Prevention of toxoplasmosis in transplant patients

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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

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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 determining 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

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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/\(\text{mm}^3\) 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 pre-transplant 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

<table>
<thead>
<tr>
<th>Type of risk</th>
<th>Clinical disease and prevention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type of risk</td>
</tr>
<tr>
<td>Haematologous stem cell transplantation</td>
<td></td>
</tr>
<tr>
<td>Autologous</td>
<td>No risk</td>
</tr>
<tr>
<td>Allogenic(^a)</td>
<td>Possible but no case reported</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid organ transplantation</td>
<td></td>
</tr>
<tr>
<td>Heart/heart–lung(^b)</td>
<td>High in mismatched D(^+)/R(^-)</td>
</tr>
<tr>
<td>Liver(^c)</td>
<td>Low in mismatched D(^+)/R(^-)</td>
</tr>
<tr>
<td>Kidney(^c)</td>
<td>Low in mismatched D(^+)/R(^-)</td>
</tr>
<tr>
<td>Intestine(^c)</td>
<td>Low in mismatched D(^+)/R(^-)</td>
</tr>
<tr>
<td>Other (eye, bone, artery)</td>
<td>No risk</td>
</tr>
</tbody>
</table>

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].

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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

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of seronegative recipients</th>
<th>Number of D+/E- mismatches</th>
<th>Clinical toxoplasmosis (prevalence)</th>
<th>Reactivation in seropositive recipients</th>
<th>Number of seropositive recipients</th>
<th>Serological reactivations (prevalence)</th>
<th>Clinical toxoplasmosis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>509 (84%)</td>
<td>32</td>
<td>4 (12.5%; 25% in the 16 patients without prophylaxis)</td>
<td>98 (16%)</td>
<td>ND</td>
<td>0/98</td>
<td>Montoya et al. [27]</td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>31 (62%)</td>
<td>4</td>
<td>3 (75%)</td>
<td>19 (38%)</td>
<td>10/19 (52.6%)</td>
<td>0</td>
<td>Luft et al. [21]</td>
<td></td>
</tr>
<tr>
<td>Switzerland</td>
<td>52 (43%)</td>
<td>18</td>
<td>3 (16.7%)</td>
<td>69 (57%)</td>
<td>5/69 (7.2%)</td>
<td>2/5</td>
<td>Gallino et al. [24]</td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>175 (70%)</td>
<td>21</td>
<td>6 (26.5%; 57% in the four of seven patients without prophylaxis)</td>
<td>75 (30%)</td>
<td>3/75 (4%)</td>
<td>2/3</td>
<td>Wregitt et al. [22]</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>23 (36%)</td>
<td>ND</td>
<td>0</td>
<td>42 (64%)</td>
<td>10 (23.8%)</td>
<td>0</td>
<td>Lavarde et al. [59]</td>
<td></td>
</tr>
</tbody>
</table>

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 reinfected 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/mm³.

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, antithymocyte 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.
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

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

ND, not documented.

a Estimated prevalence in Japan provided in [40].
b Estimated from a fraction of patients.
c Study restricted to *Toxoplasma gondii*-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.

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 pentamidine 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 folic 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 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.

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**TRANSPARENCY DECLARATION**

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

**REFERENCES**


