9th EUROPEAN CONFERENCE on INFECTIONS in LEUKAEMIA

Guidelines for the management of toxoplasma infection and disease in haematology patients and stem cell transplant recipients

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IN-PERSON CONFERENCE
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Toxo infection and disease are of rare occurrence in the hematology ward but may have a high mortality

- ✓ **Most cases were reported in allogeneic HCT recipients**
- ✓ **Very rare cases were reported after autologous HCT** (1)
- ✓ **Exceptional cases were reported in non-transplanted patients** with hematological diseases, eg. lymphoproliferative disorders, acute leukemia, ITP (2) and after CAR-T cell therapy (3)

(1) *Aoun et al., 2006; Rusinakova et al., 2009; Sumi et al., 2013; Robert-Gangneux et al., 2015; Sumi et al., 2016; Stajner et al., 2021*

(2) *Murakami et al., 2021; Omori et al. 2021Brown et al., 1991; Pagano et al. 2004; Matsuzawa et al., 2019*

(3) *Marzolini et al. 2019*

Most cases of toxo observed in hematology occur in seropositive patients

The mean seropositivity of all cases is around 95%

=>Almost all toxo cases observed after allogeneic HCT occur in R+ patients through REACTIVATION of a pretransplant, latent, infection

- Some cases were not tested

- Most cases received immunosuppressive drugs before serology assessment. A recent report mentions that 30% of children with acute leukemia (AL) and who were sero+ at diagnosis of AL became seronegative at the preHCT assessment *(Stajner et al. 2021)*

=> In case of seronegativity for toxo just before HCT, the serostatus should be checked with the serology at the diagnosis of the underlying disease (before any treatment) or before if available

Main characteristics of toxo after allogeneic HCT

Mean (or median) day of onset of toxo infection *or* disease after allogeneic HCT: **day 62,** much earlier than after solid organ transplant *(Adekunle et al., 2021)* . **However:**

- **earlier for infection than disease**
- **earlier for a first PCR toxo in prospective screening studies**
- earlier for pts receiving no prophylaxis than in pts with prophylaxis

90% of the cases occur within 6 months from transplant

PRIMO-INFECTION, occurring in R- recipients, is extremely rare and usually not observed during the first year after transplant.

Most reported cases of primo-infection in adults are likely false negative serologies rather than primary infections. In children where the seronegativity is more frequent, some cases were reported very late after HCT with similar presentation than in the healthy population

Main characteristics of toxo after autologous HCT

Rare cases (0-1%) of toxoplasmosis, mostly toxo disease, were reported both in adults and children, after different types of autoHCT, including BMT, PBSCT, CD34+ selected transplants, and tandem approach *(1)*

The clinical presentation and timing were close to the ones observed after alloHCT *(2)*

When the information was available:

- most patients were sero+ before transplant

- most patients did not receive TMP-SMX prophylaxis

Only 2 studies investigated autologous HCT recipients with a weekly PCR screening in 21 adults *(3)* and 29 children, respectively *(4)* . None of the patients was PCR+ after transplant but the small number of patients does not allow to draw any conclusion about the true incidence of infection after autoHCT

(1)Slavin et al. 1994; Geissmann et al. 1994; Yadlapati et al. 1997; Pagano et al. 2004; Matsuo et al. 2007; Prestes et al. 2018; Kitahara et al. 2021; Contopoulos-Ionannidis 2021). (2) Geissmann et al. 1994; Yadlapati et al. 1997; Nakane et al. 2001; Gonzalez-Vicent et al. 2003; Lopez-Duarte et al. 2003; Grosu et al. 2007; Voegele et al. 2013; Sumi et al. 2013; Sumi et al. 2016; van Mourik et al. 2019; Kitahara et al. 2021. (3) Edvinsson et al. 2018. (4) Stajner et al 2021

Main pediatric specificities of Toxo after allogeneic HCT

- Incidence rates range from 0.38 to 4.3%; with 5% in seropositives

  Incidence in autologous transplant recipients or hematology patients not receiving a transplant is similar to the adult population.

- 63% of evaluable cases reported were confirmed seropositive; the serostatus was negative in 8% and not reported in the remainder
- Mean age of 12.7 years, predominance of boys
- Haplo- and cord blood procedures, use of MAC and GvHD were also suggested as possible predisposing factors
- Approximately 40% of patients were receiving TMP/SMX at the time of presentation; 30% were not on TMP/SMX, and prophylaxis was not reported in a further 30%
- Clinical presentation and onset close to the one of adults

  Maybe more cases of pneumonitis than in adults

Recommendations for PCR testing

1. There are multiple in-house and commercial assays available (1). The real-time quantitative PCR (qPCR) format should be preferred because of the dramatic decrease of the risk of false positive (2), and because of the interest of quantification to follow the parasite load.
2. The qPCR assay used should comply with the MIQE recommendations (3) in providing limit of detection and in using an internal control of amplification.
3. qPCR assays targeting the repeated DNA elements rep529 have a better sensitivity as compared to B1 PCR (4).
4. qPCR can be tested on different sample types (tissue, broncho-alveolar lavage, whole blood, serum, plasma, ocular fluids, CSF, pleural effusion …)
5. Whole blood is most commonly used as sample type. There is no data comparing the performance on plasma or serum to whole blood (1-5)
6. Although probably more sensitive (5), testing PCR on buffy-coat is difficult to realize in routine practice. Moreover, the result is highly impacted by the variation of circulating white cells.
7. The center using PCR should provide the results of their participation to external quality assessment programs.

1. Robert-Gangneux DOI:10.1097/QCO.0000000000000275
2. Bretagne et al. 2005 doi:10.1016/j.cccn.2005.05.051
3. Bustin S Chem Clin 2009 DOI: 10.1373/clinchem.2008.112797
4. Reischl BMC Infect Dis . 2003 May 2;3:7. doi: 10.1186/1471-2334-3-7
5. Brenier-Pinchart MP http://dx.doi.org/10.1016/j.diagmicrobio.2015

Recommendations for blood PCR interpretation

1. There is **no minimal cut-off** to consider the clinical pertinence of a positive toxo PCR (1)
2. **The kinetics of the qPCR** (2 tests at 2-3 days interval) **may be informative about the spontaneous evolution of the infection**
3. **The kinetics of the qPCR** (2 tests at 3-7 days interval) **is informative to follow the efficacy of the treatment** although the clinical evolution may not strictly follow the parasitological cure
4. **PCR in any sample should be preferably performed before specific treatment** as the parasitic load may rapidly decrease after starting treatment

1. Neves ES et al. Parasitol Res. 2021

Summary of the data on PCR screening after allogeneic HCT (1/2)

The incidence of at least 1 PCR Toxo+ (infection and/or disease) was between :

- in all (R+ or R-) patients (8 studies): 2.6% to 18.5% (mean 8%)
- **in seropositive (R+) patients (11 studies) : 6.4 % to 32.5% (mean: 14 %)**
- **In seronegative (R-) patients (8 studies): 0.4 %**

The median time of the first positive PCR test was between day 5 and day 67 (extremes: D2-D332)

Most of PCR+ transplant patients received a specific treatment (TMP-SMX, pyrimethamine plus sulfadiazine or clindamycin) except in 2 studies:

- In one study, among 7 PCR+ patients: 3 developed toxo disease within 3-30 days after the 1st PCR and 2 of them died despite treatment. The 4 other patients who developed only 1 or 2 asymptomatic PCR+ were not treated *(Janitschke 2003)*
- In another study, 9 PCR+ patients were not treated and had a favorable outcome *(Fricker-Hidalgo 2009)*

Summary of the data on PCR screening after allogeneic HCT (2/2)

Despite the lack of randomized study, and the paucity of data on treatment of asymptomatic infection, these PCR screening studies showed that:

- **PCR screening early identifies toxo reactivation in 6 to 32% of the R+ allogeneic HCT recipients**
- **The risk is especially high before engraftment due to the usual lack of TMP-SMX prophylaxis**
- **Untreated infection mostly precedes disease** *(Janitschke 2003; Martino 2005; Paccoud 2020; Stajner 2021)*
- **The toxo-attributable mortality has been decreased with this strategy when compared to historical mortality before 2005** *(Martino 2005; Gajurel 2015; Robert-Gangneux 2015)*
- **A minority of patients may spontaneously become PCR negative** (3/37, 8% in Aerts et al. 2021; 2/52, 4% in Xhaard et al. 2022). **However, when untreated, these patients seem to be at risk of subsequent toxoplasmosis**

Data on primary Toxo prophylaxis in HCT

- Observational studies support beneficial effect of TMP/SMX prophylaxis *(Martino 2005, Au 2005, Tomonari 2008, Gajurel 2015*)

- However, Toxo infections/disease have been reported during prophylaxis (breakthrough infections) or after early stopping TMP/SMX *(Brinkman 1998, Khoury 1999, Bretagne 2000, von Lilienfeld-Toal 2007)*

- The dosing data of TMP-SMX prophylaxis in 28 HCT recipients with breakthrough infections reviewed by *Gajurel et al. 2015* suggested a **lower efficacy of TMP-SMX prophylaxis in regimens that used the drug less than 3 times a week**

- **Most randomized clinical trials for toxo prophylaxis were performed in HIV patients**

Summary of the HIV+ CDC 2021 guidelines on toxo prophylaxis in HIV+ patients

- **Preferred Prophylaxis regimen**: TMP-SMX 1 double-strength tablet/day (AII)
- Alternative regimens:
  - TMP-SMX 1 DS tablet x3/w (BIII) or
  - TMP-SMX 1 SS tablet /d (BIII) or
  - Dapsone 50 mg/d + pyrimethamine 50mg + Leucovorin 25mg PO/W (BI) or
  - Dapsone 200mg + pyrimethamine 75mg + leucovorin 25mg/w (BI) or
  - Atovaquone 1500 mg/d + pyrimethamine 25mg + leucovorin 10mg) (CIII)

- **Duration of primary prophylaxis**:
  - until CD4> 200 cells/µL for > 3 months in response to ART (AI) or
  - CD4 > 100 cells/µL for 3-6 months if HIV plasma RNA levels remain below limits of detection (BII)
- **Indication for restarting prophylaxis**: CD4 between 100-200 cells/µL
- **Secondary prophylaxis /chronic maintenance therapy**: all patients after > 6 weeks of initial treatment of toxo encephalitis (TE)(AI): until CD4 > 200 for > 6 months in response to ART

*available at https://clinicalinfo.hiv.gov/en/guidelines/adult-and-adolescent-opportunistic-infection/toxoplasma-gondii-encephalitis?view=full

GUIDELINES

I Proposals for modifying the EBMT 2000 definitions for Toxo infection and disease

II Allogeneic HCT recipients

III Autologous HCT recipients

IV Non-transplanted patients with hematological malignancies

I - ECIL proposal for modifying the EBMT 2000 definitions for toxo infection and disease after HCT

Toxoplasmosis classification	Definition
***TOXOPLASMA* DISEASE**	
Definite *toxoplasma* disease	**Histologic or cytologic demonstration of tachyzoïtes 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**
Probable *toxoplasma* disease (documented by PCR)	**Clinical and radiologic evidence suggestive of organ involvement plus at least one positive PCR test from any fluid sample, or in tissue but without histologic evidence of the presence of *T gondii***
Possible *toxoplasma* disease (documented by imaging)	**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 may explain the findings**
***TOXOPLASMA* INFECTION**	**Positive PCR in blood in a patient without evidence of organ involvement (with or without fever)**

II

ECIL recommendations

for the management of toxoplasma infection and disease in allogeneic HCT recipients

Recommendations for pretransplant toxo assessment of the RECIPIENT

Recommendations	Grading
All HCT recipients should be assessed before transplant for toxo serology in order to assess the risk of reactivation and implement a strategy In case of seronegativity at the pretransplant assessment, it is suggested to check the serology at or before the diagnosis of the underlying disease if possible	**AIIr**
In case the recipient has pretransplant IgM (whatever the IgG titer), a PCR should be performed on blood. If the recipient is PCR+, the patient should be treated and the transplant should be delayed if possible until the end of treatment plus 2 negative tests	**BIII**

Recommendations for pretransplant toxo assessment of the DONOR

Recommendations	Grading
The HCT donors can be assessed for toxo serology in order to assess: a) The risk of reactivation in a seropositive recipient (more risk for the recipient if D-) b) The unlikely risk of transmission by the graft	**BIII**
In case of the presence of IgM in the donor at the pretransplant assessment, it is suggested to perform a Toxoplasma PCR test on blood	
In case the donor is PCR+ in blood, the donation should be delayed until two negative tests, 7 days apart In case the donor was negative on pretransplant PCR or could not have a PCR before transplant, and he/she is PCR+ on graft, a preemptive treatment and a PCR screening should be initiated in the recipient (cf. Pre-emptive therapy)	**BIII** **BIII**

Recommendations for Toxo qPCR screening after allogeneic HCT (1/3)

Recommendations	Grading
A qPCR screening is recommended for allogeneic seropositive (R+) HCT recipients: ✓ Before engraftment	**AII u**
✓ After engraftment if no prophylaxis or any alternative prophylaxis to TMP-SMX is given or in case of poor compliance or absorption of TMP-SMX	**AII u**
✓ After engraftment if prophylaxis with appropriate doses of TMP-SMX are given	**BII u**
A screening is not recommended in D+/R- or in D-/R- patients	(no grading)
The screening should start from transplant (day 0)	**AII u**
The screening should be at least once weekly until day 100, then at least every two weeks until day 180, adapted to the schedule of patient follow-up and the intensity of immunosuppression	**BII u**

Recommendations for Toxo qPCR screening after allogeneic HCT (3/3)

Recommendations	Grading
HCT recipients who have developed a toxo 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	**AII u**
The negativation of qPCR is a minimal requirement to confirm the efficacy of treatment of toxo infection and disease with 2 consecutive negative tests in blood, 7 days apart, and at least 1 in any other previously qPCR+ fluid	**BII u**

ECIL recommendations for Toxo primary prophylaxis in allogeneic HCT (1/2)

Recommendations	Grading
All seropositive (R+) patients before transplant should receive primary toxo prophylaxis	**AII t u**
On the basis of the HIV trials and limited experience in HCT patients, oral or IV TMP/SMX 80/400 mg daily is recommended as the first choice Alternative is TMP/SMX 160/800 mg, 3 times / week regimen Any less frequent dosing or lower doses is not recommended	**AII t** **DII u**
Alternate options if TMP/SMX cannot be used: ✓ Pyrimethamine/sulfadiazine with folinic acid ✓ Oral atovaquone 1500 mg daily ✓ 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 grading **CIII** No grading

** Pyrimethamin-sulfadoxin no more routinely available but may be imported in most European countries*

ECIL recommendations for Toxo primary prophylaxis in allogeneic HCT (2/2)

Recommendations	Grading
Prophylaxis should ideally be started as soon as feasible after transplant but no later than neutrophil engraftment	**AII u**
Given the potential for marrow toxicity, it is advised not to start TMP/SMX prophylaxis during the pre-engraftment period	**BIIt**
If engraftment is delayed and TMP/SMX cannot be used due to myelosuppression or no PCR screening available, atovaquone 1500mg daily can be considered until engraftment followed by TMP/SMX	**CIII**
The duration of Toxo prophylaxis should be at least 6 months and eventually extended during the treatment-induced immunosuppression	**BIIu**
or in patients who no longer receive IS drugs: until the CD4+ cell count significantly increases	**CIII**
Both Toxo and PcP prophylaxis should have the same duration in a given patient	

ECIL guidelines for the treatment of asymptomatic toxo infection : « Pre-emptive therapy » (1/2)

Recommendations	Grading
In case of a toxo infection documented by a 1st qPCR+, the patient should be resampled before the initiation of anti-toxo treatment, and retested for qPCR, ideally within 48-72h Concomitantly and without delaying the treatment by waiting for the 2nd qPCR result, the patient should be assessed for any toxo disease (especially CNS, eyes and lungs) with CNS imaging (preferably MRI), chest-CT, and fundoscopy	**AII u**
If the 2nd qPCR is negative, it is the clinician choice to continue the treatment of the patient according to the IS status, or to stop treatment. However, at least this patient should receive an efficient prophylaxis to prevent later toxo and be monitored with qPCR	**BIII**
The therapeutic options are: 1) <u>In patients under TMP-SMX prophylaxis</u>: increase the dose or switch to an alternative therapy 2) <u>In patients receiving no prophylaxis or another prophylaxis than TMP-SMX</u>: -TMP-SMX, prophylactic, intermediary or therapeutic dose - Pyrimethamine + sulfadiazine or Pyrimethamine + clindamycin (cf. doses for toxo treatment)	**No grading because of lack of data**

ECIL guidelines for the treatment of asymptomatic toxo infection : « Pre-emptive therapy » (2/2)

Recommendations	Grading
Whatever the drug and dose choice, we recommend checking the parasitic load by qPCR at least once weekly, to readapt the treatment in case of persisting or increasing parasitic load	**BIII**
After starting the treatment, preemptive treatment should be continued until at least the 2nd negative qPCR, 7 days apart	**BII u**
Once 2 negative qPCR tests have been achieved, the preemptive treatment should be followed by a secondary prophylaxis and ongoing qPCR screening as long as the patient is immunosuppressed and/or has low CD4 counts	**BII u**
Lowering or discontinuing of immunosuppression is recommended, where clinically appropriate	**CIII**

ECIL guidelines for treatment of toxoplasma disease in HCT recipients (1/2)

Recommendations based on HIV data:	Grading
PYRIMETHAMINE (200mg single loading dose followed by 50—75mg PO /day) + folinic acid (10-25mg/d) is the most effective agent for toxo disease and should always be used in combination with a second active agent: - **with sulfadiazine** 4-6 g /day - **with clindamycin** 600mg x 4/day (PO or IV) *, in patients intolerant to sulfadiazine - **with atovaquone** (1500 mg/day PO) if intolerance to other regimens	**A II t** **A II t** **CII t**
TMP/SMX (10-20/50-100mg/kg/d, orally or IV) **with/without clindamycin** (600mg TiD-QiD) can be used as an alternative regimen in settings where either pyrimethamine is not available or oral route is not feasible, irrespectively of previous TMP-SMX prophylaxis	**A II t**
ATOVAQUONE in combination **with sulfadiazine** (daily doses above)	**CII t**

* Should be associated with aPcP prophylaxis

ECIL guidelines for treatment of toxoplasma disease (2/2)

Recommendations	Grading
Minimum duration of therapy is 6 weeks and/or until clinical resolution and 2 PCR negative in blood, 7 days apart, or 1 qPCR negative in a previously qPCR+ CSF Longer courses may be required if clinical disease or radiological findings are extensive or response is incomplete at 6 weeks	**BII t**
Lowering or discontinuing of immunosuppression is recommended, when possible	**CIII**
Steroids can be carefully considered in patients with severe ocular toxoplasmosis or CNS disease with radiological midline shift, progression within 48 hours of treatment or elevated intracranial pressure	**CIII**

ECIL recommendations for SECONDARY PROPHYLAXIS of toxoplasma infection /disease in HCT recipients

Recommendations	Grading
Secondary prophylaxis of toxo should be considered in patients who experienced toxo infection or disease and who have ongoing risk factors for recurrence at the end of toxo treatment (active GVHD/immunosuppression or low CD4 counts The choice of drug(s) for secondary prophylaxis depends on an eventual previous prophylaxis, the initial induction therapy and tolerance of the patient	**BII u**

III

ECIL recommendations for the management of toxoplasma infection and disease in autologous HCT recipients

ECIL recommendations for toxo prophylaxis in autologous HCT (1/2)

Recommendations	Grading
Autologous HCT recipients should receive PcP prophylaxis, preferably with TMP-SMX, for 3-6 months (previous AII grading)* . TMP-SMX, providing sufficient doses (≥ 80/400 mg/d or 160/800 mgx3/w), should offer toxo prophylaxis concomitantly, including for secondary prophylaxis in patients who developed toxo before transplant	**BII t**
In case the patient is intolerant to TMP-SMX, no other toxo prophylactic approach is recommended in routine	**CII u**
R+ patients undergoing T-cell depleted autologous HCT (e.g autoimmune disease) should receive prophylaxis for 3-6 months based on immunosuppressed nature of these transplants	**CIII**
Screening PCR in seropositive patients undergoing autologous HCT is not routinely recommended	**DII**
PCR screening may be considered for 3 months in R+ patients who cannot receive TMP-SMX, or who have received ATG in the conditioning or in patients who developed toxo before transplant, irrespectively of prophylaxis	**CIII**

** Maertens et al. JAC 2016; doi: 10.1093/jac/dkw157*

ECIL recommendations for toxo prophylaxis in autologous HCT (2/2)

In case of symptoms evocative of toxoplasmosis or unexplained fever, a diagnostic work-up should be performed (including blood PCR), especially in R+ patients who are severely immunodepressed

Autologous HCT recipients with toxo infection or disease should be treated as allogeneic HCT recipients and benefit thereafter from a secondary prophylaxis as long as they are immunosuppressed

IV

ECIL recommendations for the management of toxoplasma infection and disease in hematology patients who are not transplanted

ECIL recommendations for toxo prophylaxis in patients with haematological malignancies and not transplanted (CAR-T cell patients excluded)

Due to the rarity of toxo infection or disease in non-transplanted hematology patients, no primary prophylaxis for toxo and no qPCR screening are recommended in non-transplanted patients

In case of symptoms evocative of toxoplasmosis or persistent unexplained fever, a diagnostic work-up should be performed (including blood qPCR), especially in seropositive patients who are severely immunosuppressed

Pediatric specificities

The same recommendations are proposed for transplanted adults or children, providing adaptation of the doses of antitoxo drugs