection is the main cause of toxoplasmic encephalitis in AIDS patients, and it has been closely related to the severity and progression of immunosuppression. The precise factors that trigger tissue cyst rupture and reactivation are largely unknown, but the risk of occurrence of toxoplasmic encephalitis sharply increases when the blood CD4 count is below 200 CD4/mm^3.

In transplant recipients, this risk of reactivation also exists for recipients who are seropositive for *Toxoplasma*. It is closely related to the degree and duration of immunodeficiency and, therefore, markedly differs according to the type of transplantation (Table 1). Moreover, the incidence rate of *Toxoplasma* reactivation in transplant patients varies according to countries, as it is closely related to the prevalence of toxoplasmosis in the general population.

HSCT recipients. Allogeneic HSCT recipients are at very high risk, whereas toxoplasmosis is a rare event in autologous HSCT recipients [38–41]. Allogeneic HSCT recipients are also at much higher risk than SOT recipients, because of the severity of duration of immunosuppression experienced by these patients. The main risk factors for toxoplasmosis in HSCT recipients, identified in a recent prospective study, were: cord blood transplantation (as opposed to other sources of stem cells), advanced underlying disease, anti-thymocyte globulin during a conditioning regimen, receipt of an organ from a seronegative donor, and lack of appropriate prophylaxis. In multivariate analysis, only cord blood transplantation, which is associated with a very severe and protracted immunocompromised status, retained statistical significance [42].

From several reviews and case reports [12,42–48], the median time to disease onset can be estimated at 2 months post-HSCT, with fewer than 10% of cases occurring before 30 days and 15–20% later than day 100 (Fig. 1). Therefore, the risk of *Toxoplasma* reactivation in seropositive recipients is particularly high during the 2–4 months post transplant. It can be prolonged to 6 months, and is even longer in cases of delayed immune reconstitution and occurrence of graft-versus-host disease.

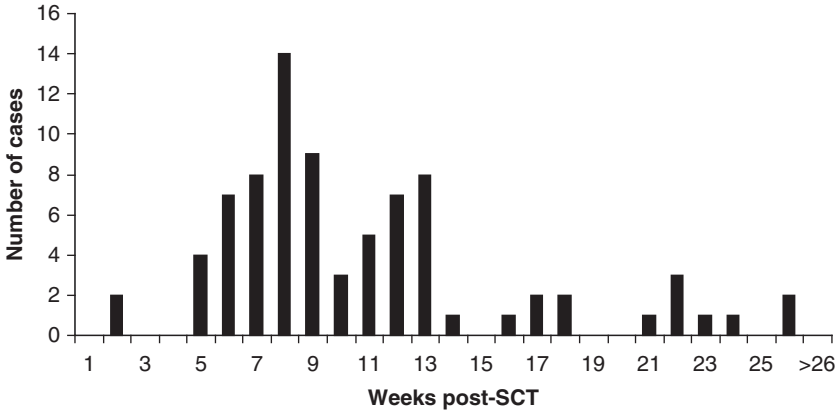

Fig. 1. Onset of toxoplasmosis in 81 allogeneic stem cell transplant (SCT) recipients; adapted from references [12,42,45–47].

The extreme severity of the disease is highlighted in most studies and case reports, and the mortality rate is high. Toxoplasmic encephalitis presenting with brain abscesses is the most frequent initial presentation [48,49], but diffuse brain lesions and meningitis have also been reported [45,50,51]. Pneumonitis, including acute distress syndrome and disseminated toxoplasmosis with multiple organ involvement, is frequently reported in autopsies [12,52,53], and has been associated with a haemophagocytic syndrome in some cases [54].

The true incidence of toxoplasmosis in HSCT recipients is difficult to estimate, because diagnosis is difficult, and autopsy records indicate that cases might have been misdiagnosed. The reported incidence markedly varies from 0.2% to 4% according to HSCT centre, with higher rates of incidence being seen in countries where the prevalence of toxoplasmosis is high in the general population. However, if only the seropositive recipients are considered, the range of incidences among countries is more narrow (1.9–5.7%) (Table 3) [40,42,44–47,55,56].

It has been suggested that the serological status of the donor could influence the risk of occurrence of reactivation, as approximately 80% of reported cases of toxoplasmosis are observed in patients whose donor was seronegative for *T. gondii* (D^-/R^+) [42,43,45]. However, the protective function of the grafted cell from a seropositive donor is very uncertain, as the immune reconstitution of the recipient by the donor's cell is delayed and incomplete. Thus, even if a D^-/R^+ mismatch represents a potentially greater risk factor, any seropositive recipient should be considered to be at high risk of reactivation.

SOT recipients. Reactivation of latent *Toxoplasma* infections occurs in SOT seropositive recipients, but is far less frequent and less severe than in HSCT recipients [57]. In cases of heart and heart–lung transplantation, the rates of reactivation markedly vary according to country and transplant centre; reactivation is mainly serological, with clinical symptoms of toxoplasmosis manifesting occasionally [21,22,24,27,58,59] (Table 2).

Table 3. Reported prevalence of toxoplasmosis in haematopoietic stem cell transplant recipients in various countries

Country	Number of patients	*Toxoplasma* seroprevalence (number of seropositive patients)	Clinical toxoplasmosis		Reference
			Number of cases	Prevalence (%) global/in seropositive patients	
Japan	925	10%[a] (93)	2	0.2/2.1	Matsuo *et al.* [40]
USA	3803	15%[b] (570)	12	0.3/2.1	Slavin *et al.* [46]
Brazil	786	60%[b] (472)	9	1.1/1.9	De Meddeiros *et al.* [47]
France	296	68%[b] (201)	7	2.4/3.5	Derouin *et al.* [45]
Germany	75	71% (53)	3	4.0/5.7	Janitschke *et al.* [56]
France	106	100% (106)[c]	6	ND/5.7	Martino *et al.* [42]

ND, not documented.
[a]Estimated prevalence in Japan provided in [40].
[b]Estimated from a fraction of patients.
[c]Study restricted to *Toxoplasma gondi*-seropositive patients.

In non-cardiac SOT recipients, clinical toxoplasmosis is a rare event. In a recent extensive review of the literature, Campbell *et al*. recorded 34 cases of clinical toxoplasmosis in kidney transplant recipients, of which eight could be related to reactivation [32]. Among liver transplant recipients, three cases of toxoplasmosis related to reactivation have been reported.

Asymptomatic serological reactivations might be much more frequent, as observed in seven of 49 seropositive renal transplant recipients and eight of 25 liver transplant recipients in two French centres [60,61].

Considering the number of SOTs performed throughout the world, the number of reported cases of clinical toxoplasmosis is very low. However, clinicians should be aware of the risk in seropositive recipients, especially those receiving aggressive immunosuppressive therapy. In these patients, the risk of re-infection has also been hypothesized but needs to be further assessed by strain genotyping.

DIAGNOSIS

The diagnosis of toxoplasmosis in transplant recipients is often difficult. From a clinical point of view, the features of reactivated or transmitted toxoplasmosis are not specific, with fever being the main sign, associated with various other signs that are dependent on the dissemination of the parasite. Imaging by computed tomography, or preferably by magnetic resonance imaging, provides strong support for a diagnosis of toxoplasmic encephalitis. Focal necrosis is the most common presentation, with single or multiple contrast-enhancing lesions with hypodensities, surrounded by oedema; a mass effect may be present, with displacement of the ventricle (Fig. 2). However, as stated earlier, lesions may be non-focal (diffuse encephalitis) or atypical. Pulmonary toxoplasmosis usually manifests as interstitial pneumonitis with alveolar condensations, and is frequently associated with signs of dissemination and with multiple organ involvement or failure.

The serological diagnosis of toxoplasmosis has important limitations, as the underlying immunosuppression alters antibody production and its kinetics. The demonstration of parasites in blood, body fluids and tissues is the mainstay of diagnosis, providing definitive proof of the disease.

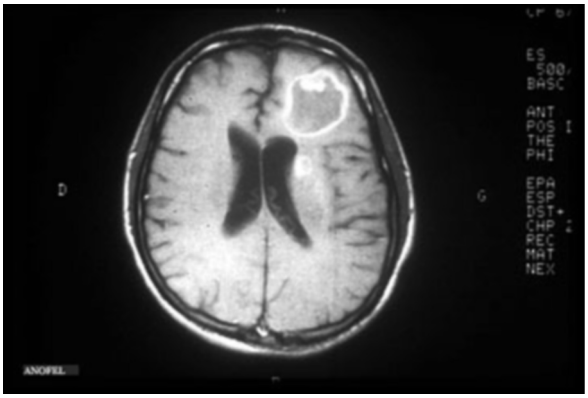

Fig. 2. Source: CD-ROM ANOFEL 3 and courtesy of Professor J. Frija, Service de Radiologie, Hôpital Saint-Louis, Paris, France.

One must not forget that the demonstration of tachyzoites by microscopic examination of Giemsa-stained smears of bone marrow aspirates or bronchoalveolar lavage fluid remains the simplest and most rapid means of diagnosis of disseminated toxoplasmosis. Diagnosis can also rely on the demonstration of tachyzoites and cysts in haematoxylin- or Giemsa-stained tissue biopsy specimens, preferentially using immunohistochemistry [62,63]. Because of the low sensitivity of direct microscopic methods, enrichment methods should be used in association with microscopy. Mouse inoculation is the reference technique, allowing strain isolation and possible genotyping, but a definitive result can only be obtained 4 weeks post inoculation. Cell culture offers the possibility of reducing this delay, but is no longer used for diagnosis, since the development of DNA-based diagnostic methods. PCR techniques, the most sensitive of which involve the repeated B1 and AF146527 gene targets, have markedly changed the strategy for diagnosis and treatment of transplant recipients [42,64–68]. Moreover, the use of real-time PCR avoids the risk of false positivity due to DNA carryover [65], and allows quantification of *T. gondii* DNA to monitor treatment efficacy [50].

In 2000, the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation defined 'probable toxoplasmosis' as the observation of 'clinical and radiological evidence suggestive of organ involvement plus at least one positive PCR result from blood, CSF, or BAL, but no histologic confirmation and absence of another pathogen that may explain the

findings' [49]. The existence of a positive PCR result from blood, without evidence of organ involvement, should be considered as only a possible diagnosis of toxoplasmosis. A negative PCR result has a good negative predictive value, as demonstrated by the results of the large prospective study by Martino *et al.* [42], in which none of the stem cell transplant recipients for whom PCR from blood was negative developed toxoplasmosis.

However, a negative PCR result cannot rule out the diagnosis of toxoplasmosis, as cases of toxoplasmosis have also been reported with negative PCR results from blood [63,65]. The negative predictive value of PCR results from blood might also be dependent on the timing and the repetition of blood sampling.

Diagnosis of organ-transmitted infection. Organ-transmitted infection can be suspected in cases of serological mismatch (D^+/R^-). A seroconversion occurring early after transplant, with demonstration of IgM and IgG antibodies, and eventually IgA and IgE [69], is a strong indication of an acquired (and probably transmitted) infection with subsequent risk of disease. In cases of profound immunosuppression, the antibody response might be lacking or atypical, and diagnosis can be based only on the demonstration of parasites or parasite DNA in blood, body fluids or biopsy specimens, guided by clinical symptoms [67]. In HSCT recipients, seroconversion with the appearance of IgG and IgM antibodies has rarely been reported, whereas a transient increase of IgG antibodies at a low titre (<100 IU/mL and without IgM) is more frequent and probably related to passive antibody transmission via the graft or blood transfusions [55].

Diagnosis of reactivation. A reactivation should be suspected upon observation of a rise in specific antibody titres, usually IgG antibodies without IgM or IgA antibodies [70]. IgG antibodies produced during reactivation have a high avidity index, indicating an immune recall rather than a primary immune response [71]. The appearance of IgM antibodies is occasionally observed in HSCT recipients, possibly as a primary immune response of grafted cells from seronegative donors. However, there is no clear relationship between serological reactivation in a seropositive recipient and clinical disease. Among cardiac transplant recipients, clinical symptoms indicative of toxoplasmosis have been reported at the time of antibody increase in two of five patients by Gallino *et al.* [24] and two of four patients by Wregitt *et al.* [22], but were not reported in other studies in which high rates of serological reactivation have been observed [21,59] (Table 2). Among renal transplant recipients, an antibody increase was observed in 5–10% of patients and was not associated with, or followed by, clinical symptoms indicative of toxoplasmosis [60,72]. Among HSCT recipients, serological reactivations that occur late after transplantation (10–18 months post-HSCT) are frequent and usually asymptomatic [55].

The diagnosis of reactivation is even more difficult in patients who develop clinical toxoplasmosis when antibody titres remain stable after transplant. This is the most frequent situation in HSCT recipients. In these cases, no conclusion can be drawn from the results of serological tests, and diagnosis must be based on imaging and the demonstration of parasites or parasitic DNA. As stated earlier, the proof of infection is provided only by the demonstration of tachyzoites in blood, bone marrow, bronchoalveolar lavage fluid, cerebrospinal fluid or tissue biopsy specimens using microscopy, mouse inoculation or PCR techniques. Indeed, a positive PCR result is indicative of reactivation, but should be interpreted in the context of clinical symptoms and with the understanding that asymptomatic transient positive reactions can be observed after HSCT [64].

PREVENTION AND PROPHYLAXIS OF TOXOPLASMOSIS IN TRANSPLANT RECIPIENTS

Toxoplasmosis represents a major infectious complication in transplant recipients that should be prevented, and three complementary approaches to prevention can be proposed.

Serological screening of donor and recipients

The determination of the *Toxoplasma* serological status of donors and recipients provides critical information concerning the potential risk of toxoplasmosis following transplantation. This test is inexpensive, and allows discrimination among three categories of risk: (i) the potential risk of

transmission in SOT recipients in cases of mismatch (D^+/R^-) and in seronegative HSCT recipients in cases of recently acquired infection of the donor; (ii) the risk of reactivation for seropositive HSCT and SOT recipients (independent of the serology of the donor); and (iii) the low risk of toxoplasmosis if both donor and recipient are seronegative. In the latter case, the only risk is contamination via ingestion of cysts with undercooked meat or oocysts with insufficiently washed foods, or via poor hand hygiene.

In Europe, Toxoplasma serology is undertaken according to the practice of the respective transplant center (ESGP information). In some countries, e.g. France, the test for *Toxoplasma* is included among the serological tests legally required for every organ donor and is strongly recommended for recipients prior to transplantation. Among 29 countries participating in the Eurotransplant network, *Toxoplasma* serology is routinely undertaken for SOT donors in 11 and is undertaken according to the characteristics of the donor in ten [73].

In the USA, serological screening of donors is not mandatory, and routine screening of donors and recipients largely depends on the transplant centre (J. S. Remington, personal communication).

Recent publications indicate that routine screening may be unnecessary because, for years, no case of toxoplasmosis has been observed among heart transplant recipients treated prophylactically with co-trimoxazole for prevention of pneumocystosis [29,74]. Indeed, the remarkable prophylactic efficacy of co-trimoxazole must be acknowledged, but there is a potential risk of toxoplasmosis if co-trimoxazole prophylaxis is suspended or replaced by pentaminide prophylaxis, which has no effect on *T. gondii.* Owing to the low cost of the serological test and the benefit provided in terms of risk management, we propose that a serological test should be performed in all transplant donors and recipients. In cases of HSCT, the serological status of both donor and recipient should be determined before transplantation, because of the early and high risk of toxoplasmosis following transplantation. In cases of SOT, which involves a lower risk of toxoplasmosis, a possible alternative would be to store both donor and recipient pre-transplant sera and perform serological testing only in cases of clinical suspicion of toxoplasmosis [74].

Serological and PCR follow-up of patients after transplant

A serological follow-up is informative in cases of SOT mismatched recipients (D^+/R^-), as the observation of a seroconversion clearly indicates transmission of *T. gondii*. In other SOT and HSCT recipients, such follow-up has little benefit, as an antibody increase does not reliably indicate a progressive disease. A PCR follow-up of patients at risk might be more informative, as a positive PCR result can be an early sign of disease [42,64,75]. However, because of the high cost of PCR testing, the indications and the timing of PCR follow-up should probably be restricted to defined high-risk periods and to patients who do not receive prophylaxis. This follow-up recommended in *Toxoplasma*-seropositive HSCT recipients who do not tolerate co-trimoxazole (or other anti-*Toxoplasma* drugs) and thus receive pentamidine for prevention of pneumocystosis, and recipients of unrelated cord blood transplants, because of their high risk of reactivating toxoplasmosis.

Chemoprophylaxis

Considering the severity and rapid progression of toxoplasmosis in SOT and HSCT recipients, administration of primary chemoprophylaxis to high-risk patients should be considered.

The ideal drug for prophylaxis would be able to eradicate cysts, would be preferentially parasiticidal against the different parasitic stages, would be well distributed in the main sites of infection, and would be well tolerated. No such drug exists. Presently, there is no drug with proven efficacy against tissue cysts. Atovaquone has been found to reduce the number of brain cysts in chronically infected mice [76], but this effect has not been (and probably cannot be) confirmed in humans.

Other drugs are effective only on the tachyzoite, which is the highly replicative stage of the parasite that is present at the acute phase of infection and during reactivation. Among these drugs, folate inhibitors (dihydrofolate reductase inhibitors, sulphonamides) as well as atovaquone are strongly parasiticidal and are rapidly effective against tachyzoites *in vitro* and *in vivo*. Macrolides (spiramycine, clarithromycin, roxithromycin, azithromycin) and related drugs

(clindamycin) have a delayed antiparasitic effect and are not adapted for the containment of an active infection [77]. The possible innate resistance of some genotypes of *T. gondii* and the risk of selecting drug-resistant parasites via long-term prophylaxis have been suggested for *T. gondii*, as for *Pneumocystis* and *Plasmodium*. Drug-resistant isolates of *T. gondii* can be obtained *in vitro* by mutagenesis, but there is no documented proof of such selection in humans. Recently, the study of 17 strains (15 of human origin) belonging to various genotypes of *T. gondii* revealed similar susceptibilities to pyrimethamine and atovaquone, whereas three strains were resistant to sulphadiazine, without evidence of mutation on the target dyhidropteroate synthase (DHPS) gene, or a relationship with the parasite genotype [78].

Presently, the combination of a dihydrofolate reductase inhibitor and a sulphonamide is the most efficient prophylaxis for toxoplasmosis, as both compounds are highly synergistic against *T. gondii*. Pyrimethamine alone has been proposed for prophylaxis for toxoplasmosis in heart transplant recipients [22,26], but this drug is at least ten-fold less efficient alone than in combination with a sulphonamide. Two drug combinations proved efficient in preventing toxoplasmosis in transplant recipients: trimethoprim–sulphamethoxazole (co-trimoxazole) [29,74,79,80] and pyrimethamine–sulphadoxine [80]. Both combinations have undesirable side effects, related to sulphonamides (skin rashes, nephrotoxicity and, rarely, Lyell syndrome with pyrimethamine–sulphadoxine) and related to the folate inhibitor (myelotoxicity). Myelotoxicity can usually be prevented by concomitant administration of folinic acid (leucovorin) and adaptation of the dosage to the renal clearance. Atovaquone alone proved efficient for secondary prophylaxis of toxoplasmosis in AIDS patients [81], and proved to be both efficient and well tolerated in autologous HSCT recipients for prevention of pneumocystosis [82]. However, its use as primary prophylaxis for toxoplasmosis is limited by its poor intestinal absorption and risk of failure under therapy [83].

Prophylaxis in HSCT recipients. Prophylaxis prior to transplantation is recommended for all recipients who are seropositive for *Toxoplasma*. Co-trimoxazole is the recommended drug in the USA [84,85] and the most widely used drug in European HSCT centres [44]. Pyrimethamine–sulphadoxine is preferred in some centres [80], as it can be administered weekly rather than once-daily or two or three times weekly, which is the regimen for co-trimoxazole. For patients who are intolerant to co-trimoxazole, a combination of clindamycin, pyrimethamine and leucovorin has been proposed [84], but this combination exposes the patient to myelosupression. Atovaquone could be a safe alternative, but its efficacy has not been evaluated. There is no consensus concerning initiation and duration of prophylaxis. Starting prophylaxis immediately after HSCT exposes the patient to some degree of toxicity with regard to grafted cells and delayed engraftment, whereas delaying prophylaxis places the patient at risk for toxoplasmosis. A delay of 30 days after HSCT before initiation of prophylaxis seems reasonable, as 90% of the cases of toxoplasmosis occur 30 days post-HSCT. If delayed prophylaxis is preferred, a weekly follow-up of the recipient using PCR with peripheral blood is recommended for high-risk patients during the entire period without prophylaxis.

With regard to the incidence rate of toxoplasmosis following HSCT, prophylaxis should be maintained for 6 months post-HSCT. It should be prolonged in cases of graft-versus-host disease, prolonged neutropenia and prolonged administration of corticosteroids. If prophylaxis must be suspended because of drug intolerance, a close follow-up by PCR should be instituted.

HSCT recipients who are seronegative are at very low risk of acquiring toxoplasmosis, but they should be advised of the risk of acquiring toxoplasmosis via ingestion of undercooked meat (possibly containing cysts) or raw vegetables (possibly contaminated by oocysts). A low but potential risk of transmission via the stem cell graft from a recently infected donor should be taken into account if the donor's serology shows a pattern of recently acquired infection, i.e. presence of IgM, low avidity of IgG antibodies, and increasing IgG titre. In this case, the proposed procedure is: (i) to treat the donor, if possible, using a short course of drugs that rapidly eradicate parasitaemia, e.g. pyrimethamine combined with sulphadiazine; (ii) to assess the collected haematopoietic stem cells by PCR; and (iii) to

initiate prophylaxis in the HSCT recipient following transplantation [86].

Prophylaxis in SOT recipients. The risk of transmission of *T. gondii* is almost restricted to mismatched patients, i.e. D^+/R^-, and is limited to a several-month period post transplantation. Co-trimoxazole is the drug of choice for prophylaxis. Its efficacy has been established in studies in which it was used for prophylaxis of pneumocystosis [29,74]. In cases of intolerance to sulphonamides, a possible alternative is the use of pyrimethamine alone [22,26]. The risk of reactivation of a latent toxoplasmosis is very low in SOT seropositive recipients, and does not justify a specific prophylaxis regimen in addition to that for pneumocystosis. However, the potential risk of reactivation of toxoplasmosis should not be ignored in patients who are no longer receiving prophylaxis for pneumocystosis.

CONCLUSION

Toxoplasmosis remains one of the most severe opportunistic infections occurring after transplantation, with a high mortality rate in cases of delayed diagnosis. It should be stressed that at least 75% of the cases occur in patients who have not received prophylaxis, emphasizing the need to make available updated information concerning the pathogenesis of toxoplasmosis, the procedures for identification of risk factors and the measures for prevention. The combination of serological screening of donors and recipients, chemoprophylaxis in patients susceptible to reactivation or exposed to organ transmission of T. gondii and PCR follow-up of high-risk patients should be effective in reducing the occurrence of toxoplasmosis in transplant recipients.

ACKNOWLEDGEMENTS

We thank the members of the executive committee of the ESCMID Study Group on Clinical Parasitology (B. Evengård, E. Petersen, F. Peyron, A. Mathis, T. van Gool, P. Chiodini, M. Junie, L. Kortbeek) for their support in preparing this article. We thank J. S. Remington for providing information on serological screening of donors and recipients in the USA, P. Ribaud, J. P. Brion and H. Talabani for critical review of the manuscript, A. Sulahian for English revision of the manuscript, and M. F. Le Gall for helpful secretarial assistance.

We are indebted to the Association Française des Enseignants de Parasitologie et Mycologie Médicales (ANOFEL) for providing the cerebral toxoplasmosis magnetic resonance image (Fig. 1), extracted from the educational CD-ROM ANOFEL 3. This work was presented in part during the ESGT Educational Workshop at the 17th ECCMID/25th ICC 2007, 31 March to 3 April 2007, Munich, Germany.

TRANSPARENCY DECLARATION

The authors have no relationship that may constitute a conflicting or dual interest.

REFERENCES

1. Demar M, Ajzenberg D, Maubon D *et al.* Fatal outbreak of human toxoplasmosis along the Maroni river: epidemiological, clinical, and parasitological aspects. *Clin Infect Dis* 2007; **45**: e88–e95.
2. Hunter CA, Remington JS. Immunopathogenesis of toxoplasmic encephalitis. *J Infect Dis* 1994; **170**: 1057–1067.
3. Raffi F, Aboulker JP, Michelet C *et al.* A prospective study of criteria for the diagnosis of toxoplasmic encephalitis in 186 AIDS patients. The BIOTOXO Study Group. *AIDS* 1997; **11**: 177–184.
4. Luft BJ, Remington JS. Toxoplasmic encephalitis in AIDS. *Clin Infect Dis* 1992; **15**: 211–212.
5. Abgrall S, Rabaud C, Costagliola D. Clinical Epidemiology Group of the French Hospital Database on HIV. Incidence and risk factors for toxoplasmic encephalitis in human immunodeficiency virus-infected patients before and during the highly active antiretroviral therapy era. *Clin Infect Dis* 2001; **33**: 1747–1755.
6. Kaplan JE, Masur H, Holmes KK, USPHS; Infectious Disease Society of America. Guidelines for preventing opportunistic infections among HIV-infected persons—2002. Recommendations of the US Public Health Service and the Infectious Diseases Society of America. *MMWR Recomm Rep* 2002; **51**: 1–52.
7. Yeni P. Prise en charge médicale des personnes infectées par le VIH. Rapport 2006. *Recommandations du groupe d'experts*.. Paris: Flammarion, 2006; 348.
8. Israelski DM, Remington JS. Toxoplasmosis in the non-AIDS immunocompromised host. In: Remington J, Swartz M, eds. *Current Clinical Topics in Infectious Diseases*. London: Blackwell Scientific Publications, 1993; 322–356.
9. Rogers NM, Peh CA, Faull R, Pannell M, Cooper J, Russ GR. Transmission of toxoplasmosis in two renal allograft recipients receiving an organ from the same donor. *Transpl Infect Dis* 2008; **10**: 71–74.
10. Renoult E, Biava MF, Hulin C, Frimat L, Hestin D, Kessler M. Transmission of toxoplasmosis by renal transplant: a report of four cases. *Transplant Proc* 1996; **28**: 181–183.
11. Jurges E, Young Y, Eltumi M *et al.* Transmission of toxoplasmosis by bone marrow transplant associated with Campath-1G. *Bone Marrow Transplant* 1992; **9**: 65–66.
12. Chandrasekar PH, Momin F. Disseminated toxoplasmosis in marrow recipients: a report of three cases and a review of the literature. Bone Marrow Transplant Team. *Bone Marrow Transplant* 1997; **19**: 685–689.
13. Paugam A, Dupouy-Camet J, Sumuyen MH, Romand S, Lamoril J, Derouin F. Detection of *Toxoplasma gondii* parasitemia by polymerase chain reaction in perorally infected mice. *Parasite* 1995; **2**: 181–184.

14. Miller MJ, Aronson WJ, Remington JS. Late parasitemia in asymptomatic acquired toxoplasmosis. *Ann Intern Med* 1969; **71**: 139–145.
15. Guy EC, Joynson DH. Potential of the polymerase chain reaction in the diagnosis of active *Toxoplasma* infection by detection of parasite in blood. *J Infect Dis* 1995; **172**: 319–322.
16. Dubey JP, Lindsay DS, Speer CA. Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. *Clin Microbiol Rev* 1998; **11**: 267–299.
17. Remington JS, Cavanaugh EN. Isolation of the encysted form of *Toxoplasma gondii* from human skeletal muscle and brain. *N Engl J Med* 1965; **273**: 1308–1310.
18. Frenkel JK, Nelson BM, Arias-Stella J. Immunosuppression and toxoplasmic encephalitis: clinical and experimental aspects. *Hum Pathol* 1975; **6**: 97–111.
19. Ryning FW, McLeod R, Maddox JC, Hunt S, Remington JS. Probable transmission of *Toxoplasma gondii* by organ transplantation. *Ann Intern Med* 1979; **90**: 47–49.
20. Stinson EB, Bieber CP, Griepp RB, Clark DA, Shumway NE, Remington JS. Infectious complications after cardiac transplantation in man. *Ann Intern Med* 1971; **74**: 22–36.
21. Luft BJ, Naot Y, Araujo FG, Stinson EB, Remington JS. Primary and reactivated *Toxoplasma* infection in patients with cardiac transplants. Clinical spectrum and problems in diagnosis in a defined population. *Ann Intern Med* 1983; **99**: 27–31.
22. Wreghitt TG, Hakim M, Gray JJ *et al.* Toxoplasmosis in heart and heart and lung transplant recipients. *J Clin Pathol* 1989; **42**: 194–199.
23. Speirs GE, Hakim M, Calne RY, Wreghitt TG. Relative risk of donor-acquired *Toxoplasma gondii* in heart, liver and kidney transplant recipients. *Clin Transpl* 1988; **2**: 225–260.
24. Gallino A, Maggiorini M, Kiowski W *et al.* Toxoplasmosis in heart transplant recipients. *Eur J Clin Microbiol Infect Dis* 1996; **15**: 389–393.
25. Michaels MG, Wald ER, Fricker FJ, del Nido PJ, Armitage J. Toxoplasmosis in pediatric recipients of heart transplants. *Clin Infect Dis* 1992; **14**: 847–851.
26. Hakim M, Esmore D, Wallwork J, English TA, Wreghitt T. Toxoplasmosis in cardiac transplantation. *BMJ (Clin Res Ed)* 1986; **292**: 1108.
27. Montoya JG, Giraldo LF, Efron B *et al.* Infectious complications among 620 consecutive heart transplant patients at Stanford University Medical Center. *Clin Infect Dis* 2001; **33**: 629–640.
28. Orr KE, Gould FK, Short G *et al.* Outcome of *Toxoplasma gondii* mismatches in heart transplant recipients over a period of 8 years. *J Infect* 1994; **29**: 249–253.
29. Baran DA, Alwarshetty MM, Alvi S *et al.* Is toxoplasmosis prophylaxis necessary in cardiac transplantation? Long-term follow-up at two transplant centers. *J Heart Lung Transplant* 2006; **25**: 1380–1382.
30. Garbino J, Romand JA, Pittet D, Giostra E, Mentha G, Suter P. Infection and rejection in liver transplant patients: a 10-year Swiss single-centre experience. *Swiss Med Wkly* 2005; **135**: 587–593.
31. Assi MA, Rosenblatt JE, Marshall WF. Donor-transmitted toxoplasmosis in liver transplant recipients: a case report and literature review. *Transpl Infect Dis* 2007; **9**: 132–136.
32. Campbell AL, Goldberg CL, Magid MS, Gondolesi G, Rumbo C, Herold BC. First case of toxoplasmosis following small bowel transplantation and systematic review of tissue-invasive toxoplasmosis following noncardiac solid organ transplantation. *Transplantation* 2006; **81**: 408–417.
33. Hommann M, Schotte U, Voigt R *et al.* Cerebral toxoplasmosis after combined liver–pancreas–kidney and liver–pancreas transplantation. *Transplant Proc* 2002; **34**: 2294–2295.
34. Castagnini M, Bernazzali S, Ginanneschi C *et al.* Fatal disseminated toxoplasmosis in a cardiac transplantation with seropositive match for Toxoplasma: should prophylaxis be extended? *Transpl Immunol* 2007; **18**: 193–197.
35. Robert-Gangneux F, Amrein C, Lavarde V, Botterel F, Dupouy-Camet J. Neosynthesized IgG detected by western blotting in Toxoplasma-seropositive heart or lung transplant recipients. *Transpl Int* 2000; **13**: 448–452.
36. Morisset S, Peyron F, Lobry JR *et al.* Serotyping of *Toxoplasma gondii*: striking homogeneous pattern between symptomatic and asymptomatic infections within Europe and South America. *Microbes Infect*, 2008; **10**: 742–747.
37. Dardé ML, Aubert D, Derouin F, Dumètre A, Pelloux H, Villena I. *Toxoplasma gondii*. In: Khan N, ed. *Emerging protozoan pathogens*. London: Taylor and Francis, 2007; 227–287.
38. Geissmann F, Derouin F, Marolleau JP, Gisselbrecht C, Brice P. Disseminated toxoplasmosis following autologous bone marrow transplantation. *Clin Infect Dis* 1994; **19**: 800–801.
39. López-Duarte M, Insunza A, Conde E, Iriondo A, Mazorra F, Zubizarreta A. Cerebral toxoplasmosis after autologous peripheral blood stem cell transplantation. *Eur J Clin Microbiol Infect Dis* 2003; **22**: 548–550.
40. Matsuo Y, Takeishi S, Miyamoto T *et al.* Toxoplasmosis encephalitis following severe graft-vs.-host disease after allogeneic hematopoietic stem cell transplantation: 17 yr experience in Fukuoka BMT group. *Eur J Haematol* 2007; **79**: 317–321.
41. Peacock JE Jr, Greven CM, Cruz JM, Hurd DD. Reactivation toxoplasmic retinochoroiditis in patients undergoing bone marrow transplantation: is there a role for chemoprophylaxis? *Bone Marrow Transplant* 1995; **15**: 983–987.
42. Martino R, Bretagne S, Einsele H *et al.* Early detection of Toxoplasma infection by molecular monitoring of *Toxoplasma gondii* in peripheral blood samples after allogeneic stem cell transplantation. *Clin Infect Dis* 2005; **40**: 79–81.
43. Small TN, Leung L, Stiles J *et al.* Disseminated toxoplasmosis following T cell-depleted related and unrelated bone marrow transplantation. *Bone Marrow Transplant* 2000; **25**: 969–973.
44. Martino R, Bretagne S, Rovira M *et al.* Toxoplasmosis after hematopoietic stem transplantation. Report of a 5-year survey from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant* 2000; **25**: 1111–1114.
45. Derouin F, Devergie A, Auber P *et al.* Toxoplasmosis in bone marrow-transplant recipients: report of seven cases and review. *Clin Infect Dis* 1992; **15**: 267–270.
46. Slavin MA, Meyers JD, Remington JS, Hackman RC. *Toxoplasma gondii* infection in marrow transplant recipients: a 20 year experience. *Bone Marrow Transplant* 1994; **13**: 549–557.

47. de Medeiros BC, de Medeiros CR, Werner B, Loddo G, Pasquini R, Bleggi-Torres LF. Disseminated toxoplasmosis after bone marrow transplantation: report of 9 cases. *Transpl Infect Dis* 2001; **3**: 24–28.
48. Mele A, Paterson PJ, Prentice HG, Leoni P, Kibbler CC. Toxoplasmosis in bone marrow transplantation: a report of two cases and systematic review of the literature. *Bone Marrow Transplant* 2002; **29**: 691–698.
49. Martino R, Maertens J, Bretagne S *et al.* Toxoplasmosis after hematopoietic stem cell transplantation. *Clin Infect Dis* 2000; **31**: 1188–1195.
50. Menotti J, Vilela G, Romand S *et al.* Comparison of PCR-enzyme-linked immunosorbent assay and real-time PCR assay for diagnosis of an unusual case of cerebral toxoplasmosis in a stem cell transplant recipient. *J Clin Microbiol* 2003; **41**: 5313–5316.
51. Mueller-Mang C, Mang TG, Kalhs P, Thurnher MM. Imaging characteristics of toxoplasmosis encephalitis after bone marrow transplantation: report of two cases and review of the literature. *Neuroradiology* 2006; **48**: 84–89.
52. Sing A, Leitritz L, Roggenkamp A *et al.* Pulmonary toxoplasmosis in bone marrow transplant recipients: report of two cases and review. *Clin Infect Dis* 1999; **29**: 429–433.
53. Ortonne N, Ribaud P, Meignin V *et al.* Toxoplasmic pneumonitis leading to fatal acute respiratory distress syndrome after engraftment in three bone marrow transplant recipients. *Transplantation* 2001; **72**: 1838–1840.
54. Duband S, Cornillon J, Tavernier E, Dumollard J-M, Guyotat D, Peoc'h M. Toxoplasmosis with hemophagocytic syndrome after bone marrow transplantation: diagnosis at autopsy. *Transpl Infect Dis* 2008; **10**: 372–374.
55. Derouin F, Gluckman E, Beauvais B *et al.* Toxoplasma infection after human allogeneic bone marrow transplantation: clinical and serological study of 80 patients. *Bone Marrow Transplant* 1986; **1**: 67–73.
56. Janitschke K, Held T, Krüiger D, Schwerdtfeger R, Schlier G, Liesenfeld O. Diagnostic value of tests for *Toxoplasma gondii*-specific antibodies in patients undergoing bone marrow transplantation. *Clin Lab* 2003; **49**: 239–242.
57. Kotton CN. Zoonoses in solid-organ and hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2007; **44**: 857–866.
58. Peleg AY, Husain S, Kwak EJ *et al.* Opportunistic infections in 547 organ transplant recipients receiving alemtuzumab, a humanized monoclonal CD-52 antibody. *Clin Infect Dis* 2007; **44**: 204–212.
59. Lavarde V, Heyer F, Guillemain R, Amrein C, Vulser C, Dreyfus G. Toxoplasmose et transplantation cardiaque. Intérêt et limites de la sérologie. *Med Mal Infect* 1990; **20**: 121–125.
60. Derouin F, Debure A, Godeaut E, Lariviere M, Kreis H. Toxoplasma antibody titers in renal transplant recipients. Pretransplant evaluation and posttransplant follow-up of 73 patients. *Transplantation* 1987; **44**: 515–518.
61. Bourrée P, Romand S, Majoube A. Toxoplasmose et transplantation hépatique: surveillance de 34 patients par deux techniques sérologiques. *Bull Soc Fr Parasitol* 1995; **13**: 155–160.
62. Conley FK, Jenkins KA, Remington JS. *Toxoplasma gondii* infection of the central nervous system. Use of the peroxidase–antiperoxidase method to demonstrate Toxoplasma in formalin fixed, paraffin embedded tissue sections. *Hum Pathol* 1981; **12**: 690–698.
63. Held TK, Krüger D, Switala AR *et al.* Diagnosis of toxoplasmosis in bone marrow transplant recipients: comparison of PCR-based results and immunohistochemistry. *Bone Marrow Transplant* 2000; **25**: 1257–1262.
64. Bretagne S, Costa JM, Foulet F, Jabot-Lestang L, Baud-Camus F, Cordonnier C. Prospective study of *Toxoplasma* reactivation by polymerase chain reaction in allogeneic stem-cell transplant recipients. *Transpl Infect Dis* 2000; **2**: 127–132.
65. Bretagne S. Molecular diagnostics in clinical parasitology and mycology: limits of the current polymerase chain reaction (PCR) assays and interest of the real-time PCR assays. *Clin Microbiol Infect* 2003; **9**: 505–511.
66. Laibe S, Ranque S, Curtillet C, Faraut F, Dumon H, Franck J. Timely diagnosis of disseminated toxoplasmosis by sputum examination. *J Clin Microbiol* 2006; **44**: 646–648.
67. Botterel F, Ichai P, Feray C *et al.* Disseminated toxoplasmosis, resulting from infection of allograft, after orthotopic liver transplantation: usefulness of quantitative PCR. *J Clin Microbiol* 2002; **40**: 1648–1650.
68. Fricker-Hidalgo H, Brion JP, Durand M, Chavanon O, Brenier-Pinchart MP, Pelloux H. Disseminated toxoplasmosis with pulmonary involvement after heart transplantation. *Transpl Infect Dis* 2005; **7**: 38–40.
69. Aubert D, Foudrinier F, Villena I, Pinon JM, Biava MF, Renoult E. PCR for diagnosis and follow-up of two cases of disseminated toxoplasmosis after kidney grafting. *J Clin Microbiol* 1996; **34**: 1347.
70. Sulahian A, Nugues C, Garin YJF *et al.* Serodiagnosis of toxoplasmosis in patients with acquired or reactivating toxoplasmosis and analysis of the specific IgA antibody responses by ELISA, agglutination and immunoblotting. *Immunol Infect Dis* 1993; **3**: 63–69.
71. Mechain B, Garin YJ, Robert-Gangneux F, Dupouy-Camet J, Derouin F. Lack of utility of specific immunoglobulin G antibody avidity for serodiagnosis of reactivated toxoplasmosis in immunocompromised patients. *Clin Diagn Lab Immunol* 2000; **7**: 703–705.
72. Renoult E, Biava MF, Aimone-Gastin I *et al.* Evolution and significance of *Toxoplasma gondii* antibody titers in kidney transplant recipients. *Transplant Proc* 1992; **24**: 2754–2755.
73. European Commission. Directorate. Human organ transplantation in Europe; an overview. Report from the General Health and Consumer Protection Public Health and Risk Assessment Directorate; 2003; 1–20. Available at: ec.europa.eu/health/ph_threats/human_substance/documents/organ_survey.pdf.
74. Gourishankar S, Doucette K, Fenton J, Purych D, Kowalewska-Grochowska K, Preiksaitis J. The use of donor and recipient screening for *Toxoplasma* in the era of universal trimethoprim sulfamethoxazole prophylaxis. *Transplantation* 2008; **85**: 980–985.
75. Edvinsson B, Lundquist J, Ljungman P, Ringden O, Evengard B. A prospective study of diagnosis of *Toxoplasma gondii* infection after bone marrow transplantation. *APMIS 1* 2008; **116**: 345–351.
76. Araujo FG, Huskinson J, Remington JS. Remarkable in vitro and in vivo activities of the hydroxynaphthoquinone 566C80 against tachyzoites and tissue cysts of *Toxoplasma gondii*. *Antimicrob Agents Chemother* 1991; **35**: 293–299.

77. Derouin F. Systematic search and analysis of preclinical published data on in vitro and in vivo activities of antitoxoplasma drugs. EUROTOXO European TOXO PREVENTION Project Prevention of Congenital Toxoplasmosis (Panel 2: treatment issues). 2005. Available at :http://eurotoxo.isped.u-bordeaux2.fr/WWW_PUBLIC/DOC/Pharmacodynamics_v26082005.pdf.
78. Meneceur P, Bouldouyre MA, Aubert D *et al.* In vitro susceptibility of various genotypic strains of *Toxoplasma gondii* to pyrimethamine, sulfadiazine, and atovaquone. *Antimicrob Agents Chemother* 2008; **52**: 1269–1277.
79. Baden LR, Katz JT, Franck L *et al.* Successful toxoplasmosis prophylaxis after orthotopic cardiac transplantation with trimethoprim–sulfamethoxazole. *Transplantation* 2003; **75**: 339–343.
80. Foot AB, Garin YJ, Ribaud P, Devergie A, Derouin F, Gluckman E. Prophylaxis of toxoplasmosis infection with pyrimethamine/sulfadoxine (Fansidar) in bone marrow transplant recipients. *Bone Marrow Transplant* 1994; **14**: 241–245.
81. Katlama C, Mouthon B, Gourdon D, Lapierre D, Rousseau F. Atovaquone as long-term suppressive therapy for toxoplasmic encephalitis in patients with AIDS and multiple drug intolerance. Atovaquone Expanded Access Group. *AIDS* 1996; **10**: 1107–1112.
82. Colby C, McAfee S, Sackstein R, Finkelstein D, Fishman J, Spitzer T. A prospective randomized trial comparing the toxicity and safety of atovaquone with trimethoprim/sulfamethoxazole as *Pneumocystis carinii* pneumonia prophylaxis following autologous peripheral blood stem cell transplantation. *Bone Marrow Transplant* 1999; **24**: 897–902.
83. Megged O, Shalit I, Yaniv I, Stein J, Fisher S, Levy I. Breakthrough cerebral toxoplasmosis in a patient receiving atovaquone prophylaxis after a hematopoietic stem cell transplantation. *Pediatr Transplant* 2008; Jan 8 [Epub ahead of print].
84. Sullivan KM, Dykewicz CA, Longworth DL *et al.* Preventing opportunistic infections after hematopoietic stem cell transplantation: the Centers for Disease Control and Prevention, Infectious Diseases Society of America, and American Society for Blood and Marrow Transplantation Practice Guidelines and beyond. *Hematology Am Soc Hematol Educ Program* 2001; 392–421.
85. Centers for Disease Control and Prevention; Infectious Disease Society of America; American Society of Blood and Marrow Transplantation; Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. *MMWR Recomm Rep* 2000; **49**: 1–125, CE1-7.
86. Brenier-Pinchart MP, Garban F, Fricker-Hidalgo H, Richard MJ, Makowski C, Pelloux H. Avoidance of *Toxoplasma gondii* transmission from a recently infected donor to the recipient of hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2005; **35**: 735–736.