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Examining the interrelationship between the levels of neuroactive cytokines and *T.gondii* exposure on the risk for schizophrenia.

By

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Degree to be awarded: MPH

Epidemiology

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By

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Bachelor Of Science, Cell Biology

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Abstract

Examining the interrelationship between the levels of neuroactive cytokines and *T.gondii* exposure on the risk for schizophrenia.

By Sadaf Saaber

Schizophrenia is a severe mental disorder that affects an individuals' everyday ability to function. While much is known about the symptoms of schizophrenia little is understood about what causes this disease. A potential route of inquiry is the possible role of infectious agents such as the parasite *T. gondii*. Mechanistically this parasitic infection may act through or in concert with immune factors such as cytokines. To examine this mechanism, I analyzed two cohorts: the Atlanta VA cohort that consists of 369 subjects and the Augusta Cohort of 123 subjects. Combining the cohorts (n=473), we saw that after adjusting for age, sex, race, and smoking status, the adjusted odds ratio for the association between *T. gondii* in the combined cohorts was 0.93 (95% CI 0.44-1.94). The adjusted odds ratio in the Augusta Cohort was 1.21 (0.21-7.00) and the Atlanta VA was 0.98 (.42-2.29). I also conducted analysis on a subgroup of our total sample that had cytokine data (n=213) and saw that after adjusting for the same factors, the odds ratio for the association between the infection and schizophrenia outcome was 1.24 (0.37-4.12). I also examined the relationship between being seropositive for *T.gondii* and cytokine levels. This analysis revealed that the cytokines Il-4, IFN-Gamma, IL-2, Il-1Beta and Il-6 were significantly different between infected and non-infected individuals (alpha=0.05). The finding with IL-4 and IFN-Gamma is relevant to the tryptophan/kynurenine pathway, which is regulated by these two cytokines and has been of considerable prior interest in schizophrenia.

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Background/Literature Review

Schizophrenia Background:

Schizophrenia is a "chronic and severe mental disorder" that affects an individual's everyday function from how they feel, think, and behave (1). Individuals with this condition are typically diagnosed between the ages of 16 to 30 years. The 12-month prevalence is 1.1% of US adults (3). Symptoms are described as both positive and negative. The positive symptoms include the following: experiencing hallucinations, delusions, thought disorder (disorganized thinking), and (sometimes) movement disorders. The negative symptoms are the following: having a flat affect (reduced facial expression of emotions), reduction of pleasure in their daily lives, difficulty in tasks and reduced speaking or fluency (2). A common measure assessing the severity of these symptoms is the Positive and Negative Syndrome Scale (PANSS), a 30 item standardized scoring system which consists of rating the positive symptoms, negative symptoms and general psychopathology of the patient.

Toxoplasmosis/ *T.gondii* Background:

While much is know about the signs and symptoms of schizophrenia, research has not yet discovered the etiology of this debilitating disease. One possible route of inquiry is the role of infectious agents like parasites, bacteria and viruses. One such parasite, *Toxoplasma gondii* (*T.gondii*), a protozoan parasite, has been hypothesized to increase an individual's risk of schizophrenia. The parasite can be transmitted through foodborne transmissions, animal to human (zoonotic) transmission, mother-to child (congenital) transmission as well as organ transplantation transmission methods. The following are the foodborne transmission methods:

1) "Eating undercooked, contaminated meat (especially pork, lamb, and venison) or shellfish.
2) Accidental ingestion of foods such as lettuce that have been contaminated with infectious oocytes.
3) Eating food that was contaminated by knives, utensils, cutting boards, or other foods that had contact with raw, contaminated meat" (4).

The zoonotic transmission route is by contact with the stool of infected cats, which contains the infectious cyst. Congenital transmission is where a woman who "is newly infected with Toxoplasma during pregnancy can pass the infection to her unborn child" (4). Sometimes infected individuals, who have their organs transplanted into another individuals, could also pass the infection to another individual.

After the parasite enters the body through the routes described above, healthy individual can have no symptoms. However in some instance, it will result in the disease known as Toxoplasmosis. Individuals diagnosed with this condition will experience flu like symptoms that can last for a few weeks. Those with compromised immune systems like those with HIV can experience "severe symptoms if they are infected with *T.gondii* while immune suppressed" (5). However, it should be noted that while the symptoms of the disease can subside, the parasite remains in the body in an inactive state as quiescent cysts that reside mainly in the brain and muscle of the host.

Link Between *T.gondii* and Schizophrenia Risk

Researchers have linked being seropositive for *T.gondii* and schizophrenia in many studies. The first route of investigating the relationship was looking at if there was a link between having antibodies produced against *T. gondii* and schizophrenia. For

example, one case control study conducted by Tamer et al found that the IgG antibodies for *T.gondii* "were significantly higher in schizophrenia patients as compared with controls" (6). This pattern was also seen in a cross-sectional study where they looked at both IgG and IgM antibodies and determined that there was a difference statistically between the first episode schizophrenia vs. controls (P=0.02 and P=0.007) (7).

There have now been several meta-analyses of this association. In the review article by Torrey et al, they saw that in "18 [out of 19 studies] reported a higher percentage of antibodies in the affected persons; in 11 studies the difference was found to be statistically significant" (8). In a meta-analysis, Arias et al. has found that being infected with *T.gondii* increases the risk of a schizophrenia diagnosis (OR =2.70; 95 CI 1.34-4.42 at p=0.005) (10). However, while this meta-analysis did seem to indicate that there is a statistically significant relationship between *T.gondii* infection and schizophrenia risk, it should be noted that when the Arias et al. pulled together 8 studies together the range of ORs went from 0.91(0.65-1.27) to 6.88 (3.13-15.11), possibly indicating the varying nature of this relationship between different populations (9). Another meta-analysis of 23 studies determined that the overall OR is 2.73 (2.1-3.60 with a P <0.000001) (11). All of these studies seem to indicate that there is a link being infected with *T.gondii* and eventually becoming an individual with schizophrenia.

Not only have researchers seen the effects of the individual becoming infected with *T.gondii* and later schizophrenia, but it has also been seen that mothers who have been infected with *T.gondii* have an ability to transfer the antibodies to their children and having these children later in their life become diagnosed with schizophrenia. In a study conducted in a Danish population showed that there is a risk for schizophrenia when

individuals are exposed to maternal IgG antibodies against *T.gondii* with an OR of 1.79 (1.01-3.15 P=0.045) adjusting place of birth, year of birth, first degree family history and gender of males vs. females (12). This would seem to indicate that a mother who has been infected with *T.gondii* could pass the antibodies to her child and thus that child could have an increased risk for schizophrenia.

Cytokines Background:

While researchers have been able to link being exposed to *T.gondii* and schizophrenia, they are currently trying to determine the mechanism by which this could occur. A possible link is through immune factors such as cytokines. Cytokines are "soluble regulatory proteins that act as intercellular messages when related by certain body cells" (13) and "are systemic mediators of host response to infection, representing a reliable marker of infections and inflammatory conditions" (14). Cytokines are secreted by a variety of immune cells and some non-immune cells. Cytokines typically show cross regulation and operate within a cytokine network. The nomenclature for cytokines is constantly evolving, but they are typically named by their origin cell, function and /or order that they were discovered (13). Cytokine groups include Interleukins (ILs), Interferons (IFNs), growth factors, tumor necrosis factors (TNFs) and chemokines.

Cytokines and Schizophrenia:

Researchers believe that since schizophrenia is a brain disease and could be related to inflammation and cytokines, the link between cytokines and schizophrenia deserves considerable attention (15, 20). For example, a case-control study was done on 91 subjects with schizophrenia in Saudi Arabia and 50 age and sex matched controls, where they found that there was an increased level of TNF-Alpha, Il-1Beta, IL-6 and a

decrease of IFN-Gamma in people with schizophrenia compared to healthy controls (16). Another case-control study, which was conducted in 47 veterans with schizophrenia and 20 controls, determined "that there was a significant association between levels of cytokines and PANSS scores for G-CSF, Il-1Beta, IL-1RA, IL-3, IL-6, IL-9, IL-10, sCD40L and TNF-Beta" (17).

Perhaps most convincing evidence for a relationship between cytokine alterations and schizophrenia comes from meta-analysis of various human studies. One such meta-analysis was conducted and reported that Il-6, TNF-Alpha and sIL-2R and Il-1RA were significantly increased in patients with schizophrenia as compared to controls (18). Another meta-analysis that was conducted on cross-sectional studies indicated that there was an increase in IL-1RA, sIL-2R and IL-6 and a decrease in IL-2 in individuals with schizophrenia (19). All these studies indicate that there is a relationship between different cytokines and schizophrenia, though the mechanisms remain to be explained.

Cytokines and *T.gondii*:

The interplay between infection with this parasite, immune responses in production cytokines, and risk of schizophrenia deserves further study. Figure 1 illustrates how the various cytokines and other cellular and molecular processes are involved in the sequence of events following infection with *T. gondii*. This cascade demonstrates the importance of the cytokines in the eventual elimination of the *T.gondii* within the body (23). Il-12, Il-18, Il-15, IFN-Gamma as well as Il-12 are the key cytokines that participate in the pathway for *T.gondii* control (Figure 1).

However, while these are the most important cytokines in the pathway, other papers have also indicated other cytokines that could be potentially important. After an

individual is infected with *T.gondii*, the immune system is activated and results in a cascade of responses of the immune system including the lymphocytes -such as B cells, Helper T cells both type 1 and type 2 (Th1 and Th2), cytotoxic T cells (Tc), and Regulatory T cells (Tr)- and the subsequent cytokines which provide the signals for these lymphocytes to act (13). Many of these events have been examined mainly through animal models and so human studies, as conducted for this thesis, are crucial.

Looking at a study conducted in mice, it has been shown that IFN-Gamma is "one of the most important cytokines for immune control of Toxoplasma infection in mice" through decreasing the synthesis of other cytokines like MCP-1, G-CSF, GM-CSF and Il-6 (22). This is further seen in another review that was conducted which illustrated the importance of IFN-Gamma in the cascade of reactions from oral infection to eventual phagocytosis of the parasites. As shown in Figure 1, various cytokines and other participants are involved in this sequence of events. This cascade demonstrates the importance of the cytokines in the control of the *T.gondii* within the body (23).

Another study of cytokines conducted in pregnant woman in individuals from United States (n =144: 48 were uninfected, 49 chronically infected and 47 were acutely infected) and Colombia (n=113) comparing *T.gondii* infected individuals to uninfected *T.gondii* individuals. It was determined through this study that in the American acute-infection group, the immune mediators, Eotaxin, FGF-Beta, CXCL1, IFN-Gamma, Il-15, Il-2, Il-4 MCSF, SCF and TNF-Alpha levels were decreased as compared to uninfected individuals. In both the American chronic population and American acute GCSF, Il-17, LIF, CCL3 and NGF were decreased as compared to uninfected individuals. In the Columbian Acute ICAM1, CXCl10 CXCL9 and TNF-beta levels were increased while in

both the Columbian Acute and American Acute HGF, IL1-alpha, CCL7, CCL5 and Trail were decreased as compared to their respective uninfected individuals. In all populations, CD40L, Il8, CCL4 and Resistin levels were decreased in all infected populations as compared to uninfected populations (30).

While there has been research into linking *T.gondii* infection with schizophrenia, little is understood about the immune mechanism linking *T.gondii* infection to increase schizophrenia risk. My thesis will examine this issue. I will be looking at the relationship between being infected with *T.gondii* and indices of immunological responses, and how these are associated with having schizophrenia.

Methods:

Research Goals:

1) Examine the relationship of being exposed to *T.gondii* and schizophrenia in Dr. Erica Duncan's dataset for the Atlanta VA as well as the Augusta VA patients recruited by Dr. Brian Miller.
2) Use the VA cohort and the Augusta cohort (Dr. Brian Miller) to test the association between *T.gondii* seropositivity and levels of cytokines.
3) Explore possible interrelationships between cytokine levels (using 29 cytokines and related immune molecules as biomarkers), *T.gondii*, and their relationship to schizophrenia.

Subjects:

The information provided by Dr. Erica Duncan is from a Veteran's Affair cohort of 369 that includes individuals with schizophrenia, controls and family members. The information provided by Dr. Brian Miller is called the Augusta Cohort that consists of 123 individuals with schizophrenia and healthy controls. The subjects in Dr. Miller study were not established as a cohort per se, but were a well-studied convenience sample.

Measurements:

Outcome/Dependent Variable: The Diagnosis and Statistical Manual of Mental Disorder-IV in conjunction with the Structured Clinical Interview IV was used to determine if an individual was diagnosed with schizophrenia.

Exposure Variable: T.gondii seropositivity was determine using the BIO-RAD Toxoplasma IgG EIA which is an enzyme immunoassay (EIA) for the detection of

Toxoplasma IgG antibodies in Human Serum. This is only to be used for in vitro diagnosis only.

Covariates: Information about covariates like age, sex and race was determined by interview. Smoking status for the Atlanta VA cohort was determined using the Fagerstorm Smoking Tolerance Questionnaire (27). For the Augusta Cohort, smoking status was determined using the Dartmouth Assessment of Lifestyle instruments (28).

Cytokines: Blood samples were collected in tubes in the presence of EDTA anticoagulant. Blood samples were then centrifuged at 4000 rpm for 10 minutes at room temperature. Plasma was extracted divided into aliquots, and stored at -70C until thawed for assay. Hemolyzed samples were not used, and the plasma was carefully removed, leaving behind a small layer of plasma to avoid disturbing the pelleted cells and platelets. For the analysis samples were thawed and cytokine/chemokine levels were measured by multiplexing with a Luminex 200 System with Human Cytokine/Chemokine 25-plex Antibody Bead Panel (CAT# LHC0009, Invitrogen). The cytokine/chemokine kit contained interleukin (IL)-1 beta (IL-1β), IL-1 receptor antagonist (IL-1RA), IL-2, soluble IL-2 receptor (sIL-2R), IL-5, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, granulocyte macrophage-colony stimulating factor (GM-CSF), interferon (IFN) alpha (IFN-α), IFN-Gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), eotaxin, IP-10, MCP-1, MIG, MIP-1α, MIP-1β, and RANTES. Plasma samples were centrifuged prior the analysis. Multiplex assays were performed according to manufacturer's instructions

SDF-1α levels were quantified using a separate sandwich ELISA kit (Quantikine Human CXCL12/SDF-1α immunoassay, R & D systems, Inc., Minneapolis, MN) according to the manufacturer's protocol.

Tryptophan, kynurenine, and kynurenic acid. High-pressure liquid chromatography (HPLC) was used to measure the concentration of tryptophan, kynurenine, and kynurenic acid in plasma samples. Briefly, samples were suspended in 15 mM acetate buffer (Sigma-Aldrich) and deproteinated using trichloroacetic acid (Sigma-Aldrich). Samples were clarified by centrifugation and filtration. Finally, samples were analyzed on a 4.6×50 mm reverse phase C18 column. All reagents where applicable are HPLC grade. The area under the curve of tryptophan, kynurenine, and kynurenic acid were integrated and converted to concentrations from a standard curve.

Statistical Analysis:

All data analysis was performed using SAS 9.4 (Cary, NC) as well as using SPSS 10.3 to confirm the results. Descriptive statistics for the study was calculated looking at both the Atlanta and Augusta datasets separately and together. Also descriptive statistics were used to look at cytokine data in the total dataset stratified by having *T. gondii* and by schizophrenia diagnosis. Logistic regression models were performed to determine the link between being exposed to T.gondii and schizophrenia risk separately in Atlanta VA cohort and the Augusta cohort as well as both cohorts together (Goal 1). Mann-Whitney Test was conducted to test the association between T.gondii and levels of cytokines, as the cytokines were not normally distributed. Also spearman test for correlation was performed to determine if they cytokines were correlated to each other.

As the cytokines were not normally distributed, in an attempt to normalize the data, we first used standard methods such as log10, Ln, and log10+1 and sqrt transformations. However, based on the Sharpiro-Wilks test statistic and P values, the cytokines were still not normally distributed. Moreover, some of the cytokines were zero-

inflated because they had values that were below the limit of detection for the assy. There are multiple possible approaches to analysis with values that are <LOD. We applied the non-detectable substitution method (31), which is commonly used when <15% of the values are <LOD, with the LOD/ √2. (25) The LOD values we used were from Bio-Rad Bio-Plex Pro Human Cytokine, Chemokine, and Growth factor Assays. However, it should be noted that this panel only had 22 out of the 25 cytokines that we were analyzing. As a result, the cytokines IFN-Alpha, IL-1RA and IL-2R were not assessed for goal 3 (24). For a few cytokines (e.g. IL-1Beta) there were a large number of samples that were <LOD. For these, we re-ran the Mann-Whitney test after eliminating all those with values <LOD. We also examined the probability of having a value of <LOD in the *T. gondii* seropositive versus seronegative groups for goal 2, including stratification by schizophrenia status. It should be noted that for the analysis for the chemokines including Kynurenine, Tryptophan, KT-Ratio and SDF1-alpha, were not normalized.

For goal 3, I focused on those few cytokines that showed evidence of association with *T. gondii* in goal 2. Exploratory logistic regression were run for all cytokines but these results are not a focus of this thesis because such results lack the context of the association of these cytokines with schizophrenia independent of *T. gondii.* This later analysis is being performed as part of another project of Dr. Miller.

Results:

Study Population

For our analysis, we started with two different cohorts, the Atlanta VA cohort that consisted of 369 individuals and the Augusta Cohort that had 123 individuals. As a result, after we merged both cohorts together we had a total of 492 individuals. However, after looking at our outcome diagnosis of schizophrenia or not as well as being exposed to *T.gondii* we determined that 18 individuals are missing both an outcome diagnosis and being exposed to *T.gondii* information (Figure 2). Also one individual within the Atlanta VA cohort was missing *T. gondii* information while still having an outcome diagnosis. Because of this missing data about outcome diagnosis and/or *T.gondii* exposure, these 19 people were excluded from our initial analysis. These individuals were included in the evaluation of goal 1. For goal 2 and 3, 260 individuals were further excluded from our analysis, as they did not have information for the 29 cytokine. As such, 213 individuals were analyzed to explore Goal 2 and 3. (Figure 2)

As reported in Table 1, the mean age is 41.88 years old with an SD of 13.04 years and they are primarily males (58.74%, N=289) who are African American (52.03%, N=256), currently not smoking (63.41%, N=312), with over half having a diagnosis of schizophrenia (57.52%, N=283), and are primarily seronegative for *T.gondii* (86.38%, N=425). After being stratified by cohort we can see that the mean age for the Augusta cohort was 41.63 years old with an SD of 12.63 years and in the Atlanta VA being 42.11 years old SD of 13.18 years. In the Augusta Cohort we can see that there is approximately the same amount of males to females while in the Atlanta VA cohort there are more males 61.52% as compared to 33.6% females. We can also see that in both cohorts there are more African American individuals with 59.35% in the Augusta cohort

and 49.59% in the Atlanta VA cohort. There are more non-smokers as compared to smokers in both the Augusta and Atlanta VA cohorts and there are more people with schizophrenia in the Augusta and Atlanta VA cohorts, 73.17% and 52.30% respectively.

However, when the data was stratified by schizophrenia diagnosis as seen in Table 2, the average age for individuals with schizophrenia is 43.87 years old with an SD of 11.06 years as compared to the controls of 38.93 years old with an SD of 15.08 years. If we look at the percent of males and females for individuals with schizophrenia it is 71.02% to 28.98% as compared to the controls of 46.07% to 53.93%. If we look at the race of the individuals with schizophrenia there are 60.78% of black individuals as compared to 43.98% of black individuals for the controls. Comparing the smoking habits there are more smokers have schizophrenia (50.88%) as compared to 8.9% in the control population. Also there are more individuals who are seropositive for *T.gondii* in the people with schizophrenia sample 11.31% as compared to 8.38% in the controls.

Relationship between schizophrenia and being seropositive for *T.gondii* (Goal 1):

To understand the relationship between schizophrenia and being seropositive for *T.gondii*, we included in our analysis 473 individuals and excluded 19 individuals who were missing either schizophrenia diagnosis and/or *T.gondii* exposure information. (Figure 2) After conducing a univariate analysis, the odds ratio for the 473 individuals in the combined cohort is 1.40 (95% CI 0.74-2.60) as seen in Table 3. Looking at the odds ratio individually by the different cohorts, in the Augusta Cohort the odds ratio was 1.94 (0.40-9.35) and in the Atlanta VA cohort was 1.31 (0.65-2.66). Putting all this information together indicates that the odds of being exposed to *T.gondii* as indicated by being seropositive for *T.gondii* is higher in individuals with schizophrenia as compared to being exposed in the controls. It is important to note that these odds ratio come from an

these unadjusted models which only looks at the relationship between schizophrenia and *T. gondii* exposure while not looking at potential confounders. While there appears to be a positive association between schizophrenia risk and *T.gondii* exposure through the high odds ratios of 1.40, 1.94 and 1.31, this relationship appears to be statistically insignificant as the confidence intervals include one. Also given the wide confidence interval indicates that our measures are not precise, this is especially seen in the Augusta cohort with a confidence interval being 8.95 indicates that there is a large variability within the data.

After adjusting for age, sex, race and smoking status, the adjusted odds ratio decreased to 1.21 (0.21-7.00) in the Augusta Cohort, 0.98(0.42-2.29) in the Atlanta VA cohort and 0.93(0.44-1.94) in the combined cohorts. (Table 3) This would seem to indicate that after adjusting for the confounders there was a reduction in the odds ratio the association between infection and schizophrenia. With the odds ratios being close to 1 in the Atlanta VA cohort and combined datasets, indicates that there is actual not a relationship between being exposed to *T.gondii* in individuals with schizophrenia as compared to controls. Also again, for the Augusta cohort we saw a large confidence interval indicating a large variability within that dataset as compared to the Atlanta VA and combined cohorts. It should also be noted that in the Atlanta VA cohort one subject was missing an age and another subject was missing a smoking status, thus these individuals were further excluded from our analysis and for the combined cohort analysis was conducted on 471 individuals and the analysis for the Atlanta VA included 350 individuals. The Augusta Cohort was not affected as it had all the information that we needed. Through conducting a collinearity analysis into the fully adjusted model, we can

determine that the variables including in this model are not related to each other due the Conditional Indices being less than 30.

Differences between being seropositive for *T.gondii* and cytokines (Goal 2):

In goal 2, we were attempting to understand if there was any difference between being seropositive for *T.gondii* and cytokine levels. For this goal we conducted our analysis on 213 individuals excluding 260 individuals who did not have any cytokine data. (Figure 2) Originally, we had intended to conduct a simple linear regression to explore this goal. However, due to the inability of effectively normalize the cytokine data and thus a violation to the assumptions of a simple linear regression, a Mann-Whitney test was conducted to determine if cytokines levels were significantly different comparing seropositive and seronegative groups *of T.gondii*. After conducting this test, the only statistically significant result based on an alpha level of 0.05, in the total sample was in IFN-Gamma (Mann-Whitney P value of 0.03) and Il-4 (Mann-Whitney P value of 0.04) as seen in Table 4, and four Il-1Beta (Mann-Whitney P value of 0.04), Il-6 (Mann-Whitney P value of 0.01), IFN-Gamma (Mann-Whitney P value of 0.03) and Il-2 (Mann-Whitney P value of 0.04), for the controls as seen in Table 5. No values had reached this statistical significance in the individuals with schizophrenia group as seen in Table 6.

We looked at the median values graphically to visualize differences between the outcome diagnosis and exposure to *T.gondii* for the median values of the IFN-Gamma, IL-4, Il-6, Il-2 and IL-1Beta. The pattern for the median values of IFN-Gamma shows the highest level of the cytokine among the control (non-schizophrenic) subjects who are seropositive, with an IFN-Gamma level of 14.51 pg/ml as seen in table 5. In the schizophrenia group there was little difference between the seropositive and seronegative

individuals. For Il-4, we can see that individuals who are both schizophrenic and seropositive for *T.gondii* have the highest median value of Il-4 with a value of 25.57 pg/ml. Also that same pattern holds for Il-6 (median value of 4.8 pg/ml), Il-2 (median value of 5.05 pg/ml) and Il-1Beta (median value of 15.54 pg/ml). This would seem to indicate that individuals who have schizophrenia and are seropositive for *T.gondii* have higher levels of these cytokines as compared to the other median values for other combinations. (Appendix 3)

We also conducted Spearman tests for correlation to determine if these cytokines are related to each other and could possible reveal a potential pathway. Looking at the spearman correlation coefficients in Table 7, we can see that IFN-Gamma and Il-4 have a correlation coefficient of 0.53 indicating that there is a moderate positive relationship, which is statistically significant (p=<0.0001). Also Il-1Beta and Il-4 has a spearman correlation coefficient of 0.48 indicating a moderate positive relationship (p=<0.0001). The Spearman correlation between IL-2 and Il-1Beta is 0.78(p=<0.0001) indicating a strongly positive correlation. This was also seen in the case of Il-1Beta and Il-4 with a Spearman coefficient of 0.49 (p=<0.0001). It is interesting to note that all of these correlations are positively related to each other and do not include a negative correlation coefficient.

In supplementary analysis, we also looked at all 29 cytokines through the Pearson and Spearman correlation coefficients. It is here that we start to see correlation coefficients being negatively correlated with most of the chemokines of Kynurenine, Tryptophan, KT-Ratio and SDF1-alpha being most negatively correlated with the other cytokines. Also there are correlation coefficients, which are about 0.90 indicating a

strong correlation between these two factors. (Appendix 1: Pearson Correlation Coefficients) If we look at the spearman correlation coefficients, the chemokines of Kynurenine, Tryptophan, KT-Ratio and SDF1-alpha have negative correlation coefficients. (Appendix 2: Spearman Correlation Coefficients)

Since for a few cytokines (Il-7, Il-1Beta, Il-2, Il-8, Il-17) there were more than 15% of those above the LOD value as seen in Appendix 6, we decided to determine if there was a substantial effect of having many values being below of the LOD value. Using a chi-squared analysis for this, we looked at the frequencies of those individuals who had values above the LOD value and those below the LOD value in seropositive and seronegative individuals. Through this analysis we determine that there was not a significant difference between the frequencies in these groups as indicated by the p-value being above 0.05 indicating a statistically insignificant relationship (Appendix 4). As the second part of this two-step modeling, we ran the Mann-Whitney test including only those individuals who were above the LOD values. In this analysis we determined that while the p-value changed slightly as compared to table 4 where we included everyone regardless of LOD value, these values were still above the alpha level of 0.05 indicating that there is not difference between being seropositive and seronegative for individuals (Appendix 4).

Relationship between schizophrenia, being seropositive for *T.gondii* and cytokines (Goal 3):

In goal 3, we were attempting to understand if there is a relationship between schizophrenia and being seropositive or seronegative for *T.gondii* with cytokines. For this goal we conducted our analysis on 213 individuals excluding anyone who did not have any cytokine data (n=260). (Figure 2) After conducting separate logistical models for the

29 cytokines and chemokines, where the covariates of age, sex, race and smoking status as well as a product term of each individual cytokines and with being seropositive for *T.gondii,* the Wald-test statistic and P-values in for the product term was seen to be statistically insignificant with a significance level of 0.05. This indicated that there was not an interaction between being seropositive for *T.gondii* and cytokines on schizophrenia risk.

However, when the model was created to include the 29 cytokines individually as covariates, and looking at the Wald-Test Statistic and p-values to determine if we needed to include this variable in a model, we saw that cytokine eotaxin was statistically significant, showing an association with a P value of 0.01 as seen in appendix 5. Thus there was a weak positive association between the cytokine and schizophrenia in models that included *T. gondii* and relevant covariates.

As described in the methods and figure 2, we only used subsample of individuals with cytokine data to accomplish this goal. Most of the odds ratios were greater than one for the association between being seropositive and schizophrenia, but they all included one. We used a univariate and adjusted models for only the 213 individuals that had cytokine data as indicated in appendix 5. For that we had a univariate odds ratio of 1.40 (0.53-3.69) that matches our original univariate odds ratio of 1.40 (0.74-2.60) for the dataset that had 473 individuals (Table 3). However after conducting an adjusted analysis, we had an odds ratio of 1.24 (0.37-4.12) that is greater than the 0.93(.44-1.94) for the dataset that had 473 individuals (Table 3).

If we use the adjusted odds ratio of 1.24 as a comparison to the other odds ratio including the individual cytokines in the model, we can see that IFN-Gamma, Il-7,

Kynurenine, Tryptophan, KT-Ratio and SDF1-alpha are all turned toward higher than this value. Also Il-2 is lower with an odds ratio of 0.72. The rest of the cytokines are close to the 1.24. However, the confidence intervals for these odds ratio contain 1 indicating that these results are statistically insignificant.

Discussion

Our study findings indicate that there is a positive correlation between being seropositive for *T.gondii* and schizophrenia, though this was only in the unadjusted models and was not statistically significant. This was in accordance with the available literature as was the fact that depending on the population that was studied there is variability in the magnitude of that relationship as seen by the differing odds ratios in those papers and as well as the different odds ratios in both the 473 subjects and the 213 subjects we analyzed in this thesis. When we looked at the 473 subjects, the confounders of age, sex, race and smoking status had a large effect and after adjusted for those variables the results became close to null. However, when we conduct the same analysis for the 213 individuals with cytokine data, we see that the odds ratio is 1.24.

A key finding of the study is the relationship between cytokine levels and being seropositive for *T.gondii*. For most of cytokines there was not a difference between cytokine levels in seronegative versus seropositive *T.gondii* groups. However, we determined that Il-4, IFN-Gamma, IL-2, Il-1Beta and Il-6 were statistically significant between seropositive versus seronegative groups. Considering that this was based on a Mann-Whitney nonparametric test, these findings need to be considered with caution. Nevertheless this finding suggests that we reject the null that the distributions of the cytokine levels are the same between seropositive and seronegative groups.

Also by looking at the correlations between them as well as the median values, there could be a potential mechanism or pathway that all of these cytokines are on that is activated or inhibited when an individual is infected with *T.gondii.* Looking at the cytokine network in figure 3, we can see that all of these cytokines are involved in the cytokine pathways that communicate with the T cell, B cell and help to coordinate an

immune response after being infected with a parasite like *T.gondii* and its subsequent entrance into the body (26). We can see that IFN-Gamma is present throughout this network and is important in interacting with each cell type involved in the immune system. Along with IFN-Gamma, Il-2, Il-4, and Il-6 are present and interact with each cell type as well.

Two most prominent cytokines in our results, IFN-Gamma and Il-4 are on the pathway of the tryptophan/kynurenine metabolism (Figure 4). IFN-Gamma is an activator of KMO enzyme and indoleamine 2,3-dioxygenase enzymes while Il-4 is an inhibitor. Looking at figure 4, it would appears as if they work in different directions in that while Il-4 inhibits the production or activity of those enzymes, IFN-Gamma activates the production of those enzymes. Mechanistically, we can speculate on potential mechanisms for the cytokines and schizophrenia and its relationship to *T. gondii.* Animal model studies indicate that the parasite itself is able to suppress IFN-Gamma, and that this gives an advantage to the parasite in evading the host immune response (34). Since patients with schizophrenia who are seropositive for the parasite seem to have a reduced IFN-Gamma level, this could indicate that they are unable to mount an effective control of the chronic infection, including the cysts that reside in the brain. This could be because the individuals with schizophrenia were infected by a serotype of *T.gondii* that was more effective in suppressing IFN-Gamma, or that they had immunogenetic differences that reduce their ability to produce the cytokine. But contrary to our original hypothesis, a low level of the cytokine should be associated with a reduced activation of the kynurenine pathway. The higher level of IL-4 among infected people with schizophrenia would also suggest less activation of the kynurenine pathway. Analogous arguments need to be

considered in how different strains of T.gondii modulate the other inflammatory responses in the human immune system. Figure 5 provides context (32) for this mechanism.

The strengths of our study were a large overall population and having our data collected in a way that limits information bias. Starting out we had 492 individuals after merging both of the datasets together. After excluding 279 individuals, we still had 213 individuals to analyze in our study. Not only did we have a large overall sample, but also since our measurements were collected using already validated tools such as the DSM-IV and SCID for schizophrenia diagnosis, and employing a blood test to determine seropositivity for *T.gondii* as well as quantitative assays to determine cytokine information, we have limited potential information biases.

However, while we had many strengths there were many weakness including the fact that our cytokine data was not normally distributed, small percentage of individuals infected with *T.gondii*, confounding bias as well as problems with multiple comparisons. For some cytokines some of the blood tested at levels below the limit of detection for the assay. There is a large literature on how to handle such zero-inflated data. For goal 2, we examined differences between *T. gondii* seropositive and seronegative groups using a nonparametric test. This is one method suggested by Delucchi and Bostrom for mildly zero inflated data (33). However it can be problematic if there is a large number of zero values in the data set. For those with a larger number of values below the LOD, we confirm our results with the two-part model that addressed the difference between groups in having a nondetectable level in the first step, and then assessed whether those levels

that were in the detectable range differed between groups in the second step. Results were similar with either method.

Another weakness of the cytokine information was the presence of statistical outliers as determined by three standard deviations away from the mean or median values. However, while statistically these are outliers biologically they could be possible and as such was not removed from the analysis as these outliers could biologically possible. Another weakness was the small percentage of the population infected with *T.gondii* in both schizophrenics as well as controls. We had an issue for confounding as after adjusted for age, race, sex and smoking status we saw a difference between the adjusted and crude OR. However this was address through adjusting for those factors. Also, we have the problem of multiple statistical comparisons. There is a substantial literature, which includes a fair amount of controversy, on potential ways to adjust for multiple comparisons. One approach potential approach is the Bonferroni correction. However this approach is far to conservatives in that the new alpha level would have to be 1-.05/29=99.8 or .02, which is too conservative.

Future Directions

This study was one of the first to explore the interrelated factors of being seropositive for *T.gondii,* cytokines and schizophrenia risk. Through this analysis we have determine that the cytokines of Il-4, IFN-Gamma, IL-2, Il-1Beta and Il-6 are possibly important in the potential immune response that results after an individuals is infected with the parasite of *T.gondii*. Not only are these cytokines important after being infected with *T.gondii* our analysis points to mechanisms by which these cytokines could relate to schizophrenia. One potential future direction is to confirm these results and determine if this pathway is indeed a causal pathway from infection with *T.gondii* to becoming schizophrenic, through mice knockout studies, for example.

Also another important item that could be further explored is using different statistical techniques to analyze this data set. Since we had some problems with normalizing the cytokine data another potential method for analysis can come from using zero-inflated poison regression techniques in analyzing for the relationship between being seropositive for *T.gondii* and schizophrenia risk with the cytokines in the model. Multiple imputation procedures for values below the limit of detection have also been described. These would be an important analysis techniques to use for future potential thesis projects.

Some future directions resulting from the findings of this thesis are to conduct genetic testing to determine if particular genes and/or single nucleotide polymorphisms or combination of them are important in the pathways where these cytokines are activation or inhibited especially in the pathway of the tryptophan/kynurenine metabolism. Along with this line, since we determined that specifically these cytokines are important in the immune response after infection with *T.gondii*, a possible treatment is

immunomodulatory therapy directed at these cytokines. This ultimately could help us determine better treatment options after infection with *T.gondii*, which could decrease an individuals' risk for schizophrenia.

References:

1. National Institute of Mental Health, US National Library of Medicine. *Schizophrenia. http://www.nimh.nih.gov/health/topics/schizophrenia/index.shtml. Accessed Sep 4, 2016.*

2. National Institute of Mental Health. *Schizophrenia*. Bethesda, MD: National Institute Mental Health, (National Institute of Health (NIH) no. 15-3517).

3. National Institute of Mental Health, US National Library of Medicine. *Schizophrenia. http://www.nimh.nih.gov/health/topics/schizophrenia/index.shtml. Accessed Feb 5, 2017.*

4. Centers of Disease Control, Global Health- Division of Parasitic Disease. *Parasites (Toxoplasmosis) Epidemiology and Risk Factors. https://www.cdc.gov/parasites/toxoplasmosis/epi.html.* Updated March 1, 2017. Accessed March 29, 2017.

5. Centers of Disease Controls, Global Health- Division of Parasitic Disease. *Parasites (Toxoplasmosis) Disease. https://www.cdc.gov/parasites/toxoplasmosis/disease.html* Updated March 1, 2017. Accessed March 29, 2017.

6. Tamer GS, Dubdar D, Yalug I, et al. The schizophrenia and Toxoplasma gondii connection: infectious, immune or both?. *Advance in Therapy*. 2008; 25(7): 703-9.

7. Yolken RH, Bachnann S, Ruslanova I, et al. Antibodies to Toxoplasma gondii in individuals with first-episode schizophrenia. *Clinical Infectious Disease.* 2001; 32(5): 842-2.

8. Torrey EF, and Yolken RH. Toxoplasmosis gondii and schizophrenia. *Emerging Infectious Disease*. 2003; 9(11): 1375-80.

9. Alvarado-Eqquivel C, Urbina-Alvarez JD, Estrada-Martinez S, et al. Toxoplasma gondii infection and schizophrenia: a case control study in a low Toxoplasma seroprevalence Mexican population. *Parasitology International.* 2011; 60(2):151-5.

10. Arias, Isabel, et al. Infectious Agents Associated with Schizophrenia: A Meta-analysis. *Schizophrenia Research*. 2012; 136(1-3):128-36.

11. Torrey EF, Bartko JJ, and Yolken RH. Antibodies to Toxoplasma gondii in patients with schizophrenia: a meta-analysis. *Schizophrenia Bulletin.* 2007; 33(3): 729-36.

12. Mortensen PB, Norgaard-Pedersen B et al. *Toxoplasma gondii* as a Risk Factor for Early-Onset Schizophrenia: Analysis of Filter Paper Blood , Samples Obtained at Birth. *Biological Psychiatry*. 2007; 61(5): 688-93.

13. Bauman RW. Microbiology with Diseases by Taxonomy. 3rd ed. San Fransisco, CA: Benjamin Cummings; 2010.

14. Altamura AC, Pozzoli S, Fiorentini A, and Dell'osso B. Neurodevelopment and inflammatory patterns in schizophrenia in relation to pathophysiology. *Progress in Neuro-Psychopharmacology and Biological Psychiatry.* 2013; 42:63-70.

15. Na KS, Jung HY, and Kim Yk. The role of pro-inflammatory cytokines in the neuroinflammation and neurogenesis of schizophrenia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry.* 2014; 48:277-86.

16. Al-Askmari AK and Khan MW. Inflammation and schizophrenia: alterations in cytokine levels and perturbation in antioxidative defense systems. *Human &Experimental Toxicology.* 2014; 33(2):115-22.

17. Dimitrov DH, Lee S et al. Differential correlations between inflammatory cytokines and psychopathology in veterans with schizophrenia: potential role for IL-17 pathway. *Schizophrenia Research. 2013; 151(1-3):29-35.*

18. Goldsmith DR, Rapaport MH, and Miller BJ. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. *Molecular Psychiatry.* 2016; 21(12) 1696-1709.

19. Potvin S, Stip E et al. Inflammatory cytokine alterations in schizophrenia: a systematic quantitative review. *Biological Psychiatry.* 2008; 63(5): 801-8.

20. Kirkpatrick B and Miller BJ. Inflammation and schizophrenia. *Schizophrenia Bulletin.* 2013; 39(6)1174-9.

21. Gaddi PJ and Yap GS. Cytokine regulation of immunopathology in toxoplasmosis *Immunology and Cell Biology*. 2007; 85(2): 155–159.

22. Mammari N, Vignoles P, et al. Interferon gamma effect on immune mediator production in human nerve cells infected by two strains of Toxoplasma gondii. *Parasite*. 2015; 22: 39. E pub.

23. Miller CM, Boulter, et al. The immunobiology of the innate response to Toxoplasma gondii. *International Journal for Parasitology*. 2009; 39(1): 23–39.

24. BIO-RAD Laboratories. Bio-Plex Pro Human Cytokine, Chemokine and Growth Factor Assays. http://www.bio-rad.com/en-us/product/bio-plex-pro-human-cytokine-chemokine-growth-factor-assays. Accessed March 30, 2017.

25. Huynh T, et al. Comparison of Methods for Analyzing Left- Censored Occupational Exposure Data. *Annals of Occupational Hygiene.* 2014; 58(9): 1126-1142.

26. Zhang JM, and An J. Cytokines, Inflammation and Pain. *International Anesthesiology Clinics*. 2007; 45(2): 27–37.

27. Fagerstrom K.O. Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. *Addictive Behaviors. 1978;* 3(3-4): 235-241.

28. Rosenberg SD, Drake RE, Wolford GL, et al. Dartmouth Assessment of Lifestyle Instrument (DALI): a substance use disorder screen for people with severe mental illness. *American Journal of Psychiatry.1998; 155(2): 232-8.*

29. Muller N, and Schwarz MJ. Immune System and Schizophrenia. *Current Immunology Reviews.* 2010; 6(3): 213-220.

30. Pernas L, et al. Immune Profiling of Pregnant Toxoplasma- Infected US and Colombia Patients Reveals Surprising Impacts of Infection on Peripheral Blood Cytokines. *The Journal of Infectious Diseases;* 2014: 210: 923–31.

31. Hornung RW, and Reed LD. Estimation of Average Concentration in the Presence of Nondetechtable Values. *Applied Occupational and Environmental Hygiene; 1990; 5: 46-51.*

32. Melo MB, Jensen KDC and Saeij JPJ. Toxoplasma gondii effectors are master regulators of the inflammatory response. *Trends in Parasitology*; 2011; (11): 487-95

33. Delucchi Kl, and Bostrom A. Methods for Analysis of Skewed Data Distributions in Psychiatric Clinical Studies: Working With Many Zero Values. *The American Journal of Psychiatry*; 2004; 161:1159-1168.

34. Bhadra R Cobb DA, Weiss LM, and Khan IA. Psychiatric Disorder in Toxoplasma Seoporitive Patients- The CD8 Connection. Schizophrenia Bulletin; 2013; 39 (3): 485-489.

Tables and Figures

Table 1: Demographic and Clinical Characteristics Stratified By Cohort

Characteristic		Total Population (n=492)	Augusta Cohort (N=123)	Atlanta VA (N=369)
Age	**Mean (SD)**	41.88(13.04)	41.26(12.63)	42.11(13.18)
Sex	**%(N)**			
Males		58.74(289)	50.41 (62)	61.52(227)
Females		37.60(185)	49.59(61)	33.6(124)
missing		3.66(18)	0	4.88(18)
Race	**%(N)**			
White		35.77(176)	34.96(43)	36.04(133)
Black		52.03(256)	59.35(73)	49.59(183)
Other		48.54(42)	5.69(7)	9.49(35)
Missing		3.66(18)	0	4.88(18)
Smoking Status	**%(N)**			
Current Smoker		32.72(161)	44.72(55)	28.73(106)
Not		63.41(312)	55.28(68)	66.12(244)
missing		3.86(19)	0	5.15(19)
Schizophrenia Diagnosis	**%(N)**			
Schizophrenic		57.52(283)	73.17(90)	52.30(193)
Control		38.83(191)	26.83(33)	42.82(158)
Missing		3.66(18)	0	4.88(18)
Seropositvity	**%(N)**			
Seropositive for T.Gondii		9.76(48)	9.76(12)	9.76(36)
Seronegative for T.Gondii		86.38(425)	90.24(111)	85.09(314)
Missing		3.86(19)	0	5.15(19)

Table 2: Demographic and Clinical Characteristics Stratified By Schizophrenia Diagnosis

Characteristic		Total Population (n=492)	Schizophrenic (N=283)	Controls (N=191)
Age	**Mean (SD)**	41.88(13.04)	43.87(11.06)	38.93(15.08)
Sex	**%(N)**			
Males		58.74(289)	71.02(201)	46.07(88)
Females		37.60(185)	28.98(82)	53.93(103)
Missing		3.66(18)	0	0
Race	**%(N)**			
White		35.77(176)	33.57(95)	42.41(81)
Black		52.03(256)	60.78(172)	43.98(84)
Other		48.54(42)	5.65(16)	13.61(26)
Missing		3.66(18)		
Smoking Status	**%(N)**			
Current Smoker		32.72(161)	50.88(144)	8.9(17)
Not		63.41(312)	49.12(139)	90.58(173)
Missing		3.86(19)	0	0.52(1)
Seropositvity	**%(N)**			
Seropositive for T.gondii		9.76(48)	11.31(32)	8.38(16)
Seronegative for T.gondii		86.38(425)	88.69(251)	91.1(174)
Missing		3.86(19)	0	0.52(1)

Table 3: Odds Ratio for the Association of Schizophrenia and Seropositivity for T.Gondii

	Univariate Odds Ratio (95% CI)	Adjusted Odds Ratio* (95% CI)
Augusta Cohort	1.94(0.40-9.35)	1.21(0.21-7.00)
Atlanta VA Cohorts	1.31 (0.65-2.66)***	0.98(0.42-2.29)*****
Both Cohorts	1.40(0.74-2.60)**	0.93(0.44-1.94)****

*Adjusted for age, sex, race and smoking status

**missing 19 idividuals from missing schizophrenia diagnosis and/or T.gondii exposure information (n=473)

***missing 19 idividuals from missing schizophrenia diagnosis and/or T.gondii exposure information (n=350)

****missing 21 individuals due to missing schizophrenia diagnosis, and/or T.gondii exposure information, age and smoking status (n=471)

*****missing 21 individuals due to missing schizophrenia diagnosis, and/or T.gondii exposure information, age and smoking status (n=348)

Table 4: Relationship Between Seropositivity for T.gondii and Cytokines for the Both Cohorts

	Seronegative for T.gondii					Seropositive for T.gondii					
Cytokine	**N**	**Median**	**IQR**	**Min**	**Max**	**N**	**Median**	**Min**	**Max**	**QRange**	**MW P value**
IL-1Beta	184	4.78	17.37	0	2170.38	19	6.87	0	48.54	15.12	0.89
IL-10	194	4.37	8.51	1.03	4005.79	19	4.3	1.2	180.23	4.79	0.76
IFN-Alpha	194	63.8	50.21	2.84	931.07	19	66.16	26.16	173.44	53.06	0.58
IL-6	194	3.35	5.5	0	756.72	19	3.77	0	129.81	5.95	0.72
IL-12	194	176.7	113.09	20.21	2658.24	19	161.49	84.18	281.89	87.78	0.54
RANTES	194	5094.35	12750.8	0	126771.4	19	9879.59	879.8	272180	12396.5	0.27
Eotaxin	194	38.76	75.95	1.04	581.96	19	51.82	8.21	133.86	68.07	0.97
IL-13	192	22.12	51.38	0	89617.62	17	24.58	7.85	470.77	47.17	0.47
IL-15	194	32.56	47.19	0	3753.77	19	30.94	0	134.41	40.94	0.93
IL-17	194	16.78	37.28	0	3390.46	19	18.8	0	181.09	18.98	0.8
MIP-1Alpha	194	50.73	27.42	0	3147.01	19	53.48	24.91	209.5	26.2	0.6
GM-CSF	193	32.8	118.27	0	24897.23	19	31.15	0	883.18	75.24	0.9
MIP-1Beta	194	51.67	71.49	0	6180.58	19	54.96	0	178.95	75.13	0.84
MCP-1	194	153.27	160.82	30.87	15748.31	19	129.42	69.1	387.52	107.96	0.45
IL-5	187	3.37	8.64	0.06	1368.93	18	5.02	0.51	45.13	8.32	0.86
IFN-Gamma	182	3.11	7.41	0	318	16	8.05	1.64	40.94	13.47	0.03
TNF-Alpha	194	6.02	8.12	0	1318.05	19	5.95	0.09	22.17	5.09	0.59
IL-1RA	194	374.46	374.28	7.4	138125.6	19	417.11	59.6	1406.25	325.11	0.85
IL-2	188	2.04	4.49	0	987.98	16	2.76	0	18.66	5.88	0.94
IL-7	179	12.19	48.31	0	1616.96	19	14.29	0	376.11	43.74	0.93
IP-10	194	52.07	57.94	5.55	3434.37	19	41.19	12.54	165.89	51.37	0.73
IL-2R	194	183.27	207.58	21.96	8629.07	19	166.52	2.79	329.38	153.76	0.3
MIG	194	76.41	70.39	0	2033.52	18	72.04	31.09	175.1	49.94	0.58
IL-4	194	12.7	14.67	2.2	1240.57	19	23.2	5.86	68.88	13.47	0.04
IL-8	194	3.68	8.77	0	217.88	19	4.98	0	54.5	9.7	1
Kynurenine	88	2.46	1.51	0.57	5.6	11	2.81	1.12	5.43	2.65	0.82
Tryptophan	88	59.82	41.25	16.86	152.3	11	57.34	34.23	150.61	66.67	0.8
KT-Ratio	88	0.04	0.02	0.02	0.09	11	0.04	0.02	0.08	0.02	0.96
SDF1-alpha	57	2101	557	1526	4470	8	2112	1523	3573	443.5	0.92

The units for the cytokines are pg/ml with the exception of Kynureine and Tryptophan being nM

Table 5: Relationship Between Seropositivity for T.gondii and Cytokines for the Controls

Cytokine	Seronegative for T.gondii					Seropositive for T.gondii					
	N	Median	IQR	Min	Max	N	Median	IQR	Min	Max	MW P value
IL-1Beta	84	4.84	24.98	0	2170.38	7	0	2.35	0	7.99	0.04
IL-10	87	4.67	12.89	1.03	3700.06	7	4.3	4.1	1.2	7.03	0.35
IFN-Alpha	87	59.49	56.14	2.84	931.07	7	65.5	31.42	42.03	83.62	0.94
IL-6	87	2.9	5.07	0	459.9	7	0.68	1.91	0	4.6	0.01
IL-12	87	158.8	124.11	53.33	2658.24	7	129.26	107.33	85.26	213.94	0.49
RANTES	87	3278	10063	0	46652	7	6148.71	7158.38	1834.8	10258	0.56
Eotaxin	87	27.2	38	1.04	200.61	7	23.32	37.59	8.95	59.33	0.83
IL-13	86	25.12	61.95	0.5	89617.6	6	31.34	31.12	7.85	59.35	0.96
IL-15	87	34.24	61.27	0	3753.77	7	27.97	15.64	6.33	59.03	0.27
IL-17	87	13.09	37.63	0	2271.86	7	7.87	14.17	0	21.86	0.54
MIP-1Alpha	87	49.22	33.74	0	3147.01	7	45.57	15.76	40.01	62.4	1.00
GM-CSF	87	46.3	232.64	0	24897.2	7	43.76	56.4	0	83.17	0.50
MIP-1Beta	87	47.54	99.64	0	6180.58	7	40.29	74.84	0	119.83	0.21
MCP-1	87	132.5	178.62	35.3	15748.3	7	92.6	71.05	73.72	215.09	0.27
IL-5	85	3.66	6.66	0.1	1368.93	7	3.72	5.46	0.98	10.79	0.77
IFN-Gamma	82	3.12	7.07	0	318	5	14.51	5.58	3.62	21.06	0.03
TNF-Alpha	87	5.17	9.88	0	1318.05	7	5.95	1.84	0.09	8.17	0.90
IL-1RA	87	369.5	455.83	22.13	138126	7	276.01	310.49	59.6	487.07	0.14
IL-2	86	2.44	7.88	0	987.98	7	0.58	2.76	0	2.76	0.04
IL-7	82	3.6	59.09	0	766.66	7	0	15.95	0	17.45	0.12
IP-10	87	43.2	48.66	5.88	194.51	7	37.36	53.96	20.32	88.78	0.44
IL-2R	87	177.4	189.58	29.62	8629.07	7	207.86	139.5	65.85	296.45	0.94
MIG	87	78.48	66.77	2.59	1813.4	7	73.65	45.94	31.09	94.65	0.28
IL-4	87	12.7	16.95	2.24	1240.57	7	18.77	6.69	8.41	68.88	0.36
IL-8	87	2.48	8.09	0	217.88	7	0	5.49	0	6.77	0.07
Kynurenine	28	2.48	1.01	1.22	4.44	2	3.15	0.68	2.81	3.49	0.27
Tryptophan	28	56.35	37.87	45.3	136.82	2	51.14	12.41	44.93	57.34	0.31
KT-Ratio	28	0.04	0.01	0.02	0.06	2	0.06	0.03	0.05	0.08	0.07
SDF1-alpha	18	1765	605	1526	2662	1	1523	0	1523	1523	0.14

The units for the cytokines are pg/ml with the exception of Kynureine and Tryptophan being nM

Table 6: Relationship Between Seropositivity for T.gondii and Cytokines for Schizophrenics

Cytokine	Seronegative for T.gondii					Seropositive for T.gondii					
	N	Median	IQR	Min	Max	N	Median	IQR	Min	Max	MW P value
IL-1Beta	100	4.62	13.43	0	1253.49	12	11.7	15.54	0	48.54	0.08
IL-10	107	3.83	6.29	1.2	4005.79	12	4.49	13.55	1.38	180.23	0.57
IFN-Alpha	107	65.5	45.13	7.72	809.91	12	73.32	59.43	26.16	173.44	0.38
IL-6	107	3.71	5.99	0	756.72	12	4.8	4.22	2.13	129.81	0.17
IL-12	107	190.68	114.01	20.21	1226.7	12	180.29	84.67	84.18	281.89	0.77
RANTES	107	6800.95	12988	0	126771.4	12	15073.32	14786.6	879.8	272180.2	0.29
Eotaxin	107	56.24	78.78	5.13	581.96	12	73.94	61.51	8.21	133.86	1.00
IL-13	106	20.41	35.57	0	14787.14	11	17.3	81.4	7.85	470.77	0.29
IL-15	107	30.94	31.8	0	2134.9	12	43.66	46.4	0	134.41	0.35
IL-17	107	20.68	36.11	0	3390.46	12	24.85	20.15	7.21	181.09	0.40
MIP-1Alpha	107	52.37	22.59	0	987.94	12	62.18	26.7	24.91	209.5	0.38
GM-CSF	106	19.41	92.85	0	23134.3	12	22.25	168.26	0	883.18	0.68
MIP-1Beta	107	53.72	48.78	0	3678.74	12	83.22	58.59	18.71	178.95	0.35
MCP-1	107	162.31	155.23	30.87	14439.41	12	156.23	93.94	69.1	387.52	0.98
IL-5	102	2.46	9.08	0.06	972.5	11	6.33	18.64	0.51	45.13	0.59
IFN-Gamma	100	3.1	7.58	0	59.83	11	4.44	9.64	1.64	40.94	0.28
TNF-Alpha	107	6.46	7.78	0	1111.84	12	7.67	10.73	1.42	22.17	0.44
IL-1RA	107	384.82	303.78	7.4	62289.52	12	507.41	362.01	106.92	1406.25	0.24
IL-2	102	1.96	3.69	0	313.79	9	5.05	4.94	0	18.66	0.06
IL-7	97	14.39	44.59	0	1616.96	12	22.33	47.35	0	376.11	0.23
IP-10	107	62.62	64.95	5.55	3434.37	12	60.88	55.82	12.54	165.89	1.00
IL-2R	107	195.63	221.61	21.96	5894.92	12	135.78	173.81	2.79	329.38	0.22
MIG	107	72.4	73.06	0	2033.52	11	70.43	73.04	42.39	175.1	0.91
IL-4	107	13.22	12.94	2.2	1024.26	12	25.57	13.68	5.86	49.88	0.06
IL-8	107	4.98	9.12	0	172.35	12	6.8	16.77	0.08	54.5	0.21
Kynurenine	60	2.31	1.6	0.57	5.6	9	1.66	2.65	1.12	5.43	0.92
Tryptophan	60	60.13	49.12	16.86	152.3	9	61.23	60.59	34.23	150.61	0.46
KT-Ratio	60	0.04	0.02	0.02	0.09	9	0.04	0.01	0.02	0.05	0.48
SDF1-alpha	39	2176	356	1635	4470	7	2163	337	1677	3573	0.95

The units for the cytokines are pg/ml with the exception of Kynureine and Tryptophan being nM

Table 7: Spearman Correlation Coefficent

	IFN_Gamma	IL_4	IL_1Beta	IL_6	IL_2
IFN_Gamma	1	0.53236	0.01367	0.2036	0.11474
		<.0001	0.8523	0.004	0.1149
	198	198	188	198	190
IL_4	0.53236	1	0.48815	0.41949	0.48279
	<.0001		<.0001	<.0001	<.0001
	198	213	203	213	204
IL_1Beta	0.01367	0.48815	1	0.58977	0.78283
	0.8523	<.0001		<.0001	<.0001
	188	203	203	203	199
IL_6	0.2036	0.41949	0.58977	1	0.5457
	0.004	<.0001	<.0001		<.0001
	198	213	203	213	204
IL_2	0.11474	0.48279	0.78283	0.5457	1
	0.1149	<.0001	<.0001	<.0001	
	190	204	199	204	204

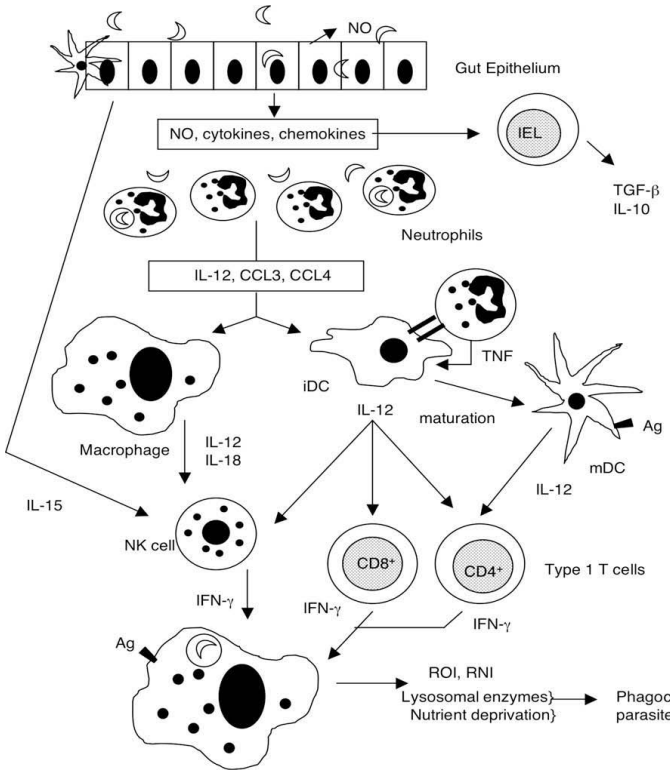


Fig. 2. Sequence of events following oral infection with *Toxoplasma gondii.* Following ingestion of *T. gondii* cysts, bradyzoites are released in the intestine. They convert to tachyzoites and move through the gut epithelium by infecting enterocytes. Infected enterocytes secrete chemokines and cytokines that attract neutrophils, macrophages and dendritic cells (DCs). Neutrophils quickly flood to the site of infection where they can phagocytose parasites and also release chemokines and cytokines such as IL-12, CCL3 and CCL4 that attract immature DCs (iDCs), macrophages and T cells. Neutrophils help in DC maturation by binding to iDCs and passing along endogenously produced TNF. Although neutrophils, macrophages and DCs all secrete IL-12, DCs are the most significant source in *T. gondii* infection. DCs also have a central role in the presentation of antigen to T cells and direct polarisation towards a Th1 adaptive response and the production of IFN-γ. Macrophages are the most important phagocytic cell and crucial in limiting initial dissemination of the parasite. Binding of antigen to their surface results in the production of endogenous TNF that, along with IFN-γ produced by natural killer (NK) cells and T cells, classically activates them to secrete toxic reactive oxygen (ROI) and nitrogen (RNI) intermediates that kill the parasite. IL-12 and IL-18 from macrophages stimulate NK cells to produce IFN-γ and up-regulate their cytotoxicity, respectively, while IL-18 together with IL-15 from enterocytes increases their proliferation. Secretion of CCL3 and CCL4 by enterocytes also attracts intraepithelial lymphocytes (IEL) that become cytotoxic for infected enterocytes and produce the anti-inflammatory cytokines TGF-β and IL-10. These two cytokines are ultimately responsible for dampening the classical Th1 response and limiting inflammatory damage to the host following infection with *T. gondii*.

Figure 1: Sequence of Events following infection with *T.gondii* (23)

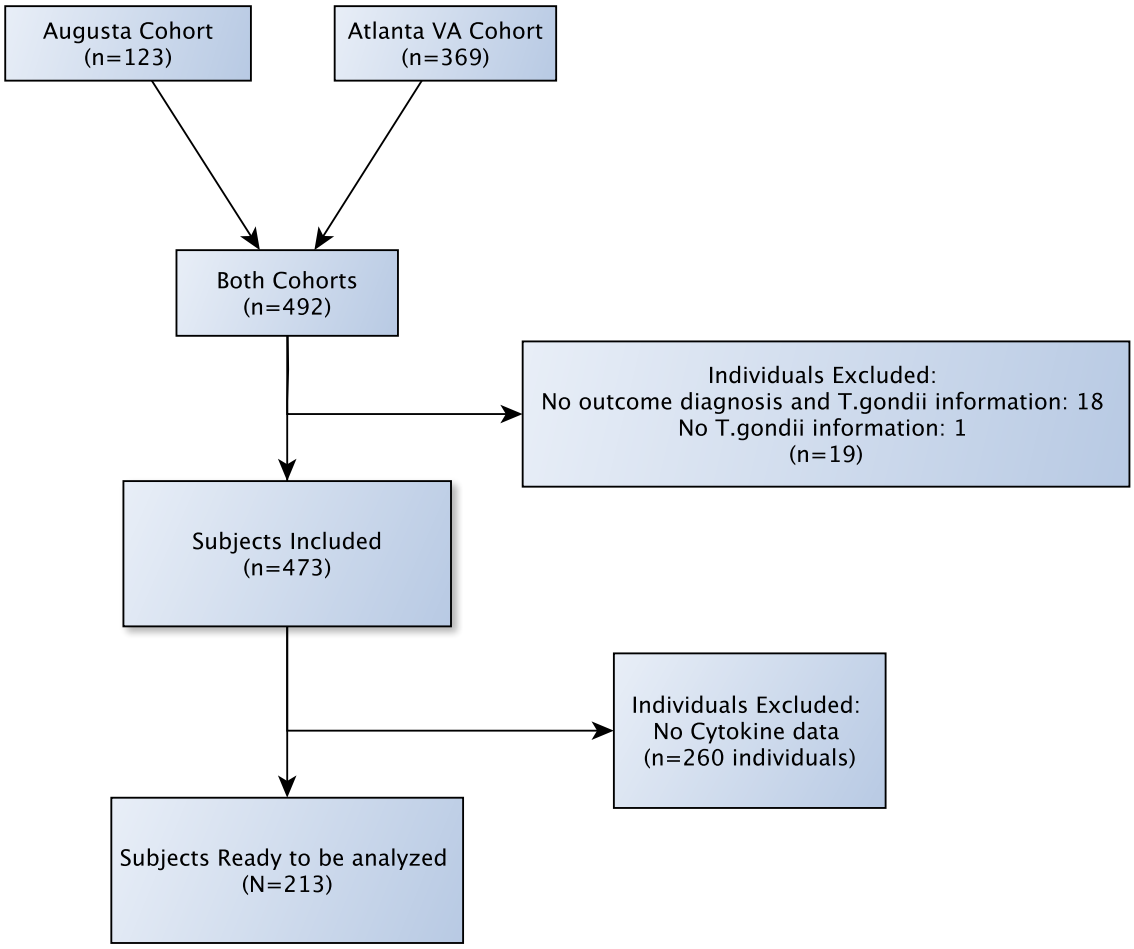


Figure 2: Flow chart describing inclusion and exclusion process of subjects

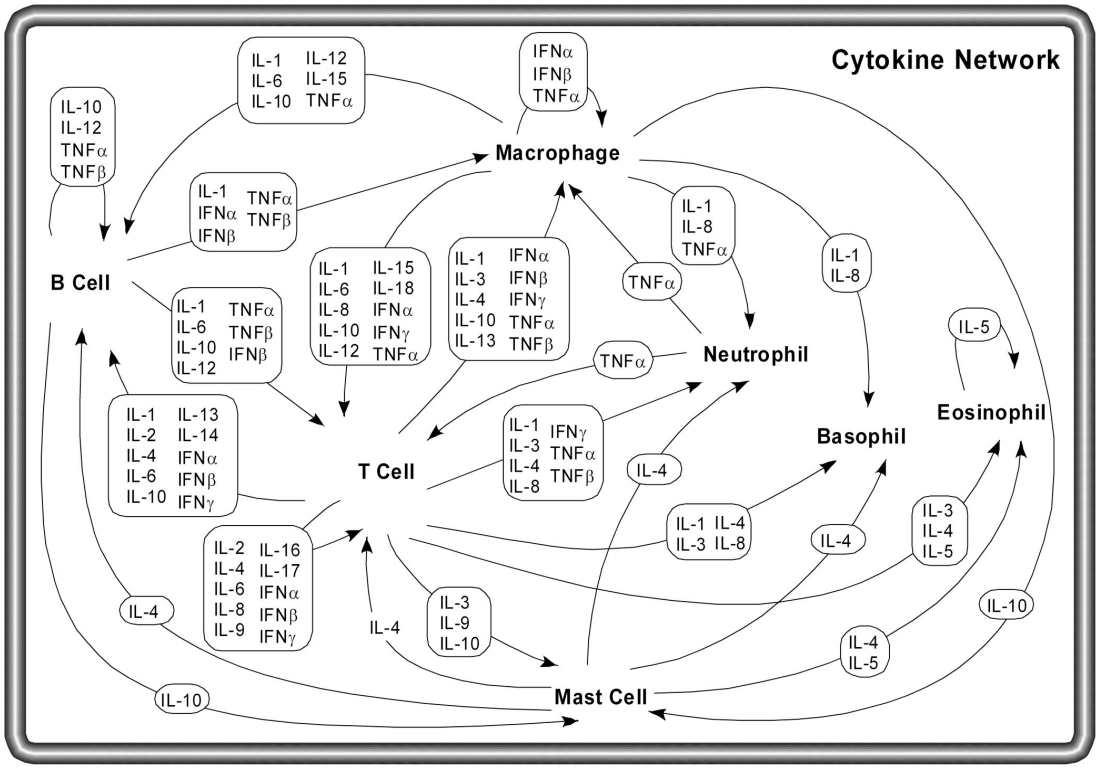


Figure 1.
Cytokine network. Several different cell types coordinate their efforts as part of the immune system, including B cells, T cells, macrophages, mast cells, neutrophils, basophils and eosinophils. Each of these cell types has a distinct role in the immune system, and communicates with other immune cells using secreted cytokines. Macrophages phagocytose foreign bodies and are antigen-presenting cells, using cytokines to stimulate specific antigen dependent responses by B and T cells and non-specific responses by other cell types. T cells secrete a variety of factors to coordinate and stimulate immune responses to specific antigen, such as the role of helper T cells in B cell activation in response to antigen. The proliferation and activation of eosinophils, neutrophils and basophils respond to cytokines as well.

Figure 3: Interaction between immune system with cytokines (26)

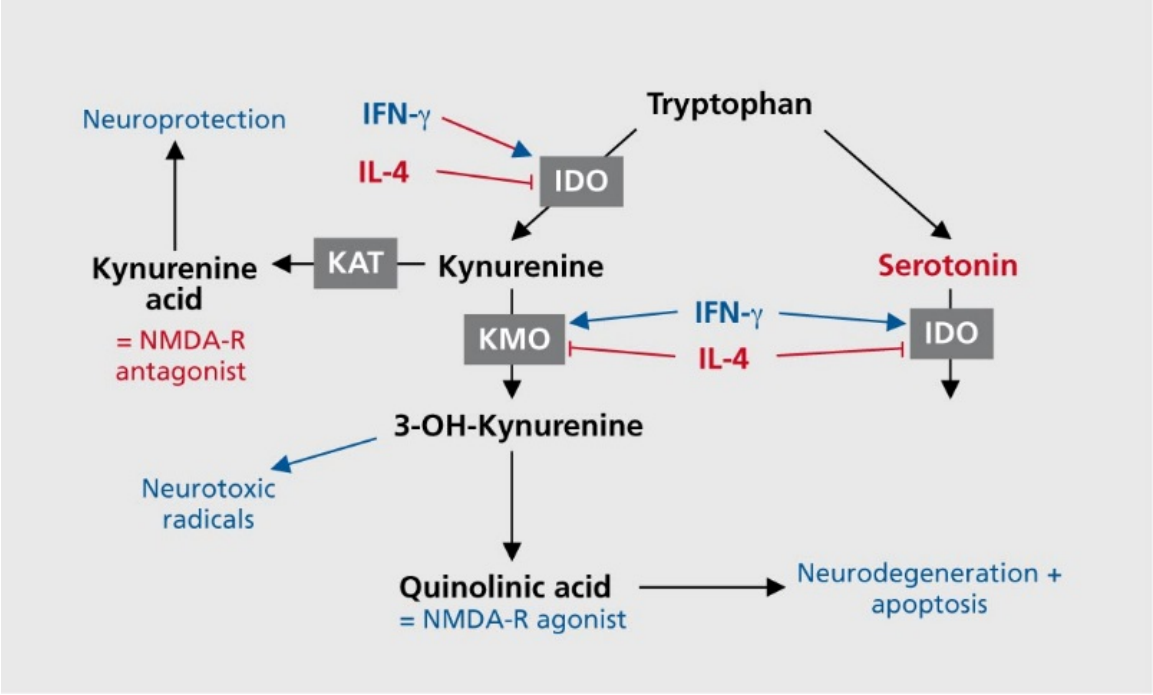


Figure 4: The tryptophan/ kynurenine metabolism pathway. Adapted from N. Muller and Schwarz 2010. (29)

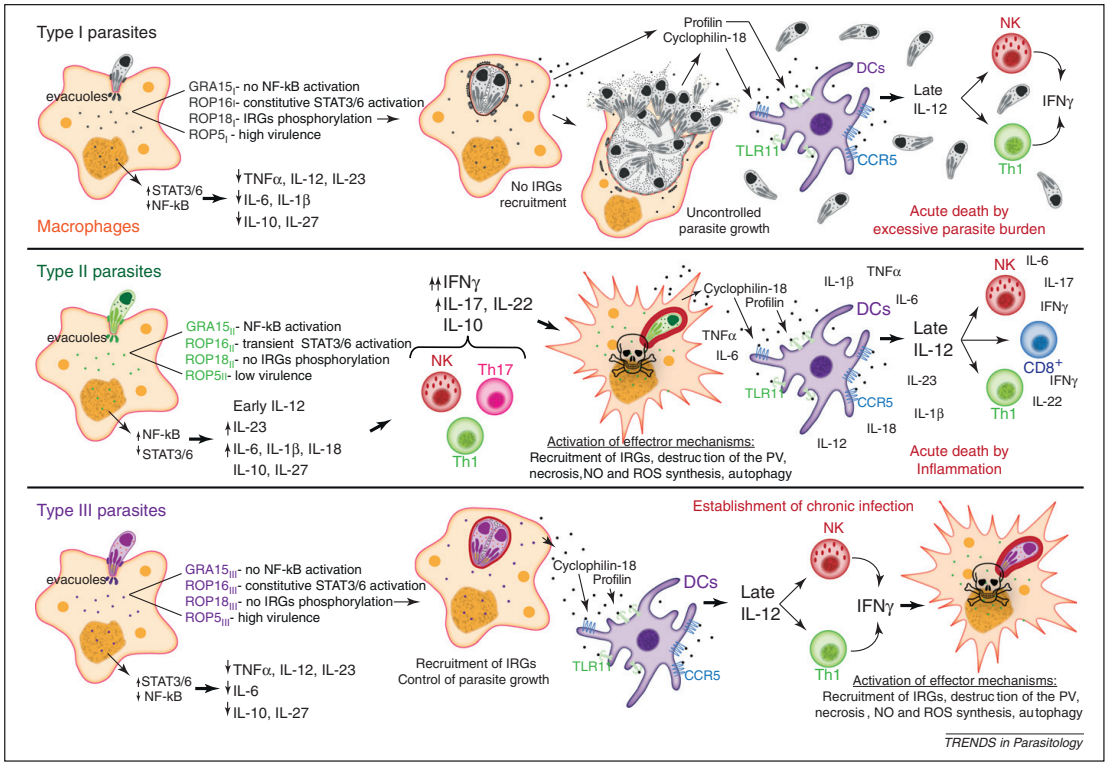


Figure 2. Overview of how *Toxoplasma* strains modulate host immune pathways. Modulation of host cell signaling pathways requires the secretion of numerous parasite proteins from specialized secretory organelles called dense granules and rhoptries. At early time points, infection with type I parasites does not activate pro-inflammatory responses. The type I (RH strain) allele of GRA15 results in a truncated and non-functional protein, allowing a 'silent' infection without activation of NF-κB [42]. On the other hand, ROP16$_I$ induces sustained activation of STAT3 and STAT6, dampening the production of IL-12, IL-1β and IL-6 [41]. Together with the ability to reduce pro-inflammatory cytokine production, type I parasites express ROP5 alleles associated with high virulence [26,37], and ROP18$_I$ phosphorylates IRGs blocking their recruitment to the PV, which is required for parasite destruction, permitting unrestricted parasite growth [28,29]. Conserved parasite proteins secreted by infected cells, profilin and cyclophylin-18, are recognized by DCs via TLR11 and CCR5 respectively, leading to late NF-κB activation and production of IL-12, which in turn activates NK and T cells to secrete IFNγ [59,95]. However, type I parasites also prevent activation of DCs [96], and by the time that the pro-inflammatory response kicks in, host survival is already compromised due to uncontrolled parasite burden. Type II parasites are very effective in activating an early response. These parasites express the active form of GRA15, which activates NF-κB in the infected cells [42], and a less functional form of ROP16, which leads to a transitory activation of STAT3/6 [41]. As a consequence there is a massive production of pro-inflammatory cytokines early after infection. The environment induced by the parasite modulates activation of several T cell subtypes, mainly directing the response towards a Th1 type [97]. Aspects of the Th17 response to *Toxoplasma* seem to have opposite effects on host survival, mainly an IL-23 driven IL-22 response by CD4+ T cells has a negative effect [98], while signaling through the IL-17 receptor can have a beneficial effect by lowering parasite burden [99]. Intracellular parasite growth is controlled due to expression of an avirulent form of ROP18, which does not block the recruitment of IRGs to the PV [28,29], and type II parasites also express ROP5 alleles associated with low virulence [26,37], but susceptible animals die of severe ileitis [69]. Like type I, type III secreted GRA15 and ROP16 do not activate NF-κB and induce a sustained activation of STAT3/6 respectively, limiting the initial production of pro-inflammatory cytokines [41,42]. Nevertheless, these parasites express an inactive ROP18, being unable to avoid intracellular killing mediated by IRGs [28,29]. In this case, late production of IL-12 by DCs triggers a Th1-type response that is sufficient to control parasite burden and induce cyst formation, leading to a chronic infection. CCR5, C-C chemokine receptor type 5; DCs; dendritic cells; GRA, dense granule protein; IRG, interferon-regulated GTPase; NK, natural killer cells; NO, nitric oxide; PV, parasitophorous vacuole; ROP, rhoptry protein; STAT, signal transducer and activator of transcription; ROS, reactive oxygen species, TLR11, Toll-like receptor 11.

Figure 5: Different Types of T.gondii and the response of the immune system as well as the cytokines (32)

Appendices

Appendix 1: Pearson Correlations for all 29 cytokines

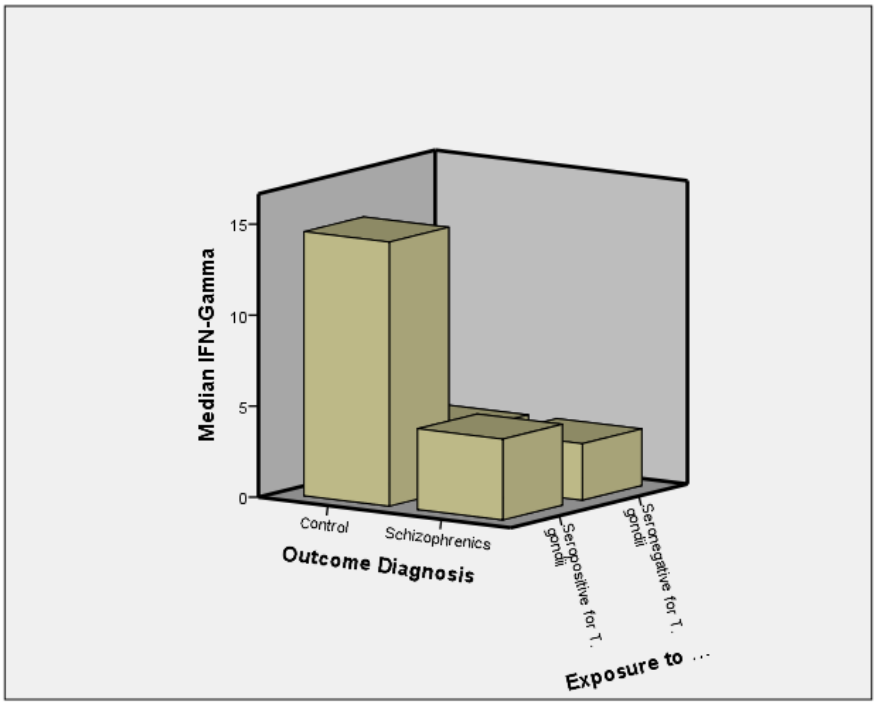
	IL_1Beta	IL_10	IFN_Alpha	IL_6	IL_12	RANTES	Eotaxin	IL_13	IL_15	IL_17	MIP_1Alpha	GM_CSF	MIP_1Beta	MCP_1
IL_1Beta	1	0.84044	0.81918	0.55181	0.72574	-0.05569	0.03293	0.67067	0.89931	0.63289	0.73506	0.86816	0.93046	0.95144
		<.0001	<.0001	<.0001	<.0001	0.43	0.6409	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	203	203	203	203	203	203	203	199	203	203	203	203	203	203
IL_10	0.84044	1	0.75363	0.61789	0.51942	-0.05773	0.10074	0.64981	0.72504	0.8021	0.45834	0.96337	0.76533	0.88084
	<.0001		<.0001	<.0001	<.0001	0.4019	0.1428	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
IFN_Alpha	0.81918	0.75363	1	0.64693	0.83096	0.04101	0.13404	0.40924	0.86495	0.82172	0.77633	0.74074	0.86235	0.79848
	<.0001	<.0001		<.0001	<.0001	0.5517	0.0508	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
IL_6	0.55181	0.61789	0.64693	1	0.65798	-0.02643	0.23202	0.24346	0.54309	0.61013	0.64667	0.48013	0.60156	0.46553
	<.0001	<.0001	<.0001		<.0001	0.7013	0.0006	0.0004	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
IL_12	0.72574	0.51942	0.83096	0.65798	1	0.01862	0.24485	0.2446	0.7574	0.60914	0.93123	0.49467	0.84375	0.68156
	<.0001	<.0001	<.0001	<.0001		0.7871	0.0003	0.0004	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
RANTES	-0.05569	-0.05773	0.04101	-0.02643	0.01862	1	0.13446	-0.03878	-0.05362	-0.01821	-0.02838	-0.0735	-0.05675	-0.0619
	0.43	0.4019	0.5517	0.7013	0.7871		0.05	0.5772	0.4363	0.7916	0.6805	0.2868	0.4099	0.3687
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
Eotaxin	0.03293	0.10074	0.13404	0.23202	0.24485	0.13446	1	0.02656	0.007	0.11611	0.07319	0.01977	0.03259	0.05075
	0.6409	0.1428	0.0508	0.0006	0.0003	0.05		0.7026	0.9191	0.091	0.2876	0.7747	0.6363	0.4613
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
IL_13	0.67067	0.64981	0.40924	0.24346	0.2446	-0.03878	0.02656	1	0.577	0.38126	0.25631	0.63809	0.51986	0.63202
	<.0001	<.0001	<.0001	0.0004	0.0004	0.5772	0.7026		<.0001	<.0001	0.0002	<.0001	<.0001	<.0001
	199	209	209	209	209	209	209	209	209	209	209	208	209	209
IL_15	0.89931	0.72504	0.86495	0.54309	0.7574	-0.05362	0.007	0.577	1	0.69019	0.80349	0.73738	0.91752	0.83315
	<.0001	<.0001	<.0001	<.0001	<.0001	0.4363	0.9191	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
IL_17	0.63289	0.8021	0.82172	0.61013	0.60914	-0.01821	0.11611	0.38126	0.69019	1	0.5315	0.74449	0.69404	0.66033
	<.0001	<.0001	<.0001	<.0001	<.0001	0.7916	0.091	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
MIP_1Alpha	0.73506	0.45834	0.77633	0.64667	0.93123	-0.02838	0.07319	0.25631	0.80349	0.5315	1	0.43596	0.86116	0.64403
	<.0001	<.0001	<.0001	<.0001	<.0001	0.6805	0.2876	0.0002	<.0001	<.0001		<.0001	<.0001	<.0001
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
GM_CSF	0.86816	0.96337	0.74074	0.48013	0.49467	-0.0735	0.01977	0.63809	0.73738	0.74449	0.43596	1	0.78436	0.93428
	<.0001	<.0001	<.0001	<.0001	<.0001	0.2868	0.7747	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001
	203	212	212	212	212	212	212	208	212	212	212	212	212	212
MIP_1Beta	0.93046	0.76533	0.86235	0.60156	0.84375	-0.05675	0.03259	0.51986	0.91752	0.69404	0.86116	0.78436	1	0.90981
	<.0001	<.0001	<.0001	<.0001	<.0001	0.4099	0.6363	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
MCP_1	0.95144	0.88084	0.79848	0.46553	0.68156	-0.0619	0.05075	0.63202	0.83315	0.66033	0.64403	0.93428	0.90981	1
	<.0001	<.0001	<.0001	<.0001	<.0001	0.3687	0.4613	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
IL_5	0.49364	0.60974	0.58737	0.49487	0.36944	-0.0343	0.05016	0.22818	0.60396	0.67409	0.35629	0.58952	0.61043	0.52
	<.0001	<.0001	<.0001	<.0001	<.0001	0.6254	0.4751	0.0011	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	196	205	205	205	205	205	205	203	205	205	205	205	205	205
IFN_Gamma	0.54041	0.50608	0.58505	0.22326	0.37577	-0.06604	0.021	0.60579	0.64019	0.53007	0.32106	0.53384	0.54301	0.55859
	<.0001	<.0001	<.0001	0.0016	<.0001	0.3553	0.7691	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	188	198	198	198	198	198	198	196	198	198	198	197	198	198
TNF_Alpha	0.76027	0.77844	0.91474	0.57909	0.77073	-0.06035	0.06616	0.39102	0.83937	0.90801	0.71164	0.76956	0.86228	0.79564
	<.0001	<.0001	<.0001	<.0001	<.0001	0.3808	0.3365	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
IL_1RA	0.81543	0.59677	0.8299	0.59377	0.93367	-0.0575	0.05002	0.39355	0.83556	0.63872	0.94809	0.60234	0.92976	0.78981
	<.0001	<.0001	<.0001	<.0001	<.0001	0.4037	0.4678	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
IL_2	0.838	0.56774	0.78743	0.69545	0.86626	-0.03786	0.06587	0.46327	0.8735	0.53655	0.94426	0.53148	0.8909	0.70129
	<.0001	<.0001	<.0001	<.0001	<.0001	0.5908	0.3492	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	199	204	204	204	204	204	204	200	204	204	204	204	204	204
IL_7	0.60472	0.79368	0.71903	0.78074	0.50068	-0.01758	0.12745	0.36225	0.63465	0.82894	0.4482	0.71745	0.64525	0.59539
	<.0001	<.0001	<.0001	<.0001	<.0001	0.8059	0.0736	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	193	198	198	198	198	198	198	195	198	198	198	197	198	198
IP_10	0.01124	0.01348	0.00317	0.03417	0.08124	0.02211	0.11066	0.01371	0.0437	0.01241	0.00859	0.00559	0.01281	0.02383
	0.8736	0.845	0.9634	0.62	0.2377	0.7484	0.1073	0.8438	0.5259	0.8571	0.9008	0.9356	0.8526	0.7295
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
IL_2R	0.72704	0.79337	0.87248	0.5174	0.66152	-0.07685	0.07307	0.43834	0.78844	0.87899	0.56604	0.79959	0.77511	0.7813
	<.0001	<.0001	<.0001	<.0001	<.0001	0.2642	0.2884	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
MIG	0.74949	0.8257	0.82738	0.60168	0.62648	-0.04329	0.06396	0.47998	0.81725	0.83366	0.58537	0.79912	0.80777	0.78187
	<.0001	<.0001	<.0001	<.0001	<.0001	0.5307	0.3541	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	202	212	212	212	212	212	212	208	212	212	212	211	212	212
IL_4	0.74614	0.82491	0.89339	0.57121	0.68354	-0.03527	0.09782	0.4972	0.79899	0.91686	0.59488	0.80697	0.78675	0.78546
	<.0001	<.0001	<.0001	<.0001	<.0001	0.6088	0.1548	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
IL_8	0.43068	0.4358	0.53211	0.20433	0.41078	0.01766	0.38393	0.44251	0.51326	0.50378	0.27718	0.43788	0.4507	0.47157
	<.0001	<.0001	<.0001	0.0027	<.0001	0.7977	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	203	213	213	213	213	213	213	209	213	213	213	212	213	213
Kynurenine	-0.02524	-0.02465	-0.19929	-0.01769	-0.10464	-0.1396	-0.06949	0.00768	-0.1727	-0.03376	-0.02419	-0.03232	0.0985	-0.02122
	0.8144	0.8087	0.048	0.862	0.3027	0.1682	0.4943	0.9411	0.0874	0.7401	0.8121	0.752	0.332	0.8348
	89	99	99	99	99	99	99	95	99	99	99	98	99	99
Tryptophan	-0.02584	-0.01362	-0.21687	-0.01244	-0.15379	-0.05355	-0.06617	-0.00087	-0.16568	-0.08541	0.04249	-0.03678	0.11265	-0.06922
	0.81	0.8936	0.0311	0.9027	0.1286	0.5986	0.5152	0.9933	0.1012	0.4006	0.6762	0.7192	0.2669	0.496
	89	99	99	99	99	99	99	95	99	99	99	98	99	99
KT_Ratio	-0.01745	-0.0301	0.00085	-0.02539	0.04679	-0.14941	-0.07598	-0.01319	-0.01046	0.05697	-0.09162	-0.01433	-0.01145	0.00177
	0.8711	0.7674	0.9934	0.803	0.6456	0.1399	0.4548	0.899	0.9181	0.5754	0.3671	0.8886	0.9104	0.9861
	89	99	99	99	99	99	99	95	99	99	99	98	99	99
SDF1_alpha	-0.16412	-0.11935	0.01488	-0.11483	-0.1163	0.33283	-0.01144	-0.13349	-0.07625	-0.06408	-0.11997	-0.12178	-0.08058	-0.12128
	0.2312	0.3436	0.9064	0.3624	0.3562	0.0067	0.9279	0.301	0.546	0.612	0.3411	0.3377	0.5234	0.3359
	55	65	65	65	65	65	65	62	65	65	65	64	65	65

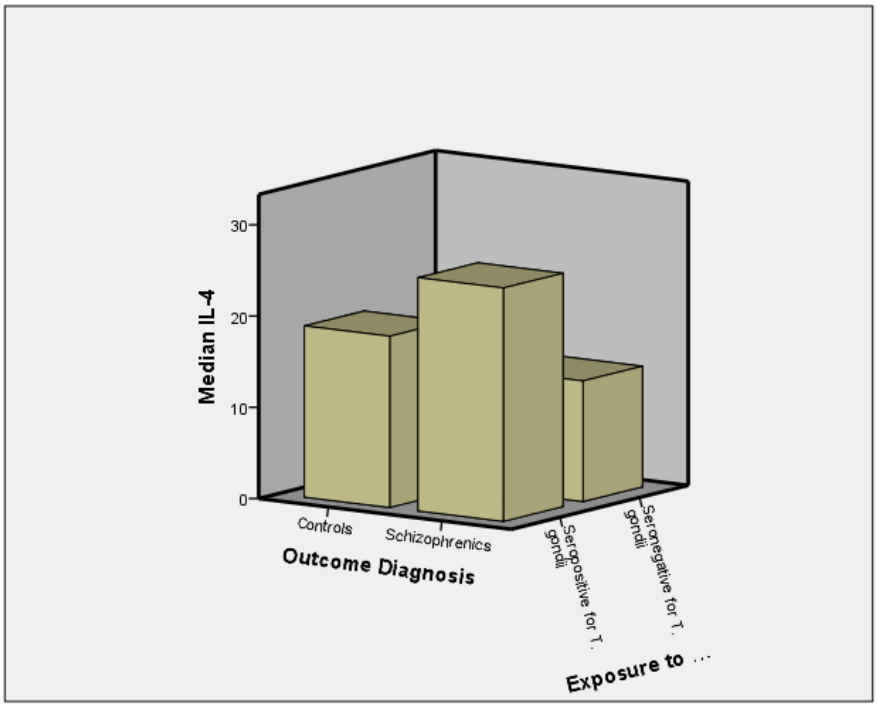
	IL_5	IFN_Gamma	TNF_Alpha	IL_1RA	IL_2	IL_7	IP_10	IL_2R	MIG	IL_4	IL_8	Kynurenine	Tryptophan	KT_Ratio	SDF1_alpha
IL_1Beta	0.49364	0.54041	0.76027	0.81543	0.838	0.60472	0.01124	0.72704	0.74949	0.74614	0.43068	-0.02524	-0.02584	-0.01745	-0.16412
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.8736	<.0001	<.0001	<.0001	<.0001	0.8144	0.81	0.8711	0.2312
	196	188	203	203	199	193	203	203	202	203	203	89	89	89	55
IL_10	0.60974	0.50608	0.77844	0.59677	0.56774	0.79368	0.01348	0.79337	0.8257	0.82491	0.4358	-0.02465	-0.01362	-0.0301	-0.11935
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.845	<.0001	<.0001	<.0001	<.0001	0.8087	0.8936	0.7674	0.3436
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IFN_Alpha	0.58737	0.58505	0.91474	0.8299	0.78743	0.71903	0.00317	0.87248	0.82738	0.89339	0.53211	-0.19929	-0.21687	0.00085	0.01488
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.9634	<.0001	<.0001	<.0001	<.0001	0.048	0.0311	0.9934	0.9064
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_6	0.49487	0.22326	0.57909	0.59377	0.69545	0.78074	0.03417	0.5174	0.60168	0.57121	0.20433	-0.01769	-0.01244	-0.02539	-0.11483
	<.0001	0.0016	<.0001	<.0001	<.0001	<.0001	0.62	<.0001	<.0001	<.0001	0.0027	0.862	0.9027	0.803	0.3624
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_12	0.36944	0.37577	0.77073	0.93367	0.86626	0.50068	0.08124	0.66152	0.62648	0.68354	0.41078	-0.10464	-0.15379	0.04679	-0.1163
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2377	<.0001	<.0001	<.0001	<.0001	0.3027	0.1286	0.6456	0.3562
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
RANTES	-0.0343	-0.06604	-0.06035	-0.0575	-0.03786	-0.01758	0.02211	-0.07685	-0.04329	-0.03527	0.01766	-0.1396	-0.05355	-0.14941	0.33283
	0.6254	0.3553	0.3808	0.4037	0.5908	0.8059	0.7484	0.2642	0.5307	0.6088	0.7977	0.1682	0.5986	0.1399	0.0067
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
Eotaxin	0.05016	0.021	0.06616	0.05002	0.06587	0.12745	0.11066	0.07307	0.06396	0.09782	0.38393	-0.06949	-0.06617	-0.07598	-0.01144
	0.4751	0.7691	0.3365	0.4678	0.3492	0.0736	0.1073	0.2884	0.3541	0.1548	<.0001	0.4943	0.5152	0.4548	0.9279
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_13	0.22818	0.60579	0.39102	0.39355	0.46327	0.36225	0.01371	0.43834	0.47998	0.4972	0.44251	0.00768	-0.00087	-0.01319	-0.13349
	0.0011	<.0001	<.0001	<.0001	<.0001	<.0001	0.8438	<.0001	<.0001	<.0001	<.0001	0.9411	0.9933	0.899	0.301
	203	196	209	209	200	195	209	209	208	209	209	95	95	95	62
IL_15	0.60396	0.64019	0.83937	0.83556	0.8735	0.63465	0.0437	0.78844	0.81725	0.79899	0.51326	-0.1727	-0.16568	-0.01046	-0.07625
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5259	<.0001	<.0001	<.0001	<.0001	0.0874	0.1012	0.9181	0.546
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_17	0.67409	0.53007	0.90801	0.63872	0.53655	0.82894	0.01241	0.87899	0.83366	0.91686	0.50378	-0.03376	-0.08541	0.05697	-0.06408
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.8571	<.0001	<.0001	<.0001	<.0001	0.7401	0.4006	0.5754	0.612
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
MIP_1Alpha	0.35629	0.32106	0.71164	0.94809	0.94426	0.4482	0.00859	0.56604	0.58537	0.59488	0.27718	-0.02419	0.04249	-0.09162	-0.11997
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.9008	<.0001	<.0001	<.0001	<.0001	0.8121	0.6762	0.3671	0.3411
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
GM_CSF	0.58952	0.53384	0.76956	0.60234	0.53148	0.71745	0.00559	0.79959	0.79912	0.80697	0.43788	-0.03232	-0.03678	-0.01433	-0.12178
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.9356	<.0001	<.0001	<.0001	<.0001	0.752	0.7192	0.8886	0.3377
	205	197	212	212	204	197	212	212	211	212	212	98	98	98	64
MIP_1Beta	0.61043	0.54301	0.86228	0.92976	0.8909	0.64525	0.01281	0.77511	0.80777	0.78675	0.4507	0.0985	0.11265	-0.01145	-0.08058
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.8526	<.0001	<.0001	<.0001	<.0001	0.332	0.2669	0.9104	0.5234
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
MCP_1	0.52	0.55859	0.79564	0.78981	0.70129	0.59539	0.02383	0.7813	0.78187	0.78546	0.47157	-0.02122	-0.06922	0.00177	-0.12128
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.7295	<.0001	<.0001	<.0001	<.0001	0.8348	0.496	0.9861	0.3359
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_5	1	0.52171	0.70254	0.39957	0.41442	0.82777	-0.00915	0.67143	0.75017	0.67025	0.43855	-0.00827	-0.0204	-0.0125	-0.11627
		<.0001	<.0001	<.0001	<.0001	<.0001	0.8964	<.0001	<.0001	<.0001	<.0001	0.938	0.8478	0.9064	0.3763
	205	195	205	205	198	192	205	205	205	205	205	91	91	91	60
IFN_Gamma	0.52171	1	0.6493	0.46679	0.38434	0.48496	0.0087	0.749	0.63301	0.7397	0.75601	-0.09173	-0.04622	-0.10724	0.14231
	<.0001		<.0001	<.0001	<.0001	<.0001	0.9032	<.0001	<.0001	<.0001	<.0001	0.4066	0.6763	0.3316	0.2954
	195	198	198	198	190	187	198	198	198	198	198	84	84	84	56
TNF_Alpha	0.70254	0.6493	1	0.8244	0.69305	0.75473	0.01263	0.95036	0.88302	0.95848	0.58053	-0.0022	-0.04062	0.01944	-0.03209
	<.0001	<.0001		<.0001	<.0001	<.0001	0.8546	<.0001	<.0001	<.0001	<.0001	0.9828	0.6897	0.8485	0.7997
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_1RA	0.39957	0.46679	0.8244	1	0.89906	0.49234	0.01164	0.70898	0.68489	0.73184	0.40192	-0.09483	-0.10528	-0.00841	-0.12755
	<.0001	<.0001	<.0001		<.0001	<.0001	0.8659	<.0001	<.0001	<.0001	<.0001	0.3505	0.2997	0.9342	0.3113
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_2	0.41442	0.38434	0.69305	0.89906	1	0.53471	0.00241	0.56702	0.62927	0.60564	0.31404	-0.05967	-0.05309	-0.01259	-0.14621
	<.0001	<.0001	<.0001	<.0001		<.0001	0.9727	<.0001	<.0001	<.0001	<.0001	0.5764	0.6192	0.9062	0.2778
	198	190	204	204	204	193	204	204	204	204	204	90	90	90	57
IL_7	0.82777	0.48496	0.75473	0.49234	0.53471	1	0.02373	0.74153	0.79182	0.77708	0.4248	0.00489	-0.00977	-0.00002	-0.15205
	<.0001	<.0001	<.0001	<.0001	<.0001		0.74	<.0001	<.0001	<.0001	<.0001	0.9648	0.9297	0.9999	0.2918
	192	187	198	198	193	198	198	198	197	198	198	84	84	84	50
IP_10	-0.00915	0.0087	0.01263	0.01164	0.00241	0.02373	1	0.00664	0.12433	0.00391	0.027	0.10193	0.11917	-0.01125	-0.10296
	0.8964	0.9032	0.8546	0.8659	0.9727	0.74		0.9232	0.0708	0.9548	0.6952	0.3154	0.2401	0.912	0.4144
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_2R	0.67143	0.749	0.95036	0.70898	0.56702	0.74153	0.00664	1	0.86566	0.97265	0.66212	-0.09536	-0.07866	-0.05626	-0.14866
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.9232		<.0001	<.0001	<.0001	0.3478	0.439	0.5802	0.2372
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
MIG	0.75017	0.63301	0.88302	0.68489	0.62927	0.79182	0.12433	0.86566	1	0.87379	0.5148	0.05654	-0.04987	0.2107	-0.07204
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0708	<.0001		<.0001	<.0001	0.5803	0.6258	0.0373	0.5685
	205	198	212	212	204	197	212	212	212	212	212	98	98	98	65
IL_4	0.67025	0.7397	0.95848	0.73184	0.60564	0.77708	0.00391	0.97265	0.87379	1	0.64888	-0.17843	-0.17573	-0.01875	-0.05134
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.9548	<.0001	<.0001		<.0001	0.0772	0.0819	0.8538	0.6846
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_8	0.43855	0.75601	0.58053	0.40192	0.31404	0.4248	0.027	0.66212	0.5148	0.64888	1	-0.11739	-0.07294	-0.07874	0.10831
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.6952	<.0001	<.0001	<.0001		0.2472	0.4731	0.4385	0.3904
	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
Kynurenine	-0.00827	-0.09173	-0.0022	-0.09483	-0.05967	0.00489	0.10193	-0.09536	0.05654	-0.17843	-0.11739	1	0.72639	0.33618	-0.00216
	0.938	0.4066	0.9828	0.3505	0.5764	0.9648	0.3154	0.3478	0.5803	0.0772	0.2472		<.0001	0.0007	0.9863
	91	84	99	99	90	84	99	99	98	99	99	99	99	99	65
Tryptophan	-0.0204	-0.04622	-0.04062	-0.10528	-0.05309	-0.00977	0.11917	-0.07866	-0.04987	-0.17573	-0.07294	0.72639	1	-0.33305	-0.14398
	0.8478	0.6763	0.6897	0.2997	0.6192	0.9297	0.2401	0.439	0.6258	0.0819	0.4731	<.0001		0.0008	0.2525
	91	84	99	99	90	84	99	99	98	99	99	99	99	99	65
KT_Ratio	-0.0125	-0.10724	0.01944	-0.00841	-0.01259	-0.00002	-0.01125	-0.05626	0.2107	-0.01875	-0.07874	0.33618	-0.33305	1	0.20259
	0.9064	0.3316	0.8485	0.9342	0.9062	0.9999	0.912	0.5802	0.0373	0.8538	0.4385	0.0007	0.0008		0.1056
	91	84	99	99	90	84	99	99	98	99	99	99	99	99	65
SDF1_alpha	-0.11627	0.14231	-0.03209	-0.12755	-0.14621	-0.15205	-0.10296	-0.14866	-0.07204	-0.05134	0.10831	-0.00216	-0.14398	0.20259	1
	0.3763	0.2954	0.7997	0.3113	0.2778	0.2918	0.4144	0.2372	0.5685	0.6846	0.3904	0.9863	0.2525	0.1056	
	60	56	65	65	57	50	65	65	65	65	65	65	65	65	65

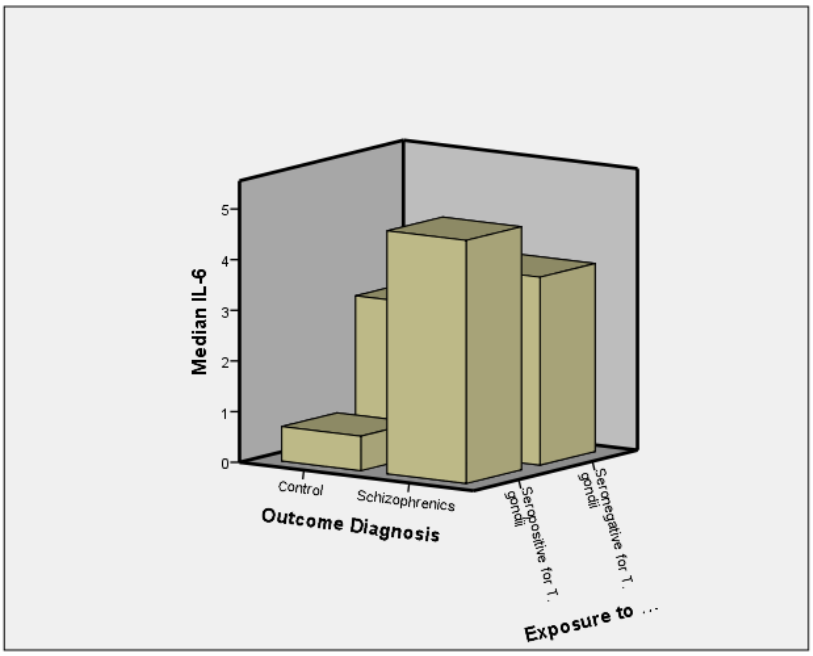
Appendix 2: Spearman Correlation Coefficient for all 29 cytokines

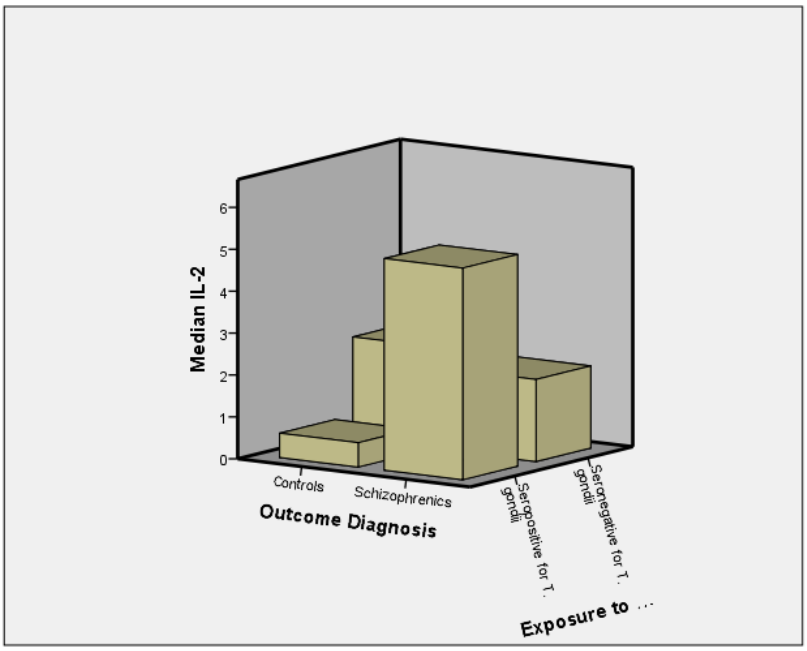
	IL_1Beta	IL_10	IFN_Alpha	IL_6	IL_12	RANTES	Eotaxin	IL_13	IL_15	IL_17	MIP_1Alpha	GM_CSF	MIP_1Beta	MCP_1	IL_5	IFN_Gamma	TNF_Alpha	IL_1RA	IL_2	IL_7	IP_10	IL_2R	MIG	IL_4	IL_8	Kynurenine	Tryptophan	KT_Ratio	SDF1_alpha
IL_1Beta	1	0.16269	0.60882	0.58977	0.61472	0.23968	0.2835	0.2539	0.49394	0.61088	0.58249	0.34156	0.57788	0.68839	0.08264	0.01367	0.4637	0.78086	0.78283	0.6084	0.33073	0.11435	0.18699	0.48815	0.48318	0.18687	0.17038	0.01923	-0.17606
		0.0204	<.0001	<.0001	<.0001	0.0006	<.0001	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2495	0.8523	<.0001	<.0001	<.0001	<.0001	<.0001	0.1043	0.0077	<.0001	<.0001	0.0795	0.1104	0.858	0.1985
	203	203	203	203	203	203	203	199	203	203	203	203	203	203	196	188	203	203	199	193	203	203	202	203	203	89	89	89	55
IL_10	0.16269	1	0.23679	0.32624	0.06606	-0.30197	-0.31467	0.76947	0.44417	0.2242	0.33043	0.73954	0.43029	0.21077	0.75086	0.53407	0.4833	0.28751	0.22022	0.34754	-0.17974	0.61316	0.69474	0.34533	0.037	0.11217	0.10459	-0.06924	-0.07025
	0.0204		0.0005	<.0001	0.3373	<.0001	<.0001	<.0001	<.0001	0.001	<.0001	<.0001	<.0001	0.002	<.0001	<.0001	<.0001	0.0016	<.0001	0.0086	<.0001	<.0001	<.0001	0.5913	0.269	0.3029	0.4959	0.5781	
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IFN_Alpha	0.60882	0.23679	1	0.53062	0.61084	0.24993	0.3112	0.33309	0.50417	0.69031	0.66946	0.47368	0.53436	0.66452	0.27729	0.33284	0.66128	0.73005	0.60725	0.72691	0.29271	0.20543	0.34196	0.7337	0.48055	-0.14063	-0.27549	0.198	0.12508
	<.0001	0.0005		<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0026	<.0001	<.0001	<.0001	0.165	0.0058	0.0495	0.3208	
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_6	0.58977	0.32624	0.53062	1	0.53409	0.26833	0.28206	0.32834	0.37945	0.52345	0.52916	0.45106	0.51628	0.62279	0.19076	0.2036	0.44667	0.61615	0.5457	0.63041	0.34818	0.21198	0.28168	0.41949	0.43793	0.24137	0.30855	0.01522	0.21821
	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0061	0.004	<.0001	<.0001	<.0001	<.0001	0.0019	<.0001	<.0001	<.0001	0.0161	0.0019	0.8811	0.8808	
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_12	0.61472	0.06606	0.61084	0.53409	1	0.43713	0.53389	0.0315	0.36872	0.49504	0.50814	0.15851	0.42332	0.71597	-0.03663	0.00383	0.36235	0.75719	0.57135	0.52261	0.59227	0.09783	0.1875	0.40384	0.56798	-0.13216	-0.27076	0.12018	-0.01899
	<.0001	0.3373	<.0001	<.0001		<.0001	<.0001	0.6507	<.0001	<.0001	<.0001	0.0209	<.0001	<.0001	0.6021	0.9573	<.0001	<.0001	<.0001	<.0001	<.0001	0.1548	0.0062	0.1922	0.0067	0.2361	0.8806	
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
RANTES	0.25968	-0.30197	0.24993	0.26833	0.43713	1	0.56188	-0.21101	0.01103	0.17616	0.30488	-0.14176	0.16082	0.30546	-0.27527	-0.08006	0.05287	0.30083	0.12491	0.17672	0.38715	-0.21176	-0.14174	0.18391	0.39375	0.03912	0.08237	-0.09529	0.13036
	0.0006	<.0001	0.0002	<.0001	<.0001		<.0001	0.0022	0.8729	0.01	<.0001	0.0392	0.0188	<.0001	<.0001	0.2622	0.4427	<.0001	0.0751	0.0128	<.0001	0.0019	0.0392	0.0071	<.0001	0.7007	0.4176	0.3481	0.3007
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
Eotaxin	0.2835	-0.31467	0.3112	0.28206	0.53389	0.56188	1	-0.37444	-0.02092	0.22362	0.29396	-0.25777	0.09576	0.45298	-0.37057	-0.12044	0.01481	0.36892	0.10945	0.19554	0.63225	-0.21639	-0.17492	0.11413	0.43117	-0.12308	-0.0431	-0.11698	0.19264
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	0.7615	0.001	<.0001	0.0001	0.1638	<.0001	<.0001	0.091	0.8298	<.0001	0.1191	0.0248	<.0001	0.0015	0.0107	0.0967	<.0001	0.2249	0.6718	0.2489	0.1242
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_13	0.2539	0.76947	0.33309	0.32834	0.0315	-0.21101	-0.37444	1	0.48577	0.38713	0.39629	0.77206	0.52118	0.2187	0.81648	0.61408	0.62673	0.29726	0.29972	0.50871	-0.30812	0.58032	0.61099	0.56762	0.15511	0.37299	0.32714	0.10375	-0.07561
	0.0003	<.0001	<.0001	<.0001	0.6507	0.0022	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	0.0015	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0249	0.0002	0.0012	0.3171	0.5592
	199	209	209	209	209	209	209	209	209	209	209	208	209	209	203	196	209	209	200	195	209	209	208	209	209	95	95	95	62
IL_15	0.49394	0.44417	0.50417	0.37945	0.36872	0.01103	-0.02092	0.48577	1	0.5091	0.55699	0.51781	0.51185	0.50243	0.44173	0.3472	0.59646	0.55573	0.52933	0.50746	0.24685	0.4165	0.49509	0.567	0.27681	-0.09168	-0.21887	0.13322	-0.07028
	<.0001	<.0001	<.0001	<.0001	<.0001	0.8729	0.7615	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003	<.0001	<.0001	<.0001	<.0001	0.3668	0.0295	0.1887	0.578	
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_17	0.61088	0.2242	0.69031	0.52345	0.49504	0.17616	0.22362	0.38713	0.5091	1	0.65586	0.4332	0.56922	0.55683	0.31047	0.31477	0.71266	0.67262	0.59155	0.77127	0.20036	0.26653	0.32572	0.67014	0.46503	0.01133	-0.13501	0.16201	-0.04532
	<.0001	0.001	<.0001	<.0001	<.0001	0.01	0.001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0033	<.0001	<.0001	<.0001	<.0001	0.9113	0.1827	0.1091	0.72	
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
MIP_1Alpha	0.58249	0.33043	0.66946	0.52916	0.50814	0.30488	0.29396	0.39629	0.55699	0.65586	1	0.47097	0.71216	0.51825	0.37118	0.48599	0.68928	0.68914	0.56115	0.61974	0.18766	0.52118	0.49394	0.69883	0.41059	0.11123	0.15878	-0.12211	-0.00724
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.006	<.0001	<.0001	<.0001	<.0001	0.273	0.1165	0.2286	0.9543
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
GM_CSF	0.34156	0.73954	0.47368	0.45106	0.15851	-0.14176	-0.25777	0.77206	0.51781	0.4332	0.47097	1	0.51639	0.3493	0.70157	0.52553	0.634	0.42559	0.40046	0.56837	-0.14577	0.4831	0.49792	0.58941	0.15524	0.06864	0.06474	0.03454	-0.12989
	<.0001	<.0001	<.0001	<.0001	0.0209	0.0392	0.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0339	<.0001	<.0001	<.0001	0.0238	0.5517	0.5265	0.7357	0.3063
	203	212	212	212	212	212	212	208	212	212	212	212	212	212	205	197	212	212	204	197	212	212	211	212	212	98	98	98	64
MIP_1Beta	0.57788	0.43029	0.53436	0.51628	0.42332	0.16082	0.09576	0.52118	0.51185	0.56922	0.71216	0.51639	1	0.58517	0.359	0.34086	0.62552	0.68168	0.53835	0.57133	0.07126	0.46085	0.44532	0.53652	0.4261	0.22884	0.25961	-0.06797	0.20103
	<.0001	<.0001	<.0001	<.0001	<.0001	0.0188	0.1638	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.3005	<.0001	<.0001	<.0001	<.0001	0.0227	0.0095	0.5039	0.1083	
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
MCP_1	0.68839	0.21077	0.66452	0.62279	0.71597	0.30546	0.45298	0.2187	0.50243	0.55683	0.51825	0.3493	0.58517	1	0.09972	0.09938	0.48461	0.79316	0.56521	0.60078	0.54704	0.23187	0.232	0.52358	0.53584	-0.02984	-0.04742	0.02237	0.02587
	<.0001	0.002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.1549	0.1636	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.7694	0.6412	0.8261	0.8379
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_5	0.08264	0.75086	0.27729	0.19076	-0.03663	-0.27527	-0.37057	0.81648	0.44173	0.31047	0.37118	0.70157	0.359	0.09972	1	0.72914	0.54773	0.19444	0.1924	0.36248	-0.31194	0.55284	0.65507	0.49234	0.02618	0.07628	0.05019	0.04822	0.0147
	0.2495	<.0001	<.0001	0.0061	0.6021	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1549		<.0001	<.0001	0.0052	0.0066	<.0001	<.0001	<.0001	<.0001	<.0001	0.7094	0.4723	0.6366	0.6499	0.9113	
	196	205	205	205	205	205	205	203	205	205	205	205	205	205	205	195	205	205	198	192	205	205	205	205	205	91	91	91	60
IFN_Gamma	0.01367	0.53407	0.33284	0.2036	0.00383	-0.08006	-0.12044	0.61408	0.3472	0.31477	0.48599	0.52553	0.34086	0.09938	0.72914	1	0.55655	0.18944	0.11474	0.31837	-0.16557	0.09938	0.14174	0.33837	0.16621	0.33816	0.32095	0.00002	0.16127
	0.8523	<.0001	<.0001	0.004	0.9573	0.2622	0.091	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	0.0075	0.1149	<.0001	0.0197	<.0001	<.0001	<.0001	0.0086	0.0017	0.0029	0.9999	0.2351	
	188	198	198	198	198	198	198	196	198	198	198	197	198	198	195	198	198	198	190	187	198	198	198	198	198	84	84	84	56
TNF_Alpha	0.4637	0.4833	0.66128	0.44667	0.36235	0.05287	0.01481	0.62673	0.59646	0.71266	0.68928	0.634	0.62552	0.48461	0.54773	0.55655	1	0.58772	0.50886	0.65919	-0.00958	0.42401	0.55938	0.7464	0.33602	0.10869	-0.03197	0.11325	0.04852
	<.0001	<.0001	<.0001	<.0001	<.0001	0.4427	0.8298	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	0.8894	<.0001	<.0001	<.0001	<.0001	0.2842	0.7534	0.2644	0.7011	
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_1RA	0.78086	0.28751	0.73005	0.61615	0.75719	0.30083	0.36892	0.29726	0.55573	0.67262	0.68914	0.42559	0.68168	0.79316	0.19444	0.18944	0.58772	1	0.66533	0.69721	0.42998	0.25384	0.33637	0.61894	0.55647	-0.07019	-0.25467	0.16398	-0.06706
	<.0001	<.0001	<.0001	<.0001	<.0001	0.0751	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0052	0.0075	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.49	0.011	0.1048	0.5956	
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_2	0.78283	0.22022	0.60725	0.5457	0.57135	0.12491	0.10945	0.29972	0.52933	0.59155	0.56615	0.40046	0.53835	0.56521	0.1924	0.11474	0.50886	0.66533	1	0.62001	0.21152	0.13505	0.2859	0.48279	0.42055	0.01018	0.01317	0.03877	-0.08332
	<.0001	0.0016	<.0001	<.0001	<.0001	0.0751	0.1191	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0066	0.1149	<.0001	<.0001		<.0001	0.0024	0.0541	<.0001	<.0001	<.0001	0.9241	0.902	0.7167	0.5378	
	199	204	204	204	204	204	204	200	204	204	204	204	204	204	198	190	204	204	204	193	204	204	204	204	204	90	90	90	57
IL_7	0.6084	0.34754	0.72691	0.63041	0.52261	0.17672	0.13954	0.50873	0.50746	0.77127	0.61974	0.56837	0.57133	0.60078	0.36248	0.31837	0.65919	0.69721	0.62001	1	0.18135	0.28655	0.34554	0.6518	0.54149	0.22442	0.07374	0.1988	-0.10706
	<.0001	<.0001	<.0001	<.0001	<.0001	0.0128	0.0248	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.0106	<.0001	<.0001	<.0001	<.0001	<.0001	0.5091	0.0699	0.4593	
	193	198	198	198	198	198	198	195	198	198	198	197	198	198	192	187	198	198	193	198	198	198	197	198	198	84	84	84	50
IP_10	0.33073	-0.17974	0.29271	0.34818	0.59227	0.38715	0.63225	-0.30812	0.24685	0.20036	0.18766	-0.14577	0.07126	0.54704	-0.31194	-0.16557	-0.00958	0.42998	0.21152	0.18135	1	-0.15305	-0.079	0.07018	0.34668	-0.02114	-0.2096	0.22575	-0.00552
	<.0001	0.0086	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003	0.0033	0.006	0.0339	0.3005	<.0001	<.0001	0.0197	0.8894	<.0001	0.0024	0.0106		0.0255	0.2521	0.308	<.0001	0.8354	0.0373	0.0247	0.9652	
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_2R	0.11435	0.61316	0.20543	0.21198	0.09783	-0.21176	-0.21639	0.58032	0.4165	0.26653	0.35728	0.4831	0.46085	0.23187	0.55284	0.35771	0.42401	0.25384	0.13505	0.29655	-0.15305	1	0.64349	0.35842	0.03128	-0.03976	-0.06355	-0.01904	-0.01982
	0.1043	<.0001	0.0026	0.0019	0.1568	0.0019	0.0015	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0006	<.0001	<.0001	<.0001	<.0001	0.0002	0.0141	0.0248	0.0255	<.0001	<.0001	0.6499	0.696	0.5321	0.8516	0.8755	
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
MIG	0.18699	0.69474	0.34196	0.28168	0.1875	-0.14174	-0.17492	0.61099	0.49509	0.32572	0.49394	0.49792	0.44532	0.232	0.65507	0.64178	0.55938	0.31637	0.2859	0.34554	-0.079	0.64349	1	0.42936	0.10603	0.23152	0.16738	0.01883	0.15838
	0.0077	<.0001	<.0001	<.0001	0.0062	0.0392	0.0107	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0007	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2521	<.0001		<.0001	0.1238	0.0218	0.0995	0.854	0.2076	
	202	212	212	212	212	212	212	208	212	212	212	211	212	212	205	198	212	212	204	197	212	212	212	212	98	98	98	65	
IL_4	0.48815	0.34533	0.7337	0.41949	0.40384	0.18391	0.11413	0.50762	0.567	0.67014	0.69883	0.58941	0.53652	0.52358	0.49234	0.53236	0.7464	0.61894	0.48279	0.6518	0.07018	0.35842	0.42936	1	0.40913	-0.24215	-0.32627	0.09199	-0.03502
	<.0001	<.0001	<.0001	<.0001	<.0001	0.0071	0.0967	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.308	<.0001	<.0001		0.0157	0.001	0.3652	0.7818	
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65
IL_8	0.48318	0.037	0.48055	0.43793	0.56798	0.39375	0.43117	0.15511	0.27681	0.46503	0.41059	0.15524	0.4261	0.53584	0.02618	0.18621	0.33602	0.55647	0.42055	0.54149	0.34668	0.03128	0.10603	0.40913	1	0.10541	0.07906	0.01412	0.33232
	<.0001	0.5913	<.0001	<.0001	<.0001	<.0001	<.0001	0.0249	<.0001	<.0001	<.0001	0.0238	<.0001	<.0001	0.7094	0.0086	<.0001	<.0001	<.0001	<.0001	<.0001	0.6499	0.1238	<.0001		0.2991	0.4366	0.8897	0.0068	
	203	213	213	213	213	213	213	209	213	213	213	212	213	213	205	198	213	213	204	198	213	213	212	213	213	99	99	99	65	
Kynurenine	0.18687	0.11217	-0.14063	0.24137	-0.13216	0.03912	-0.12308	0.37299	-0.09168	0.01133	0.11123	0.06084	0.22884	-0.02984	0.07628	0.33816	0.10869	-0.07019	0.01018	0.22442	-0.02114	-0.03976	0.23152	-0.24215	0.10541	1	0.64768	0.44137	0.06375
	0.0795	0.269	0.165	0.0161	0.1922	0.7007	0.2249	0.0002	0.3668	0.9113	0.273	0.5517	0.0227	0.7694	0.4723	0.0017	0.2842	0.49	0.9241	0.0401	0.8354	0.696	0.0218	0.0157	0.2991		<.0001	<.0001	0.6139	
	89	99	99	99	99	99	99	95	99	99	99	98	99	99	91	84	99	99	90	84	99	99	98	99	99	99		99	99	65	
Tryptophan	0.17038	0.10459	-0.27549	0.30855	-0.27076	0.08237	-0.0431	0.32714	-0.21887	-0.13501	0.15878	0.06474	0.25961	-0.04742	0.05019	0.32095	-0.03197	-0.25467	0.03137	0.07374	-0.2096	-0.06355	0.16738	-0.32627	0.07906	0.64768		0.44137	0.06375
	0.1104	0.3029	0.0058	0.0019	0.0067	0.4176	0.6718	0.0012	0.0295	0.1827	0.1165	0.5265	0.0095	0.6412	0.6366	0.0029	0.7534	0.011	0.902	0.505	0.0373	0.5321	0.0995	0.001	0.4366	<.0001		0.0008	0.8338	
	89	99	99	99	99	99	99	95	99	99	99	98	99	99	91	84	99	99	90	84	99	99	98	99	99	99	99		99	65	
KT_Ratio	0.01923	-0.06924	0.198	0.01522	0.12018	-0.09529	-0.11698	0.10375	0.13322	0.16201	-0.12211	0.03454	-0.06797	0.02237	0.04822	0.00002	0.11325	0.16398	0.03877	0.1988	0.22575	-0.01904	0.01883	0.09199	0.01412	0.44137	-0.33074	1	0.04771
	0.858	0.4959	0.0495	0.8811	0.2361	0.3481	0.2489	0.3171	0.1887	0.1091	0.2286	0.7357	0.5039	0.8261	0.6499	0.9999	0.2644	0.1048	0.7167	0.0699	0.0247	0.8516	0.854	0.3652	0.8897	<.0001	0.0008		0.7059	
	89	99	99	99	99	99	99	95	99	99	99	98	99	99	91	84	99	99	90	84	99	99	98	99	99	99	99	99		65	
SDF1_alpha	-0.17606	-0.07025	0.12508	0.21821	-0.01899	0.13036	0.19264	-0.07561	-0.07028	-0.04532	-0.00724	-0.12989	0.20103	0.02587	0.0147	0.16127	0.04852	-0.06706	-0.08332	-0.10706	-0.00552	-0.01904	0.20763	0.03502	0.33232	0.06375	-0.02653	0.04771	1
	0.1985	0.5781	0.3208	0.0808	0.8806	0.3007	0.1242	0.5592	0.578	0.72	0.9543	0.3063	0.1083	0.8379	0.9113	0.2351	0.7011	0.5956	0.5378	0.4593	0.9652	0.8755	0.2076	0.7818	0.0068	0.6139	0.8338	0.7059	
	55	65	65	65	65	65	65	62	65	65	65	64	65	65	60	56	65	65	57	50	65	65	65	65	65	65	65	65	65	

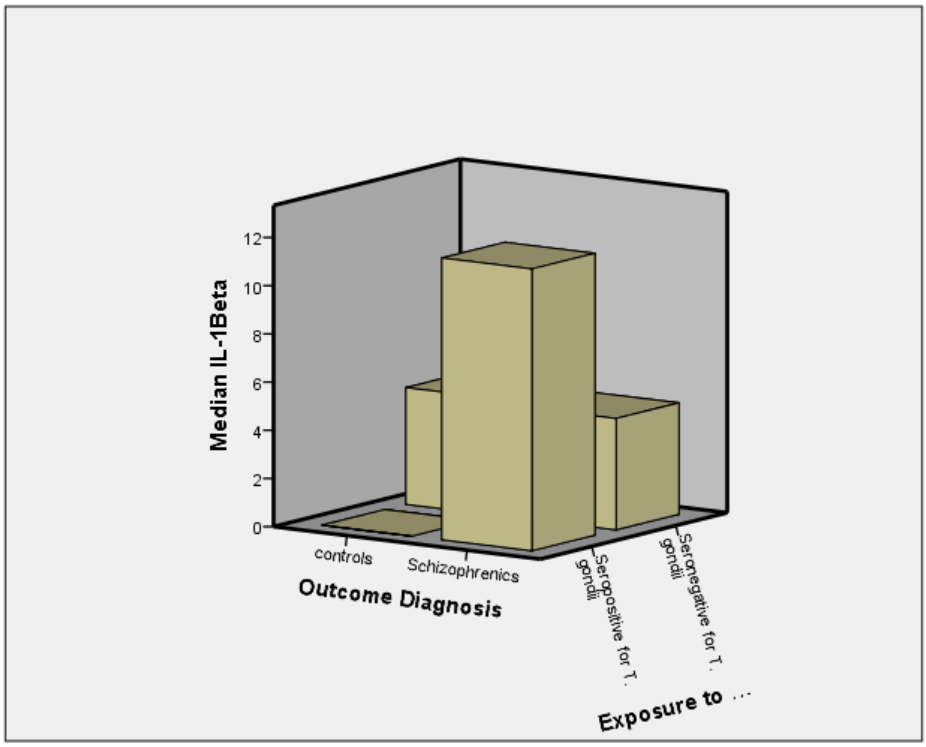
Appendix 3: Median values of outcome diagnosis and exposure of T.gondii to different cytokines (units are pg/ml)






Median IL-6
0
1
2
3
4
5
Control
Schizophrenics
Outcome Diagnosis
Seropositive for T. gondii
Seronegative for T. gondii
Exposure to ...


Median IL-2
0
1
2
3
4
5
6
Controls
Schizophrenics
Outcome Diagnosis
Seropositive for T. gondii
Seronegative for T. gondii
Exposure to ...



* The units for the cytokines are pg/ml

Appendix 4: Output for the further analysis on il-7, Il-1Beta, il-2, il-8, il-17 for those individuals who had more than 15% of their cytokine data above the LOD value with SAS code

The SAS System

The FREQ Procedure

Table of new_result by yesnoIL_7

Frequency / **Expected** / **Percent** / **Col Pct**	**new_result(new_result)**	**yesnoIL_7**		
		n	**y**	**Total**
	0	121	58	179
		121.14	57.859	
		61.11	29.29	90.40
		90.30	90.63	
	1	13	6	19
		12.859	6.1414	
		6.57	3.03	9.60
		9.70	9.38	
	Total	134	64	198
		67.68	32.32	100.00

Frequency Missing = 294

Statistics for Table of new_result by yesnoIL_7

Statistic	**DF**	**Value**	**Prob**
Chi-Square	1	0.0053	0.9418
Likelihood Ratio Chi-Square	1	0.0053	0.9417
Continuity Adj. Chi-Square	1	0.0000	1.0000
Mantel-Haenszel Chi-Square	1	0.0053	0.9420
Phi Coefficient		-0.0052	
Contingency Coefficient		0.0052	

Statistic	DF	Value	Prob
Cramer's V		-0.0052	

Fisher's Exact Test

Cell (1,1) Frequency (F)	121
Left-sided Pr <= F	0.5826
Right-sided Pr >= F	0.6205
Table Probability (P)	0.2031
Two-sided Pr <= P	1.0000

Effective Sample Size = 198
Frequency Missing = 294

WARNING: 60% of the data are missing.

The SAS System

The FREQ Procedure

Table of new_result by yesnoIl1beta

Frequency Expected Percent Col Pct	new_result(new_result)	yesnoIl1beta n	 y	Total
	0	130	54	184
		129.62	54.384	
		64.04	26.60	90.64
		90.91	90.00	
	1	13	6	19
		13.384	5.6158	
		6.40	2.96	9.36
		9.09	10.00	

Total	143	60	203
	70.44	29.56	100.00

Frequency Missing = 289

Statistics for Table of new_result by yesnoll1beta

Statistic	DF	Value	Prob
Chi-Square	1	0.0412	0.8392
Likelihood Ratio Chi-Square	1	0.0407	0.8401
Continuity Adj. Chi-Square	1	0.0000	1.0000
Mantel-Haenszel Chi-Square	1	0.0410	0.8396
Phi Coefficient		0.0142	
Contingency Coefficient		0.0142	
Cramer's V		0.0142	

Fisher's Exact Test

Cell (1,1) Frequency (F)	130
Left-sided Pr <= F	0.6875
Right-sided Pr >= F	0.5123
Table Probability (P)	0.1998
Two-sided Pr <= P	0.7976

Effective Sample Size = 203
Frequency Missing = 289

WARNING: 59% of the data are missing.

Table of new_result by yesnoIL_2

Frequency / Expected / Percent / Col Pct	new_result(new_result)	yesnoIL_2 n	yesnoIL_2 y	Total
	0	158	30	188
		156.67	31.333	
		77.45	14.71	92.16
		92.94	88.24	
	1	12	4	16
		13.333	2.6667	
		5.88	1.96	7.84
		7.06	11.76	
	Total	170	34	204
		83.33	16.67	100.00

Frequency Missing = 288

Statistics for Table of new_result by yesnoIL_2

Statistic	DF	Value	Prob
Chi-Square	1	0.8681	0.3515
Likelihood Ratio Chi-Square	1	0.7839	0.3759
Continuity Adj. Chi-Square	1	0.3391	0.5604
Mantel-Haenszel Chi-Square	1	0.8638	0.3527
Phi Coefficient		0.0652	
Contingency Coefficient		0.0651	
Cramer's V		0.0652	

WARNING: 25% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Fisher's Exact Test

Cell (1,1) Frequency (F)	158
Left-sided Pr <= F	0.8954

Fisher's Exact Test	
Right-sided Pr >= F	0.2659
Table Probability (P)	0.1613
Two-sided Pr <= P	0.3136

Effective Sample Size = 204
Frequency Missing = 288

WARNING: 59% of the data are missing.

The SAS System

The FREQ Procedure

Table of new_result by yesnoIL_8

Frequency / **Expected** / **Percent** / **Col Pct**	**new_result(new_result)**	**yesnoIL_8**		
		n	**y**	**Total**
	0	163	31	194
		162.12	31.878	
		76.53	14.55	91.08
		91.57	88.57	
	1	15	4	19
		15.878	3.1221	
		7.04	1.88	8.92
		8.43	11.43	
	Total	178	35	213
		83.57	16.43	100.00

Frequency Missing = 279

Statistics for Table of new_result by yesnoIL_8

Statistic	DF	Value	Prob
Chi-Square	1	0.3244	0.5690
Likelihood Ratio Chi-Square	1	0.3051	0.5807
Continuity Adj. Chi-Square	1	0.0601	0.8063
Mantel-Haenszel Chi-Square	1	0.3228	0.5699
Phi Coefficient		0.0390	
Contingency Coefficient		0.0390	
Cramer's V		0.0390	

WARNING: 25% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Fisher's Exact Test

Cell (1,1) Frequency (F)	163
Left-sided Pr <= F	0.8183
Right-sided Pr >= F	0.3815
Table Probability (P)	0.1997
Two-sided Pr <= P	0.5253

Effective Sample Size = 213
Frequency Missing = 279

WARNING: 57% of the data are missing.

The SAS System

The FREQ Procedure

Table of new_result by yesnoil_17

Frequency / Expected / Percent	new_result(new_result)	yesnoil_17 n	yesnoil_17 y	Total

Col Pct	**0**	153	41	194
		155.75	38.254	
		71.83	19.25	91.08
		89.47	97.62	
	1	18	1	19
		15.254	3.7465	
		8.45	0.47	8.92
		10.53	2.38	
Total		171	42	213
		80.28	19.72	100.00

Frequency Missing = 279

Statistics for Table of new_result by yesnoil_17

Statistic	**DF**	**Value**	**Prob**
Chi-Square	1	2.7535	0.0970
Likelihood Ratio Chi-Square	1	3.5599	0.0592
Continuity Adj. Chi-Square	1	1.8422	0.1747
Mantel-Haenszel Chi-Square	1	2.7406	0.0978
Phi Coefficient		-0.1137	
Contingency Coefficient		0.1130	
Cramer's V		-0.1137	

WARNING: 25% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Fisher's Exact Test

Cell (1,1) Frequency (F)	153
Left-sided Pr <= F	0.0775
Right-sided Pr >= F	0.9875
Table Probability (P)	0.0651

Fisher's Exact Test

Two-sided Pr <= P	0.1321

Effective Sample Size = 213
Frequency Missing = 279

WARNING: 57% of the data are missing.

Reruning the mann whitney for these values and determining if the p value changes

The SAS System

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable IL_7
Classified by Variable new_result

new_result	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
0	121	8203.0	8167.50	133.016139	67.793388
1	13	842.0	877.50	133.016139	64.769231

Average scores were used for ties.

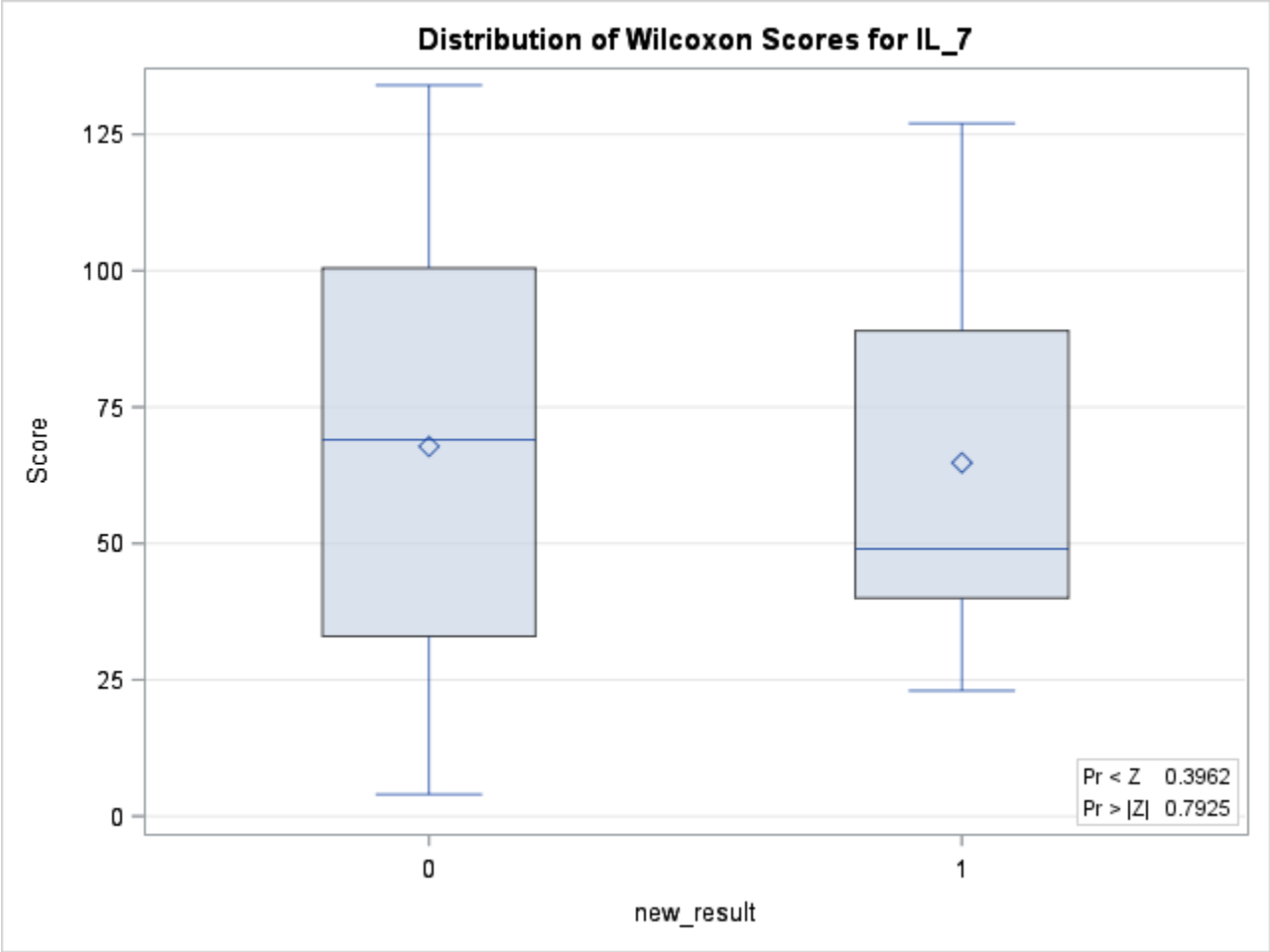
Wilcoxon Two-Sample Test

Statistic	842.0000
Normal Approximation	
Z	-0.2631
One-Sided Pr < Z	0.3962
Two-Sided Pr > |Z|	0.7925
t Approximation	
One-Sided Pr < Z	0.3964
Two-Sided Pr > |Z|	0.7929

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	0.0712
DF	1
Pr > Chi-Square	0.7896



The SAS System

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable IL_1Beta Classified by Variable new_result

new_result	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
0	130	9354.50	9360.0	142.389162	71.957692
1	13	941.50	936.0	142.389162	72.423077

Wilcoxon Scores (Rank Sums) for Variable IL_1Beta
Classified by Variable new_result

new_result	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	941.5000

Normal Approximation

Z	0.0351
One-Sided Pr > Z	0.4860
Two-Sided Pr > |Z|	0.9720

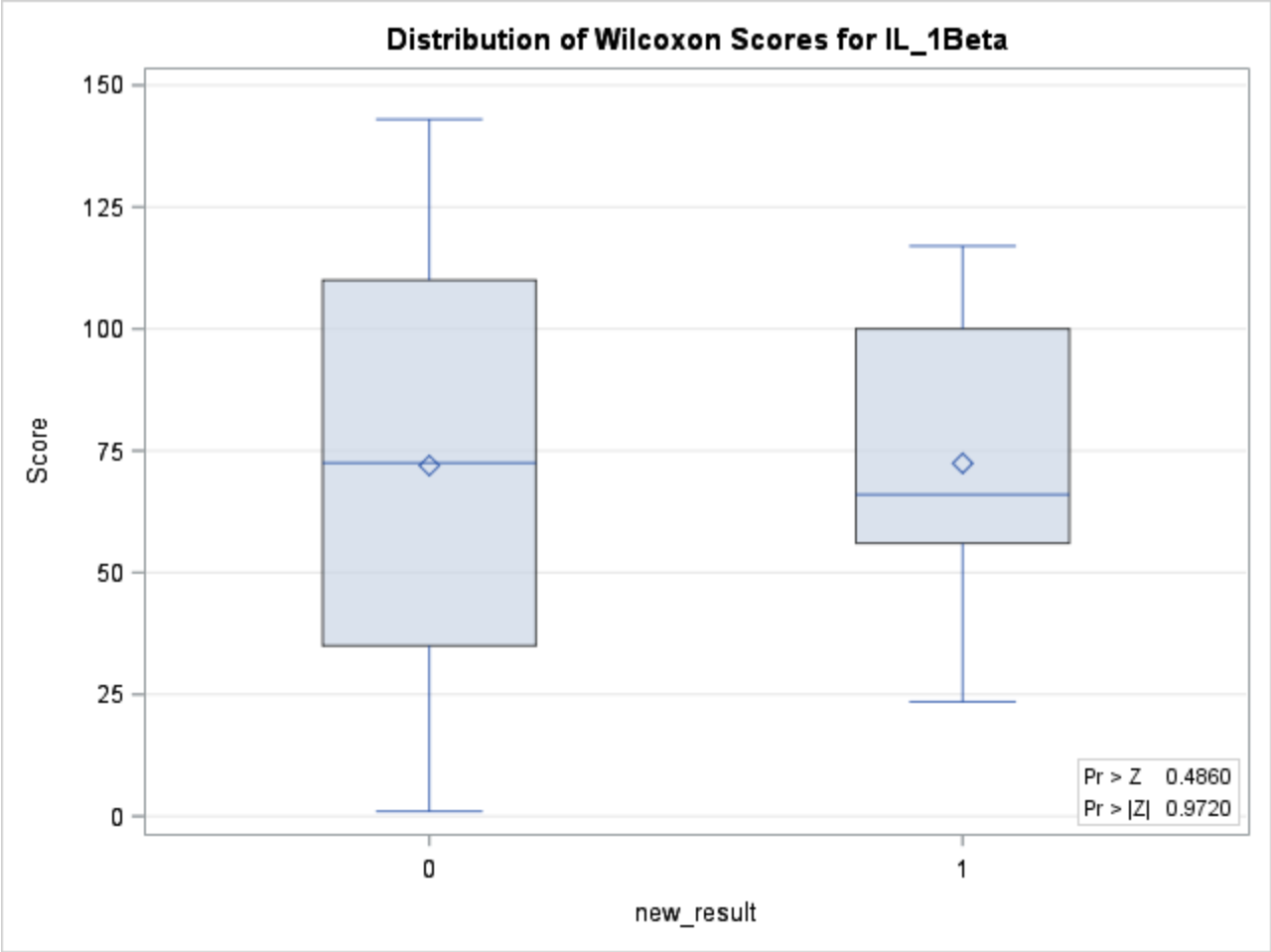
t Approximation

One-Sided Pr > Z	0.4860
Two-Sided Pr > |Z|	0.9720

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	0.0015
DF	1
Pr > Chi-Square	0.9692



The SAS System

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable IL_2
Classified by Variable new_result

new_result	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
0	158	13390.0	13509.0	164.341717	84.746835
1	12	1145.0	1026.0	164.341717	95.416667

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	1145.0000

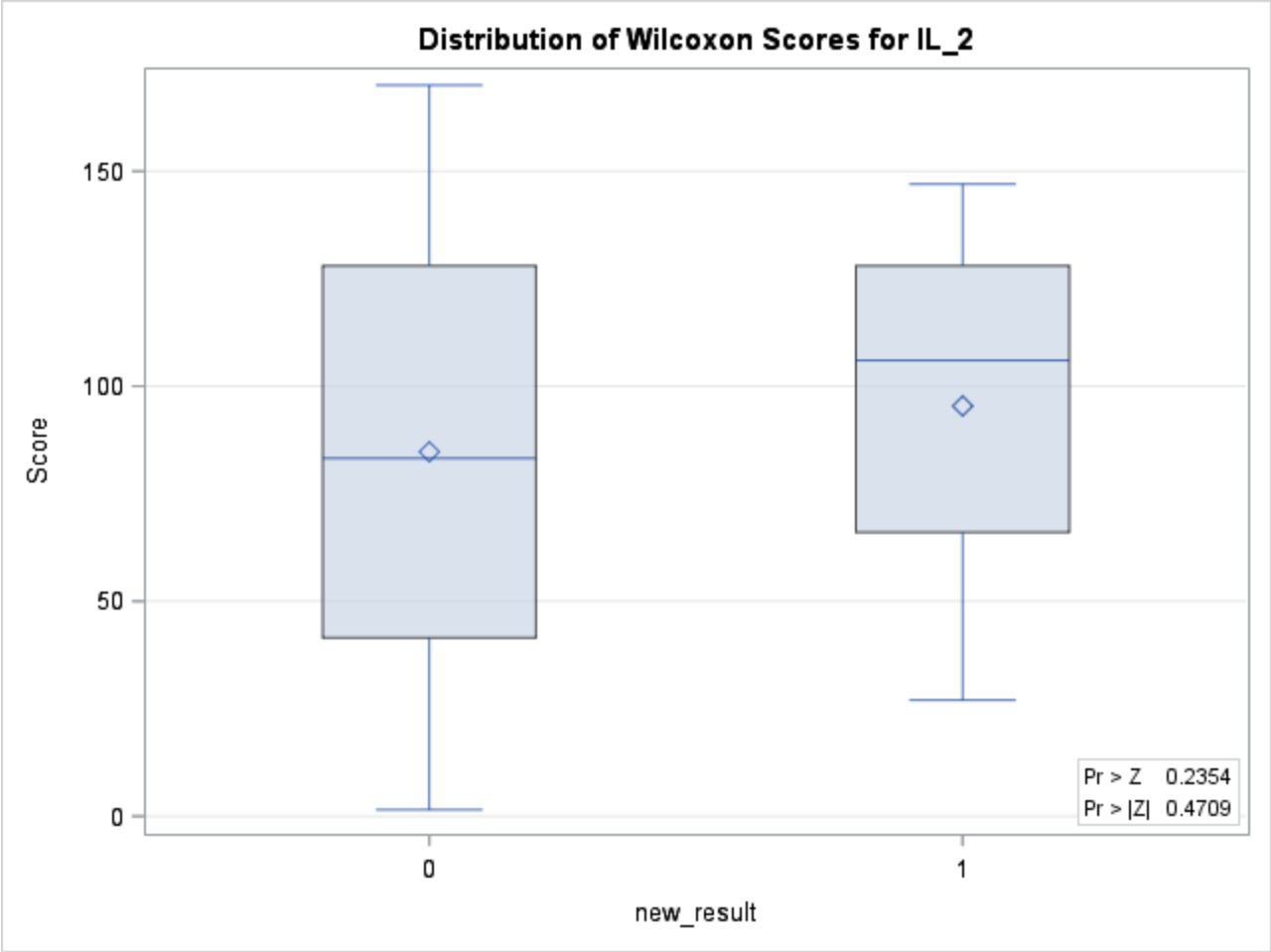
Wilcoxon Two-Sample Test

Normal Approximation	
Z	0.7211
One-Sided Pr > Z	0.2354
Two-Sided Pr > |Z|	0.4709
t Approximation	
One-Sided Pr > Z	0.2359
Two-Sided Pr > |Z|	0.4719

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	0.5243
DF	1
Pr > Chi-Square	0.4690



The SAS System

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable IL_8
Classified by Variable new_result

new_result	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
0	163	14495.50	14588.50	190.902942	88.929448
1	15	1435.50	1342.50	190.902942	95.700000

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	1435.5000

Normal Approximation

Wilcoxon Two-Sample Test

Z	0.4845
One-Sided Pr > Z	0.3140
Two-Sided Pr > |Z|	0.6280

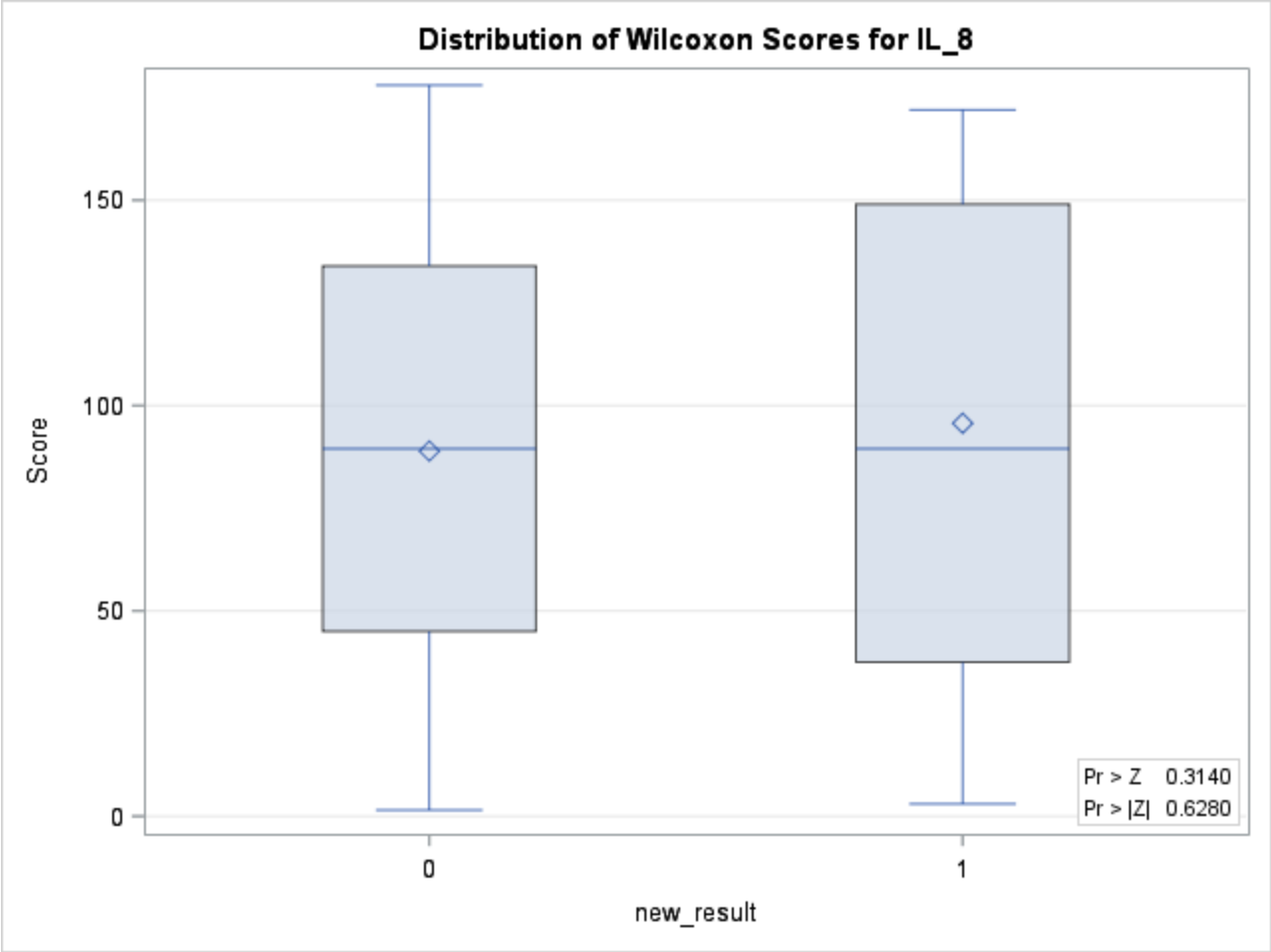
t Approximation

One-Sided Pr > Z	0.3143
Two-Sided Pr > |Z|	0.6286

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	0.2373
DF	1
Pr > Chi-Square	0.6261



The SAS System

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable IL_17
Classified by Variable new_result

new_result	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
0	153	13386.0	13158.0	198.676238	87.490196
1	18	1320.0	1548.0	198.676238	73.333333

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	1320.0000

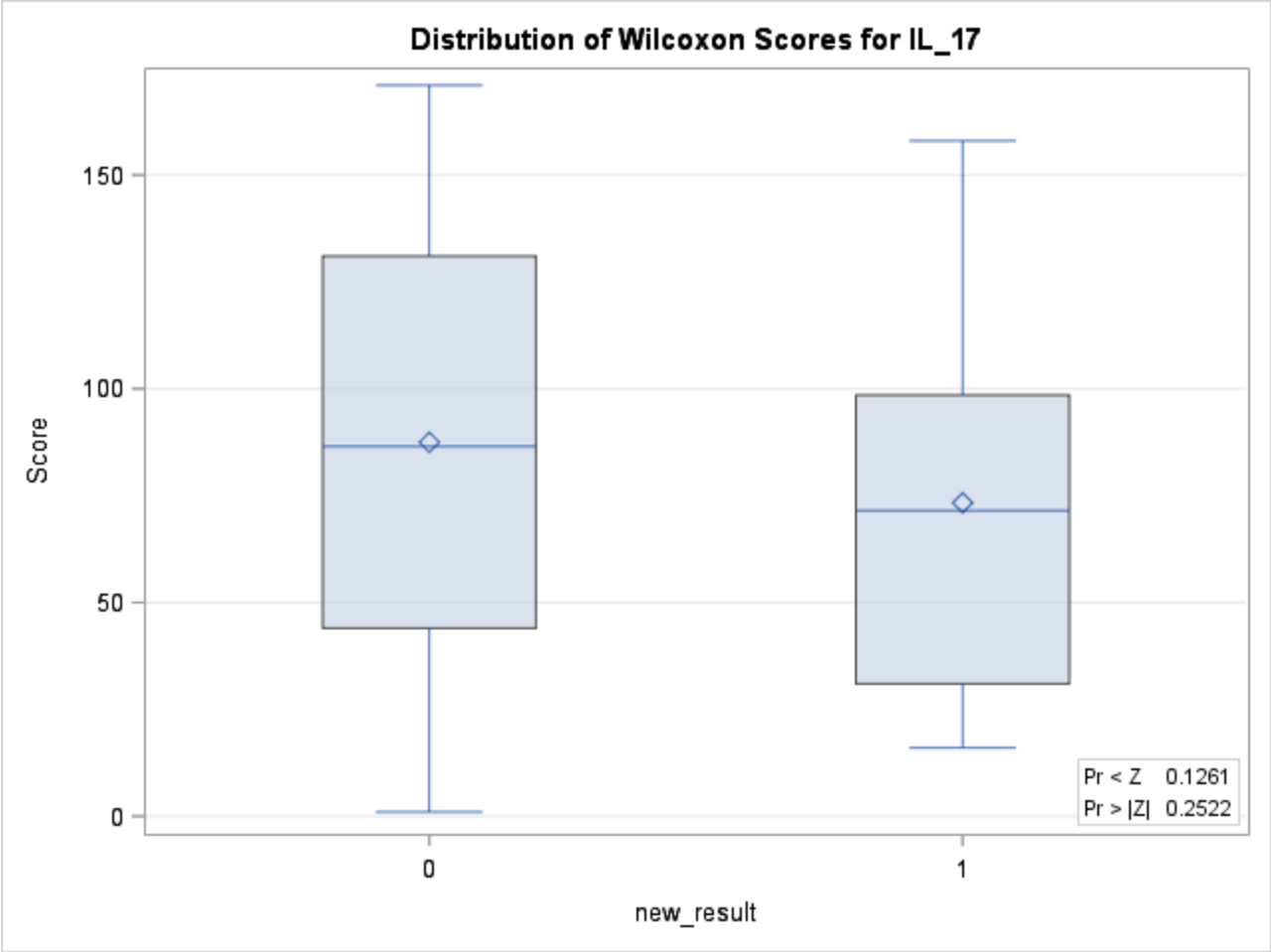
Wilcoxon Two-Sample Test

Normal Approximation	
Z	-1.1451
One-Sided Pr < Z	0.1261
Two-Sided Pr > |Z|	0.2522
t Approximation	
One-Sided Pr < Z	0.1269
Two-Sided Pr > |Z|	0.2538

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	1.3170
DF	1
Pr > Chi-Square	0.2511




```
*importing the first file which has exposure code #1;

proc import datafile="T:\epiprojs\Grad Students\Sadaf
Saaber\updated_SAS_Files\Mergedfile3.sav" dbms=sav out=work.mergedfile;
run;

proc freq data=mergedfile;
tables subject_ID/list missing;
run;

proc contents data=mergedfile position;
run;

*creating a new dataset which has exposure code #2;
data toxonewcat;
set mergedfile;

if kitresult eq "negative" then new_result=0;
else if kitresult eq "positive" then new_result=1;
else if kitresult eq "inconclusive" then new_result=.;

if toxo_4cat eq 0 then new_result=0;
else if toxo_4cat eq 1 then new_result=0;
else if toxo_4cat eq 2 then new_result=1;
```

```
else if toxo_4cat eq 3 then new_result=1;

run;

*creating the new variables of missing values as well as intensity
value for the new analysis;

data newvar;
set toxonewcat;

if IL_1Beta eq . then yesnoIl1beta=. ;
else if IL_1Beta eq 0 then yesnoIl1beta= 0;
else if IL_1Beta ne 0 then yesnoIl1beta= 1;

if il_17  eq . then yesnoil_17= . ;
else if il_17  eq 0 then yesnoil_17= 0;
else if il_17  ne 0 then yesnoil_17= 1;

if IL_2  eq . then yesnoIL_2 =. ;
else if IL_2 eq 0 then yesnoIL_2= 0;
else if IL_2 ne 0 then yesnoIL_2= 1;

if IL_7  eq . then yesnoIL_7 =. ;
else if IL_7  eq 0 then yesnoIL_7= 0;
else if IL_7 ne 0 then yesnoIL_7= 1;

if  IL_8   eq . then yesnoIL_8 = . ;
else if  IL_8  eq 0 then yesnoIL_8=0;
else if  IL_8 ne 0 then yesnoIL_8= 1;

run;

proc freq data=newvar;
tables IL_1Beta*yesnoIl1beta/list missing;
run;

proc freq data=newvar;
tables il_17*yesnoil_17/list missing;
run;


proc freq data=newvar;
tables  IL_2*yesnoIL_2/list missing;
run;

proc freq data=newvar;
tables IL_7*yesnoIL_7/list missing;
run;

proc freq data=newvar;
tables IL_8*yesnoIL_8/list missing;
run;

*running the chisquared tests for each of the cytokines (yes/no
category);

proc freq data=newvar;
```

```
tables new_result*yesnoIl1beta/expected chisq norow;
run;

proc freq data=newvar;
tables new_result*yesnoIl10/expected chisq norow;
run;

proc freq data=newvar;
tables new_result*yesnoIFN_Alpha/expected chisq norow;
run;

proc freq data=newvar;
tables new_result*yesnoIL6/expected chisq norow;
run;

proc freq data=newvar;
tables new_result*yesnoIL12/expected chisq norow;
run;

*determining the items il_7, Il-1Beta, il_2, il-8, il-17 with being
seropositive and negativing using chiquared;

proc freq data=newvar;
tables new_result*yesnoIL_7/ expected chisq norow;
run;

proc freq data=newvar;
tables new_result*yesnoIl1beta/ expected chisq norow;
run;

proc freq data=newvar;
tables new_result*yesnoIL_2/ expected chisq norow;
run;

proc freq data=newvar;
tables new_result*yesnoIL_8/ expected chisq norow;
run;

proc freq data=newvar;
tables new_result*yesnoil_17/ expected chisq norow;
run;


*rerunning the mann-whitney for these values (Il-1Beta, il_2, il-8, il-
17);

proc npar1way data=newvar wilcoxon;
class new_result;
var il_7;
where yesnoIL_7 eq 1;
run;

proc freq data=newvar;
tables yesnoIL_7/list missing;
run;

proc npar1way data=newvar wilcoxon;
```

```
class new_result;
var il_1beta;
where yesnoIl1beta eq 1;
run;

proc freq data=newvar;
tables yesnoIl1beta/list missing;
run;

proc npar1way data=newvar wilcoxon;
class new_result;
var il_2;
where yesnoIL_2 eq 1;
run;

proc freq data=newvar;
tables yesnoIL_2/list missing;
run;

proc npar1way data=newvar wilcoxon;
class new_result;
var il_8;
where yesnoIL_8 eq 1;
run;

proc freq data=newvar;
tables yesnoIL_8/list missing;
run;

proc npar1way data=newvar wilcoxon;
class new_result;
var il_17;
where yesnoil_17 eq 1;
run;

proc freq data=newvar;
tables yesnoil_17/list missing;
run;
```

Appendix 5: Relationship between seropositivity for T.gondii and Schizophrenia with Specific Cytokines

Table 8: Relationship between seropositivity for T.gondii and Schizophrenia with Specific Cytokines

Cytokine	N^	Odd Ratio For being seropositive for T.gondii*	CI	Odds Ratio for Cytokines*	CI	Wald Test Statistic	P value
univariate model**	**213**	1.4	0.53-3.69	N/A	N/A	N/A	N/A
Adjusted Model**	**211**	1.24	0.37-4.12	N/A	N/A	N/A	N/A
IL-1Beta	**201**	1.19	0.36-3.90	1.00	1.00- 1.00	2.76	0.10
IL-10	**211**	1.23	0.37- 4.11	1.00	1.00- 1.00	0.20	0.65
IFN-Alpha***							
IL-6	**211**	1.24	0.37-4.13	1.00	1.00- 1.00	0.01	0.90
IL-12	**211**	1.24	0.37-4.11	1.00	1.00- 1.00	0.03	0.85
RANTES	**211**	1.12	0.33-3.84	1.00	1.00- 1.00	3.57	0.06
Eotaxin	**211**	1.25	0.37-4.25	1.01	1.00-1.02	7.77	0.01
IL-13	**207**	1.39	0.39-4.95	1.00	1.00- 1.00	0.50	0.48
IL-15	**211**	1.18	0.35-3.91	1.00	1.00- 1.00	2.58	0.11
IL-17	**211**	1.24	0.37-4.18	1.00	1.00- 1.00	0.03	0.87
MIP-1Alpha	**211**	1.22	0.37- 4.05	1.00	1.00- 1.00	0.94	0.33
GM-CSF	**210**	1.23	0.37-4.07	1.00	1.00- 1.00	0.75	0.39
MIP-1Beta	**211**	1.2	0.36-3.98	1.00	1.00- 1.00	2.07	0.15
MCP-1	**211**	1.21	0.36-4.02	1.00	1.00- 1.00	1.33	0.25
IL-5	**203**	1.16	0.35-3.89	1.00	1.00- 1.00	0.80	0.37
IFN-Gamma	**196**	1.64	0.44-6.10	0.99	0.99-1.01	1.22	0.27
TNF-Alpha	**211**	1.22	0.37-4.06	1.00	1.00- 1.00	0.66	0.42
IL-1RA***							
IL-2	**202**	0.72	0.20- 2.68	1.00	0.99-1.00	2.26	0.13
IL-7	**196**	1.32	0.40- 4.37	1.00	1.00-1.00	0.04	0.84
IP-10	**211**	1.21	0.36- 4.07	1.01	1.00-1.02	3.87	0.05
IL-2R***							
MIG	**210**	1.17	0.35- 3.97	1.00	1.00-1.00	0.44	0.51
IL-4	**211**	1.23	0.37-4.01	1.00	1.00-1.00	0.42	0.52
IL-8	**211**	1.24	0.37-4.14	1.00	0.98-1.01	0.11	0.75
Kynurenine**	**99**	1.87	0.28-12.52	0.89	0.57-1.39	0.25	0.61
Tryptophan**	**99**	1.74	0.27-11.31	1.00	0.98-1.01	0.19	0.66
KT-Ratio**	**99**	1.79	0.27-12.01	0.09	N/A	0.02	0.89
SDF1-alpha**	**65**	2.23	0.12-40.38	1.00	1.00-1.00	3.61	0.06

*adjusted for age, race, sex, smoking status and specific cytokine

**chemokine were not normalized through beta substitution, rather actual values were used

***cytokines not assessed as did not have LOD values to normalize

****these are the ORS for the models I ran for the 213 individuals

^ missing individuals due to either not having *T.gondii* exposure information, schizophrenia diagnosis, age, smoking status or cytokine information

Appendix 6: Percentages of values below LOD value for the cytokines

Cytokines	Missing Values	Number of Zero values	% of zero values
IL_10	261 (53.05%)	n/a	n/a
IFN_Alpha	261 (53.05%)	n/a	n/a
IL_12	261 (53.05%)	n/a	n/a
Eotaxin	261 (53.05%)	n/a	n/a
MCP_1	261 (53.05%)	n/a	n/a
IL_1RA	261 (53.05%)	n/a	n/a
IP_10	261(53.05%)	n/a	n/a
IL_2R	261(53.05%)	n/a	n/a
IL_4	261(53.05%)	n/a	n/a
IL_7	276(56.1%)	73	33.80%
IL_1Beta	271 (55.08%)	74	33.48%
IL_2	270 (54.88)	53	23.87%
IL_8	261(53.05%)	52	22.51%
IL_17	261 (53.05%)	47	20.35%
GM_CSF	262(53.25%)	31	13.48%
IFN_Gamma	276 (56.10%	20	9.26%
IL_6	261 (53.05%)	19	8.23%
MIP_1Beta	261 (53.05%)	13	5.63%
IL_15	261 (53.05%)	12	5.19%
TNF_Alpha	261 (53.05%)	11	4.76%
IL_5	269(54.67%)	5	2.24%
RANTES	261 (53.05%)	4	1.73%
MIP_1Alpha	261 (53.05%)	4	1.73%
MIG	262(53.25%)	2	0.87%
IL_13	265(53.86%)	1	0.44%