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Signaling networks in inflammatory pathways and risk for suicidal behavior

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ABSTRACT

Background: Suicide is a leading cause of death in the young adult population, with few biological markers identified thus far to be associated with suicidality. Cytokines (including IL-6 and TNF α) may contribute to increased risk for depression and suicidality. Few studies have examined the associations of cytokine mRNA expression with depression and suicidal ideation and behavior. This study examines these associations and whether cytokine signaling networks differentiate suicide attempters (SA), suicide ideators (SI), and healthy controls (HC).

Methods: Cytokine pathway marker (CPM; e.g. cytokines and proteins in cytokine signaling pathways) mRNA gene expression in whole blood was examined in suicide attempters (n = 38), suicide ideators (n = 38), and healthy controls (n = 36). Between-group differences in CPM gene expression were examined. We also examined association of the mRNA of these genes with the severity of depression and suicidal ideation. Novel Gaussian Graphical Model (GGM) techniques were utilized to examine between-network partial correlation differences in cytokine signaling networks relevant to IL-6 and TNF α signaling pathways.

Results: The severity of depression symptoms was positively associated with TNF α mRNA levels and negatively associated with IL-10 mRNA levels, but CPM expression was not associated with suicidal ideation severity. There were no between-group differences in CPM markers among healthy controls, SI and SA groups after correcting for multiple comparisons. In network analyses, we found suggestive results of between-group network differences between SI and control groups in gene pairs with IL-6R and STAT3 as common nodes.

Discussion: In a cohort of suicide attempters and ideators, TNF α and IL-10 mRNA levels appear to be associated with depressive symptomatology, consistent with elevation of pro-inflammatory cytokine production and reduction of anti-inflammatory cytokine production. Additionally, cytokine signaling networks may differentiate suicide ideators from healthy controls based on between-network differences, with differences possibly related to relationships of IL6R or STAT3 with other components of cytokine signaling networks.

1. Introduction

Suicide ranks as the second leading cause of death worldwide in the 15–29 year old age group, according to the latest estimates from the World Health Organization (WHO, 2019). However, significant difficulty exists in detecting individuals at high-risk for suicidal behavior in order to offer preemptive intervention (Franklin et al., 2017). Current prognostic indicators are largely psychosocial and do not reliably identify individuals at risk for suicidal behavior (May et al., 2012; Dawes et al., 1989). Accordingly, researchers have begun to examine whether

biological markers improve risk assessment and potentially offer novel understanding to the pathophysiology of suicidal behavior (Sudol and Mann, 2017).

Recent meta-analyses and post-mortem studies have identified elevated levels of pro-inflammatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF α) in depressed individuals, suicidal individuals and suicide attempters, both in plasma and in depression-relevant brain regions (Pandey et al., 2018; Black and Miller, 2015). Thus, peripheral levels of cytokines may provide insight into biological differences between suicidal and non-suicidal individuals and whether

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inflammation is specific to those with suicidal ideation and/or behavior or driven by underlying depression and mood disorders. Growing evidence suggests that dysregulation in interrelationships amongst cytokines, apart from variation in cytokine levels themselves, may be associated with neuroinflammation and subsequent neurological or psychiatric pathology (Becher et al., 2017). Cytokine networks examine the relationships between cytokines and their multiple signaling pathway components (e.g. protein transcription factors such as STAT, Signal Transducer and Activator of Transcription). These cytokine networks have been used to understand the pathology of illnesses such as sepsis and asthma (Matsumoto et al., 2018; de Llano et al., 2018).

Given that proinflammatory cytokine levels are elevated in individuals with suicidal ideation/attempt and depression (Ganança et al., 2016), cytokine signaling networks may be of value in further understanding the pathophysiology of suicidal behavior. Examination of intracellular inflammatory signaling pathways is methodologically difficult at the protein level given poor detection of intracellular signaling pathway proteins in plasma, but whole blood mRNA analysis provides a unique opportunity to examine the expression of cytokine genes, transcription factors, and post-transcriptional regulators involved in a cytokine signaling network. One prior study attempted to examine various inflammatory markers associated with suicidality in a network model, but no other studies to our knowledge have attempted to examine cytokine signaling networks or cytokine networks in high-risk suicidal individuals (Keaton et al., 2019). In that study (which analyzed protein associations using a weighted correlation network analysis in the statistical program R), suicide risk (defined as presence of recent/current suicidal ideation or behavior) was associated with a heterogeneous cluster of inflammatory proteins/cell types which included cytokines such as IL-6. Another study examined mitogen-stimulated cytokine levels for five cytokines, finding lower IL-6 and IL-2 in suicide attempters compared to those with suicidal ideation and non-suicidal depressed patients, although this study did not examine associations of cytokine levels with the severity of depression and suicidal ideation, or between-group differences in cytokine signaling pathways (Kim and Kim, 2006).

Thus, no prior studies to our knowledge have attempted to distinguish suicide attempters from those with suicidal ideation on inflammatory markers in cytokine signaling pathways, which is important given the higher risk of death by suicide associated with prior attempts (Ganança et al., 2016; Bostwick et al., 2016). We have previously found increased C-Reactive Protein (CRP) levels and TNF α mRNA in suicide attempters (SA), but not those with suicidal ideation (SI), as compared to healthy controls, providing valuable information to potentially use these markers to predict suicide risk in the future (Melhem et al., 2017). Gaussian Graphical Models (GGMs) are a recent advanced method of examining differences in multiple markers together since they are likely to function interactively to affect diseases. GGMs are models comprised of nodes (comprised of a set of related variables) connected by lines that reflect the strength of partial correlations controlling for other markers/variables.

In this paper, we examined whether cytokine pathway marker (CPM) mRNA expression was associated with depression symptoms and suicidal ideation and behavior through analysis of whole blood CPM mRNA expression levels. We then sought to determine whether there were differences in CPM mRNA expression networks between psychiatric inpatients admitted for suicide attempt (SA), those admitted for suicidal ideation but with no prior history of attempt (SI), and healthy controls (HC), through GGMs. This study is the first to examine, cross-sectionally, associations of both CPM mRNA expression and cytokine mRNA expression in suicidal populations using these advanced GGM network analyses techniques. We primarily focused on pathways involved in the IL-6 and TNF α signaling pathways, given prior evidence for dysregulation of these cytokines in suicidal ideation and behavior. We hypothesized that (1) mRNA expression of proinflammatory CPMs would be positively associated with the severity of depression and suicidal ideation, (2) proinflammatory CPMs mRNA expression would be elevated in SA and SI

groups compared to healthy controls and (3) proinflammatory CPMs mRNA expression interrelationships, as defined by network model derived edge weight, would be enhanced among psychiatric inpatients (SA and SI groups) compared to healthy controls.

2. Material and methods

2.1. Participant sample

The sample included 76 psychiatric inpatients, between 15 and 30 years of age, who were admitted to Western Psychiatric Hospital following a suicide attempt (SA group, $n = 38$) or admitted for suicidal ideation (SI group, $n = 38$). The SI group had no lifetime history of attempt. Details of our recruitment were previously reported (Melhem et al., 2017). We recruited healthy controls ($n = 36$) from the University of Pittsburgh Clinical and Translational Science Institute Research, which recruits participants who are in contact with the University of Pittsburgh Medical Center (UPMC) at points of routine clinical care. Healthy controls had no history of psychiatric disorders, suicidal ideation or behavior, self-harm, or childhood abuse, which may affect immune markers (Goldsmith et al., 2016; O'Dushlaine et al., 2015). For all cohorts, we excluded patients who were taking immunomodulatory medications (e.g. corticosteroids) or antibiotic medications, patients with chronic conditions potentially affecting the HPA axis or immune system (e.g. Cushing's disease), and pregnant women. Those with an acute infection (e.g. cold or flu) in the two weeks prior to the blood draw were rescheduled. All participants were recruited in accordance with the Institutional Review Board of the University of Pittsburgh.

2.2. Assessment

Participants' psychiatric and medical history were obtained during the clinical interview. The self-report Patient Health Questionnaire-9 (PHQ9) was administered to assess the severity of depressive symptoms. This nine item measure assesses depressive symptomatology over the past two weeks, with total scores ranging from 0 to 27 (Löwe et al., 2004). The adult Suicidal Ideation Questionnaire (SIQ) is a 25-item self-reported questionnaire assessing severity of suicidal ideation in the past month, with total scores ranging from 0 to 150 (Reynolds, 1987). Height and weight were collected to calculate body mass index (BMI).

2.3. Whole blood mRNA selection and analysis

Whole blood collection was collected using PAXgene Blood RNA Tubes, and RNA isolation and purification were done with the PAXgene Blood RNA Kit to determine CPM mRNA levels. Human HT-12 v4 Expression BeadChip Kit (Illumina) was utilized to run samples. Meta-analyses and recent studies report elevations of IL-6 and TNF α in depressed and suicidal individuals (Black and Miller, 2015). Given these associations, we specifically sought to identify CPMs involved in IL-6 and TNF α signaling pathways. Thus, we extracted those CPMs interacting with both the IL-6 and TNF α pathways that had either theoretical or clinical evidence for alterations in depression and suicidal ideation or behavior, prior to any data analysis (Black and Miller, 2015; Guo et al., 2012; Heinrich et al., 2003; Park and Bowers, 2010) (see Supplemental Table A). Within the IL-6 signaling pathway, gp130 (IL6ST), soluble IL-6 receptor (sIL6R), and IL-6 were identified as canonical interactive proteins (see Supplemental Figure A: IL-6 and TNF α Signaling Pathway for diagram/information on CPM markers). Plasma IL-6 typically binds to sIL6R (termed trans-signaling) and then binds to membrane bound gp130 which dimerizes to activate broad-based JAK-STAT pathways (Luo and Zheng, 2016). Within the TNF α pathway, we examined TNF α , TNF α R1, and TNF α R2. TNF α binds to TNF α R1 and TNF α R2 which activate both pro-inflammatory and anti-inflammatory pathways via NF- κ B1, STAT, and MAPK pathways (Sheng et al., 2018; Ortí-Casañ et al., 2019; Van Herreweghe et al., 2010). We also selected a number of key

secondary messengers in both these pathways, including NF- κ B1/2, STAT3, AP-1 subunits (including FOS, FOSB, and JUN), while also examining other regulators of cytokine activity including ADAM17, SOCS3, and C/EBP β . Lastly, we selected cytokines or receptors that have been associated with IL-6 and TNF α pathway activity, including IL-17, IL-8, IL-10, IL-1 β , IL-4, glucocorticoid receptor alpha (GR α), and glucocorticoid receptor beta (GR β).

Given potential signal effects of non-specific probe hybridization, a background adjustment to eliminate such effects was conducted. Similarly, data normalization utilizing the average normalization method was performed to adjust for confounding systematic processing biases (Schmid et al., 2010). Only CPM genes that passed our quality control (QC) analysis and demonstrated a detection p-value which was significant in at least 50% of the sample were included in our analysis. The following genes did not pass QC analysis for any isoform of the gene and thus were not included: *IL-6*, *TNFA1*, *IL-17* and *IL-4*. If a CPM gene had multiple probes, the Ensemble Genome Browser was used to identify uniqueness of transcript variants in encoding for unique isoforms. If two or more transcript variants encoded the same isoform, then, we averaged intensities if they were highly correlated, which was done for *IL6R*, *NFKB2*, *SOCS3*, and *STAT3 (isoform 3)* (r 's > 0.5).

2.4. Statistical analysis

All CPM mRNA expression levels (obtained through the whole blood analyses steps described above) were normalized using a natural logarithmic transformation. We examined relationships of PHQ9 and SIQ scores, dimensional measures of depression and suicidal ideation, respectively, with CPM mRNA expression through linear regression analysis. Whole blood assays were conducted in two batches, and thus we statistically adjusted for batch in all analysis that included CPMs. We also controlled for age and sex given these are known to be associated with CPMs (Michaud et al., 2013; O'Brien et al., 2007). For any CPMs found to have significant associations with PHQ9 or SIQ scores, sensitivity analysis including BMI as a covariate was conducted. However, BMI was not a significant covariate in any regression analyses. Similarly, to calculate potential effects of variation in immune cell counts on CPM mRNA expression, percentages of different immune cell types were individually entered into regression models (in addition to batch, age, and sex). We used the CIBERSORT deconvolution algorithm to determine the percentage of immune cells, which utilizes an immune gene signature matrix expression of 547 genes to determine percentages of 22 hematopoietic cell types in an individual participant's whole blood sample. This approach has high accuracy in discriminating cell subsets in blood samples and showed similar results in our analyses to those not accounting for percentage of immune cell types (see Supplemental Table G for further methodology and specific cell types) (Newman et al., 2015).

Similarly, we examined the relationships of CPM mRNA expression with group status (healthy control (HC), those with suicidal ideation (SI), and suicide attempters (SA)) using linear regression analysis. Group status (SA or SI) was coded as a dummy variable, with the healthy control group as the reference group. We used the Benjamini-Hochberg false discovery rate to correct for multiple comparisons given 19 CPMs that were analyzed per outcome (Benjamini and Hochberg, 1995). The Benjamini-Hochberg correction was used both to optimize the power to detect rejection of the null hypothesis and also efficiently control for the false discovery rate (FDR), consistent with prior analyses that have examined inflammatory pathways (Nograla et al., 2008; Thissen et al., 2002). Given our sample size, we have power to detect medium effect sizes in the order of $d = 0.655$ or larger. Prior studies (Black and Miller, 2015) have suggested that effect size differences in cytokines between suicidal individuals and healthy controls are low to moderate (e.g. 0.09 for TNF alpha and 0.3 for IL-6); however, there are no studies looking at effect sizes for mRNA in this population. Unless otherwise described, participant characteristics and data analysis reflect participants with peripheral blood measures.

We utilized a high-dimensional Gaussian Graphical Model (GGM) to construct our gene-gene network with the Statistical Inference of Large-Scale Gaussian Graphical Model in Gene Networks (SILGGM) package, which identifies reliable gene co-expression networks through the statistical program R, allowing better understanding of interactive biological processes (Zhang et al., 2018; Bhushan et al., 2019). The SILGGM package estimates partial correlations for conditionally dependent gene pairs, conducts statistical inference, and provides significant gene pairs. Although other gene network R packages such as Weighted Gene Co-Expression Network Analysis (WGCNA) are commonly used in gene network analysis, SILGGM is more suitable to study the direct relationships between specific gene-pairs, given that GGM analyses control for all other genes in the network (Wang and Huang, 2014; Li et al., 2016). In particular, SILGGM uniquely allows statistical inference between gene pairs and conduct between-network comparisons based on such inferences. Because gene pairs are conditionally dependent, we wanted to take this into account to identify key gene pairs that could be driving differences between groups. We selected the CPMs as nodes and estimated the network with bivariate nodewise scaled Lasso algorithm ("B_NW_SL") in SILGGM given our small set of sample genes. Three networks were estimated for SA, SI, and control groups, respectively; partial correlations and z scores representing difference of partial correlations were obtained. We examined between-group pairwise comparisons between SA, SI and control groups. We report both unadjusted and adjusted p values. Network visualization was done by R's *ggnet2* package, using significant partial correlation differences unadjusted for multiple comparisons.

To take into account the potential effects of age, gender, percentage of different immune cell types (individually entered as previously described in the methods) and batch, these were entered into regression models as independent variables with CPMs as dependent variables and resulting residuals for each CPM were used for nodal values. All gene pairs found significant in the primary analysis (see Table 3) were significant in these sensitivity analyses (without adjustment for multiple comparisons). In all analyses, both unadjusted p values and FDR adjusted p values were reported unless otherwise indicated. An alpha level of significance of 0.05 was adopted for all analyses. The statistical program R version 3.5.2 was used in all analyses (R Core Team, 2018).

3. Results

3.1. Demographic and clinical characteristics

Participants were 42.9% female, average age of 22.87 ± 3.4 years, and with average BMI of 24.1 ± 4.2 kg/m² (see Supplemental Table B for demographic/clinical characteristics table). There were no differences between SA (n = 38), SI (n = 38), and controls (n = 36) on sex, age, or BMI (p 's > 0.05). The average time between attempt and blood draw for SA was 5.3 days (SD = 2.7). There were no significant differences in cytokine pathway marker mRNA levels by sex, age, or BMI, except for *STAT3 (isoform 1)*, which was greater in males ($p = 0.024$) and *TNFA*, which was positively associated with age ($p = 0.012$), after adjusting for batch. SA and SI were similar and significantly different from controls on PHQ9 (18.1 ± 7 , 18.6 ± 6.7 , and 1.1 ± 1.6 in SA, SI, and controls, respectively, $F = 109.9$, $df = 109$, $p < 0.001$) and SIQ scores (82.9 ± 40 , 81.4 ± 38.1 , and 0.6 ± 0.9 in SA, SI, and controls respectively, $F = 78.4$, $df = 107$, $p < 0.001$) (See Supplemental Table B). We have previously reported that the SA and SI groups did not differ on primary psychiatric diagnoses or use of medications prior to admission (Melhem et al., 2017).

3.2. Relationships of cytokine pathway marker mRNA with PHQ-9, SIQ, and group

We conducted regression analysis controlling for age, sex, and batch and found PHQ9 scores to be significantly and positively associated with *TNFA* mRNA levels ($\beta = 0.004$, 95% CIs: 0.001 to 0.007, unadjusted $p =$

0.005, adjusted $p = 0.048$; r ($TNF\alpha$, PHQ9) = 0.3, unadjusted $p < 0.001$ and negatively associated with $IL-10$ mRNA levels ($\beta = -0.002$, 95% CIs: -0.004 to -0.001 , unadjusted $p = 0.004$, adjusted $p = 0.048$; r ($IL-10$, PHQ9) = -0.27 , unadjusted $p = 0.004$) (Table 1). SIQ scores were positively associated with $TNF\alpha$ mRNA levels ($\beta < 0.001$, unadjusted $p = 0.019$) and negatively associated with $IL-10$ ($\beta < 0.001$, unadjusted $p = 0.028$), $IL-6R$ ($\beta < 0.001$, unadjusted $p = 0.045$), and $STAT3$ (isoform 3) ($\beta < 0.001$, unadjusted $p = 0.024$) mRNA levels, although these results were not significant after adjustment for multiple comparisons (Supplemental Table C: CPM and Suicidal Ideation). When comparing between-group differences SA, SI, and control groups, differences existed in $GR\alpha$ mRNA levels ($F = 3.4$, unadjusted $p = 0.037$), $IL-10$ mRNA levels ($F = 3.27$, unadjusted $p = 0.042$), and $TNF\alpha$ mRNA levels ($F = 4.22$, unadjusted $p = 0.017$), although these results were not significant after adjustment for multiple comparisons (Table 2).

3.3. Exploratory network analysis results

To examine cytokines network differences between groups, we examined our cytokine pathway proteins as part of a Gaussian Graphical Model utilized in gene network analysis, using a bivariate nodewise scaled Lasso method, finding significant differences in partial correlations of gene pairs in between-network comparisons (see Fig. 1). Prior to adjustment for multiple comparisons, we found several between-group network differences in gene pairs (see Supplemental Tables D, E, and F). After correction for multiple comparisons and with significance at a trend level, $STAT3-GR\alpha$ (r [SI] = -0.14 , r [HC] = 0.52 , unadjusted $p = 0.001$, adjusted $p = 0.077$) and $FOS-STAT$ (isoform 3) (r [SI] = -0.21 , r [HC] = 0.44 , unadjusted $p = 0.001$, adjusted $p = 0.077$) were less correlated in SI as compared to healthy controls. On the other hand, $IL6R-SOCS$ (r [SI] = 0.49 , r [HC] = -0.14 , unadjusted $p = 0.002$, adjusted $p = 0.089$) and $IL6R-STAT$ (isoform 3) (r [SI] = 0.61 , r [HC] = -0.03 , unadjusted $p = 0.001$, adjusted $p = 0.077$) were more correlated in SI as compared to healthy controls, at a trend level (see Table 3).

Of note, $FOS-STAT$ (isoform 3) and $NF\kappa B2-TNF\alpha R2$ gene pairs had significantly less correlation, while $IL6R-SOCS$ gene pair had

significantly greater correlation, in both SA and SI groups as compared to healthy controls (prior to multiple correction adjustment).

Supplemental Tables D, E, and F detail between-network comparisons contrasting SI vs HC, SA vs HC, and SI vs SA groups respectively.

4. Discussion

We sought to identify cytokine pathway marker (CPM) mRNA associations with the severity of depression and suicidal ideation, and differences in CPM networks between psychiatric inpatients at the time of an acute suicidal crisis due to suicide attempt or ideation. Replicating prior findings and consistent with our hypothesis, we found that $TNF\alpha$ mRNA expression was positively associated with depressive symptoms (as measured by the PHQ-9) and $IL-10$ was negatively associated with depressive symptoms, after adjustment for relevant confounding variables. We found associations of the severity of suicidal ideation and certain CPM markers (e.g. $IL-10$, $TNF\alpha$, $IL-6R$, $STAT3$ (isoform 3)), along with differences in CPM markers between SA, SI, and control groups, although these results were not significant after multiple corrections.

We discuss these results in the context of the strengths and limitations of our study. To our knowledge, no prior studies have specifically examined cytokine signaling networks in populations of patients with psychiatric illness or suicidal ideation or behavior. However, this study is limited by its cross-sectional design, relatively small sample size and stringent corrections for multiple comparisons, and inability to include all desired cytokine markers in our analysis (e.g. $IL-6$) due to our stringent quality control protocol. It is possible that inflammatory changes are secondary to the medical effects of a suicide attempt in the SA group; however, common methods of suicide attempt (e.g. overdose, hanging, jumping) are often associated with increase across multiple cytokines, which was not suggested by our findings (Janelidze et al., 2011; James et al., 2005; Pandey et al., 2012). Additionally, mRNA analysis was restricted to whole blood analysis, which may not be an ideal representation of protein-level inflammatory changes occurring in the blood, brain, or cerebrospinal fluid that might be implicated in suicidal ideation and behavior. We were unable to comprehensively control for confounds

Table 1
Associations of cytokine pathway marker expression and depression severity.

Cytokine Pathway Marker	Unstandardized β	SE	t value	Unadjusted p value	Adjusted p value
<i>ADAM17</i>	-0.001	0.001	-1.279	0.204	0.316
<i>C/EBPβ</i>	0	0.002	-0.008	0.994	0.994
<i>FOS</i>	0.001	0.003	0.304	0.762	0.852
<i>FOSB</i>	-0.001	0.001	-1.689	0.094	0.29
<i>GRα</i>	-0.001	0.001	-1.587	0.116	0.29
<i>GRβ</i>	0.001	0.001	1.626	0.107	0.29
<i>IL-10</i>	-0.002	0.001	-2.908	0.004	0.048*
<i>IL1β</i>	0.005	0.003	1.716	0.089	0.29
<i>IL-6R</i>	-0.001	0.001	-1.558	0.122	0.29
<i>IL-6ST</i> (gp130)	0	0	-0.22	0.826	0.872
<i>IL-8</i>	0.002	0.001	1.083	0.281	0.356
<i>JUN</i>	-0.001	0.001	-1.164	0.247	0.335
<i>NF-κB1</i>	-0.002	0.002	-0.942	0.348	0.413
<i>NF-κB2</i>	-0.001	0.001	-1.429	0.156	0.316
<i>SOCS3</i>	-0.001	0.001	-1.288	0.201	0.316
<i>STAT3</i> (isoform 1)	-0.002	0.002	-1.244	0.216	0.316
<i>STAT3</i> (isoform 3)	-0.005	0.002	-2.609	0.01	0.063
<i>TNFα</i>	0.004	0.002	2.863	0.005	0.048*
<i>TNFαR2</i>	-0.003	0.002	-1.386	0.168	0.316

Note: Results of multivariate linear regression model modeling cytokine pathway marker expression (log-transformed) as dependent variable and depression severity (PHQ9 scores) as independent variable. Age, gender, and batch were included as covariates. Highlighted variables in gray have significant associations (* indicates $p < 0.05$). Df for all variables is 107.

Table 2

Descriptive differences of cytokine pathway marker expression between suicide attempters, suicide ideators, and healthy controls.

Cytokine Pathway Marker	HC	SI	SA	F statistic	P value	Adjusted P value
<i>ADAM17</i>	2.801	2.773	2.772	1.03	0.36	0.622
<i>C/EBPβ</i>	8.248	8.261	8.23	0.15	0.862	0.862
<i>FOS</i>	5.936	5.973	5.898	0.45	0.637	0.805
<i>FOSB</i>	2.138	2.109	2.117	2.71	0.071	0.337
<i>GRα</i>	4.693	4.711	4.659	3.4	0.037	0.266
<i>GRβ</i>	2.152	2.197	2.177	1.99	0.141	0.507
<i>IL-10</i>	4.011	3.968	3.967	3.27	0.042	0.266
<i>IL-1β</i>	5.981	6.043	6.062	0.71	0.493	0.781
<i>IL-6R</i>	2.272	2.261	2.243	1.86	0.16	0.507
<i>IL-6ST (gp130)</i>	2.045	2.057	2.043	1.27	0.286	0.557
<i>IL-8</i>	2.17	2.198	2.199	0.39	0.678	0.805
<i>JUN</i>	4.858	4.849	4.845	0.3	0.742	0.813
<i>NF-κB1</i>	7.755	7.731	7.748	0.26	0.77	0.813
<i>NF-κB2</i>	2.272	2.247	2.252	0.57	0.569	0.805
<i>SOCS3</i>	4.739	4.734	4.713	1.3	0.276	0.557
<i>STAT3 (isoform 1)</i>	5.352	5.352	5.327	0.45	0.64	0.805
<i>STAT3 (isoform 3)</i>	6.329	6.275	6.262	1.44	0.241	0.557
<i>TNFα</i>	5.209	5.315	5.3	4.22	0.017	0.266
<i>TNFαR2</i>	8.068	8.006	8.013	1.24	0.293	0.557

Note: Descriptive raw CPM level means and results of ANCOVA analysis controlling for batch between different groups, comparing different levels of cytokine pathway marker expression (log-transformed). ANCOVA F statistic and p values are presented, and CPMs with significant p values (prior to multiple correction adjustment) are highlighted in gray. No differences existed between groups after multiple comparison correction, and sensitivity analysis adjusting for age, gender, or race did not alter these results. HC = healthy controls; SI = suicide ideators; SA = suicide attempters.

Table 3

Gene 1	Gene 2	Correlation Difference	Correlation (SI)	Correlation (HC)	Z score	Unadjusted P value	Adjusted P value
<i>IL6R</i>	<i>STAT3 (isoform 3)</i>	0.64	0.61	-0.03	3.27	0.001	0.077
<i>GRA</i>	<i>STAT3 (isoform 1)</i>	-0.65	-0.14	0.52	-3.24	0.001	0.077
<i>FOS</i>	<i>STAT3 (isoform 3)</i>	-0.66	-0.21	0.44	-3.2	0.001	0.077
<i>IL6R</i>	<i>SOCS</i>	0.63	0.49	-0.14	3.08	0.002	0.089

Note. Significant gene pair partial correlation differences as estimated by GGM. Difference in partial correlations between SI and HC are provided, with unadjusted p values and adjusted p values (using BH FDR correction). See [Supplemental Table D](#) for full results.

such as medication use, sleep, or diet. However, we did conduct sensitivity analyses examining BMI and BMI was not a significant covariate. We have also previously reported our inpatient groups to be similar on medication use (Melhem et al., 2017). Lastly, we did not have a psychiatric control group without recent SI/SA, which limit generalizability of these findings to patients with recent SI/SA.

The positive relationship of *TNFα* and negative relationship of *IL-10* with depressive symptoms is consistent with prior studies reporting pro-inflammatory cytokine activation in depression (Köhler et al., 2017). However, prior meta-analyses reported elevated peripheral blood *IL-10*, an anti-inflammatory cytokine, to be associated with depressive disorders and suicidal ideation or behavior (Black and Miller, 2015; Köhler et al., 2018). Yet, our study sample is unique in including patients who are acutely ill and recruited at the time of a suicidal crisis while prior studies examining cytokine elevation in depression or suicidal ideation or behavior may have included more patients with chronic symptoms. Several studies also indicate that these relationships are bidirectional (Ganança et al., 2016; Liu et al., 2016). Future studies are needed to examine differences between acute suicide attempters and individuals with past history of suicide attempts, given that acute suicide attempters may have state-related cytokine alterations beyond the effects of acute stress related to psychosocial stressors or the inpatient admission process. Future longitudinal studies are also needed to examine whether these inflammatory markers predict future suicide attempts.

We found associations of the severity of suicidal ideation and certain CPM markers (e.g. *IL-10*, *TNFα*, *IL-6R*, *STAT3 (isoform 3)*) and differences in CPM markers between SA, SI, and control groups, although these results were not significant after multiple corrections. Our correction for multiple comparisons is stringent given our relatively small sample size. This study is unique in including patients across the spectrum of psychiatric disorders. Prior studies have examined suicidality in the context of major depression or examined suicide attempters only, which may limit generalizability to the general construct of suicidality (Black and Miller, 2015; Dowlati et al., 2010). Finally, we have previously reported that the severity and variability in self-reported depression symptoms is a strong predictor of suicide attempts in high-risk individuals (Melhem et al., 2019). Future studies are needed to examine whether *TNFα* or *IL-10* mRNA, which we find to be associated with depression symptoms, predicts suicide attempts prospectively.

We utilized a novel Gaussian Graphical Model designed for gene networks to assess cytokines networks differences between groups. Gene expression levels in these networks were significantly different between controls, SI, and SA groups but did not reach statistical significance after correcting for multiple comparisons (see [Table 2](#)), though these findings should be taken in context of previously described limitations of low study power. We found gene pairs with *IL6R*, specifically *IL6R-STAT3 (isoform 3)* and *IL6R-SOCS*, were more correlated in the SI group compared to healthy control group. Although a few prior studies have

Figure 1a.
SI vs SA

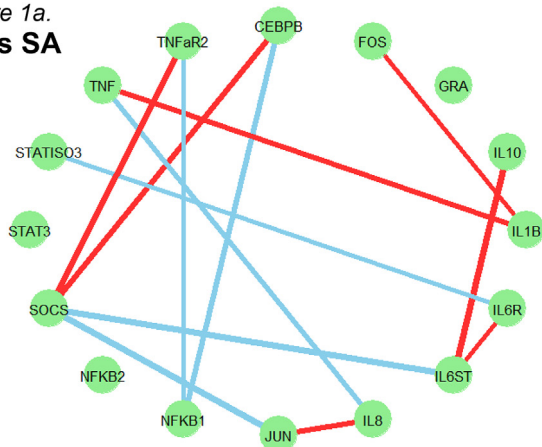


Figure 1b.
SI vs HC

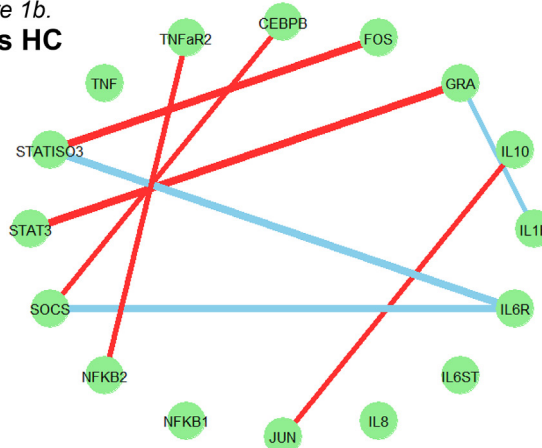


Figure 1c.
SA vs HC

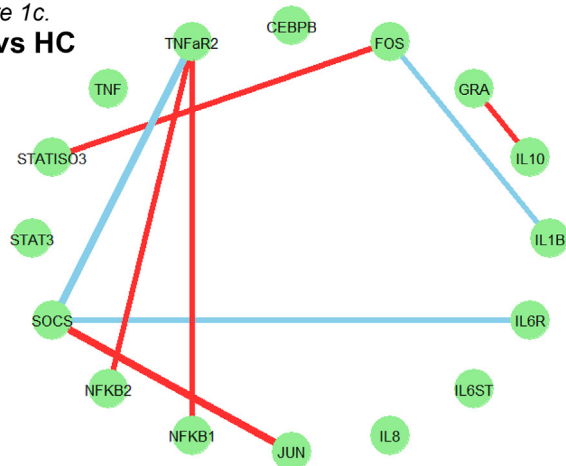


Fig. 1. Cytokine Pathway Markers Networks for suicide attempters, suicide ideators, and healthy controls

Note. Between-network partial correlation comparisons diagrams for CPMs networks are provided below. Edges with significant differences (prior to multiple corrections) in between-network comparisons are provided. Blue lines indicate greater partial correlations and red lines indicate lower partial correlations in the first comparator group (e.g. SI in Fig. 1a) as compared to the second comparator group (e.g. SA in Fig. 1a). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

noted the role of IL-6 (Rengasamy et al., 2018) and IL6R in patients with high suicide risk (e.g. treatment-resistant depression) (Yamasaki et al., 2020), our findings of differential *IL6R* relationship with *SOCS* and *STAT3* (isoform 3) suggest potential specific mechanisms by which *IL6R* may be associated with suicidal ideation or behavior. Genes pairs with *STAT3* isoforms were differentially correlated between SI and healthy controls groups, both positively and negatively, suggesting this CPM may also be implicated in suicidal ideation and behavior, consistent with one prior study noting the role of *STAT3* activation in neuronal apoptosis in suicidal patients (Hoyo-Becerra et al., 2013). As previously described, these relationships between CPMs and suicidal ideation and behavior are likely bidirectional.

In conclusion, our findings show pro-inflammatory cytokine *TNF α* mRNA levels to be positively associated with depression severity, and anti-inflammatory cytokine *IL-10* mRNA levels to be negatively associated with depression severity. In a novel network analysis of cytokine pathway proteins, our results suggest that *IL6R* and *STAT3* (isoform 3) may be potentially important in suicidal ideation. Future studies with larger sample sizes and longitudinal studies are needed to examine these relationships prospectively and whether they predict suicide attempt, ideation, or the transition from ideation to attempt and to better understand the biological mechanisms implicated in suicidal behavior.

Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbih.2020.100122>.

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