Detection and treatment of cerebral toxoplasmosis in an aplastic pediatric post-allogeneic hematopoietic cell transplant patient: a case report

Danielle Brewer1, Margaret L. MacMillan2, Mark R. Schleiss3, Satja Issaranggoon Na Ayuthaya3, Jo-Anne Young4 and Christen L. Ebens2*

Abstract
Background: Cerebral toxoplasmosis infection presents with non-specific neurologic symptoms in immunocompromised patients. With lack of measurable adaptive immune responses and reluctance to sample affected brain tissue, expedient diagnosis to guide directed treatment is often delayed.

Case presentation: We describe the use of cerebrospinal fluid polymerase chain reaction and plasma cell-free DNA technologies to supplement neuroimaging in the diagnosis of cerebral toxoplasmosis in an immunocompromised pediatric patient following allogeneic hematopoietic cell transplantation for idiopathic severe aplastic anemia. Successful cerebral toxoplasmosis treatment included antibiotic therapy for 1 year following restoration of cellular immunity with an allogeneic stem cell boost.

Conclusions: Plasma cell-free DNA technology provides a non-invasive method of rapid diagnosis, improving the likelihood of survival from often lethal opportunistic infection in a high risk, immunocompromised patient population.

Keywords: Toxoplasmosis, Allogeneic hematopoietic cell transplantation, Severe aplastic anemia, Immune mediated cytopenia, Cell-free DNA, Case report

Background
Cerebral toxoplasmosis is a rare but serious complication of allogeneic hematopoietic cell transplantation (alloHCT). Caused by the protozoan parasite Toxoplasma gondii, toxoplasmosis most often results from reactivation of latent infection in immunocompromised patients [1]. It is one of the most common opportunistic infections of the central nervous system (CNS) [2], with greatest prevalence in those with acquired immunodeficiency syndrome (AIDS) [3]. The incidence of toxoplasmosis after alloHCT ranges from 0.3 to 9% [2, 4], with variation based on population seroprevalence. Although the incidence and treatment of toxoplasmosis in adult alloHCT patients has been reported extensively, few studies have focused specifically on cerebral toxoplasmosis in pediatric patients [5–17]. Furthermore, cerebral toxoplasmosis diagnosis is usually based on a combination of radiologic imaging abnormalities and clinical symptoms such as seizures, headaches, and altered mental status, non-specific findings contributing to delays in diagnosis and treatment [18]. This case reviews the successful management of cerebral toxoplasmosis in a pediatric alloHCT patient...
following diagnosis with the use of cerebrospinal fluid (CSF) polymerase chain reaction (PCR) and microbial cell free DNA (cfDNA) technology.

**Case presentation**

A 13-year-old male with idiopathic severe aplastic anemia was treated with a human leukocyte antigen (HLA)-matched unrelated donor alloHCT on an Institutional Review Board-approved protocol with parental consent. His transplant course was complicated by Epstein-Barr virus (EBV) viremia (day +21, successfully treated with rituximab), immune-mediated cytopenias versus inadequate graft function (beginning at day +100, refractory to granulocyte-colony stimulating factor (GCSF), corticosteroids, intravenous immunoglobulin (IVIG), plasmapheresis and bortezomib), and right cervical lymphadenopathy concerning for EBV-post-transplant lymphoproliferative disease (day +188, surgically excised, negative for infection or malignancy). With persistent pancytopenia, he required blood product transfusions and prophylactic anti-infective agents (valacyclovir, itraconazole, and intravenous pentamidine). Eight months after alloHCT, he was hospitalized locally for a severe gastrointestinal hemorrhage requiring superior mesenteric artery branch embolization.

Nine months after alloHCT, he was readmitted to our hospital with refractory pancytopenia. He denied night sweats and weight loss, but endorsed 2 weeks of intermittent headaches. With no financial, cultural or social barriers to care, the patient was promptly evaluated. A bone marrow biopsy was hypocellular (5–10%), with 93% donor chimerism. On day 3 of hospitalization, his severe headache recurred, accompanied by somnolence, nausea, fever, and hypertension. While head CT and ophthalmologic exams were unchanged, his LP opening pressure was again elevated at 55 cm H2O. Improvement in mental status/alertness following the LP (closing pressure of 26.5) prompted initiation of acetazolamide and serial therapeutic LPs (16 times over 58 days). Atovaquone (1500 mg twice daily) was added when an MRI at 4 weeks of therapy (day +337 post-alloHCT) showed decreased cerebral edema but unchanged toxoplasmosis lesions.

In the context of persistent cytopenias and poor graft function despite multi-modal therapy (Fig. 1), the patient received 4 days of immunosuppressive fludarabine followed by a CD34+ selected peripheral blood stem cell boost from his previous bone marrow donor (day +349 after alloHCT). After 6 weeks of toxoplasmosis treatment showing both clinical and radiologic response, and to avoid bone marrow suppression after his stem cell boost, sulfadiazine was transitioned to oral clindamycin 600 mg 3 times/day for chronic maintenance therapy. One month after the stem cell boost, peripheral blood donor chimerism was 100% in the CD33+ myeloid compartment and 87% in the CD3+ lymphoid compartment. Transfusion independence was achieved at 42 days, eltrombopag discontinued at 60 days, and GCSF discontinued at 100 days. Fifty-five days following his stem cell boost—3 months of hospitalization—he was discharged on maintenance pyrimethamine and clindamycin. Adherence to oral therapies was monitored by nursing while inpatient and by the patient’s mother while outpatient. The patient himself reported no intolerance or adverse toxicities.

After 5 months of cerebral toxoplasmosis therapy, comprehensive neuropsychologic evaluations were completed. Compared to pre-alloHCT 14 months earlier, he displayed fine motor speed, dexterity and visuomotor
integration deficiencies. From 6 to 12 months following cerebral toxoplasmosis diagnosis, his course was complicated by a single 30 s partial seizure. A brain MRI at 12.5 months of therapy revealed residual hypointense right posterior temporal lesions, resolution of associated vasogenic edema, and no new lesions. A bone marrow evaluation at that time was remarkable for 30–40% cellularity, trilineage hematopoiesis with no dysplasia and 98% donor contribution. With reassuring MRI findings and a CD4 count > 400 cells/microliter, toxoplasmosis therapy was discontinued. A 4 month off-therapy brain MRI was stable with no new lesions and interval improvement in mild ventriculomegaly.

Discussion and conclusions

This case demonstrates the successful diagnosis and management of cerebral toxoplasmosis in a pediatric alloHCT patient. While seroprevalence of *Toxoplasma* exceeds 50% in some regions of the world, in both the United States and China (where this patient resided for
Toxoplasma is less common (~10%) [19, 20]. As such, surveillance for Toxoplasma is not routine prior to alloHCT at our institution and the serostatus of this patient was unknown. Risk factors for opportunistic reactivation included 4–6 months of preceding cytopenias and medication-associated immunosuppression from graft-versus-host disease prophylaxis, EBV treatment, and immune-mediated cytopenia therapies. Notably, routine prophylaxis against Pneumocystis jirovecii pneumonia with trimethoprim-sulfamethoxazole (TMP-SMX) until at least 1 year post-alloHCT and recovery of CD4+ lymphocyte count to >200 cell/mm³ additionally protects against Toxoplasma reactivation and infection. However, to avoid further myelosuppression from TMP-SMX in this patient with concurrent cytopenias, his Pneumocystis jirovecii pneumonia prophylaxis had been transitioned to pentamidine, an agent with no activity against Toxoplasma [21]. Without standard alloHCT population recommendations, toxoplasmosis treatment and duration was based on U.S. Department of Health and Human Services “Guidelines for prevention and treatment of opportunistic infection in adults and adolescents with HIV” (available at https://aidsinfo.nih.gov/2019).

PCR as a diagnostic tool for CSF samples of immunocompromised patients with suspected cerebral toxoplasmosis demonstrates wide variability in sensitivity [22–27]. Variations are attributable to laboratory variability, sample processing efficiency, and patient level differences in CSF protein and cellularity [27–29]. Regardless, CSF PCR remains less invasive than brain biopsy and provides rapid detection of parasite DNA. Moreover, CSF PCR expanded gene targets to detect Toxoplasma DNA [17, 28] are increasing accuracy of this methodology.

Microbial cfDNA sequencing technology provides a novel, non-invasive approach to the diagnosis of thousands of infectious organisms [30], including detection of opportunistic infection in immunocompromised hosts [31, 32]. However, cfDNA studies to date are limited by small sample sizes, lack of control groups, and cohort heterogeneity. Clinical indications for this novel approach remain to be clearly established. There is no published medical literature reporting the use of cfDNA to identify cerebral toxoplasmosis in an immunocompromised host. Prior to CSF PCR and plasma cfDNA sequencing results, the infectious differential diagnosis for our teenage alloHCT patient's brain lesions included a broad group of neurotropic viruses, fungi and parasites. In our case, cfDNA sequencing provided rapid evidence of cerebral toxoplasmosis despite negative blood serologies and ophthalmologic examination. Thus, cfDNA sequencing emerges as a useful adjunct to diagnosis for toxoplasmosis, particularly when tissue diagnosis is not feasible [33].

Of note, while Toxoplasma serologies are often useful to assess for prior or current immune response to infection, they are unreliable before adequate immune reconstitution after alloHCT. This particularly patient was profoundly immune suppressed from treatment of immune mediated cytopenias after alloHCT and had recently undergone plasmapheresis, further reducing the likelihood of production of circulating antibodies. Interpretation of positive serologies, had they been found, would also be challenging as he had recently received IVIG.

While mortality of cerebral toxoplasmosis in post-alloHCT patients is reported from 38 to 67% [34], little is known about long term sequelae in adult or pediatric survivors [14]. While promptly initiated on antibiotics, our patient only displayed definitive clinical improvement after a CD34+ stem cell boost restored the cellular immunity essential for Toxoplasma clearance. Clinical and radiographic signs of recovery persisted at follow-up 4 months following completion of maintenance antibiotics. Future studies exploring the incidence and outcomes of cerebral toxoplasmosis in pediatric post-alloHCT patients are needed.

Patient perspective
Fortunately during the time I was most ill as a patient I don’t really remember how I felt in the hospital and only have hazy memories. However, as I began to heal I do have memories of some nurses that especially helped me laugh during this time. I also remember enjoying integrative healing therapies in the form of music, aromatherapy, and massages. I am currently doing great, finishing my Freshman year of high school, playing in fantasy sports leagues, and also relieved to not be on clindamycin anymore.

Abbreviations
HCT: Allogeneic hematopoietic cell transplantation; CNS: Central nervous system; AIDS: Acquired immunodeficiency syndrome; CSF: Cerebrospinal fluid; PCR: Polymerase chain reaction; HLA: Human leukocyte antigen; EBV: Epstein-Barr virus; GCSF: Granulocyte colony stimulating factor; IVIG: Intravenous immunoglobulin; CT: Computed tomography; MRI: Magnetic resonance imaging; LP: Lumbar puncture; CMV: Cytomegalovirus; cfDNA: Cell free DNA; TMP-SMX: Trimethoprim-sulfamethoxazole.

Acknowledgements
We thank our patient and his family for their patience and perseverance during his complicated alloHCT and cerebral toxoplasmosis course.

Authors’ contributions
DB and CLE contributed to conception of the report and drafted the manuscript, DB, MLM, SIN, MRS, JY, and CLE all contributed to data analysis and critical revision of the manuscript, CLE interpreted data and created the figure. All authors read and approved the final manuscript.
Funding
This research was supported by the National Institutes of Health's National Center for Advancing Translational Sciences, Grants K23TR002492, funding research effort for CLE. The content is the sole responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health's National Center for Advancing Translational Sciences which had no role in study design, data collection, analysis, interpretation or writing of the manuscript.

Availability of data and materials
Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study. All relevant data are herein included.

Declarations
Ethics approval and consent to participate
The patient’s care was provided on a University of Minnesota IRB approved allogeneic hematopoietic cell transplant protocol.

Consent for publication
Verbal and written consent for publication of de-identified clinical data was obtained from the patient’s parent with the patient’s assent.

Competing interests
The authors report no competing interests.

Author details
1 Medical School, University of Minnesota, Minneapolis, MN, USA. 2 Department of Pediatrics, Division of Blood and Marrow Transplantation and Cellular Therapy, University of Minnesota, Minneapolis, MN, USA. 3 Department of Pediatrics, Division of Infectious Diseases, University of Minnesota, Minneapolis, MN, USA. 4 Department of Medicine, Division of Infectious Diseases and International Medicine, University of Minnesota, Minneapolis, MN, USA.

Received: 14 May 2021 Accepted: 1 September 2021
Published online: 10 September 2021

References
1. Martino R, Cordonnier C, European Group for B, Marrow Transplantation Infectious Diseases Working P. Toxoplasmosis following allogeneic hematopoietic stem cell transplantation. Bone Marrow Transpl. 2003;31(7):617–8.
2. Maschke M, Dietrich U, Prumbach M, Kastrup O, Turowski B, Schaefer UW, et al. Opportunistic CNS infection after bone marrow transplantation. Bone Marrow Transpl. 1999;23(11):1167–76.
3. Belanger F, Derouin F, Grangeot‑Keros L, Meyer L. Incidence and risk factors of toxoplasmosis in a cohort of human immunodeficiency virus-infected patients: 1988–1995. HEMOCO and SEROCO Study Groups. Clin Infect Dis. 1999;28(3):575–81.
4. Martino R, Bretagne S, Rovira M, Ullmann AJ, Maertens J, Held T, et al. Toxoplasmosis after hematopoietic stem transplantation. Report of a 5-year survey from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. Bone Marrow Transpl. 2000;25(10):1111–4.
5. Hirsch R, Burke BA, Kersey JH. Toxoplasmosis in bone marrow transplant recipients. J Pediatr. 1984;105(3):426–8.
6. Jurges E, Young Y, Etum M, Hollman RE, Vellodi A, Rogers TR, et al. Transmission of toxoplasmosis by bone marrow transplant associated with Campath-1G. Bone Marrow Transpl. 1992;9(1):65–6.
7. Slavin MA, Meyers JD, Remington JS, Hackman RC. Toxoplasma gondii infection in marrow transplant recipients: a 20 year experience. Bone Marrow Transpl. 1994;13(3):549–57.
8. Duzovalli O, Chorszcy MS, Chan KW. Hypoponatremia as the presenting feature of cerebral toxoplasmosis. Bone Marrow Transpl. 2005;35(12):1221–2.
9. Goebel WS, Conway JH, Faught P, Vakili ST, Haut PR. Disseminated toxoplasmosis resulting in graft failure in a cord blood stem cell transplant recipient. Pediatr Blood Cancer. 2007;48(2):222–6.
10. Megged O, Shalit I, Yaniv I, Stein J, Fisher S, Levy I. Breakthrough cerebral toxoplasmosis in a patient receiving atovaquone prophylaxis after a hematopoietic stem cell transplantation. Pediatr Transpl. 2008;12(8):902–5.
11. Fricker-Hidalgo H, Bulabois CE, Brenier-Pinchart MP, Hamidfar R, Garban F, Briot JP, et al. Diagnosis of toxoplasmosis after allogeneic stem cell transplantation: results of DNA detection and serological techniques. Clin Infect Dis. 2009;49(2):e9–15.
12. Caselli D, Andreoli E, Paolocci O, Savelli S, Guidi S, Peccie P, et al. Acute encephalopathy in the immune-compromised child: never forget toxoplasmosis. J Pediatr Hematol Oncol. 2012;34(5):383–6.
13. Bautista G, Ramos A, Fores R, Regidor C, Ruiz E, de Laiglesia A, et al. Toxoplasmosis in cord blood transplantation recipients. Transpl Infectious Dis: Off J Transpl Soc. 2012;14(5):496–501.
14. Keri K, Ehleit K, Bentrup A, Schiborr M, Keyvani K, Becker K, et al. Cerebral toxoplasmosis in an adolescent post allogeneic hematopoietic stem cell transplantation: successful outcome by antiprotozoal chemotherapy and CD4+ T-lymphocyte recovery. Transpl Infectious Dis: Off J Transpl Soc. 2015;17(1):119–24.
15. Decembrino N, Comelli A, Genco F, Vitullo A, Recupero S, Zecca M, et al. Toxoplasmosis disease in paediatric hematopoietic stem cell transplantation: do not forget it still exists. Bone Marrow Transpl. 2017;52(9):1326–9.
16. Czyzewski K, Fraczekiewicz J, Salamonowicz M, Pieczonka A, Zajac‑Spychala O, Zaucha‑Prazmo A, et al. Low serorelevance and low incidence of infection with Toxoplasma gondii (Nicolle et Manceaux, 1908) in pediatric hematopoietic cell transplantation donors and recipients: polish nationwide study. Folia Parasitol (Praha). 2019;66.
17. Zaucha‑Prazmo A, Samardakiewicz M, Dubiełt J, Kowalczyk JR. Cerebral toxoplasmosis after haematopoietic stem cell transplantation. Ann Agric Environ Med. 2017;24(2):237–9.
18. Hakkio E, Ozkaz HA, Karaman K, Gubuz Z. Analysis of cerebral toxoplasmosis in a series of 170 allogeneic hematopoietic stem cell transplant patients. Transpl Infectious Dis: Off J Transpl Soc. 2013;15(6):575–80.
19. Pappas G, Rousou N, Falagas ME. Toxoplasmosis snapshots: global status of Toxoplasma gondii seroprevalence and implications for pregnancy and congenital toxoplasmosis. Int J Parasitol. 2009;39(12):1385–94.
20. Wang T, Han Y, Pan Z, Wang H, Yuan M, Lin H. Serorelevance of Toxoplasma gondii infection in blood donors in mainland China: a systematic review and meta-analysis. Parasite. 2018;25:36.
21. Bocozza SA, Finkelstein DM, Spector SA, Frame P, Powdrick WG, He W, et al. A randomized trial of three antipneumycotists agents in patients with advanced human immunodeficiency virus infection. NIAID AIDS Clinical Trials Group. N Engl J Med. 1995;332(11):693–9.
22. Costa JM, Pautas C, Ernaud P, Foulet F, Cordonnier C, Bretagne S. Real-time PCR for diagnosis and follow-up of Toxoplasma reactivation after allogeneic stem cell transplantation using fluorescence resonance energy transfer hybridization probes. J Clin Microbiol. 2000;38(8):2929–32.
23. Buchbinder S, Blatz R, Rodloff AC. Comparison of real-time PCR detection methods for B1 and P30 genes of Toxoplasma gondii. Diagn Microbiol Infect Dis. 2003;45(4):269–71.
24. Hiel T, Reischl U, Lang P, Hebart H, Stark M, Kyme P, et al. Preliminary evaluation of one conventional nested and two real-time PCR assays for the detection of Toxoplasma gondii in immunocompromised patients. J Med Microbiol. 2004;53(Pt 7):629–32.
25. Edvinsson B, Lappalainen M, Evengard B. Toxoplasmosis ESfG. real-time PCR targeting a 529-bp repeat element for diagnosis of toxoplasmosis. Clin Microbiol Infect. 2006;12(2):131–6.
26. Brenier‑Pinchart MP, Morand‑Bui V, Fricker‑Hidalgo H, Euy‑V, Marlu R, Pelloux H. Adapting a conventional PCR assay for Toxoplasma gondii detection to real-time quantitative PCR including a competitive internal control. Parasite. 2007;14(2):149–54.
27. Angulo LM, Villar FC, Lima JE, Yamamoto AT, Bollela VR, Takayagami OU. Usefulness and limitations of polymerase chain reaction in the etiologic diagnosis of neurotoxoplasmosis in immunocompromised patients. J Neurol Sci. 2013;346(1–2):231–4.
28. Robert‑Gangneux F, Belaz S. Molecular diagnosis of toxoplasmosis in immunocompromised patients. Curr Opin Infect Dis. 2016;29(4):330–9.
29. Correa CC, Melo HR, Costa VM. Influence of neurotoxoplasmosis characteristics on real-time PCR sensitivity among AIDS patients in Brazil. Trans R Soc Trop Med Hyg. 2010;104(1):24–8.
30. Blauwkamp TA, Thair S, Rosen MJ, Blair L, Lindner MS, Vilfan ID, et al. Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease. Nat Microbiol. 2019;4(4):663–74.
31. Camargo JF, Ahmed AA, Lindner MS, Morris MI, Anjan S, Anderson AD, et al. Next-generation sequencing of microbial cell-free DNA for rapid noninvasive diagnosis of infectious diseases in immunocompromised hosts. F1000Res. 2019;8:1194.
32. Armstrong AE, Rossoff J, Hollemon D, Hong DK, Muller WJ, Chaudhury S. Cell-free DNA next-generation sequencing successfully detects infectious pathogens in pediatric oncology and hematopoietic stem cell transplant patients at risk for invasive fungal disease. Pediatr Blood Cancer. 2019;66(7):e27734.
33. Hong DK, Blauwkamp TA, Kertesz M, Bercovici S, Truong C, Banaei N. Liquid biopsy for infectious diseases: sequencing of cell-free plasma to detect pathogen DNA in patients with invasive fungal disease. Diagn Microbiol Infect Dis. 2018;92(3):210–3.
34. Gajurel K, Dhakal R, Montoya JG. Toxoplasma prophylaxis in haematopoietic cell transplant recipients: a review of the literature and recommendations. Curr Opin Infect Dis. 2015;28(4):283–92.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.