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Toxoplasma gondii IgG Serointensity Is Positively Associated With Frailty

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Abstract

Background: Persistent inflammation related to aging ("inflammaging") is exacerbated by chronic infections and contributes to frailty in older adults. We hypothesized associations between *Toxoplasma gondii* (*T. gondii*), a common parasite causing an oligosymptomatic unremitting infection, and frailty, and secondarily between *T. gondii* and previously reported markers of immune activation in frailty.

Methods: We analyzed available demographic, social, and clinical data in Spanish and Portuguese older adults [*N* = 601; age: mean (*SD*) 77.3 (8.0); 61% women]. Plasma *T. gondii* immunoglobulin G (IgG) serointensity was measured with an enzyme-linked immunosorbent assay. The Fried criteria were used to define frailty status. Validated translations of Mini-Mental State Examination, Geriatric Depression Scale, and the Charlson Comorbidity Index were used to evaluate confounders. Previously analyzed biomarkers that were significantly associated with frailty

in both prior reports and the current study, and also related to *T. gondii* serointensity, were further accounted for in multivariable logistic models with frailty as outcome.

Results: In *T. gondii*-seropositives, there was a significant positive association between *T. gondii* IgG serointensity and frailty, accounting for age ($p = .0002$), and resisting adjustment for multiple successive confounders. Among biomarkers linked with frailty, kynurenine/tryptophan and soluble tumor necrosis factor receptor II were positively associated with *T. gondii* serointensity in seropositives ($p < .05$). Associations with other biomarkers were not significant.

Conclusions: This first reported association between *T. gondii* and frailty is limited by a cross-sectional design and warrants replication. While certain biomarkers of inflammaging were associated with both *T. gondii* IgG serointensity and frailty, they did not fully mediate the *T. gondii*–frailty association.

Keywords: Chronic toxoplasmosis, Inflammaging, Kynurenine to tryptophan ratio, sTNF-RII

There has been a substantial rise in the global aging population in the past century leading to a shift from discussing "chronological age" to individual sensitivity to pathophysiological challenges, known as frailty (1). Frailty is a multifactorial syndrome with many possible causes and reciprocal interactions. Biological mechanisms increasingly implicate low-grade chronic inflammation (2), specifically "inflammaging" (3,4), and endothelial dysfunction (5). Frailty is associated with decreased quality of life (6), and increased morbidity and mortality (3,7–9). The Fried frailty criteria are commonly used to evaluate frailty status. The presence of 3 or more of the following 5 criteria—unintentional weight loss, exhaustion, low physical activity, slowness, and weakness—defines the frailty phenotype; prefrailty is defined by manifesting 1 or 2 out of the 5 criteria, and nonfrail status is defined by the absence of all 5 criteria (10).

The highly prevalent and chronic infection with *Toxoplasma gondii* (*T. gondii*) is epidemiologically associated with increased allostatic load (11), a multidimensional risk factor of frailty (12). *Toxoplasma gondii* cycles between 2 phases: (i) the tachyzoite, the active, invasive form; and (ii) the bradyzoite, the slow-growing, insidious form, encapsulated by a cyst. The tachyzoite form has a strong tropism for muscle and brain tissues (13,14), the cardinal tissues implicated in frailty. The definitive host of *T. gondii* is any member of the cat family and intermediate hosts are any other warm-blooded animal, including humans (15). *Toxoplasma gondii* is more commonly transmitted via ingestion of undercooked meat (which contains tissue cysts) and fecal–oral transmission of oocysts (Figure 1) (16).

During acute infection, *T. gondii* infects intestinal epithelial cells. Dendritic cells and monocytes are subsequently infected (17). The parasite stimulates migration of these cells, which carry *T. gondii* through the bloodstream to peripheral tissues, including the striated muscle, and, after crossing the blood–brain barrier (BBB), to the central nervous system (CNS) (18,19). Immunocompetent hosts are able to suppress and control the growth of the parasite (20), with transformation of fast-growing tachyzoites into slow-growing bradyzoites and, in the brain, formation of intraneuronal cysts (13,20). In order to contain *T. gondii* to tissue cysts in its slow-growing bradyzoite form, a persistent level of low-grade immune activation with upregulation of proinflammatory cytokines is necessary (21–23). For example, higher levels of interleukin 6 (IL-6) (24) and tumor necrosis factor alpha (TNFα) were reported among individuals that are *T. gondii*-seropositive (25,26). Some of the immune mediators upregulated during *T. gondii* infection are also components of inflammaging and associated with frailty, including TNFα, IL-6, and the 75-kDa soluble TNF receptor II (sTNF-RII) (27). While the parasite is protected from immune recognition and attack inside the cysts, it does intermittently
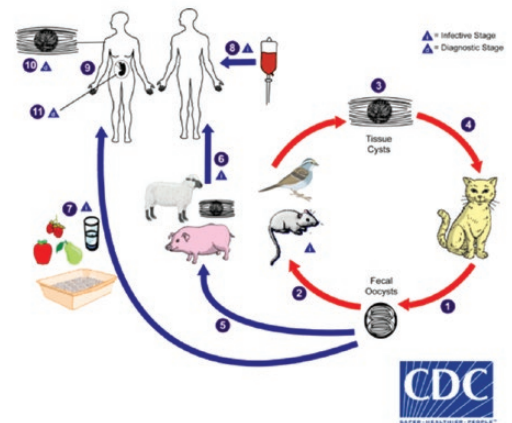

Figure 1. Members of the Felidae family are the only known definitive hosts for *Toxoplasma gondii* (domestic cats and their relatives). The cat's waste contains unsporulated oocysts❶. Oocysts normally only shed for 1–3 weeks; however, they can shed in huge numbers. Oocysts take 1–5 days to sporulate in the environment and become infective. After consuming contaminated soil, water, or plant matter in nature, intermediate hosts (such as birds and rodents) get infected❷. In the digestive tract, oocysts quickly change into tachyzoites after being consumed. These tachyzoites ride dendritic cells and monocytes to settle in muscle and neural tissue, where they become tissue cyst bradyzoites❸. Cats become infected after consuming intermediate hosts harboring tissue cysts❹. Cats may also become infected directly by ingestion of sporulated oocysts, just as any warm-blooded intermediate host❺. There are several ways humans can get infected: (a) consuming raw or undercooked meat containing tissue cysts❻; (b) consuming food or water contaminated with cat excrement or by exposure to oocysts in soil or changing a cat's litter box, in conjunction to poor hand hygiene❼; (c) organ transplantation or blood transfusion❽; and (d) devastatingly for the fetus, from the pregnant mother during gestation, transplacentally❾. The parasites create tissue cysts in the human host, most frequently in the skeletal muscle, heart, brain, and eyes; these cysts may last the entirety of the host's life. Serology is typically used for diagnosis, though stained biopsy specimens may show tissue cysts as well❿. *Toxoplasma gondii* DNA in amniotic fluid can be uncovered using molecular techniques like PCR to diagnose congenital infections⓫. [Based on: https://www.cdc.gov/parasites/toxoplasmosis/biology.html (accessed on October 7, 2022)].

reactivate, multiply, emerge from the cyst, invade local tissue, and translocate at distant sites, further triggering innate and adaptive immune responses (20).

Chronic infections are known to contribute to inflammaging, immunosenescence, and frailty (28–30). Although other neurotropic microorganisms that also establish a chronic infection with intermittent exacerbations have been previously associated with frailty (30–35), there are, to our knowledge, no similar studies on *T. gondii*. Moreover, while

chronic infections perpetuate and fuel inflammaging, they also accelerate T cell senescence (36), deficits in immune surveillance and defense functions leading to vulnerability to infections and impaired response to vaccines (36–38), and thus decrease the host's ability to prevent subsequent reactivations of *T. gondii*. Ex vivo stimulation of peripheral blood mononuclear cells from older *T. gondii*-seropositives with *T. gondii* tachyzoite antigens results in robust secretion of interferon gamma and IL-2 and activation of specific gene network and regulatory microRNA affecting immune gene networks with potentially broad behavioral and health implications (39). Furthermore, studies in mice demonstrate that *T. gondii* secretes proteins such as dense granule protein (GRA15), which modulates nuclear factor kappa B (NF-κB), a pathway with a key role in aging (40), via TNF receptor-associated factors (41).

Immune response to *T. gondii* infection activates the enzyme indoleamine 2,3-dioxygenase 1 (IDO-1), which is 1 of the 2 key enzymes responsible for metabolizing tryptophan (TRP) to kynurenine (KYN) (22). IDO-1 is upregulated by proinflammatory cytokines. Therefore, *T. gondii*, and other chronicity or latency-establishing microbes have less access to available TRP, which is an essential amino acid for the human and critical for the growth of the microorganism (22). Furthermore, KYN freely crosses the BBB and is further catabolized to neuroactive compounds—such as quinolinic acid and kynurenic acid—which act as N-methyl-D-aspartate (NMDA) receptor neuromodulators (42,43). The ratio of KYN to TRP (KYN/TRP), an estimate of IDO-1 activity if other immune activation markers are increased, has been reported to be elevated in *T. gondii* infected rodents (44), in frail individuals as compared to nonfrail controls (45), in older versus younger individuals and particularly in those with higher mortality (46).

For all these reasons, we hypothesized that frailty is positively associated with *T. gondii* immunoglobulin G (IgG) seropositivity and, among individuals who are seropositive, with *T. gondii* IgG serointensity. We further expected, as secondary outcomes, to find that plasma levels of certain immune-related biomarkers, including cytokines (27), and markers of TRP degradation (KYN pathway) (45), previously linked with frailty in Iberic populations (27,45), are also positively associated with *T. gondii* serointensity and seropositivity.

Method

Study Design

This was a cross-sectional secondary analysis of data and plasma samples collected in Spain and Portugal for parent projects examining links between frailty and inflammation (45), cognitive dysfunction (47), social factors (48,49), and environmental factors (50).

Recruitment

All participants were 65 years or older. For participants from the first 2 Spanish cohorts (referred to as Spain 1 and Spain 2), that is, from A Coruña, and Vigo, the Ethics Committee at the University of A Coruña approved the protocol for this study (reference number CE 18/2014), required to be in compliance with the Declaration of Helsinki (45); participants signed an informed consent form after a full presentation

of the study. The procedures have been previously reported (45). For Spanish participants from the third Spanish cohort (referred to as Spain 3—a different sample in A Coruña, Spain), the study was approved by the Autonomic Research Ethics Committee of Galicia, Spain (code 2018/049). Additional inclusion and exclusion criteria and procedures have been previously reported in detail (47). For Portuguese participants (metropolitan area of Porto and Cávado subregion), this study was approved by the Ethics Committee of the Instituto de Saúde Pública da Universidade do Porto (No. CE17081) and was authorized by the National Committee for Data Protection (Comissão Nacional de Proteção de Dados; Authorization No. 5446/2018). Inclusion and exclusion criteria and procedures have been previously described (50).

Sample

We used data collected from 601 individuals. Participants were aged 65–102, [mean (*SD*) 77.3 (8.0); 61% women]. Spain 1 consisted of 258 participants [aged 65–102; mean (*SD*) 79.5 (8.8); 67% women], Spain 2 consisted of 83 participants [aged 66–96; mean (*SD*) 80.3 (6.2); 73% women], and Spain 3 consisted of 60 participants [aged 65–89; mean (*SD*) 72.5 (6.1); 60% women], and Portugal consisted of 200 participants [aged 65–94, mean (*SD*) 74.6 (6.4); 49% women].

Blood Sample Collection, Storage, and Analysis

Venipuncture was performed between 9:30 am and 12:30 pm, using vacuum blood collection tubes with ethylenediaminetetraacetic acid. After centrifugation, resultant plasma was aliquoted and stored at –80°C. Plasma samples were transported in dry ice from the original labs to the Institute of Human Virology, University of Maryland School of Medicine for the *T. gondii* serology analysis. The *T. gondii* IgG serointensity was determined with an enzyme-linked immunosorbent assay (ELISA) kit, per manufacturer's instructions, with the laboratory personnel being blind to the frailty status assignment of the participants but not to the study hypothesis; seropositivity was determined as an Antibody Index ≥ 1.1 (IBL International GmbH, Hamburg, Germany). The immune-related biomarkers were selected based on their previously reported significant associations with frailty in Spanish older adults and included neopterin, KYN, TRP, KYN to TRP ratio (KYN/TRP) (45), IL-6, TNFα, and sTNF-RII (27). Commercial kits (all from R&D Systems Inc., Minneapolis, MN) for quantitative sandwich ELISA were used to measure plasma levels of IL-6, TNFα, and sTNF-RII in accordance with the manufacturer's instructions. Samples were required to be diluted 10-fold for sTNF-RII analysis. The maximum intra- and inter-assay coefficients of variation were 3.0% and 8.4% for TNFα, 4.8% and 5.1% for sTNF-RII, and 4.2% and 6.4% for IL-6. Serum neopterin concentration was determined following manufacturer's instructions with a commercially available ELISA kit (BRAHMS, Hennigsdorf, Germany), with a sensitivity of 2 nmol/L. Serum TRP and KYN concentrations were measured using high-performance liquid chromatography; 3-nitro-L-tyrosine was used as an internal standard (51). Limits of detection were 0.1 μmol/L for TRP and 0.5 μmol/L for KYN. The calculated KYN/TRP, expressed as micromoles of KYN per millimole of TRP, was used to estimate TRP breakdown.

Measures

Frailty was determined based on the Fried frailty criteria, as previously described (10).

Depression was measured with the shorter version (15-item) of the original 30-item Geriatric Depression Scale (GDS) for older adults (>65 years old) (52,53). Cognitive function was assessed with the 30-point Mini-Mental State Examination (MMSE). Obesity was defined as a body mass index (BMI) ≥30. Comorbidity was quantified via the Charlson Comorbidity Index (CCI) (54). Validated translations of all measures were used. Nonnormally distributed continuous variables were log-transformed. Cognitive impairment was defined as an MMSE score of ≤24. GDS score ≥5 was characterized as "depressed," and <5 as "euthymic" (52,53). Basic education was defined as ≤8 years of school for the Spanish populations and as completing less than junior high school in the Portuguese population, according to the International Standard Classification of Education (ISCED) 2011 guidelines (55). For this study, we defined any education beyond basic education as "higher" education. Comorbidity was defined as a CCI score >0 (54,56).

Statistical Analysis

The demographic and clinical characteristics of the sample between the 2 groups were compared using 2-sample *t* tests for normally distributed original or transformed continuous variables, and with chi-squares for categorical variables. All analyses were performed with SYSTAT 13 (Systat Software Inc., Chicago, IL). The primary dependent variable was frailty status (binary). We compared "frail" individuals (≥3 frailty criteria) versus "controls" (2 or less frailty criteria). The controls included "prefrail" individuals (positive for 1–2 frailty criteria) and nonfrail individuals (no frailty criteria), as previously reported (27). The 2 primary independent variables were *T. gondii* seropositivity for all participants, and serointensity in those individuals who were seropositive.

We used multivariable logistic regressions to evaluate whether there was an association between frailty (as dependent variable) and, sequentially, seropositivity in the entire sample, and serointensity in seropositives only. All primary analyses were adjusted for age and then successively adjusted for gender, depression, cognitive impairment, "sleeping at home" (defined as sleeping at night in a family home or independently), residing in a nursing home, level of education, BMI, obesity status, and comorbidity. To assess any influence of country on these findings, we examined the interaction between country and *T. gondii* serology while adjusting for age, with the intent to stratify by country if the interaction was significant. The interaction between serointensity in seropositives and country was not significant and subsequent adjustment and stratification was not pursued. Criterion α was set at 0.05, 2-tailed. Linear regressions were always adjusted for age, and in addition, successively, for gender, depression, cognitive impairment, sleeping at home, nursing home status, and level of education.

Biomarker (inflammation and KYN pathway) secondary analysis

We reviewed biomarkers that were significantly associated with frailty in prior studies in Spain (neopterin, KYN/TRP, IL-6, TNFα, and sTNF-RII) (27,45,50). We then confirmed which of these biomarkers were significantly associated with frailty in our sample with logistic regressions. Of these biomarkers, we identified those that manifested a significant association with *T. gondii* IgG serointensity in seropositives using linear regression. Finally, the biomarkers that were significantly associated with both frailty and *T. gondii* serointensity in positives were adjusted for within a logistic regression model with *T. gondii* IgG serointensity in seropositives and age as independent variables and frailty as the dependent variable. For all biomarkers, we used the log of the variable if they were not normally distributed (all biomarkers except TRP required log transformation). For readability, we will not use "log" in the name of the variables that were log-transformed.

Results

Descriptive Analyses

There were 601 older adult participants aged 65–102 years old, with a mean (*SD*) age of 77.3 (8.0) with 368 (61%) women and 233 (39%) men. There were 403 (67%) *T. gondii*-seropositive individuals. *T. gondii* serointensity was higher in the frail than in the control group (*p* = .0005, Table 1). Differences between seropositive and seronegative individuals are also presented in Table 1.

Seropositivity–Frailty Analysis Results

We hypothesized a positive association between frailty and *T. gondii* seropositivity. We failed to confirm this hypothesis (odds ratio [OR] = 0.84, 95% CI 0.45–1.54, *p* = .56, age-adjusted). However, with secondary stratification, in depressed (but not in euthymic) participants, seropositivity was significantly associated with frailty (OR = 0.21, 95% CI 0.06–0.75, *p* = .016, age-adjusted). Furthermore, in individuals with a basic (but not higher) education, seropositivity was significantly associated with frailty (OR = 0.37, 95% CI 0.16–0.86, *p* = .021, age-adjusted).

Serointensity–Frailty Analysis Results

We hypothesized a positive association between frailty and *T. gondii* serointensity in the seropositive group. This hypothesis was confirmed (OR = 2.28, 95% CI 1.48–3.50, *p* = .0002, age-adjusted). The significant positive association between frailty and serointensity in seropositives resisted successive adjustments for gender (OR = 2.29, 95% CI 1.49–3.54, *p* = .0002), depression (OR = 2.00, 95% CI 1.11–3.62, *p* = .022), cognitive impairment (OR = 1.80, 95% CI 1.15–2.83, *p* = .010), sleeping at home (OR = 2.23, 95% CI 1.38–3.62, *p* = .001), nursing home status (OR = 2.23, 95% CI 1.38–3.62, *p* = .001), level of education (OR = 2.09, 95% CI 1.30–3.36, *p* = .002), comorbidity (OR = 1.89, 95% CI 1.13–3.15, *p* = .016), BMI (OR = 2.25, 95% CI 1.46–3.47, *p* = .0002), and obesity (OR = 2.26, 95% CI 1.47–3.48, *p* = .0002; Table 2). We examined the interaction between country (Spain vs Portugal) and the association between *T. gondii* serointensity in seropositives with frailty (adjusted for age) and found no significant interaction. Thus, we did not perform further adjustments or stratifications by country.

Biomarkers Associated With Frailty in Our Current Sample

Among seropositives, KYN/TRP (OR = 7.52, 95% CI 3.15–17.94, *p* = .000005), IL-6 (OR = 1.58, 95% CI 1.11–2.25,

Table 1. Description of Study Sample, With Comparisons Between Frail Versus Control Participants, and Toxopositive Versus Toxonegative Participants

Variable	Frail				Control				Total				p Value
	Toxopositive	Toxonegative	Total	p Value	Toxopositive	Toxonegative	Total	p Value	Toxopositive	Toxonegative	Total	p Value	
Age													
Age Mean (SD)	86.1 (7.2)	84.1 (8.5)	85.6 (7.4)	.28	75.5 (6.9)	74.2 (6.3)	75.2 (6.8)	.06	77.5 (8.0)	75.8 (7.6)	77.3 (8.0)	.024	<.000001
Age—younger/older													
Younger (65–84) N (%)	26 (27.1)	12 (12.5)	44 (7.5)	.16	293 (65.8)	113 (25.4)	432 (73.8)	.33	324 (59.2)	125 (22.9)	489 (81.4)	.09	<.000001
Older (≥85) N (%)	47 (49.0)	11 (11.5)	65 (11.1)		31 (7.0)	8 (1.8)	44 (7.5)		79 (14.4)	19 (3.5)	112 (18.6)		
Gender													
Women N (%)	53 (55.2)	15 (15.6)	78 (13.3)	.50	187 (42.0)	70 (15.7)	276 (47.2)	1.0	246 (45.0)	85 (15.5)	368 (61.2)	.67	.009
BMI													
BMI Mean (SD)	27.4 (7.2)	26.8 (3.6)	27.7 (6.5)	.70	28.7 (4.3)	29.2 (4.6)	28.8 (4.4)	.28	28.5 (5.0)	28.9 (4.6)	28.6 (4.8)	.41	.036
Obesity status													
Not Obese (<30) N (%)	51 (54.8)	17 (18.3)	75 (12.9)	.36	214 (48.2)	77 (17.3)	312 (53.7)	.61	267 (49.5)	94 (17.4)	389 (66.7)	.82	.32
Obese (≥30) N (%)	21 (22.6)	4 (4.3)	31 (5.3)		109 (24.5)	44 (9.9)	163 (28.1)		130 (24.1)	48 (8.9)	194 (33.3)		
Depression													
GDS score Mean (SD)	5.3 (4.2)	5.5 (3.3)	5.4 (4.0)	.89	2.5 (2.6)	2.6 (2.5)	2.5 (2.6)	.79	2.8 (2.9)	2.9 (2.7)	2.8 (2.9)	.70	<.000001
Depressed—yes/no													
Yes N (%)	13 (28.3)	11 (23.9)	26 (5.0)	.10	60 (13.7)	20 (4.6)	85 (16.3)	.57	78 (15.9)	31 (6.3)	118 (21.9)	.89	<.000001
No N (%)	17 (37.0)	5 (10.9)	26 (5.0)		258 (58.8)	101 (23.0)	385 (73.8)		276 (56.2)	106 (21.6)	420 (78.1)		
Cognition													
MMSE score Mean (SD)	17.3 (9.1)	19.8 (8.5)	17.8 (8.9)	.27	27.0 (3.8)	27.9 (2.3)	27.2 (3.4)	.015	25.2 (6.3)	26.7 (4.8)	25.5 (6.0)	.013	<.000001
Cognitive impairment—yes/no													
Yes N (%)	49 (55.1)	12 (13.5)	72 (12.5)	.20	55 (12.4)	8 (1.8)	70 (12.1)	.005	110 (20.4)	20 (3.7)	153 (25.8)	.001	<.000001
No N (%)	19 (21.3)	9 (10.1)	30 (5.2)		269 (60.4)	113 (25.4)	406 (70.2)		288 (53.3)	122 (22.6)	441 (74.2)		
Higher education—yes/no													
Yes N (%)	51 (58.0)	5 (5.7)	64 (12.4)	.00004	81 (20.9)	36 (9.3)	133 (25.8)	.54	132 (27.7)	41 (8.6)	197 (38.3)	.16	<.000001
No N (%)	17 (19.3)	15 (17.0)	36 (7.0)		196 (50.5)	75 (19.3)	282 (54.8)		213 (44.7)	90 (18.9)	318 (61.7)		

Table 1. Continued

Variable	Frail				Control				Total				p Value
	Toxopositive	Toxonegative	Total	p Value	Toxopositive	Toxonegative	Total	p Value	Toxopositive	Toxonegative	Total	p Value	
Sleeps at home—yes/no													
Yes N (%)	25 (27.8)	6 (6.7)	34 (7.4)	.52	224 (65.5)	96 (28.1)	333 (72.7)	.10	249 (57.6)	102 (23.6)	367 (80.1)	.22	<.000001
No N (%)	44 (48.9)	15 (16.7)	68 (14.8)		19 (5.6)	3 (0.9)	23 (5.0)		63 (14.6)	18 (4.2)	91 (19.9)		
Nursing home—yes/no													
Yes N (%)	44 (48.9)	15 (16.7)	68 (14.8)	.52	19 (5.6)	3 (0.9)	23 (5.0)	.10	63 (14.6)	18 (4.2)	91 (19.9)	.22	<.000001
No N (%)	25 (27.8)	6 (6.7)	34 (7.4)		224 (65.5)	96 (28.1)	333 (72.7)		249 (57.6)	102 (23.6)	367 (80.1)		
Comorbidity—yes/no													
Yes N (%)	26 (35.1)	3 (4.1)	35 (13.6)	.52	28 (17.8)	12 (7.6)	45 (17.5)	.99	54 (23.4)	15 (6.5)	80 (31.1)	.50	.019
No N (%)	38 (51.4)	7 (9.5)	51 (19.8)		82 (52.2)	35 (22.3)	126 (49.0)		120 (51.9)	42 (18.2)	177 (68.9)		
T gondii IgG serointensity													
Mean (SD)	2.6 (0.8)	0.5 (0.3)	2.1 (1.2)	<.000001	2.1 (0.7)	0.5 (0.3)	1.7 (1.0)	<.000001	2.2 (0.7)	0.5 (0.3)	1.8 (1.0)	<.000001	.0005

Notes: BMI = body mass index; GDS = Geriatric Depression Scale; MMSE = Mini-Mental Status Examination; *SD* = standard deviation. The *p* value columns under the headings "Frail, Control, and Total" refer to seropositive versus seronegative. The *p* value column (last column on the right) refers to frail versus control individuals (Frail "total" column and Control "total" column). The *p* value columns under the headings "Frail, Control, and Total" refer to seropositive versus seronegative.

Table 2. *Toxoplasma gondii* Seurointensity Is Positively Associated With Frailty Among Seropositives; the Association Is Robust to Multiple Successive Adjustments

Adjustments for	Odds Ratio	95% CI	*p* Value
Age	2.28	1.48–3.50	.0002
Gender	2.29	1.49–3.54	.0002
Depression	2.00	1.11–3.62	.022
Cognitive impairment	1.80	1.15–2.83	.010
Sleeping at home	2.23	1.38–3.62	.001
Nursing home status	2.23	1.38–3.62	.001
Level of education	2.09	1.30–3.36	.002
Comorbidity	1.89	1.13–3.15	.016
BMI	2.25	1.46–3.47	.0002
Obesity status	2.26	1.47–3.48	.0002
Neopterin	2.36	1.52–3.65	.0001
KYN/TRP	2.19	1.35–3.53	.001
IL-6	1.99	1.23–3.22	.005
sTNF-RII	1.82	1.09–3.05	.022
TNFα	2.39	1.07–5.34	.034

Notes: BMI = body mass index; CI = confidence interval; IL-6 = interleukin 6; KYN/TRP = kynurenine/tryptophan; sTNF-RII = soluble TNF receptor II; TNFα = tumor necrosis factor α. All biomarkers except for TRP (which was normally distributed) were log-transformed. Factors adjusted for in logistic regression models (with age as a covariate in all models), in succession, are listed above.

$p = .012$), and sTNF-RII (OR = 21.15, 95% CI 6.81–65.65, $p < .000001$) were all positively associated with frailty. KYN (OR 0.16, 95% CI 0.05–0.52, p .003) was negatively associated with frailty.

TNFα (OR = 1.49, 95% CI 0.60–3.66, $p = .39$), neopterin (OR 1.26, 95% CI 0.74–2.15, $p = .40$), and TRP (OR = 0.98, 95% CI 0.96–1.00, $p = .084$) were not significantly associated with frailty.

Associations Between Immune-Related Biomarkers and *T. gondii* Serointensity in Seropositives

Among seropositives, KYN/TRP [$B = 0.077$, $F_{(2,301)} = 54.80$, $p = .017$], sTNF-RII [$B = 0.08$, $F_{(2,227)} = 55.75$, $p = .019$], and TNFα [$B = 0.27$, $F_{(2,59)} = 4.55$, $p = .048$] were positively associated with serointensity. All other biomarkers were not significantly associated with serointensity in seropositives: IL-6 [$B = 0.06$, $F_{(2,225)} = 13.21$, $p = .54$], KYN [$B = -0.0002$, $F_{(2,304)} = 4.94$, p 1.00], TRP [$B = 0.31$, $F_{(2,304)} = 2.41$, p .79], and neopterin [$B = -0.03$, $F_{(2,360)} = 37.49$, $p = .36$]. All linear regressions were adjusted for age.

No Significant Associations Between Immune-Related Biomarkers and *T. gondii* Seropositivity

Among the total sample, no biomarkers were significantly associated with *T. gondii* seropositivity. The results are listed as follows: IL-6 [$F_{(1,294)} = 0.43$, $p = .51$], neopterin [$F_{(1,488)} = 0.17$, $p = .68$], TNFα [$F_{(1,84)} = 2.20$, $p = .14$], sTNF-RII [$F_{(1,296)} = 0.20$, $p = .66$], TRP [$F_{(1,419)} = 0.18$, $p = .67$], KYN [$F_{(2,425)} = 12.70$, p .99], KYN/TRP [$F_{(1,419)} = 2.02$, $p = .16$]. All analysis of covariance (ANCOVAs) were adjusted for age.

Frailty–*T. gondii* IgG Serointensity in Seropositives Association: Robust to Adjustment for Immune-Related Biomarkers Significantly Associated With Both Frailty and *T. gondii* Serointensity

Among seropositives, the association between serointensity and frailty was adjusted for individual biomarkers that were significantly associated with both frailty and *T. gondii* serointensity (Table 2). Only KYN/TRP and sTNF-RII met these criteria. Though still significant after adjustment, the association between *T. gondii* IgG serointensity and frailty diminished somewhat after adjustment for KYN/TRP (OR 2.19, 95% CI 1.35–3.53, $p = .001$) and sTNF-RII (OR 1.82, 95% CI 1.09–3.05, $p = .022$).

Discussion

To the best of our knowledge, this is the first study linking frailty with markers of chronic *T. gondii* infection, specifically, with IgG serointensity in IgG-seropositive individuals. This finding was resilient to adjustment for gender, cognitive impairment, depression, as well as other potential confounders. Additionally, the IgG serointensity in seropositives was associated with the KYN/TRP ratio, an indicator of increased activity of IDO-1 (57), an enzyme induced by proinflammatory molecules previously linked with aging (58), frailty (45,59), and overall mortality (46) in older adults.

Our significant associations with serointensity but not seropositivity are consistent with other studies focused on cognitive function, including worse cognitive performance on Trails test (60) and worse reasoning with serointensity but not seropositivity (60). Consistently, serointensity, but not seropositivity, is significantly associated with smaller gray matter volume in middle-aged and older adults (61). A higher serointensity in *T. gondii* positives may be the result of a more virulent infection resulting from parasite-related factors, such as a more virulent strain, a fecal–oral oocyst transmission rather than tissue cyst transmission, a more recent infection or reinfection with another strain (62,63), or from increased host susceptibility, which could be, in part, heritable (64). A higher spread or reactivation of *T. gondii*, or reinfection with another strain, results in higher serointensity, may upregulate inflammation, and exacerbate inflammaging, manifested by increased levels of certain proinflammatory cytokines (3) mechanistically implicated in frailty (28,65). In sum, a higher virulence, spread, and reactivation (66) may be necessary for *T. gondii* infection to contribute to certain neuroanatomical (e.g., gray matter volume) and functional (cognitive impairment, frailty) deficits highly relevant for aging.

Analyzing the immune biomarkers that were previously associated with frailty, in relationship to *T. gondii* serointensity in seropositives, uncovered positive associations with KYN/TRP, sTNF-RII, and TNFα, while KYN, TRP, IL-6, and neopterin were not significantly associated. However, in our own data, only KYN/TRP and sTNF-RII were significantly associated with both frailty and serointensity in seropositives. The KYN/TRP and sTNF-RII finding implicates balancing the immune control of *T. gondii* with limiting overly reactive and unremitting low-grade inflammation. These mechanisms modulate the NF-κB pathways activated by the microorganism (41). Additionally, they also limit *T. gondii*-induced autoimmunity (67) by downregulating autoreactive immune cells (68).

The association between IgG serointensity in seropositives and frailty remained significant, yet slightly diminished after adjustment for analyzed biomarkers associated with both frailty and *T. gondii* IgG serointensity in seropositives (KYN/TRP and sTNF-RII). All in all, these results suggest that biomarkers of immune activation that have been associated with both frailty and *T. gondii* IgG serointensity in seropositives do not fully mediate the association between serointensity and frailty. Moreover, it appears that, because KYN is negatively, rather than positively, associated with frailty and not significantly associated with serointensity, and TRP is not significantly associated with any biomarker, the KYN/TRP ratio is more likely an indicator of increased activation of IDO in chronic *T. gondii* infection rather than a mediator of the *T. gondii* serointensity and frailty association. Future longitudinal designs will be necessary for formal mediation analyses.

Consistent with our results, prior studies have documented impaired psychomotor performance in chronic toxoplasmosis that, so far, have been mainly attributed to CNS involvement in mice (69) and humans (70). However, of considerable relevance to frailty, *T. gondii* also induces sustained damage to skeletal musculature (13,14), and thus potentially precipitating and exacerbating the cardinal motor deficits of frailty. This is based on recent experimental animal data demonstrating that *T. gondii* infection induces acute as well as ongoing skeletal muscle damage, inhibits muscle regeneration by dysregulating the macrophage M1/M2 balance by promoting the accumulation of predominantly M1 macrophages in skeletal muscle, and by aberrant Treg-perpetuated inhibition of muscle regeneration (14).

Chronic infection of the brain by *T. gondii* results in state-dependent epigenetic modulation of multiple host cellular processes and signaling pathways including apoptosis, oxidative stress, and inflammation. This occurs, in part, by altering global microRNA expression, which contributes to the parasite's immunological resistance, persistence, and tissue damage (71). In a mouse model of chronic *T. gondii* infection, neurodegenerative changes in CNS activation are accompanied by marked increases in expression of proinflammatory cytokines and chemokines (CX3CL1), upregulated complement proteins (C1q and C3), and locally activated microglia (72).

The cross-sectional design is a major limitation that precludes causal inferences. The hypothesized direction of causality may be erroneous, as frailty risk factors, or frailty-associated conditions such as depression and cognitive deficits, via self-neglect and compromised hand and food preparation hygiene, could contribute rather than be caused by *T. gondii* infection. Longitudinal and interventional designs are important for future research for improving the estimation of the directions of causality and allowing a proper mediation analysis differentiating direct from indirect (immune-mediated effects). The relatively high seropositivity in our sample, while representing an asset of the study through maintaining statistical power even when analyzing subgroups, is also a liability, by decreased generalizability of the results. Thus, the results call for replication in other populations, with longitudinal design, and including biomarkers of immunosenescence.

Conclusion

Our study, performed in an Iberian older adult population with high *T. gondii* seroprevalence, is the first, to the best of our knowledge, to link serological markers of chronic *T. gondii* infection with frailty. We identified an association between *T. gondii* IgG serointensity in *T. gondii* positive individuals and frailty, resisting sequential adjustments for multiple variables including demographic factors, depression, cognitive deficits, medical comorbidities, and immune-related biomarkers individually associated with both *T. gondii* serointensity and frailty. This study provides justification for future projects identifying, better understanding, and addressing less known and potentially modifiable targets exacerbating frailty, such as markers of a more active and widespread infection with *T. gondii*, and leading to developing novel preventative and treatment approaches.

Conflict of Interest

None.

Author Contributions

Study concept and design: B.L., L.L.-L., J.P.T., S.C., L.A., T.T.P. Acquisition of data: V.V., N.F.-B., C.L.-A., A.M., A.T.-G., S.C. Serological analysis: N.C. Kynurenine pathway analysis: D.F., J.M.G. Analysis and interpretation of data: E.P., J.C.M.-C., T.T.P., N.C., A.D., H.K.O., C.A.L., A.J.H., E.J.K., J.A.R., L.A.B., D.F., J.M.G. Drafting of the manuscript: H.M., T.T.P., C.M. Critical revision of the manuscript for important intellectual content: B.L., V.V., E.P., L.L.-L., J.C.M.-C., A.M., J.P.T., S.C., H.M., T.T.P., N.C., A.D., H.K.O., B.T., L.A., P.Y., C.M., C.A.L., A.J.H., J.A.R., H.K.O., L.A.B., D.F., J.M.G. Statistical analysis: P.Y., H.M., T.T.P. Obtained funding: B.L., E.P., L.L.-L., J.C.M.-C., A.M., J.P.T., T.T.P., E.J.K. Administrative, technical, and material support: N.F.-B., C.L.-A., A.T.-G., L.A.B., T.T.P., J.A.R.,

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