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Monocyte activation detected prior to a diagnosis of schizophrenia in the US Military New Onset Psychosis Project (MNOPP)

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Abstract

Low-grade inflammation is present in some cases of schizophrenia, particularly in the early stages of this disorder. The inflammation source is not known but may be the result of dysbiotic processes occurring in the gut. We examined peripheral biomarkers of bacterial translocation, soluble CD14 (sCD14) and lipopolysaccharide binding protein (LBP), and of general inflammation, C-reactive protein (CRP), in a unique, pre-onset study of schizophrenia. This sample was composed of 80 case-control matched pairs of US military service members from whom blood samples were obtained at time of entry to service, before a psychiatric diagnosis was made. Elevated levels of sCD14 in individuals who were subsequently diagnosed with schizophrenia generated odds ratios of 1.22 for association with disease ($p < 0.02$). Conversely, LBP levels for those who developed schizophrenia were unchanged or very marginally decreased compared to controls ($p = 0.06$). No significant changes were found for CRP in schizophrenia compared with their matched controls. This diversity of patterns suggests that a dysregulated immune system is present prior to a diagnosis of schizophrenia. In particular, sCD14 elevation and discordant LBP decrease in cases support a more generalized monocyte activation rather than a

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Conflicts of Interest

The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies. None of the other authors have any associations, financial or otherwise, that may present a conflict of interest.

Contributors

Drs. Weber, Cowan, Niebuhr, Yolken, and Severance designed the study and wrote the protocol. Dr. Weber, Dr. Severance and Ms. Gressitt collected data and performed the data analyses. Drs. Weber and Severance wrote the first drafts of the manuscript. All authors contributed to and have approved the final manuscript.

specific translocation of gut bacteria into circulation. The corresponding absence of general inflammation as measured by CRP may indicate that this monocyte activation or related immune dysfunction precedes the early inflammatory stage frequently evident in schizophrenia.

Keywords

immune system; prodromal symptoms; biomarkers; diagnosis; psychiatric disorders; microbes

1. Introduction

A low-level systemic inflammation is a hypothesized harbinger of schizophrenia and other psychoses, as dysregulated inflammatory markers are often found in early vs. later stages of these disorders (Bechter, 2013; Li et al., 2013; Niebuhr et al., 2011; Niebuhr et al., 2008; Severance et al., 2012; Suvisaari and Mantere, 2013). It is not known if this inflammation is a source or a consequence of the disease state, but it is a condition that may be associated in part with co-occurring defects in the gastrointestinal, immune and vascular systems (Severance et al., 2015; Severance et al., 2016). The convergence of these dysfunctions is reflected in a model of the gut-brain axis by which microbial dysbiosis in the gut promotes an inflammatory environment that in turn results in compromised vascular endothelial cytoarchitecture in the intestinal tract and at the blood brain barrier. As such, this process allows for (1) the translocation of gut-derived bacteria, toxins and food peptides into systemic circulation, and (2) access of these gut-based products and associated systemic immune factors to the brain (Severance et al., 2013; Severance et al., 2015; Severance et al., 2016). As understanding and acceptance of a dynamic gut-brain interaction gains traction, an imbalanced gut microbiome may be an important environmental factor to consider in studies of the pathophysiology of schizophrenia.

Microbial dysbiosis related to schizophrenia has been documented in deep sequencing studies and biomarker examinations of microbial translocation (Castro-Nallar et al., 2015; Severance et al., 2013; Severance et al., 2015; Yolken et al., 2015). Previously, we found that people with schizophrenia had increased levels of an often-used surrogate marker for bacterial translocation, soluble CD14 (sCD14), as compared to controls (Severance et al., 2013). Recent studies, however, show that sCD14 is less specific a marker of the bacterial translocation process than lipopolysaccharide-based markers (Barbosa et al., 2012; Romero-Sanchez et al., 2012). Indeed, we found that although the lipopolysaccharide binding protein (LBP) was moderately correlated with sCD14 in both cases and controls, it was not differentially upregulated in individuals with schizophrenia, as was sCD14. Both markers were also associated with levels of C-reactive protein (CRP) only in schizophrenia, suggesting that bacterial translocation may differentially contribute in part to inflammation related to this disorder (Severance et al., 2013).

The ability to predict a forthcoming diagnosis based on a blood sample is an extremely valuable tool and gives the opportunity for early possible preventive treatment. Our longitudinal studies of a unique population cohort from the United States (US) military have enabled this possibility and led to the identification of a number of immune related

biomarkers that are altered prior to a formal diagnosis of schizophrenia (Li et al., 2013; Niebuhr et al., 2011; Niebuhr et al., 2008; Weber et al., 2015). We sought to examine the hypothesis that gut permeability and monocyte activation may be part of the predictive pathogenesis of this psychiatric disorder. We tested this hypothesis by measuring sCD14 and LBP in this population and compared these marker patterns to a generalized, systemic measure of inflammation, CRP.

2. Materials and methods

2.1 Study Population

We used a subset (80 cases with schizophrenia and their matched controls) of the Military New Onset Psychosis Project (MNOPP), a large nested case-control study of the US active component military service members who developed schizophrenia (855 cases and 1,165 matched controls) and received medical discharges from the military between 1992 and 2005. The MNOPP study population was individually paired and matched on many demographic and technical factors, as described below. The subset includes first available serum specimens and it was assembled so that we could test and identify promising biomarkers in a representative case-control study pilot sample. For those biomarkers deemed promising, future full cohort screening tests are planned.

The details of the MNOPP, the diagnostic process leading to medical discharge from military service and the validity of the psychiatric diagnosis have been provided elsewhere (Millikan et al., 2007; Niebuhr et al., 2008). Briefly, individuals whose stored blood samples were evaluated in this study were identified based on a retrospective analysis of physical disability and medical records. Military medical records were screened for incidences of hospitalization for a psychiatric disorder, as diagnosed using the International Classification of Disease 9th Revision (ICD-9-CM) codes (290–319).

Prospective members of the Armed Forces are medically screened at accession for both currently present, and histories of, physical and mental conditions that may be disqualifying for accession. All applicants are interviewed by a physician, and those exhibiting symptoms of mental illness are further evaluated. It is possible that applicants deny medical history or symptoms of mental illness. It is extremely unlikely that anyone floridly psychotic would be permitted to enlist. At this time, blood is drawn for human immunodeficiency virus (HIV) testing. The United States Military Entrance Processing Command (USMEPCOM), has processes in place to conduct these screenings and to identify individuals who do not meet the standards outlined in Department of Defense Instruction (DoDI) 6130.03 “Medical Standards for Appointment, Enlistment, or Induction in the Military Services.” If, at the time of this screening, a physical or mental condition is identified that may be disqualifying, the prospective member can be referred for additional assessment by a medical specialist consultant prior to a final medical qualification decision being made by USMEPCOM (Report on Preliminary Mental Health Screenings for Individuals Becoming Members of the Armed Forces, <https://health.mil/Reference-Center/Reports/2017/01/11/Report-on-Preliminary-Mental-Health-Screenings>).

In more detail, the MNOPP cases were selected from the data provided by each of the U.S. military disability review agencies: the Army Physical Disability Agency, the Secretary of the Navy Council of Review Boards, and the Air Force Personnel Center/U.S. Air Force Physical Disability Division. The medical and demographic data from 1989 to 2006 (released in 2007) were provided by the Defense Medical Surveillance System (DMSS), the Armed Forces Health Surveillance Branch (AFHSB), Silver Spring, MD. Serum specimens from 1988 to 2006 (released in 2007) were retrieved from the Department of Defense Serum Repository (DoDSR), AFHSB, Silver Spring, MD. The mission of the DoDSR includes storage of serum that remains following mandatory HIV- and operational deployment-related testing. Those aged 18 and older who were on active component at the time of their schizophrenia diagnosis and who had at least one serum sample of 0.5 ml or greater in the DoDSR obtained prior to diagnosis were selected as potential MNOPP cases. The time of disease onset was estimated as the earliest date of either the first hospitalization with psychiatric disorder, International Classification of Disease 9th Revision (ICD-9-CM) codes (290–319) in any diagnostic position, or the date when the medical or physical evaluation board was initiated. Control subjects, who were over the age of 18 with no inpatient or outpatient psychiatric disorder diagnoses, were selected from the active component U.S. military service population. All MNOPP control subjects were matched to their cases on sex, race, branch of military service, date of birth (+/–12 months), year of military entrance (+/–12 months), and serum specimen collection time (+/–90 days). Multiple matched (+/–90 days) serum specimens, stored at –30°F, were obtained for the MNOPP from the DoDSR. Demographic and other study population characteristics are described in Table 1.

For this study, we have randomly (without replacement) selected a sample of 80 MNOPP subjects who later were diagnosed with schizophrenia. Their individually matched (1:1) controls were also pulled from the MNOPP study. The subjects' first available serum specimens (usually from the time of military entrance) were identified from the existing pool of sera obtained for the MNOPP and tested.

The work was performed under approved human subjects protocol WRAIR #2140 approved by the Walter Reed Army Institute of Research Institutional Review Board and the Institutional Review Board of the Johns Hopkins School of Medicine.

2.2 Laboratory Procedures

sCD14, LBP and CRP levels were measured according to the manufacturer's protocol using commercially available kits (Human sCD14 Quantikine ELISA kit, R&D Systems, Minneapolis, MN, U.S.A.; Multispecies Lipopolysaccharide Binding Protein ELISA kit, Cell Sciences, Canton, MA, U.S.A.; High Sensitivity C-Reactive Protein ELISA kit, IBL America, Minneapolis, MN, U.S.A.). Serum dilutions were 1:200 for sCD14, 1:300 for LBP and 1:100 for CRP.

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were defined as three times and ten times the standard deviation of the blank absorbances, respectively. For the sCD14 assay, these values were 0.002 for LOD and 0.006 for LOQ. For the LBP assay, these values were 0.007 for LOD and 0.02 for LOQ. For the CRP assay, these values were 0.02 for LOD

and 0.06 for LOQ. Mean level absorbances for each biomarker fell above these limits and significant differences were adequately detectable.

2.3 Data Analyses

We have calculated means and p-values of the paired case-control differences of the standardized marker levels for cases and controls overall and stratified by gender. Student's t-test was used when the sample data were normally distributed. When sample data distributions were not normal, we used signed or signed rank test depending on the symmetry of the distribution. Spearman correlation was applied to find strength and significance of inter-variable relationships of CRP, LBP and sCD14. Odds ratios (OR) for association with schizophrenia were determined with conditional logistic regression using case-control status as the dichotomous dependent or outcome variable and quantitative marker levels as the continuous independent or predictor variable. For this pilot study, we relied on the high level of individual matching across demographic and technical variables as an internal control to minimize the influence of potentially confounding factors. More stringent multivariate modelling will be undertaken in large-scale studies once an array of promising markers is identified.

3. Results

The schizophrenia study samples were representative of the study population from which the samples were derived. As shown in Table 1, cases and controls were well-matched across all of the listed study population characteristics. This sample set predominantly contained young males, serving in the Army, with the great majority of soldiers serving fewer than 5 years before discharge from the military. Because the first available specimens were selected for those with multiple specimens, the majority of the sera were collected more than a year before schizophrenia diagnosis was established.

Serum biomarkers were associated with distinct case-control patterns, as shown in Table 2. Mean levels of sCD14 were significantly elevated in blood samples from individuals who subsequently developed schizophrenia compared to controls ($\mu=0.73$; $p=0.02$). This case-control difference in serum sCD14 levels was confirmed by logistic regressions which generated an OR for disease association of 1.22 (95% CI 1.01–1.46, $z = 2.16$, $p<0.02$). Conversely, we observed trends toward lower mean LBP levels in schizophrenia compared to controls ($\mu=-2.50$, $p=0.06$), and this was associated with an OR of 0.97 (95% CI 0.93–1.01, $z = -1.42$, $p<0.15$). No significant differences in mean levels or OR generation were found for CRP in cases compared with their matched controls. Basic differences in quantitative marker levels were not influenced by gender.

Significant correlations were also detected between marker levels in case-control patterns, particularly when individuals were stratified by gender, as shown in Table 3. For example, while CRP and LBP were well correlated in male cases with schizophrenia ($r = 0.46$, $p = 0.0001$) and in male controls ($r = 0.48$, $p < 0.0001$), the correlation in females was strong and significant only for controls ($r = 0.67$, $p = 0.02$). The correlation of CRP and LBP for female cases with schizophrenia was not even suggestively significant ($r = -0.24$, $p = 0.46$). A significant but weak correlation was also found between levels of LBP and sCD14 overall

for schizophrenia cases ($r = 0.23$, $p = 0.04$) compared to controls ($r = 0.17$, $p = 0.15$), but there were no case-control differences with gender stratification: male cases ($r = 0.23$, $p = 0.06$); male controls ($r = 0.22$, $p = 0.07$); female cases ($r = 0.17$, $p = 0.59$); female controls ($r = -0.15$, $p = 0.65$). sCD14 was not significantly correlated with CRP in any of the case or control groups.

4. Discussion

In this unique, closely matched, case-control study sample of individuals in the US military for whom blood samples were available before a psychiatric diagnosis was made, we found that blood levels of sCD14 were significantly elevated prior to the onset of schizophrenia. Other studies previously reported increases in this marker peripherally and in the CNS of individuals already diagnosed with schizophrenia (Arion et al., 2007; Johansson et al., 2017; Severance et al., 2013; Tanaka et al., 2017) thus, our findings identify an even earlier time period during which immune pathologies may begin in schizophrenia. These results also have mechanistic implications regarding the timing and order of inflammation and immune anomalies leading to and characterizing the prodromal stage of this disorder. Discordant patterns of immune and inflammatory biomarkers in this population of pre-onset schizophrenia point toward a distinct dysregulation of the immune system even prior to induction of a low-grade inflammatory state.

A primary objective of the current study was to evaluate gut-related inflammation and specifically the translocation of gut bacteria into systemic circulation as a source of low grade inflammation that may precede the diagnosis of schizophrenia. Our findings here replicate those from an earlier study of individuals with established schizophrenia (Severance et al., 2013), where two often-used surrogate markers of the translocation process, sCD14 and LBP, were discordantly altered. In both the previous and present studies, sCD14 levels were elevated in the schizophrenia group compared to controls, with minor LBP decreases in the comparison of these same individuals. Because (1) sCD14 is not strictly a marker for bacterial translocation and also reflects a generalized monocyte activation triggered by nonbacterial ligands, and (2) LBP is selective for serological Gram-negative gut-based bacteria, our results support a more focused role toward monocyte activation or monocyte dysfunction that may be independent of bacterial translocation in early schizophrenia pathophysiology. Furthermore, the marker of general inflammation, CRP, which should detect any source of systemic inflammation including from the gut, was itself unaltered between cases and controls. The absence of statistically significant differences in levels of CRP suggests that early monocyte alterations, as reflected by sCD14 elevations, may precede, then subsequently trigger disease-associated inflammation. Interestingly, this early sCD14 effect appears specific to schizophrenia, as none of the three markers were altered in the US military bipolar disorder sample (data not shown).

Impaired monocyte activation in schizophrenia has been previously reported (Uranova et al., 2017) and could result in a diminished ability to clear pathogens that in turn could lead to a state of low-grade systemic inflammation (Muller et al., 2012). In a previous study of this military cohort, significant downregulation of a pattern recognition protein, long pentraxin PTX3, was detected in individuals who subsequently developed schizophrenia compared to

controls (Weber et al., 2015), another indication of early dysfunction of the innate immune response associated with this psychiatric disorder. As mentioned, our findings of elevated peripheral sCD14 prior to a diagnosis of schizophrenia complement studies in people with already established schizophrenia whose blood sCD14 levels were also increased compared to controls (Severance et al., 2013; Tanaka et al., 2017). These increases in sCD14 may reflect an upregulated, but ineffective response by monocytes to address either the presence of an endogenous or environmental antigen. Furthermore, a recent investigation of microglial and neurodegenerative markers in twins discordant for schizophrenia demonstrated elevated sCD14 in the cerebrospinal fluids of the affected twin compared to the unaffected twin and in association with worse psychiatric symptoms (Johansson et al., 2017). sCD14 elevations in the central nervous system (CNS) may indicate microglial activation, and interestingly, overexpression of CD14 transcripts was also detected in postmortem prefrontal cortex from individuals with schizophrenia compared to controls (Arion et al., 2007).

The inter-marker associations provided additional mechanistic insight, since case-control differences in marker correlation patterns were evident. We had expected that the two markers of bacterial translocation would be highly correlated, but this was not the case, further indicating that sCD14 and LBP reflect changes in different, although overlapping, physiologic pathways. LBP was well correlated with CRP in most groups, supporting a link between systemic inflammation as indicated by CRP and endotoxemic disturbances as reflected by LBP. The absence of a significant association of these two markers in women with schizophrenia could indicate possible sex-specific disease pathophysiologies and further suggests that the endocrine system may contribute to or compound immune dysfunction. However, there is no statistical power for this comparison, so no definitive interpretation could be made at this point. This finding requires further investigation to explore correlation between LBP and CRP in a larger population of females. The sCD14 levels did not correlate with CRP levels overall or in any stratified subgroups of schizophrenia, again supportive of a more selective monocyte aberration associated with schizophrenia before inflammation may be present.

Our study has a number of limitations, predominantly with respect to our study design and presence of potentially confounding variables. This study was a retrospective retrieval of serum samples for Service Members medically retired with schizophrenia and their matched controls. Serum samples taken before the diagnosis of schizophrenia were retrieved for cases and temporally matched with the stored samples of controls. We do not have further clinical information available regarding subsequent symptom measures or other clinical comorbidities occurring at the time of diagnosis, only that a diagnosis of schizophrenia was made according to standard diagnostic criteria (ICD-9-CM). Similarly, we cannot rule out the presence of self-managed prodromal symptoms or psychiatric disorder diagnoses that were intentionally concealed at the time of entry to the military. However, at the time of accession, prospective Service Members are screened for learning, psychiatric, and behavioral issues inclusive with screenings for all physical systems. Military Entrance Processing Station (MEPS) medical providers can medically qualify or disqualify the applicant based on their clinical judgment and, if necessary, request an additional assessment by a medical specialist consultant.

Etiopathogenic mechanisms might be heterogeneous by race, gender and/or prevalent symptoms of psychiatric disorder as well as associated comorbid diseases. Cases and controls were individually matched in this study population based on age, sex, race, branch of military service, date of birth, year of military entrance and serum specimen collection time. It is still possible, however, that untested demographic, lifestyle, genetic or other environmental variables might shape these study findings. Such factors include cigarette smoking, other substance use and comorbid medical illness. However, the prevalence of medical comorbidity is very low in the military as a result of the accession and retention screening, ready access to medical care, and preventive measures such as routine vaccinations. Serum based marker analyses of schizophrenia cohorts can also be influenced by the potential effects of medication and particularly antipsychotics. In our case, however, the pre-onset status of our population renders the antipsychotic medication issue improbable. Of note, body mass index (BMI) is an important consideration when testing inflammatory markers, and it is becoming evident that an altered gut microbiome may contribute not only to inflammation but to fat deposition, metabolism regulation and insulin resistance (Torres-Fuentes et al., 2017). While the absence of BMI information limits the present analysis, our study population contained mostly young adults between the ages of 18 and 40 years, all of whom underwent medical and fitness screening before entering and periodically during their service. Thus, the range in BMI for service members is likely quite low compared to a civilian population, and none of the subjects had morbid obesity. Future investigations of the entire military study populations with schizophrenia as well as bipolar disorder are planned, so we can have more power to explore gender, race and other differences and importantly evaluate relative longitudinal changes in these and other immune markers. Future studies are also needed to examine associations of these markers with the presence of specific psychiatric symptoms, symptom severity, disease remission status and treatment response.

In conclusion, the early sCD14 upregulation in peripheral circulation suggests the promise of this biomarker as an aid for diagnosing the schizophrenia prodrome. sCD14 is persistently elevated across studies of individuals with schizophrenia at different stages of disease ranging from pre-onset, as established in the present study, to post-mortem time periods. Thus, these sCD14 alterations in schizophrenia may reflect an underlying lifelong genetic-based and/or neurodevelopmentally-primed immune insufficiency in pathways that impact monocyte responses to bacterial and non-bacterial antigens. The presence of sCD14 in the CNS and its association with microglia raise an intriguing possibility that aberrant expression of sCD14 in schizophrenia may compromise microglial functions at synapses, a subject that will be addressed in future studies.

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Abbreviations

AFHSB	Armed Forces Health Surveillance Branch
CRP	C-reactive protein
DMSS	Defense Medical Surveillance System
DoDSR	Department of Defense Serum Repository
ELISA	Enzyme-linked immunosorbent assays
IgG	Immunoglobulin G
LBP	Lipopolysaccharide binding protein
MNOPP	Military New Onset Psychosis Project
OR	Odds ratio
sCD14	soluble CD14

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Table 1

Matched characteristics of the study population

Characteristic	Case/Control Pairs	%
Gender		
Male	68	85.0
Female	12	15.0
Age at Case Diagnosis		
18 – 20	17	21.3
21 – 25	35	43.8
26 – 30	17	21.3
31 – 35	7	8.8
> 35	4	5.0
Race		
White	40	50.0
Black	32	40.0
Other	8	10.0
Branch of Military		
Army	64	80.0
Air Force	7	8.8
Marines	0	0.0
Navy	9	11.3
Time [*] in Service before Discharge (for cases only)		
1	21	26.3
> 1 to 3	28	35.0
> 3 to 5	13	16.3
> 5 to 10	11	13.8
>10	7	8.8
Time [*] from Serum Collection to Case Diagnosis		
> 3	30	37.5
2 – 3	18	22.5
1 – 2	17	21.3
0.5 – 1	7	8.8
0.5	8	10.0

^{*}
Time in years

Table 2

Biomarker data of population

	Cases (mean absorbance+SD)	Controls (mean absorbance+SD)	Difference in mean levels	Paired t-test or sign test p-value	OR	95% CI	CLR p-value
sCD14	2.03+2.58	1.42+2.09	0.73	0.02	1.22	1.02–1.46	0.02
LBP	17.76+7.15	19.97+8.16	–2.50	0.06	0.97	0.93–1.01	0.15
CRP	0.49+0.72	0.45+0.52	0.03	1.00	1.11	0.65–1.90	0.71

SD refers to standard deviation

OR refers to odds ratio

CI refers to confidence interval

CLR refers to conditional logistic regressions

Table 3

Correlations of biomarker data by gender

	All			Female		Male	
	sCD14	LBP	sCD14	LBP	sCD14	LBP	LBP
Cases							
LBP	0.23 *	1.00	0.17	1.00	0.23 [#]	1.00	
CRP	0.14	0.36 **	-0.31	-0.24	0.21	0.46 ***	
Controls							
LBP	0.17	1.00	-0.15	1.00	0.22	1.00	
CRP	0.13	0.53 ***	-0.12	0.67 *	0.22	0.48 **	

SD refers to standard deviation

OR refers to odds ratio

CI refers to confidence interval

CLR refers to conditional logistic regressions

NS refers to not statistically significant

Asterisk (*) refers to p<0.05;

**
p<0.001;

p<0.0001;

[#]
p<0.06