Published in final edited form as:

J Affect Disord. 2019 March 15; 247: 57–65. doi:10.1016/j.jad.2018.12.100.

An inflammatory profile linked to increased suicide risk

Sarah A Keaton^{1,2}, Zachary B Madaj³, Patrick Heilman², LeAnn Smart⁴, Jamie Grit⁵, Robert Gibbons⁶, Teodor Postolache^{7,8}, Kimberly Roaten⁹, Eric Achtyes^{4,10,*}, Lena Brundin^{2,*,#}

¹Department of Physiology, Michigan State University, East Lansing, MI, USA

²Center for Neurodegenerative Science, Van Andel Research Institute, Grand Rapids, MI, USA

³Bioinformatics and Biostatistics Core, Van Andel Research Institute, Grand Rapids, MI, USA

⁴Pine Rest Christian Mental Health Services, Grand Rapids, MI, USA

⁵Center for Cancer and Cell Biology, Van Andel Research Institute, Grand Rapids, MI, USA

⁶Center for Health Statistics, Departments of Medicine and Public Health Sciences, University of Chicago, Illinois, USA

⁷Department of Psychiatry, University of Maryland-Baltimore School of Medicine, Baltimore, MD, USA

⁸Rocky Mountain Mirecc, Denver, CO, USA

⁹Department of Psychiatry, University of Texas Southwestern, Dallas, TX, USA

¹⁰Division of Psychiatry & Behavioral Medicine, Michigan State University College of Human Medicine, Grand Rapids, Michigan, USA

Abstract

Background: Suicide risk assessments are often challenging for clinicians, and therefore, biological markers are warranted as guiding tools in the assessments. Suicidal patients display increased cytokine levels in peripheral blood, although the composite inflammatory profile in the subjects is still unknown. It is also not yet established whether certain inflammatory changes are specific to suicidal subjects. To address this, we measured 45 immunobiological factors in peripheral blood and identified the biological profiles associated with cross-diagnostic suicide risk and depression, respectively.

#Corresponding author Lena.Brundin@vai.org, Van Andel Research Institute, 333 Bostwick Ave NE, Grand Rapids MI-49503, USA.

*Shared last authors

Author contributions

SK; performed analyte measurements and prepared the first draft of the manuscript. ZM; performed the statistical analysis. JG; performed biological analyses. PH, TP; contributed to concept development and manuscript writing. LS; was the clinical research coordinator, enrolling and assessing study subjects. RG; developed the computerized assessment tool for mental health (CAT-MH™). EA; was the psychiatrist responsible for the clinical design and clinical conduct of the study. LB; was responsible for the overall study design, concept development, and manuscript writing. All authors commented and assisted in writing of the manuscript and approved of the final version.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Methods: 66 women with mood disorders underwent computerized adaptive testing for mental health, assessing depression and suicide risk. Weighted correlation network analysis was used to uncover system level associations between suicide risk, depression, and the immunobiological factors in plasma. Secondary regression models were used to establish the sensitivity of the results to potential confounders, including age, BMI, treatment and symptoms of depression and anxiety.

Results: The biological profile of patients with increased suicide risk differed from that associated with depression. At the system level, a biological cluster containing increased levels of IL-6, lymphocytes, monocytes, WBC and PMN significantly impacted suicide risk, with the latter two inferring the strongest influence. The cytokine IL-8 was independently and negatively associated with increased suicide risk. The results remained after adjusting for confounders.

Limitations: This study is cross-sectional and not designed to prove causality.

Discussion: A unique immunobiological profile was linked to increased suicide risk. The profile was different from that observed in patients with depressive symptoms, and indicates that granulocyte mediated biological mechanisms could be activated in patients at risk for suicide.

Keywords

depression; suicide; white blood cells; network analysis; cytokine; granulocyte

Introduction

Inflammation first emerged as a potential trigger of depression in the late 1980s, when treatment with interferons for diseases such as cancer and hepatitis was found to cause depression and suicidal behavior (Capuron et al., 2000; Capuron et al., 2004; Dieperink et al., 2004). It is now known that what we consider to be primary depression is also associated with peripheral inflammatory changes (Dowlati et al., 2010; Howren et al., 2009; Liu et al., 2012; Valkanova et al., 2013). Moreover, there is mounting evidence that patients with suicidal ideation and behavior have pronounced inflammatory changes in both blood and cerebrospinal fluid (CSF) (Black and Miller, 2015; Lindqvist et al., 2009; Janelidze et al., 2011; O'Donovan et al., 2013). Inflammatory factors in the blood can reach the central nervous system (CNS) via several pathways, including passive or active transport across the blood brain barrier, immune cell transmigration and vagal nerve signaling (Banks, 2014; Hosoi et al., 2002). Within the brain, they may play a role in the progression of psychiatric diseases, triggering symptoms by acting on neural structure and function (Udina et al., 2012). As such, inflammatory factors may be targets for future therapies and interventions. They might also serve as biomarkers, reflecting the severity of symptoms and indicating suicide risk (Falcone et al., 2010; Sudol and Mann, 2017).

The purpose of this study was to identify immunobiological factors associated with increased suicide risk in a psychiatric outpatient setting. We focused on a patient sample with mood disorders in different stages, as this represents one of the most common populations encountered in an outpatient clinic. The critical question we addressed was whether we could detect a biological profile indicative of cross-diagnostic suicide risk among these women. The cross-diagnostic design was adapted from the DSM-V criteria, which recognizes suicidality as a cross-diagnostic entity (American Psychiatric Association,

2013). Thus, the sample, women with mood disorders, was chosen for clinical relevance. They were assessed for suicide risk, the unifying factor, for which we aimed to identify biological risk-indicators.

Previous studies, by us and others, have often focused on the association of a limited number of inflammatory factors with depressive symptoms and suicide risk (Lindqvist et al., 2009; Adhikari et al., 2018; Yang et al., 2018). However, inflammatory factors interact in complex networks, and it is still not known what specific inflammatory factor, or groups of inflammatory factors, best might reflect depressive and suicidal symptoms. To address this knowledge gap, we designed this study to measure 45 immunobiological factors in peripheral blood from women with mood disorders. The complete list of immunobiological analytes is found in Table 1. Some of the biological analytes, including cytokines, kynurenine pathway metabolites, VEGF and antibody titers, have previously been implicated in depression and suicidal behavior (Arling et al., 2009; Ganança et al., 2016; Isung et al., 2012; Ling et al., 2011; Meier et al., 2016b; Okusaga et al., 2011; Simanek et al., 2014). A well-replicated finding is that the pro-inflammatory cytokines IL-6 and TNF- α are elevated in suicidal patients compared to healthy controls, which was confirmed in a meta-analysis (Black and Miller, 2015). However, other immunobiological factors may be decreased in suicidality. As one example, we and others have found that levels of IL-8, a cytokine with neuroprotective functions, might be lower in suicidal patients (Isung et al., 2012 and Janelidze et al., 2015).

Here, we used network analysis to identify the composite immunobiological profiles associated with depression and suicide risk. Our hypothesis was that we would find a distinct biological profile associated with suicide risk. Our primary outcomes were the immunobiological networks associated with suicide risk and depression, respectively, as calculated using weighted co-expression network analysis (WGCNA). Next, we performed sensitivity analyses to establish whether the outcomes were affected by potential confounders including age, treatment and the degree of current symptoms of anxiety and depression. As a result of this systems level approach, we observed a unique proinflammatory cell profile in women with increased suicide risk, which was different from our findings in patients with depressive symptoms.

Materials and Methods

Participants

The study was approved by the Mercy Health Saint Mary's Institutional Review Board. A total of 66 women, age 18–67, with mood disorders, active or in remission to cover a full range of symptom severity, were included in this study (see Table 2). Study participants were recruited by advertisement in the outpatient clinics of Pine Rest Christian Mental Health Services (Pine Rest) located in Grand Rapids, Michigan, USA. The past/current psychiatric diagnoses of the subjects were established using the SCID for DSM-IV-TR (First et al., 2002). The psychiatric and somatic diagnoses of the patients as well as ongoing medication are listed in Tables 2 and 3. No patients in this study had abnormal liver status or signs of an on-going infection. Blood samples were collected at Pine Rest, between July 9, 2012 and March 29, 2013. The exclusion criteria included a history of psychotic disorders; drug or

alcohol dependence in the three months prior to study enrollment; organic mood disorder due to a general medical condition or substance use; current inpatient treatment; or a diagnosis of major/minor neurocognitive disorders. Additionally, no patients with borderline personality disorder/emotionally unstable personality disorder were included. This study is an extension of the analysis of subjects described in Bryleva et al. (Bryleva et al., 2017) which looked at single markers in relation to traditional diagnostic tools.

Assessment of psychiatric symptoms

CAT-MH™ is a computerized assessment of psychiatric symptoms that draws questions related to signs and symptoms (Gibbons et al., 2012). Convergent validity has been confirmed against the Patient Health Questionnaire-9 (PHQ-9), Hamilton Depression Rating Scale (HAMD), and Center for Epidemiologic Studies Depression Scale (CES-D) (Gibbons et al., 2012). Adaptive scale score significantly predicts current DSM-5 diagnoses of major depressive disorder, based on SCID interviews (Achtyes et al., 2015; Gibbons et al., 2012). There are four mandatory suicide-risk screening questions, which are derived from the Columbia Suicide Severity Rating Scale (C-SSRS). The questions are the following: I) In the past month, have you actually had any thoughts of killing yourself? II) Have you had any intention of acting on these thoughts of killing yourself? As opposed to you have the thoughts, but you definitely would not act on them? III) Have you started to work out, or actually worked out, the specific details of how to kill yourself and did you actually intend to carry out the details of your plan? IV) In the past 3 months, have you done anything, started to do anything, or prepared to do anything to end your life? Examples: Collected pills, obtained a gun, gave away valuables, wrote a will or suicide note, took out pills but didn't swallow any, held a gun but changed your mind about hurting yourself or it was grabbed from your hand, went to the roof to jump but didn't; or actually took pills, tried to shoot yourself, cut yourself, tried to hang yourself. A binary yes/no *suicide warning* (referred to here as "suicide risk") is generated if the patient responds yes to one or several of these four questions.

Blood Draws

Blood draws for all participants were done fasting between 8–10 AM within 5 days of completing the psychiatric evaluations. Plasma was separated by centrifugation at 700×g at 4°C, aliquoted, and immediately placed in a –80°C freezer until time of analysis. A complete cell count blood panel (CBC) was performed using a Beckman Coulter LH755 automated analyzer (BC, Southfield, MI, USA).

Inflammatory- and growth factors

Pro- and anti-inflammatory factors, acute-phase reactants and growth factors in plasma were analyzed on the Meso Scale Discovery Sector 6000 imager according to the manufacturer's protocol (MESO SCALE DIAGNOSTICS, LLC, Rockville, Maryland). All samples were run in duplicate and the mean values were used for statistical analysis. IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IFN-γ and TNF-α were analyzed using the Pro-inflammatory I multiplex panel. Inter-assay coefficients of variation (CV): IL-1β (7.6%), IL-2 (8.9%), IL-4 (9.5%), IL-6 (3.7%), IL-8 (5.3%), IL-10 (2.9%), IL-12p70 (5.9%), IL-13 (3.4%), IFN-γ (3.5%) and TNF-α (6.3). The Vascular Injury Panel 2 Kit was utilized for

the detection of SAA, CRP, VCAM-1, and ICAM-1. Inter-assay (CV): SAA (10.9%), CRP (9.3%), VCAM-1 (6.2%), and ICAM-1 (4.8%). The Human Growth Factor I Kit was utilized to detect bFGF, sFLT-1, PIGF, and VEGF. Inter-assay (CV): bFGF (10.3%), sFLT-1 (7.3%), PIGF (7.8%), and VEGF (7.6%).

Measures of immunity towards pathogen and pathogen activity

HSV1 and HSV2 IgG titer and IgG avidity were analyzed by the manufacturer's instructions using the SERION ELISA classic Herpes Simplex Virus IgG kits (Institut Virion\Serion GmbH, Würzburg, Germany). All kits were run on an Infinite 200 PRO plate reader (Tecan Group Ltd., Männedorf, Switzerland) using the Tecan i-control application. To measure antibody avidity, a 6 M urea wash step was included to remove low avidity antibodies from the plate prior to titer quantification as described previously (Hashido et al., 1997). Avidity index was calculated from the ratio of high-avidity IgG to total IgG, with an AI higher than 0.6 indicating recurrent infection (Blackburn et al., 1991; Hashido et al., 1997). HSV 1 & 2 IgM, *T. gondii* IgM and IgG, and cytomegalovirus CMV IgM, IgG, and IgG avidity were analyzed by the manufacturer's recommendations using IBL Internationals kits (IBL International Corp GmbH, Hamburg, and Germany). All plates were read on a Synergy HT plate reader (BioTek, Winooski, VT) using software version 2.04.11. A 6 M urea wash step was included to measure CMV IgG avidity. All samples were run in duplicate and the mean values of the duplicates were used for analysis.

Measures of kynurenine pathway metabolites

Plasma from the patients was analyzed using gas chromatography-mass spectrometry to determine PIC and QUIN concentrations. Plasma proteins were precipitated out using 10% trichloroacetic acid and centrifuged. 50 µL of the solution were added to a glass tube along with deuterated internal standard and the previously published protocol was performed (Smythe et al., 2002). The sample was injected into a Thermo Trace GC Ultra gas chromatograph interfaced to a Thermo DSQ II mass spectrometer. The inter-assay CV's were 1.85% for PIC and 4.46% for QUIN. KYN and TRP were analyzed by high-performance liquid chromatography (HPLC). Plasma proteins were precipitated out using the method stated above. The supernatant was then filtered through a 0.22 µm PTFE filter into an HPLC polypropylene vial, and 20 µL was injected into the Thermo Scientific Dionex UltiMate 3000 (Meier et al., 2016a). The inter-assay CV's were 3.16% (KYN) and 0.81% (TRP).

Statistical analysis

All analyses were performed using R v 3.4.3 (<https://cran.r-project.org/>). The systems level analyses were assessed using weighted co-expression network analysis (WGCNA). We used the WGCNA R software package to carry out module construction, hub analyte selection and network statistics. The method WGCNA is a data reduction method that serves to reduce large amounts of biological information into modules; forming a network. The network preserves the continuous nature of the underlying correlation information and is highly robust compared to alternative methods. Standard practice in WGCNA is to calculate the first principal component of the modules (called "the eigengene" in WGCNA). The eigengenes are the most representative immunobiological profiles of the respective modules

(consisting of the cumulative signal of the analytes in the module). Next, two sets of Spearman correlation analyses are conducted with the eigengenes. In the first, eigengenes association with the patient characteristics (CAT-MH depression and suicide risk) is established. The results are visualized in Figure 2 and shows whether a module (and therefore the analytes within it) is significantly associated with the patient characteristics. In the next step, the association of the eigengenes with the individual analytes within the module is established. The resulting coefficients are denoted module membership values. Analytes with high module membership indicate high connectivity to the other analytes contained within the module and are identified as hub markers. These hubs are generally good candidates for biomarkers and focus for inferring pathology, as they are linked to the most representative expression profile of the module. The soft threshold for the scale-free topology of WGCNA was assessed by fitting over an array of powers from 1–30 for a signed-hybrid network. Good scale-free topology (minimum correlation of 80%) was achieved using a power of 6. The minimum number of markers needed to create a module was set to 1. No modules were merged. All p-values in the results section are reported after treatment and demographic correction unless otherwise stated.

We next performed sensitivity analyses to test whether the relationship between the immunobiological markers and suicide risk or depression were impacted by potential confounders, including treatment, demographics and comorbid depressive/anxiety symptoms by ridge regression (RiR) models. The models were adjusted for BMI, age, anti-inflammatory medications, antiepileptics, antipsychotic, psychostimulants, benzodiazepines, serotonin-norepinephrine reuptake inhibitors, selective serotonin reuptake inhibitors, tricyclic antidepressant treatment, anxiety scores derived from HAMD (sum of item 10 and 11) and HAMD17 total scores for depression. RiR was chosen specifically to address multicollinearity in the covariates (de Vlaming and Groenen, 2015). P-values were calculated using a p-value trace, a plot of the negative logarithm of the p-values of RiR coefficients with increasing shrinkage parameters, developed by Cule and colleagues (Cule et al., 2011) to reduce computational cost. Linear RiR was used for analysis of depression severity, while logistic RiR was used for the binary suicide warning outcome. RiR with only treatment adjustment and standard regression with only the cytokine and outcome were also fit to assess the sensitivity of the results to the exclusion of demographic and treatment adjustments. Measured analytes were log transformed as necessary. N-fold cross validation was used to establish the value of the tuning parameter, which was set as one standard deviation from the value that minimized the mean-squared error.

Results

System level analysis and module identification

Demographics of the cohort, based on “suicide risk”, is shown in Table 4. Using WGCNA, we identified three separate modules, i.e. clusters of highly interconnected biological analytes, among the 45 immunobiological factors. The first module was comprised primarily of red blood cell factors, the second module consisted of kynurenine pathway analytes, and the third module was comprised of white blood cell-related factors (Fig. 1).

A biological profile associated with suicide risk

At the network level, WGCNA identified “suicide risk” to be positively associated with the blue module, containing IL-6, LYMPH, MONO, WBC and PMN (WGCNA, $r=0.27$, $p<0.05$) (Fig. 2). WBC (Module membership (MM)=0.94) and PMN (MM=0.86) constituted hubs (a hub consists of analytes inside a module that have high connectivity, typically of pathophysiological relevance) and had the largest influence on “suicide risk”. Module membership information can be found in the supplementary data (Fig. S1). There was no system level significant association between “depression” and any of the identified immunobiological modules (WGCNA, Fig. 2).

Impact of potential confounders

In order to determine if the associations between the immunobiological factors and outcome “suicide risk” and “depression” were influenced by potential confounders, we performed sensitivity analyses where we introduced age, BMI, treatment, depression and anxiety scores into regression models (Table 5). The results show that the effect on “suicide risk” by the immunobiological markers was not influenced symptoms of depression and anxiety. Similarly, the immunobiological profile associated with “depression” was not influenced by symptoms of anxiety. Moreover, to control for potential confounding effects of medications, we included anti-inflammatory medications, antiepileptics, antipsychotic, psychostimulants, benzodiazepines, serotonin-norepinephrine reuptake inhibitors, selective serotonin reuptake inhibitors, and tricyclic antidepressant treatment as covariates in regression models. The results were not sensitive to the treatment covariates, as illustrated by the regression models (Table 5). The independent exploratory analyses also revealed that the IL-8 levels were negatively associated with increased suicide risk (RiR, $\beta=-0.03\pm0.02$, $p<0.05$), and there were significant positive associations between “depression” and the acute phase reactant SAA (RiR, $\beta=0.05\pm0.02$ SE, $p=0.01$) and platelet count (RiR, $\beta=0.05\pm0.02$ SE, $p<0.01$) (Table 5).

Discussion

In this study, we measured 45 immunobiological markers in peripheral blood and analyzed their associations with cross-diagnostic suicide risk in a population of 66 women with mood disorders. We discovered a distinct immunobiological profile in subjects with increased suicide risk, composed of IL-6 and white blood cells, in particular granulocytes. There was also a module-independent negative association between IL-8 and suicide risk. We did not identify any composite network of immunobiological factors linked to depression severity, but independent positive associations between depression and the acute phase mediator SAA as well as increased platelet count. Our results add support to the mounting number of studies demonstrating a significant dysregulation of the immune system in patients with suicidal ideation and behavior. The results are novel in several ways. First, we used an unbiased way to generate the suicide risk assessment (the CAT-MH™ generated *suicide warning*) which was next matched with the biological data. Second, we subjected a large number of immunobiological factors to robust statistical methods, the network analysis WGCNA and sensitivity analyses using ridge regression models. Our significant results indicate that depression and suicide risk are associated with distinct immunobiological

profiles in peripheral blood, confirming our hypothesis. Third, our data analysis show that granulocyte mediated immune reactions could potentially be central among the biological mechanisms responsible for triggering suicidal ideation and behavior.

As strengths of our study, we analyzed a high number of biological analytes in clinically well-characterized patients and subjected the data to robust network analysis, and showed that the data was not impacted by important potential confounders in sensitivity analyses. Thus, our analysis showed that the data was not sensitive to pharmacological treatment, BMI, age or the severity of symptoms of anxiety or depression. However, as a limitation; even if the statistical methods we used are not sensitive to sample size or data heterogeneity, the biological features are cohort specific and need to be confirmed in future studies. Since the population in this study was limited to women with mood disorders, there is a need to assess the biological networks connected to suicide risk in additional cohorts before generalizing the results. Other limitations of this study include a lack of data regarding the number of past episodes or disease duration. Such data may provide further insight into specific immunobiological factors that are important during different stages of mood disorders (Brietzke et al., 2009).

We found that suicide risk was associated with increased pro-inflammatory factors and white blood cell counts. CRP is a marker of acute inflammation that has previously been associated with suicidal ideation and behavior (Gibbs et al., 2016; Loas et al., 2016). During the acute-phase response, an elevation of CRP is often accompanied by increased numbers of lymphocytes (Barzilay et al., 2016; Gans et al., 2015). At the network level, we found that suicide risk was linked to a module containing IL-6, total white blood cell count, lymphocytes, monocytes and in particular polymorphonuclear cells (granulocytes). There are some previous reports indicating that suicidality (ideation and behavior) in depressive patients correlates with increased white blood cell counts (Endres et al., 2016). Additionally, impulsivity, which is linked to suicide (Mann et al., 1999), is associated with increased white blood cell counts (Sutin et al., 2012). A recent study which evaluated over 300,000 women of which 1,000 later died by suicide, higher levels of white blood cell counts was predictive of later suicide (Batty et al., 2018). Interestingly, our network analysis indicated granulocytes to be the cell type with strongest influence on suicide risk. Granulocytes are cells of the innate immune that produce a large variety of proinflammatory cytokines, both constitutively, and in increased amounts after stimulation. They fulfill several functions in the immune response including responses towards pathogens, including parasitic infections, and also mediate allergic reactions. Allergies and certain parasitic infections have been linked with increased suicide risk in previous epidemiological studies, and could thus be hypothesized to be triggers of the involved immunobiological mechanisms (Lund-Sorensen et al., 2016; Pedersen et al., 2012; Zhang et al., 2012).

In addition to increased white blood cell counts, we observed a reduction of IL-8 in women with increased suicide risk. IL-8 is a chemoattractant cytokine that has strong target specificity for neutrophils (Bickel, 1993). The cytokine serves pleiotropic and tissue specific functions, including a role in the development and establishment of synaptic plasticity in the central nervous system (Willette et al., 2013). Isung and colleagues reported lower levels of IL-8 in the CSF of suicide attempters compared to health controls (Isung et al., 2012).

Additionally, our group has previously found low levels of IL-8 in the plasma and CSF to be associated with increased anxiety levels in suicide attempters (Janelidze et al., 2015). Our current study thus provides additional support, in a third clinical cohort, that low levels of the cytokine IL-8 are associated with suicidal ideation and behavior. The potential biological mechanism(s) impacted by IL-8 in suicidal patients appears to be separate from the module identified by our network analysis, as IL-8 was not a member of the immunobiological network associated with suicide risk. Instead, we identified IL-6, a pro-inflammatory cytokine that has previously been linked to suicidality in multiple studies (Isung et al., 2014; Janelidze et al., 2011; Lindqvist et al., 2009; Mina et al., 2015), to be part of the immunobiological network linked to suicide risk. IL-6 is a pro-inflammatory and regulatory cytokine that can be secreted by many cells of the immune system, in particular monocytes and granulocytes of the innate immune system (Ericson et al., 1998; Oishi and Machida, 1997; Zimmermann et al., 2016). The cytokine can also be secreted by adipose tissue and in response to exercise (Hallberg et al., 2010; Kern et al., 2001; Lyngso et al., 2002; Makki et al., 2013). However, we did not find any impact of BMI on our data, and the close connection between the IL-6 levels and immune cells in the WGCNA module demonstrates that the cytokine is likely being secreted by immune cells rather than by other cell types in the body in individuals with suicide risk. In addition to being a mediator of immune reactions, IL-6 can bind directly to receptors on neurons in the CNS and has been shown to directly impact the generation of action potentials, which may influence changes in emotion and behavior, including increased risk for suicidal intent and behavior (Gruol, 2015; Xia et al., 2015).

Different immunobiological mediators were implicated in depression. While the network analysis did not find any biological network associated with depression, we detected independent associations for the acute phase mediator SAA and platelet counts in our exploratory sensitivity analyses. SAA is an acute phase mediator that can activate macrophages and glial cells, and is increased in patients with multiple, chronic inflammatory diseases (Muhlebach et al., 2016; Scarpioni et al., 2016; Ye and Sun, 2015). SAA induces the production of pro-inflammatory cytokines from microglia, including those associated with depression (Eklund et al., 2012), and we previously found that elevated levels of SAA were associated with depressive symptoms as measured by HAMD (Bryleva et al., 2017). Platelets are also known to increase in acute inflammatory conditions, and play a role in the inflammatory cascade by increasing vascular permeability (Gros et al., 2014; Kapur et al., 2015; Williams, 2012), detecting and responding to vascular injury. A “platelet hypothesis of depression” has been proposed, as depression is a risk factor for cardiovascular mortality and morbidity, and studies have demonstrated that increased platelet reactivity and aggregation are associated with increased risk for depression (Lederbogen et al., 2001; Musselman et al., 1996; Williams, 2012).

A network analysis approach to finding associated blood markers may be more insightful than analyses focusing on only one or a few markers, as there are often synergistic and distinct effects, depending on what type of immunobiological network is active; for example; the cytokine IL-6 can be elevated as part of a pro-inflammatory cascade, as part of a cytokine release from muscular tissue in response to exercise, or as part of a regulatory anti-inflammatory response. Whether the biological mechanisms implicated here are specific

for depressive and suicidal individuals need to be confirmed in additional populations. Causative effects of the proposed mechanisms can be tested in experimental models. Suicidal symptoms and behavior can be dissected into components such as impulsivity, hopelessness and aggression that can be tested further in relation to the distinct immunobiological profiles, in both experimental models and in human populations.

Conclusion

In this study, we integrated several innovative approaches to assess the immunobiological profiles of women with mood disorders, to identify a unique biological profile in subjects with increased suicide risk. Our study highlights a couple of immunological mediators that may be of specific importance in the pathophysiology of suicidal behavior, including the cytokines IL-6 and IL-8 and granulocyte mediated mechanisms. Replication studies in independent cohorts will be necessary to further the development of clinical biomarkers of suicide risk. Additional studies are warranted using network analysis in larger populations of carefully clinically characterized patients, in order to advance our understanding of the complex immunobiological networks underlying depressive illness and suicide risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We are deeply thankful to all our subjects for devoting their time and blood samples to this research project. We also thank Stan Krzyzanowski and Colt Capan in the Brundin laboratory for proof reading and editing the manuscript. Van Andel Research Institute and Pine Rest Christian Mental Health Services in Grand Rapids, Michigan, USA, are both greatly acknowledged for providing facilities and resources for this project.

Role of the funding source

Grant support for this project was provided by NIH R01-MH104622 (L. Brundin, E. Achtyes and T. Postolache); NIH R01-MH66302 (R. Gibbons); the Pine Rest Foundation (E. Achtyes, L. Smart); a Distinguished Investigator Award from the American Foundation for Suicide Prevention, DIG 1-162-12 (T. Postolache); P30 DK072488 NIDDK NORC pilot/developmental grant (T. Postolache); the Joint Institute for Food Safety and Applied Nutrition, and the US FDA, cooperative agreement FDU.001418 (T. Postolache); and the 1101CX001310-01, Merit Award from VA CSR&D (T. Postolache and L. Brundin). None of these institutions had any further role in the design of the study or in the decision to submit the work for publication.

References

- Achtyes ED, Halstead S, Smart L, Moore T, Frank E, Kupfer DJ, Gibbons R, 2015 Validation of Computerized Adaptive Testing in an Outpatient Nonacademic Setting: The VOCATIONS Trial. *Psychiatric services* (Washington, D.C.) 66, 1091–1096.
- Adhikari A, Dikshit R, Karia S, Sonavane S, Shah N, De Sousa A, 2018 Neutrophil-lymphocyte Ratio and C-reactive Protein Level in Patients with Major Depressive Disorder Before and After Pharmacotherapy. *East Asian archives of psychiatry : official journal of the Hong Kong College of Psychiatrists* = *Dong Ya jing shen ke xue zhi* : *Xianggang jing shen ke yi xue yuan qi kan* 28, 53–58.
- American Psychiatric Association, 2013 *Diagnostic and Statistical Manual of Mental Disorders* 5th ed.
- Arling TA, Yolken RH, Lapidus M, Langenberg P, Dickerson FB, Zimmerman SA, Balis T, Cabassa JA, Scrandis DA, Tonelli LH, Postolache TT, 2009 *Toxoplasma gondii* antibody titers and history of

suicide attempts in patients with recurrent mood disorders. *The Journal of nervous and mental disease* 197, 905–908. [PubMed: 20010026]

- Banks W, 2014 *The Blood-brain Barrier in Neuroimmunology: Tales of Separation and Assimilation*
- Barzilay R, Lobel T, Krivoy A, Shlosberg D, Weizman A, Katz N, 2016 Elevated C-reactive protein levels in schizophrenia inpatients is associated with aggressive behavior. *European psychiatry: the journal of the Association of European Psychiatrists* 31, 8–12. [PubMed: 26657596]
- Batty GD, Jung KJ, Lee S, Back JH, Jee SH, 2018 Systemic inflammation and suicide risk: cohort study of 419 527 Korean men and women. *Journal of epidemiology and community health* 72, 572–574. [PubMed: 29572361]
- Bickel M, 1993 The role of interleukin-8 in inflammation and mechanisms of regulation. *Journal of periodontology* 64, 456–460. [PubMed: 8315568]
- Black C, Miller BJ, 2015 Meta-Analysis of Cytokines and Chemokines in Suicidality: Distinguishing Suicidal Versus Nonsuicidal Patients. *Biological psychiatry* 78, 28–37. [PubMed: 25541493]
- Blackburn NK, Besselaar TG, Schoub BD, O'Connell KF, 1991 Differentiation of primary cytomegalovirus infection from reactivation using the urea denaturation test for measuring antibody avidity. *Journal of medical virology* 33, 6–9. [PubMed: 1849983]
- Bryleva EY, Keaton SA, Grit J, Madaj Z, Sauro-Nagendra A, Smart L, Halstead S, Achtyes E, Brundin L, 2017 The acute-phase mediator serum amyloid A is associated with symptoms of depression and fatigue. *Acta psychiatrica Scandinavica* 135, 409–418. [PubMed: 28374419]
- Brietzke E, Stertz L, Fernandes BS, Kauer-Sant'Anna M, Mascarenhas M, Escosteguy Vargas A, Chies JA, Kapczinski F, 2009 Comparison of cytokine levels in depressed, manic and euthymic patients with bipolar disorder. *Journal of Affective Disorders* 116, 214–217. [PubMed: 19251324]
- Capuron L, Ravaud A, Dantzer R, 2000 Early depressive symptoms in cancer patients receiving interleukin 2 and/or interferon alfa-2b therapy. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 18, 2143–2151. [PubMed: 10811680]
- Capuron L, Ravaud A, Miller AH, Dantzer R, 2004 Baseline mood and psychosocial characteristics of patients developing depressive symptoms during interleukin-2 and/or interferon-alpha cancer therapy. *Brain, behavior, and immunity* 18, 205–213.
- Cule E, Vineis P, De Iorio M, 2011 Significance testing in ridge regression for genetic data. *BMC bioinformatics* 12, 372. [PubMed: 21929786]
- de Vlaming R, Groenen PJ, 2015 The Current and Future Use of Ridge Regression for Prediction in Quantitative Genetics. *BioMed research international* 2015, 143712. [PubMed: 26273586]
- Dieperink E, Ho SB, Tetrick L, Thuras P, Dua K, Willenbring ML, 2004 Suicidal ideation during interferon-alpha2b and ribavirin treatment of patients with chronic hepatitis C. *General hospital psychiatry* 26, 237–240. [PubMed: 15121353]
- Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lancot KL, 2010 A meta-analysis of cytokines in major depression. *Biological psychiatry* 67, 446–457. [PubMed: 20015486]
- Eklund KK, Niemi K, Kovanen PT, 2012 Immune functions of serum amyloid A. *Critical reviews in immunology* 32, 335–348. [PubMed: 23237509]
- Endres D, Perlov E, Dersch R, Baumgartner A, Hottenrott T, Berger B, Stich O, Tebartz van Elst L, 2016 Evidence of cerebrospinal fluid abnormalities in patients with depressive syndromes. *Journal of affective disorders* 198, 178–184. [PubMed: 27017374]
- Ericson SG, Zhao Y, Gao H, Miller KL, Gibson LF, Lynch JP, Landreth KS, 1998 Interleukin-6 production by human neutrophils after Fc-receptor cross-linking or exposure to granulocyte colony-stimulating factor. *Blood* 91, 2099–2107. [PubMed: 9490696]
- Falcone T, Fazio V, Lee C, Simon B, Franco K, Marchi N, Janigro D, 2010 Serum S100B: a potential biomarker for suicidality in adolescents? *PloS one* 5, e11089. [PubMed: 20559426]
- First M, Spitzer RL, Gibbon ML, Williams J, 2002 *Structured clinical interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient Edition*
- Ganança L, Oquendo MA, Tyrka AR, Cisneros-Trujillo S, Mann JJ, Sublette ME, 2016 The Role of Cytokines in the Pathophysiology of Suicidal Behavior. *Psychoneuroendocrinology* 63, 296–310. [PubMed: 26546783]

- Gans SL, Ateman JJ, Stoker J, Toorenvliet BR, Laurell H, Boermeester MA, 2015 C-reactive protein and white blood cell count as triage test between urgent and nonurgent conditions in 2961 patients with acute abdominal pain. *Medicine* 94, e569. [PubMed: 25738473]
- Gibbons RD, Weiss DJ, Pilkonis PA, Frank E, Moore T, Kim JB, Kupfer DJ, 2012 Development of a Computerized Adaptive Test for Depression. *Archives of general psychiatry* 69, 1104–1112. [PubMed: 23117634]
- Gibbs HM, Davis L, Han X, Clothier J, Eads LA, Caceda R, 2016 Association between C-reactive protein and suicidal behavior in an adult inpatient population. *Journal of psychiatric research* 79, 28–33. [PubMed: 27135541]
- Gros A, Ollivier V, Ho-Tin-Noe B, 2014 Platelets in inflammation: regulation of leukocyte activities and vascular repair. *Frontiers in immunology* 5, 678. [PubMed: 25610439]
- Grønlund DL, 2015 IL-6 regulation of synaptic function in the CNS. *Neuropharmacology* 96, 42–54. [PubMed: 25445486]
- Hallberg L, Janelidze S, Engstrom G, Wisen AG, Westrin A, Brundin L, 2010 Exercise-induced release of cytokines in patients with major depressive disorder. *Journal of affective disorders* 126, 262–267. [PubMed: 20347489]
- Hashido M, Inouye S, Kawana T, 1997 Differentiation of primary from nonprimary genital herpes infections by a herpes simplex virus-specific immunoglobulin G avidity assay. *Journal of clinical microbiology* 35, 1766–1768. [PubMed: 9196189]
- Hosoi T, Okuma Y, Nomura Y, 2002 The mechanisms of immune-to-brain communication in inflammation as a drug target. *Current drug targets. Inflammation and allergy* 1, 257–262. [PubMed: 14561190]
- Howren MB, Lamkin DM, Suls J, 2009 Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosomatic medicine* 71, 171–186. [PubMed: 19188531]
- Isung J, Aeieneband S, Mobarrez F, Martensson B, Nordstrom P, Asberg M, Piehl F, Jokinen J, 2012 Low vascular endothelial growth factor and interleukin-8 in cerebrospinal fluid of suicide attempters. *Translational psychiatry* 2, e196. [PubMed: 23168996]
- Isung J, Aeieneband S, Mobarrez F, Nordstrom P, Runeson B, Asberg M, Piehl F, Jokinen J, 2014 High interleukin-6 and impulsivity: determining the role of endophenotypes in attempted suicide. *Translational psychiatry* 4, e470. [PubMed: 25335166]
- Janelidze S, Mattei D, Westrin A, Traskman-Bendz L, Brundin L, 2011 Cytokine levels in the blood may distinguish suicide attempters from depressed patients. *Brain, behavior, and immunity* 25, 335–339.
- Janelidze S, Suchankova P, Ekman A, Erhardt S, Sellgren C, Samuelsson M, Westrin A, Minthon L, Hansson O, Traskman-Bendz L, Brundin L, 2015 Low IL-8 is associated with anxiety in suicidal patients: genetic variation and decreased protein levels. *Acta psychiatrica Scandinavica* 131, 269–278. [PubMed: 25251027]
- Kapur R, Zufferey A, Boilard E, Semple JW, 2015 Nouvelle cuisine: platelets served with inflammation. *Journal of immunology (Baltimore, Md. : 1950)* 194, 5579–5587.
- Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G, 2001 Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *American journal of physiology. Endocrinology and metabolism* 280, E745–751. [PubMed: 11287357]
- Lederbogen F, Gilles M, Maras A, Hamann B, Colla M, Heuser I, Deuschle M, 2001 Increased platelet aggregability in major depression? *Psychiatry research* 102, 255–261. [PubMed: 11440776]
- Lindqvist D, Janelidze S, Hagell P, Erhardt S, Samuelsson M, Minthon L, Hansson O, Bjorkqvist M, Traskman-Bendz L, Brundin L, 2009 Interleukin-6 is elevated in the cerebrospinal fluid of suicide attempters and related to symptom severity. *Biological psychiatry* 66, 287–292. [PubMed: 19268915]
- Ling VJ, Lester D, Mortensen PB, Langenberg PW, Postolache TT, 2011 Toxoplasma gondii seropositivity and suicide rates in women. *The Journal of nervous and mental disease* 199, 440–444. [PubMed: 21716055]
- Liu Y, Ho RC, Mak A, 2012 Interleukin (IL)-6, tumour necrosis factor alpha (TNF-alpha) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression. *Journal of affective disorders* 139, 230–239. [PubMed: 21872339]

- Loas G, Dalleau E, Lecointe H, Yon V, 2016 Relationships between anhedonia, alexithymia, impulsivity, suicidal ideation, recent suicide attempt, C-reactive protein and serum lipid levels among 122 inpatients with mood or anxious disorders. *Psychiatry research* 246, 296–302. [PubMed: 27744231]
- Lund-Sorensen H, Benros ME, Madsen T, Sorensen HJ, Eaton WW, Postolache TT, Nordentoft M, Erlangsen A, 2016 A Nationwide Cohort Study of the Association Between Hospitalization With Infection and Risk of Death by Suicide. *JAMA psychiatry* 73, 912–919. [PubMed: 27532502]
- Lyngso D, Simonsen L, Bulow J, 2002 Interleukin-6 production in human subcutaneous abdominal adipose tissue: the effect of exercise. *The Journal of physiology* 543, 373–378. [PubMed: 12181307]
- Makki K, Froguel P, Wolowczuk I, 2013 Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. *ISRN inflammation* 2013, 139239. [PubMed: 24455420]
- Mann JJ, Waternaux C, Haas GL, Malone KM, 1999 Toward a clinical model of suicidal behavior in psychiatric patients. *The American journal of psychiatry* 156, 181–189. [PubMed: 9989552]
- Meier TB, Drevets WC, Wurfel BE, Ford BN, Morris HM, Victor TA, Bodurka J, Teague TK, Dantzer R, Savitz J, 2016a Relationship between neurotoxic kynurenine metabolites and reductions in right medial prefrontal cortical thickness in major depressive disorder. *Brain, behavior, and immunity* 53, 39–48.
- Meier TB, Drevets WC, Wurfel BE, Ford BN, Morris HM, Victor TA, Bodurka J, Teague TK, Dantzer R, Savitz J, 2016b Relationship between neurotoxic kynurenine metabolites and reductions in right medial prefrontal cortical thickness in major depressive disorder. *Brain, behavior, and immunity* 53, 39–48.
- Mina VA, Lacerda-Pinheiro SF, Maia LC, Pinheiro RF Jr., Meireles CB, de Souza SI, Reis AO, Bianco B, Rolim ML, 2015 The influence of inflammatory cytokines in physiopathology of suicidal behavior. *Journal of affective disorders* 172, 219–230. [PubMed: 25451421]
- Muhlebach MS, Clancy JP, Heltshe SL, Ziady A, Kelley T, Accurso F, Pilewski J, Mayer-Hamblett N, Joseloff E, Sagel SD, 2016 Biomarkers for cystic fibrosis drug development. *Journal of cystic fibrosis : official journal of the European Cystic Fibrosis Society* 15, 714–723. [PubMed: 28215711]
- Musselman DL, Tomer A, Manatunga AK, Knight BT, Porter MR, Kasey S, Marzec U, Harker LA, Nemeroff CB, 1996 Exaggerated platelet reactivity in major depression. *The American journal of psychiatry* 153, 1313–1317. [PubMed: 8831440]
- O'Donovan A, Rush G, Hoatam G, Hughes BM, McCrohan A, Kelleher C, O'Farrelly C, Malone KM, 2013 Suicidal ideation is associated with elevated inflammation in patients with major depressive disorder. *Depression and anxiety* 30, 307–314. [PubMed: 23504697]
- Oishi K, Machida K, 1997 Some plasma component is essential for IL-6 secretion by Neutrophils. *Environmental health and preventive medicine* 2, 89–92. [PubMed: 21432460]
- Okusaga O, Yolken RH, Langenberg P, Lapidus M, Arling TA, Dickerson FB, Scrandis DA, Severance E, Cabassa JA, Balis T, Postolache TT, 2011 Association of seropositivity for influenza and coronaviruses with history of mood disorders and suicide attempts. *Journal of affective disorders* 130, 220–225. [PubMed: 21030090]
- Pedersen MG, Mortensen PB, Norgaard-Pedersen B, Postolache TT, 2012 *Toxoplasma gondii* infection and self-directed violence in mothers. *Archives of general psychiatry* 69, 1123–1130. [PubMed: 22752117]
- Scarpioni R, Ricardi M, Albertazzi V, 2016 Secondary amyloidosis in autoinflammatory diseases and the role of inflammation in renal damage. *World journal of nephrology* 5, 66–75. [PubMed: 26788465]
- Simanek AM, Cheng C, Yolken R, Uddin M, Galea S, Aiello AE, 2014 Herpesviruses, inflammatory markers and incident depression in a longitudinal study of Detroit residents. *Psychoneuroendocrinology* 50, 139–148. [PubMed: 25218654]
- Smythe GA, Braga O, Brew BJ, Grant RS, Guillemin GJ, Kerr SJ, Walker DW, 2002 Concurrent quantification of quinolinic, picolinic, and nicotinic acids using electron-capture negative-ion gas chromatography-mass spectrometry. *Analytical biochemistry* 301, 21–26. [PubMed: 11811963]

- Sudol K, Mann JJ, 2017 Biomarkers of Suicide Attempt Behavior: Towards a Biological Model of Risk. *Current psychiatry reports* 19, 31. [PubMed: 28470485]
- Sutin AR, Milanese Y, Cannas A, Ferrucci L, Uda M, Schlessinger D, Zonderman AB, Terracciano A, 2012 Impulsivity-related traits are associated with higher white blood cell counts. *Journal of behavioral medicine* 35, 616–623. [PubMed: 22190235]
- Udina M, Castellvi P, Moreno-Espana J, Navines R, Valdes M, Fornis X, Langohr K, Sola R, Vieta E, Martin-Santos R, 2012 Interferon-induced depression in chronic hepatitis C: a systematic review and meta-analysis. *The Journal of clinical psychiatry* 73, 1128–1138. [PubMed: 22967776]
- Valkanova V, Ebmeier KP, Allan CL, 2013 CRP, IL-6 and depression: a systematic review and meta-analysis of longitudinal studies. *Journal of affective disorders* 150, 736–744. [PubMed: 23870425]
- Willette AA, Coe CL, Birdsill AC, Bendlin BB, Colman RJ, Alexander AL, Allison DB, Weindruch RH, Johnson SC, 2013 Interleukin-8 and interleukin-10, brain volume and microstructure, and the influence of calorie restriction in old rhesus macaques. *Age (Dordrecht, Netherlands)* 35, 2215–2227.
- Williams MS, 2012 Platelets and depression in cardiovascular disease: A brief review of the current literature. *World Journal of Psychiatry* 2, 114–123. [PubMed: 24175177]
- Xia W, Peng GY, Sheng JT, Zhu FF, Guo JF, Chen WQ, 2015 Neuroprotective effect of interleukin-6 regulation of voltage-gated Na(+) channels of cortical neurons is time- and dose-dependent. *Neural regeneration research* 10, 610–617. [PubMed: 26170823]
- Yang C, Tiemessen KM, Bosker FJ, Wardenaar KJ, Lie J, Schoevers RA, 2018 Interleukin, tumor necrosis factor-alpha and C-reactive protein profiles in melancholic and non-melancholic depression: A systematic review. *Journal of psychosomatic research* 111, 58–68. [PubMed: 29935756]
- Ye RD, Sun L, 2015 Emerging functions of serum amyloid A in inflammation. *Journal of leukocyte biology* 98, 923–929. [PubMed: 26130702]
- Zhang Y, Traskman-Bendz L, Janelidze S, Langenberg P, Saleh A, Constantine N, Okusaga O, Bay-Richter C, Brundin L, Postolache TT, 2012 Toxoplasma gondii immunoglobulin G antibodies and nonfatal suicidal self-directed violence. *The Journal of clinical psychiatry* 73, 1069–1076. [PubMed: 22938818]
- Zimmermann M, Arruda-Silva F, Bianchetto-Aguilera F, Finotti G, Calzetti F, Scapini P, Lunardi C, Cassatella MA, Tamassia N, 2016 IFNalpha enhances the production of IL-6 by human neutrophils activated via TLR8. *Scientific reports* 6, 19674. [PubMed: 26790609]

Highlights

- We identified a distinct immunobiological profile linked to cross-diagnostic suicide risk in women with mood disorders, attending a psychiatric outpatient clinic.
- We analyzed a high number of biological analytes and subjected the data to robust statistical models to understand inflammatory factors in their composite biological networks.
- Granulocyte mediated immune reactions could potentially be central among the biological mechanisms triggering suicidal ideation and behavior.

Figure 1.

Dendrogram of WGCNA biological outcome measures. Three biological analyte clusters (modules) were identified, coded here by: turquoise, red blood cell related factors; brown, kynurenine pathway metabolites; and blue, white blood cell- related factors. Analytes with a lower height number have a greater impact on their respective module. The color gray denotes no association of an analyte with a specific module.

Figure 2.

Heat map of WGCNA biological outcome measures. Module-trait relationships are listed for suicide risk and depression. Module significance was determined by the average of absolute analyte significance for all analytes in the module. The blue module, comprising white blood cell-related factors, was significantly associated with increased suicide risk ($R=0.27$, $p<0.05$)

Table 1.

Complete list of the immunobiological measures

Immunobiological analyte	Abbreviation
Picolinic Acid	PIC
Quinolinic Acid	QUIN
Picolinic Acid/ Quinolinic Acid Ratio	PIC/QUIN ratio
Tryptophan	TRP
Kynurenine	KYN
Kynurenine/ Tryptophan Ratio	KYN/TRP ratio
C-reactive Protein	CRP
Serum Amyloid A	SAA
Intercellular Adhesion Molecule 1	ICAM-1
Vascular Cell Adhesion Molecule 1	VCAM-1
Placental Growth Factor	PIGF
Vascular Endothelial Growth Factor	VEGF
Basic Fibroblastic Growth Factor	bFGF
Soluble Fms-like Tyrosine Kinase 1	sFLT-1
Interferon- γ	IFN- γ
Interleukin-10	IL-10
Interleukin-12p70	IL-12p70
Interleukin-13	IL-13
Interleukin-1 β	IL-1 β
Interleukin-2	IL-2
Interleukin-4	IL-4
Interleukin-6	IL-6
Interleukin-8	IL-8
Tumor Necrosis Factor- α	TNF- α
Herpes Simplex Virus Type 1 IgG titer	HSV1 IgG t
Herpes Simplex Virus Type 1 Avidity	HSV1 Av.t
Herpes Simplex Virus Type 1 Positivity	HSV1 pos.
Herpes Simplex Virus Type 2 Positivity	HSV2 pos.
Cytomegalovirus IgG Titer	CMV IgG t
Cytomegalovirus Avidity	CMV Av.t
Cytomegalovirus Positivity	CMV pos.
<i>Toxoplasma Gondii</i> IgG Positivity	<i>T. Gondii</i> IgG pos.
White Blood Cell Count	WBC
Red Blood Cell Count	RBC
Hemoglobin	HGB
Hematocrit	HCT
Mean Cell Volume	MCV

Immunobiological analyte	Abbreviation
Mean Cell Hemoglobin Concentration	MCHC
Red Blood Cell Distribution Width	RDW
Platelet Count	PLT
Polymorphonuclear Leukocyte Count	PMN
Lymphocyte Count	LYMPH
Monocyte Count	MONO
Eosinophil Count	EOS
Basophil Count	BASO

Table 2.

Primary DSM-IV-TR diagnoses of the study participants

Psychiatric Diagnosis	Current PTE	Remission PTE	Somatic conditions								
			Allergy or asthma PDC	Cardiovasc. PDC	Digestive PDC	Endocrine PDC	MSN PDC	Pain PDC	RE PDC	Repr PDC	Sleep PDC
MDD	25 (37.9%)	8 (12.1%)	4 (12.1%)	6 (18.2%)	4 (12.1%)	2 (6.1%)	8 (24.2%)	3 (9.1%)	2 (6.1%)	2 (6.1%)	
Depressive disorder NOS	3 (4.5%)	2 (3.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Dysthymic disorder	10 (15.2%)	0 (0.0%)	2 (20.0%)	2 (20.0%)	2 (20.0%)	0 (0.0%)	4 (40.0%)	1 (10.0%)	0 (0.0%)	1 (10.0%)	
Anxiety	23 (34.9%)	0 (0.0%)	3 (13.0%)	5 (21.7%)	2 (8.7%)	5 (21.7%)	6 (26.1%)	3 (13.0%)	0 (0.0%)	3 (13.0%)	
Bipolar I	8 (12.1%)	2 (3.0%)	2 (20.0%)	2 (20.0%)	0 (0.0%)	2 (20.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)	
Bipolar II	6 (9.1%)	7 (10.6%)	2 (15.4%)	2 (15.4%)	0 (0.0%)	1 (7.7%)	3 (23.1%)	2 (15.4%)	0 (0.0%)	1 (7.7%)	
Bipolar NOS	2 (3.03%)	0 (0.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	

MDD, major depressive disorders; NOS, not otherwise specified. PTE, Percentage of total enrolled; PDC, Percentage of people in diagnostic category. The major comorbid somatic disorders, grouped per system are shown. MSN, Musculoskeletal and nervous system disorders; RE, respiratory disorders; Repr, reproductive disorders.

Table 3.

Types of medication used by study participants

Medication	N
Anti-inflammatory	25
Lithium	3
Antiepileptic	27
Antipsychotic	17
Psychostimulants	12
Benzodiazepines	30
SNRI	28
SSRI	25
TCA	5

N, number of patients; SNRI, serotonin–norepinephrine reuptake inhibitors; SSRI, selective serotonin re-uptake inhibitors; TCA, tricyclic antidepressant

Table 4.

Study participant demographics.

Diagnosis	Total Subjects	Age	HAMD 17 Total Score	HAMD 25 Total score	CES-D Total Score	PHQ-9 Total Score
Suicide risk	<i>(Count)</i>	<i>Median (IQR)</i>	<i>Median (IQR)</i>	<i>Median (IQR)</i>	<i>Median (IQR)</i>	<i>Median (IQR)</i>
No	51	45.0 (39.0–55.0)	12.0 (7.0–18.0)	15.0 (8.0–21.0)	25.0 (13.0–30.0)	6.5 (4.0–14.0)
Yes	15	43.0 (33.0–52.0)	18.0 (12.0–24.0)	25.0 (13.0–26.0)	38.0 (21.0–42.0)	18.0 (9.0–22.0)

IQR, interquartile range

Table 5.

Sensitivity analyses, checking the influence of potential confounders on the effect of the biological factors on suicide risk and depression.

Significant biological predictors for outcome depression (top rows) and suicide risk (bottom rows) analyzed by four separate regression models. Column (model) 1 shows RiR adjusted for anxiety levels, treatment and demographics for depression, and adjusted for both depression and anxiety scores, treatment and demographics for suicide risk. Column 2 shows RiR adjusted for treatment and demographics only. Column 3 shows the RiR adjusted only for treatments. Column 4 shows the unadjusted regression model, only measuring the impact of the biological factors on the outcomes of depression or suicide risk without any adjustment.

Blood Analyte	Dependent: Depression					
	1. Treatment, Demographics, and Anxiety Included	2. Treatment and Demographics Included		3. Treatment Included		4. Unadjusted regression
	Regression coefficient ($\pm$ SEM)	p	Regression coefficient ($\pm$ SEM)	p	Regression coefficient ($\pm$ SEM)	p
PLT	0.132 ($\pm$ 0.05)	0.01	0.05 ($\pm$ 0.02)	0.008	0.05 ($\pm$ 0.02)	0.008
SAA	0.035 ($\pm$ 0.01)	0.01	0.05 ($\pm$ 0.02)	0.01	0.05 ($\pm$ 0.02)	0.01
Blood Analyte	Dependent: Suicide Risk					
	1. Treatment, Demographics, Depression, and Anxiety Included	2. Treatment and Demographics Included		3. Treatment Included		4. Unadjusted regression
	Regression coefficient (SEM)	p	Regression coefficient (SEM)	p	Regression coefficient ($\pm$ SEM)	p
CRP	0.03 ($\pm$ 0.01)	0.007	0.03 ($\pm$ 0.01)	0.007	0.03 ($\pm$ 0.01)	0.006
EOS	0.03 ($\pm$ 0.01)	0.01	0.03 ($\pm$ 0.01)	0.01	0.03 ($\pm$ 0.01)	0.01
IL-8	-0.03 ($\pm$ 0.01)	0.04	-0.03 ($\pm$ 0.02)	0.04	-0.03 ($\pm$ 0.02)	0.04
LYMPH	0.03 ($\pm$ 0.01)	0.03	0.03 ($\pm$ 0.01)	0.03	0.03 ($\pm$ 0.01)	0.03
PMN	0.03 ($\pm$ 0.01)	0.04	0.03 ($\pm$ 0.01)	0.04	0.03 ($\pm$ 0.01)	0.04
WBC	0.03 ($\pm$ 0.01)	0.004	0.03 ($\pm$ 0.01)	0.004	0.03 ($\pm$ 0.01)	0.004
					0.38 ($\pm$ 0.15)	0.009