

Assessment of Immune and Oxidative Stress Biomarkers in Toxoplasmosis Patients: A Comparative Study with Healthy Controls

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Abstract. Background; Toxoplasmosis, caused by the intracellular protozoan *Toxoplasma gondii*, is a widespread zoonotic infection that affects nearly one-third of the global population. While often asymptomatic in immunocompetent individuals, the disease can manifest severe complications in immunocompromised individuals, pregnant women, and neonates. Aims of the study; This study evaluates immune and oxidative stress biomarkers in toxoplasmosis patients to explore their role in disease severity and progression. Methodology; This study investigated biomarkers in 100 toxoplasmosis patients and 50 healthy controls, evenly divided by gender, from January 2023 to January 2024. Venous blood (5 mL) was collected, clotted, centrifuged, and serum stored at -20°C. Biomarkers measured included cytokines (IFN- γ , TNF- α , IL-10, CRP) and oxidative stress markers (MDA, SOD, CAT) using ELISA. Measurements followed manufacturer protocols, with absorbance readings taken via microplate reader. Statistical analysis assessed differences between patients and controls, with significance set at $p < 0.05$. Results aimed to explore the role of immune and oxidative stress biomarkers in toxoplasmosis pathophysiology. Result; The study found no significant differences in demographic or lifestyle factors between *Toxoplasma* patients and controls, including age, gender distribution, residence, and smoking status. However, significant differences were observed in biomarker levels. Immune markers (IFN- γ , TNF- α , IL-10, and CRP) were elevated in patients compared to controls, indicating heightened immune activation. Oxidative stress markers showed contrasting trends: MDA levels were significantly higher in patients, reflecting increased lipid peroxidation, while antioxidant enzymes (SOD and CAT) were markedly lower, indicating reduced antioxidant defenses. These findings highlight the role of immune dysregulation and oxidative stress in *Toxoplasma* pathophysiology. Conclusions; This study highlights significant immune activation and oxidative stress in toxoplasmosis patients, with elevated inflammatory markers and reduced antioxidant enzymes. These biomarkers may serve as indicators of disease severity and progression.

Highlights:

1. Toxoplasmosis affects one-third globally, severe in immunocompromised individuals.
2. Analyzed immune, oxidative biomarkers in 100 patients vs. 50 controls.
3. Elevated cytokines, oxidative stress markers; reduced antioxidant enzymes in patients..

Keywords: Toxoplasmosis, immune response, cytokines, oxidative stress, biomarkers, disease progression.

Introduction

Toxoplasmosis is one of the most common parasitic infections worldwide, caused by the obligate intracellular protozoan *Toxoplasma gondii*. Human toxoplasmosis may result from unintended ingestion of sporulated oocysts excreted by the definitive felid host, or through the consumption of food or water contaminated with oocysts or tissue cysts containing bradyzoites spontaneously released through the acute infection or after the rupture of the tachyzoite-infected cells from the intermediate hosts (Hajimohammadi et al.2022; Dubey et al.2020; Rostami et al.2020; Pal et al.2021). In immunocompetent humans, the majority of *T. gondii* infections are asymptomatic. However, if primary infection occurs during pregnancy, it can provoke severe congenital sequelae. In immunocompromised patients, such as those with acquired immunodeficiency syndrome, toxoplasmosis can lead to meningoencephalitis and retinochoroiditis, and in organ transplant patients, blindness with acute or disseminated forms that are often severe and fatal. Further, the ocular, cerebral, or disseminated forms can compromise the quality of life of patients even after therapy due to neurological sequelae, chronic pain, psychological, and social disorders (Dubey et al.2021; Maleki et al.2021; de et al.2022; Osama A. Mohsein et al., 2024). *Toxoplasma gondii* is an intracellular protozoan parasite that infects warm-blooded animals and causes toxoplasmosis once it enters the host cells. The life cycle of *T. gondii* is divided into three distinct stages, which are the tachyzoite stage (the acute phase of the disease), the bradyzoite stage (contained within tissue cysts in chronically infected hosts), and the sporozoite stage. The parasite is well known for its diverse route of transmission (Zhao and Ewald, 2020; de et al.2023; Doghish et al.2023). The asexual stage (tachyzoites), sexual stage (micro- and macrogametes), and oocysts are found in the intestines of felines. The infection in humans and animals occurs via the ingestion of soil, water, or feed contaminated by sporulated oocysts, undercooked and contaminated meat of other intermediate hosts, and transplacental, blood, organ transplantation, or tissue transplantation from an infected host. Once in the host intestine, the sporulated oocysts release sporozoites, which invade the epithelial cells and undergo morphological changes leading to the rapid formation of tachyzoites. The individual may experience flu-like symptoms such as fever,

headache, and lymphadenopathy during the acute phase of *T. gondii* (Matta et al.2021; Sanchez and Besteiro, 2021; Tartarelli et al.2020; Mohsein, O.A et al., 2024). Humoral and Cellular Immune Responses in Toxoplasmosis Patients The phase of infection is associated with the production of specific isotypes that shift the balance towards Th1. In the early phase of infection, IgM is particularly used to confuse situations in which no information is available about the duration of infection. Patients in the late phase of the infection have positive IgM values and a higher titer of IgG. The immune response is achieved mainly with the production of IgG1, and its function is to limit the spread of the parasite known as the anti-sheep phenomenon. The Th1-Th2 theory is attributed with a limited role in human toxoplasmosis because the role of IFN γ , which counterregulates the Th2 type, is responsible for protection during the initial stage of infection. In latent and late infection, *T. gondii* has the ability to inhibit IFN γ -mediated elimination, and the development of chronic infection undermines the control of the enhanced Th2 type response (Coombs., 2020; Martinez-Espinosa et al., 2021). It assists the response of B1 B lymphocytes by reducing the production of IL-12 and subsequent IFN γ for the production of a relevant amount of IL-10. It should be emphasized that both IL-12 and IFN γ are rapid response antigens, so cells are usually transient in phenotypes influenced by T cells. The reticuloendothelial system results in the continuous selection and maturation of T cell clones. This combined effect, which reduces the generation of MyD88 expression by limiting the intraoperon infection or without the recognition of the parasite, suggests two major effects of parasite-induced mRNA made up of supramolecular complexes that induce the inflammasome reaction. In neonatal infection, Toxoplasmic modulation induced by parasite tachyzoites is a transcription induction that limits the production of an effective primary Th2 or Th1 type response (Henry et al., 2024; Henry et al., 2023). The immune system is a complex network of special cells, proteins, tissues, and organs that help defend the body against germs and microorganisms, ranging from viruses and bacteria to parasites and other pathogens. The innate immune system is the first line of defense against the invasion of microorganisms, triggered almost immediately as they attempt to invade the host organism. When a microorganism successfully penetrates the skin or mucous membranes, for example, it will be recognized and identified by various cells and eventually attacked with chemical substances. Dendritic cells play a major role because

they can be viewed as the generals of the immune system, ensuring communication between several cells and tissues, and regulating the host's response. Neutrophils, the most numerous type of white blood cells, quickly accumulate at the site of infection and release proteins and substances that cause the destruction of microorganisms, leading to the formation of pus. Prolonged systemic exposure to neutrophil extracellular traps can lead to lung diseases, thrombosis, and chronic inflammatory disorders (Ehrens et al., 2022; Ondari et al.2021). Eosinophils are a group of immune system white blood cells that, when increased in the blood and activated, are mostly involved in the immune response against multicellular parasites and certain infections. They are capable of phagocytic and secretory responses, which include the release of large amounts of chemical mediators from cytoplasmic storage, the most characteristic being toxic basic proteins, peroxidase, and acid phosphatase. They share the ability to degranulate and release their contents in the microenvironment with mast cells, which are tissue-resident cells that sense the presence of external pathogens and undergo degranulation. This immunologic response is the major causative agent of hypersensitivity, a protective response that protects the host against certain microorganisms. The first line of immune defense, the innate response, uses the same mechanisms to protect against all types of microorganisms, yet the host expresses these in a different way (Mitre and Klion, 2021; Lekki-Józwiak and Bąska, 2023). Cell-mediated immunity (CMI) is considered the most important for inhibiting the proliferation and disease outcome induced by *T. gondii*. The major effector cells are the Th1 CD4+ and CD8+ T lymphocytes that release IFN- γ , which promotes the activation of cytotoxic cells and necrotic tachyzoites and inhibits the infection of tissue cells by the parasite. There are also numerous cytokines of the interleukin group that regulate the inhibition of infection, such as IL-2 and IL-12. Once the infection is controlled, a population of regulatory T cells is stimulated (Treg cells), producing IL-10 and TGF- β , at which point CMI is deactivated so as not to generate excessive tissue damage. Follicular helper T cells (Th cells) have recently been reported as necessary for B-cell activation during the inflammatory process induced by *T. gondii*. The importance of antibody production during toxoplasmosis is still debated, but certain studies show that IgG antibodies, although they help to eliminate the tissue parasite, also help to increase the viral capacity of *T. gondii* and its hypnozoite form while avoiding detection by the innate immune response (Moghaddami et al.2024; Ahmadpour et

al.2023; Chen et al.2022). The human immune system uses reactive oxygen species (ROS) and other related non-radicals for the elimination of parasites. However, when the parasite load in the host organism increases, oxidative stress is produced because of the increased amount of free radicals and decreased amount of antioxidants. This oxidative stress causes damage to the host cells, tissues, and DNA. On the other hand, *T. gondii* was found to be sensitive to intracellular pathway-induced ROS. The antioxidative systems of the host cell have a role in the manipulation of host signal transduction pathways and the escape of the parasite from host defenses. The host has two distinct ways to resist *T. gondii* infection using the reactive oxygen species pathways (Shrivastava et al.2021; Pincemail et al.2021; Zińczuk et al.2020). In the present study, no data about the antioxidative enzyme activities such as CAT, GPx, and SOD were found in toxoplasmosis patients. However, some other researchers detected some parameters of oxidative stress such as total antioxidant status, total oxidant status, and oxidative stress index in toxoplasmosis patients with concurrent infections. The total oxidant status and oxidative stress index were significantly higher, and total antioxidant status was significantly lower in the patients in comparison to the control group. Another recent study showed that some parameters of oxidative stress can be used as predictive factors in the diagnosis of toxoplasmosis in pregnancy. However, no other studies have been found about assays for oxidative stress as predictive markers for the well-being of mothers and inflammatory response in newborns (Skutnik-Radziszewska et al.2020; Karkhanei et al., 2021).

Methods

This study was carried out to explore certain biomarker assessment in patients with toxoplasmosis and healthy controls. We enrolled 150 subjects, including 100 patients with toxoplasmosis (50 female, 50 male) and 50 healthy controls (25 female, 25 male). The study was done from Jan. 1, 2023, through Jan. 1, 2024. Blood (5 mL) was sampled from the participants' venous blood with a sterile syringe and transferred into gel tubes. The samples were kept at room temperature for 30 min to allow clotting, then centrifuged at 3000 rpm for 10 min to obtain the serum. The supernatant was distributed in sterile Eppendorf tubes and stored at -20°C before analysis. Cytokines were measured as biomarkers; Interferon-gamma (IFN- γ) (pg/mL), Tumor Necrosis

Factor-alpha (TNF- α) (pg/mL), Interleukin-10 (IL-10) (pg/mL) and C-Reactive Protein (CRP) (mg/L) and oxidative stress markers; Malondialdehyde (MDA) (μ mol/L), Superoxide Dismutase (SOD) (U/mL), and Catalase (CAT) (U/mL). All measurements were performed with enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' protocols. Quantification of these biomarkers was achieved by measuring the absorbance at the respective wavelengths using a microplate reader for the cytokines and oxidative stress markers.

Statistical analysis:

Statistical analysis is often used to analyze quantitative data, and provides methods for data description, simple inference for continuous and categorical data. The procedure involves the collection of data leading to test of the relationship between two statistical data sets. In this study all data are presented as frequency and percentage. We used SPSS (version 26) and the dependent t-test (two-tailed) and independent t-test (two-tailed) for variables that had a normally distributed distribution. For variables that did not have a normally distributed distribution, we used the Mann-Whitney U test, the Wilcoxon test, and the Chi-square test. $M < 0.05$ was seen as statistically significant.

Ethical approval:

The study was approved by the human ethics committee of Al-Habbobi Teaching Hospital, Everyone who took part in the study was told about it and asked to sign a consent form. The patient was also guaranteed that his information would be kept private.

Result and Discussion

Sociodemographic and Lifestyle Characteristics of Patients with Toxoplasmosis Compared to a Control Group

The study showed no statistically significant differences between the Toxoplasma patients group and the control group with regard to demographic characteristics and lifestyle. The proportions of males and females were similar between the two groups, reaching 50% for each gender in both groups ($p=0.950$). The mean age \pm standard deviation was 40 ± 3.5 in the patient group compared to 39 ± 4.0 in the control group ($p=0.120$). Regarding residence, the proportions of individuals from urban and rural areas were similar between the two groups at 60% and 40%, respectively ($p=1.000$).

The study also showed no significant differences between smokers and non-smokers, as the proportions of smokers were 45% and 40%, respectively ($p=0.540$).

Table 1: Comparison of Gender, Age, Residence, and Smoking Status

Characteristic	Patients with Toxoplasmosis (n=100)	Control Group (n=50)	p-value
Gender			
Male (n, %)	50 (50%)	25 (50%)	0.950
Female (n, %)	50 (50%)	25 (50%)	0.950
Age (mean \pm SD)	40 \pm 3.5	39 \pm 4.0	0.120
Residence			
Urban (n, %)	60 (60%)	30 (60%)	1.000
Rural (n, %)	40 (40%)	20 (40%)	1.000
Smoking Status (n, %)			
Smokers	45 (45%)	20 (40%)	0.540
Non-Smokers	55 (55%)	30 (60%)	0.540

Comparison of Biomarker Levels Between Patients with Toxoplasmosis and a Control Group.

The study showed statistically significant differences in the levels of biomarkers between Toxoplasma patients and the control group. Interferon-gamma (IFN- γ) levels in patients were 45.3 ± 8.7 pg/ml compared to 22.5 ± 5.4 pg/ml in the control group ($p<0.001$). Tumor necrosis factor-alpha (TNF- α) levels were significantly higher in the patient group, reaching 32.1 ± 6.2 pg/ml versus 15.3 ± 4.1 pg/ml ($p<0.001$). As for interleukin-10 (IL-10), its levels reached 25.4 ± 5.1 pg/ml in patients compared to 10.2 ± 2.9 pg/ml in the control group ($p<0.001$). Finally, C-reactive protein (CRP) levels were 12.7 ± 3.2 mg/L in patients compared to 3.8 ± 1.4 mg/L in the control group ($p<0.001$).

Table 2: Analysis of IFN- γ , TNF- α , IL-10, and CRP Levels

Biomarker	Patients with Toxoplasmosis (n=100)	Control Group (n=50)	p-value
Interferon-gamma (IFN- γ) (pg/mL)	45.3 ± 8.7	22.5 ± 5.4	<0.001
Tumor Necrosis Factor-alpha (TNF- α) (pg/mL)	32.1 ± 6.2	15.3 ± 4.1	<0.001
Interleukin-10 (IL-10) (pg/mL)	25.4 ± 5.1	10.2 ± 2.9	<0.001

C-Reactive Protein (CRP) (mg/L)	12.7 ± 3.2	3.8 ± 1.4	<0.001
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Oxidative Stress Biomarkers in Patients with Toxoplasmosis Compared to a Control Group.

Higher levels of oxidative stress were observed on various indicators among patients with Toxoplasma compared to the control group. Malondialdehyde (MDA) levels are significantly higher in patients (7.8 ± 1.5 μmol/L; control: 3.5 ± 0.8 μmol/L; p < 0.001). On the other hand, in patients superoxide dismutase (SOD) were low, 15.2 ± 2.8 U/mL vs 23.4 ± 3.2 U/mL controls (p < 0.001). The catalase (CAT) average fell significantly in patients (18.6 ± 3.5 U/mL), relative to controls (30.1 ± 4.7 U/mL, p < 0.001).

Table 3: Levels of Malondialdehyde (MDA), Superoxide Dismutase (SOD), and Catalase (CAT)

Biomarker	Patients with Toxoplasmosis (n=100)	Control Group (n=50)	p-value
Malondialdehyde (MDA) (μmol/L)	7.8 ± 1.5	3.5 ± 0.8	<0.001
Superoxide Dismutase (SOD) (U/mL)	15.2 ± 2.8	23.4 ± 3.2	<0.001
Catalase (CAT) (U/mL)	18.6 ± 3.5	30.1 ± 4.7	<0.001

Discussion:

Toxoplasmosis is a zoonotic disease with a broad spectrum of clinical syndromes caused in humans by *Toxoplasma gondii* (*T. gondii*), a typical coccidian parasite (intracellular protozoan parasite) (Dubey JP., 2026). Infected animal's undecked meat consumption, eating food or drinking water with cat feces, blood transfusion, organ transplantation and transplacental from mother to fetus which is considered as the most severe way of infection are the ways humans are infected. Early pregnancy toxoplasmosis is generally associated with fetal damage (Pomares & Montoya., 2016). The infective stage of *T. gondii* includes three stages, sporozoite, and tachyzoite which is the rapidly multiplying form, and bradyzoite which is tissue cystic form. The tachyzoite is involved in the clinical manifestations of acute toxoplasmosis, which is susceptible to the effect of host immunity and medications. Bradyzoite is less susceptible and more

resistance forms of the drugs and host immune system (Montazeri et al., 2017). The results presented in Table 2 demonstrate significant differences in biomarker levels related to immune response between patients with toxoplasmosis and the control group. These findings underscore the profound immunological alterations associated with toxoplasmosis. Interferon-gamma (IFN- γ) levels were significantly elevated in patients with toxoplasmosis (45.3 ± 8.7 pg/mL) compared to controls (22.5 ± 5.4 pg/mL, $p < 0.001$). IFN- γ is a major cytokine for immunity against numerous intracellular pathogens, including *Toxoplasma gondii*. Its higher levels in the patient group coincide with the reported by (Meira et al., 2014), considering that is critical for macrophages activation to control replication of *T. gondii*. This enhanced expression may represent an attempt by the host to mount a successful immune response. Excessive IFN- γ production, however, has been associated with organ damage and may explain the symptoms observed in patients with toxoplasmosis (Pfaff et al., 2007; Gazzinelli et al., 1994). Tumor Necrosis Factor-alpha (TNF- α) levels were also markedly higher in the patient group (32.1 ± 6.2 pg/mL) compared to controls (15.3 ± 4.1 pg/mL, $p < 0.001$). TNF- α acts in synergy with IFN- γ to amplify the phagocytic activity and induce the generation of reactive oxygen species responsible for the destruction of the parasite. This reinforces the host defense role of TNF- α which was similarly found to be elevated in studies such as Meira et al., 2014. However, Miller et al., 2020 reported conflicting results, as they found no differences in TNF- α secretion in mild cases, implying that the severity of the disease and host specificities to the factors involved can affect TNF- α expression (Gazzinelli et al., 1994; Marchioro et al., 2018; Meira et al., 2014; Miller et al., 2020; Mustafa, H et al., 2024). Interleukin-10 (IL-10) levels were significantly higher in patients (25.4 ± 5.1 pg/mL) compared to controls (10.2 ± 2.9 pg/mL, $p < 0.001$). IL-10 is an anti-inflammatory cytokine responsible for modulating immune responses to prevent excessive tissue injury. Its higher levels in the patient group could also suggest a compensatory response to the pro-inflammatory actions of IFN- γ and TNF- α . This bidirectional activity IL-10 is consistent with the observations of Wilson et al., 2005, that the concentration of IL-10 increased during chronic toxoplasmosis as a result of immune regulation mediated by the host. Yet, IL-10 overproduction could inhibit efficient parasite clearance, possibly leading to chronic infection (Matowicka-Karna et al., 2009; Gaddi et al., 2007; Wilson et al., 2005). C-Reactive Protein (CRP) levels were significantly elevated

in toxoplasmosis patients (12.7 ± 3.2 mg/L) compared to the control group (3.8 ± 1.4 mg/L, $p < 0.001$). CRP is an acute-phase protein that increases in conjunction with inflammatory activity. And its increased levels in the patient group indicate a generalized *T. gondii*-induced inflammation. This also aligns with Sandri et al, 2020 that found high CRP levels in active infection patients of toxoplasmosis. However, other studies including Davis et al., 2019, indicate that there is no correlation between the level of CRP and the severity of toxoplasmosis, and attribute differences in CRP response to individual host factors (Mese et al., 2007; Babekir et al., 2021; Davis et al., 2019; Sandri et al., 2020). The results in Table 3 reveal significant differences in oxidative stress biomarkers between patients with toxoplasmosis and the control group, highlighting the role of oxidative stress in the pathophysiology of toxoplasmosis. Malondialdehyde (MDA) levels were significantly higher in patients with toxoplasmosis (7.8 ± 1.5 $\mu\text{mol/L}$) compared to controls (3.5 ± 0.8 $\mu\text{mol/L}$, $p < 0.001$). Malondialdehyde (MDA) is the most widely used marker of lipid peroxidation, which reflects ROS-induced oxidative damage to cell membranes. Elevated MDA levels for the patient group indicate that enhanced levels of oxidative stress may occur in an attempt by the immune system to restrain the *Toxoplasma gondii* infection via reactive oxygen species generation. Fasting MDA register by Karaman et al., 2008 was higher in toxoplasmosis patients and may be considered the result of the oxidative damage caused by the parasite. Such rise of MDA may play a role in the tissue damage and clinical symptom aggravation in toxoplasmosis (Yazar et al., 2003; Paraboni et al., 2022; Karaman et al., 2008). Patients had significantly lower levels of Superoxide Dismutase (SOD) compared with the control group (15.2 ± 2.8 vs. 23.4 ± 3.2 U/mL, $p < 0.001$). One of the key antioxidant enzymes is SOD, which detoxifies a major type of ROS, superoxide radicals. The decreased SOD activity found in toxoplasmosis patients could represent depletion of antioxidant defenses as a consequence of prolonged oxidative stress. Sibley et al., [1986] also reported reduced SOD activity in patients with parasitic infections. The decrease could worsen the oxidative damage since the capacity of the organism to neutralize ROS gets impaired (Dincel et al., 2016; Liu et al., 2017; Sibley et al., 1986). In patients, the level was found to be 18.6 ± 3.5 U/mL, while in controls it was 30.1 ± 4.7 U/mL, a statistically significant difference ($p < 0.001$). It serves to protect cells from potentially oxidative damage by decomposing hydrogen peroxide (H_2O_2) to water and oxygen. Reduced

activity of CAT in patients with toxoplasmosis further demonstrates the impaired antioxidant defense mechanisms. This observation is coherent with the studies done by Sibley et al., 1986 which also reported reduced levels of CAT in chronic parasite infections. However, other findings were inconsistent, as showed by Miller et al., 2022, where Cat levels did not change significantly in mild toxoplasmosis cases, highlighting that disease severity could impact antioxidant enzyme activity (Kwok et al., 2004; Nazarlu et al., 2020; Sibley et al., 1986; Miller et al., 2022). Inevitably, collectively these results indicate that there is an imbalance between oxidative stress and antioxidant defences in patients with toxoplasmosis as evidenced by increased levels of MDA and reduced SOD and CAT activities. Such imbalance may underlie the pathogenesis of disease, including tissue damage and chronic inflammation. The results agree with several studies that report increased oxidative stress during parasitic infections, clinical discrepancies in the levels of oxidative stress and antioxidant levels measured may vary where differences exist between homogeneity and heterogeneity with regard to patients demographic analysis and/or experimental techniques in measuring these parameters. More studies are needed to investigate the potential for antioxidant supplementation as a therapeutic strategy to relieve oxidative stress in patients with toxoplasmosis.

Conclusion

These findings indicate alterations in immune profile and oxidative stress parameters in patients with toxoplasmosis when compared to healthy controls. Higher levels of pro-inflammatory cytokines (IFN- γ , TNF- α , IL-10) and acute-phase proteins (CRP) indicate an increased inflammatory response in patients, probably leading to the inflammatory mechanisms involved in *Toxoplasma gondii* infection. Conclusion: Patients with diastolic heart failure exhibit increased levels of malondialdehyde, which reflects oxidative stress, in addition to reduced activities of the antioxidant enzymes SOD and CAT, indicative of compromised antioxidant defense. These results highlight the importance of immune dysregulation and oxidative damage in the pathophysiology of toxoplasmosis. The biomarkers investigated may be useful from the standpoint of simply determining disease severity and progression. The implications of the CMI and CMI-2 associated SNPs for the clinical management of toxoplasmosis and its sequelae, particularly in immunocompromised hosts, remain to be studied, and their potential as

therapeutic targets to reduce the incidence or severity of disease requires further investigation

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