ORIGINAL RESEARCH

Key Words: cytokines, major depressive disorder, antidepressant treatment, predicting treatment outcome

Macrophage Migration Inhibitory Factor and Interleukin 1-β mRNA Levels as Predictors of Antidepressant Treatment Response in Major Depression

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ABSTRACT ~ ***Background:*** *Immunologic measures have been studied as predictors of who will respond to standard antidepressants. Two previous, small studies of pre-treatment leukocyte mRNA expression levels of the cytokines macrophage migration inhibitory factor (MIF) and interleukin 1-beta (IL1-β) identified antidepressant treatment responders.* ***Methods:*** *We tested these findings in 1,299 patients from the PRIME Care study, a multi-center pharmacogenetic depression treatment trial. Patients underwent 5 depression-symptom assessments over 24 weeks. mRNA was extracted from peripheral blood, purified, and assayed with TaqMan gene expression assays and a known copy number calibrator to yield relative quantification and copy numbers for each sample. In generalized estimating equations models, we regressed the repeated depression measures and a binary treatment response measure on the baseline MIF and IL-1β measures and relevant covariates.* ***Results:*** *Participants' depression scores decreased monotonically during treatment, with the treatment response percentage increasing concomitantly. We found no significant associations of the cytokine concentrations with either the change in depression scores or the likelihood of a treatment response. A secondary analysis limited to a subsample of 126 participants selected to remove the potential for confounding also showed no significant associations.*

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Limitations: *Despite efforts to control for sample and assay method differences, these could have contributed to the lack of replication of prior research.* ***Conclusions:*** *We did not replicate prior findings that pre-treatment expression levels for two cytokines predicted antidepressant treatment response. This raises questions about the clinical utility of using these biomarkers in treating depression.* Psychopharmacology Bulletin. *2025;55(1):8–25.*

Introduction

Major depressive disorder (MDD) is common in the U.S. adult population, with a past-year prevalence estimated at 10.4%.[1] It is also one of the most common conditions associated with military service. A meta-analysis of 25 epidemiological studies estimated the prevalence of current DSM-IV MDD to be 12.0% among currently deployed U.S. military personnel, 13.1% among previously deployed personnel, and 5.7% among individuals who were never deployed.[2] MDD results in costs to individuals, families, and society, including decreased work productivity. It also increases risk for and adversely affects outcomes in many other conditions, including diabetes and heart disease, where it contributes to higher healthcare costs and mortality rates.[3,4]

Greater risk of MDD among deployed military personnel is attributable in part to their exposure to traumatic experiences, including witnessing combat and separation from family during deployment or military training.[5] In a cross-sectional survey of active duty soldiers the 30-day prevalence of MDD was 4.8%, compared to <1% among a civilian comparison group.[6] Among Veterans seeking care in the Veterans Health Administration (VHA), an estimated 21% of post-9/11 Veterans suffer from MDD, a prevalence that compares with that of post-traumatic stress disorder (PTSD).[7]

Depression treatment guidelines developed by the U.S. Department of Veterans Affairs and the Department of Defense[8] recommend either antidepressants or psychotherapy as first-line treatments for mild-to-moderate depression. However, in clinical practice, both forms of treatment yield relatively low remission rates. In a large outpatient study of depression treatment, for example, only about one-third of patients achieved remission with the first treatment and with each successive treatment trial there was a decrement in the odds of remission.[9] Thus, predicting who will respond to standard treatment could inform a personalized approach to antidepressant therapy and substantially enhance outcomes for patients with MDD.

Biological heterogeneity of depressive states could account for lower-than-expected remission rates in depression. Plasma concentrations of pro-inflammatory cytokines vary among people,[10] with remission of

depression following conventional antidepressant treatment associated with lower plasma concentrations of pro-inflammatory proteins[11,12] and mRNA.[13,14]

In a study of 74 participants from the GENDEP study—a randomized comparison of the selective serotonin reuptake inhibitor escitalopram and the tricyclic antidepressant nortriptyline for treating MDD—the pretreatment leukocyte mRNA expression levels of three of nine cytokines assayed were elevated among individuals who did not respond to antidepressant treatment.[13] The three cytokines whose higher levels were associated with treatment non-response were interleukin-1beta (IL-1β), macrophage migration inhibitory factor (MIF), and tumor necrosis factor-alpha (TNF-α). In a subsequent analysis of GENDEP data, absolute pretreatment values of MIF and IL1-β had a positive predictive value of 100% in identifying antidepressant non-responders.[15] This finding was validated in an independent sample of 68 depressed patients treated with a variety of antidepressants.[15] Further, among patients from the GENDEP study, those with higher pretreatment levels of C-reactive protein (CRP) – an inflammatory cytokine levels of which are widely used as a marker of inflammation – were less responsive to escitalopram, whereas the converse was true for those treated with nortriptyline.[16]

We examined whether pretreatment plasma concentrations of CRP and MIF and IL-1-β mRNA were associated with antidepressant treatment response in a subsample of patients from the PRIME Care study, a multi-center pharmacogenetic trial for treating depression.[17] We hypothesized that higher pretreatment mRNA levels of the two inflammatory cytokines, MIF and IL-1-β, would be associated with a poorer antidepressant treatment response, consistent with the previous studies of these cytokines.[15] Given prior contradictory findings of an association of CRP levels with a positive response to nortriptyline treatment but a negative response to escitalopram treatment,[16] we also hypothesized that CRP levels in the PRIME Care sample, which was treated with a variety of antidepressants would not be associated with treatment outcome.

Methods

Patients

Patients were recruited from among participants in the PRIME Care study, a single-blind trial conducted at 22 Department of Veterans Affairs (VA) medical centers.[17] The treatment trial protocol and informed consent form, including those for the sub-study reported

here, were approved by the VA Central Institutional Review Board. All patients and their healthcare providers gave written informed consent to participate. Patients were randomly assigned 1:1 to receive pharmacogenetic test results within 2–3 business days after randomization (pharmacogenomic-guided group) or 24 weeks later (usual care group). Patients assigned to the usual care group were to start treatment on the day of randomization as directed by their provider, while providers in the pharmacogenomic-guided group were asked to wait until the pharmacogenetic test results were available for discussion with their patients before prescribing an antidepressant. Pharmacogenetic testing was conducted by Myriad Genetics using their Genesight panel and a combinatorial interpretation of genetic variation that predicts medication levels and treatment outcome better than single-variant assays.[18]

Participants were enrolled from July 2017 through February 2021, with follow-up ending November 2021. Eligible patients were 18–80 years old and had a clinical diagnosis of MDD and a score >9 on the Patient Health Questionnaire (PHQ-9).[19] The treating provider identified patients and confirmed their diagnosis of current MDD and prior receipt of one or more antidepressants. Patients were excluded if they were diagnosed with a current substance use disorder, bipolar illness, psychosis, or borderline or antisocial personality disorder; were in treatment with an antipsychotic medication, methadone, buprenorphine, or naltrexone; were determined to be actively using substances; or were receiving 2 or more antidepressants at the time of randomization. A study coordinator at each site obtained written, informed consent from patients prior to having them complete the PHQ-9.

Assessments and Endpoints

Outcomes were assessed at 4, 8, 12, 16, and 24 weeks after randomization by interviewers who were blind to clinical care and study group employed by a centralized call center to reduce variability in data collection and potential unblinding of group assignment. The primary outcome measures were repeated measures of depression outcomes based on the PHQ-9[19] and a binary measure of response to treatment defined as a 50% decrease from the baseline PHQ-9 score.

Sample Collection and Handling and Laboratory Methods

For the measurement of CRP concentrations, patients' peripheral blood samples were collected and centrifuged. Plasma was extracted,

frozen, and shipped on dry ice monthly to the coordinating site in Philadelphia, where samples were kept at −80° C until they were shipped in batches to LabCorp for assay. Peripheral blood for measuring MIF and IL-1β mRNA concentrations was collected in PAXgene blood RNA tubes (PreAnalytiX). After mixing and equilibration at room temperature for 2 h, samples were frozen and shipped on dry ice to Philadelphia, where they were stored at −80° C prior to overnight batch shipment on dry ice to the Pharmacogenomics Analysis Laboratory in Little Rock, AR. After receipt, samples were inspected and stored at −80° C until RNA was extracted and purified.

RNA Purification

Blood samples were equilibrated at room temperature overnight. Samples were extracted using the PAXgene blood RNA kit according to the manufacturer's instructions with the following modifications: 1) the sample was added to the first column along with 4 μL of VETMAX Xeno Internal Positive Control RNA (Thermo Fisher) at 10,000 copies per μL, 2) 80 μL were eluted from the second column and 50 μL of this eluate was then passed through the same column to obtain a higher yield of total RNA.

RNA samples were quantified by UV spectrometry (NanoDrop). For quality control, 10% of the samples, chosen at random, and any sample that appeared atypical were analyzed by electrophoresis using an Agilent BioAnalyzer. After quantification, 200 ng of total RNA per sample was converted to cDNA using the High-Capacity RNA-to-cDNA Kit (Applied Biosystems). Controls included samples converted with and without reverse transcriptase enzyme.

Gene Expression Assays

Modifications were made to a published method[15] using TaqMan assays for IL-1β, MIF, and the housekeeping gene β-actin. Three validated TaqMan probe mixes (Applied Biosystems) were used: IL-1β (Hs_01555410_m1), MIF (Hs_00236988_g1), and β-actin (Hs_99999903_m1). A VetMAX Xeno TaqMan assay (ThermoFisher A29767) was also used to detect the Xeno RNA controls.

A synthetic DNA sequence was designed to serve as a known copy number calibrator for the gene expression assays. The calibrator contained the exact TaqMan probe target sequences for IL-1β, MIF, and β-actin (listed above) with spacer sequences between the targets. The calibrator DNA sequence was cloned into the pMK-RQ plasmid (Invitrogen). Quality control sequencing and quantification of the plasmid were performed by GeneArt (Invitrogen). The pMK-RQ plasmid

was linearized by digestion with PstI. After linearization, 10,000 copies of calibrator were mixed with 10,000 copies of VetMAX Xeno DNA to formulate a calibrator/control for each TaqMan assay.

Each 384-well plate contained samples converted to cDNA in groups of 48 along with corresponding controls for each sample and a Xeno RNA to cDNA conversion control. TaqMan assays were performed in triplicate on the QuantStudio 12K Flex as a comparative Ct reaction. The manufacturer's preset PCR program was used: 50° C for 2 min, 95° C for 10 min, and 40 cycles of 95 C° for 15 sec, and 60° C for 1 min. Three assay plates were run for each sample group of 48: 1) IL-1β, 2) MIF, and 3) a control plate of β-actin and Xeno assays. The calibrator, samples, and controls were loaded at a volume of 2 μl in each well in a 10 μl reaction. Of a total of 1,530 patient samples, 11 samples yielded an inadequate volume of total RNA for conversion to cDNA.

Calculation of Gene Expression

Using the calibrator and a housekeeping gene allows for the calculation of the relative quantification (RQ) and the copy number of each gene of interest for each sample. For the relative quantification, we made the following calculations: ΔCt (gene of interest) = Ct (gene of interest) − Ct (β-actin); ΔCt (Calibrator) = Ct (Calibrator) − Ct (β-actin); ΔΔCt = ΔCt (gene of interest) − ΔCt (Calibrator); $RQ = 2^{-(\Delta\Delta Ct)}$. Calculation of the copy number was done as follows: Ratio (reference/target) = $2^{Ct(ref)-Ct(target)}$, where the reference is the calibrator, and the target is the gene of interest. The value of the ratio (reference/target) was multiplied by 10,000 to calculate the copy number. Replicates with a standard deviation greater than 0.5 were omitted from the calculation. Formulas for the calculations can be found at https://genomique.iric.ca/resources/files/Understanding_qPCR_results.pdf and in Mahboudi et al.[20]

High-sensitivity C-Reactive Protein (CRP) concentrations were measured using a particle-enhanced immunoturbidimetric assay (Roche Diagnostics) that measures the agglutination of latex particles coated with monoclonal anti-CRP antibodies with CRP.

Statistical Analysis

Generalized estimating equations (GEE) models were used to regress each of the repeated PHQ-9-based depression measures on the baseline MIF, IL-1β and CRP measures, with study week included as a five-level categorical variable. We used linear GEE models to evaluate the decrease in PHQ-9 score and logistic regression GEE models to evaluate the binary measure (i.e., >50% decrease in PHQ-9 score

from baseline); in each case, a compound symmetry working correlation was specified. The primary models used the full sample of 1,299 participants on whom the mRNA and CRP measures were collected. To account for site effects, a categorical factor for site was included in the models, and a binary factor for randomization group (i.e., early vs late receipt of PGx results) was also included. The models also included the baseline PHQ-9 score, BMI, current smoking status (smoker/non-smoker), and gender as covariates. To examine the possible moderating effects of autoimmune or other inflammatory disease, the use of anti-inflammatory drugs, race (European ancestry vs. Other), and PTSD diagnosis at baseline, the models were then extended to examine interactions between these four variables and each of the mRNA and CRP variables. In supplementary analyses, we also examined the possible moderating effects of the presence of treatment-refractory depression, and the presence of active prescriptions for tricyclic, Non-SRI, SSRI and SNRI antidepressant medications. Finally, to provide a direct comparison to previous literature, the initial analyses were repeated on the subsample of N = 126 participants that excluded individuals diagnosed with an inflammatory disease (e.g., cancer, autoimmune disease), those who were being treated with an anti-inflammatory medication (e.g., corticosteroids, non-steroidal anti-inflammatory drugs), PTSD, and those of non-European ancestry to remove the potential for confounding by any of these factors.

Results

The average age of the 1,299 individuals who gave informed consent and provided a blood sample for RNA extraction was about 47 years with three-quarters being male, two-thirds being White, and more than one-quarter current smokers (Table 1). The sample's average PHQ-9 score was 17.5, thus most participants were experiencing moderate-to-severe levels of depressive symptoms. Almost one-third of the sample had treatment refractory depression, defined as having had more than one prior treatment with an antidepressant or having received electroconvulsive therapy, vagus nerve stimulation, or transcranial magnetic stimulation. Rates of prescriptions for tricyclic medications were between 3% and 4% at baseline and after one month; rates for non-SRI, SSRI and NSRI prescriptions increased over the month after randomization, from 14%, 25% and 13% to 23%, 42% and 32%, respectively (Supplementary Table 1). Participants' PHQ-9 scores across the 24 weeks of treatment decreased monotonically, with concomitant increases in the percentage showing a treatment response (Table 2).

TABLE 1

Baseline Characteristics in the Full Sample and the Subsample with Exclusions (*)

	FULL SAMPLE (N = 1,299)		SUBSAMPLE (N = 126)	
VARIABLES	MEAN	SD	MEAN	SD
Age (yr)	47.59	14.78	42.10	14.52
Male (%)	74.50	43.60	78.23	41.44
Race: White (%)	67.39	46.90	100.0	0.00
Race: Black (%)	19.40	39.56	0.00	0.00
Race: Other/Missing/ Refused (%)	13.21	33.88	0.00	0.00
Post-traumatic Stress Disorder Diagnosis (%)	58.91	49.22	0.00	0.00
Current Smoker (%)	27.28	44.56	26.61	44.37
ICD-10 Infectious Disease Diagnosis (%)	7.42	26.22	0.00	0.00
ICD-10 Inflammatory Disease Diagnosis (%)	41.42	49.28	0.00	0.00
ICD-10 Cancer Diagnosis (%)	9.74	29.66	0.00	0.00
Medical Exclusion (120 days)	50.31	50.02	0.00	0.00
Body Mass Index	30.63	5.87	29.22	5.28
Patient Health Questionnaire-9 Total Score	17.49	4.29	15.97	4.08
IL-1β Concentration	23.47	0.82	23.32	0.81
MIF Concentration	22.31	0.68	22.29	0.71
CRP (adjusted)	3.37	6.96	2.10	2.08

(*) Exclusions: inflammatory diagnoses (e.g., cancer, autoimmune disease), receipt of an anti-inflammatory medication (e.g., corticosteroids, non-steroidal anti-inflammatory drugs), post-traumatic stress disorder, and non-European ancestry; SD = standard deviation; ICD-10 = International Classification of Diseases, Tenth Revision, Clinical Modification; IL-1β = interleukin-1-beta; MIF = macrophage migration inhibitory factor; CRP = C-reactive protein.

TABLE 2

Change in Outcomes Over Time for the Full Sample (N = 1,299)

		DECREASE IN PHQ-9 SCORE		PERCENTAGE WITH >50% REDUCTION IN PHQ-9 SCORE	
WEEK	N	MEAN	SD	MEAN	SD
4	1,240	3.32	5.00	17.10	37.66
8	1,125	4.43	5.35	23.64	42.51
12	1,095	4.86	5.49	26.76	44.29
18	1,041	5.06	5.65	28.05	44.95
24	1,049	5.21	5.71	30.22	45.94

PHQ-9 = Patient Health Questionnaire-9; SD = standard deviation.

Table 3 describes the (unadjusted) effect sizes for the mRNA and CRP variables for each study week, expressed as correlations with the decrease in PHQ-9 score, and as odds ratios for depression response associated with a one-SD increase in the cytokine measures. These effects are small, with all correlations <0.05 in magnitude and odds ratios that range between 0.79 and 1.16, with only one (for MIF at week 12) significantly different from the null value of 1.

The GEE model score statistics for tests of interactions of the mRNA and CRP concentrations with time, and for the main effects of these measures on each of the two depression responses show no significant effects for any the associations (Table 4). Score statistics for the tests of the moderator hypotheses showed no significant interactions with the proposed moderator variables (Table 5). Similarly, evaluating the moderator effects of the treatment refractory measure or those reflecting the day-28 prescriptions showed no significant interactions (Supplementary Table 2). Inclusion of the treatment refractory or day-28 prescription measures as covariates in the models had little effect on the estimated cytokine effects or cytokine by time effects of Table 4 (see Supplementary Table 3).

Table 6 reports the adjusted effects corresponding to 1-SD increases on the mRNA and CRP concentrations on the depression responses in the subsample of N = 126. The estimated changes on the PHQ-9 were less than one unit and the odds ratios for the IL-1β and MIF variables were close to 1. The CRP variable shows an OR of 1.90, which is larger than the effects observed in the full sample but is not significantly different from 1 in this small sample.

TABLE 3

Unadjusted Effect Sizes for the mRNA and CRP Variables for each Study Week

	Correlations of Baseline Biological Variables with Decreased Score			Odds Ratios for Baseline Biological Variables with Treatment Response		
Week	IL-1-β	MIF	CRP	IL-1-β	MIF	CRP
4	0.04	0.01	−0.01	0.89	1.09	0.86
	(−0.02,0.10)	(−0.05,0.07)	(−0.07,0.05)	(0.77,1.03)	(0.94,1.26)	(0.68,1.09)
8	−0.01	0.05	0.00	0.99	1.10	1.01
	(−0.07,0.05)	(−0.01,0.11)	(−0.06,0.06)	(0.87,1.14)	(0.96,1.26)	(0.89,1.15)
12	0.03	0.02	−0.05	0.97	1.16	0.79
	(−0.03,0.09)	(−0.04,0.08)	(−0.11,0.01)	(0.85,1.11)	(1.02,1.33)	(0.63,1.00)
18	0.05	0.03	−0.02	0.98	1.09	1.00
	(−0.01,0.11)	(−0.03,0.09)	(−0.08,0.04)	(0.86,1.13)	(0.95,1.24)	(0.88,1.13)
24	0.01	0.02	−0.03	0.95	1.04	0.88
	(−0.05,0.07)	(−0.04,0.08)	(−0.09,0.03)	(0.84,1.09)	(0.91,1.18)	(0.73,1.05)

IL-1β = interleukin-1-beta; MIF = macrophage migration inhibitory factor; CRP = C-reactive protein.

TABLE 4

Regression Results for Depression Responses on IL-1β and MIF mRNA and CRP Concentrations

			PHQ-9 DECREASE			DEPRESSION RESPONSE	
VARIABLE	EFFECT	DF	χ^2 VALUE	P-VALUE	DF	χ^2 VALUE	P-VALUE
IL-1β	Interaction by time	4	7.17	0.127	4	3.83	0.430
	Main effect	1	0.05	0.821	1	0.44	0.508
MIF	Interaction by time	4	1.92	0.750	4	2.41	0.660
	Main effect	1	0.68	0.410	1	1.23	0.268
CRP	Interaction by time	4	3.22	0.521	4	1.62	0.804
	Main effect	1	0.59	0.444	1	0.48	0.488

IL-1β = interleukin-1-beta; MIF = macrophage migration inhibitory factor; CRP = C-reactive protein; DF = degrees of freedom.

TABLE 5

Results of Moderator Analyses

			PHQ-9 DECREASE			DEPRESSION RESPONSE	
PREDICTOR	INTERACTION TERM	DF	χ^2 VALUE	P-VALUE	DF	χ^2 VALUE	P-VALUE
IL-1β	ICD-10 diagnosis	1	0.35	0.554	1	0.60	0.438
	Medication	1	0.47	0.493	1	0.55	0.457
	Race	1	0.00	0.960	1	0.19	0.661
	PTSD	1	2.67	0.102	1	1.38	0.239
MIF	ICD-10 diagnosis	1	0.00	0.973	1	0.02	0.888
	Medication	1	0.09	0.762	1	0.00	0.967
	Race	1	0.32	0.570	1	0.35	0.553
	PTSD	1	1.89	0.169	1	0.17	0.680
CRP	ICD-10 diagnosis	1	0.60	0.440	1	1.56	0.211
	Medication	1	0.04	0.848	1	0.39	0.531
	Race	1	1.14	0.286	1	0.13	0.715
	PTSD	1	0.11	0.743	1	1.16	0.281

IL-1β = interleukin-1-beta; MIF = macrophage migration inhibitory factor; CRP = C-reactive protein; DF = degrees of freedom; ICD-10 diagnosis of an inflammatory disease; Medication = anti-inflammatory medications; Race = European ancestry vs. other; PTSD = post-traumatic stress disorder.

TABLE 6

Estimated (Adjusted) Effects, and Hypothesis Tests, for Cytokine Effects on Depression Treatment Response (N = 126)

	DECREASED SCORE		TREATMENT RESPONSE	
VARIABLE	BETA (95% CI)	P-VALUE	BETA (95% CI)	P-VALUE
IL-1β	0.24 (−0.48, 0.95)	0.51	0.90 (0.66, 1.24)	0.53
MIF	0.18 (−0.55, 0.91)	0.63	0.94 (0.71, 1.25)	0.68
CRP	−0.41 (−3.47, 2.66)	0.80	1.90 (0.55, 6.60)	0.26

IL-1β = interleukin-1-beta; MIF = macrophage migration inhibitory factor; CRP = C-reactive protein.

Discussion

In a sample of nearly 1,300 individuals who received an antidepressant medication for treatment of major depression, there was a monotonic reduction in the PHQ-9 score from baseline and an increase in the treatment response rate that exceeded 50% at 24 weeks. However, we found no evidence that pretreatment plasma concentrations of MIF and IL-1β mRNA and CRP were associated with either depression treatment outcome measure. This is notable given that our sample was more than an order of magnitude larger than the 74-patient study on which the initial findings regarding MIF and IL-1β were based and the 68-patient study in which those findings were validated.[15] The larger sample here enabled us to examine a range of moderator variables as potential moderators of the relations between the cytokines and depression treatment outcome. Although one of these analyses yielded a nominally significant effect, a correction for multiple comparisons renders it non-significant. Thus, as with main effects of the cytokines, none of the moderator analyses showed significant effects on depression treatment outcome.

Limitations

There are multiple potential explanations for the lack of replication. The demographics of our study sample differed substantially from those of that of Cattaneo et al.[15] Because we recruited participants from the VA, our sample was three-quarters male, while more than half of both the discovery and validation samples in Cattaneo et al[15] were female. Further, our sample was nearly 10 years older than those in the prior studies (47.6 years vs. 38–39 years) and only about two-thirds of our sample were of European ancestry, while the samples studied by Cattaneo et al,[15] although not specified, were recruited exclusively in Europe and likely of predominantly European ancestry. Despite these demographic differences, the clinical features of the samples were comparable, with depression symptoms (although measured with different instruments) of moderate or greater severity and response rates (defined as a 50% reduction from baseline) comparable at about 55%. Participants in the PRIME Care sample were required to have had at least one prior depressive episode treated with an antidepressant. Although there was not a similar requirement for the samples in Cattaneo et al,[15] the population from which those samples were drawn was, on average, in their second episode of moderately severe depression. Finally, many of the patients in the study by Cattaneo et al[15] were treated with the tricyclic antidepressant nortriptyline, which was not true for the VA sample.

To account for potential demographic or clinical confounders that could explain the lack of association in our sample, we conducted a secondary analysis in a subsample of 126 patients who were selected to exclude the presence of inflammatory diseases (e.g., cancer, autoimmune disease), receipt of an anti-inflammatory medication (e.g., corticosteroids, non-steroidal anti-inflammatory drugs), a diagnosis of PTSD, and non-European ancestry. Findings obtained from that sample paralleled those in the full sample, with no evidence of an association between the depression outcome measures and pretreatment concentrations of IL-1-β and MIF mRNA or CRP.

Method variance in quantifying mRNA concentrations is another potential explanation for the difference in findings between our study and those of Cattaneo et al.[15] The latter prepared standard curves from cDNA clones of human *MIF* and *IL-1*β genes. In contrast to the assertion that they quantified absolute mRNA, the quantities they reported are based on standard curves that use cDNA not RNA, so their method provides a relative measure of mRNA. We also measured relative gene expression using real-time detection, but our method included an endogenous control (i.e., the "housekeeping gene" β-actin), which was expressed at moderate-to-high levels in all analyzed blood samples. Ct scores ranged from 13–21 (mean = 16.33 +/− 0.22 S.D.). Second, a known copy number calibrator constructed and quantified by a commercial vendor was used in the calculations of both RQ and Copy Number per sample. These calculations (i.e., the 2-ΔΔCT method) have been extensively used as a relative quantification strategy for quantitative real-time polymerase chain reaction (qPCR) data analysis and are more accurate than those using standard curve slopes and y-intercepts, which fluctuate randomly due to a variety of errors during preparation, such as pipetting and mixing. Third, we used validated real-time PCR assays from a commercial source for the housekeeping gene and genes of interest rather than assays produced within the laboratory. Last, we validated the gene expression results from the QuantStudio 12K Flex (qPCR) by running a small subset of samples (n = 47) on a Bio-Rad QX200 Digital Droplet System, which shows that gene expression followed the same trends in copy number as in the original experiment (data not shown).

Conclusions

Among patients with MDD who are treated with at least one of a variety of antidepressant medications, we found no association between MIF and IL-1β mRNA levels and antidepressant treatment response. Similarly, CRP levels were not associated with treatment outcome despite efforts to maximize the likelihood of showing an effect. ✤

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Disclaimer

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The Department of Veteran Affairs had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

Declaration of Interests

Dr. Kranzler is a scientific advisory board member for Altimmune, Clearmind Medicine, Dicerna Pharmaceuticals, Enthion Pharmaceuticals, Eli Lilly and Company, and Sophrosyne Pharmaceuticals; a consultant for Sobrera Pharmaceuticals; the recipient of research funding and medication supplies for an investigator-initiated study from Alkermes; a member of the American Society of Clinical Psychopharmacology's Alcohol Clinical Trials Initiative (ACTIVE Group), which over the past three years was supported by Alkermes, Dicerna, Ethypharm, Lundbeck, Mitsubishi, and Otsuka; and holder of U.S. patent 10,900,082 titled: "Genotype-guided dosing of opioid agonists," issued 26 January 2021. Dr. Oslin received grant support from Myriad Genetics, which supplied genetic tests for the study; was the co-chair of the VA/DoD Clinical Practice Guideline for Major Depressive Disorder during the study; and received grant support from Janssen Pharmaceuticals, Inc. for work on depression. Dr. Thase, during the last three years, served as a consultant for Acadia Inc.,

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Supplementary Material

SUPPLEMENTARY TABLE 1

Baseline Treatment Refractory Status and Antidepressant Prescriptions, and Week 4 Antidepressant Prescriptions in the Full Sample and the Subsample after Exclusions (*)

	FULL SAMPLE (N = 1,299)			SUBSAMPLE (N = 126)		
VARIABLES	N	MEAN	SD	N	MEAN	SD
TxRefract	1,299	30.95	46.25	126	29.37	45.73
Day −7: TCA (%)	1,299	1,299	17.28	126	3.17	17.60
Day −7: Non-SRI (%)	1,299	14.24	34.96	126	13.49	34.30
Day −7: SSRI (%)	1,299	25.48	43.59	126	15.87	36.69
Day −7: SNRI (%)	1,299	13.24	33.91	126	13.49	34.30
Day 28: TCA (%)	1,299	3.85	19.25	126	1.59	12.55
Day 28: Non-SRI (%)	1,299	23.17	42.21	126	24.60	43.24
Day 28: SSRI (%)	1,299	41.65	49.32	126	42.86	49.68
Day 28: SNRI (%)	1,299	31.64	46.52	126	27.78	44.97
Day 28: TCA (%)	**1,240**	**3.95**	**19.49**	**120**	**1.67**	**12.86**
Day 28: Non-SRI (%)	**1,240**	**23.15**	**42.19**	**120**	**24.17**	**42.99**
Day 28: SSRI (%)	**1,240**	**41.37**	**49.27**	**120**	**41.67**	**49.51**
Day 28: SNRI (%)	**1,240**	**31.85**	**46.61**	**120**	**28.33**	**45.25**

(*) Exclusions: inflammatory diagnoses (e.g., cancer, autoimmune disease), receipt of an anti-inflammatory medication (e.g., corticosteroids, non-steroidal anti-inflammatory drugs), post-traumatic stress disorder, and non-European ancestry; SD = standard deviation; ICD-10 = International Classification of Diseases, Tenth Revision, Clinical Modification; IL-1β = interleukin-1-beta; MIF = macrophage migration inhibitory factor; CRP = C-reactive protein. TxRefract = Treatment refractory, which indicates having had more than one prior treatment with an antidepressant or having received electroconvulsive therapy, vagus nerve stimulation or transcranial magnetic stimulation. TCA = Tricyclic antidepressant, Non-SRI = Non-serotonin reuptake inhibitor, SSRI = selective serotonin reuptake inhibitor, SNRI = serotonin-norepinephrine reuptake inhibitor. N = 1,299 refers to the baseline sample, while the numbers in bold are for the 1,240 people who provided a PHQ-9 score at week 4, i.e., those who contribute to the longitudinal analyses. Losing 59 people during follow-up does not change the "baseline = 28" distribution very much.

SUPPLEMENTARY TABLE 2

Results of Moderator Analyses that Include Depression Treatment Variables

		PHQ-9 DECREASE			DEPRESSION RESPONSE		
PREDICTOR	INTERACTION TERM	DF	χ^2 VALUE	P-VALUE	DF	χ^2 VALUE	P-VALUE
IL-1β	ICD-10 Dx	1	0.12	0.726	1	0.27	0.601
	Medication	1	0.18	0.669	1	0.88	0.347
	Race	1	0.05	0.827	1	0.60	0.439
	PTSD	1	2.99	0.084	1	1.55	0.213
	Tx Refract	1	0.01	0.918	1	0.15	0.703
	TCA-28	1	0.27	0.603	1	0.50	0.481
	Non-SRI-28	1	2.23	0.135	1	1.03	0.311
	SSRI-28	1	0.27	0.603	1	0.00	0.990
	SNRI-28	1	4.06	0.044	1	3.10	0.078
MIF	ICD-10 Dx	1	0.00	0.951	1	0.00	0.974
	Medication	1	0.04	0.846	1	0.00	0.982
	Race	1	0.19	0.663	1	0.26	0.607
	PTSD	1	1.89	0.169	1	0.22	0.642
	Tx Refract	1	0.00	0.948	1	0.05	0.832
	TCA-28	1	0.18	0.670	1	0.00	0.957
	Non-SRI-28	1	0.01	0.926	1	0.46	0.499
	SSRI-28	1	3.68	0.055	1	0.66	0.415
	SNRI-28	1	0.53	0.466	1	0.23	0.634
CRP	ICD-10 Dx	1	0.55	0.460	1	1.81	0.179
	Medication	1	0.25	0.619	1	0.00	0.958
	Race	1	1.81	0.179	1	0.53	0.468
	PTSD	1	0.86	0.355	1	2.53	0.111
	Tx Refract	1	2.64	0.104	1	2.26	0.132
	TCA-28	1	1.80	0.180	1	2.80	0.094
	Non-SRI-28	1	0.02	0.901	1	0.05	0.818
	SSRI-28	1	1.00	0.318	1	1.20	0.274
	SNRI-28	1	0.20	0.652	1	1.01	0.315

IL-1β = interleukin-1-beta; MIF = macrophage migration inhibitory factor; CRP = C-reactive protein; DF = degrees of freedom; ICD-10 Dx = ICD-10 = International Classification of Diseases, Tenth Revision, Clinical Modification diagnosis of major depression; PTSD = post-traumatic stress disorder; TxRefract = Treatment refractory (i.e., having had more than one prior treatment with an antidepressant or electroconvulsive therapy, vagus nerve stimulation or transcranial magnetic stimulation); TCA = Tricyclic antidepressant, Non-SRI = Non-serotonin reuptake inhibitor, SSRI = selective serotonin reuptake inhibitor, SNRI = serotonin-norepinephrine reuptake inhibitor (each within the 28 days prior to the follow-up interview).

SUPPLEMENTARY TABLE 3

Regression Results for Depression Responses on IL-1β and MIF mRNA and CRP Concentrations that Include Depression Treatment Variables in The Model

			PHQ-9 DECREASE			DEPRESSION RESPONSE	
VARIABLE	EFFECT	DF	χ^2 VALUE	P-VALUE	DF	χ^2 VALUE	P-VALUE
IL-1β	Interaction by time	4	7.33	0.120	4	3.82	0.431
	Main effect	1	0.05	0.832	1	0.51	0.477
MIF	Interaction by time	4	2.06	0.724	4	2.47	0.651
	Main effect	1	0.74	0.391	1	1.32	0.251
CRP	Interaction by time	4	3.25	0.518	4	1.63	0.803
	Main effect	1	0.99	0.320	1	0.91	0.341

IL-1β = interleukin-1-beta; MIF = macrophage migration inhibitory factor; CRP = C-reactive protein; DF = degrees of freedom.