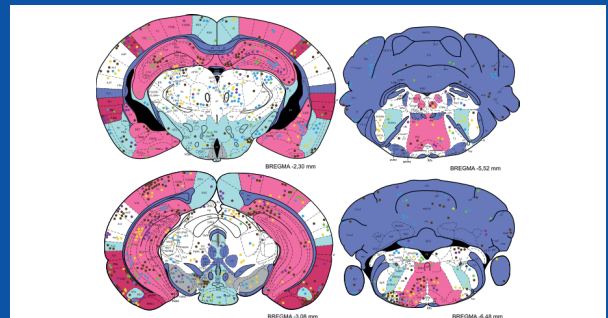
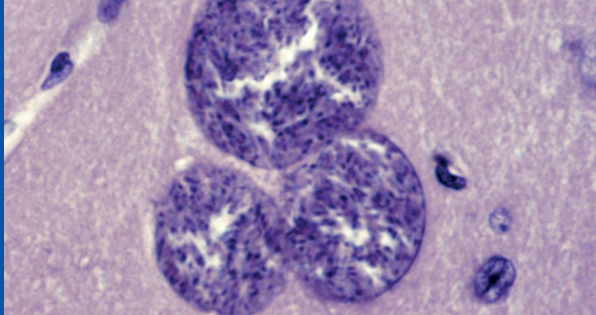




Special Issue

Effects of Latent Toxoplasmosis: Three Decades of Studies

Guest Editors:

Jaroslav Flegr

Faculty of Science, Charles University, Prague, Czech Republic

Ivan Fiala

Institute of Parasitology, Biology Centre of the Czech Academy of Sciences
České Budějovice, Czech Republic

Flegr J., Ullmann J., Španiel F., Toman J., Hůla M., Šebánková B., Petrusová J., Novotný P., Včelák J., Kaňková Š. 2026: Does latent *Toxoplasma* infection mimic the immune profile of schizophrenia? Sex-specific cytokine and brain-marker alterations suggest partial overlap. Special Issue on Toxoplasmosis. Folia Parasitol. 73: 006. Doi: 10.14411/fp.2026.006

Special Issue on Toxoplasmosis

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Does latent *Toxoplasma* infection mimic the immune profile of schizophrenia? Sex-specific cytokine and brain-marker alterations suggest partial overlap

Jaroslav Flegr¹ , Jana Ullmann¹ , Filip Španiel^{2,3} , Jan Toman¹, Martin Hůla¹ , Blanka Šebánková¹ ,
Jana Petrusová^{4,5} , Petr Novotný^{4,5} , Josef Včelák⁶  and Šárka Kaňková¹ 

¹ Laboratory of Evolutionary Biology, Department of Philosophy and History of Sciences, Faculty of Science, Charles University, Prague, Czech Republic;

² National Institute of Mental Health, Klecany, Czech Republic;

³ Third Faculty of Medicine, Charles University, Prague, Czech Republic;

⁴ ESSENCE LINE s.r.o., Praha, Czech Republic;

⁵ BIOCEV, First Faculty of Medicine, Charles University, Vestec, Czech Republic;

⁶ Department of Molecular Endocrinology, Institute of Endocrinology, Prague, Czech Republic

Abstract: Schizophrenia often features low-grade neuroinflammation. Because latent toxoplasmosis (LT) is more prevalent among individuals with schizophrenia, we tested whether LT yields a biomarker pattern resembling that reported in schizophrenia. We quantified 15 cytokines and 15 blood markers of brain injury in 65 LT-positive individuals and 103 matched LT-negative controls using multiplex immunoassays. Multivariate effects of infection, age, sex, and their interaction were assessed by MANCOVA and PERMANOVA. Effects on individual biomarkers were tested by partial Kendall correlation (controlling for age and sex). Differences in the internal correlation structure were evaluated with Mantel tests on dissimilarity matrices derived from partial correlations. LT was associated with higher KLK6, S100B, and TDP-43, and lower MIF; several other markers showed nonsignificant but sizable trends. Cytokines showed reduced IFN- γ , IL-1 β , and MCP-1, and elevated IL-13 and IL-17 in the infected group. Sex-stratified analyses suggested stronger effects on brain-injury markers in women and on cytokines in men. Correlation structure also diverged: infected individuals exhibited more negative links between brain-injury markers and cytokines, whereas controls showed predominantly positive associations (Mantel $r = 0.461$, $p = 0.043$). The LT profile overlapped with schizophrenia in elevated KLK6 and S100B and, in men, reduced GDNF, but contrasted for MIF and for the overall cytokine pattern (no consistent IL-6/TNF- α elevation). LT entails neuroinflammatory and neuroimmune alterations that only partly recapitulate schizophrenia; the biomarker pattern and interrelationships differ, arguing against LT as the main driver of schizophrenia-related neuroinflammation.

Keywords: Chronic toxoplasmosis; neuroimmune communication; HPA axis dysregulation; inflammatory signaling; glial activation markers; brain injury biomarkers; sex-specific immune modulation; aging.

Toxoplasma gondii (Nicolle et Manceaux, 1908), a parasitic protozoan estimated to infect approximately one-third of the global population, is the causative agent of several clinically significant disease manifestations (Tenter et al. 2000, Pappas et al. 2009). The most clinically serious form is often considered to be congenital toxoplasmosis. It arises when a pregnant woman acquires the infection and transmits it to the developing foetus. Depending on the trimester in which the infection occurs, the consequences may range from miscarriage to varying degrees of foetal damage. Postnatally, infected infants may suffer from sensory and cognitive impairments, as well as severe neuro-

logical manifestations such as encephalitis, chorioretinitis, hydrocephalus, microcephaly, and intracerebral calcification (Jones et al. 2001a).

Among other severe manifestations observed in specific cases is ocular toxoplasmosis caused by postnatal infection with virulent strains – particularly prevalent in regions such as South America – which may lead to blindness (Miyagaki et al. 2024). In immunocompromised individuals, such as AIDS patients or immunosuppressed organ transplant recipients, cerebral toxoplasmosis may develop after infection. This condition requires urgent medical intervention and is otherwise often fatal (Aerts et al. 2024).

Address for correspondence: Jaroslav Flegr, Laboratory of Evolutionary Biology, Department of Philosophy and History of Sciences, Faculty of Science, Charles University, Prague, Czech Republic. E-mail: flegr@cesnet.cz; ORCID: [0000-0002-0822-0126](https://orcid.org/0000-0002-0822-0126)

In immunocompetent individuals, acute toxoplasmosis usually progresses over the course of several weeks to a month and is often accompanied by cervical lymphadenopathy and a range of nonspecific symptoms like fever, joint pain, headache and tiredness (Jones et al. 2001b). The infection then spontaneously transitions into a chronic phase. During this phase, the parasite persists throughout the host's lifetime as slowly replicating bradyzoites within so-called tissue cysts in various organs, including the brain. This chronic stage of infection is mostly considered asymptomatic (Markell et al. 1999, Roberts et al. 2000) and is commonly referred to as latent toxoplasmosis.

However, the term 'latent' is somewhat misleading, as infected individuals exhibit a range of more or less specific symptoms. Among the best-documented are behavioural changes, alterations in psychological profiles, and usually – but not always – impaired cognitive performance (for review, see Latifi and Flegr 2025). Over the past 30 years, these symptoms have primarily been investigated in the context of the manipulation hypothesis, which proposes that the parasite modifies host behaviour to enhance its own transmission. In the past decade, however, it has become increasingly evident that many of the observed changes are unlikely to result from parasite-induced manipulation. Instead, they may represent side effects of deteriorated health in infected individuals (Flegr et al. 2024).

Since the mid-20th century, it has been known that *Toxoplasma*-infected individuals show increased prevalence of certain psychiatric disorders, especially schizophrenia. Initially anecdotal, these observations have since been supported by targeted epidemiological studies and, eventually, by several meta-analyses (Torrey et al. 2007, 2012, Sutherland et al. 2015, Contopoulos-Ioannidis et al. 2022). Moreover, it has become clear that individuals with latent toxoplasmosis not only have an increased risk of mental health disorders but also a higher likelihood of physical health problems and exhibit numerous symptoms indicative of poorer general health.

A large cross-sectional study involving 333 *Toxoplasma*-infected and 1,486 uninfected individuals found significantly worse outcomes on 28 out of 29 health-related measures in the infected group (Flegr and Escudero 2016). These individuals reported more frequent use of medications, hospitalisations, and medical complaints, with the strongest associations seen for musculoskeletal, neurological, immune, and metabolic disorders.

Similarly, an ecological study using WHO data from 88 countries found that *Toxoplasma* seroprevalence was significantly associated with disease burden in 23 of 128 disease categories and with mortality in 12 categories (Flegr et al. 2014a). For example, robust correlations were observed for cardiovascular disease, perinatal conditions, epilepsy, and certain endocrine and psychiatric disorders. In European countries, seroprevalence accounted for up to 23% of the variance in overall disease burden. In addition to these statistical findings, the study included an extensive literature review on the health effects of *Toxoplasma* infection. These and numerous other results demonstrate that

latent toxoplasmosis may not be truly asymptomatic, as it has both direct and especially indirect effects on the health of infected individuals. Many of these effects may be related to changes in immune system function.

The existence of pronounced immunological changes associated with both acute and chronic *Toxoplasma* infection has been demonstrated in numerous studies. In acutely infected women, Matowicka-Karna et al. (2009) reported elevated levels of IL-6, IL-5, and a fivefold increase in IL-10, with no significant changes in IL-12 or TNF- α , suggesting a shift toward a Th2-dominated, anti-inflammatory immune profile.

Mohyuddin et al. (2023) observed that in older adults, higher anti-*Toxoplasma* antibody titres were associated with increased kynurenine-to-tryptophan ratio and elevated soluble TNF receptor II, indicating chronic immune activation. In pregnant women with chronic *Toxoplasma* infection, elevated levels of IL-1 β , IL-2, FGF-basic, and PDGF-BB were found (Ullmann et al. 2025). Another study in pregnant women showed increased TNF, IL-6, and IL-10 levels, but only among those with comorbid depression (Lopes et al. 2025).

Flegr and Stríž (2011) showed that chronically infected men had lower counts of NK cells, monocytes, and leukocytes, whereas infected women had elevated counts of these cells. Both sexes showed a reduction in B-cell numbers. Interestingly, the same study found lower *Toxoplasma* seroprevalence among men with certain immune-mediated diseases, potentially reflecting immunosuppressive effects of the parasite that mitigate immunopathology.

Also, *in vitro* and *in vivo* studies in mice have demonstrated that TNF- α , IFN- γ , and IL-12 help control parasite replication, while IL-10 may subsequently contribute to the downregulation of inflammatory mediators, including IL-2 and nitric oxide, depending on cellular context (Roberts et al. 1995, Kaňková et al. 2010, Doherty et al. 2024). Consistent with these immune dynamics, Egorov et al. (2021) reported elevated CRP and sVCAM-1 levels in infected individuals.

Altogether, the findings suggest that chronically infected individuals experience mild, chronic systemic inflammation, which may also extend into brain tissue. In support of this hypothesis, histological studies have revealed inflammatory lesions in the brain. Notably, these lesions are not co-localised with tissue cysts but are instead located near blood vessels, suggesting that the observed neuroinflammation is likely driven by immune responses rather than directly cyst-induced damage (Berenreiterova et al. 2011).

At the same time, the inflammatory response during chronic *Toxoplasma* infection appears to be tightly regulated in a way that may limit – though not necessarily prevent – immune-mediated damage to brain structure or function. Infected hosts exhibit strong pro-inflammatory responses (e.g., IFN- γ , TNF- α), but also elevated levels of some anti-inflammatory cytokines. For example, Cekana-viciute et al. (2014) demonstrated that TGF- β signalling is activated in astrocytes during toxoplasmic encephalitis and that blocking this pathway leads to greater immune cell infiltration and neuronal injury. This suggests the possible

existence of an intrinsic check on inflammation within the CNS, potentially preventing excessive neuroinflammation.

It remains unclear whether this immune downregulation primarily serves the parasite – by maintaining a dormant yet transmissible bradyzoite population – or represents a host adaptation aimed to limit neuroinflammation-associated damage. One interpretation is that the parasite actively induces or exploits anti-inflammatory pathways to promote its own survival. The latent bradyzoite stage of *T. gondii* encysted in neurons is thought to be an immune-evasion strategy – a way for the parasite to persist in a dormant, transmissible form while avoiding complete immune clearance (Eberhard et al. 2025). Most likely, immune system dynamics in infected individuals results from a complex host-parasite interplay.

However, this delicate equilibrium may come at a cost. Even when overt pathology is avoided, chronic neuroinflammation and subtle immune dysregulation could affect brain function in more insidious ways. One striking example is the robust association between *Toxoplasma* infection and schizophrenia. Earlier meta-analyses reported total odds ratios as high as 2.72 (Torrey et al. 2007, 2012), a considerably greater effect than that of any genetic factor identified to date. However, more recent studies typically report lower effect sizes (Sutterland et al. 2015, Contopoulos-Ioannidis et al. 2022).

This most likely reflects the fact that, given the current emphasis on patient rights, individuals with the most severe symptoms of schizophrenia are frequently excluded from scientific studies due to their unwillingness or inability to provide informed consent (Flegr et al. 2014b). Yet, such severe cases are strongly over-represented among individuals infected with *Toxoplasma* (Wang et al. 2006, Amminger et al. 2007, Horáček et al. 2012, Holub et al. 2013). On top of that, *T. gondii* infection is also associated with broader structural and functional brain alterations, further implicating its role in the neurobiology and pathophysiology of schizophrenia (Horáček et al. 2012, Andreou et al. 2025).

There is a plausible mechanistic explanation for the observed association between *Toxoplasma* infection and schizophrenia. Many symptoms of schizophrenia have been linked to increased dopamine concentrations in specific brain regions. As early as 2003, it was proposed that certain behavioural effects of latent toxoplasmosis – particularly personality trait alterations – may also be related to elevated dopamine levels, and that this could represent the mechanistic link between *Toxoplasma* infection and schizophrenia (Flegr et al. 2003). This hypothesis was supported several years later by the discovery that *Toxoplasma* possesses two unique genes encoding enzymes homologous to tyrosine hydroxylase, which may affect host dopamine metabolism (Gaskell et al. 2009). A follow-up study showed high dopamine concentrations in tissue cysts and surrounding neural tissue (Prandovszky et al. 2011).

An alternative to dopamine-centred models may lie in convergent inflammatory mechanisms. Schizophrenia is associated with marked immune system abnormalities (Meyer et al. 2011, Aricioglu et al. 2016), many of which resemble those induced by *T. gondii*. Increasing evidence suggests

that innate immunity-driven inflammation represents a final common pathway in schizophrenia (Jacomb et al. 2018, Özdin and Böke 2019, Llorca-Bofi et al. 2024). *Toxoplasma* infection has been shown to activate the innate immune system, promoting the release of proinflammatory cytokines such as IL-1 β , IL-6, TNF- α , and IFN- γ (Sher et al. 2017).

Because acute psychosis, a symptom associated with schizophrenia, is similarly characterised by immune activation, recent or reactivated toxoplasmosis may contribute to its pathophysiology. Supporting this link, a meta-analysis of 16 studies found significantly higher odds of anti-*T. gondii* IgM seropositivity in acutely psychotic patients, indicating an association between recent *T. gondii* infection and acute psychosis (Monroe et al. 2015).

Any potential link between *T. gondii* and schizophrenia should be considered within the broader context of immune-inflammatory mechanisms implicated in the disorder. Meta-analytic study of Miller et al. (2011) reported elevated levels of multiple pro- and anti-inflammatory cytokines, namely IL-6, IL-12, TNF- α , IL-1 β , IFN- γ and TGF- β , in the blood and cerebrospinal fluid of patients with schizophrenia. Consistent with this immune activation, patients often have increased absolute and relative counts of total white blood cells, particularly monocytes (Meyer et al. 2011). Biomarkers of astrocyte activation, such as protein S100B, provide further evidence of neuroinflammation.

The presence of activated microglia in certain brain regions (notably the hippocampus) has been demonstrated using multiple techniques, including *postmortem* analyses and neuroimaging (PET scans) (Fineberg and Ellman 2013). Further evidence comes from flow cytometric analyses of blood samples from patients not previously treated with antipsychotic medications (i.e., drug-naïve individuals) experiencing acute psychosis, which have shown elevated CD4⁺ T cells and B cells, reduced CD8⁺ T cells, and increased plasma IL-6 (Zhuo et al. 2023). It should be emphasised, however, that these immunological findings are not uniform. A growing body of review literature points to considerable variability in immune profiles across studies, with cytokine and immune cell alterations showing dependence on clinical phase, treatment exposure, and possibly other yet-unidentified confounding variables (Miller et al. 2011, Ayyildiz et al. 2019, Reale et al. 2021).

Patients with schizophrenia also exhibit alterations in several biomarkers of neural injury, many of which are closely linked to neuroinflammatory responses or tissue repair processes. Elevated levels have been reported for Fibroblast Growth Factor 21 (FGF-21), a hepatokine that regulates glucose and lipid metabolism (Qing et al. 2015), Kallikrein-6 (KLK6), a brain-expressed serine protease involved in amyloid metabolism and upregulated during neuroinflammation, Macrophage Migration Inhibitory Factor (MIF), a pleiotropic cytokine with roles in immunoregulation and neuroprotection (Yu et al. 2022), and S100 calcium-binding protein B (S100B), an astrocyte-derived molecule widely regarded as a marker of glial activation, neural damage, and neuroinflammation (Aleksovska et al. 2014, Schümberg et al. 2016).

Conversely, patients with schizophrenia typically exhibit reduced levels of Brain-Derived Neurotrophic Factor (BDNF), a key molecule involved in neurogenesis, synaptic plasticity, and neuronal survival (Gören 2016, Libersona et al. 2024), Ciliary Neurotrophic Factor (CNTF), a neurotrophic factor with protective effects on neurons and retinal cells (Liu et al. 2020), Glial Cell Line-Derived Neurotrophic Factor (GDNF), essential for the survival and function of dopaminergic neurons (Davarinejad et al. 2025), and Ubiquitin C-Terminal Hydrolase L1 (UCHL1), a neuron-specific protein involved in removing damaged proteins (Demirel et al. 2017).

The overall immune profile in schizophrenia is characterised by immune activation – manifested by elevated pro-inflammatory cytokines, activated microglia, and abnormal lymphocyte responses – and is paralleled by structural and functional brain alterations. It is important to note, however, that only 30–40% of schizophrenia patients, depending on the markers used and the patient subgroup examined, exhibit clear signs of inflammation (Murphy et al. 2021). This raises the possibility that the inflammatory subgroup may overlap substantially with individuals infected with *Toxoplasma* (Příplatová et al. 2014).

Comparing the immune alterations observed in schizophrenia with those in *Toxoplasma*-infected individuals reveals that patients with schizophrenia exhibit a range of immune system changes that significantly overlap with those seen in latent or chronic *T. gondii* infection. Both conditions are marked by a pro-inflammatory milieu (e.g., elevated IL-6, IL-1 β , TNF- α), Th1-type immune activation (IFN- γ -driven), and microglia-associated neuroinflammation. While immune abnormalities in schizophrenia have been studied in great detail and documented in numerous systematic studies, focused research on the immune effects of chronic *Toxoplasma* infection in humans remains in its early stages. Furthermore, whereas schizophrenia has been linked to various molecular markers of brain injury, comparable data on corresponding brain injury biomarkers in *Toxoplasma*-infected humans are limited and fragmentary.

The aim of the present study was to test the hypothesis that previously reported differences in cytokine levels and brain injury biomarkers between individuals with and without schizophrenia might be partly explained by the higher prevalence of latent *T. gondii* infection in patients with schizophrenia. To this end, we measured and compared the concentrations of cytokines and brain injury markers in the blood of 65 individuals chronically infected with *T. gondii* and 103 uninfected controls. We then examined whether the differences observed between infected and uninfected participants were consistent with those reported in the literature for individuals with and without schizophrenia. Finding such a concordance would support the hypothesis that some of the reported biomarker differences associated with schizophrenia could result from a higher prevalence of latent toxoplasmosis in this patient population.

MATERIALS AND METHODS

Participants

Most participants were former students of the Faculty of Science, Charles University, who had previously taken part in studies investigating the effects of *Toxoplasma gondii* infection on human behaviour. We primarily invited individuals with a documented history of *Toxoplasma* seropositivity, as determined by earlier serological testing. The final sample was then supplemented with seronegative individuals to reach the target sample size, ensuring that age and gender distributions were matched between the two groups. As a result, the proportion of infected individuals in the sample does not reflect the seroprevalence in the general population. For further details on the participants, see Section Descriptive statistics.

Potential participants were contacted via email and invited to the Faculty of Science at Charles University. After signing informed consent, they completed a battery of performance-based tests as part of a separate longitudinal study. A trained physician then collected venous blood samples for serological analyses. Serum samples were obtained after centrifugation for 2 min at 3000 g at 21°C. Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific Inc., Waltham, MA, USA) were added to the serum samples for biomarker and cytokine analyses. All serum samples were immediately stored at -20°C until analysed. Participants received financial compensation of 500 CZK (20 EUR) for their involvement.

A total of 168 participants without acute health problems took part in the study. Serum samples from 162 participants (six samples were excluded due to test kit capacity, specifically those for whom no optimal *Toxoplasma*-positive counterpart of the same sex and similar age could be identified) were tested for brain injury biomarkers, but one participant was excluded retrospectively after showing extreme concentrations (up to 100-fold higher than average) of multiple brain damage biomarkers, suggesting a possible early-stage Alzheimer's disease diagnosis. Serum samples from 165 participants were tested for cytokine levels (three samples were excluded due to insufficient serum volume).

The study was approved by the Institutional Review Board of the Faculty of Science, Charles University (approval number 2021/4), and conducted in accordance with applicable regulations and ethical guidelines.

Immunoassays

Concentrations of the following 15 human blood biomarkers associated with brain injury, stress, or neuroplasticity were measured using the commercially available ProcartaPlex™ Human Neuroscience Panel, 18-plex (Cat. No. EPX180-15837-901, Thermo Fisher Scientific): Amyloid- β (A β), Brain-Derived Neurotrophic Factor (BDNF), Ciliary Neurotrophic Factor (CNTF), Fibroblast Growth Factor 21 (FGF-21), Glial Cell Line-Derived Neurotrophic Factor (GDNF), Kallikrein-6 (KLK6), Macrophage Migration Inhibitory Factor (MIF), Neural Cell Adhesion Molecule 1 (NCAM-1), Neurogranin (NRGN), S100 Calcium-Binding Protein B (S100B), TAR DNA-Binding Protein 43 (TDP-43), Total Tau (Tau, Total), Phosphorylated Tau (Tau, pT181), Ubiquitin C-Terminal Hydrolase L1 (UCHL1), and Chitinase-3-like protein 1 (YKL-40).

This multiplex immunoassay is based on bead-based Luminex xMAP technology, which allows simultaneous quantification of multiple analytes in small-volume samples. Three markers from the original 18-plex panel were excluded from further analysis. One (Glial Fibrillary Acidic Protein, GFAP) was omitted due to the manufacturer's inability to supply the necessary reagent, and two others, Neurofilament heavy chain (NF-H) and Nerve Growth Factor β (NGF- β), exhibited near-zero concentrations across almost all samples.

Similarly, concentrations of the following 15 human cytokines and chemokines were measured using the commercially available Bio-Plex Pro Human Cytokine Panel 17-Plex (Cat. No. M5000031YV, Bio-Rad): Granulocyte Colony-Stimulating Factor (G-CSF), Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Interferon-gamma (IFN- γ), Interleukin-1 β (IL-1 β), Interleukin-5 (IL-5), Interleukin-6 (IL-6), Interleukin-7 (IL-7), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Interleukin-12 (IL-12), Interleukin-13 (IL-13), Interleukin-17 (IL-17), Monocyte Chemoattractant Protein-1 (MCP-1), Macrophage Inflammatory Protein-1 β (MIP-1 β), and Tumor Necrosis Factor- α (TNF- α). Two cytokines from this 17-plex panel (IL-2, IL-4) were excluded from analysis because their concentrations were consistently undetectable (near-zero) across almost all samples.

The presence of anti-*Toxoplasma gondii* IgG antibodies was assayed using an ELISA IgG kit (TestLine Clinical Diagnostics, Brno, Czech Republic). Examinations were carried out at the National Reference Laboratory for Toxoplasmosis at the National Institute of Public Health in Prague.

All assays were performed according to the manufacturers' instructions. Detection limits, intra-assay, and inter-assay coefficients of variation for all kits are provided in the manufacturers' documentation.

Statistical analysis

All statistical analyses were performed using R version 4.4.2. Descriptive statistics (means, standard deviations, counts, and percentages) were calculated for the study cohort characteristics. Differences in age between males and females, and between infected and non-infected individuals, were assessed using independent sample *t*-tests. Differences in *Toxoplasma* prevalence between males and females were assessed using Pearson's Chi-square test.

A global Multivariate Analysis of Covariance (MANCOVA) was initially performed to assess the effects of age, *Toxoplasma* infection status (infected vs. non-infected), sex, and infection-sex interaction on the concentrations of blood biomarkers of brain damage and cytokines. This analysis was conducted on a subset of 146 individuals for whom complete data across all specified biomarkers and cytokines were available. Parametric MANCOVA and its assumptions (e.g., multivariate normality, homogeneity of variance-covariance matrices) were evaluated using functions from base R and the dplyr and MVN packages.

Due to observed violations of parametric MANCOVA assumptions, a robust permutational Multivariate Analysis of Variance (PERMANOVA) was subsequently performed as a non-parametric alternative to re-evaluate the multivariate effects of age, *Toxoplasma* infection status (TOXO), sex, and the interaction between *Toxoplasma* infection and sex on the concentrations of the 30 blood biomarkers of brain damage and cytokines.

This analysis was conducted on the same 146-participant subset as above. Prior to performing the PERMANOVA, all biomarker and cytokine concentrations were rank-transformed (using base R rank() function with ties.method = 'average') to enhance robustness against non-normal distributions and outliers. Subsequently, Euclidean distances were calculated on these rank-transformed data, generating a dissimilarity matrix that captured the overall multivariate differences between individuals.

The PERMANOVA was carried out using the adonis2 function from the vegan package. The model specified was distance_matrix_rank ~ age + SEX + TOXO:SEX + TOXO. Statistical significance was assessed using 9,999 permutations, with effects tested by sequential (Type I) sums of squares (by = "terms"), meaning the contribution of each term was evaluated after accounting for the variance explained by preceding terms in the model formula.

Given the violation of parametric assumptions, the influence of *Toxoplasma* infection on individual blood biomarkers of brain damage and cytokines was further analysed using non-parametric partial Kendall correlation tests, a rank-based method robust to non-normality and outliers. This approach allowed us to utilise data from the full cohort of 168 individuals, controlling for the confounding effects of sex and age. These partial Kendall correlations were computed using Explor v.1 script employing the tcltk2, ppcor, corpcor, pcaPP, and psych packages.

To assess whether the internal cross-correlation structure of 30 blood biomarkers of brain damage and cytokine concentrations differed between *Toxoplasma*-positive and *Toxoplasma*-negative individuals, we constructed two dissimilarity matrices (R_{tox}: *Toxoplasma*-infected and R_{ctrl}: *Toxoplasma*-free) by calculating one minus the partial Spearman's correlation for all pairwise biomarker combinations within each respective group. The observed Mantel correlation coefficient (*r*), assessing the similarity of the partial correlation-derived dissimilarity matrices (R_{tox} and R_{ctrl}) between groups, was then calculated. To test the hypothesis that these matrices differed more than expected by random chance, we performed a permutation test. The TOXO status was randomly permuted 1,000 times, generating a null distribution of Mantel *r* values (*r*_i). The one-tailed *p*-value was determined as the proportion of permuted *r*_i values less than or equal to the observed *r*: $p = (\text{sum}(r_i \leq r_{\text{observed}}) + 1) / (1000 + 1)$. All data are available at figshare.com/s/21941788d5c0591caf69.

RESULTS

Descriptive statistics

The study cohort comprised 168 individuals (97 females and 71 males). The mean age of males (31.0 ± 8.1 years, mean $\pm$ S.D.) did not significantly differ from that of females (31.4 ± 7.7 years), as indicated by a *t*-test ($t_{166} = 0.33$, $p = 0.740$). Among males, 24 (33.8%) were *Toxoplasma*-infected, while among females, 41 (42.3%) were infected. However, this difference in prevalence was not statistically significant (Pearson Chi-square: 1.238, *df* = 1, $p = 0.266$). Age distributions did not differ significantly between infected and uninfected individuals (31.4 ± 9.7 years vs. 31.2 ± 6.6 years, respectively; $t_{166} = 0.136$, $p = 0.892$).

Data on the concentration of blood biomarkers of brain damage were available for 161 individuals, and cytokine concentration data were available for 165 individuals. Ta-

Table 1. Concentrations of blood biomarkers of brain damage and cytokines by infection with *Toxoplasma gondii* and their association with sex and age.

Variable	Non-infected		Infected		Women		Men		Sex	Age
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Tau	Tau
Amyloid β	1.1	1.51	1.43	2.38	1.23	1.77	1.23	2.06	-0.029	-0.088
BDNF	669.23	531.62	608.09	453.62	678.32	523.67	603.26	473.76	-0.075	0.122
CNTF	6.19	23.35	8.78	23.26	8.8	26.18	5.09	18.82	-0.105	0.104
FGF-21	6.35	23.71	11.72	32.58	10.45	30.29	5.77	23.31	-0.155	0.037
GDNF	25.5	103.55	45.81	135.16	39.89	132.84	24.79	91.99	-0.066	0.14
Kallikrein-6	678.28	251.42	771.24	301.76	668.77	260.45	772.99	283.46	0.18	-0.122
MIF	89.73	43.84	78.18	38.81	84.24	38.29	86.64	47.11	0.024	0.213
NCAM-1	40174.4	15995.26	36023.78	15110.23	36016.4	16087.04	41903.53	14741.63	0.139	-0.02
Neurogranin	412.86	551.04	489.46	575.85	339.93	435.38	575.52	669.77	0.157	-0.043
S100B	0.64	1.63	0.93	1.51	0.6	1.19	0.95	1.98	0.036	-0.15
TDP-43	149.25	504.88	301.17	749.92	247	679.93	156.74	514.09	-0.087	0.045
Tau (Total)	6.05	4.67	6.5	6.17	5.65	4.44	6.97	6.17	0.083	0.034
Tau (pT181)	0.09	0.47	0.26	0.98	0.13	0.58	0.19	0.85	0.007	0.019
UCHL1	53.74	347.14	35.16	219.43	82.42	401.26	0.0	0.0	-0.156	0.032
YKL-40	6320.31	3512.14	5525.86	2656.33	6350.13	3045.78	5577.89	3415.27	-0.131	0.154
G-CSF	1.59	8.2	0.49	3.88	1.09	6.37	1.31	7.65	0.001	0.121
GM-CSF	0.95	7.37	0.07	0.45	0.07	0.47	1.4	9.06	0.042	-0.057
IFN-γ	10.14	7.16	13.11	38.11	12.46	30.59	9.54	7.76	-0.068	0.12
IL-1β	0.22	0.5	0.09	0.27	0.15	0.37	0.2	0.51	0.065	0.087
IL-5	21.69	124.57	5.88	12.26	8.27	15.15	26.42	152.9	0.166	0.103
IL-6	3.12	22.46	0.63	2.15	0.73	3.55	4.25	27.39	-0.096	0.008
IL-7	2.81	6.3	4.46	12.63	3.82	10.38	2.87	7.23	0.017	0.035
IL-8	15.62	60.65	26.97	77.53	16.5	55.13	24.71	82.17	-0.04	0.055
IL-10	2.22	2	2.11	1.33	2.3	1.48	2.01	2.13	0.117	0.21
IL-12	11.63	25.7	8.4	9.69	9.4	9.34	11.87	31.11	-0.164	-0.12
IL-13	1.14	2.23	1.44	1.57	1.26	1.53	1.24	2.55	-0.173	0.086
IL-17	0.91	1.33	1.83	3.69	1.22	1.64	1.3	3.42	-0.106	-0.118
MCP-1	27.18	19.15	20.97	15.69	22.97	15.26	27.52	21.41	-0.093	0.022
MIP-1β	38.84	56.72	36.54	39.4	33.41	39.07	44.49	63.67	0.072	0.156
TNF-α	33.66	107.14	23.34	21.27	35.01	108.24	22.32	32.63	0.078	0.054

All concentrations are in pg/ml. The last two columns present the partial Kendall Tau correlation coefficients, controlled for either age or sex, between the biomolecule concentrations and sex (coded 0 for women, 1 for men) or age, respectively. A positive Tau value indicates that men or older individuals generally exhibit higher concentrations of the biomolecule compared to women or younger individuals. Significant Tau values are bolded.

Table 2. Effects of age, infection with *Toxoplasma gondii*, and sex on blood biomarkers of brain damage and cytokines.

Effect	Test	Parametric MANCOVA					PER-MANOVA	
		Value	F	Effect	Error	p	Partial eta-squared	p permut.
Intercept	Wilks	0.401	6.427	29	125	0.000	0.599	NA
Age	Wilks	0.669	2.132	29	125	0.002	0.331	0.0011
Infection	Wilks	0.727	1.616	29	125	0.037	0.273	0.0178
Sex	Wilks	0.698	1.867	29	125	0.010	0.302	0.0002
Infection × sex	Wilks	0.799	1.085	29	125	0.366	0.201	0.9003

The main part of the table displays MANCOVA results; the last column shows p-values computed with robust permutation MANCOVA.

ble 1 presents the mean concentrations, stratified by infection status and sex, along with the correlations of these concentrations with age.

Effects of *Toxoplasma* infection on concentrations of blood biomarkers of brain damage and cytokines

A global Multivariate Analysis of Covariance (MANCOVA) was performed on a subset of 146 individuals for whom data on all blood biomarkers of brain damage and all cytokines were available (Table 2).

While the initial parametric MANCOVA indicated significant effects of age, infection, and sex, but not infection × sex interaction, it was noted that the data violated

most assumptions for the MANCOVA test. Therefore, a non-parametric alternative – permutational multivariate analysis of variance (PERMANOVA) – was used to assess the same multivariate effects. PERMANOVA also revealed significant effects for *Toxoplasma* infection, age, and sex (Table 2, last column).

Therefore, in the subsequent phase of the study, the influence of infection on individual blood biomarkers of brain damage and cytokines was analysed separately using non-parametric partial Kendall correlation tests, controlling for the effects of sex and age. This approach allowed us to revert to the original cohort of 168 individuals. Tables 3 and 4 present the results of these analyses for the entire cohort, as well as separately for males and females.

Of the 15 blood biomarkers of brain damage studied, the concentrations of four demonstrated significant correlations with *Toxoplasma* infection even after correction for multiple comparisons: Kallikrein-6, S100B, and TDP-43 correlated positively, while MIF correlated negatively. Several other markers exhibited non-significant but sizable trends: positive for Tau (pT181); negative for GDNF, NCAM-1, and YKL-40. The effects of *Toxoplasma* infection (as indicated by partial Kendall Tau coefficients) were predominantly stronger in women than in men (e.g., Kallikrein-6: 0.217 vs. 0.070; MIF: -0.152 vs. -0.064; Neurogranin: 0.178 vs. -0.063; S100B: 0.211 vs. 0.070).

Table 3. Effects of infection with *Toxoplasma gondii* on concentrations of blood biomarkers of brain damage.

	All		Men		Women	
	Tau	p	Tau	p	Tau	p
Amyloid β	0.002	0.969	-0.096	0.246	0.078	0.277
BDNF	-0.033	0.540	-0.084	0.310	0.011	0.874
CNTF	0.082	0.125	0.035	0.675	0.110	0.123
FGF-21	0.079	0.138	0.106	0.198	0.067	0.352
GDNF	-0.095	0.074	-0.191	0.020	-0.023	0.744
Kallikrein-6	0.137	0.010	0.070	0.392	0.217	0.002
MIF	-0.123	0.021	-0.064	0.440	-0.152	0.034
NCAM-1	-0.098	0.067	-0.071	0.388	-0.130	0.069
Neurogranin	0.077	0.151	-0.063	0.443	0.178	0.013
S100B	0.149	0.005	0.070	0.392	0.211	0.003
TDP-43	0.114	0.032	0.144	0.079	0.098	0.171
Tau (Total)	0.007	0.900	0.084	0.309	-0.037	0.601
Tau (pT181)	0.088	0.098	0.088	0.286	0.092	0.199
UCHL1	-0.005	0.920	NA	NA	-0.006	0.936
YKL-40	-0.096	0.073	-0.084	0.310	-0.108	0.132

Table shows partial Tau, reflecting effect size and direction, and significance of effects computed with partial Kendall correlation controlled for sex and age. Positive Tau indicates that infected subjects have higher concentrations of a marker. Effects (Tau) significant after correction for multiple (15) tests with Benjamini-Hochberg procedure are bolded. The False Discovery Rate was set to 0.2, implying that 20% of significant tests (4 of 20 observed significant Tau values) are likely false positive results.

An exception was GDNF, where the effect of *Toxoplasma* infection was not evident in women, but a significant decrease was observed in men (Tau = -0.191). The concentration of Amyloid β , a peptide associated with Alzheimer's pathology, showed no significant association with *Toxoplasma* infection overall. However, it showed a negative trend in men (Tau = -0.096) and a positive trend (Tau = 0.078) in women.

Table 4 illustrates the effects of *Toxoplasma* infection on cytokine concentrations. Again, it was found that 5 out of the 15 studied cytokines correlated with *Toxoplasma* infection: IFN- γ , IL-1 β , and MCP-1 correlated negatively, while IL-13 and IL-17 correlated positively. For cytokines, the effects and trends were generally stronger in men than in women, with the exception of IL-1 β , where the negative effect was approximately three times stronger in women (Tau = -0.164) than in men (Tau = -0.056, non-significant). Notably, GM-CSF concentration correlated negatively with *Toxoplasma* infection in men (Tau = -0.163) but positively in women (Tau = 0.123).

Cross-correlation between concentration of markers and cytokines

In the next part of the study, we analysed the correlation between the concentrations of individual biomolecules (Table 5). Generally, blood biomarkers of brain damage exhibited positive correlations with each other, with correlation strengths typically being high. Out of 105 pairwise comparisons, 57 showed significant positive correlations. Conversely, all 13 negative correlations were non-significant ($p > 0.077$). Similarly, positive and strong correlations were observed among the concentrations of individual cytokines. Of 105 cytokine pairs, 89 correlated positively, with 70 being significant, and only 16 correlated negatively, with only three negative correlations (IL-8 : IL-10, IL-8 : IL-13, and IL-8 : IL-17) reaching statistical significance.

Table 4. Effects of infection with *Toxoplasma gondii* on concentrations of cytokines.

	All		Men		Women	
	Tau	p	Tau	p	Tau	p
G-CSF	-0.059	0.263	-0.110	0.187	-0.025	0.715
GM-CSF	-0.002	0.964	-0.163	0.051	0.123	0.075
IFN- γ	-0.111	0.035	-0.149	0.074	-0.086	0.216
IL-1 β	-0.114	0.030	-0.056	0.502	-0.164	0.018
IL-5	-0.077	0.147	-0.063	0.449	-0.078	0.260
IL-6	0.074	0.159	0.027	0.745	0.110	0.112
IL-7	-0.013	0.803	-0.027	0.747	-0.004	0.957
IL-8	0.000	1.000	0.027	0.748	-0.014	0.836
IL-10	0.016	0.757	-0.031	0.709	0.057	0.409
IL-12	-0.020	0.700	-0.027	0.750	-0.027	0.692
IL-13	0.153	0.004	0.200	0.017	0.130	0.060
IL-17	0.132	0.012	0.178	0.033	0.106	0.124
MCP-1	-0.127	0.016	-0.228	0.006	-0.070	0.311
MIP-1 β	0.040	0.450	0.058	0.491	0.023	0.743
TNF alfa	0.022	0.681	-0.044	0.596	0.053	0.441

Table shows partial Tau, reflecting effect size and direction, and significance of effects computed with partial Kendall correlation controlled for sex and age. Positive Tau indicates that infected subjects have higher concentrations of a marker. Effects (Tau) significant after correction for multiple (15) tests with Benjamini-Hochberg procedure are bolded. The False Discovery Rate was set to 0.2, implying that 20% of significant tests (3–4 of 18 significant Tau values) are likely false positive results.

Among the 225 possible blood biomarker-cytokine pairs, 48 showed significant positive correlations and 20 exhibited significant negative correlations; only MIF and Total Tau showed no statistically significant correlations with any of the cytokines tested. Notably, similar correlation matrices calculated separately for *Toxoplasma*-infected and non-infected participants revealed substantial structural differences. For infected individuals, among the 225 blood biomarkers of brain damage-cytokine correlations, 8 significant positive correlations and 31 significant negative correlations were observed (with a similar number approaching significance). In contrast, among non-infected individuals, there were 63 significant positive correlations and only 6 negative correlations (Table 6).

To investigate whether the pattern of pairwise correlations among the studied biomolecules differed between *Toxoplasma*-positive and *Toxoplasma*-negative individuals, a Mantel test, a permutation-based significance test, was performed by comparing the two corresponding dissimilarity matrices. The observed Mantel correlation coefficient (r) between the *Toxoplasma*-positive and *Toxoplasma*-negative cross-correlation matrices was 0.461. A permutation test, where individuals were randomly assigned to either group 1,000 times, revealed that only 0.043 (or 4.3%) of the randomly generated Mantel r values were lower than the observed r of 0.461. This indicates a statistically significant difference in the cross-correlation matrix structures between *Toxoplasma*-positive and *Toxoplasma*-negative individuals ($p = 0.043$). The relatively low observed Mantel r value of 0.461, compared to the distribution of r values obtained from permuted datasets, suggests that the internal relational patterns of the biomolecules are indeed significantly less similar between the actual *Toxoplasma*-defined groups than would be expected by chance.

Table 5. Matrix of pairwise correlations between individual blood biomarkers of brain damage and cytokines.

	Amyloid- β	BDNF	CNTF	FGF-21	GDNF	Kallikrein-6	MIF	NCAM-1	Neurogranin	SI100B	TDP-43	Tau (Total)	Tau (pT181)	UCHL1	YKL-40	G-CSF	GM-CSF	IFN- γ	IL-16	IL-5	IL-6	IL-7	IL-8	IL-10	IL-12	IL-13	IL-17	MCP-1	MIP-16	TNF- α
Amyloid- β	NA	0.970	0.000	0.008	0.004	0.000	0.607	0.904	0.000	0.001	0.000	0.178	0.000	0.000	0.290	0.360	0.021	0.896	0.180	0.307	0.118	0.087	0.164	0.281	0.453	0.369	0.508	0.929	0.582	0.378
BDNF	-0.002	NA	0.717	0.133	0.025	0.266	0.000	0.008	0.967	0.167	0.340	0.017	0.031	0.176	0.000	0.410	0.297	0.621	0.037	0.668	0.829	0.204	0.243	0.980	0.881	0.314	0.756	0.049	0.706	0.605
CNTF	0.405	0.019	NA	0.000	0.000	0.817	0.772	0.710	0.254	0.000	0.000	0.598	0.000	0.000	0.441	0.078	0.888	0.330	0.251	0.240	0.101	0.347	0.437	0.338	0.000	0.000	0.004	0.515	0.484	0.060
FGF-21	0.136	0.077	0.289	NA	0.000	0.253	0.752	0.562	0.183	0.000	0.002	0.935	0.000	0.000	0.138	0.000	0.003	0.828	0.015	0.921	0.003	0.921	0.328	0.029	0.075	0.000	0.000	0.490	0.173	0.009
GDNF	0.149	0.115	0.415	0.203	NA	0.265	0.000	0.000	0.344	0.478	0.000	0.319	0.000	0.000	0.001	0.007	0.177	0.003	0.952	0.031	0.013	0.273	0.737	0.098	0.001	0.018	0.050	0.002	0.986	0.076
Kallikrein-6	0.226	0.057	-0.012	0.059	-0.057	NA	0.319	0.086	0.000	0.000	0.027	0.010	0.015	0.013	0.055	0.024	0.331	0.272	0.438	0.001	0.084	0.291	0.596	0.039	0.001	0.089	0.942	0.069	0.009	0.011
MIF	0.026	0.180	0.015	0.016	0.231	0.051	NA	0.010	0.597	0.292	0.002	0.000	0.013	0.021	0.000	0.892	0.440	0.355	0.326	0.745	0.494	0.703	0.644	0.596	0.868	0.151	0.730	0.102	0.165	0.762
NCAM-1	-0.006	0.135	0.019	-0.030	0.200	0.088	0.131	NA	0.788	0.118	0.959	0.002	0.163	0.759	0.001	0.792	0.791	0.505	0.809	0.703	0.814	0.766	0.315	0.051	0.870	0.198	0.047	0.005	0.066	0.480
Neurogranin	0.315	-0.002	0.058	0.068	-0.048	0.220	0.027	0.014	NA	0.000	0.001	0.076	0.047	0.045	0.133	0.533	0.321	0.035	0.142	0.030	0.130	0.120	0.890	0.001	0.001	0.678	0.095	0.005	0.716	0.122
SI100B	0.178	0.071	0.179	0.269	0.036	0.132	0.054	0.080	0.249	NA	0.000	0.010	0.000	0.000	0.185	0.346	0.120	0.087	0.218	0.070	0.104	0.331	0.341	0.620	0.091	0.004	0.000	0.020	0.035	0.291
TDP-43	0.213	-0.049	0.464	0.160	0.346	0.113	0.155	0.003	0.012	0.170	NA	0.382	0.000	0.000	0.335	0.251	0.045	0.130	0.016	0.061	0.006	0.047	0.654	0.025	0.042	0.000	0.000	0.017	0.000	0.390
Tau (Total)	0.069	0.122	-0.027	-0.004	0.051	0.132	0.323	0.159	0.091	0.132	0.045	NA	0.934	0.764	0.000	0.415	0.690	0.774	0.725	0.838	0.869	0.284	0.942	0.442	0.278	0.515	0.357	0.617	0.959	0.556
Tau (pT181)	0.320	0.110	0.438	0.331	0.429	0.124	0.127	0.072	0.102	0.203	0.361	0.004	NA	0.000	0.123	0.000	0.000	0.014	0.402	0.119	0.001	0.571	0.032	0.629	0.174	0.559	0.004	0.863	0.024	0.350
UCHL1	0.197	0.069	0.257	0.431	0.313	0.128	0.118	-0.016	0.102	0.240	0.327	-0.015	0.524	NA	0.101	0.000	0.000	0.006	0.219	0.668	0.011	0.028	0.001	0.065	0.252	0.228	0.002	0.023	0.000	0.628
YKL-40	0.054	0.264	0.039	0.076	0.178	0.098	0.272	0.171	-0.077	0.068	0.049	0.127	0.079	0.084	NA	0.292	0.031	0.995	0.038	0.239	0.995	0.276	0.145	0.615	0.294	0.230	0.223	0.000	0.792	0.586
G-CSF	-0.047	0.042	0.090	0.205	0.137	-0.115	0.007	-0.014	-0.032	0.048	0.059	-0.042	0.224	0.278	-0.054	NA	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.002	0.001	0.220	0.812	0.130	0.359	0.001
GM-CSF	-0.118	-0.053	0.007	0.152	0.069	-0.050	0.040	-0.014	-0.051	0.080	0.103	-0.020	0.195	0.249	-0.111	0.663	NA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.002	0.685	0.013	0.000	0.000
IFN- γ	0.007	-0.025	0.050	0.011	0.151	-0.056	0.047	-0.034	-0.108	-0.088	0.077	0.015	0.126	0.140	0.000	0.166	0.289	NA	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IL-16	-0.069	-0.107	-0.059	-0.125	-0.003	0.040	-0.050	-0.012	-0.075	-0.063	0.123	-0.018	-0.043	0.063	-0.106	0.233	0.371	0.341	NA	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IL-5	-0.052	-0.022	0.060	0.005	0.110	-0.163	-0.017	0.020	-0.111	-0.093	0.096	0.010	0.080	0.022	-0.060	0.273	0.438	0.389	0.218	0.165	0.464	NA	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IL-6	-0.080	-0.011	0.084	0.152	0.028	-0.088	0.035	0.012	-0.077	0.083	-0.102	-0.008	0.173	0.130	0.000	0.412	0.602	0.338	0.317	0.141	0.003	0.443	NA	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IL-7	-0.088	-0.065	-0.048	0.005	0.056	-0.054	-0.020	0.015	-0.080	-0.050	-0.102	-0.055	-0.029	0.113	-0.056	0.334	0.319	0.240	0.391	0.275	0.177	NA	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000
IL-8	-0.071	-0.060	-0.040	-0.050	0.017	0.027	0.024	0.051	-0.007	-0.049	-0.023	0.004	0.110	0.174	-0.075	0.225	0.200	0.338	0.317	0.141	0.003	0.443	NA	0.003	0.889	0.000	0.002	0.323	0.008	0.098
IL-10	-0.035	0.001	0.049	0.111	0.085	-0.106	0.027	-0.100	-0.167	-0.025	0.115	-0.039	-0.025	-0.094	0.026	0.160	0.246	0.127	0.107	0.456	0.345	0.129	-0.007	0.366	NA	0.000	0.000	0.000	0.000	0.000
IL-12	0.038	-0.008	0.200	0.091	0.178	-0.169	0.009	0.008	-0.176	-0.086	0.104	-0.056	0.070	-0.059	0.054	0.177	0.260	0.267	0.107	0.247	0.249	0.142	0.409	NA	0.000	0.000	0.000	0.000	0.000	0.000
IL-13	0.046	0.052	0.190	0.249	0.121	-0.087	0.074	-0.066	-0.021	0.148	0.261	0.033	0.030	0.062	0.061	0.063	0.165	-0.064	-0.096	0.081	0.219	0.026	-0.274	0.341	0.211	0.000	0.000	0.000	0.000	0.000
IL-17	-0.034	0.016	0.148	0.249	0.100	0.004	0.018	-0.101	-0.085	0.199	0.202	-0.047	0.148	0.159	0.062	0.012	0.158	0.018	-0.033	0.051	0.211	0.015	-0.159	0.292	0.172	0.391	NA	0.427	0.010	0.009
MCP-1	0.005	0.101	0.033	-0.035	0.161	-0.093	0.084	0.145	-0.115	-0.119	-0.122	-0.026	0.009	-0.116	0.186	0.077	-0.021	0.062	-0.054	0.059	0.065	0.017	-0.051	0.026	0.162	-0.059	-0.041	NA	0.082	0.008
MIP-16	0.028	-0.019	0.036	0.007	-0.001	0.133	0.071	-0.094	0.019	0.108	0.236	0.003	0.115	0.189	0.014	0.047	0.127	0.168	0.108	0.092	0.127	-0.036	0.135	0.072	-0.013	0.050	0.131	-0.089	NA	0.363
TNF- α	-0.045	0.027	0.096	0.133	0.091	-0.130	-0.016	-0.036	-0.079	-0.054	0.044	-0.030	-0.048	-0.025	0.028	0.164	0.251	0.232	0.194	0.363	0.223	0.238	0.085	0.283	0.382	0.232	0.134	0.136	0.047	NA

Cells below the diagonal report Kendall's Tau (effect size and direction); cells above the diagonal show the corresponding p-values from the partial Kendall's Tau test adjusted for age and sex. Colour intensity encodes $|\tau|$: red indicates positive associations and blue negative ones (deeper colours = stronger effects). Significant τ values are bolded. No correction for multiple comparisons was applied in this exploratory analysis.

Table 6. Matrix of pairwise correlations between individual blood biomarkers of brain damage and cytokines for *Toxoplasma*-infected and non-infected subjects.

	Amyloid- β	BDNF	CNTF	FGF-21	GDNF	Kallikrein-6	MIF	NCAM-1	Neuro-granin	S100B	TDP-43	Tau (Total)	Tau (pT181)	UCHL1	YKL-40	G-CSF	GM-CSF	IFN- γ	IL-16	IL-5	IL-6	IL-7	IL-8	IL-10	IL-12	IL-13	IL-17	MCP-1	MIP-16	TNFr
Amyloid- β	NA	-0.111	0.381	0.177	0.068	0.187	-0.042	-0.036	0.354	0.119	0.213	0.071	0.271	0.192	-0.033	-0.042	-0.052	0.021	-0.043	-0.022	-0.043	-0.083	-0.053	-0.050	0.077	0.085	0.049	-0.072	0.018	0.001
BDNF	0.161	NA	0.014	0.079	0.112	-0.019	0.241	0.172	-0.001	0.177	-0.015	0.173	0.087	0.028	0.246	0.150	0.051	-0.020	-0.091	0.023	0.017	0.064	0.058	-0.024	-0.015	0.033	0.062	0.111	-0.007	0.062
CNTF	0.457	0.034	NA	0.352	0.368	-0.021	-0.048	-0.014	0.051	0.059	0.461	-0.035	0.400	0.303	-0.053	0.223	0.116	0.096	-0.001	0.098	0.164	-0.010	0.049	0.054	0.264	0.175	0.131	0.001	0.052	0.163
FGF-21	0.094	0.076	0.201	NA	0.186	0.091	0.078	-0.119	0.099	0.305	0.288	0.018	0.441	0.469	0.088	0.284	0.329	0.054	-0.063	0.079	0.276	0.007	-0.022	0.144	0.120	0.284	0.305	0.041	0.152	0.204
GDNF	0.274	0.112	0.509	0.262	NA	-0.016	0.224	0.201	-0.076	0.005	0.323	0.087	0.390	0.283	0.182	0.259	0.179	0.229	0.119	0.172	0.233	0.167	0.160	0.096	0.231	0.060	0.092	0.201	0.031	0.179
Kallikrein-6	0.287	0.192	-0.010	0.006	-0.112	NA	0.048	0.082	0.172	0.177	0.130	0.073	0.184	0.134	0.066	-0.085	0.071	0.114	0.071	-0.141	-0.047	0.008	0.015	-0.043	-0.157	-0.070	0.139	-0.034	0.143	-0.103
MIF	0.119	0.089	0.138	-0.013	0.209	0.080	NA	0.126	-0.011	0.028	0.095	0.328	0.131	0.089	0.311	-0.013	0.032	0.061	-0.032	-0.030	0.025	0.023	0.116	0.037	0.004	0.084	0.069	0.146	0.039	0.054
NCAM-1	0.046	0.049	0.110	0.097	0.205	0.126	0.118	NA	0.013	0.045	-0.056	0.079	0.007	-0.020	0.154	0.006	-0.028	0.004	0.000	0.016	0.005	0.023	0.075	-0.100	-0.114	-0.100	0.163	-0.101	-0.054	0.001
Neurogranin	0.254	0.012	0.057	0.046	0.013	0.298	0.104	0.017	NA	0.207	0.024	0.032	0.122	0.120	-0.099	0.003	0.030	-0.055	-0.113	-0.079	0.017	-0.118	0.008	-0.144	-0.172	0.056	0.026	-0.080	0.070	-0.064
S100B	0.266	-0.062	0.353	0.243	0.114	0.186	0.112	0.153	0.275	NA	0.158	0.063	0.213	0.327	-0.024	0.198	0.288	-0.089	0.022	-0.050	0.228	-0.038	-0.118	0.098	-0.086	0.244	0.344	-0.142	0.168	-0.052
TDP-43	0.237	-0.105	0.478	0.001	0.426	0.069	0.282	0.127	-0.023	0.184	NA	0.006	0.367	0.304	0.009	0.186	0.237	0.130	0.276	0.120	0.202	-0.060	-0.004	0.196	0.133	0.289	0.272	-0.189	0.260	0.192
Tau (Total)	0.068	0.050	0.000	-0.029	0.006	0.231	0.355	0.291	0.203	0.236	0.096	NA	0.016	0.008	0.141	-0.080	0.020	0.110	0.021	0.061	0.020	0.003	0.030	-0.006	-0.065	0.089	0.067	-0.008	-0.070	-0.004
Tau (pT181)	0.390	0.145	0.503	0.194	0.510	0.028	0.140	0.188	0.061	0.180	0.374	-0.003	NA	0.609	0.069	0.347	0.321	0.223	0.004	0.103	0.226	-0.015	0.146	0.021	0.109	0.040	0.196	0.001	0.162	0.089
UCHL1	0.208	0.139	0.197	0.331	0.385	0.134	0.173	-0.019	0.091	0.152	0.152	-0.057	0.399	NA	0.067	0.369	0.350	0.134	0.114	0.069	0.246	0.151	0.189	-0.045	0.002	0.129	0.193	-0.051	0.193	0.074
YKL-40	0.183	0.283	0.182	0.073	0.157	0.178	0.202	0.188	-0.019	0.241	0.116	0.100	0.093	0.101	NA	0.008	-0.072	-0.020	-0.125	-0.042	0.053	0.044	-0.042	0.064	0.056	0.102	0.114	0.184	0.013	-0.007
G-CSF	-0.056	-0.133	-0.111	0.096	-0.064	-0.168	0.033	-0.045	-0.065	-0.142	-0.119	0.025	0.038	0.099	-0.187	NA	0.683	0.134	0.206	0.270	0.451	0.318	0.214	0.174	0.185	0.141	0.046	0.025	0.017	0.206
GM-CSF	-0.219	-0.211	-0.147	-0.090	-0.091	-0.243	0.053	0.015	-0.182	-0.193	-0.076	-0.077	0.011	0.069	-0.193	0.632	NA	0.279	0.410	0.411	0.622	0.298	0.148	0.288	0.246	0.230	0.157	-0.081	0.178	0.243
IFN- γ	0.031	-0.065	0.026	-0.052	0.026	-0.114	0.026	-0.122	-0.138	-0.005	0.058	-0.131	0.016	0.137	-0.006	0.202	0.295	NA	0.331	0.376	0.243	0.292	0.343	0.161	0.293	-0.042	0.005	0.053	0.211	0.235
IL-16	-0.122	-0.161	-0.151	-0.222	-0.264	0.021	-0.149	-0.050	0.021	-0.171	-0.105	-0.093	-0.130	-0.068	-0.095	0.291	0.312	0.346	NA	0.310	0.225	0.451	0.233	0.238	0.119	0.052	0.067	-0.072	0.087	0.236
IL-5	-0.081	-0.104	0.019	-0.112	0.028	-0.164	0.004	0.030	-0.133	-0.119	0.088	-0.081	0.063	-0.075	-0.126	0.255	0.478	0.370	0.377	NA	0.418	0.351	0.128	0.255	0.457	0.088	0.010	-0.021	0.117	0.394
IL-6	-0.116	-0.050	-0.021	-0.012	0.023	-0.161	0.068	0.046	-0.204	-0.092	0.072	-0.050	0.112	-0.047	-0.081	0.339	0.566	0.176	0.087	0.535	NA	0.233	0.043	0.269	0.307	0.195	0.184	-0.009	0.267	0.242
IL-7	-0.092	-0.281	-0.110	-0.019	-0.132	-0.146	-0.073	0.010	0.004	-0.050	-0.171	-0.151	-0.047	0.037	-0.236	0.351	0.351	0.127	0.294	0.116	0.069	NA	0.397	0.148	0.210	-0.001	-0.017	0.086	0.009	0.312
IL-8	-0.089	-0.181	-0.144	-0.087	-0.164	0.052	-0.045	0.050	0.002	0.031	-0.040	-0.042	0.076	0.164	-0.108	0.213	0.258	0.317	0.490	0.156	-0.071	0.495	NA	-0.144	0.010	-0.257	-0.213	0.055	0.047	0.104
IL-10	-0.043	0.064	0.057	0.079	0.121	-0.211	0.036	-0.049	-0.210	-0.177	0.015	-0.101	-0.093	-0.183	-0.046	0.120	0.152	0.065	-0.146	0.224	0.188	0.108	-0.198	NA	0.367	0.330	0.292	-0.037	0.181	0.336
IL-12	-0.014	-0.001	0.114	0.044	0.127	-0.170	0.029	0.054	-0.157	-0.070	0.087	-0.050	0.017	-0.181	0.030	0.140	0.273	0.205	0.077	0.434	0.381	0.166	-0.078	0.351	NA	0.148	0.106	0.061	0.087	0.333
IL-13	-0.002	0.093	0.192	0.205	0.247	-0.154	0.102	0.033	-0.168	-0.001	0.213	-0.050	0.015	-0.013	0.045	-0.064	0.063	-0.059	-0.341	0.080	0.227	-0.050	-0.302	0.399	0.326	NA	0.364	-0.140	0.117	0.270
IL-17	-0.135	-0.031	0.171	0.178	0.148	-0.215	-0.021	-0.062	-0.256	-0.010	0.087	-0.210	0.082	0.131	0.018	-0.054	0.149	0.060	-0.177	0.127	0.215	0.066	-0.099	0.295	0.274	0.431	NA	-0.064	0.210	0.130
MCP-1	0.104	0.076	0.063	-0.022	0.056	-0.169	-0.066	0.075	-0.153	-0.053	-0.029	-0.059	-0.014	-0.259	0.155	0.156	0.090	0.027	-0.081	0.170	0.213	-0.101	-0.222	0.179	0.324	0.100	0.019	NA	-0.008	0.092
MIP-16	0.047	-0.040	0.017	-0.052	-0.038	0.091	0.153	-0.058	-0.069	0.022	0.205	0.103	0.036	0.192	0.035	0.101	0.048	0.128	0.165	0.063	-0.069	-0.111	0.272	-0.137	-0.187	-0.067	0.003	-0.192	NA	0.178
TNFr	-0.093	-0.019	-0.005	0.040	-0.025	-0.178	-0.090	0.018	-0.102	-0.033	-0.147	-0.095	-0.238	-0.186	0.085	0.075	0.261	0.176	0.118	0.302	0.166	0.143	0.013	0.199	0.440	0.181	0.159	0.248	-0.170	NA

Cells below the diagonal display Kendall's Tau coefficient for the non-infected subjects. For additional information regarding the interpretation of these values, please refer to the legend of Table 5.

DISCUSSION

This study investigated the association of infection with *Toxoplasma gondii* with blood levels of 30 analytes – 15 cytokines and 15 brain-injury markers – in a cohort of 168 individuals. A parametric MANCOVA (controlling for age and sex) indicated a significant overall effect of infection. As several model assumptions were violated, we confirmed this result with a non-parametric multivariate approach (PERMANOVA). To identify which molecules contributed to the multivariate signal, we then ran *post hoc* partial Kendall correlations (τ), again adjusting for age and sex. Key findings include specific significant associations between *Toxoplasma* infection, multiple blood biomarkers of brain damage and a subset of cytokines.

Furthermore, notable differences were observed in the patterns of correlation matrices for blood biomarkers of brain damage and cytokines when comparing *Toxoplasma*-infected and non-infected participants. While in 103 *Toxoplasma*-free subjects, the concentration of the majority of blood biomarkers of brain damage (63 of 69) correlated positively with cytokine levels across all possible combinations of biomarkers and cytokines, among 65 *Toxoplasma*-infected individuals, the majority (31 of 39) of significant correlations were negative (see Table 6).

Our results corroborate accumulating evidence that latent *Toxoplasma* infection is not truly asymptomatic, but is associated with chronic low-grade neuroinflammation and disruption of the neuroimmune system. The significant increase in KLK6, S100B, and TDP-43 in infected individuals may reflect low-grade cellular stress and subclinical damage within the central nervous system (CNS). S100B is a well-established marker of astrocyte activation or injury (Langeh and Singh 2021), and its elevation is consistent with the known neurotropism of *T. gondii*, which is unable to establish persistent cysts within glial cells, leading to reactive astrogliosis (Everett and Elsheikha 2025). However, under certain conditions, S100B also exhibits neuroprotective activity – for example, by reducing TNF- α production (Langeh and Singh 2021, Everett and Elsheikha 2025).

The increase in KLK6, a serine protease implicated in neuroinflammatory processes and the degradation of CNS components (Patra et al. 2018, Mella et al. 2020), further supports this interpretation. The rise in TDP-43, another protein linked to neurodegenerative diseases like amyotrophic lateral sclerosis, frontotemporal lobar degeneration and Alzheimer's disease, may also signify a subclinical stress response within neurons. The significant decrease in Macrophage Migration Inhibitory Factor (MIF) is intriguing. As a potent pro-inflammatory cytokine, MIF is critical for host resistance against *T. gondii* (Flores et al. 2008, Terrazas et al. 2010). It is possible that its suppression in chronically infected individuals could reflect an evolved immune evasion strategy by the parasite, aimed at dampening an effective host response and ensuring parasite's long-term survival. Increased, rather than decreased, MIF levels were observed in schizophrenia patients (Yu et al. 2022). However, Yu et al.'s study was performed on first episode schizophrenia patients only.

Generally, the observed cytokine profile in infected individuals is paradoxical and is consistent with the hypothesis of parasite-induced modulation of host immunity. The cornerstone of protective immunity against *T. gondii* is a robust Th1 response, driven primarily by IFN- γ and IL-12 (Sturge and Yarovsky 2014, Ihara and Yamamoto 2024). Our finding of significantly lower levels of IFN- γ , along with the pro-inflammatory cytokine IL-1 β and the monocyte-recruiting chemokine MCP-1, in chronically infected subjects is counterintuitive from a host-defense perspective.

However, it is in line with the known ability of *T. gondii* to actively suppress IFN- γ signaling pathways to evade clearance and establish lifelong latency (Kim et al. 2007, Rosowski et al. 2014). This suggests that the parasite establishes a state of modulated, 'cold' inflammation, sufficient to prevent uncontrolled propagation but not to achieve sterile immunity. This is further corroborated by the concurrent increase in IL-13 and IL-17. Elevated IL-13 indicates a shift toward a Th2-type response, which is known to antagonise the protective Th1 immunity (Wynn 2015). The increase in IL-17, a key cytokine of the Th17 lineage, may play a dual role: while it can be protective during acute infection, its sustained elevation in the chronic phase may promote persistent low-grade neuroinflammation and compromise the integrity of the blood-brain barrier (Cua and Tato 2010).

Perhaps the most notable finding of this study is the reversal of the correlation patterns between brain damage biomarkers and cytokines in infected versus non-infected individuals. In the uninfected cohort, the network behaves as expected: markers of cellular stress are positively correlated with inflammatory cytokines, reflecting a coordinated physiological response to maintain homeostasis. In the *Toxoplasma*-infected cohort, this network appears markedly altered, dominated by negative correlations. This suggests that latent *Toxoplasma* infection does not merely alter the levels of specific molecules but profoundly disrupts the communication and regulatory logic of the entire neuroimmune system. Such systemic dysregulation, in which the usual relationship between damage signals and immune response is disrupted, could underlie certain long-term neurological and psychiatric effects associated with *Toxoplasma* infection. Additionally, this 'rewiring' may represent the ultimate host manipulation by the parasite, resulting in a dysregulated immune environment that favors parasite persistence while potentially impairing host neuroimmune integrity.

We observed sex-specific differences in the associations of brain damage markers and cytokines with infection. Specifically, the association between *Toxoplasma* infection and brain injury biomarkers was stronger in women, whereas cytokine alterations were more pronounced in men. These findings are in line with the growing body of literature on sexual dimorphism in immunity to *Toxoplasma* (Roberts et al. 1995, Walker et al. 1997). Hormonal differences are known to shape immune responses, with females generally mounting stronger humoral and males stronger cellular responses (Gay et al. 2021).

Studies in mice have shown that males can produce IFN- γ more rapidly, potentially leading to a better initial control of the parasite but different long-term inflammatory sequelae (Walker et al. 1997). The pronounced reduction of GDNF in infected men is particularly notable, given its role in supporting dopaminergic neurons (Bourque and Trudeau 2000, Mätlik et al. 2022) and the reported increase in dopaminergic activity in *Toxoplasma*-infected individuals (Flegr et al. 2003, Prandovszky et al. 2011) – a phenomenon that is itself modulated by sex-specific neuroendocrine factors (Woodcock et al. 2020, Williams et al. 2021). These findings suggest that *Toxoplasma* may exploit baseline sex-specific immune and neuroendocrine differences, resulting in distinct pathophysiological pathways and potentially different neuropsychiatric outcomes in men and women.

A potentially important sex-specific observation concerns the opposing trends in Amyloid β concentrations, a key marker of Alzheimer's disease, between infected and uninfected individuals. While the differences are not statistically significant in either sex, the directionally opposing trends are noteworthy despite relatively small effect sizes (Kendall's Tau = -0.096 for men; 0.078 for women). This potential sex-specific divergence might help reconcile the often contradictory findings regarding the association between toxoplasmosis and Alzheimer's disease (Bayani et al. 2019, Nayeri Chegeni et al. 2019), as well as the higher prevalence of Alzheimer's disease in women compared to men (Fiest et al. 2016, Matthews et al. 2016, Podcasy and Epperson 2016).

Some studies have also demonstrated that, while infected women tend to perform worse than uninfected controls in certain cognitive tests, infected men perform better than uninfected men on certain cognitive tasks (Flegr et al. 2012, Stock et al. 2014, Wyman et al. 2017). In this context, the results of studies using a mouse model of Alzheimer's disease (5xFAD strain) may be relevant. Experimental infection with *T. gondii* was associated with a marked reduction in amyloid plaque density throughout the brain, particularly in the cortex and hippocampus (Yanes et al. 2024). This was likely due to the recruitment of Ly6C^{hi} monocytes and enhancement of phagocytosis and degradation of soluble Amyloid β (Möhle et al. 2016).

Comparison of our findings with previously published data is challenging, particularly in the case of brain damage markers. A recent study – whose Russian authors explicitly declared that no dataset was generated during the study – reports highly implausible statistical results (e.g., 15 out of 34 low-powered tests significant, 13 of which reportedly reached $p < 0.001$ after correction for multiple comparisons). Owing to the questionable credibility of these findings, we have chosen not to cite the study directly. However, the reference is available upon request.

For cytokines, two recent studies have been published, both conducted on pregnant women. The first, originating from our institution, reported increased concentrations of cytokines such as IL-1 β , IL-1ra, IL-2, FGF-basic, TNF- α and PDGF-BB in infected women (Ullmann et al. 2025).

The second study found elevated levels of TNF- α , IL-6, and IL-10, and decreased levels of IL-8 and MIF, although

these changes reached statistical significance only in the subgroup of pregnant women with depression (Lopes et al. 2025). The cytokine shifts observed in depressed women largely corresponded to our current findings in terms of direction, although not always in magnitude. In contrast, the results published in our earlier study (Ullmann et al. 2025) – except for the consistently elevated TNF- α – differed from our present findings and, in some cases, even contradicted them in direction.

Although our previous study employed a different cytokine detection kit from the same manufacturer, both analyses were performed in the same laboratory using the same instrumentation approximately two years apart. However, the study populations differed substantially, particularly in age, and more importantly, in pregnancy status. The earlier data were collected from pregnant women. It is well established that pregnancy induces substantial shifts in immune system functioning due to the need to maintain immune tolerance toward the semi-allogeneic foetus while preserving antimicrobial defence (Guleria and Sayegh 2007, Abu-Raya et al. 2020, Li et al. 2020).

The primary aim of our study was to seek empirical support for the hypothesis that the increased level and incidence of neuroinflammation observed in patients with schizophrenia may be related to the elevated prevalence of latent toxoplasmosis in this population. Our findings suggest that the cytokine and brain injury biomarker profiles in *Toxoplasma*-infected individuals share notable similarities with those reported in patients with schizophrenia. Notably, elevated levels of S100B, a trend toward increased FGF-21, and a pronounced trend toward decreased GDNF levels (statistically significant in men) are fully consistent with this pattern.

However, some notable discrepancies with previously published findings were also observed. In our study, *Toxoplasma*-infected individuals exhibited decreased levels of MIF, whereas previous research has reported elevated MIF levels in patients with schizophrenia (Yu et al. 2022). On the other hand, it is important to emphasize that the elevated MIF levels were detected in drug-naïve, first-episode schizophrenia patients and that 10 weeks of risperidone treatment led to a significant reduction in MIF concentration. If schizophrenia onset and *Toxoplasma* infection are indeed related, the reported elevation of MIF may reflect a transient response linked to the waning acute phase of infection rather than to latent infection. It is therefore plausible that in a cohort composed primarily of individuals with chronic infections lasting over a decade (originally diagnosed 10–13 years ago in a longitudinal study), the concentration of this molecule had already declined significantly.

The immunological hypothesis of schizophrenia posits that neuroinflammation plays a key role in the etiology of this severe mental disorder as well as in the expression of many of its symptoms (Aricioglu et al. 2016). It is virtually certain that the causes of neuroinflammation are diverse, and that latent toxoplasmosis may be only one of many contributing factors. Warm-blooded animals, including primates, serve as intermediate hosts for *T. gondii*, facilitating the transmission of the parasite to its definitive host,

felids, via predation (Tenter et al. 2000). From an evolutionary perspective, it is therefore in the parasite's interest not to kill its host prematurely but rather to allow it to survive long enough to accumulate tissue cysts, ideally from genetically diverse parasite strains, thereby increasing the genetic diversity of gametes during sexual reproduction of the parasite in the feline host. Therefore, unlike other agents capable of inducing neuroinflammation (and thereby, potentially, schizophrenia), *T. gondii* may have evolved specific adaptations to modulate neuroimmune processes in a way that preserves host viability. This may explain why the neuroinflammatory patterns observed in patients with schizophrenia – potentially arising from heterogeneous causes – differ in several respects from those associated with latent toxoplasmosis.

The primary limitation of this study was the relatively small sample size. However, the study followed a case-control design and was intentionally enriched for *Toxoplasma*-infected individuals to increase statistical power for detecting infection-related effects. Uninfected controls were selected from a larger cohort to match infected individuals in terms of age and sex, thereby minimising potential confounding effects. While the sample size was sufficient to detect significant effects of infection on several biomarkers and cytokines, other comparisons – despite showing substantial differences in mean concentrations between infected and uninfected individuals – did not reach statistical significance. This suggests that some biologically meaningful differences may have remained undetected due to limited statistical power. Future research, particularly if it follows an age-stratified design, would benefit from larger sample sizes.

The selection of biomarkers analysed in this study was constrained by the composition of commercially available assay kits. As a result, some potentially relevant molecules could not be included. Furthermore, for certain biomarkers, the assay sensitivity was insufficient for reliable detection in a non-clinical population, leading to their exclusion from the statistical analyses.

A large proportion of the individuals willing to participate in our study had already taken part in other time-consuming, non-compensated studies more than a decade ago. The sample is therefore almost certainly not a random

cross-section of the general population but is more likely composed of curious and altruistic individuals (as the rewards offered to participants were not substantial this time either). It is thus uncertain to what extent our findings can be generalised to the broader population. Nevertheless, given that we investigated concentrations of brain damage markers and cytokines, it seems unlikely that curiosity or altruism would systematically influence how these molecular parameters relate to *Toxoplasma* infection.

In conclusion, this study tested whether previously reported differences in cytokine and brain injury biomarker levels between individuals with and without schizophrenia could partly reflect the higher prevalence of latent *T. gondii* infection in the affected individuals. Our findings indicate that chronically infected individuals exhibit signs of mild but persistent neuroinflammation and neuroimmune dysregulation. Several observed changes – such as elevated S100B and KLK6 or trends toward increased FGF-21 – mirror those reported in schizophrenia. However, other findings differ markedly. For instance, we detected decreased MIF concentrations in infected subjects, while studies on schizophrenia have reported increased MIF levels, at least in early-stage, untreated patients.

Taken together, our findings suggest that although latent toxoplasmosis may contribute to certain immune abnormalities seen in schizophrenia, it is insufficient to account for the full neuroinflammatory profile associated with the disorder. The observed immunological alterations in schizophrenia likely stem from a range of causes, and chronic toxoplasmosis represents only one possible contributing factor. Taken together, our findings do not support the hypothesis that the elevated prevalence of latent toxoplasmosis among patients with schizophrenia fully explains their neuroinflammatory profile, as the pattern observed in infected individuals diverges in several important respects.

Acknowledgments. The study was supported by the Czech Science Foundation (grant no. 22-20785S).

Author contribution statement. JF, ŠK and FŠ designed the study, JU, JT, MH, BŠ, JP, PN and JV performed the study, JF analysed data, JF drafted the paper, and all coauthors participated in writing the paper.

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Received 22 October 2025

Accepted 9 March 2026

Published online 25 June 2026

Cite this article as: Flegr J., Ullmann J., Španiel F., Toman J., Hůla M., Šebánková B., Petrusová J., Novotný P., Včelák J., Kaňková Š. 2026: Does latent *Toxoplasma* infection mimic the immune profile of schizophrenia? Sex-specific cytokine and brain-marker alterations suggest partial overlap. Special Issue on Toxoplasmosis. *Folia Parasitol.* 73: 006.