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The Role of *Toxoplasma gondii* Infection on the Development of Neurocognitive Disorders in Traumatic Brain Injury Patients

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Abstract

The Role of *Toxoplasma gondii* Infection on the Development of Neurocognitive Disorders in Traumatic Brain Injury Patients

By Lauren Shanker

Neurocognitive disorders are an important adverse health outcome for Traumatic Brain Injury (TBI) patients. The etiology of neurocognitive disorders in TBI patients is currently not well understood. This study was conducted to test for the association between *Toxoplasma gondii* (TOXO) infection and the odds of psychosis and mood disorders in TBI patients. The data are a subset of 100 TBI patients attending the Atlanta Veteran's Affairs Medical Center from 2014 to 2015. TOXO is under investigation because it is thought to increase the risk of schizophrenia and suicide worldwide. There is minimal data that relates any infectious agents with worsening mental health outcomes for TBI patients. TOXO titer (the amount of anti-*Toxoplasma* IgG antibodies) was analyzed as a dichotomous variable and as a continuous variable due to evidence that TOXO exposure is not normally distributed and cut-off levels for seropositivity often differ by study. For the dichotomous variable, TOXO titer was categorized into two groups; seropositive (TOXO titer greater than or equal to 27 IU/mL) and seronegative (TOXO titer less than 27 IU/mL). Statistical analysis revealed a positive relationship between TOXO seropositivity and severity of head injury and age. Log-risk models using TOXO seropositivity or TOXO titer were fit to investigate the effect of TOXO on psychosis and mood disorders. The Crude Odds Ratio for the effect of TOXO seropositivity on psychosis was 3.50 (95% Confidence Interval: 0.46, 18.43). After adjusting for age and severity of head injury, the estimated effect of TOXO seropositivity on psychosis is 2.93 (95% Confidence Interval: 0.32, 2.05). The Crude Odds Ratio for the effect of TOXO titer on psychosis is 1.03 (95% Confidence Interval: 1.00, 1.06). The data suggest that increasing TOXO titer has a worsening effect on psychosis in TBI patients. Public health interventions that call for better treatment options for TOXO or reduce the likelihood of TOXO infection can have the potential to reduce the burden of neurocognitive disorders in TBI patients.

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Introduction

The degree to which an infection can cause adverse health effects for brain trauma patients is not well known. In particular, the role of chronic central nervous system infection on adverse neuropsychological symptoms for trauma survivors has yet to be systemically investigated. The purpose of this research is to elucidate the relative importance of *Toxoplasma gondii* (TOXO) infection on the mental health of Traumatic Brain Injury (TBI) patients. This research introduces a novel target for disease control to improve the negative neuropsychological symptoms attributed to this parasite.

TBI

Traumatic Brain Injury (TBI) is an important and often under addressed public health problem. By definition, a TBI is a physical and external injury to brain tissue that may temporarily or permanently cause brain function impairment (1). The major causes of TBI are “falls, motor vehicle crashes, struck by or against events, and assaults” (2). Active military personnel are at an increased risk of sustaining a TBI due to blast injuries in war zones (2). Overall, men are twice as likely as females to experience a TBI, with a four times greater risk of a fatal head injury (3). TBI is a cause of major global concern because of the sheer quantity of injuries that occur and the lack of treatment options.

TBI is the leading cause of death and disability in persons under 45 years of age (3). In the United States alone, an average of 1.4 million TBIs occur each year (2). This includes 1.1 million emergency department visits, 50,000 deaths, 230,000 hospitalizations, and 80,000–90,000 long-term disabilities (2). Blast-related TBIs are common in the Iraq and Afghanistan wars with an estimated 320,000 troops (19.5%) reporting experiencing TBI during deployment (4). There are numerous challenges for the 5.3 million Americans living with long-term TBI-related disabilities and for those treating TBI’s (2).

TBI and neuropsychiatric disorders

Neuropsychiatric disorders may vary in severity from subtly deficits to severe intellectual and emotional disturbances as the direct result of a TBI (3). The literature suggests that TBI increases the likelihood of developing many Axis I and II Psychiatric Disorders including cognitive impairments, mood disorders, anxiety disorders, psychosis, and behavioral problems (5, 3). In fact, psychiatric disorders are a major cause of disability after TBI (5). Early age of injury, left hemispheric lesions, non-penetrating (closed) head injury, and an underlying genetic or environmental vulnerability pose as risk factors for development of psychosis post injury (7).

Current data on the severity of TBI in relation to risk for psychosis is inconclusive. While some studies suggest that individuals who develop a psychosis after TBI had generally sustained moderate to severe head injuries, many other case studies report development of a psychosis after mild brain injuries with no loss of consciousness (6). One retrospective follow-up study found a variety of newly arising disorders after TBI including major depression, substance abuse or dependence, panic disorder, bipolar disorder, generalized anxiety disorder, posttraumatic stress disorder (PTSD), and psychotic disorders (5). The key finding of the study was the high rate of many axis I and II disorders during the 30 years post TBI (5). Another clinical study found that a large series of patients developed “schizophrenia-like psychosis (SLP)” for the first time following a head injury (7). Predominantly, those who develop SLP have a gradual onset of paranoid-hallucinatory psychosis initially with a “subacute or chronic course” (7). For many people, TBI can cause long-term vulnerability to psychiatric disorders. People are particularly vulnerable to “depressive episodes, delusional disorder, and personality disturbances” post TBI (5). Neuropsychological disorders commonly occur post-TBI and should be an area of public health concern.

Issues with current treatment

Many issues arise when trying to treat and diagnose TBI due to the limited information on TBI pathophysiology and problems in access to quality healthcare. The factors that determine disability from a TBI for an individual are still poorly understood. The consequence of this is “a large gap in knowledge related to how extensive the problem is or how to address it” (4). Over half of OEF (Operation Enduring Freedom) and OIF (Operation Iraqi Freedom) service-members who report having a probable TBI never get evaluated by a physician for their injury (4). The lack of people seeking and receiving treatment may stem from societal pressures as well as lack of quality care options.

Unlike physical traumas that are easily recognizable to society, TBIs often presents itself as an “invisible disability”, where the condition remains unperceivable to others (4). According to the CDC, these disabilities originate from “cognitive, emotional, sensory, and motor impairments”, which can permanently change person's attitudes and have profound effects on interpersonal relationships (8). Like other invisible disabilities, seeking out treatment for a TBI requires extra effort by the patient. Often it is easier for doctors to treat visible and physical wounds because psychological disorders are not universally talked about or the focus of treatment efforts. People fear being ostracized if they speak publically about their brain injury and might not feel comfortable seeking treatment. For example, some health officials believe that soldiers coming home from deployment “often minimize mental health symptoms for fear that admitting any problem could delay their return home” (4).

For those individuals that are seeking care for their TBI, lack of access to “quality care” options inhibits adequate treatment. Both the Department of Defense and Department of Veteran’s Affairs “have come under congressional and public scrutiny regarding their capacity to address PTSD and TBI” (4). Indeed, only half of those OEF

and OIF service-members who did receive mental health services (including those caused by TBI) are receiving quality care, or “treatment that has been demonstrated to be effective” (4). Better and accessible treatment options are necessary for the many people who are struggling with TBI and not receiving quality care.

Cost to society

The burden of TBI in America is substantial. Health costs from TBI are estimated to be approximately $35 billion per year (3). This estimate included costs for hospital care, extended care, and other medical care and services, injury-related work loss and disability, and loss of income from premature death (4). The cost per case varies according to the severity of injury. The one-year cost of mild TBI is estimated to be from $27,260 to $32,760 per case while moderate to severe TBI is estimated to be from $268,900 to $408,520 per case (4). Also, the non-quantifiable emotional burden “stemming from lost productivity, reduced quality of life, homelessness, domestic violence, the strain on families, and suicide” has a great impact on the injured person, their loved ones, and society (4). Treatment options that improve symptoms and reduce disability would alleviate the great financial and emotional burden of this complex injury.

Toxoplasma gondii

Toxoplasmosis is a common zoonosis caused by *Toxoplasma gondii* (TOXO), an intracellular protozoan parasite that can infect virtually all warm-blooded animals (9). TOXO is prevalent in most of the world and can cause disease and disability in humans (9). The prevalence of toxoplasmosis in humans is high, “with estimates of chronic infection among adults of 15%-85%, depending on geographic location” (10). TOXO is of clinical importance because eliminating this parasite might lead to better health outcomes for those infected.

Molecular basis of the disease

TOXO undergoes sexual reproduction in the intestinal epithelial cells of cats, where the parasite produces oocysts that are shed via feces into the environment (10). A variety of hosts can become infected and support the asexual life cycle phase of this parasite (10). The majority of horizontal transmissions of TOXO to humans is caused by ingestion of oocysts from food or water contaminated with feline fecal matter or ingestion of tissue cysts from a chronically infected host (9). Vertical transmission occurs when a fetus acquires congenital infection by tachyzoites via the placenta (9). Tachyzoites are haploid cells that proliferate in the initial acute phase of infection (10). In chronic infection, tachyzoites develop into a slow-growing form known as bradyzoites that are contained within cysts that persist for the life of the host (10). Active infection can occur after a latent period when cysts reinitiate and release viable parasites (12). Tissue cysts can develop in intermediate hosts as early as 6–7 days after infection (9). While TOXO is not typically thought of as a lethal parasite, the full effect of infection in humans has yet to be established.

Important clinical characteristics

While TOXO does not typically cause overt disease in healthy adults, it is an "increasingly important agent of human disease" (10). Specifically, TOXO infection can be pathogenic and lead to death in the congenitally infected and immunocompromised (10). In newly infected pregnant woman, TOXO can cross the placenta and cause devastating effects for the fetus like intellectual disability, blindness, epilepsy, and death (11).

TOXO can persist without presenting symptoms for many years and then reactivate to cause morbidity and mortality if a patient becomes immunocompromised (10). One of the most severe impacts of TOXO is in late AIDS, where up to 25% of

patients develop Toxoplasmic Encephalitis (TE) from the reactivation of a previously latent infection (12). This underscores the neurotropic nature of this parasite in humans. Although immunocompetent human hosts are usually asymptomatic, they have a life-long latent infection “in the form of quiescent tissue cysts within which the parasite apparently continues to replicate very slowly” (12).

A growing body of epidemiological data has demonstrated the role of infectious pathogens as etiological agents of some cases of chronic disease (13). Currently, the role of TOXO in the development of mental health conditions is at the focus of many clinical research studies. TOXO seropositivity is positively associated with risk for bipolar disorder. Additionally, TOXO has emerged as a prime candidate as a partial cause for schizophrenia. Studies have reported that individuals with schizophrenia, compared to controls, have a higher prevalence of antibodies to TOXO and have had greater exposure to cats in childhood (13). Furthermore, drugs used to treat the psychotic symptoms of schizophrenia are known to have antiprotozoal effects (13).

Current studies are focusing on the relationship between TOXO seropositivity and suicide. Indeed, a positive association was found between lifelong history of suicide attempts and TOXO titer (the amount of anti-*Toxoplasma* IgG antibodies) in women (14). Additionally, the prevalence of TOXO was positively associated with national suicide rates for men and women in a sample of 20 European nations (14). Lastly, the seropositivity level for TOXO among 200 people with suicide attempts was significantly higher than for 200 healthy controls (15).

Risk factors for toxoplasmosis

Clinical studies have found a variety of risk factors for TOXO infection including: owning cats, handling cat litter, increased age, being foreign-born, having low education levels, living in crowded conditions, poor hand hygiene, infrequent washing of kitchen

knives, and working in soil-related occupations (11). Ingestion of shellfish is also a strong risk factor for TOXO infection. Protective factors include vegetarianism, living at a high altitude or in an arid climate, and living in a region with frequent freezing and thawing (11). Historically, outbreaks of toxoplasmosis have been attributed to ingestion of raw or undercooked meat, consumption of unpasteurized goat's milk, and exposure to contaminated water, soil, or aerosolized soil (11).

Considering that Toxoplasma infection occurs in diverse areas around the world, the source of infection likely varies with differences in climate, culture, and eating habits. (9). In France, where many people commonly consume raw meat, pregnant women have a TOXO seroprevalence of 71 percent (11). TOXO seroprevalence is also high where environmental conditions (like temperature, humidity, and wind) favor the sporulation of oocysts in the environment (9). For example, in tropical Panama (where meat is typically well cooked), seroprevalence has been reported to be 90 percent by age 60 (11). Furthermore, in a study done in Ibadan, Nigeria, seroprevalence was reported to be 78 percent among pregnant women. These women were mostly living in overcrowded areas with poor sanitation conditions including exposure to considerable amount of cat feces (16). In summary, TOXO seroprevalence is typically high in parts of the world that have tropical climates, an abundance of cat feces, or where eating raw meat is commonplace (11). There are different strains of the parasite, which differ geographically; their prevalence could explain discrepancies in the literature concerning neuropsychiatric sequela. Environmental factors and the different habits of humans impact the epidemiology of TOXO infections (9).

Treatment and prevention

To prevent against infection from infected meat and developing Toxoplasmosis, freezing meat to -12 degrees Celsius or cooking meat to an internal temperature of 67

degrees Celsius will kill any tissue cysts present (17). In order to treat TOXO infection or to provide prophylaxis measures, a combination of chemotherapy with sulfadiazine (Sdz) and pyrimethamine (PM) is used (12). These drugs act to provide a synergistic blockade to the folate biosynthetic pathway (12). However, there are currently no drugs available that target the quiescent cysts.

TOXO is a unique target for those experiencing symptoms of psychosis. Eliminating TOXO from the body might be a useful tool to treat and prevent psychotic symptoms. Furthermore, infectious disease control efforts could be used to reduce the likelihood of TOXO entering the body. Reducing the prevalence of Toxoplasmosis would lessen the burden of TOXO on society.

What the literature does not address

What the literature does not yet address is how TOXO infection might lead to the progression of disease and disability in TBI patients. TBI can result in neuronal injury and can disrupt the blood brain barrier (18). This could make TBI patients especially at risk for chronic reactivation of TOXO infection due to the trauma of their head injury. One study has shown that increased microglial activation can be present up to 17 years after TBI, which suggests that TBI triggers a chronic inflammatory response (18). Inflammatory mediators are also produced in response to TOXO infection (14). It is unknown if TBI and TOXO exacerbate each other. A head injury might lead to the subsequent inability to keep latent TOXO infection dormant. The development of psychosis in some TBI patients and not others calls into question the role of environmental factors in the etiology of mental health disorders. To address this gap in the literature, herein we report the results of a retrospective cohort study of TOXO-linked psychosis in TBI patients. Establishing a link between TOXO and psychosis in TBI patients could lead to the development of better treatment options in the future.

Methods and Materials

To examine the relationship between prevalence of immunoglobulin G (IgG) antibodies to TOXO and neuropsychological disorders in TBI patients, data were collected from the Atlanta Veteran's Affairs Medical Center between July 2014 and March 2015. The study was a retrospective cohort type; the outcome of interest already occurred at the time the study was initiated. The population from which participants were recruited included U.S Veterans aged 18-60 with positive results on screening for TBI. Subjects were prescreened for age and TBI diagnosis using the VA electronic medical records. All subjects' medical histories were obtained by questionnaire and through Computerized Patient Record System (CPRS) chart review. Subjects were excluded if they were born outside of the U.S (because rates of TOXO infection vary somewhat by country. Furthermore, patients were excluded if they had any autoimmune diseases including HIV (this condition is known to affect response to TOXO infection), and/or significant medical illness or treatment known to affect immune status (such as rheumatoid arthritis and other autoimmune disorders). All subjects provided written informed consent before enrollment.

Diagnosis of TBI was performed by clinicians at the TBI clinic at the Atlanta VA Medical Center and based on U.S. military clinical criteria for TBI. Severity of injury was based on self-reported information and categorized as an ordinal variable with mild, moderate, and severe categorization. Mild injury was coded as 1, moderate injury was coded as 2, and severe injury was coded as 3. Mood disorders such as Major Depressive Disorder (MDD), Post Traumatic Stress Disorder (PTSD), and being high risk for suicide, were categorized as "Yes" or "No". Variables categorized as "Yes" were coded as 1 and variables coded as "No" were coded as 0. Diagnosis of these disorders were made by the Mental Health clinicians at the Atlanta VA prior to inclusion into study. A history of suicide attempts was ascertained by an Atlanta VA Mental Health clinician

(specifically described in the “suicide behavior report”) and included patients with any history of suicide attempts or suicide gestures. Psychosis was defined as any history of schizophrenia, bipolar disorder, or MDD with psychotic features. All diagnoses were confirmed by chart review of the Atlanta VA electronic medical records. According to the BIO-RAD “TOXOPLASMA IgG EIA” kit used to test anti-*Toxoplasma gondii* IgG in this study, titer greater than or equal 27 IU/mL is considered an “equivocally” TOXO seropositive and 33 IU/mL is considered “positive”. Given the sample size of this study, it was not practical to form a categorical variable for “equivocal”, thus TOXO seropositivity was defined by the authors as TOXO titer greater than or equal to 27 IU/mL.

Sociodemographic factors like age, race, and gender were also assessed. Age was grouped as 18-25, 26–30, 31–35, 36-40, 41-45, 46-50, 51-55, and 56-60 years. Race/ethnicity was based on self-reported information and categorized as non-Hispanic White, non-Hispanic Black, Hispanic White, and Asian/Pacific Islander; no other race/ethnicities or missing data were reported in this cohort. Non-Hispanic Whites were coded as 1, non-Hispanic Blacks were coded as 2, Hispanic Whites were coded as 3, Asian/Pacific Islanders were coded as 4. Lastly, males were coded as 1 and females were coded as 0.

Collection of blood samples and laboratory assays

Testing subjects for prior exposure to TOXO was accomplished using a fingerstick for capillary blood collection. Blood was collected in a capillary blood collection microtainer tube (BD365963), centrifuged, and isolated for plasma. BIO-RAD’s commercially available “TOXOPLASMA IgG EIA” (*Toxoplasma gondii* Immunoglobulin G “IgG” Enzyme- Immunosoassay) kit was used to conduct quantitative measurement of IgG class antibodies against *Toxoplasma gondii* in Human plasma. Quality control of the TOXO assay was done using known positive and negative controls provided in the kit.

This would reveal if contamination occurred and assures the accuracy of the absorbance levels found.

Statistical Analysis

Statistical analyses were conducted with SAS (version 9.4) and R (programming language). Ninety-five percent confidence limits were estimated with the exact binomial method. Where TOXO serology was defined dichotomously: Mann-Whitney-Wilcoxon test was used to assess the correlation between age on both TOXO seropositivity and each outcome variable; Pearson's Chi-Square used to assess the relationship of gender, race, and severity of head injury on both TOXO seropositivity and each outcome variable. Where TOXO exposure was defined as a continuous exposure: Spearman's R was used to assess the correlation of age and TOXO titer; ANOVA was used to assess the relationship of gender, race, and severity of head injury on TOXO titer. Logistic regression and multivariate logistic regression were used to examine the association between TOXO (both as a categorical variable and a continuous variable) and odds of neuropsychological disorders. Four models for each neuropsychological disorder outcome were examined with TOXO seropositivity as the exposure variable: a univariate model with only TOXO, a model adjusting for age, a model adjusting for severity of head injury, and a model with both age and severity of head injury. Two models for each neuropsychological disorder outcome were examined using TOXO titer as the exposure variable: a univariate model with only TOXO and a model adjusting for age. A Kologorov-Smirnov test was used to see if age was normally distributed and additional analysis explored the relationship between age and psychosis in a logistic model stratified by TOXO seropositivity and by psychosis outcome. For all analyses, a p-value of .05 from every statistic (U, Chi-Square, etc.) were considered significant.

The cohort consisted of individuals with complete data on all mood disorder diagnoses who were tested for TOXO antibody. Specifically, 100 individuals aged 18-58 years were included in the study, and 100 (100% of those interviewed) were examined for TOXO. Of the 100 individuals examined, 100 (100%) had complete neurocognitive disorder data.

Results

Demographic information of the 100 eligible TBI patients from the Atlanta Veteran's Affairs Medical Center were assessed in Table 1. The study cohort was well racially balanced (50% black, 46% white), had a high prevalence of MDD and PTSD (54% and 61% respectively), and was predominantly composed of males (88%) and mild head trauma patients (82%). Furthermore, all 5-year age categories from 18 to 55 were well represented (only 1 subject was aged 56-60) and there was a high prevalence of being high risk for suicide and having a history of psychosis (15% and 10% respectively).

A bar plot of TOXO titer for each patient was created to visualize the TOXO titer levels in this cohort. As seen in Figure 1, a TOXO seropositivity cut-off of 27 IU/mL or greater excludes many patients with high titer levels relative to the rest of the cohort. Two shoulders can be seen, at 14 IU/mL and 6 IU/mL, where TOXO titer spikes and then increases at a more constant rate.

A histogram of TOXO titer levels allows for the visualization of the frequency of titer levels. As seen in Figure 2, TOXO titer was not normally distributed and right skewed. The majority of the cohort had titer levels below 20 IU/mL.

In Table 2, statistical analyses were performed to assess the relationship of the potential confounders (age, race, severity of injury, gender) with the exposure (TOXO seropositivity) and with each neurocognitive disorder (psychosis, MDD, PTSD, and being high risk for suicide). TOXO seropositive status is a dichotomous variable; seropositive subjects (8/100) had anti-*Toxoplasma gondii* IgG titer greater than or equal to 27 IU/mL and seronegative subjects had titer less than 27 IU/mL. As seen in Table 2, a Mann-Whitney-Wilcoxon test revealed a significant association of age with TOXO seropositivity as well as with psychosis, PTSD, and MDD ($p<.05$). Furthermore, a Chi-Square test

indicated that severity of head injury (an ordinal variable) was associated with TOXO seropositivity and PTSD.

To further assess the statistically significant relationships between the covariates and outcome variables, cross tabulations were created in Table 3 and Table 4. In Table 3, a comparison of levels of head injury with TOXO seropositivity, there was an overrepresentation of severe head injury patients who are TOXO seropositive. While 50% of the patients with severe head injury (2 people) were TOXO seropositive, none of the patients with moderate head injury were TOXO seropositive, and 7.3% of those with mild TBI were seropositive. In Table 4, a comparison of levels of head injury with PTSD, there was an underrepresentation of patients with severe head injury and PTSD (0 people). An additional statistical analysis was performed and no significant relationship between the different age categories and head injury severity were found.

Four logistic regression models were created to analyze the association of TOXO seropositivity with and without confounders for each neuropsychological disorder. We examined for potential confounders and found that age and severity of injury were associated with the exposure and outcome variables. Thus, age and severity of injury were forced into the models both together and separately to account for confounding.

As presented in Table 5, crude OR reveal that TOXO seropositivity was associated with increased risk for psychosis (crude OR 3.50) and MDD (crude OR 1.46) and decreased odds for PTSD (crude OR 0.19) and being high risk for suicide (crude OR 0.80), though only the results for PTSD reached statistical significance ($p<0.05$). In models adjusted for covariates, similar trends were observed and none of the models reached statistical significance.

In Table 6, the distribution of age both for the psychosis outcome and TOXO seropositivity exposure were assessed. Age was not normally distributed among TOXO

seronegative patients (Kolmogorov-Smirnov D=0.11, p<0.01) or non-psychotic patients (Kolmogorov-Smirnov D=0.13, p<0.01).

TOXO exposure was considered and assessed as a continuous variable in Figure 3 and Tables 7 and 8. Figure 3, a scatterplot with a line of best fit, illustrates the relationship between TOXO titer and age. Figure 3 shows that age and TOXO titer are positively correlated. In Table 7, Spearman's R and ANOVA were performed to analyze the relationship of each potential confounder (age, race, severity of injury, gender) with the exposure (TOXO titer). The correlation between age and TOXO titer was assessed using a Spearman's correlation coefficient due to the abnormal distribution of these variables.

In Table 8, two logistic regression models were created to analyze the association of TOXO titer with each neuropsychological disorder outcome variable. To predict the change in odds of each outcome per one unit change in TOXO titer, two logistic regression models were created. Logistic regression Model 1 included TOXO titer with no covariates and Model 2 included TOXO titer and age. Although age was not significantly associated with TOXO titer, it was forced into Model 2 because a relationship between the two variables was evident (Figure 3). In Model 1, TOXO titer was statistically significantly associated with an increased odds of psychosis (according to both the p-value and CI). In Model 2, where age was included as a covariate, TOXO titer was marginally associated with an increased odds of psychosis (p-value .07; CI did not cross 1). These findings are in concordance with the findings that age is not a significant confounder. The relationships between TOXO titer on MDD, PTSD, and being high risk for suicide were all non-significant (p>.05).

The same trend towards an increased odds of psychosis can be seen when TOXO is analyzed a continuous exposure variable and when it is defined as a

categorical variable. The protective effect of TOXO on PTSD was only seen when TOXO was defined as a categorical exposure.

Discussion

Several remarkable findings derived from this first systematic study of Toxoplasma serology among veterans with TBI. We found a high prevalence of psychiatric morbidity among this group of veterans, and indications of a positive association between TOXO and risk for psychosis.

The prevalence of psychotic disorders in our sample (10%) was similar to what have been found in previous studies on psychosis after TBI. In a retrospective follow up study conducted 30 years post TBI, 6.7% of patients were diagnosed with psychosis (5). The prevalence of Post-traumatic Stress Disorder (PTSD) and Major Depressive Disorder (MDD) in this cohort, 61% and 54% respectively, were high for TBI patients. In the same 30 year follow up study, 26.7% of TBI patients were diagnosed with major depression (5). Other studies have demonstrated a large range of the rate of major depression from 14% to 77% and a range of PTSD from 5% to 28% (5). The prevalence of PTSD in this cohort might have been higher than what has been reported in the literature because the patients in this study were all U.S. Veteran's. The exposure to war and active combat might be contributing the high prevalence of MDD and PTSD in this specific patient population.

The finding that TOXO seropositivity is significantly associated with severity of head injury is interesting to consider. The high prevalence of TOXO seropositivity in severe head injury patients can be explained by three different hypotheses. First, evidence has indicated that TOXO seropositive people have slower reaction times and are more likely be involved in an accident (14). Having high levels of TOXO might increase the likelihood of sustaining a severe head injury and thus lead to the relationship between this type of injury and infection. Next, latent toxoplasmosis is known to effect cognition and personality (14). Those who are positive for TOXO might self-report head injuries as more severe than those who are not infected. Last, severe

head trauma might make a person more vulnerable to TOXO infection or cyst burden due to the immunomodulatory effects of TBI or behavioral factors that heighten exposure to TOXO. Immunomodulatory factors could be extended to leakiness of the blood brain barrier, enhanced reactivation of TOXO, or abnormal antibody responses. The high titer levels seen in severe head trauma patients are germane to the mechanism.

Crude OR for the association of TOXO seropositivity with psychotic disorder outcomes was 3.5, but did not reach statistical significance. After adjustment for various covariates, OR ranged from 2.6 to 4.9, although 95% confidence intervals still included 1. While we are aware of no previous studies examining this infection among people with TBI, the literature on TOXO and schizophrenia could be informative. In 54 studies which examined TOXO and mental health outcomes, 49 reported that individuals with schizophrenia and other psychoses had a higher prevalence of antibodies to TOXO when compared with controls (13).

Using 27 IU/mL as the cut-off for seropositivity, there were few patients that were considered positive for TOXO (8%). Literature review has revealed differences in the amount of titer that is considered seropositive, which is likely due in part to the use of different assay methods or kits. For example, one study investigating antibody titers and suicide attempts in patients with recurrent mood disorders defined TOXO seropositive as titer levels greater than or equal to 10 IU/mL (19).

TOXO titer was also examined as a continuous variable. In this study and larger clinical studies, TOXO tends to be right skewed, where the majority of people have very low titer levels. Some studies have used log-transformed TOXO titer levels, and considered titer itself as the exposure variable rather than determine seropositivity (20). Other analytic methods include using regression residuals to help determine cut-points for seropositivity. Logistic regression revealed a significant association of TOXO titer as

an exposure variable on psychosis prevalence. For every one unit increase in TOXO titer (a continuous variable, with no covariates in the model) the odds of psychosis increased 1.03 times (95%CI 1.00-1.06, p=0.03). When age was added to the model, the odds of psychosis stayed the same, however the p-value demonstrated only marginal significance (95%CI 1.00-1.06, p=0.07).

Strengths

We collected a purposeful sample using an a priori design that leveraged the Veteran's Affairs Computerized Patient Record System (CPRS) to extract information, including access to complete patient medical history. One of the strengths of this study is the completeness of data and lack of missing observations.

One person was responsible for collecting all the blood samples and running the TOXO immunoassays. This can have an impact on the precision of a study and likely reduced the amount of variability that would have occurred if the project passed through multiple hands. The human plasma samples did not go through any freeze-thaw cycles, which can result in a loss of detection of TOXO.

Limitations

The study was designed *a priori* based on an expected TOXO seropositive rate of at least 12%. Among the 100 patients that were recruited and eligible to participate, only 8% were seropositive. A larger cohort would have provided more power to the study, and possibly led to more conclusive results.

The psychosis variable was difficult to extract from patient medical records because of the varying features of the illness. Due to time constraints, only subjects with DSM-IV diagnoses of psychosis were considered as having psychosis in this study. This

eliminated subjects who lacked a clinical diagnosis but might have experienced symptoms of psychosis, like hallucinations or illusions.

Conclusion

In conclusion, the findings, taken in context with those from previous studies, suggest that higher TOXO titer may increase odds for psychosis. Establishing a link between TOXO and psychosis in TBI patients could lead to the development of better treatment options for TOXO or better infection control methods to reduce the prevalence of TOXO in the future.

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Tables

Table 1. Characteristics of a Cohort of U.S., Veterans, attending the Traumatic Brain Injury Clinic at the Atlanta Veteran's Affairs Medical Center

	Enrolled[a] (n=100)	
	No.	**%**
Sex		
Male	88	88.0
Female	12	12.0
Age, years		
18-25	9	9.0
26-30	27	27.0
31-35	13	13.0
36-40	17	17.0
41-45	12	12.0
46-50	10	10.0
51-55	11	11.0
56-60	1	1.0
Race/Ethnicity		
White, Non-Hispanic	46	46.0
Black, Non-Hispanic	50	50.0
White, Hispanic	3	3.0
Asian/Pacific Islander	1	1.0
Missing	0	0.0
Post-Traumatic Stress Disorder		
Yes	61	61.0
No	39	39.0
Missing	0	0.0
Major Depressive Disorder		
Yes	54	54.0
No	46	46.0
Missing	0	0.0
Psychosis		
Yes	10	10.0
No	90	90.0
Missing	0	0.0
Severity of Injury		
Mild	82	82.0
Moderate	11	11.0
Severe	4	4.0
Missing	3	3.0
High Risk for Suicide		
No	85	85.0
Yes	15	15.0
Missing	0	0.0

[a]100 patients who enrolled and met inclusion criteria were eligible

Table 2. Assessment of Potential Confounding Variables in a Model with TOXO Seropositivity[a]

	Exposure	Outcomes			
	TOXO Seropositivity	**MDD**	**PTSD**	**Suicide**	**Psychosis**
Age[b]	$U = 181.5$, $p = .018$	$U = 783$, $p = .002$	$U = 1508$, $p = .025$	$U = 554$, $p = .423$	$U = 328.5$, $p = .164$
Gender[c]	$\chi^2 = 0.00$, $p = .964$	$\chi^2 = 0.88$, $p = .348$	$\chi^2 = 0.18$, $p = .668$	$\chi^2 = 0.03$, $p = .863$	$\chi^2 = 1.52$, $p = .218$
Race[d]	$\chi^2 = 2.44$, $p = .486$	$\chi^2 = 1.9$, $p = .594$	$\chi^2 = 2.69$, $p = .442$	$\chi^2 = 1.33$, $p = .723$	$\chi^2 = 0.47$, $p = .926$
Severity of Injury[e]	$\chi^2 = 10.30$, $p = .006$	$\chi^2 = 1.49$, $p = .475$	$\chi^2 = 6.48$, $p = .039$	$\chi^2 = 0.63$, $p = .730$	$\chi^2 = 1.81$, $p = .404$

[a] TOXO seropositive is defined as anti-*Toxoplasma gondii* IgG titer greater than or equal to 27 IU/mL

[b] Mann-Whitney-Wilcoxin Test used to assess age

[c,d,e] Pearson's Chi-Square used to assess gender, race, and severity of Injury

Table 3. The Relationship Between TOXO Seropositivity and Severity of Head Injury

	Severity of Injury			
	Mild	**Moderate**	**Severe**	**Total**
TOXO+[a]				
No	76	11	2	89
Yes	6	0	2	8
Total	82	11	4	97

[a] TOXO+(TOXO Seropositive) is defined as anti-*Toxoplasma gondii* IgG titer greater than or equal to 27 IU/mL

Table 4. The Relationship Between PTSD and Severity of Head Injury

	Severity of Injury			
	Mild	**Moderate**	**Severe**	**Total**
PTSD				
No	30	4	4	38
Yes	52	7	0	59
Total	82	11	4	97

Table 5. The Association of TOXO Seropositivity with Patient Outcome Characteristics in Two Models, Assessed with Logistic Regression

	Model 1. TOXO Seropositivity[a], no covariates				Model 2. TOXO Seropositivity and Age				Model 3. TOXO Seropositivity and Severity of Injury				Model 4. TOXO Seropositivity, Severity of Injury, and Age			
Characteristics	**Odds[b]**	**95% CI**	***p*-Value**	**Nagelkerke R^2**	**Odds[c]**	**95% CI**	***p*-Value**	**Nagelkerke R^2**	**Odds[d]**	**95% CI**	***p*-Value**	**Nagelkerke R^2**	**Odds[e]**	**95% CI**	***p*-Value**	**Nagelkerke R^2**
Psychosis	3.50	(0.46, 18.43)	0.162	0.0344	2.63	(0.96, 1.12)	0.301	0.0555	4.93	(0.61, 30.58)	0.094	0.1211	2.93	(0.32, 2.05)	0.291	0.1657
MDD	1.46	(0.33, 7.47)	0.617	0.0034	1.07	(0.17, 4.40)	0.790	0.1249	1.69	(0.37, 9.38)	0.509	0.0196	0.99	(0.19, 6.22)	0.991	0.1543
PTSD	0.19	(0.03, 0.86)	0.047	0.0618	0.26	(0.91, 1.00)	0.118	0.1080	0.21	(0.17, 1.16)	0.075	0.0980	0.30	(0.04, 1.55)	0.177	0.1420
High Risk for Suicide	0.80	(0.49, 4.99)	0.084	0.0008	0.67	(0.03, 4.46)	0.717	0.0101	0.75	(0.17, 1.16)	0.801	0.0980	0.65	(0.03, 4.49)	0.706	0.0087

[a] TOXO seropositive is defined as anti-*Toxoplasma gondii* IgG titer greater than or equal to 27 IU/mL

[b] Difference in odds of each characteristic comparing TOXO+ to TOXO-

[c,d,e] Difference in odds of each characteristic comparing TOXO+ to TOXO-, after controlling for the confounding effects of the covariates in the model

Table 6. Summary Statistics for Age for All Eligible Subjects, Stratified on Psychosis Outcome, and Stratified on TOXO Exposure

		Psychosis Outcome		TOXO Exposure	
	All	**Psychosis[a]**	**No Psychosis**	**TOXO+[b]**	**TOXO-**
n	100	10	90	8	92
mean	36.63	40.40	36.21	44.50	35.95
s.d.	9.54	9.40	9.51	7.63	9.41
median	36	41	35	45	34
minimum	21	28	21	37	21
maximum	58	53	58	58	53
Skewness	0.30	-0.01	0.34	0.69	0.35
Kurtosis	-1.10	-1.68	-1.04	-0.27	-1.15
Kolmogorov-Smirnov Test for Normality					
Statistic	0.12	0.19	0.11	0.22	0.13
p-value	<0.010	>0.150	<0.010	>0.150	<0.010

[a]Psychosis includes bipolar disorder, schizophrenia, and Major Depressive Disorder with Psychotic Features

[b]TOXO+ have anti-Toxoplasma gondii IgG titer greater than or equal to 27 IU/mL ; TOXO- have anti-Toxoplasma gondii IgG titer less than 27 IU/mL

Table 7. Assessment of Potential Confounding Variables in a Model with TOXO Titer

	Degrees of freedom	**Sum Square**	**Mean Square**	**Statistic[a]**	***p* value**
Variable				0.05	0.645
Age					
Race	1	509	509	2.03	0.158
Residuals	98	24612	251		
Severity of Injury	1	337	337	1.29	0.258
Residuals	95	24727	261		
Gender	1	72	72	0.28	0.597
Residuals	98	25049	256		

[a] Spearman's R statistic used to assess age; ANOVA F value used to assess Gender, Race, and Severity of Injury

Table 8. The Association of TOXO Titer (IU/mL) with Patient Characteristics, in Two Models, Assessed with Logistic Regression

Characteristic	Model 1. TOXO Titer, no covariates				Model 2. TOXO Titer and Age			
	Odds[a]	95% CI	*p*-Value	Nagelkerke R^2	Odds[b]	95% CI	*p*-Value	Nagelkerke R^2
Psychosis	1.03	(1.00, 1.06)	0.0285	0.086	1.03	(1.00, 1.06)	0.0704	0.097
MDD	1.01	(0.98, 1.03)	0.6589	0.003	1.00	(0.97, 1.03)	0.7834	0.086
PTSD	0.98	(0.95,1.00)	0.1207	0.036	0.98	(0.95,1.01)	0.2660	0.090
High Risk for Suicide	0.99	(0.94,1.03)	0.0779	0.001	0.99	(0.94,1.02)	0.6737	0.011

[a]Change in the odds of each characteristic per 1 unit change in TOXO titer

[b]Change in the odds of each characteristic per 1 unit change in TOXO titer after controlling for age

Figures

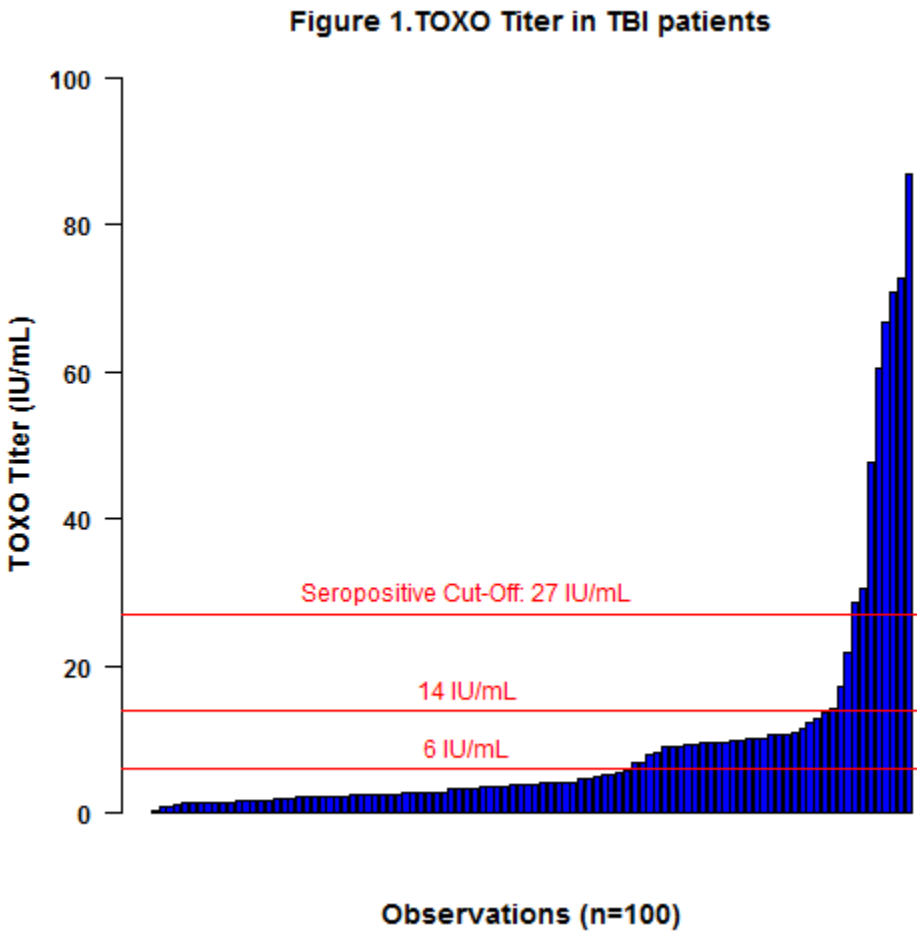

Figure 1. A bar graph of TOXO titer in a cohort of 100 veteran's attending the TBI clinic at the Atlanta Veteran's Affairs Medical Center 2014-2015.

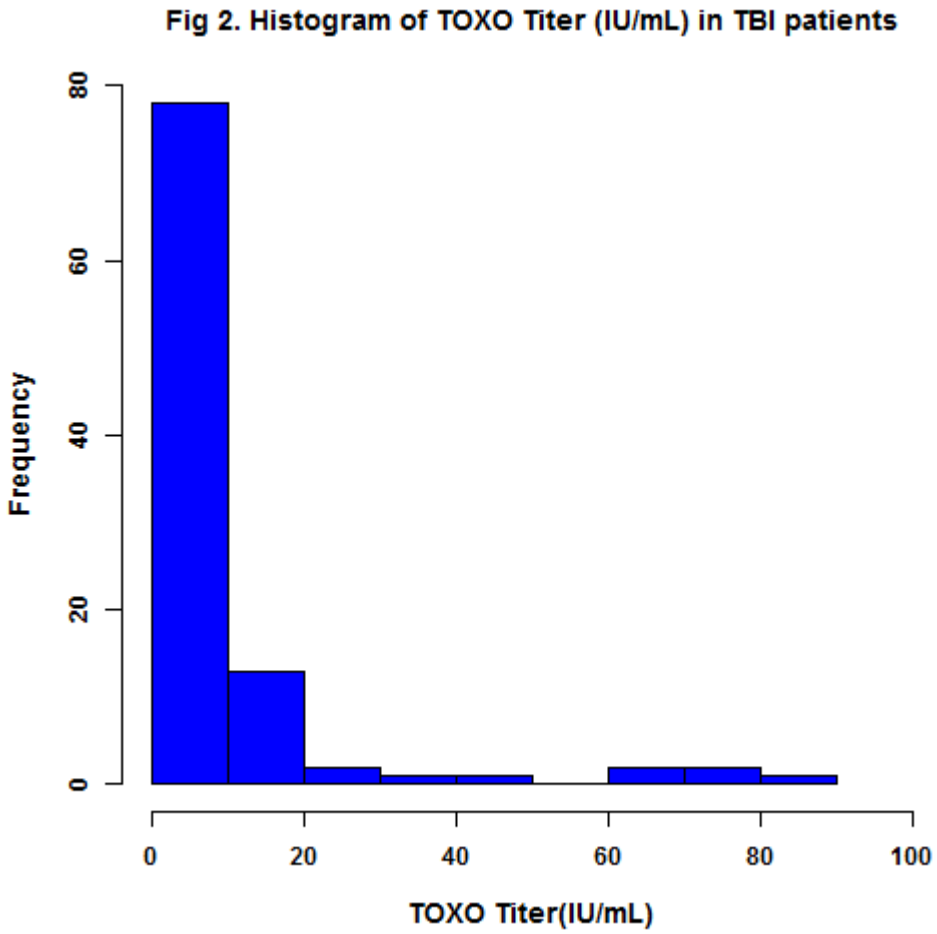

Figure 2. A histogram illustrating the frequency of TOXO titer in a cohort of 100 veteran's attending the TBI clinic at the Atlanta Veteran's Affairs Medical Center 2014-2015.

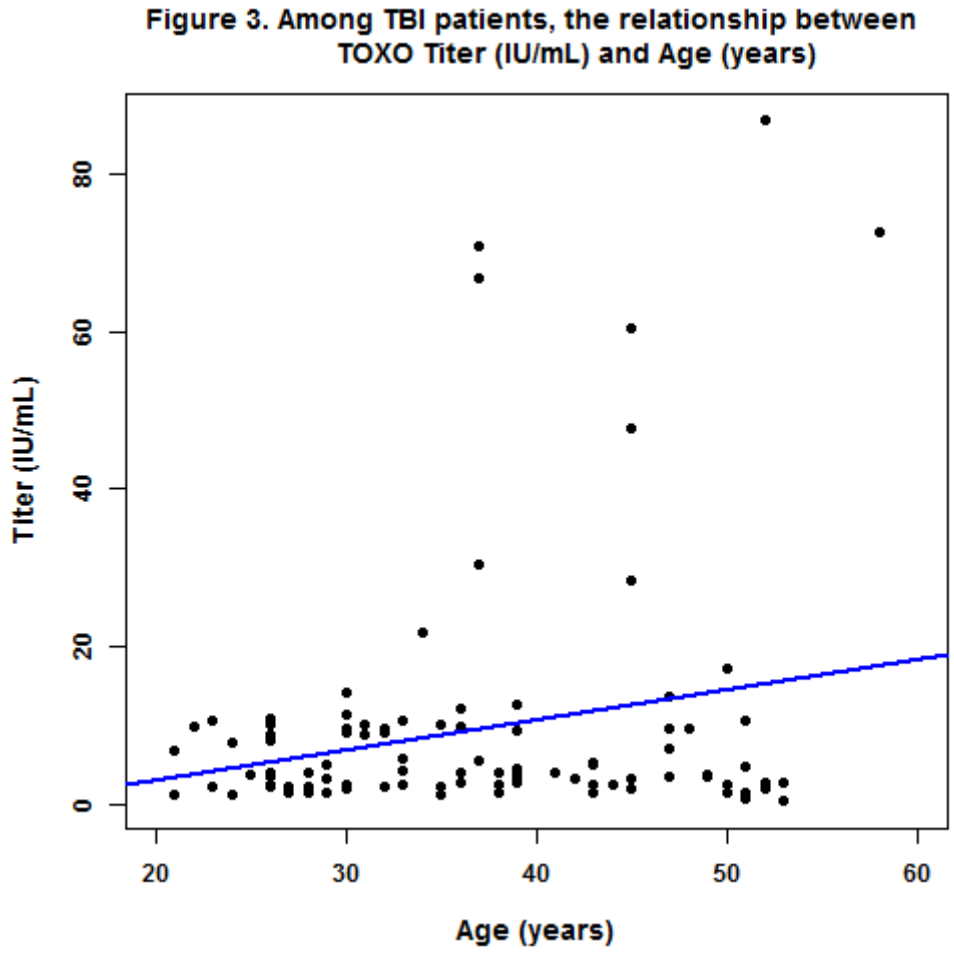

Figure 3. A scatterplot with a line of best fit illustrates the positive relationship between TOXO titer and age in a cohort of 100 veteran's attending the TBI clinic at the Atlanta Veteran's Affairs Medical Center 2014-2015.

Appendices

R code output

#Logistic Regression Models

#Logistic Models with MDD as outcome variable

#Exposure= seropositivity, Outcome= MDD

```
> glm1 <- glm(MDD ~ toxoresults, data = TOXO2, family = "binomial")
> summary(glm1)$coef
             Estimate Std. Error   z value  Pr(>|z|)
(Intercept) 0.1306202  0.2089593 0.6250988 0.5319062
toxoresults 0.3802054  0.7596034 0.5005315 0.6167009
> exp(glm1$coef[2])
toxoresults
   1.462585
> exp(confint(glm1))
Waiting for profiling to be done...
                2.5 %   97.5 %
(Intercept) 0.7568879 1.721934
toxoresults 0.3387885 7.466512
> NagelkerkeR2(glm1)
$N
[1] 100

$R2
[1] 0.003419498
```

#Exposure= seropositivity, age Outcome= MDD

```
> glm2 <- glm(MDD ~ toxoresults + age, data = TOXO2, family = "binomial
")
> summary(glm2)$coef
                Estimate Std. Error   z value    Pr(>|z|)
(Intercept) -2.40529322 0.88138369 -2.728997 0.006352733
toxoresults -0.21470220 0.80659624 -0.266183 0.790098287
age          0.07116478 0.02422687  2.937432 0.003309432
> exp(glm2$coef["toxoresults"])
toxoresults
  0.8067817
> exp(glm2$coef["age"])
     age
1.073758
> exp(confint(glm2))
Waiting for profiling to be done...
                 2.5 %   97.5 %
(Intercept) 0.01494886 0.483703
toxoresults 0.16711333 4.401001
age         1.02569476 1.128654
> NagelkerkeR2(glm2)
$N
[1] 100

$R2
[1] 0.1249075
```

Exposure= seropositivity, severity of injury Outcome= MDD

```
> glm19 <- glm(MDD ~ toxoresults + severity, data = TOXO2, family = "bi
nomial")
> summary(glm19)$coef
             Estimate Std. Error    z value  Pr(>|z|)
(Intercept)  0.7082441  0.5478374  1.2927998 0.1960803
toxoresults  0.5247169  0.7937704  0.6610437 0.5085843
severity    -0.4713222  0.4332261 -1.0879359 0.2766234
> exp(glm19$coef["toxoresults"])
toxoresults
    1.68998
> exp(confint(glm19))
Waiting for profiling to be done...
                2.5 %   97.5 %
(Intercept) 0.7011852 6.161761
toxoresults 0.3729779 9.384780
severity    0.2543423 1.444100
> NagelkerkeR2(glm19)
$N
[1] 97

$R2
[1] 0.01962848
```

Exposure= seropositivity, severity of injury, age Outcome= MDD

```
> glm23 <- glm(MDD ~ toxoresults + severity +age, data = TOXO2, family
= "binomial")
> summary(glm23)$coef
                Estimate Std. Error     z value    Pr(>|z|)
(Intercept) -1.837344396 0.98918393 -1.85743454 0.063249356
toxoresults -0.009874587 0.86189359 -0.01145685 0.990858955
severity    -0.658853170 0.47855232 -1.37676309 0.168585505
age          0.077079225 0.02530644  3.04583416 0.002320358
> exp(glm23$coef["toxoresults"])
toxoresults
   0.990174
> exp(confint(glm23))
Waiting for profiling to be done...
                2.5 %   97.5 %
(Intercept) 0.02142349 1.071822
toxoresults 0.18888457 6.222988
severity    0.19093499 1.291429
age         1.02989282 1.138147
> NagelkerkeR2(glm23)
$N
[1] 97

$R2
[1] 0.1542532
```

#Switching from seropositivity to titer as the exposure

Exposure= titer, Outcome= MDD

```
> glm9 <- glm(MDD ~ Titer, data = TOXO2, family = "binomial")
> summary(glm9)$coef
               Estimate Std. Error   z value  Pr(>|z|)
(Intercept) 0.106511819 0.23412927 0.4549274 0.6491615
Titer       0.005734301 0.01298991 0.4414425 0.6588927
> exp(glm9$coef["Titer"])
   Titer
1.005751
> exp(confint(glm9))
```

```
Waiting for profiling to be done...
                2.5 %   97.5 %
(Intercept) 0.7025455 1.764962
Titer       0.9808122 1.034432
> NagelkerkeR2(glm9)
$N
[1] 100

$R2
```

#Exposure= titer,age Outcome= MDD

```
> glm13 <- glm(MDD ~ Titer+ age, data = TOXO2, family = "binomial")
> summary(glm13)$coef
                 Estimate Std. Error    z value    Pr(>|z|)
(Intercept) -2.383636104 0.87068740 -2.7376486 0.006188017
Titer       -0.003849758 0.01400370 -0.2749101 0.783385294
age          0.071097206 0.02412696  2.9467955 0.003210855
> exp(glm13$coef["Titer"])
    Titer
0.9961576
> exp(confint(glm13))
Waiting for profiling to be done...
                 2.5 %    97.5 %
(Intercept) 0.01562429 0.4845599
Titer       0.96949862 1.0263794
age         1.02581636 1.1283350
> NagelkerkeR2(glm8)
$N
[1] 100

$R2
[1] 0.03440595
```

#Logistic Models with PTSD as outcome variable

#Exposure= seropositivity, Outcome= PTSD

```
> glm3 <- glm(PTSD ~ toxoresults, data = TOXO2, family = "binomial")
> summary(glm3)$coef
             Estimate Std. Error   z value    Pr(>|z|)
(Intercept)  0.5810299  0.2173757  2.672930 0.007519198
toxoresults -1.6796422  0.8449365 -1.987892 0.046823681
> exp(glm3$coef["toxoresults"])
toxoresults
  0.1864407
> exp(confint (glm3))
Waiting for profiling to be done...
                 2.5 %    97.5 %
(Intercept) 1.17576565 2.7658261
toxoresults 0.02627998 0.8613041
> NagelkerkeR2(glm3)
$N
[1] 100

$R2
[1] 0.06176382
```

#Exposure= seropositivity,age Outcome= PTSD

```
>
> glm4 <- glm(PTSD ~ toxoresults + age, data = TOXO2, family = "binomia
l")
> summary(glm4)$coef
              Estimate Std. Error   z value   Pr(>|z|)
(Intercept)  2.17565488 0.88569441  2.456440 0.01403214
toxoresults -1.35187339 0.86574002 -1.561524 0.11840028
age         -0.04375051 0.02323377 -1.883057 0.05969264
> exp(glm4$coef["toxoresults"])
toxoresults
  0.2587551
> exp(glm4$coef["age"])
      age
0.9571927
> exp(confint(glm4))
Waiting for profiling to be done...
                 2.5 %    97.5 %
(Intercept) 1.62172683 53.361380
toxoresults 0.03551261  1.259553
age         0.91347822  1.001183
> NagelkerkeR2(glm4)
$N
[1] 100

$R2
[1] 0.1080329
```

#Exposure= seropositivity, severity of injury Outcome= PTSD

```
> glm20 <- glm(PTSD ~ toxoresults + severity, data = TOXO2, family = "b
inomial")
> summary(glm20)$coef
              Estimate Std. Error   z value   Pr(>|z|)
(Intercept)  1.4617331  0.6064111  2.410466 0.01593217
toxoresults -1.5412081  0.8657674 -1.780164 0.07504908
severity    -0.7499765  0.4773123 -1.571249 0.11612481
> exp(glm20$coef["toxoresults"])
toxoresults
  0.2141223
> exp(confint(glm20))
Waiting for profiling to be done...
                 2.5 %    97.5 %
(Intercept) 1.37305425 15.207495
toxoresults 0.02941677  1.049708
severity    0.17147691  1.161323
> NagelkerkeR2(glm20)
```

Exposure= seropositivity, severity of injury, age Outcome= PTSD

```
> glm24 <- glm(PTSD ~ toxoresults + severity +age, data = TOXO2, family
= "binomial")
> summary(glm24)$coef
              Estimate Std. Error   z value   Pr(>|z|)
(Intercept)  2.99682114 1.05990794  2.827435 0.00469225
toxoresults -1.19605033 0.88606081 -1.349851 0.17706369
severity    -0.69361927 0.48087018 -1.442425 0.14918245
age         -0.04374405 0.02383789 -1.835064 0.06649626
> exp(glm24$coef["toxoresults"])
toxoresults
  0.3023862
> exp(confint(glm24))
Waiting for profiling to be done...
```

```
                 2.5 %     97.5 %
(Intercept) 2.71369194 179.403096
toxoresults 0.04044317   1.554321
severity    0.18158188   1.246903
age         0.91236052   1.002381
> NagelkerkeR2(glm24)
$N
[1] 97

$R2
[1] 0.1419785
```

#Switching from seropositivity to titer as the exposure

#Exposure= titer, Outcome= PTSD

```
> glm10 <- glm(PTSD ~ Titer, data = TOXO2, family = "binomial")
> summary(glm10)$coef
               Estimate Std. Error   z value   Pr(>|z|)
(Intercept)  0.65244189 0.24376889  2.676477 0.00744006
Titer       -0.02139722 0.01378852 -1.551814 0.12070666
> exp(glm10$coef["Titer"])
    Titer
0.9788301
> exp(confint(glm10))
Waiting for profiling to be done...
                2.5 %   97.5 %
(Intercept) 1.2006833 3.134117
Titer       0.9490243 1.004190
> NagelkerkeR2(glm10)
$N
[1] 100

$R2
[1] 0.03575108
```

Exposure= titer, age Outcome= PTSD

```
> glm14 <- glm(PTSD ~ Titer+ age, data = TOXO2, family = "binomial")
> summary(glm14)$coef
               Estimate Std. Error   z value    Pr(>|z|)
(Intercept)  2.32698375 0.87824150  2.649594 0.008058846
Titer       -0.01598766 0.01437348 -1.112302 0.266008114
age         -0.04660801 0.02308474 -2.018996 0.043487603
> exp(glm14$coef["Titer"])
    Titer
0.9841395
> exp(confint(glm14))
Waiting for profiling to be done...
                2.5 %    97.5 %
(Intercept) 1.9224407 61.427622
Titer       0.9531663  1.010790
age         0.9110903  0.997976
> NagelkerkeR2(glm14)
$N
[1] 100

$R2
```

```
[1] 0.09007026
```

#Logistic Models with psychosis as outcome variable

#Exposure= seropositivity, Outcome= psychosis

```
> glm5 <- glm(psych ~ toxoresults, data = TOXO2, family = "binomial")
> summary(glm5)$coef
             Estimate Std. Error   z value     Pr(>|z|)
(Intercept) -2.351375  0.3700064 -6.354958 2.084832e-10
toxoresults  1.252763  0.8964214  1.397516 1.622586e-01
> exp(glm5$coef["toxoresults"])
toxoresults
        3.5
> exp(confint(glm5))
Waiting for profiling to be done...
                  2.5 %     97.5 %
(Intercept) 0.04243802  0.1845605
toxoresults 0.46214976 18.4322404
> NagelkerkeR2(glm5)
$N
[1] 100

$R2
[1] 0.03440595
```

#Exposure= seropositivity,age Outcome= psychosis

```
> glm7 <- glm(psych ~ toxoresults + age, data = TOXO2, family = "binomi
al")
> summary(glm7)$coef
               Estimate Std. Error   z value  Pr(>|z|)
(Intercept) -3.74381156 1.47297080 -2.541674 0.0110323
toxoresults  0.96730795 0.93588297  1.033578 0.3013335
age          0.03731424 0.03683422  1.013032 0.3110449
> exp(glm7$coef["toxoresults"])
toxoresults
   2.630853
> exp(confint(glm7))
Waiting for profiling to be done...
                   2.5 %     97.5 %
(Intercept) 0.0009949268  0.3598306
toxoresults 0.3268704899 15.0796973
age         0.9651386186  1.1185478
> NagelkerkeR2(glm7)
$N
[1] 100

$R2
[1] 0.05545897
```

#Exposure= seropositivity, severity of injury Outcome= psychosis

```
> glm18 <- glm(psych ~ toxoresults + severity, data = TOXO2, family = "
binomial")
```

```
Warning message:
glm.fit: fitted probabilities numerically 0 or 1 occurred
> summary(glm18)$coef
             Estimate   Std. Error      z value   Pr(>|z|)
(Intercept)  14.218384 2204.9068832  0.006448519 0.99485486
toxoresults   1.595049    0.9525492  1.674505891 0.09403122
severity    -16.506580 2204.9067762 -0.007486294 0.99402686
> exp(glm18$coef["toxoresults"])
toxoresults
   4.928571
> exp(confint(glm18))
Waiting for profiling to be done...
                    2.5 %       97.5 %
(Intercept) 3.884834e-84           NA
toxoresults 6.075944e-01 3.058330e+01
severity              NA 8.489482e+65
There were 31 warnings (use warnings() to see them)
> NagelkerkeR2(glm18)
$N
[1] 97

$R2
[1] 0.1211284
```

Exposure= seropositivity, severity of injury, age Outcome= psychosis

```
> glm22 <- glm(psych ~ toxoresults + severity +age, data = TOXO2, famil
y = "binomial")
Warning message:
glm.fit: fitted probabilities numerically 0 or 1 occurred
> summary(glm22)$coef
               Estimate   Std. Error      z value  Pr(>|z|)
(Intercept)  12.26020088 2175.1032986  0.005636606 0.9955027
toxoresults   1.07609723    1.0180751  1.056991961 0.2905153
severity    -16.79872756 2175.1027556 -0.007723188 0.9938378
age           0.05993483    0.0415399  1.442825621 0.1490696
> exp(glm22$coef["toxoresults"])
toxoresults
    2.93321
> exp(confint(glm22))
Waiting for profiling to be done...
                    2.5 %       97.5 %
(Intercept) 2.610486e-74           NA
toxoresults 3.216580e-01 2.046962e+01
severity              NA 4.094509e+71
age         9.796209e-01 1.157333e+00
There were 43 warnings (use warnings() to see them)
> NagelkerkeR2(glm22)
$N
[1] 97

$R2
[1] 0.1657317
```

#Switching from seropositivity to titer as the exposure

>#Exposure= titer, age Outcome= psychosis

```
> glm12 <- glm(psych ~ Titer + age, data = TOXO2, family = "binomial")
> summary(glm12)$coef
              Estimate Std. Error    z value   Pr(>|z|)
(Intercept) -3.65929222 1.47957271 -2.4732088 0.01339059
Titer        0.02738139 0.01513287  1.8093980 0.07038920
age          0.02893939 0.03793473  0.7628732 0.44553902
```

```
> exp(glm12$coef["Titer"])
  Titer
1.02776
> exp(confint(glm12))
Waiting for profiling to be done...
                  2.5 %    97.5 %
(Intercept) 0.001066045 0.3970378
Titer       0.996313344 1.0594871
age         0.954241469 1.1109894
> NagelkerkeR2(glm12)
$N
[1] 100

$R2
[1] 0.09733198
```

#Logistic Models with suicide as outcome variable

#Exposure= seropositivity, Outcome= suicide

```
> glm6 <- glm(Suicide ~ toxoresults, data = TOXO2, family = "binomial")
> summary(glm6)$coef
             Estimate Std. Error    z value     Pr(>|z|)
(Intercept) -1.7176515  0.2902569 -5.9176934 3.264878e-09
toxoresults -0.2282587  1.1077442 -0.2060572 8.367462e-01
> exp(glm6$coef["toxoresults"])
toxoresults
  0.7959184
> exp(confint(glm6))
Waiting for profiling to be done...
                 2.5 %    97.5 %
(Intercept) 0.09740539 0.3066245
toxoresults 0.04093605 4.9868400
> NagelkerkeR2(glm6)
$N
[1] 100

$R2
[1] 0.0007810607
```

> >#Exposure= seropositivity,age Outcome= suicide

```
> glm8 <- glm(Suicide ~ toxoresults + age, data = TOXO2, family = "bino
mial")
> summary(glm8)$coef
               Estimate Std. Error    z value   Pr(>|z|)
(Intercept) -2.52243861 1.15418495 -2.1854718 0.02885427
toxoresults -0.41081571 1.13540176 -0.3618241 0.71748349
age          0.02197763 0.02994933  0.7338269 0.46305427
> exp(glm8$coef["toxoresults"])
toxoresults
  0.6631091
> exp(confint(glm8))
Waiting for profiling to be done...
                 2.5 %    97.5 %
(Intercept) 0.007354096 0.7180684
toxoresults 0.033098663 4.4593552
age         0.963042233 1.0847450
> NagelkerkeR2(glm8)
$N
[1] 100

$R2
```

```
[1] 0.01013873
```

#Exposure= seropositivity, severity of injury Outcome= suicide

```
>
> glm21 <- glm(Suicide ~ toxoresults + severity, data = TOXO2, family =
"binomial")
> summary(glm21)$coef
              Estimate Std. Error     z value   Pr(>|z|)
(Intercept) -1.73533427  0.7440176 -2.33238339 0.01968053
toxoresults -0.28409545  1.1270056 -0.25207990 0.80097931
severity     0.04856757  0.5828371  0.08332958 0.93358948
> exp(glm21$coef["toxoresults"])
toxoresults
  0.7526948
> exp(confint(glm20))
Waiting for profiling to be done...
                2.5 %    97.5 %
(Intercept) 1.37305425 15.207495
toxoresults 0.02941677  1.049708
severity    0.17147691  1.161323
> NagelkerkeR2(glm20)
$N
[1] 97

$R2
[1] 0.09800905
```

Exposure= seropositivity, severity of injury, age Outcome= suicide

```
> glm25 <- glm(Suicide ~ toxoresults + severity+age, data = TOXO2, fami
ly = "binomial")
> summary(glm25)$coef
              Estimate Std. Error     z value   Pr(>|z|)
(Intercept) -2.41485427 1.29572282 -1.86371207 0.06236215
toxoresults -0.43151968 1.14506441 -0.37685188 0.70628368
severity     0.01529249 0.59064388  0.02589122 0.97934410
age          0.01956116 0.03014976  0.64880000 0.51646765
> exp(glm25$coef["toxoresults"])
toxoresults
  0.6495213
> exp(confint(glm25))
Waiting for profiling to be done...
                 2.5 %   97.5 %
(Intercept) 0.006544153 1.144473
toxoresults 0.032087306 4.490544
severity    0.252747735 2.903411
age         0.960479710 1.082762
> NagelkerkeR2(glm25)
$N
[1] 97

$R2
[1] 0.008699613
```

#Switching from seropositivity to titer as the exposure

Exposure= titer, Outcome= suicide

```
> glm11 <- glm(Suicide ~ Titer, data = TOXO2, family = "binomial")
> summary(glm11)$coef
               Estimate Std. Error    z value     Pr(>|z|)
(Intercept) -1.684853277 0.32693960 -5.1534084 2.557941e-07
```

```
Titer       -0.005520454 0.01967841 -0.2805336 7.790681e-01
> exp(glm11$coef["Titer"])
    Titer
0.9944948
> exp(confint(glm11))
Waiting for profiling to be done...
                2.5 %    97.5 %
(Intercept) 0.09402966 0.3432646
Titer       0.94331986 1.0267422
> NagelkerkeR2(glm11)
$N
[1] 100

$R2
[1] 0.001485971
```

> > > **#Exposure= titer, age Outcome= suicide**

```
> glm15 <- glm(Suicide ~ Titer+ age, data = TOXO2, family = "binomial")
> summary(glm15)$coef
               Estimate Std. Error    z value   Pr(>|z|)
(Intercept) -2.484800460 1.14535064 -2.1694670 0.03004724
Titer       -0.008308737 0.01973178 -0.4210840 0.67369377
age          0.022162540 0.02982594  0.7430625 0.45744382
> exp(glm15$coef["Titer"])
    Titer
0.9917257
> exp(confint(glm15))
Waiting for profiling to be done...
                 2.5 %    97.5 %
(Intercept) 0.007747557 0.7298105
Titer       0.941456337 1.0247426
age         0.963527213 1.0847632
> NagelkerkeR2(glm15)
$N
[1] 100

$R2
[1] 0.01108399
```

#Assessing the relationship between suicide and gender
#Exposure= suicide, Outcome= gender

```
> glm7 <- glm(gender ~ Suicide, data = TOXO2, family = "binomial")
> summary(glm7)$coef
             Estimate Std. Error    z value     Pr(>|z|)
(Intercept)  2.0149030  0.3366465  5.9852183 2.160998e-09
Suicide     -0.1431008  0.8308145 -0.1722416 8.632476e-01
> exp(glm7$coef["Suicide"])
  Suicide
0.8666667
> exp(confint(glm7))
Waiting for profiling to be done...
                2.5 %    97.5 %
(Intercept) 4.0715769 15.456563
Suicide     0.1979304  6.056035
 [1] 0.002658585
```

#Finding Spearman correlation coefficients between age and outcome variables

```
> rcorr(TOXO2$age, TOXO2$MDD, type="spearman")
     x    y
```

```
x 1.00 0.32
y 0.32 1.00

n= 100


P
  x      y
x        0.0012
y 0.0012
> rcorr(TOXO2$age, TOXO2$PTSD, type="spearman")
      x     y
x  1.00 -0.23
y -0.23  1.00

n= 100


P
  x      y
x        0.0235
y 0.0235
> rcorr(TOXO2$age, TOXO2$Suicide, type="spearman")
     x    y
x 1.00 0.08
y 0.08 1.00

n= 100


P
  x      y
x        0.4225
y 0.4225
> rcorr(TOXO2$age, TOXO2$psych, type="spearman")
     x    y
x 1.00 0.14
y 0.14 1.00

n= 100


P
  x      y
x        0.1634
y 0.1634
> rcorr(TOXO2$age, TOXO2$toxoresults, type="spearman")
     x    y
x 1.00 0.24
y 0.24 1.00

n= 100
```

```
> rcorr(TOXO2$age, TOXO2$Titer, type="spearman")
     x    y
x 1.00 0.05
y 0.05 1.00

n= 100


P
  x      y
x        0.6452
y 0.6452
```

#Assessment of Potential Confounding Variables in a Model with TOXO Titer using ANOVA

```
Analysis of Variance Table

Response: Titer
          Df  Sum Sq Mean Sq F value Pr(>F)
racecat    1   508.9  508.89  2.0263 0.1578
Residuals 98 24611.6  251.14
```

```
Response: Titer
          Df  Sum Sq Mean Sq F value Pr(>F)
severity   1   336.8  336.83  1.2941 0.2582
Residuals 95 24726.5  260.28
```

```
Response: Titer
          Df Sum Sq Mean Sq F value Pr(>F)
gender     1     72  71.993  0.2817 0.5968
Residuals 98  25049 255.597
```

 # Pearson's Chi-Square used to assess the relationship of gender, race, and severity of head injury on both TOXO seropositivity and each outcome variable, *note SAS was used for final chi-square test analysis (with no Yates' continuity correction)

```
Pearson's Chi-squared test with Yates' continuity correction

data:  TOXO2$gender and TOXO2$toxoresults
X-squared = 0, df = 1, p-value = 1

>
> chsq_gender2 <- chisq.test(TOXO2$gender, TOXO2$MDD)
> chsq_gender2

        Pearson's Chi-squared test with Yates' continuity correction

data:  TOXO2$gender and TOXO2$MDD
X-squared = 0.3966, df = 1, p-value = 0.5288

>
> chsq_gender3 <- chisq.test(TOXO2$gender, TOXO2$PTSD)
Warning message:
```

```
In chisq.test(TOXO2$gender, TOXO2$PTSD) :
  Chi-squared approximation may be incorrect
> chsq_gender3

        Pearson's Chi-squared test with Yates' continuity correction

data:  TOXO2$gender and TOXO2$PTSD
X-squared = 0.0129, df = 1, p-value = 0.9096

>
> chsq_gender4 <- chisq.test(TOXO2$gender, TOXO2$Suicide)
Warning message:
In chisq.test(TOXO2$gender, TOXO2$Suicide) :
  Chi-squared approximation may be incorrect
> chsq_gender4

        Pearson's Chi-squared test with Yates' continuity correction

data:  TOXO2$gender and TOXO2$Suicide
X-squared = 0, df = 1, p-value = 1

>
> chsq_race1 <- chisq.test(TOXO2$racecat, TOXO2$toxoresults)
Warning message:
In chisq.test(TOXO2$racecat, TOXO2$toxoresults) :
  Chi-squared approximation may be incorrect
> chsq_race1

        Pearson's Chi-squared test

data:  TOXO2$racecat and TOXO2$toxoresults
X-squared = 2.4429, df = 3, p-value = 0.4857

>
> chsq_race2 <- chisq.test(TOXO2$racecat, TOXO2$MDD)
Warning message:
In chisq.test(TOXO2$racecat, TOXO2$MDD) :
  Chi-squared approximation may be incorrect
> chsq_race2

        Pearson's Chi-squared test

data:  TOXO2$racecat and TOXO2$MDD
X-squared = 1.897, df = 3, p-value = 0.5941

>
> chsq_race3 <- chisq.test(TOXO2$racecat, TOXO2$PTSD)
Warning message:
In chisq.test(TOXO2$racecat, TOXO2$PTSD) :
  Chi-squared approximation may be incorrect
> chsq_race3

        Pearson's Chi-squared test

data:  TOXO2$racecat and TOXO2$PTSD
X-squared = 2.6886, df = 3, p-value = 0.4422

>
> chsq_race4 <- chisq.test(TOXO2$racecat, TOXO2$Suicide)
Warning message:
In chisq.test(TOXO2$racecat, TOXO2$Suicide) :
  Chi-squared approximation may be incorrect
> chsq_race4
```

```
        Pearson's Chi-squared test

data:  TOXO2$racecat and TOXO2$Suicide
X-squared = 1.3256, df = 3, p-value = 0.7231

>
> chsq_severity1 <- chisq.test(TOXO2$Severity, TOXO2$toxoresults)
Warning message:
In chisq.test(TOXO2$Severity, TOXO2$toxoresults) :
  Chi-squared approximation may be incorrect
> chsq_severity1

        Pearson's Chi-squared test

data:  TOXO2$Severity and TOXO2$toxoresults
X-squared = 10.8563, df = 3, p-value = 0.01253

>
> chsq_severity2 <- chisq.test(TOXO2$Severity, TOXO2$MDD)
Warning message:
In chisq.test(TOXO2$Severity, TOXO2$MDD) :
  Chi-squared approximation may be incorrect
> chsq_severity2

        Pearson's Chi-squared test

data:  TOXO2$Severity and TOXO2$MDD
X-squared = 2.0167, df = 3, p-value = 0.569

>
> chsq_severity3 <- chisq.test(TOXO2$Severity, TOXO2$PTSD)
Warning message:
In chisq.test(TOXO2$Severity, TOXO2$PTSD) :
  Chi-squared approximation may be incorrect
> chsq_severity3

        Pearson's Chi-squared test

data:  TOXO2$Severity and TOXO2$PTSD
X-squared = 6.53, df = 3, p-value = 0.08849

>
> chsq_severity4 <- chisq.test(TOXO2$Severity, TOXO2$Suicide)
Warning message:
In chisq.test(TOXO2$Severity, TOXO2$Suicide) :
  Chi-squared approximation may be incorrect
> chsq_severity4

        Pearson's Chi-squared test

data:  TOXO2$Severity and TOXO2$Suicide
X-squared = 1.1913, df = 3, p-value = 0.7551
```