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Serological Responses to *Toxoplasma gondii* and Matrix Antigen 1 Predict the Risk of Subsequent Toxoplasmic Encephalitis in People Living With Human Immunodeficiency Virus (HIV)

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Background. Clinically useful predictors for fatal toxoplasmosis are lacking. We investigated the value of serological assays for antibodies to whole *Toxoplasma* antigens and to peptide antigens of the *Toxoplasma* cyst matrix antigen 1 (MAG1), for predicting incident toxoplasmic encephalitis (TE) in people living with human immunodeficiency virus (HIV; PLWH).

Methods. We performed a nested case control study, conducted within the Multicenter AIDS Cohort Study (MACS), using serum samples obtained 2 years prior to diagnosis of TE from 28 cases, and 37 HIV disease-matched *Toxoplasma* seropositive controls at matched time-points. Sera were tested for *Toxoplasma* antibodies using a commercial assay and for antibodies to MAG1_4.2 and MAG1_5.2 peptides in enzyme-linked immunosorbent assay (ELISA).

Results. Two years prior to clinical diagnosis, 68% of TE cases were MAG1_4.2 seropositive compared with 16% of controls (odds ratio [OR] 25.0, 95% confidence interval [CI] 3.14–199.18). Corresponding results for MAG1_5.2 seropositivity were 36% and 14% (OR 3.6, 95% CI .95–13.42). Higher levels of antibody to MAG1_4.2 (OR 18.5 per doubling of the optical density [OD] value, 95% CI 1.41–242) and to *Toxoplasma* (OR 2.91 for each OD unit increase, 95% CI 1.48–5.72) were also associated with the risk of TE. When seropositivity was defined as the presence of MAG1 antibody or relatively high levels of *Toxoplasma* antibody, the sensitivity was 89% and specificity was 68% for subsequent TE.

Conclusions. Antibodies to MAG1 showed predictive value on the occurrence of TE in PLWH, and the predictive performance was further improved by adding the levels of *Toxoplasma* antibody. These measures could be clinically useful for predicting subsequent diseases in multiple at-risk populations.

Keywords. toxoplasmic encephalitis; serological responses; predict; HIV; *Toxoplasma gondii*; matrix antigen 1.

Toxoplasmic encephalitis (TE) is a brain infection with high morbidity and mortality, which almost always represents reactivation of a previously acquired parasitic infection with *Toxoplasma gondii* [1]. Exposure to *Toxoplasma* is common in the general population, but the incidence of TE is low and the risk factors for it have been difficult to characterize. The most important risk factor for TE is immunosuppression, although some *Toxoplasma*-infected AIDS patients never developed TE despite being severely immunocompromised [1]. Other factors such as host genetics, differences in virulence of *Toxoplasma* strains, and the residual burden of the parasite in the brain may also contribute to the risk of disease [2–4]. Elevated levels of antibody to the whole *Toxoplasma* organism have also been associated with increased risk for TE [5–7]. The clinical signs and symptoms of TE are nonspecific; therefore, definitive diagnosis requires either a brain biopsy or unequivocal response to *Toxoplasma* specific therapy. The poor predictive value of known risk factors for TE among *Toxoplasma*-infected individuals and the challenges of making a definitive diagnosis argue for the need to develop robust prediagnostic markers for TE to better manage patients at risk.

Previously, we developed a serological assay for antibodies to the *Toxoplasma* matrix antigen 1 (MAG1) [8]. The level of MAG1 antibodies was highly correlated with the number of tissue cysts in the brain of infected mice and with signs of chronic infection, including lower body weight, behavioral changes, altered gene expression, and immune activation [9]. Herein we retrospectively identified samples that had been collected 2 years prior to the diagnosis of TE from *Toxoplasma* seropositive people living with human immunodeficiency virus

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(HIV; PLWH) to evaluate the predictive value of serum antibodies to MAG1 and the whole *Toxoplasma* organism for development of TE, alone or in combination. The choice of two years was intended to balance a reasonably long lead time before diagnosis and a clinically pertinent interval where therapeutic interventions, such as administration of anti-microbial prophylaxis, could be deployed.

METHODS

Study Population

The study was a matched case-control design nested within the Multicenter AIDS Cohort Study (MACS), a prospective study of HIV/AIDS in men who have sex with men (MSM) in the United States, begun in 1984 [10, 11]. MACS recruited men from 4 US cities (Baltimore/Washington D.C.; Chicago, Illinois; Pittsburgh, Pennsylvania; Los Angeles, California) and had 4 enrollment cohorts: 1984–1985, 1987–1991, 2001–2003, and 2010–2018. MACS is a longitudinal study, where data and serum samples were collected at study entry and semiannual visits via interviewer-administered and computer-assisted questionnaires and physical examinations. Clinical outcomes were reported at the time of diagnosis by the attending physician. The present study is restricted to PLWH. Figure 1 is a flowchart showing case and control identification. There were 90 cases of TE reported in the MACS outcomes database between 1984 and 2002, diagnosed by clinical signs and symptoms, radiologic criteria for TE, or a self-report of TE. Cases were excluded if the diagnosis was based on self-report (n = 5), sera from recent HIV seroconverters (n = 9), or prediagnostic serum samples were not available (n = 11). After exclusions, 65 cases were screened for *Toxoplasma* antibodies. *Toxoplasma* seropositivity was an eligibility criterion because TE is the result of reactivation of a prior *Toxoplasma* infection. Thirteen of the 65 cases were seronegative and were excluded from the study, leaving a total of 52 cases.

Because the risk of *Toxoplasma* reactivation is related to the degree and duration of immunosuppression, controls were matched to cases for (1) CD4+ T-cell count (± 100 cells/mm^3 cells) at MACS enrollment and at the time of serum collection, and (2) length of time (± 3.5 years) between MACS enrollment and serum collection. To account for potential demographic and/or socioeconomic differences, controls were also matched for the MACS enrollment cohort. For each case, 4 controls matched on the criteria above were selected and then screened for *Toxoplasma* antibodies. Among 260 eligible controls (for the 65 cases), 45 (17.3%) were seropositive for *Toxoplasma* antibody, consistent with the expected seroprevalence of *Toxoplasma* in this population [12]. Among the 45 eligible *Toxoplasma* seropositive controls, 37 were matched to 28 TE cases. Some cases had more than 1 control serum samples (20 cases had 1 control, 7 cases had 2 controls, and 1 case had 3 controls). The 28 TE

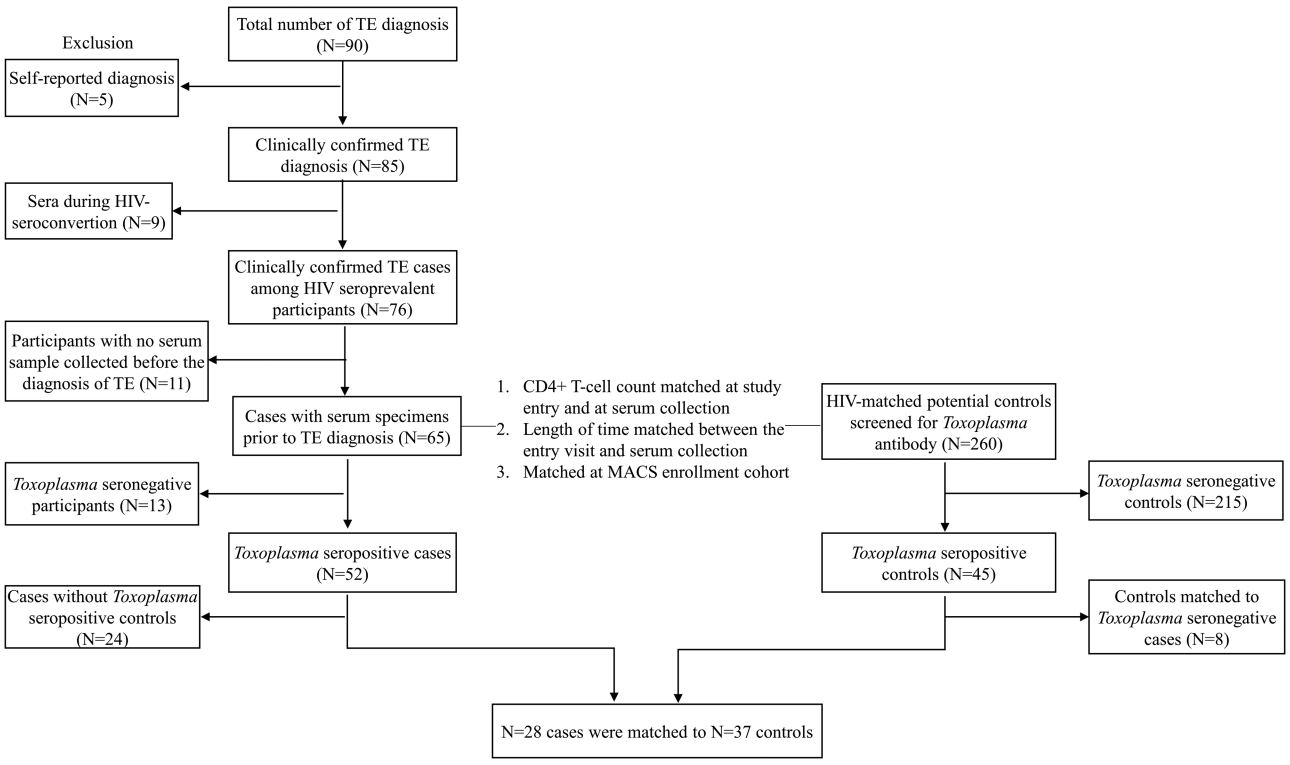

Figure 1. Flowchart to show case and control identification. Abbreviations: HIV, human immunodeficiency virus; TE, toxoplasmic encephalitis.

cases included in the final analysis were drawn from the first 2 MACS cohorts. Twenty-four of the 52 *Toxoplasma*-seropositive TE cases were not included in the final analysis because their HIV disease matched controls were *Toxoplasma*-seronegative. All controls were diagnosed with an AIDS-defining condition, other than TE, subsequent to the time of serum collection.

Serological Assays

Sera were analyzed in a blinded manner for antibodies to whole *Toxoplasma* organism, MAG1, and strain-specific antigens derived from 3 *Toxoplasma* strains. Total anti-*Toxoplasma* IgG antibodies were measured using a commercial ELISA kit (IB19213, IBL America, Minneapolis, Minnesota, USA) according to the manufacturer's protocol. Antibody against *Toxoplasma* cyst antigen MAG1 was measured using a peptide ELISA as previously described with newly designed peptide antigens [8]. The new peptides consisted of discontinuous amino acid sequence of the MAG1 open reading frame. The amino acid sequence of MAG1_4.2 includes aa 342–361 and aa 422–452, with an added proline (P) between the discontinuous segments (EKQLRRVEPEHEDNTRVEAR/P/RALLE AKTKELVEPTSKEAEEARQILAEQAA) and MAG1_5.2 includes aa 66–82 and aa 107–148 (YENSEDVAVPSDSASTP/DCEEQQEQGDTTLSDHDFHSGGTEQEGLPETEVAHQHE TEEQ). The amino acid numbers are from accession number XP_002365700 and the slash (/) indicates the separation point between the 2 discontinuous amino acid regions. Peptides were chemically synthesized to 90% purity (GenScript, Piscataway, New Jersey, USA).

The *Toxoplasma* strain type of patients infected with was determined by a previously developed serological assay [13]. Six polymorphic peptides (GRA5-II, GRA6-I, GRA6-II, GRA6-III, GRA7-II, and GRA7-III) specific to 3 clonal parasite lineages and derived from 3 dense granule antigens (GRA5, GRA6, and GRA7) were used for serological typing, as described previously [13]. Briefly, the *Toxoplasma* serotype was determined by a 2-step screening process: first, serum samples were tested to distinguish type II from type I/III infection using 5 peptides (GRA6-I, GRA6-III, GRA5-II, GRA6-II and GRA7-II). Second, type III was distinguished from type I infection using the GRA7-III peptide.

Statistical Analysis

For each participants, MAG1_4.2 and MAG1_5.2 antibodies were measured 3 times on 3 different days, and results were expressed as the average optical density (OD) value of the replicates. Because antibody results were not normally distributed within the data set, median values with interquartile ranges (IQR) were reported, and comparisons between cases and controls were based on Wilcoxon signed-rank statistics for continuous covariates. Differences in proportions across persons with and without TE were assessed by Mantel-Haenszel statistics. In order to evaluate the seroprevalence of MAG1 antibodies, we performed receiver operating characteristic (ROC) curve analysis to determine an optimal cutoff point. The area under the curve (AUC) was .78 (.67, .89) for MAG1_4.2 and .54 (.39, .68) for MAG1_5.2. According to Youden's J Index, ROC-derived cutoff was an OD value of .046 for MAG1_4.2, and .063 for MAG1_5.2. The robustness of the cutoffs was further assessed by sensitivity analyses performed using cutoffs 10% above and below the ROC-defined optimum. We calculated the odds ratios (ORs) for having a positive MAG1 test (reactivity greater than the cutoffs) using either the Mantel-Haenszel test or conditional logistic regression, with or without adjustment for age and race. The Mantel-Haenszel test was the primary analysis because the small sample size made estimates from conditional logistic regression less reliable. Conditional logistic regression analysis was also used to assess the association between antibody levels and TE. Sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR−) were estimated for each marker or combination of markers. For analyses involving the combined use of markers, individuals were considered seropositive if they were seropositive for MAG1_4.2 or MAG1_5.2, or the level of *Toxoplasma* IgG was in the upper tertile of seroreactivity. P values < .05 were considered statistically significant. All statistical calculations were performed using SAS software (SAS, Cary, North Carolina, USA), and graphs were made in GraphPad Prism 8 (GraphPad, San Diego, California, USA).

RESULTS

Participant Population

Demographic characteristics of matched cases and controls were similar at the time of serum collection (Table 1). Of note, the dates of the study preceded the introduction of antiretroviral therapy (ART), with only 1 case and 3 controls having a history of ART use. The use of anti-*Toxoplasma* drug prophylaxis was rare with only 1 case receiving pyrimethimine before the diagnosis of TE. Among the 28 cases and 37 matched controls, confounding factors that potentially could have contributed to disease status were matched accurately using the matching criteria described in Methods section (Table 1). The median time between serum collection and TE diagnosis was 2.16 years (IQR: 1.9–3 years) for cases. For controls, the median time was 2.93 years (IQR: 1.8–4 years) between serum collection and the time corresponding to the TE diagnosis of the matched case.

Antibody Distributions in Cases and Controls Prior to TE Diagnosis

The median level of antibody to MAG1_4.2 was significantly higher in cases than controls (P < .001, Figure 2A), but this was not true for antibody to MAG1_5.2 (Figure 2B). By design all cases and controls were seropositive for *Toxoplasma* antibodies.

Table 1. Characteristics Between Matched Cases and Controls

Variable	Control (n = 37)	Case (n = 28)	*P* value
Detectable viral load (HIV RNA >50 copies/mL), n (%)			.32
Yes	14 (38)	12 (43)	
No	1 (3)	0 (0)	
Not available	22 (59)	16 (57)	
Age (years), median (IQR)`	38 (34–45)	36 (34–38)	.07
Race/ethnicity, n (%)			.03
White	34 (92)	23 (82)	
Black	1 (3)	5 (18)	
Other	2 (5)	0 (0)	
CD4+ T-cell count at study entry (cells/mm^3), median (IQR)	533 (451–642)	544 (407–677)	.18
CD4+ T-cell count at serum collection (cells/mm^3), median (IQR)	313 (176–477)	303 (155–479)	.24
Time between study entry and serum collection (years), median (IQR)	2.6 (1.4–5.1)	3.2 (1.3–5.1)	.16
Anti-HIV therapy, n (%)			.18
No therapy	16 (43)	15 (53)	
Mono therapy (simply ART)	5 (14)	2 (7)	
ART therapy (combination ART)	3 (8)	1 (4)	
Data not available	13 (35)	10 (36)	
Pyrimethamine prophylaxis, n (%)			.32
No	37 (100)	27 (96)	
Yes	0 (0)	1 (4)	

P values were obtained by nonparametric Wilcoxon signed-rank test or Mantel–Haenszel test, as appropriate.

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range.

However, *Toxoplasma* seroreactivity was significantly higher in cases compared to controls (3.65 vs 1.43 OD units) ($P < .001$, Figure 2C).

MAG1 Seropositivity in Relation to the Diagnosis of TE

MAG1 seropositivity was significantly associated with the risk of TE (Table 2). MAG1_4.2 seropositivity was 67.8% and

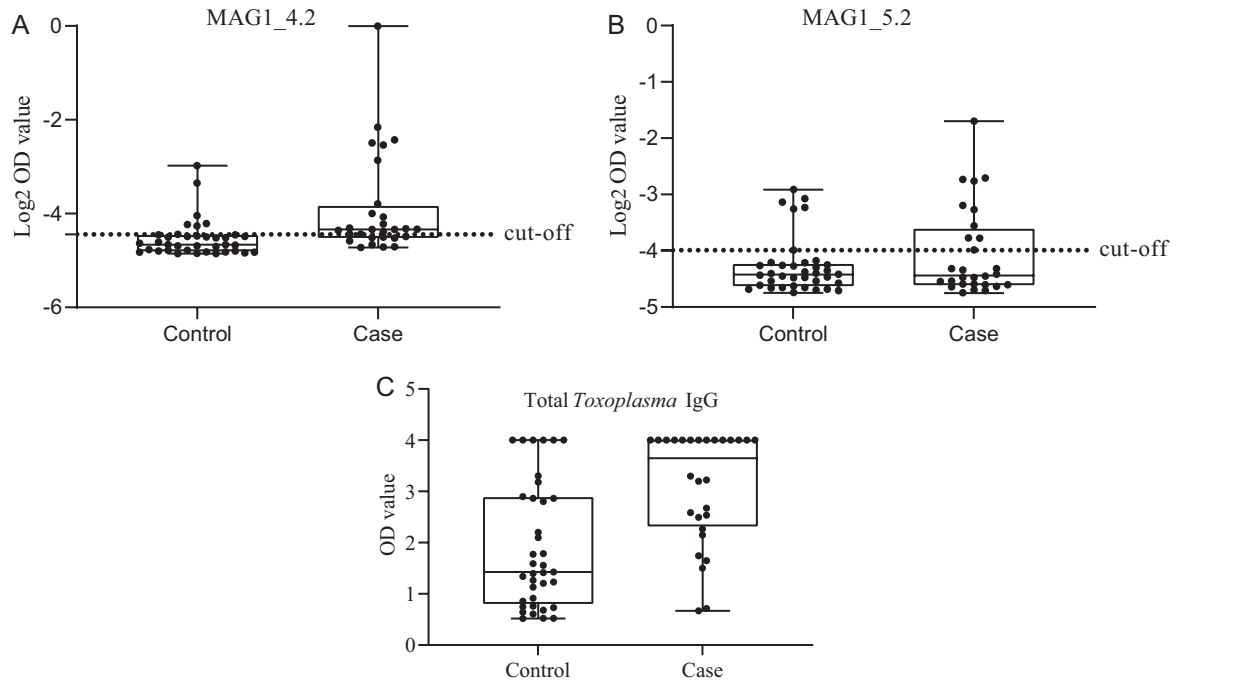

Figure 2. Distribution of antibodies to MAG1_4.2 (*A*), MAG1_5.2 (*B*), and *Toxoplasma* (*C*) by cases and controls 2 years before the diagnosis of toxoplasmic encephalitis. The length of the box corresponds to the interquartile range with the upper boundary of the box representing the 75th percentile and the lower boundary the 25th percentile. The horizontal line in the box represents the median value. The whiskers represent the minimum and maximum. The scatter plot shows individual values. Log_2-transformed values for MAG1 data were used. Abbreviations: MAG1, matrix antigen 1.

Table 2. Mantel–Haenszel Analysis of MAG1 Seropositivity in Relation to Toxoplasmic Encephalitis

	Cases (n = 28)		Controls (n = 37)			
MAG1 Assay	n	%	n	%	OR (95% CI)	*P* value
MAG1_4.2					25.0 (3.14–199.18)	<.001
Positive (>.046)	19	67.8	6	16.2		
Negative (<.046)	9	32.2	31	83.8		.033
MAG1_5.2					3.6 (.95–13.42)	
Positive (>.063)	10	35.7	5	13.5		
Negative (<.063)	18	64.3	32	86.5		
Combined[a]					6.7 (1.67–26.97)	.001
Positive	20	71.4	10	27.0		
Negative	8	28.6	27	73.0		

Abbreviations: CI, confidence interval; MAG1, matrix antigen 1; OR, odds ratio.

[a]Seropositivity in either MAG1_4.2 or MAG1_5.2 test.

16.2% and MAG1_5.2 seropositivity was 35.7% and 13.5% for cases and controls, respectively. Seropositivity in either test was 71.4% for cases and 27.0% for controls (Mantel-Haenszel OR, 6.71; 95% confidence interval [CI]: 1.67, 26.97).

Conditional logistic regression analysis also showed the association between MAG1 seropositivity and the risk of TE (Supplementary Table 1). In an unadjusted model, the odds of MAG1_4.2 seropositive was 19.3 (95% CI: 2.52, 145.41) times higher among men with TE diagnosed within a 2-year time frame compared to that among controls. Similarly, after adjusting for age and race, the odds of MAG1_5.2 seropositive was 6.71 (95% CI: 1.12, 40.28) times higher among men who subsequently developed TE compared to that among controls.

Sensitivity Analyses of the ROC-derived Seroprevalence for the Diagnosis of TE

To ensure the robustness of our findings, we conducted sensitivity analyses—testing the effect of different "cutoffs" on the observed findings. We chose cutoffs that were 10% above and below the ROC cut-off for MAG1_4.2 and MAG1_5.2 seropositivity. The results (Supplementary Table 2) showed that the cutoff for MAG1_5.2 was robust with minimal differences in odds ratios compared to that obtained using the ROC cutoff. Although the cutoff for MAG1_4.2 was more sensitive to variation, the direction of the association was the same for all cut-off values.

Antibody Levels in Relation to the Diagnosis of TE

Levels of antibodies were also analyzed in relation to the odds of developing TE (Table 3). Using log2-transformed OD values, we found that the odds of being a case increased 18.51 (95% CI: 1.41, 242.7) times per doubling of the MAG1_4.2 antibody OD value (P = .026). For MAG1_5.2, the odds of being a case increased 2.29 (95% CI: .78, 6.71) times per doubling of the OD value (P = .131). For each OD unit increase of *Toxoplasma* antibodies, the odds of developing TE increased 2.91 (95% CI: 1.48, 5.72) fold (P = .002).

Predictive Value of *Toxoplasma* IgG and MAG1 Antibodies

To assess the clinical usefulness of the serological tests, we calculated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR–) (Table 4). For the MAG1 assays the ROC cut point for seropositivity was used, and for the *Toxoplasma* antibody assay, a positive

Table 3. Conditional Logistic Regression Analysis of Increase in Levels of MAG1 and *Toxoplasma* Antibodies in Relation to Toxoplasmic Encephalitis

			Multivariate					
	Univariate		MAG1_4.2		MAG1_5.2		*Toxoplasma* IgG	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
MAG1_4.2[a]	7.56 (1.2, 47)	.030	18.51 (1.4, 242)	.026				
MAG1_5.2[a]	2.07 (.8, 5.3)	.129			2.29 (.78, 6.71)	.131		
Toxoplasma IgG	2.45 (1.3, 4.5)	.004					2.91 (1.5, 5.7)	.002
Age	.95 (.9, 1.0)	.237	.91 (.8, 1.0)	.113	.95 (.87, 1.0)	.188	.9 (.8, 1.0)	.089
White[b]	.31 (.06, 1.7)	.180	.1 (.01, 2.1)	.139	.35 (.05, 2.4)	.284	.09 (.01, 1.5)	.095

Abbreviations: CI, confidence interval; IgG, immunoglobulin G; MAG1, matrix antigen 1; OR, odds ratio.

[a]Per doubling.

[b]Reference: Black/Other.

Table 4. Sensitivity, Specificity, PPV, NPV, LR+, and LR– of Serological Markers for Prediction of Toxoplasmic Encephalitis

Markers	Sensitivity	Specificity	PPV	NPV	LR+	LR–
MAG1_4.2	68 (48~84)[a]	84 (68~94)	76 (59~87)	78 (66~86)	4.25 (1.9~9.1)	0.38 (.2~.7)
MAG1_5.2	36 (19~56)	86 (71~95)	67 (44~84)	64 (57~71)	2.57 (1.0~6.9)	0.74 (.6~1.0)
Third tertile of *Toxo* IgG[b]	57 (37~76)	81 (65~92)	70 (52~83)	71 (61~80)	3.00 (1.4~6.3)	0.53 (.3~.8)
MAG1_4.2 or third tertile of *Toxo* IgG[b]	86 (67~96)	70 (53~84)	69 (56~78)	87 (72~94)	2.87 (1.7~4.8)	0.2 (.1~.5)
MAG1_5.2 or third tertile of *Toxo* IgG[b]	64 (44~81)	78 (62~90)	69 (53~82)	74 (63~83)	2.91 (1.5~5.8)	0.46 (.3~.8)
MAG1_4.2 or MAG1_5.2 or third tertile of *Toxo* IgG[b]	89 (72~98)	68 (50~82)	68 (56~77)	89 (74~96)	2.79 (1.7~4.5)	0.16 (.05~.5)

Abbreviations: IgG, immunoglobulin G; LR+, positive likelihood ratio; LR–, negative likelihood ratio; MAG1, matrix antigen 1; NPV, negative predictive value; PPV, positive predictive value; *Toxo*, *Toxoplasma*.

[a]Number ( ): % (95% confidence interval).

[b]*Toxoplasma* IgG between 3.22 and 4.00 optical density (OD) units.

test was defined as seroreactivity in the upper tertile of OD values. The single marker with the highest sensitivity was MAG1_4.2 (68%) and sensitivity increased when the marker was combined with the other 2 markers (89%). Specificity of single markers was 81~86% and that of combined markers was 68–78%. Overall, PPVs were comparable for different single markers or combination of markers (67~76%). Combined markers generally had better NPVs compared to single markers (74~89% vs 64~78%). The LR+ ranged between 2.57 and 4.25, with MAG1_4.2 having the highest LR+. The LR– ranged between .16 and .74, with the combination of 3 markers having the lowest LR–. Based on sensitivity and LR– criteria, the combination of 3 markers had the best performance characteristics, with a sensitivity of 89% and an LR– of .16.

Toxoplasma Serotypes in Relation to the Diagnosis of TE

Because the strain of parasite may influence pathological outcome [2], we determined the infecting strain serologically using a previously developed assay (Table 5) [13]. Among cases, 9 (32%) were type II, 1 (4%) was type III, 3 (11%) were atypical, and 15 (53%) were indeterminate. Among controls, 3 (8%) were type I, 8 (22%) were type II, 1 (3%) was atypical, and 25 (67%) were indeterminate. There were no statistically significant differences among serotypes by case and control status (χ^2 value 6.436, $P = .17$). Serotype II was the most common strain among cases and controls, with a prevalence of 32% and 22% in cases and controls, respectively.

DISCUSSION

Although profound immunosuppression is the strongest predictor for development of TE, the fact that only one-third of *Toxoplasma* seropositive AIDS patients during the pre-ART era developed TE indicates the importance of other risk factors [1]. We explored the pre-diagnostic value of serological responses to *Toxoplasma* and matrix antigen 1 in a matched case-control study of PLWH. We found that antibodies to MAG1 and high levels of *Toxoplasma* antibodies are present more commonly in PLWH who developed TE compared with controls approximately 2 years prior to diagnosis. The combination of the serological assays had high sensitivity for predicting the risk of subsequent TE. In a high-risk population, tests with high sensitivity may have clinical value for screening even when specificity is only modest.

This is the first report of antibodies to MAG1 serving as a predictive biomarker for TE in PLWH. As described previously [9], measurement of MAG1 antibodies offers an approach for indirectly ascertaining parasite burden. Tissue cysts, the hallmark of chronic *Toxoplasma* infection, are predominantly located in the brain, making direct measurement of parasite burden difficult. Using highly sensitive quantitative polymerase chain reaction (qPCR) assays that target the multicopy B1 gene and AF146527 element, *Toxoplasma* DNA can be detected in cerebral spinal fluid (CSF) and blood from symptomatic patients [14, 15]. The utility of these assays is generally limited to the time of diagnosis. In contrast, antibodies to MAG1 are

Table 5. Distribution of *Toxoplasma* Serotype in Relation to Toxoplasmic Encephalitis

	Case (n = 28)		Control (n = 37)		Total		Fisher Exact Test
Serotype	n	%	n	%	n	%	*P*
I	0	0.00	3	8.11	3	4.62	.167
II	9	32.14	8	21.62	17	26.15	
III	1	3.57	0	0.00	1	1.54	
Atypical	3	10.71	1	2.70	4	6.15	
Indeterminate	15	53.57	25	67.57	40	61.54	

predictive of TE approximately 2 years before the clinical diagnosis in PLWH and can be measured in readily available body fluids such as serum.

Serologic measurement of *Toxoplasma* associated antibodies is a noninvasive method for predicting the risk of TE. Although the use of ART has greatly reduced the incidence of TE in PLWH, they still remain at risk [16–20]. A systematic review and meta-analysis reported that even after the introduction of ART, TE accounted for 6% of hospital admissions among AIDS-related illnesses for PLWH [20]. One case from the current study progressed to TE despite receiving combination ART. In many countries where combination ART is not readily available or there are issues of medication adherence, *Toxoplasma* remains an important opportunistic infection [21]. In view of the rapidly progressive and fatal outcome of *Toxoplasma* reactivation [22, 23], early diagnosis is also crucial for other at-risk populations. For instance, these tests could be useful in prevention of ocular toxoplasmosis in individuals with ocular involvement or toxoplasmosis in organ and stem cell transplant recipients [23–25]. Congenital toxoplasmosis usually results from primary infection during pregnancy. However, mothers infected before conception are also at risk of transmitting this disease to the fetus in the setting of immunodeficiency [26–29]. In clinical practice, the ability to serologically identify individuals whose infections might progress to severe disease should allow closer clinical monitoring and, where appropriate, treatment with aggressive prophylactic therapy.

To conclude, we suggest that relatively high levels of *Toxoplasma* antibodies combined with the presence of MAG1 antibodies in PLWH can be highly predictive of TE 2 years prior to the clinical diagnosis. Studies employing more participants, as well as participants from other at-risk populations, are required to further test the predicative value of these antibodies.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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