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Longitudinal Association Between Depression and Inflammatory Markers: Results From the Netherlands Study of Depression and Anxiety

Femke Lamers, Yuri Milaneschi, Johannes H. Smit, Robert A. Schoevers, Gayle Wittenberg, and Brenda W.J.H. Penninx

ABSTRACT

BACKGROUND: While cross-sectional associations of inflammatory markers interleukin-6 (IL-6) and C-reactive protein with major depressive disorder are well established, evidence for longitudinal associations mostly comes from studies on depression symptoms, not diagnoses. This study examined cross-sectional and bidirectional longitudinal associations between depression diagnosis and symptoms in an adult sample over a 6-year period.

METHODS: Data were obtained from the baseline (n = 2416) and 2- and 6-year follow-up assessments (n = 1925 and n = 1924, respectively) of the Netherlands Study of Depression and Anxiety. C-reactive protein and IL-6 were assessed at each wave, as were the Composite International Diagnostic Interview and Inventory of Depressive Symptomatology. Linear mixed models and generalized estimating equation models with a binomial distribution were used to study longitudinal associations between depression and inflammation and vice versa.

RESULTS: There was a consistent cross-sectional association between current depressive disorder (vs. no current disorder) and symptoms with IL-6 across all follow-up measurements (Cohen's $d_{\text{depression diagnosis}}$ = 0.06, p = .017; $B_{\text{standardized Inventory of Depressive Symptomatology}}$ = 0.029, SE = 0.011, p = .008). In longitudinal analyses, higher IL-6 levels predicted subsequent chronic course in those with a diagnosis at baseline in women but not in men (odds $\text{ratio}_{\text{women}}$ = 1.13, 95% confidence interval = 1.04–1.23), and both depressive disorder and high severity predicted higher IL-6 levels at the subsequent follow-up (p values $<$ .01). In contrast, C-reactive protein was not associated with current depression in cross-sectional and longitudinal analyses.

CONCLUSIONS: In this longitudinal study, cross-sectional and bidirectional longitudinal associations were found between depression and IL-6 levels. This underlines the importance of targeting inflammation pathways in the treatment of major depressive disorder. IL-6 could be a potential marker for patient profiling in personalized medicine approaches.

Keywords: CRP, Depression severity, Depressive disorder, Epidemiology, IL-6, Longitudinal

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It is well known that the immune system plays a role in major depressive disorder (MDD). Meta-analyses of cross-sectional studies have consistently linked depression with increased levels of inflammatory markers (i.e., C-reactive protein [CRP], interleukin-6 [IL-6], IL-1, tumor necrosis factor alpha, soluble IL-2 receptor) (1–3), and gene expression studies have found upregulation of inflammatory pathways in depression (4–6). IL-6 and CRP are the most often studied markers. Both are easy to measure in serum and are linked; IL-6 is produced in the initial stage of inflammation and triggers other inflammatory responses, including production of the acute phase protein CRP in the liver (7). CRP has important roles in regulating inflammatory processes, including regulating the complement system and cytokine production (8).

Mechanisms through which inflammation is linked to depression include the promotion of sickness behavior by inflammatory markers as well as effects of inflammation in the brain such as alterations in the metabolism of neurotransmitters, activation of corticotropin-releasing hormone, and disruptions in synaptic plasticity (9). Inflammation and sickness behavior also play a role in the pathogen host defense hypothesis that poses depression as an evolutionary adaptation and integral part of the immune-mediated host defense against pathogens (10).

A meta-analysis of longitudinal studies showed only a weak association of high baseline CRP levels with depressive symptoms at follow-up and showed no relation between baseline IL-6 and depressive symptoms at follow-up (11). Whether depressive symptoms predicted inflammation at follow-up was not evaluated. A recent meta-analysis in samples from elderly adults found that CRP—but not IL-6—predicted depressive symptoms at follow-up in adjusted models, but depressive symptoms at baseline did not predict CRP at follow-up (12).

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Because most studies evaluate samples from elderly adults, it remains unclear whether associations hold in younger adults, who experience less somatic comorbidity and generally experience less inflammation than elderly persons. In addition, while these studies mostly show an effect of high CRP on subsequent depressive symptoms, they focused only on depressive symptoms rather than on actual diagnoses of depression. Studies on depressive symptoms are valuable because depression more likely presents as a dimensional spectrum rather than the "yes/no" dichotomization that is used in clinical practice. Nevertheless, comparing groups with and without depression diagnosis will yield information that can be relevant in clinical practice and play a role in personalized medicine.

While few studies have used depression diagnoses in their analyses, four studies evaluated the effect of IL-6 or CRP on subsequent (onset of a) depression diagnosis (13–16), and two of these studies were conducted on children (14,15). These studies found mixed results; in one of four studies CRP predicted onset (13), and one of two studies evaluating IL-6 found it to be associated with onset of depression (15).

This current study therefore explored both cross-sectional associations and bidirectional longitudinal associations between depression (diagnosis as well as symptoms) and inflammation in a large adult sample, using three waves over a 6-year period, while taking into account important confounders (alcohol intake, smoking, and number of somatic diseases). Based on current literature, we expected both CRP and IL-6 to predict depression status at follow-up.

METHODS AND MATERIALS

Study Design

Data from the Netherlands Study of Depression and Anxiety (NESDA) were used. NESDA is an ongoing longitudinal cohort study aimed at studying the course and consequences of depressive and anxiety disorders. Persons with and without depressive and anxiety disorders (N = 2981) were recruited from the community and from primary care and secondary care settings in three cities in the Netherlands (Amsterdam, Groningen, and Leiden). Exclusion criteria were 1) not being fluent in Dutch and 2) having a primary clinical diagnosis of bipolar disorder, psychotic disorder, obsessive-compulsive disorder, or severe addiction disorder. Participants were enrolled for the baseline assessment between September 2004 and February 2007. Follow-up assessments were conducted at 2, 4, and 6 years, and the 2- and 6-year waves were used in the current study; the 4-year follow-up was not included because no inflammatory markers were assessed in this wave. The response rates for the 2- and 6-year waves were 87.1% (n = 2596) and 75.7% (n = 2256), respectively. Nonresponse at the 2-year follow-up was associated with younger age, fewer years of education, non-North European descent, being recruited in Amsterdam, no previous participation in research, and having MDD at baseline (17); nonresponse at the 6-year follow-up was associated with fewer years of education, non-North European descent, being recruited in Amsterdam, no previous participation in research, and having MDD or anxiety disorders at baseline.

Each assessment included a comprehensive diagnostic interview, a medical examination, and self-administered questionnaires. Fasting blood was collected at baseline and the 2- and 6-year follow-ups. A complete overview of the study aims and procedures is reported elsewhere (18). The study was approved by the ethical review boards of participating centers, and all participants provided informed consent.

Sample Selection

From each wave (baseline and 2- and 6-year follow-ups), we selected all participants with current or remitted MDD as well as healthy control participants (i.e., no lifetime history of depressive or anxiety disorders) who also had inflammatory data available. At baseline, 2577 persons fell within the diagnostic groups of interest, and 2539 of them (98.5%) had inflammatory data. At the 2-year follow-up 2001 (87.5%) of 2288 eligible persons had data on inflammatory markers, and at the 6-year follow-up 1999 (89.6%) of 2231 eligible persons had inflammatory data. Of these participants, we then excluded persons with CRP levels >10 mg/L, indicative of acute infection or an active autoimmune disorder (baseline n = 123, 2-year follow-up n = 76, 6-year follow-up n = 75), leaving 2416, 1925, and 1924 persons per wave for analyses, respectively. In total, 2799 unique participants contributed to the analyses; of these, 1379 had data for all three waves, 708 had data for two waves, and 712 had data for one wave.

Diagnostic Assessment

At each interview, conducted by specially trained clinical research staff, the presence of DSM-IV depressive and anxiety disorders (generalized anxiety disorder, panic disorder, agoraphobia, and social phobia) was assessed with the Composite International Diagnostic Interview (version 2.1) (19). For the current study, we made a dichotomous variable for each wave indicating presence of a current MDD diagnosis (during the past 6 months—yes/no). Among those without current MDD, we initially distinguished those with and without a prior history of MDD. But because inflammatory marker levels did not differ between these two groups, similar to previous reports of this sample (20,21), we combined them in the no current MDD group. In Supplemental Tables S2 and S3, we nevertheless also report analyses by the three groups for models where diagnostic group was a significant predictor. We also evaluated depression severity with the Inventory of Depressive Symptomatology (IDS-30 self-reported version) (22) at each wave. In analyses where the IDS score was a predictor, IDS scores were standardized.

Inflammatory Markers

At each wave, inflammatory markers were determined from fasting morning blood plasma. Plasma levels of CRP at baseline were measured in duplicate by an in-house, high-sensitivity enzyme-linked immunosorbent assay (ELISA) based on purified protein and polyclonal anti-CRP antibodies (Dako, Glostrup, Denmark). The lower detection limit of CRP is 0.1 mg/L, and the sensitivity is 0.05 mg/L. Intra- and interassay coefficients of variation were 5% and 10%, respectively. Plasma levels of CRP in the follow-up waves were measured in duplicate by a high-sensitivity particle-enhanced immunoturbidimetric assay

(CRPHS; Roche Diagnostics, Indianapolis, IN). The lower detection limit of CRP in this kit is 0.15 mg/L, and the sensitivity is 0.3 mg/L. Intra- and interassay coefficients of variation were as follows: Intra-assay 2-year follow-up 5%, 6-year follow-up 7%; interassay 2-year follow-up 4%, 6-year follow-up 9%. Plasma IL-6 levels at baseline were measured in duplicate by a high-sensitivity ELISA (PeliKine Compact ELISA; Sanquin, Amsterdam). The lower detection limit of IL-6 is 0.35 pg/mL, and the sensitivity is 0.10 pg/mL. Intra- and interassay coefficients of variation were 8% and 12%, respectively. At the 2- and 6-year follow-ups, IL-6 was measured in duplicate by a high-sensitivity solid-phase ELISA (Human IL-6 Quantikine HS Kit; R&D Systems, Minneapolis, MN). The lower detection limit of IL-6 in this kit is 0.08 pg/mL, and the sensitivity range is 0.016 to 0.110 pg/mL. Intra- and interassay coefficients of variation were 7.8% and 7.2%, respectively.

Covariates

Sociodemographic variables included gender, age, and years of education at baseline. Other covariates were measured at all waves. Health and lifestyle variables included current smoking (yes/no), body mass index (BMI) (weight in kilograms divided by height in square meters), number of alcoholic drinks per week as assessed with the Alcoholic Use Disorders Identification Test (23), and number of chronic diseases for which a person received treatment based on a self-report list of 20 common chronic diseases. Medication use was assessed by drug container inspection of drugs taken during the past month. All medication was coded according to the World Health Organization Anatomical Therapeutic Chemical (ATC) classification. Use of anti-inflammatory medication (ATC codes: M01A, M01B, A07EB, and A07EC) and statins (ATC codes: C10AA and C10B) was coded in two separate binary variables. For sensitivity analyses, we created four binary variables indicating frequent use (>50% of time) of different types of antidepressants: selective serotonin reuptake inhibitors (ATC code N06AB), serotonin–norepinephrine reuptake inhibitors (SNRIs) (ATC codes N06AX 16 and N06A 21), tricyclic antidepressants (ATC code N06AA), and tetracyclic antidepressants (ATC codes N06AX03, N06AX06, and N06AX11). Presence of comorbid 6-month anxiety diagnoses (generalized anxiety disorder, panic disorder [with/without agoraphobia], and social phobia) was based on the Composite International Diagnostic Interview.

Statistical Analyses

All analyses were conducted in SPSS (version 22; IBM Corp., Armonk, NY). Descriptive statistics (mean and SD, median [interquartile range], and frequency) were used to describe the sample at each wave. Because of non-normal distributions of CRP and IL-6, CRP and IL-6 were natural logarithmic transformed in all analyses with these markers as outcome.

To study the cross-sectional association of depression status and inflammation using all available data, we combined the three waves by stacking the waves in one cross-sectional dataset. To account for the fact that one person can have multiple observations in this combined cross-sectional dataset, we analyzed the association between depression status and inflammation (measured in the same wave) using linear mixed models (for an explanation of such models and data structures, see (24) and Supplemental Figure S1) with wave as repeated effect and patient identifier as within-subject effect and using an unstructured correlation matrix. Included covariates were time varying (e.g., measures at the same time of depression and inflammation assessment). Estimates from this model thus represent the cross-sectional association between predictor and outcome.

For longitudinal analyses to evaluate the effect of baseline current depression (yes/no) on inflammation at the 2- and 6-year follow-ups, we used linear mixed models with wave as repeated effect and patient identifier as within-subject effect and using an unstructured correlation matrix. Baseline predictor by time interaction (time coded as 0, 2, or 6) was included to test differences in slope but was retained in models only when statistically significant. Baseline values of the inflammatory markers were included as covariates, and all other covariates were measured at baseline as well. For evaluating the effect of baseline inflammation levels on current depression status (yes/no) at the 2- and 6-year follow-ups, we used generalized estimating equation models with a binomial distribution with wave as repeated effect and patient identifier as within-subject effect and using an unstructured correlation matrix. Of the covariates, BMI was added in a second step of modeling to be able to separately assess its effect because adipose tissue produces inflammatory markers (25). Sensitivity models were run with additional correction for antidepressants for those models showing significant effects of the main predictor. Cohen's *d* values were calculated for significant results in the main analysis of MDD diagnosis in fully corrected models. Interaction terms for main predictor by gender were added to check for modification by gender.

RESULTS

Sample characteristics across the three waves are reported in Table 1. The baseline sample had a mean age of 41.9 years (SD = 12.9), and 66.3% was female. At baseline, 1035 (42.8%) of participants had a current MDD, and as expected the number of current MDD cases decreased over time. Supplemental Table S1 presents mean and median values of CRP and IL-6 across waves and correlations.

Cross-sectional Associations

In the cross-sectional analyses, including 2799 unique individuals with in total more than 6000 observations, IL-6 levels were higher in current MDD cases than in control participants (0.96 vs. 0.91 pg/mL, $p = .020$, $d = 0.06$), but no difference between groups was observed for CRP (Table 2). Effects remained significant after additional correction for BMI (0.96 vs. 0.91 pg/mL, $p = .017$, $d = 0.06$). Similarly, analyses with a dimensional severity score showed an association with IL-6 ($B_{\text{standardized IDS}} = 0.029$, SE = 0.011, $p = .008$), but not with CRP. Sensitivity analyses showed that only SNRIs had a significant, positive association with IL-6 levels; in models with correction for SNRIs, the main effects of MDD diagnosis and depression severity on IL-6 remained statistically significant (data not shown). No significant interaction effects of gender were observed. Sensitivity analyses also correcting for the

Table 1. Sample Characteristics Across Three Waves

	Baseline, *n* = 2416	2-Year Follow-up, *n* = 1925	6-Year Follow-up, *n* = 1924
Demographics (Baseline)			
Age, years, mean (±SD)	41.9 (±12.9)	42.5 (±13.0)	42.4 (±13.0)
Female, *n* (%)	1603 (66.3)	1249 (64.9)	1248 (64.9)
Education, years, mean (±SD)	12.2 (±3.2)	12.3 (±3.3)	12.5 (±3.2)
Health and Lifestyle			
Current smoker, *n* (%)	939 (38.9)	602 (31.3)	532 (27.7)
BMI, mean (±SD)[a]	25.4 (±4.7)	25.6 (±4.6)	26.1 (±4.9)
Number of diseases under treatment, median (IQR)[a]	0 (0–1)	0 (0–1)	0 (0–1)
Number of alcoholic drinks per week, median (IQR)[a]	3.7 (0.2–8.7)	3.7 (0.2–8.2)	3.7 (0.2–8.2)
Anti-inflammatory medication use, *n* (%)	100 (4.1)	95 (4.9)	99 (5.1)
Statin use, *n* (%)	167 (6.9)	147 (7.6)	199 (10.3)
Clinical Characteristics			
Diagnostic group			
No current MDD, *n* (%)	1381 (57.2)	1448 (75.2)	1617 (84.0)
Current MDD, *n* (%)	1035 (42.8)	477 (24.8)	307 (16.0)
IDS severity score, mean (±SD)	21.7 (±14.6)	16.0 (±12.3)	15.2 (±11.9)
Antidepressant Use, *n* (%)			
SSRIs	430 (17.8)	271 (14.1)	228 (11.9)
SNRIs	102 (4.2)	81 (4.2)	73 (3.8)
TCAs	63 (2.6)	59 (3.1)	57 (3.0)
TeCas	43 (1.8)	33 (1.7)	32 (1.7)

BMI, body mass index; IDS, Inventory of Depressive Symptomatology; IQR, interquartile range; MDD, major depressive disorder; SNRI, serotonin–norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant; TeCa, tetracyclic antidepressant.

[a]Missing values imputed with within-person mean of wave mean.

presence of comorbid anxiety disorders showed similar results for MDD and no significant effect of anxiety on marker levels (not shown).

Longitudinal Associations: Baseline Current Depression Predicting Inflammation

Those with current MDD at baseline had higher levels of IL-6 at both the 2- and 6-year follow-ups in multivariable analyses independent of baseline IL-6 levels (Table 3). This effect remained statistically significant when also correcting for BMI (p = .002, d = 0.10, 95% confidence interval [CI] = 0.03–0.17). In contrast, current MDD did not predict CRP levels at follow-up. There were no significant time by group interaction effects, indicating that the slopes did not differ between baseline depression groups. The same pattern of results was observed when using baseline IDS severity instead of the dichotomous depression indicator ($B_{\text{standardized IDS}}$ = 0.040, SE = 0.014, p = .005) (Table 3). To summarize, having current depression or having more severe depressive symptoms was linked to higher IL-6 levels at both the 2- and 6-year follow-ups (correcting for baseline IL-6 levels). A graphical representation of IL-6 results is shown in Figure 1. Of the baseline antidepressant classes, only selective serotonin reuptake inhibitor use and SNRI use were associated with significantly higher IL-6 levels; when also correcting for these

Table 2. Cross-sectional Associations Between Depression and CRP and IL-6 Over Three Waves (N = 2799, $N_{\text{observations}}$ = 6261)

	CRP[a] (mg/L)				IL-6[a] (pg/mL)			
	Model 1		Model 2		Model 1		Model 2	
Depression Severity	*B* (SE)	*p* Value	*B* (SE)	*p* Value	*B* (SE)	*p* Value	*B* (SE)	*p* Value
zIDS	0.020 (0.016)	.24	0.001 (0.015)	.95	0.034 (0.011)	.002	0.029 (0.011)	.008
Diagnostic Group	Mean (95% CI)	*p* Value	Mean (95% CI)	*p* Value	Mean (95% CI)	*p* Value	Mean (95% CI)	*p* Value
No MDD	1.03 (0.99–1.08)	ref	1.02 (0.98–1.06)	ref	0.91 (0.89–0.94)	ref	0.91 (0.89–0.94)	ref
Current MDD	1.02 (0.96–1.08)	.70	1.01 (0.96–1.07)	.75	0.96 (0.93–1.00)	.020	0.96 (0.92–1.00)	.017

Model 1: Adjusted for wave, age, gender, years of education, number of chronic diseases under treatment, anti-inflammatory medication use, statin use, smoking status, and alcohol intake. Model 2: Model 1 + body mass index.

CI, confidence interval; CRP, C-reactive protein; IL-6, interleukin-6; MDD, major depressive disorder; ref, reference group; zIDS, standardized Inventory of Depressive Symptomatology score.

[a]Natural logarithmic transformed markers used (back transformed to display mean levels by diagnostic group).

Table 3. Longitudinal Associations[a] Between Depression Status at Baseline and Inflammation During Subsequent Follow-ups (*N* = 2383)

	CRP[b]		IL-6[b]	
	B (SE)	*p* Value	*B* (SE)	*p* Value
Model 1: Baseline Severity				
zIDS	0.012 (0.022)	.59	0.047 (0.014)	.001
Model 2: Baseline Severity				
zIDS	0.004 (0.021)	.85	0.040 (0.014)	.005
Model 1: Baseline Diagnostic Group				
No MDD	ref		ref	
cMDD	0.067 (0.041)	.11	0.083 (0.027)	.002
Model 2: Baseline Diagnostic Group				
No MDD	ref		ref	
cMDD	0.068 (0.041)	.098	0.083 (0.027)	.002

Model 1: Adjusted for age, gender, years of education, baseline value of marker, number of chronic diseases under treatment, anti-inflammatory medication use, statin use, smoking status, and alcohol intake. Model 2: Model 1 + body mass index.

cMDD, current major depressive disorder at baseline; CRP, C-reactive protein; IL-6, interleukin-6; no MDD, no current major depressive disorder at baseline; ref, reference group; zIDS, standardized Inventory of Depressive Symptomatology score.

[a]Main effects from model without interaction.

[b]Natural logarithmic transformed markers used.

antidepressant classes, the main effects of diagnostic group and depression severity remained statistically significant (data not shown). No significant interaction effects of gender were observed. A sensitivity analysis also correcting for the presence of comorbid anxiety disorders at baseline showed similar results for MDD and no significant effect of baseline anxiety on marker levels (not shown).

Longitudinal Associations: Baseline Inflammation Predicting Depression Status

For the analyses of inflammation predicting depression at follow-up, we stratified the sample by baseline diagnostic status so that we could separately assess the effect of inflammation on 1) new onset in a group of patients without current depression (n = 1287) and on 2) chronicity in persons with current depression (n = 881). Models showed that higher baseline levels of IL-6 predicted having MDD at follow-up in those with a current baseline MDD diagnosis (odds ratio = 1.08, 95% CI = 1.03–1.14) (Table 4 and Figure 2). In other words, IL-6 levels predicted chronicity of depression, but this was not observed for CRP. The effect was not explained by baseline severity, given that additional correction for baseline IDS score did not alter results (not tabulated). Because a significant gender by IL-6 interaction was observed, stratified analyses revealed a significant effect in women with current MDD (odds $\text{ratio}_{\text{women}}$ = 1.13, 95% CI = 1.04–1.23, p = .003) but not in men (odds $\text{ratio}_{\text{men}}$ = 0.95, 95% CI = 0.83–1.09, p = .48) (not tabulated). Again, the effect in women was not explained by baseline depression severity, given that the effect remained statistically significant after correction for baseline IDS score (not tabulated). With respect to inflammation predicting onset of disorders, no effects were observed of

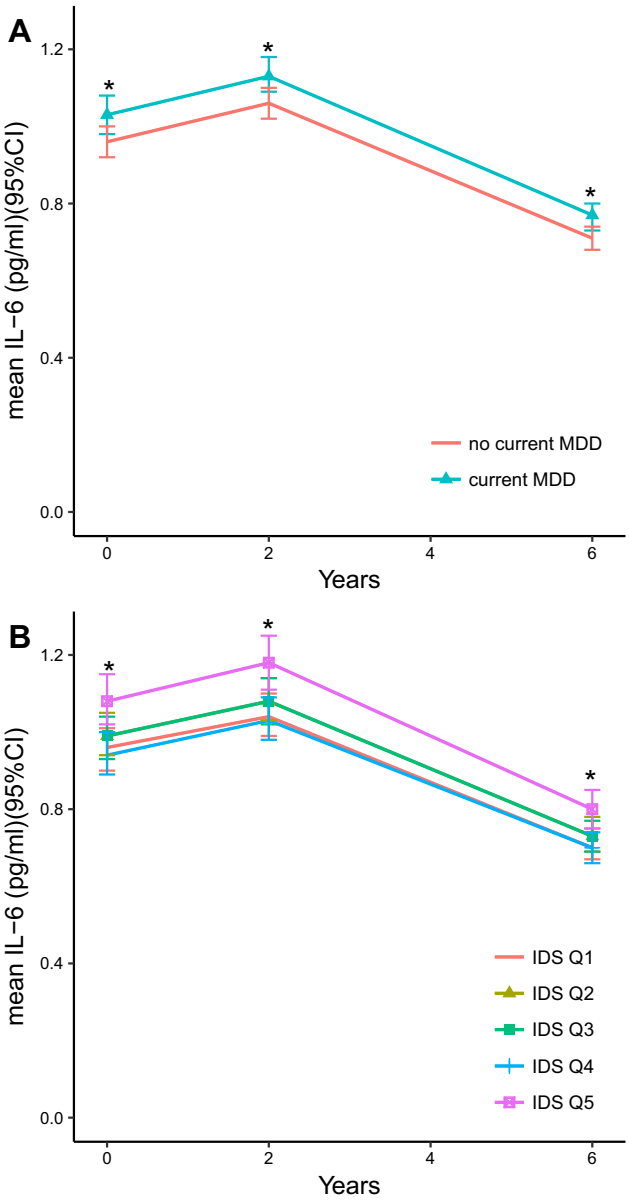

Figure 1. Longitudinal associations between baseline depression and interleukin-6 (IL-6). **(A)** Baseline depression status predicting IL-6 at follow-up. **(B)** Baseline Inventory of Depressive Symptomatology (IDS) severity quintiles predicting IL-6 at follow-up. Analysis based on natural logarithmic transformed IL-6 and back transformed for the figure. Models include baseline outcome as well for illustrative purposes. IDS is grouped into quintiles (Q1–Q5). *Main effect of group ($p < .05$). CI, confidence interval; MDD, major depressive disorder.

baseline IL-6 or CRP on onset in persons without current MDD at baseline. The analyses with outcome IDS were stratified by IDS scores at the cutoff for mild symptoms (<14 and ≥14). Inflammatory marker levels at baseline did not predict IDS depression severity scores at follow-up in either IDS stratum at baseline. Additional correction for antidepressant use did not change the effects, nor were significant gender interactions observed (data not shown).

Table 4. Associations Between Baseline Inflammatory Markers and Current MDD at Follow-up Across Baseline Diagnostic Groups

Stratum	Baseline Inflammation Marker	Current MDD at Follow-up[a]			
		Model 1		Model 2	
		OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
No MDD at Baseline (*n* = 1287)	CRP	1.03 (0.95–1.11)	.45	1.02 (0.94–1.11)	.58
Current MDD at Baseline (*n* = 881)	CRP	1.03 (0.98–1.09)	.25	1.04 (0.98–1.10)	.22
No MDD at Baseline (*n* = 1287)	IL-6	0.97 (0.91–1.03)	.29	0.97 (0.91–1.03)	.29
Current MDD at Baseline (*n* = 881)	IL-6	1.08 (1.03–1.14)	.003	1.08 (1.03–1.14)	.003
		IDS During Follow-up[a]			
		B (SE)	*p* Value	*B* (SE)	*p* Value
IDS < 14 at Baseline (*n* = 811)	CRP	−0.114 (0.095)	.23	−0.121 (0.100)	.23
IDS ≥ 14 at Baseline (*n* = 1324)	CRP	0.105 (0.112)	.37	0.173 (0.126)	.17
IDS < 14 at Baseline (*n* = 811)	IL-6	0.041 (0.053)	.43	0.042 (0.053)	.43
IDS ≥ 14 at Baseline (*n* = 1324)	IL-6	0.081 (0.093)	.38	0.085 (0.093)	.36

Binomial generalized estimating equation model, time continuous, unstructured correlation matrix, and mixed model for IDS outcome. Model 1: Adjusted for baseline age, gender, years of education, number of chronic diseases under treatment, statin use, anti-inflammatory medication use, smoking status, and alcohol intake (and model with IDS as outcome also adjusted for baseline IDS). Model 2: Model 1 + adjusted for baseline body mass index.

CI, confidence interval; CRP, C-reactive protein; IDS, Inventory of Depressive Symptomatology; IL-6, interleukin-6; MDD, major depressive disorder; OR, odds ratio.

[a]Main effects from model without interaction.

DISCUSSION

The main finding of this study is that both cross-sectionally and longitudinally we found small but consistent associations between IL-6 and current MDD. Having depression at baseline was associated not only with higher IL-6 levels at baseline but also with higher IL-6 levels after the 2- and 6-year follow-ups. Furthermore, higher levels of baseline IL-6 predicted depression chronicity over time.

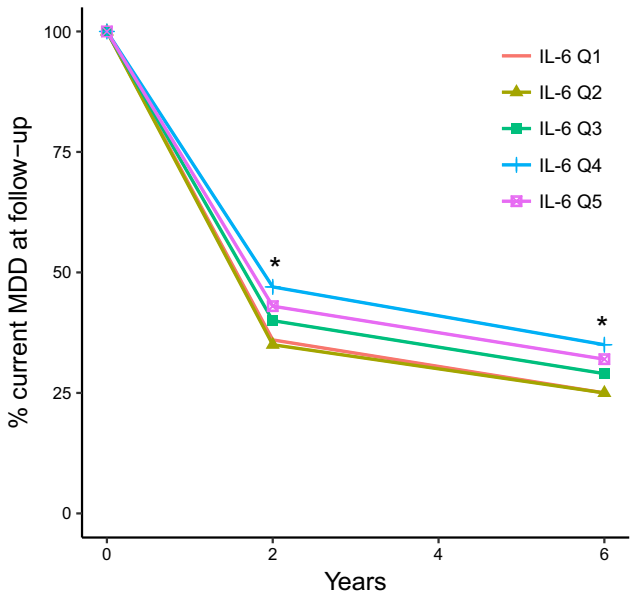

Figure 2. Baseline interleukin-6 (IL-6) quintiles (Q1–Q5) predicting major depressive disorder (MDD) at follow-up. Models include baseline outcome as well for illustrative purposes. *Main effect of group ($p < .05$).

In the cross-sectional findings, where we combined the three waves of data (with >6000 observations), we found associations with depression diagnosis and symptoms only for IL-6—but not for CRP. The null finding for CRP is also in contrast to the meta-analysis of CRP (1), but notably in this meta-analysis the effect size in cases with clinical depression versus control cases for CRP was substantially lower than that for IL-6 (d_{CRP} = 0.26, 95% CI = 0.11–0.40; $d_{IL\text{-}6}$ = 0.71, 95% CI = 0.46–0.97). The differences in findings for IL-6 and CRP may contrast with what might be expected given that IL-6 induces CRP production. In our sample, however, Spearman rank correlations between IL-6 and CRP were not high, ranging from .33 to .43 across waves, slightly lower than a correlation of .54 reported in a sample of MDD cases (26). Relevant to note is that post-transcriptional mechanisms seem to exist that modulate CRP expression beyond the downstream effect of IL-6, such as RNA-binding protein human antigen R (27), and could contribute to divergent IL-6 and CRP associations. The effect size for IL-6 we observed in the current study of $d = 0.07$ is much smaller than the effect size reported by Howren *et al.* (1), but this may be explained by our study's having a mixed sample of cases recruited from the community, primary care, and secondary care, as opposed to studies that include only clinical cases. In community samples, Howren *et al.* reported a significant effect size for IL-6 of $d = 0.09$ (95% CI = 0.04–0.15), which is much closer to our finding. In addition, the smaller effect sizes can also be the result of more stringent correction for potential confounders in our analyses, which was recently also demonstrated in a large tiered meta-analysis of CRP and depression (28). Correction for BMI could be viewed as an overcorrection because it may be a mediator rather than a confounder; however, we added BMI in a separate step of the analyses, and it did not seem to have much of an impact on results. Another explanation for finding an association only for IL-6 but not for CRP comes from a recent work, including a

meta-analysis, showing stress-related increases in IL-6 levels but not in CRP levels (29,30). In addition, in gene expression work, IL-6 pathways were found to be upregulated in depression versus controls (4), which may point to this pathway as the root of the inflammation in depression and therefore most pronouncedly present as opposed to more downstream markers such as CRP. The results from cross-sectional analyses combining three waves are also in contrast to earlier analyses of the baseline data (20) given that no significant gender interactions with depression status or marked effects of antidepressants were observed.

As opposed to previous longitudinal literature that observed CRP to predict subsequent depressive symptoms, we did not find that CRP predicted depression, nor did we find that depression predicted subsequent CRP levels. However, we found baseline current depression and higher depression severity to be associated with higher IL-6 at follow-up. Moreover, we found that among MDD cases, those having higher baseline levels of IL-6 were at greater risk for a chronic course of depression. This latter finding is in line with observations that low-grade inflammation is associated with treatment-resistant depression (31,32) and with poor treatment response to antidepressants (33,34); this could have implications for personalized psychiatry, such as in patient profiling, especially because the effect was independent of baseline depression severity. Nevertheless, these results, and results of other longitudinal studies on depression and inflammation, are mixed and do not give an unequivocal answer on pathways and direction of effects. In addition, the moderating effects of gender require more investigation. The observed effect seemed limited to women, but the gender interaction within the group with high IDS scores (≥14) failed to demonstrate a significant moderation effect, nor were there any significant gender interaction terms in any of the other analyses. Despite this, and despite the effect sizes observed here being small, the observed associations of depression with the inflammatory system may nevertheless be relevant to treatment.

The inflammatory system has been a target in depression treatment trials, such as in adjuvant nonsteroidal anti-inflammatory drug administration (35). However, it has been observed that not all patients with depression exhibit increased inflammation. There is large heterogeneity, such as in symptom presentation, clearly demonstrated by the calculation by Galatzer-Levy and Bryant that there are 227 different symptom profiles that meet diagnostic criteria for MDD (36). Of the symptoms, particularly atypical symptoms of depression are linked to increased levels of inflammatory markers (37). Furthermore, post hoc analyses of randomized controlled trials evaluating therapies that affect the inflammatory system (e.g., add-on tumor necrosis factor alpha antagonist, add-on bupropion, exercise therapy) seem to suggest that these are specifically effective in persons with depression with high baseline levels of inflammatory markers (38–40). A post hoc analysis of two anti-IL-6 antibodies in rheumatoid arthritis and multicentric Castleman's disease suggested improvement in depressive symptoms after adjusting for the improvement in the underlying diseases (41). Atypical forms of depression cluster not only with inflammation but also with obesity (42,43). In line with this, a recent study reporting that treatment-resistant depression was associated with increased levels of CRP also reported that both treatment-resistant depression and inflammation were also associated with obesity and with symptoms of fatigue and sleep disturbance (32). BMI may therefore be an easier patient stratification method in treatment trials (44,45). More research is needed to optimize patient profiling and patient stratification methods that capture this so-called immunometabolic form (37) of depression for such treatments in order to improve personalized medicine for patients with depression.

Moderately elevated levels of IL-6 and CRP have generally been interpreted as a state of chronic low-grade inflammation. While there thus are some indications that stratification by baseline levels of inflammatory markers may be helpful in clinical practice, it should be noted that IL-6 and CRP are not only proinflammatory; they exert anti-inflammatory effects as well and play a role in tissue maintenance and repair and in immune readiness (46).

When interpreting the results of this study, some limitations need to be taken into account. Different kits were used for the baseline assays; however, in the longitudinal analyses, this was taken into account by using an analysis of covariance approach (correcting for baseline). In addition, we choose not to correct for plate because samples of cases and controls were randomly distributed on plates, and plates were standardized using a positive and negative control that was included on each plate. While loss to follow-up was relatively low, it could have biased our estimates. However, the statistical models employed maximum likelihood methods that can account for missing data. While it would be interesting to evaluate whether antidepressants are immunosuppressants or are a modifying factor in the associations of IL-6 with chronicity, the naturalistic design of our study did not allow for such analyses (e.g., no pretreatment and posttreatment assays of inflammatory markers, confounding by indication). However, Strawbridge *et al.* (31) reported in their meta-analysis that, regardless of treatment response, antidepressant treatment seems to reduce IL-6 levels. They also reported that elevated inflammatory markers predicted a poorer response to antidepressants, and nonresponders to antidepressant treatments had persistently elevated inflammatory markers (31). Strengths of the study include the large and well-phenotyped sample making it possible to look at characteristics and subtypes. To date, this is one of the largest studies on longitudinal effects of depression and inflammation. Besides evaluating effects of inflammation on subsequent depression, we also evaluated the opposite direction—baseline diagnostic status predicting inflammation.

This study shows that IL-6, but not CRP, seems to be linked to depression, albeit with small effect sizes. Cross-sectional analyses showed associations of depression diagnoses and symptoms with IL-6. In addition, current MDD and higher symptom severity predicted IL-6 at follow-up, while higher IL-6 in women with current depression at baseline was predictive of a more chronic course. The results highlight the importance of investigating IL-6 as a potential marker for patient profiling in personalized medicine approaches to improve personalized medicine for patients with depression.

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According to European law (EU General Data Protection Regulation), data containing potentially identifying or sensitive patient information are restricted; our data involving clinical participants are not freely available in a public repository. However, data are available on request via the NESDA Data Access Committee (nesda@ggzingeest.nl).

ARTICLE INFORMATION

From the Department of Psychiatry (FL, YM, JHS, BWJHP), Amsterdam Public Health Research Institute, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, and Department of Psychiatry (RAS), University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; and Janssen Research & Development (GW), Titusville, New Jersey.

Address correspondence to Femke Lamers, Ph.D., Oldenaller 1, 1081 HJ Amsterdam, The Netherlands; E-mail: f.lamers@vumc.nl.

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REFERENCES

1. Howren MB, Lamkin DM, Suls J (2009): Associations of depression with C-reactive protein, IL-1, and IL-6: A meta-analysis. Psychosom Med 71:171–186.
2. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctot KL (2010): A meta-analysis of cytokines in major depression. Biol Psychiatry 67:446–457.
3. Liu Y, Ho RC-M, Mak A (2012): Interleukin (IL)-6, tumour necrosis factor alpha (TNF-α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: A meta-analysis and meta-regression. J Affect Disord 139:230–239.
4. Jansen R, Penninx BWJH, Madar V, Xia K, Milaneschi Y, Hottenga JJ, *et al.* (2016): Gene expression in major depressive disorder. Mol Psychiatry 21:339–347.
5. Mostafavi S, Battle A, Zhu X, Potash JB, Weissman MM, Shi J, *et al.* (2014): Type I interferon signaling genes in recurrent major depression: Increased expression detected by whole-blood RNA sequencing. Mol Psychiatry 19:1267–1274.
6. Leday GGR, Vértes PE, Richardson S, Greene JR, Regan T, Khan S, *et al.* (2018): Replicable and coupled changes in innate and adaptive immune gene expression in two case-control studies of blood microarrays in major depressive disorder. Biol Psychiatry 83:70–80.
7. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S (2011): The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta 1813:878–888.
8. Sproston NR, Ashworth JJ (2018): Role of C-reactive protein at sites of inflammation and infection. Front Immunol 9:754.
9. Raison CL, Capuron L, Miller AH (2006): Cytokines sing the blues: Inflammation and the pathogenesis of depression. Trends Immunol 27:24–31.
10. Raison CL, Miller AH (2013): The evolutionary significance of depression in pathogen host defense (PATHOS-D). Mol Psychiatry 18:15–37.
11. Valkanova V, Ebmeier KP, Allan CL (2013): CRP, IL-6 and depression: A systematic review and meta-analysis of longitudinal studies. J Affect Disord 150:736–744.
12. Smith KJ, Au B, Ollis L, Schmitz N (2018): The association between C-reactive protein, interleukin-6 and depression among older adults in the community: A systematic review and meta-analysis. Exp Gerontol 102:109–132.
13. Pasco JA, Nicholson GC, Williams LJ, Jacka FN, Henry MJ, Kotowicz MA, *et al.* (2010): Association of high-sensitivity C-reactive protein with de novo major depression. Br J Psychiatry 197:372–377.
14. Copeland WE, Shanahan L, Worthman C, Angold A, Costello EJ (2012): Cumulative depression episodes predict later C-reactive protein levels: A prospective analysis. Biol Psychiatry 71:15–21.
15. Khandaker GM, Pearson RM, Zammit S, Lewis G, Jones PB (2014): Association of serum interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult life: A population-based longitudinal study. JAMA Psychiatry 71:1121–1128.
16. Glaus J, von Känel R, Lasserre AM, Strippoli M-PF, Vandeleur CL, Castelao E, *et al.* (2018): Mood disorders and circulating levels of inflammatory markers in a longitudinal population-based study. Psychol Med 48:961–973.
17. Lamers F, Hoogendoorn AW, Smit JH, Van Dyck R, Zitman FG, Nolen WA, Penninx BWJH (2012): Socio-demographic and psychiatric determinants of attrition in the Netherlands Study of Depression and Anxiety (NESDA). Compr Psychiatry 53:63–70.
18. Penninx BWJH, Beekman ATF, Smit JH, Zitman FG, Nolen WA, Spinhoven P, *et al.* (2008): The Netherlands Study of Depression and Anxiety (NESDA): Rationale, objectives and methods. Int J Methods Psychiatr Res 17:121–140.
19. World Health Organization (1997): CIDI-Auto, Version 2.1: Administrator's Guide. Sydney, New South Wales, Australia: World Health Organization.
20. Vogelzangs N, Duivis HE, Beekman ATF, Kluft C, Neuteboom J, Hoogendijk W, *et al.* (2012): Association of depressive disorders, depression characteristics and antidepressant medication with inflammation. Transl Psychiatry 2:e79.
21. Verduijn J, Milaneschi Y, Schoevers RA, van Hemert AM, Beekman ATF, Penninx BWJH (2015): Pathophysiology of major depressive disorder: Mechanisms involved in etiology are not associated with clinical progression. Transl Psychiatry 5:e649.
22. Rush AJ, Gullion CM, Basco MR, Jarrett RB, Trivedi MH (1996): The Inventory of Depressive Symptomatology (IDS): Psychometric properties. Psychol Med 26:477–486.
23. Babor TF, Kranzler HR, Lauerman RJ (1989): Early detection of harmful alcohol consumption: Comparison of clinical, laboratory, and self-report screening procedures. Addict Behav 14:139–157.
24. Twisk J (2006): Applied Multilevel Analysis: A Practical Guide for Medical Researchers. Cambridge, UK: Cambridge University Press.
25. Galic S, Oakhill JS, Steinberg GR (2010): Adipose tissue as an endocrine organ. Mol Cell Endocrinol 316:129–139.
26. Felger JC, Haroon E, Patel TA, Goldsmith DR, Wommack EC, Woolwine BJ, *et al.* (2018): What does plasma CRP tell us about peripheral and central inflammation in depression? [published online ahead of print Jun 12]. Mol Psychiatry.
27. Kim Y, Noren Hooten N, Dluzen DF, Martindale JL, Gorospe M, Evans MK (2015): Posttranscriptional regulation of the inflammatory marker C-reactive protein by the RNA-binding protein HuR and microRNA 637. Mol Cell Biol 35:4212–4221.
28. Horn SR, Long MM, Nelson BW, Allen NB, Fisher PA, Byrne ML (2018): Replication and reproducibility issues in the relationship between C-reactive protein and depression: A systematic review and focused meta-analysis. Brain Behav Immun 73:85–114.
29. Lima BB, Hammadah M, Wilmot K, Pearce BD, Shah A, Levantsevych O, *et al.* (2019): Posttraumatic stress disorder is associated with enhanced interleukin-6 response to mental stress in subjects with a recent myocardial infarction. Brain Behav Immun 75:26–33.
30. Marsland AL, Walsh C, Lockwood K, John-Henderson NA (2017): The effects of acute psychological stress on circulating and stimulated

inflammatory markers: A systematic review and meta-analysis. Brain Behav Immun 64:208–219.

31. Strawbridge R, Arnone D, Danese A, Papadopoulos A, Herane Vives A, Cleare AJ (2015): Inflammation and clinical response to treatment in depression: A meta-analysis. Eur Neuropsychopharmacol 25:1532–1543.
32. Chamberlain SR, Cavanagh J, de Boer P, Mondelli V, Jones DNC, Drevets WC, *et al.* (2019): Treatment-resistant depression and peripheral C-reactive protein. Br J Psychiatry 214:11–19.
33. Uher R, Tansey KE, Dew T, Maier W, Mors O, Hauser J, *et al.* (2014): An inflammatory biomarker as a differential predictor of outcome of depression treatment with escitalopram and nortriptyline. Am J Psychiatry 171:1278–1286.
34. Vogelzangs N, Beekman AT, van Reedt Dortland AK, Schoevers RA, Giltay EJ, de Jonge P, Penninx BW (2014): Inflammatory and metabolic dysregulation and the 2-year course of depressive disorders in antidepressant users. Neuropsychopharmacology 39:1624–1634.
35. Kohler O, Benros ME, Nordentoft M, Farkouh ME, Iyengar RL, Mors O, Krogh J (2014): Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse effects: A systematic review and meta-analysis of randomized clinical trials. JAMA Psychiatry 17:1381–1391.
36. Galatzer-Levy IR, Bryant RA (2013): 636,120 Ways to have posttraumatic stress disorder. Perspect Psychol Sci 8:651–662.
37. Lamers F, Milaneschi Y, de Jonge P, Giltay EJ, Penninx BWJH (2018): Metabolic and inflammatory markers: Associations with individual depressive symptoms. Psychol Med 48:1102–1110.
38. Rethorst CD, Moynihan J, Lyness JM, Heffner KL, Chapman BP (2011): Moderating effects of moderate-intensity physical activity in the relationship between depressive symptoms and interleukin-6 in primary care patients. Psychosom Med 73:265–269.
39. Jha MK, Minhajuddin A, Gadad BS, Greer T, Grannemann B, Soyombo A, *et al.* (2017): Can C-reactive protein inform antidepressant medication selection in depressed outpatients? Findings from the CO-MED trial. Psychoneuroendocrinology 78:105–113.
40. Raison CL, Rutherford RE, Woolwine BJ, Shuo C, Schettler P, Drake DF, *et al.* (2013): A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: Role of baseline inflammatory biomarkers. JAMA Psychiatry 70:31–41.
41. Sun Y, Wang D, Salvadore G, Hsu B, Curran M, Casper C, *et al.* (2017): The effects of interleukin-6 neutralizing antibodies on symptoms of depressed mood and anhedonia in patients with rheumatoid arthritis and multicentric Castleman's disease. Brain Behav Immun 66:156–164.
42. Lamers F, de Jonge P, Nolen WA, Smit JH, Zitman FG, Beekman AT, Penninx BW (2010): Identifying depressive subtypes in a large cohort study: Results from the Netherlands Study of Depression and Anxiety (NESDA). J Clin Psychiatry 71:1582–1589.
43. Levitan RD, Davis C, Kaplan AS, Arenovich T, Phillips DI, Ravindran AV (2012): Obesity comorbidity in unipolar major depressive disorder: Refining the core phenotype. J Clin Psychiatry 73:1119–1124.
44. Jha MK, Wakhlu S, Dronamraju N, Minhajuddin A, Greer TL, Trivedi MH (2018): Validating pre-treatment body mass index as moderator of antidepressant treatment outcomes: Findings from CO-MED trial. J Affect Disord 234:34–37.
45. Uher R, Mors O, Hauser J, Rietschel M, Maier W, Kozel D, *et al.* (2009): Body weight as a predictor of antidepressant efficacy in the GENDEP project. J Affect Disord 118:147–154.
46. Del Giudice M, Gangestad SW (2018): Rethinking IL-6 and CRP: Why they are more than inflammatory biomarkers, and why it matters. Brain Behav Immun 70:61–75.