Guidelines for the management of *Toxoplasma gondii* infection and disease in patients with haematological malignancies and after haematopoietic stem-cell transplantation: guidelines from the 9th European Conference on Infections in Leukaemia, 2022

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Patients with haematological malignancies might develop life-threatening toxoplasmosis, especially after allogeneic haematopoietic stem-cell transplantation (HSCT). Reactivation of latent cysts is the primary mechanism of toxoplasmosis following HSCT; hence, patients at high risk are those who were seropositive before transplantation. The lack of trimethoprim–sulfamethoxazole prophylaxis and various immune status parameters of the patient are other associated risk factors. The mortality of toxoplasma disease—eg, with organ involvement—can be particularly high in this setting. We have developed guidelines for managing toxoplasmosis in haematology patients, through a literature review and consultation with experts. In allogeneic HSCT recipients seropositive for *Toxoplasma gondii* before transplant, because *T gondii* infection mostly precedes toxoplasma disease, we propose weekly blood screening by use of quantitative PCR (qPCR) to identify infection early as a pre-emptive strategy. As trimethoprim–sulfamethoxazole prophylaxis might fail, prophylaxis and qPCR screening should be combined. However, PCR in blood can be negative even in toxoplasma disease. The duration of prophylaxis should be a least 6 months and extended during treatment-induced immunosuppression or severe CD4 lymphopenia. If a positive qPCR test occurs, treatment with trimethoprim–sulfamethoxazole, pyrimethamine–sulfadiazine, or pyrimethamine–clindamycin should be started, and a new sample taken. If the second qPCR test is negative, clinical judgement is recommended to either continue or stop therapy and restart prophylaxis. Therapy must be continued until a minimum of two negative PCRs for infection, or for at least 6 weeks for disease. The pre-emptive approach is not indicated in seronegative HSCT recipients, after autologous transplantation, or in non-transplant haematology patients, but PCR should be performed with a high level of clinical suspicion.

Introduction

Toxoplasma gondii is an ubiquitous intracellular protozoan infecting up to one-third of the world's population.[1] *T gondii* is acquired via ingestion of oocysts present in food or water contaminated by the droppings of felids (the definitive hosts) and ingestion of cysts in the undercooked meat of intermediate hosts (primarily lamb and pork).[1] Although most infected healthy individuals are asymptomatic, the infection can be life-long, persisting in the brain and muscle tissue as cysts containing dormant parasites (bradyzoites).[2] With immunosuppression, bradyzoites can reactivate into rapidly growing invasive tachyzoites that cause necrosis of affected tissues and inflammation. Reactivation is the primary mechanism for toxoplasmosis in immuno-compromised patients in whom *T gondii* infection and disease (ie, toxoplasmosis) might cause death.[3]

Almost all cases of toxoplasmosis in haematology have been reported following allogeneic haematological stem-cell transplantation (HSCT), with only anecdotal cases in other settings.[4–8] The large variation in seroprevalence across countries might explain the varying levels of awareness of toxoplasmosis within the transplant community. The considerable number of single-case reports suggests that toxoplasmosis is unanticipated in most HSCT centres. Although there are many guidelines for managing post-HSCT infections, there were previously no specific guidelines for toxoplasmosis in patients with haematological malignancies or who are HSCT recipients. A group of ten experts were appointed, and after defining clinical issues and doing an extensive literature search, a list of recommendations was developed and discussed with all participants attending the 9th European Conference on Infections in Leukaemia (ECIL 9; September 2022, France). Here, we present a summary of the ECIL 9 guidelines for patients with haematological disorders (primarily allogeneic HSCT recipients), with the aim of providing an evidence-based resource for clinicians managing these patients.

T gondii infection and disease definitions

Until the 2000s, toxoplasmosis was usually diagnosed based on evidence of tachyzoites or cysts in the tissues, primarily via biopsy or autopsy.[9,10] The availability of toxoplasma PCR at many centres from the 2000s revealed the possibility of asymptomatic toxoplasma PCR positivity in blood samples from HSCT recipients.[11,12] In 2000, on behalf of the European Society for Blood and Marrow Transplantation (EBMT), Martino and colleagues proposed definitions (table 1) distinguishing infection

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	Definitions of the EBMT[14]	Definitions proposed by ECIL 9	Summary of changes
Toxoplasma disease			
Proven toxoplasma disease	Histological or cytological demonstration of tachyzoites in tissue samples obtained either by biopsy or BAL, or at autopsy; isolation of the parasite by culture in these samples would be evidence of disease	Histological or cytological demonstration of tachyzoites in tissue samples obtained by biopsy or at autopsy, or in a fluid sample; in case of non-characteristic pattern, a positive PCR in the sample can confirm the identification of the pathogen	Tachyzoite presence should be considered diagnostic in any body fluid, not only BAL; routine culture is no longer performed; PCR might help when there is a strong suspicion of toxoplasmosis on histology or cytology, but the pattern is not characteristic, or the smears are of poor quality
Probable toxoplasma disease (documented by PCR)	Clinical and radiological evidence suggestive of organ involvement plus at least one positive PCR test from blood, cerebrospinal fluid, or BAL, but no histological confirmation and absence of another pathogen that might explain the findings	Clinical and radiological evidence suggestive of organ involvement plus at least one positive PCR test from any fluid sample, or in tissue, but without histological evidence of the presence of *T gondii*	PCR test from any fluid sample or tissue suggests at least probable toxoplasmosis
Possible toxoplasma disease (documented by imaging)	CT or MRI highly suggestive of CNS toxoplasmosis (as considered by each hospital's neuroradiologist) and response to anti-toxoplasma therapy, but no laboratory evidence of toxoplasmosis and absence of another pathogen that might explain the findings	Imaging (preferably MRI) highly suggestive of CNS toxoplasmosis (as considered by each hospital's neuroradiologist) without any laboratory evidence of toxoplasmosis and absence of another pathogen that might explain the findings	Response to treatment is no longer a criterion; the possible definition should not be used for therapeutic trials; MRI is preferred to CT for imaging of CNS toxoplasmosis
Toxoplasma infection	Positive PCR in blood in a patient without evidence of organ involvement or seroconversion for *T gondii* after transplantation in a previously seronegative patient (with or without fever)	Positive PCR in blood in a patient without evidence of organ involvement (with or without fever)	Serology has been removed as a diagnostic criterion because of its unreliability after HSCT

BAL=bronchoalveolar lavage. EBMT=European Society for Blood and Marrow Transplantation. ECIL 9=9th European Conference on Infections in Leukaemia. HSCT=haematopoietic stem-cell transplantation. *T gondii=Toxoplasma gondii*.

Table 1: **Definitions for *T gondii* infection and disease in HSCT recipients**

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See Online for appendix

(PCR positivity alone, with or without fever) from disease (organ involvement) at different levels of certainty.[12] Since 2000, these definitions have been widely used for HSCT recipients.[13–18] Although any seropositive patient can be considered infected, notwithstanding blood PCR test results, only active infection (primary or reactivation) is considered an infection herein, similar to the model of cytomegalovirus infection and disease following HSCT. Several concerns were raised at ECIL 9, leading to the following changes in the definitions (table 1). First, for proven toxoplasma disease, direct demonstration of the parasite is mandatory. For years, this evidence was established via pathological examinations of biopsies with haematoxylin and eosin stains.[19] When the pathologist is uncertain, the nature of the suspected elements must be assessed using immunohistochemistry or PCR. Tachyzoite presence should be considered diagnostic in any body fluid, not only bronchoalveolar lavage; thus, this latter point has been added to the previous EBMT definitions. Because routine culture is no longer done, it has been deleted from the EBMT definitions. Second, for probable toxoplasma disease, the significance of a PCR-positive test is the same for blood and any body fluid (eg, pleural or pericardial effusion). Therefore, the importance of PCR has been extended to any body fluid. Third, for possible cases, there is no microbiological evidence. For imaging of the CNS, although often non-specific, MRI is preferred to CT.[12,20,21] Positive response to anti-toxoplasma treatment as a diagnostic criterion in the previous definitions was debated because CNS disease mortality is extremely high notwithstanding specific therapy.[12,22,23] This criterion could create a negative bias toward eliminating possible cases in non-responders. The decision was to remove this criterion. Fourth, for *T gondii* infection, positive PCR in blood in a patient without evidence of organ involvement remains the only criterion of diagnosis. Due to the unreliability of antibody testing following transplantation, serology has been removed from the *T gondii* infection diagnostic criteria.

Epidemiology and clinical presentation of toxoplasmosis in haematology patients

Allogeneic HSCT recipients

Until the late 1990s, toxoplasmosis was infrequently detected following HSCT,[24–26] in contrast with its high incidence in people living with HIV. Approximately 300 cases have been reported since the 1990s (appendix pp 4–8). A systematic review identified 46 studies with a median toxoplasmosis prevalence of 2·14% (0–67%) among 38751 HSCT recipients; however, the studies all used different diagnostic approaches.[27] Because reactivation of latent cysts is the primary cause of toxoplasmosis, the large variability in the frequency of toxoplasmosis following HSCT is explained by the considerable variance in seroprevalence across countries[28] and even between regions in a given country.[29,30] In the healthy population, seroprevalence has been decreasing over time, probably as a consequence of decreasing meat consumption, growing bottled water consumption, and the adoption of feeding cats with dry food,[31–35] lowering seroprevalence in HSCT candidates.[30,33] However, this decrease in seroprevalence mainly affects the younger population. As the median

age of allogeneic HSCT has steadily increased over the last 30 years, we have to consider that toxoplasmosis will remain a concern in this setting. Meanwhile, the use of toxoplasma PCR has considerably improved the diagnosis of *T gondii* infection and disease, increasing the number of identified cases.

Factors associated with T gondii infection and disease

The primary risk factor for developing toxoplasmosis following HSCT is cysts harboured in tissue; 95% of HSCT recipients who developed toxoplasmosis were seropositive for *T gondii* (hereon referred to as R-positive) before transplant. In exceptional cases, toxoplasmosis might develop in seronegative recipients, indicating two possible scenarios: (1) loss of specific immunoglobulins during pre-HSCT treatments (a recent study reported that 30% of children seropositive at diagnosis of acute leukaemia had become seronegative at pretransplant assessment[6]); or (2) development of a primary infection via ingestion in previously uninfected transplant recipients.

The second most important associated factor is not receiving cotrimoxazole (trimethoprim–sulfamethoxazole), typically administered for *Pneumocystis jirovecii* pneumonia prophylaxis. Although some cases of breakthrough toxoplasmosis have been reported under trimethoprim–sulfamethoxazole prophylaxis,[13,16,36–40] most cases occurred in patients who were not on prophylaxis or were receiving prophylaxis with atovaquone or aerosolised pentamidine.[7,13,16,40–44]

Other factors implicated in the development of toxoplasmosis are presented in the appendix (pp 9–11); most are linked to the level of immunosuppression after transplantation. Furthermore, one study has proposed T-cell depletion as a possible risk factor.[45] However, multivariate analyses yield only two associated factors: acute graft-versus-host disease (GVHD)[46] and cord-blood transplantation.[16]

Clinical presentation and imaging

T gondii infection begins a median of 62 days after transplantation (appendix pp 4–8) and within the first 6 months in 90% of the cases. However, both early[16–18,39,47] and late cases[21,48–56] occur. During the initial reactivation phase, most patients have an isolated fever. If untreated, the fever progresses to clinical disease within a median of 7–14 days.[16,17,43,57,58] This is the rationale for a pre-emptive treatment strategy.

The most common toxoplasma disease is acute encephalitis, with symptoms such as headache or altered mental status and focal neurological signs of subacute onset, including motor weakness, cranial nerve palsies, speech, sensory or visual field disturbances, cerebellar signs, seizures, and movement disorders. Meningeal signs are very rare. Hyponatraemia due to inappropriate secretion of antidiuretic hormone might occur.[59,60] When available, cerebrospinal fluid samples might show slight mononuclear pleocytosis, increased protein, and normal glucose concentrations, with a positive toxoplasma PCR test result.

Imaging by CT often shows multiple bilateral lesions in the corticomedullary junction and the basal ganglia. They are generally hypodense and show ring enhancement and peripheral oedema after intravenous contrast administration. MRI shows high signal abnormalities on T2-weighted images, although other non-specific space-occupying lesions could be seen.[22,23,61–63] MRI is more sensitive than CT scan for early diagnosis.[23]

The lungs are the second most often involved organs, frequently as part of disseminated disease and often presenting as acute respiratory distress syndrome. Clinical and radiological features are non-specific and might mimic interstitial pneumonitis of any cause and stage.[18] Diagnosis requires either finding characteristic tachyzoites or toxoplasma PCR positivity, or both, in bronchoalveolar lavage fluid. In most cases, PCR blood tests are also positive.[8,26] Pulmonary involvement is more prominent early after transplant (ie, before day 100), whereas neurological involvement is usually observed later.[18,57]

Eye involvement, mainly chorioretinitis, is rare, whereas in patients with HIV or AIDS, eye involvement is much more common.[41,64–66] It characteristically presents as cottony white retinal lesions, but the presentation might be atypical.[67,68] PCR testing of the vitreous or aqueous fluid samples allows for diagnosis and differentiation from cytomegalovirus retinitis.[67]

Prognosis

Pre-2000 studies show that 90% of patients with toxoplasmosis died from or with the disease (appendix pp 9–11). However, more recent studies with earlier diagnoses made with PCR suggest that, if appropriately treated, up to 60% of patients with toxoplasmosis might be cured,[5,13,58] although CNS involvement might lead to debilitating late effects.[69]

Autologous HSCT recipients

Very rare cases of toxoplasmosis have been reported following various types of autologous HSCT, including bone marrow or peripheral blood stem-cell transplant, CD34-selected transplant, use of anti-thymocyte globulins, and tandem approaches.[39,44,54,69–76] A recent review of 13 studies estimates the number of cases at five per 12 542 (0·03%) autologous HSCT recipients,[27] with clinical presentation and timing similar to those observed after allogeneic HSCT. Most of the patients were R-positive and did not receive trimethoprim–sulfamethoxazole. Patients who developed toxoplasma chorioretinitis before transplantation seemed to be especially at risk of post-transplant relapse.[66] The rarity of the disease in this setting precludes identifying predisposing factors besides seropositivity.

Non-transplant haematology patients

Exceptional cases of toxoplasmosis have been reported in patients with lymphoproliferative disorders,[77–81] acute

myeloid leukaemia,[44,82,83] myelodysplastic syndrome,[84] immune thrombocytopenia,[85] or following chimeric antigen receptor T-cell therapy.[86] Most cases were diagnosed late and experienced high mortality.

Paediatric specificities

52 cases (mean age 12·7 years, male 56%) with sufficient data were identified.[87,88] Most patients were transplanted for haematological malignancies (62%). Reported incidence rates of toxoplasma disease ranged from 0·38% to 4·0%,[6,14,38,89,90] and were 0·95%, 3·8%, and 4·3% in matched related HSCT for haemoglobinopathies,[91] haplo-identical HSCT for Fanconi anaemia,[92] and cord-blood HSCT for myelodysplastic syndrome,[93] respectively. Only two-thirds of the paediatric patients were seropositive, a lower proportion than adults, and two-thirds had relevant comorbidities, including GVHD (37%). Approximately 40% of patients were receiving trimethoprim–sulfamethoxazole at diagnosis. 80% of the cases occurred before day 100 post transplantation. Encephalitis alone (38·5%), or with pneumonitis (17·3%) or chorioretinitis (11·5%) were the most frequent presentations. Sepsis-like features were also reported. Overall survival was 55% in the 38 patients who received a specific treatment and 0% in those who did not.

Three cases of toxoplasmosis were reported at 12 days, 36 days, and 148 days after autologous HSCT with fever, pneumonitis (n=1), and encephalitis (n=2) at presentation.[69,71,94] Furthermore, individual cases have been reported in non-transplant children with leukaemia[95,96] or lymphoma.[97–99] The data suggest no differences in associated risk factors and clinical presentation relative to adults besides a lower rate of seropositivity and perhaps a higher frequency of pneumonitis.

Biological diagnosis of toxoplasmosis

Serology

Pre-transplant serology of the recipient is mandatory to evaluate the risk of toxoplasma reactivation after transplantation because a positive serology result indicates the presence of living cysts. Toxoplasma IgG antibodies are produced within 1–2 weeks after infection and persist for life in most cases.[1,100] However, IgG serology can be falsely negative before HSCT due to a decline of antibody titres following chemotherapy, use of anti-B-cell monoclonal antibodies,[13] a previous HSCT,[101] or dilution by blood transfusions. In the case of pretransplant seronegativity, reassessing serological examinations at the time of initial diagnosis, and if not conclusive, testing a previous stored serum sample if available, are recommended. IgM antibodies are produced within the first weeks of infection and typically disappear within 6 months; although rare, they can persist for years. If IgM is detected, a PCR should be performed on blood; if positive, the patient should be treated for toxoplasmosis and the transplant delayed, if possible, until the end of treatment and two consecutive negative PCR tests. In the case of indeterminate results, the HSCT recipient should be considered seropositive.

Serology of the HSCT donor (D) is recommended because D-negative and R-positive indicates a more elevated risk of reactivation than D-positive and R-positive (appendix pp 9–11).[11,16,43] Furthermore, donor testing can evaluate the potential risk of toxoplasma transmission via the graft.[102] In case of donor IgM positivity, performing toxoplasma PCR on a blood sample from the donor is recommended; if positive, the donation should be delayed until two negative PCR tests, 7 days apart, are obtained. If the donor's blood PCR is negative or not performed, and the graft tested PCR-positive, pre-emptive treatment and PCR screening of the recipient should be initiated. After transplantation, relying on serology for toxoplasmosis diagnosis is not advised,[103] as serology can be impacted by hypogammaglobulinaemia, digestive loss of antibodies, use of anti-B monoclonal antibodies, or antibody transmission via transfusions or Ig infusion.

Toxoplasma PCR

Only the quantitative PCR (qPCR) format should be used to control for the risk of false positives and false negatives, and for monitoring parasite burden.[104] Technical recommendations are available for assessing the robustness of a qPCR test.[105] Currently, the DNA fragment most widely used to improve sensitivity is the repetitive 529-bp fragment.[106,107] Several commercial kits are available[106] and laboratories must validate their methods via external quality controls (Quality control for Molecular Diagnostics, Glasgow, Scotland, UK). PCR can be used as a diagnostic tool to verify parasite identification in any specimen (eg, tissue, bone marrow, cerebrospinal fluid, bronchoalveolar lavage fluid, and white blood cell pellets).

Currently, in a screening strategy, PCR is also used to diagnose *T gondii* infection following allogeneic HSCT. Although buffy coat might be more sensitive than blood,[108,109] whole blood is more convenient for laboratory use.

Because no PCR positivity is expected in healthy individuals,[110] every positive result in a transplant patient should be considered relevant, even in the absence of symptoms. Blood PCR should be then repeated 2–3 days later to monitor the parasite load.[111,112] The repeat test might be negative, indicating that the patient's immune system has controlled the parasite's growth. In other cases, the load persists or increases, strongly indicating parasite multiplication.

After treatment, the parasite load should decrease[111,112] and a minimum of two consecutive negative blood test results a week apart suggests the cessation of parasite replication. In other fluids that cannot easily be resampled, one negative PCR test result is required if the patient initially tested positive. These negative qPCR test results should be accompanied by the resolution of symptoms, if initially present.

Notably, neither a negative cerebrospinal fluid PCR, negative blood PCR, nor negative results for both blood and cerebrospinal fluid conclusively exclude the diagnosis of cerebral toxoplasmosis.[38,113–115] Using well validated qPCR tests, however, should decrease the risk of false negatives.

Genotyping of *T gondii* could be offered in South America, where new virulent genotypes have been reported.[116,117] Otherwise, genotyping is not recommended, as there is no known association between cotrimoxazole resistance and overexpression or polymorphisms in genes for therapeutic targets or ATP-binding cassette transporters, and thus any genotype.[118]

Toxoplasma PCR screening in HSCT recipients

Because DNA detection in blood samples might precede symptoms,[11,16,17,119,120] blood PCR screening following HSCT has been proposed, similar to the approach taken with cytomegalovirus infection[121,122] to detect infection early and adopt a pre-emptive strategy that can prevent the onset of toxoplasma disease. This strategy has yielded a decreased mortality from toxoplasmosis compared with pre-2005 data.[11,16,42,58] We define PCR screening as blood PCR performed regularly—usually weekly—in an asymptomatic patient.

For these recommendations, we selected 11 studies: six prospective studies[6,11,16,41,111,119] and five retrospective studies[17,18,46,57,112] assessing the benefits of blood PCR screening following HSCT. The selected studies (all published in English) provided information on pretransplant serology, timing of the screening, and development of toxoplasma infection and disease. All 11 studies were on allogeneic HSCT recipients, and two included autologous HSCT recipients.[6,41] Ten studies were published by European teams: only one study was purely assessing children,[6] and four studies screened only R-positive patients.[6,16–18] Most of the studies screened weekly following transplant or engraftment until at least day 100, with most screening until day 180. Different qualitative or quantitative PCRs were used but the PCR techniques were often not well described. Although most recent series used quantitative PCRs, only two studies considered quantitative results.[111,112]

The most common definitions used for infection and disease were either the EBMT definitions,[6,12,16–18,112] or modified EBMT definitions that included IgM presence[57] or used two consecutive positive PCR tests instead of one to define infection.[46] Although most patients received a specific anti-toxoplasma treatment when PCR-positive, the exact treatment was not always reported.

Toxoplasma PCR screening in allogeneic HSCT recipients

The mean incidence of *T gondii* infection was 9·6% (range 6·4–32·5) in R-positive patients, 0·4% in R-negative patients (0·0–3·3), and 5·5% (4·0–18·5) in series where R-positive and R-negative were both included (table 2). The mean incidence of toxoplasma disease was 1·4% (0–6) in the entire cohort of R-positive and R-negative HSCT recipients. The median onset of the first positive PCR test was between days 2 and 332. When the data were available, only 20 (17%) of 117 patients were receiving trimethoprim–sulfamethoxazole at diagnosis.[11,16–18,57,112] Most of the PCR-positive patients received anti-toxoplasma therapy except in two studies.[57,119] In one study, three of seven PCR-positive untreated patients developed toxoplasma disease within 3–30 days.[119] In another study, nine asymptomatic PCR-positive patients were not treated and did not develop disease, but whether they were receiving prophylactic trimethoprim–sulfamethoxazole is unknown.[57] A small proportion of PCR-positive patients might spontaneously become negative; rates of 8%[46] and 4% have been reported;[112] however, when untreated, these patients are at risk of subsequent toxoplasmosis and thus require prophylaxis.[46]

More than two-thirds of the *T gondii* infection occurred before 3 months after transplantation, and 90% within 6 months. Several studies have highlighted the risk of early infection before engraftment, with 41–56% of the cases occurring before day 30, when patients typically do not receive trimethoprim–sulfamethoxazole.[6,17,18]

The incidence of PCR-documented infection varies depending on the following: whether it was assessed in all HSCT patients or only in R-positive patients; whether trimethoprim–sulfamethoxazole prophylaxis was given and the administration schedule and doses; which definition of infection was used (eg, one or two consecutive positive PCR tests); timing and technique of the PCR tests; and the length of follow-up. Several studies report that PCR screening is not informative and likely not cost-effective in D-positive and R-negative patients as the risk of transmission via the graft is close to zero, even without trimethoprim–sulfamethoxazole prophylaxis.[46,111] Similarly, with an incidence of 0·4% infections in 1611 patients (table 2), PCR screening might also be considered unnecessary in D-negative and R-negative allogeneic HSCT recipients, provided the pretransplant serology is not falsely negative.[6] Analogous to the management of cytomegalovirus infection after allogeneic HSCT, the toxoplasma pre-emptive approach must be decided on an individual basis considering the pretransplant toxoplasma serology of the HSCT candidate, irrespective of the toxoplasma seroprevalence in the area or country.

Finally, PCR screening is a sensitive method of detecting infection early in R-positive patients. Although some cases of toxoplasma disease can be concomitant with the first positive PCR test,[16,17,43] infection mostly preceded toxoplasmosis by several days or weeks.[6,16,17,119] Since toxoplasma disease can occur under trimethoprim–sulfamethoxazole prophylaxis, this indicates that prophylaxis and PCR screening are not exclusive approaches and should be combined. Whether the parasite load is substantially higher in toxoplasma

	Study characteristics	PCR test	Definition of infection and disease*	Most relevant observations	Incidence of infection or disease among R-positive recipients (n/N; %)	Incidence of infection or disease among R-negative recipients	Patients with toxoplasma infection or disease in the whole population when R-positive and R-negative were included (n/N; %)	Patients with toxoplasma disease (all patients; n/N, %)	Time of first positive PCR result (median, range)	Patients receiving TMP–SMX at the time of first positive PCR (n/N; %)
Bretagne et al (2000), France[11]	Prospective single-centre cohort study investigating reactivation by PCR in blood in 32 R-positive or R-negative adult HSCT recipients, with monitoring at days 21, 30, 45, 60, 90, 120, and 150 post transplant	Competitive assay, B1 gene; 500 µL	NA	The three PCR-positive patients were febrile but had no fundoscopic or cranial imaging abnormalities; the PCR signal disappeared when the patients were given TMP–SMX	3/24 (12·5%)	0/8	3/32 (9·4%)	0/32	Day 42 (21–90)	0/3
Janitschke et al (2003), Germany[119]	Prospective single-centre cohort study to investigate PCR in blood and testing for antibodies against *T gondii* in 75 adult R-positive or R-negative patients; timing: at least five PCR test results for at least 2 months	Semi-nested and quantitative assays; 18SrDNA; 200 µL	NA	Routine testing for IgG, IgM, and IgA antibodies or testing IgG avidity is not helpful after HSCT	7/53 (13%)	0/22	7/75 (9·3%)	3/53 (5·7%; all cases in R-positive)	Day 48 (15–280)	NA
Martino et al (2005), Belgium, France, Germany, and Spain[16]	Prospective multicentre cohort study of PCR monitoring in blood in 106 R-positive adult HSCT recipients; PCR done once per week until day 100 and every 2 weeks from days 100 to 180; age 16–65 years	Participants' own in-house assay; two quality controls performed during the study[123]	EBMT definitions[12]	Patients were pre-emptively treated if one PCR test was positive; in two cases, the first PCR-positive test was concomitant with toxoplasmosis	16/106 (15%)	NA	NA	6/106 (6%)	Day 42 (2–178)	5/16, including three intermittent (not daily) TMP–SMX prophylaxis
Edvinsson et al (2008), Sweden[41]	Prospective study of 33 adult HSCT recipients including 12 allogeneic HSCT recipients (R-positive 4/12) and 21 autologous HSCT recipients; one PCR per week until 6 months then one PCR per month until 12 months; adults only	Conventional assay; B1 gene and quantitative assay; 529 bp element; blood volume NA	NA	Small-sized study	1/4 (25%)	0/8	1/12 (8·3%)	0/12	Day 5	NA
Fricker-Hidalgo et al (2009), France[57]	Retrospective single-centre study of PCR screening combined with IgM serology in 70 adult HSCT recipients (40 R-positive and 30 R-negative)	Conventional assay, B1 gene; blood volume NA	EBMT definitions or presence of IgM, or both	One patient was documented only by the presence of IgM; no pre-emptive treatment was started in nine infected patients who had a favourable outcome (no toxoplasma disease)	13/40 (32·5%)	1/30 (3·3%)	13/70 (18·5%)	4/70 (5·7%)	Day 83 for infection; day 56 for disease	1/13
Isa et al (2016), USA[111]	Prospective study of PCR monitoring in two hospitals in New York in 189 adult allogeneic, R-positive (n=71) or D-positive and R-negative (n=118) HSCT recipients; PCR twice per week from day 14 until discharge, then once per week until day 100	Quantitative assay, 529 bp element; blood volume NA	NA	In all reactivating patients, parasitaemia was successfully managed with pre-emptive treatment; no attributable death was observed while five of six patients with toxoplasma disease died during the historical period without PCR screening	5/71 (7%)	0/118 (all D-positive and R-negative)	5/189 (2·6%)	1/189 (0·5%)	NA	NA

(Table 2 continues on next page)

	Study characteristics	PCR test	Definition of infection and disease*	Most relevant observations	Incidence of infection or disease among R-positive recipients (n/N; %)	Incidence of infection or disease among R-negative recipients	Patients with toxoplasma infection or disease in the whole population when R-positive and R-negative were included (n/N; %)	Patients with toxoplasma disease (all patients; n/N, %)	Time of first positive PCR result (median, range)	Patients receiving TMP–SMX at the time of first positive PCR (n/N; %)
(Continued from previous page)										
Robin et al (2019), France[18]	Retrospective single-centre study in 419 adult allogeneic HSCT recipients; qPCR performed in R-positive patients, once per week from engraftment to day 100, then every 1–2 weeks	Quantitative assay, 529 bp element; blood volume NA	EBMT definitions	Comparison between early (before day 30; n=7) and later (from day 30; n=10) cases; highlights the benefit of screening from transplant, and not from engraftment	NA	NA	17/419 (4%)	6/419 (1·4%)	Day 45 (6–332)	2/17 (early cases 0/7)
Paccoud et al (2020), France[17]	Retrospective single-centre study to evaluate toxoplasma prevention strategies by PCR screening in 138 R-positive adult HSCT recipients; blood PCR was performed once a week until 6 months after HSCT	Quantitative assay, B1 gene; blood volume NA	EBMT definitions	Two-thirds of the cases of toxoplasma disease were first diagnosed by blood PCR	16/138 (11·6%)	0/105 (but not screened)	..	7/243 (2·9%)	Day 28 (14–180)	1/16
Aerts et al (2022), Belgium[46]	Retrospective single-centre study in 775 allogeneic HSCT recipients, R-positive (n=658) or D-positive and R-negative; qPCR screening was performed once per week from days 7 to 100, then every 1–2 weeks; case-control study for risk factors	Quantitative assay, 18SrDNA; blood volume NA	Modified EBMT definitions with two consecutive PCR-positive for infection	Infection and disease were assessed 1 year after transplant	58/658 (8·8%)	0/797 (0/117 in D-positive and R-negative)	58/775 (7·5%)	20/1455 (1·4%); in R-positive 20/658 (3%)	Day 40 (13–246)	0
Štajner et al (2022), Serbia[6]	Prospective study in 104 R-positive paediatric patients including 75 allogeneic HSCT recipients; qPCR was performed once per week during hospitalisation then once per month after discharge until day 104	Quantitative assay, 529 bp element; blood volume NA	EBMT definitions	10/17 (59%) cases occurred before day 30, but the PCR done 1 month after discharge might have underestimated the reactivations after day 30; two patients with a positive PCR test result before transplant are not presented here	17/73 (23%)	..	..	3/73 (4%); 2/3 cases preceded by infection 3 months before	Day 21 (7–90)	NA
Xhaard et al (2023), France[112]	Retrospective single-centre study in 1257 allogeneic HSCT recipients; R-positive (n=734) or R-negative (n=523); qPCR screening was performed once per week from transplant to day 100, then at the discretion of the physician	Quantitative assay, 529 bp element; 1 mL	EBMT definitions	qPCR kinetics were shown for 24 patients; 56% of the patients became PCR negative just by starting or continuing TMP–SMX prophylactic doses	45/734 (6·1%)	5/523 (1·0%)	52/1257 (4·1%)	7/1257 (0·5%)	Day 19 (extremes NA; IQR 14–25 without prophylaxis); Day 48 (IQR 28–61) with prophylaxis	11/52 (21%)
Total	11 studies in allogeneic HSCT including only one purely paediatric study[6]				181/1901 (9·5%)	6/1611 (0·4%)	156/2829 (5·5%)	57/3909 (1·4%)	Range: day 2–day 332	20/117 (17%)

When the study included both allogeneic HSCT and autologous HSCT recipients,[6,41] only the results for allogeneic HSCT recipients are presented. D=donor. D-positive=donor seropositive for *T gondii* before donation. D-negative–=donor seronegative for *T gondii* before donation. EBMT=European Society for Blood and Marrow Transplantation. HSCT=haematopoietic stem-cell transplantation. NA=not available. R=recipient. R-positive=recipient seropositive for *T gondii* before transplant. R-negative=recipient seronegative for *T gondii* before transplant. *T gondii*=*Toxoplasma gondii*. TMP–SMX=trimethoprim–sulfamethoxazole. *When definitions of the study were not available, the EBMT definitions were applied.

Table 2: **Summary of selected clinical studies reporting on peripheral blood PCR screening for toxoplasmosis after allogeneic HSCT**

disease when compared with *T gondii* infection is unclear;[16,43] however, an increasing parasitic load might be linked to disease onset.[16]

The fact that treating *T gondii* infection prevents the occurrence of toxoplasma disease is supported by the rarity of the disease in these 11 studies. The benefit of PCR screening is especially high during the pre-engraftment period, during which trimethoprim–sulfamethoxazole is typically not administered,[17,18,39,47,112] or when safety issues preclude its use (table 3).

Toxoplasma PCR screening in autologous HSCT recipients

Only two PCR-screening studies investigated autologous adult[41] or paediatric[6] HSCT recipients. None of the participants (21 adults[41] and 29 children[6]) became PCR-positive or developed toxoplasma disease; however, the small number of patients precludes a definitive conclusion. We found no data on children transplanted for solid tumour, which is the main indication for autologous HSCT in this population.

	Recommendation	Grade
Pretransplant assessment of the recipient		
(1)	All HSCT recipients should be assessed before transplant for toxoplasma serology to assess the risk of reactivation and implement a strategy; in the case of pretransplant seronegativity, it is suggested to check the serology at or before the diagnosis of the underlying disease if possible	A, II, r
(2)	In case the recipient has pretransplant IgM (whatever the IgG titre), a PCR should be performed on blood; if the recipient is PCR-positive, the patient should be treated and the transplant should be postponed if possible until the end of treatment plus two negative tests	B, III
Pretransplant assessment of the donor		
(1)	HSCT donors can be assessed for toxoplasma serology to assess: the risk of reactivation in a seropositive recipient (higher risk if the donor is seronegative); the unlikely risk of transmission by the graft; and in case of donor IgM seropositivity, it is recommended to perform a toxoplasma PCR test on blood of the donor	B, III
(2)	If the donor is PCR-positive in blood, the donation should be delayed until two negative tests, 7 days apart	B, III
(3)	If the donor's blood PCR is negative or not performed and the graft tested PCR-positive, pre-emptive treatment and PCR screening of the recipient should be initiated in the recipient*	B, III
Recommendations for toxoplasma qPCR screening		
(1)	qPCR screening is recommended for allogeneic seropositive (R-positive) HSCT recipients	
(1a)	Before engraftment	A, II, u
(1b)	After engraftment if no prophylaxis or any alternative prophylaxis to trimethoprim–sulfamethoxazole is given or in case of poor compliance or absorption of trimethoprim–sulfamethoxazole	A, II, u
(1c)	After engraftment if prophylaxis with appropriate doses of trimethoprim–sulfamethoxazole is given	B, II, u
(2)	Screening is not recommended in D-positive and R-negative or in D-negative and R-negative patients	No grade
(3)	The screening should start from transplant (day 0)	A, II, u
(4)	The screening should be at least once per week until day 100, then at least every 2 weeks until day 180, adapted to the schedule of patient follow-up and the intensity of immunosuppression	B, II, u
(5)	HSCT recipients who have developed toxoplasma infection or disease identified with qPCR should be monitored at least once a week to check for the decrease of the parasitic load under treatment	A, II, u
(6)	Two consecutive negative tests in blood, 7 days apart, and at least one in any other previously qPCR-positive fluid is the minimal requirement to confirm the efficacy of treatment of toxoplasma infection and disease	B, II, u
Recommendations for toxoplasma primary prophylaxis		
(1)	All seropositive (R-positive) patients before transplant should receive primary toxoplasma prophylaxis	A, II, t, u
(2)	On the basis of the HIV trials and limited experience in HSCT patients, oral or intravenous trimethoprim–sulfamethoxazole (80–400 mg) per day is recommended as the first choice	A, II, t
(2a)	Alternative is trimethoprim–sulfamethoxazole 160 mg and 800 mg, three times per week regimen	A, II, t
(2b)	Any less frequent dosing or lower doses is not recommended	D, II, u
(3)	Alternate options if trimethoprim–sulfamethoxazole cannot be used	
(3a)	Pyrimethamine–sulfadiazine with folinic acid (pyrimethamine 25–50 mg/day orally plus sulfadiazine 2000–4000 mg/day orally two to four divided doses plus folinic acid 10–25 mg/day orally)	No grade
(3b)	Oral atovaquone 1500 mg/day	C, III
(3c)	Dapsone, azithromycin, or clindamycin have no or only limited activity for prevention when used alone, but can be used in combination with pyrimethamine and folinic acid	No grade
(4)	Prophylaxis should ideally be started as soon as feasible after transplant, but no later than neutrophil engraftment	A, II, u
(5)	Given the potential for marrow toxicity, it is advised not to start trimethoprim–sulfamethoxazole prophylaxis during the pre-engraftment period	B, II, t
(6)	If engraftment is delayed and trimethoprim–sulfamethoxazole cannot be used due to myelosuppression or no PCR screening is available, atovaquone 1500 mg daily can be considered until engraftment followed by trimethoprim–sulfamethoxazole	C, III
(7a)	The duration of toxoplasma prophylaxis should be at least 6 months and extended during treatment-induced immunosuppression; or, in patients who no longer receive immunosuppressive drugs: until the CD4 cell count significantly increases	B, II, u
(7b)	Both toxoplasmosis and *P jirovecii* pneumonia prophylaxis should have the same duration in an individual patient	C, III

(Table 3 continues on next page)

	Recommendation	Grade
(Continued from previous page)		
Recommendations for the treatment of asymptomatic toxoplasma infection (ie, pre-emptive therapy)		
(1)	In case of toxoplasma infection documented by a first qPCR-positive result, the patient should be resampled for qPCR before the initiation of anti-toxoplasma treatment, ideally within 48–72 h; concomitantly and without delaying the treatment by waiting for the second qPCR result, the patient should be assessed for any toxoplasma disease (especially CNS, eyes, and lungs) with CNS imaging (preferably MRI), chest CT, and fundoscopy	A, II, υ
(2)	If the second qPCR is negative, it is the clinician's choice to either continue the treatment of the patient according to the immunosuppression status, or to stop treatment. However, this patient should at least receive an efficient prophylaxis to prevent later toxoplasmosis and be monitored with qPCR	B, III
(3)	In patients under trimethoprim–sulfamethoxazole prophylaxis, increase the dose or switch to an alternative therapy; in patients receiving no prophylaxis or prophylaxis other than trimethoprim–sulfamethoxazole, administer (1) trimethoprim–sulfamethoxazole prophylactic, intermediate, or therapeutic dose or (2) pyrimethamine plus sulfadiazine or pyrimethamine plus clindamycin†	No grade
(4)	Whatever the drug and dose choice, we recommend checking the parasitic load by qPCR at least once per week, to readapt the treatment in case of persisting or increasing parasitic load	B, III
(5)	After starting, pre-emptive treatment should be continued until at least the second negative qPCR, 7 days apart	B, II, υ
(6)	Once two negative qPCR tests have been achieved, pre-emptive treatment should be followed by secondary prophylaxis and ongoing qPCR screening for as long as the patient is immunosuppressed, or has low CD4 cell counts, or both	B, II, υ
(7)	Lowering or discontinuation of immunosuppression is recommended, where clinically appropriate	C, III
Recommendations for the treatment of toxoplasma disease		
(1)	Pyrimethamine (200 mg single loading dose followed by 50–75 mg/day orally) plus folinic acid (10–25 mg/day) is the most effective agent for toxoplasma disease and should always be used in combination with a second active agent	
(1a)	With sulfadiazine 4–6 g/day	A, II, t
(1b)	With clindamycin 600 mg four times per day (orally or intravenously), in patients intolerant to sulfadiazine	A, II, t
(1c)	With atovaquone (1500 mg/day orally) if intolerant to other regimens	C, II, t
(2)	Trimethoprim–sulfamethoxazole (10–20 mg/kg per day and 50–100 mg/kg per day, respectively, orally or intravenously) with or without clindamycin (600 mg three to four times per day) can be used as an alternative regimen in settings where either pyrimethamine is not available or oral route is not feasible, irrespective of previous trimethoprim–sulfamethoxazole prophylaxis	A, II, t
(3)	Atovaquone might also be used in combination with sulfadiazine (daily doses above)	C, II, t
(4)	Minimum duration of therapy is 6 weeks or until clinical resolution, or both, and two negative PCR tests in blood, 7 days apart, or one qPCR negative in a previously qPCR-positive cerebrospinal fluid; longer courses might be required if clinical disease or radiological findings are extensive or response is incomplete at 6 weeks	B, II, t
(5)	Lowering or discontinuation of immunosuppression is recommended, when possible	C, III
(6)	Steroids can be carefully considered in patients with severe ocular toxoplasmosis or CNS disease with radiological midline shift, progression within 48 h of treatment or elevated intracranial pressure	C, III
Recommendations for toxoplasma secondary prophylaxis		
(1)	Secondary prophylaxis of toxoplasmosis should be considered in patients who had toxoplasma infection or disease and who have ongoing risk factors for recurrence at the end of toxoplasmosis treatment (active GVHD or immunosuppression, or low CD4 counts); the choice of drugs for secondary prophylaxis depends on previous prophylaxis, the initial induction therapy and tolerance of the patient	B, II, υ

These recommendations are graded for the level of proof and the strength of recommendation according to the European Society of Clinical Microbiology and Infectious Diseases grading system (appendix p 3).[124] They apply both for adults and children, providing adaptation of the doses of anti-toxoplasma drugs. The paediatric doses for prophylaxis and treatment of toxoplasmosis are shown in the appendix (pp 12–14). D=donor. GVHD=graft-versus-host disease. HSCT=haematopoietic stem-cell transplantation. *P jirovecii=Pneumocystis jirovecii.* qPCR=quantitative PCR. r=meta-analysis or systematic review of RCT. R=recipient. υ=based on uncontrolled trials. t=transferred evidence, that is, results from different patient cohorts or similar immune-status situation. *Refer to the section "Recommendations for the treatment of asymptomatic toxoplasma infection (ie, pre-emptive therapy)". †Refer to the section "Recommendations for the treatment of toxoplasma disease" for the doses.

Table 3: **Recommendations for the management of toxoplasmosis after allogeneic HSCT from the 9th European Conference on Infections in Leukaemia**

Prophylaxis of *T gondii* infection and disease after allogeneic HSCT

There is ongoing research on developing drugs that are active against toxoplasma tachyzoites and bradyzoites, but this goal has not yet been achieved.[125] Among the clinically available drugs, folate inhibitors (eg, pyrimethamine and trimethoprim) and sulfonamides (eg, sulfamethoxazole, sulfadiazine, and sulfadoxine) are effective and cidal to tachyzoites. However, significant toxicity issues limit their use after transplantation.

Much of the evidence for the primary prevention of toxoplasmosis is derived from trials evaluating *P jirovecii* pneumonia prevention in people living with HIV. The US Centre for Disease Control and Prevention guidelines for primary toxoplasma prophylaxis in HIV patients[126] recommend double-strength trimethoprim–sulfamethoxazole (160–800 mg) per day (Grade AII), with dapsone–pyrimethamine as alternative (Grade BI), based primarily on a meta-analysis of 22 randomised studies.[127] However, serious side-effects, including myelotoxicity, might be observed when using similar doses in HSCT patients, especially before engraftment. In HSCT recipients, omitting trimethoprim–sulfamethoxazole prophylaxis has been linked with toxoplasmosis in R-positive patients.[13,15,18,44] However, there are no prospective randomised studies of trimethoprim–sulfamethoxazole in allogeneic HSCT solely for toxoplasma prophylaxis because of the risk of *P jirovecii* pneumonia for every HSCT recipient and the recommendation for first-line trimethoprim–sulfamethoxazole prophylaxis.[128,129]

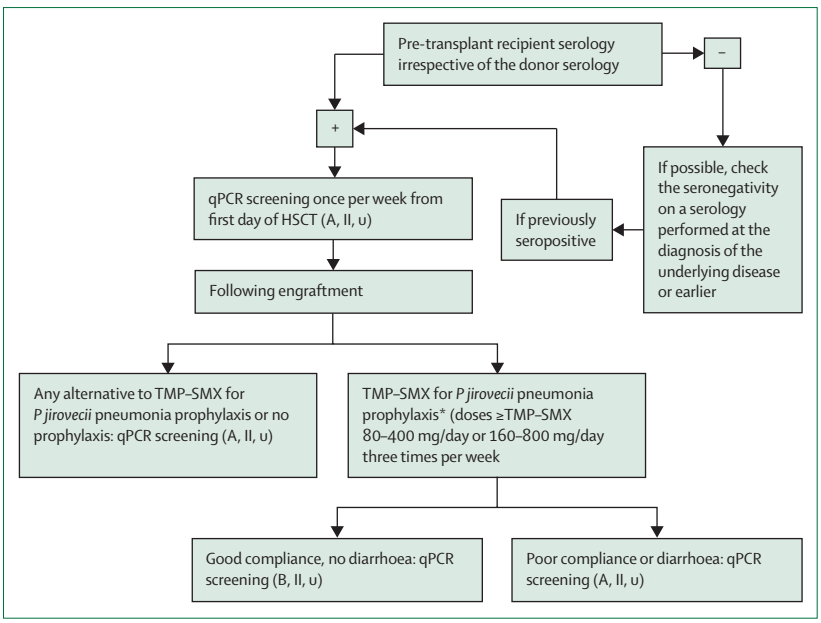

Figure: **Algorithm of the 9th European Conference on Infections in Leukaemia guidelines for primary qPCR-screening in allogeneic HSCT recipients according to pretransplant serology and prophylaxis**
This algorithm is graded according to the European Society of Clinical Microbiology and Infectious Diseases grading system (appendix p 3).[124] HSCT=haematopoietic stem-cell transplantation. *P jirovecii*=*Pneumocystis jirovecii*. qPCR=quantitative PCR. TMP–SMX=trimethoprim–sulfamethoxazole. u=uncontrolled trials. *Data from Maertens et al.[129]

Trimethoprim–sulfamethoxazole administered for *P jirovecii* pneumonia prophylaxis probably provides a reasonable prevention from toxoplasma reactivation after allogeneic HSCT. Although there is no evidence for a minimal dose of trimethoprim–sulfamethoxazole for preventing *P jirovecii* pneumonia in haematology patients,[129,130] some data support a minimal dose of double-strength trimethoprim–sulfamethoxazole three times per week for toxoplasma prophylaxis.[44] Breakthrough toxoplasmosis has been observed with trimethoprim–sulfamethoxazole prophylaxis,[15,18,29,44,57,90] either due to suboptimal dosing of trimethoprim–sulfamethoxazole, poor compliance, or inadequate oral absorption (in patients with intestinal GVHD). Although atovaquone is used in patients intolerant to trimethoprim–sulfamethoxazole, there is limited evidence for its efficacy for toxoplasma prophylaxis.[44,131–133] Other regimens, including azithromycin, clindamycin, or pyrimethamine–sulfadoxine, have little or no clinical activity in toxoplasma prevention,[44,134] but have been used in combinations for treatment of toxoplasa disease in people living with HIV.[135] With very little evidence and toxicity issues, these regimens might not be useful for primary toxoplasma prophylaxis in HSCT patients. Moreover, sulfadoxine is no longer available in many countries.

Based on observations of infections following early discontinuation of prophylaxis,[5] HIV experience, and *P jirovecii* pneumonia prophylaxis guidelines,[128,129] the duration of prophylaxis should be a least 6 months and extended during treatment-induced immunosuppression or severe CD4 lymphopenia. Furthermore, HSCT candidates must be informed of precautions to avoid primary infection.[136]

Pre-emptive treatment of PCR-documented infection after allogeneic HSCT

A pre-emptive treatment approach for *T gondii* infection (eg, a first positive PCR with or without fever) requires early resampling for qPCR within 48–72 h. Because the patient can promptly develop severe toxoplasma disease,[18] treatment should start immediately. Concurrently, patients should be evaluated clinically and radiologically for evidence of disease, especially the CNS, lungs, and eyes because disease might be already present. If the second qPCR is negative, clinical judgement is recommended to either continue therapy, depending on immunosuppression status, or to stop it. However, as a minimum, trimethoprim–sulfamethoxazole prophylaxis and weekly qPCR screening should be continued.

There is no randomised study on the outcome of treating asymptomatic PCR-positive patients versus no treatment. In most PCR screening studies, the PCR-positive patients were pre-emptively treated with various drugs and regimens.[13,18–20,43,48,113,114] If patients were not already on prophylaxis, treatment regimens often included low-dose or intermediate-dose trimethoprim–sulfamethoxazole (160–800 mg from three times per week to 480–2400 mg/day). For patients already on prophylaxis or who had persistent positive qPCR, high-dose trimethoprim–sulfamethoxazole or classical regimens (eg, pyrimethamine–sulfadiazine or pyrimethamine–clindamycin) were used. Sometimes, only adherence to prophylaxis was reinforced.[18,114,137] However, no conclusion can be made on the optimal drug, dose, or duration of pre-emptive therapy.

Notwithstanding the drug and dose choice, the parasite load usually decreases within 7–14 days of successful treatment.[113,114] After starting treatment, qPCR should be performed at least once per week, and a persisting or increasing parasitic load should inform consideration of treatment modification.

At least two consecutive negative qPCR, 7 days apart, appears to be a reasonable outcome indicating control of the infection.[18,114] Thereafter, because of the risk of toxoplasma relapse,[45,48] these patients should receive secondary prophylaxis and ongoing qPCR screening for as long as they are immunosuppressed.[115,138,139] Lowering or discontinuation of immunosuppression might be beneficial.

Treatment of toxoplasma disease

Treatment guidance is primarily based on data from reports on people living with HIV. There are no randomised treatment studies on HSCT patients. Pyrimethamine is considered the most effective agent;[140,141] to maximise synergistic effect, this drug is almost always combined with a second active agent and

requires folinic acid supplementation to reduce myelotoxicity.

Notwithstanding significant toxicity, pyrimethamine–sulfadiazine was established as the front-line therapy for toxoplasma encephalitis in HIV patients over a pyrimethamine–clindamycin regimen, which is used in patients who cannot tolerate sulfadiazine or for whom a response is not observed with a first-line pyrimethamine—sulfadiazine regimen.[142] Of note, additional *P jirovecii* pneumonia prophylaxis is required with the pyrimethamine–clindamycin regimen.[126]

Trimethoprim–sulfamethoxazole alone has been evaluated in non-randomised studies of people living with HIV for toxoplasma treatment, showing similar efficacy as pyrimethamine—sulfadiazine, but with a better tolerance profile,[143,144] and in combination with clindamycin.[145] Trimethoprim–sulfamethoxazole is the only intravenous choice. Two meta-analyses of 32 studies show that trimethoprim–sulfamethoxazole has a similar efficacy as pyrimethamine-containing regimens and a reduced discontinuation rate.[146,147] There is little evidence on other therapeutic strategies, including atovaquone with or without pyrimethamine in HSCT patients[63] (atovaquone elicits only modest responses with macrolides).[148,149] Based on HIV studies, the recommended minimum duration of initial therapy is 6 weeks.[126] Longer courses might be required for incomplete response at 6 weeks. Steroids are generally contraindicated in immunocompromised patients but can be carefully considered in patients with severe ocular toxoplasmosis, CNS disease with elevated intracranial pressure, or rapid progression on treatment.[126,150]

Based on HIV guidance, secondary prophylaxis can be considered in treated HSCT recipients who have persisting risk factors for recurrence at the end of treatment. The drug choice for secondary prophylaxis depends on previous prophylaxis, initial induction therapy, and tolerability.

ECIL recommendations for allogeneic HSCT recipients, autologous HSCT recipients, and non-transplanted patients with haematological malignancies

For allogeneic HSCT recipients, the ECIL recommendations are summarised in table 3. The figure summarises the algorithm proposed by ECIL 9 for the indication of PCR screening at various phases of allogeneic HSCT based on pre-transplant serology and *P jirovecii* pneumonia prophylaxis. For autologous HSCT recipients, due to the rarity of toxoplasmosis following autologous HSCT, no specific measure is recommended for prophylaxis or PCR screening, except in very specific populations (table 4). However, in case of toxoplasmosis-related symptoms or unexplained fever (especially in R-positive patients), a diagnostic test should be performed promptly (including blood PCR). In the case of toxoplasmosis, patients should be treated similarly to allogeneic HSCT recipients. For non-transplanted patients with haematological malignancies, no specific primary prophylaxis or qPCR screening are recommended.

	Recommendation	Grade
Recommendations for toxoplasma qPCR screening		
(1)	Screening PCR in seropositive patients undergoing autologous HSCT is not routinely recommended	D, II
(2)	PCR screening might be considered for 3 months in R-positive patients who cannot receive trimethoprim–sulfamethoxazole, or who have received anti-thymocyte globulins in the conditioning, or in patients who developed toxoplasmosis before transplant, irrespective of prophylaxis	C, III
Recommendations for toxoplasma primary prophylaxis		
(1)	Autologous HSCT recipients should receive *P jirovecii* pneumonia prophylaxis, preferably with trimethoprim–sulfamethoxazole, for 3–6 months (previous AII grading);[129] trimethoprim–sulfamethoxazole given for *P jirovecii* pneumonia prophylaxis, provided sufficient doses (80–400 mg/day or 160–800 mg, three times per week) are administered, should offer toxoplasma prophylaxis concomitantly, including for secondary prophylaxis in patients who developed toxoplasmosis before transplant	B, II, t
(2)	When the patient is intolerant to trimethoprim–sulfamethoxazole, no other routine toxoplasma prophylactic approach is recommended	C, II, u
(3)	R-positive patients undergoing T cell-depleted autologous HSCT (eg, autoimmune disease) should receive prophylaxis for 3–6 months based on immunosuppressed nature of these transplant recipients	C, III

These recommendations have been graded according to the European Society of Clinical Microbiology and Infectious Diseases grading system (appendix p 3).[124] They apply both for adults and children, providing adaptation of the doses of anti-toxoplasma drugs. GVHD=graft-versus-host disease. HSCT=haematopoietic stem cell transplantation. *P jirovecii=Pneumocystis jirovecii*. qPCR=quantitative PCR. R=recipient. u=based on uncontrolled trials. t=transferred evidence, that is, results from different patient cohorts or similar immune-status situation.

Table 4: **Recommendations for the management of toxoplasmosis after autologous HSCT from the 9th European Conference on Infections in Leukaemia**

Search strategy and selection criteria

We searched PubMed (no anterior limitation and until Sept 14, 2022) for publications in English using the terms "toxoplasma", "*toxoplasma gondii*", "toxoplasmosis", or "toxoplasma reactivation" in combination with one of the following keywords: "haematology", "transplantation", "haematopoietic cell or bone marrow transplantation", "infection", "disease", "risk factors", "PCR", "screening", "prophylaxis", and "treatment". Due to the low number of studies on toxoplasma after HSCT or in the haematology population, all studies, reviews, cases, and case-series were included. When no information was available on the HSCT population for certain topics, the search was extended to other patient populations (patients with HIV or AIDS, other transplant populations). Based on an extensive review of the literature and a list of questions with clinical impact prepared by the expert panel, guidelines were developed. The ECIL method for guideline development is summarised in the appendix (pp 1–2), along with the European Society of Clinical Microbiology and Infectious Diseases grading system (appendix p 3).[124]

Conclusions

Toxoplasmosis following HSCT remains a concern, especially in countries with high seroprevalence.

Toxoplasma disease is, however, often preventable. The pre-emptive approach using qPCR screening during the first months of transplant might identify early *T gondii* infection, which usually precedes the development of the disease. Despite the lack of randomised studies on the benefits of this approach, we consider pre-emptive therapy is—together with drug prophylaxis—the only way to decrease the incidence of toxoplasma disease and subsequent morbidity and mortality. Because drug prophylaxis is not optimal, even with trimethoprim–sulfamethoxazole, PCR screening and prophylaxis should be combined in R-positive patients.

qPCR is the key tool for managing these patients. Transplant centres with no PCR on site should consider sending samples to a reference centre. We strongly encourage prospective studies on optimising the minimal dose of trimethoprim–sulfamethoxazole, providing a dual prevention for *P jirovecii* pneumonia and toxoplasmosis, evaluating the kinetics of immune reconstitution against toxoplasma, assessing new anti-toxoplasma drugs, and integrating toxoplasma markers into laboratory panels investigating infectious symptoms in immunocompromised patients.

Contributors

CC recruited the experts, coordinated the literature analysis, and compiled the recommendations. All authors were involved in the literature search, development of recommendations, and conception of the Review. All authors revised the Review and gave final approval.

Declaration of interests

We declare no competing interests.

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