

Meta-analysis

Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies


Köhler CA, Freitas TH, Maes M, de Andrade NQ, Liu CS, Fernandes BS, Stubbs B, Solmi M, Veronese N, Herrmann N, Raison CL, Miller BJ, Lanctôt KL, Carvalho AF. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies.

Objective: To conduct a systematic review and meta-analysis of studies that measured cytokine and chemokine levels in individuals with major depressive disorder (MDD) compared to healthy controls (HCs).

Method: The PubMed/MEDLINE, EMBASE, and PsycINFO databases were searched up until May 30, 2016. Effect sizes were estimated with random-effects models.

Result: Eighty-two studies comprising 3212 participants with MDD and 2798 HCs met inclusion criteria. Peripheral levels of interleukin-6 (IL-6), tumor necrosis factor (TNF)-alpha, IL-10, the soluble IL-2 receptor, C-C chemokine ligand 2, IL-13, IL-18, IL-12, the IL-1 receptor antagonist, and the soluble TNF receptor 2 were elevated in patients with MDD compared to HCs, whereas interferon-gamma levels were lower in MDD (Hedge's $g = -0.477$, $P = 0.043$). Levels of IL-1 β , IL-2, IL-4, IL-8, the soluble IL-6 receptor (sIL-6R), IL-5, CCL-3, IL-17, and transforming growth factor-beta 1 were not significantly altered in individuals with MDD compared to HCs. Heterogeneity was large ($I^2: 51.6-97.7\%$), and sources of heterogeneity were explored (e.g., age, smoking status, and body mass index).

Conclusion: Our results further characterize a cytokine/chemokine profile associated with MDD. Future studies are warranted to further elucidate sources of heterogeneity, as well as biosignature cytokines secreted by other immune cells.

C. A. Köhler^{1,*}, T. H. Freitas^{1,*},
M. Maes^{2,3,4,5,6},
N. O. de Andrade¹, C. S. Liu^{7,8},
B. S. Fernandes^{2,9},
B. Stubbs^{10,11}, M. Solmi^{12,13},
N. Veronese^{12,14},
N. Herrmann^{8,15},
C. L. Raison^{16,17}, B. J. Miller¹⁸,
K. L. Lanctôt^{7,8,15},
A. F. Carvalho¹ 

¹Translational Psychiatry Research Group and Department of Clinical Medicine, Faculty of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil, ²Deakin University, IMPACT Strategic Research Centre, School of Medicine, Geelong, Australia, ³Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, ⁴Department of Psychiatry, Faculty of Medicine, State University of Londrina, Londrina, PR, Brazil, ⁵Department of Psychiatry, Medical University Plovdiv, Plovdiv, Bulgaria, ⁶Revitalis, Waalre, The Netherlands, ⁷Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada, ⁸Neuropsychopharmacology Research Group, Hurvitz Brain Sciences Program Sunnybrook Research Institute, Toronto, ON, Canada, ⁹Laboratory of Calcium Binding Proteins in the Central Nervous System, Department of Biochemistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil, ¹⁰Physiotherapy Department, South London and Maudsley NHS Foundation Trust, Denmark Hill, London, UK, ¹¹Health Service and Population Research Department, Institute of Psychiatry, Psychology and Neuroscience (IoPPN), King's College London, London, UK, ¹²Department of Neurosciences, University of Padova, Padova, Italy, ¹³Institute of Clinical Research and Education in Medicine (IREM), Padova, Italy, ¹⁴Department of Medicine, DIMED, Geriatrics Section, University of Padova, Padova, Italy, ¹⁵Department of Psychiatry, University of Toronto, Toronto, ON, Canada, ¹⁶Department of Human Development and Family Studies, School of Human Ecology, University of Wisconsin-Madison, Madison, WI, USA, ¹⁷Department of Psychiatry, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI, USA and ¹⁸Department of Psychiatry & Health Behavior, Augusta University, Augusta, GA, USA

Key words: cytokines; chemokines; inflammation meta-analysis; depression

André F. Carvalho, Department of Clinical Medicine, Faculty of Medicine, Federal University of Ceará, Rua Prof. Costa Mendes, 1608, 4° andar, 60430-040, Fortaleza, CE, Brazil.
E-mail: andrefc7@terra.com.br or andrefc7@hotmail.com

*Contributed equally as first authors.

Accepted for publication December 29, 2016

Summations

- Evidence indicates that peripheral immune activation may be involved in the pathophysiology of major depressive disorder.
- Herein, we conducted an updated meta-analytic review of 82 studies that measured cytokines and/or chemokines in individuals with major depressive disorder and healthy controls.
- Levels of IL-6, TNF- α , 10, the soluble IL-2 receptor, C-C chemokine ligand 2, IL-13, IL-18, IL-12, the IL-1 receptor antagonist, and the soluble TNF receptor 2 were elevated, whereas interferon- γ levels were reduced in individuals with major depressive disorder compared to controls. These results add in the characterization of a putative cytokine/chemokine profile for major depressive disorder.

Considerations

- A large degree of heterogeneity was evident in this literature. Potential sources of heterogeneity were not consistently reported across included studies.
- Methodological quality has varied across included studies.

Introduction

In the past two decades, an increasing body of evidence indicates that aberrations in immune-inflammatory pathways and activation of cell-mediated immunity represent important pathophysiological pathways for the development of major depressive disorder (MDD) (1, 2). In addition, converging experimental and clinical research points that reciprocal neuroimmune interactions may contribute to the neurobiology of MDD (3, 4). A low-grade inflammatory response characterized by increased numbers of granulocytes and monocytes (1), as well as the elevated levels of acute phase reactants (e.g., C-reactive protein and haptoglobin) (5, 6), inflammatory cytokines (7), and possibly chemokines (8), has been demonstrated in groups of individuals with MDD compared to healthy controls (HCs). These peripheral immune abnormalities may influence brain function through several mechanisms. For example, evidence indicates that cytokines may cross the blood-brain barrier, while certain cytokines (e.g., IL-1 β) may convey signals to the brain via afferent nerves like the vagus (2, 4). The pathophysiological role of proinflammatory

cytokines in MDD is further supported by preclinical research indicating that proinflammatory cytokines may promote depressive-like behaviours, whereas TNF- α and IL-6 receptor knockout mice exhibit resilience to stress-induced depressive-like behaviours (9–11). In addition, a recent meta-analysis estimates that ~25% of patients with chronic hepatitis C develop depression after treatment with the proinflammatory cytokine interferon- α (IFN- α) (12). The common denominator among these findings is that peripheral immune dysregulation may represent an important pathway for inducing functional and structural brain changes that underpin the pathophysiology of MDD. Perhaps as a consequence of this, peripheral inflammatory mediators have emerged as promising candidate biomarkers for MDD (13), although evidence of bias may limit inferences derived from the literature on peripheral biomarkers for MDD (14).

A meta-analysis that included 24 studies provided evidence that peripheral levels of tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6) are significantly elevated in individuals with MDD compared to healthy controls (HCs) (7). However,

between-study heterogeneity for these estimates was high (7). A more recent cumulative meta-analysis confirmed that peripheral levels of IL-6 are elevated in individuals with MDD and HCs, whereas no consistent evidence of changes in TNF- α and IL-1 β in patients with MDD compared to controls was found (5). This significant degree of heterogeneity could be explained by a number of factors including but not limited to the following: differences in assay methods across laboratories, medication status, and potential confounders (e.g., body mass index and smoking). Furthermore, it has been increasingly recognized that the phenotypic heterogeneity of MDD may contribute to discrepant findings. For example, melancholic depression is associated with elevated HPA axis activity (15, 16), whereas individuals with atypical depression appear to have higher levels of proinflammatory markers (16). In addition, each individual cytokine/chemokine may have different functions relevant to the pathophysiology of MDD. For example, some cytokines/chemokines are predominantly proinflammatory, whereas others are mainly anti-inflammatory, and some of these immune mediators have been increasingly implicated in neuroplasticity mechanisms (17, 18). Therefore, the characterization of peripheral levels of a wider array of cytokines and chemokines may be of particular relevance to this field.

Aims of the study

Since the publication of these previous meta-analyses (5, 7, 19, 20), additional studies have been conducted examining a wider range of immune biomarkers. Therefore, the aims of this large, collaborative meta-analysis were to investigate differences in peripheral levels of a wider range of cytokines and chemokines among individuals with major depressive disorder compared to healthy controls and to explore potential sources of heterogeneity across studies. We anticipated that the large number of new studies would allow for a more precise characterization of the role of cytokines and chemokines as peripheral biomarkers for major depressive disorder.

Methods

This study comprised a between-group meta-analysis of studies that compared cytokine or chemokine levels between adults with MDD and healthy controls. We complied with the Preferred Reported Items for Systematic Reviews and Meta-analysis (PRISMA) statement (21). The literature search, title/abstract screening, final decision on eligibility after full-text review, and data extraction were independently performed by two investigators. An *a priori* defined yet unpublished protocol was followed.

Search strategy

A systematic search was conducted in the PubMed/MEDLINE, EMBASE, and PsycINFO databases from inception up until May 30, 2016. The detailed search strings used in this review are presented in the supporting information that accompanies the online version of this article. This search strategy was augmented through tracking the citation of included articles in Google Scholar (22).

Study selection

We included original references published in any language. Eligible studies had to measure peripheral cytokine or chemokine levels in adult subjects (age \geq 18 years old) that met either DSM (23) or ICD (24) criteria for MDD, and a comparison group of healthy controls (HCs). The following exclusion criteria were adopted: (i) studies that reported that participants had medical and/or psychiatric comorbidities were excluded (except current smoking); (ii) studies that included pregnant women or women in the postpartum period; (iii) case reports or case series ($N < 10$); (iv) studies that assayed the mediators in specimens/tissues other than blood; and (v) studies in animals. The authors of meeting abstracts that met inclusion criteria were contacted by e-mail to provide data for analysis (no additional data were provided).

Data extraction

For each cytokine/chemokine, we extracted the means, variance estimates [standard deviation (SD), standard error of the mean (SEM), or 95% confidence interval (CI)], and sample sizes for both MDD and HC groups. From studies that presented only results of the comparison of the MDD and HC groups, we extracted the appropriate measure (z-score or t-score). In studies that provided median \pm IQR or median \pm range, we estimated the mean \pm SD following a standard method (25). We also extracted the following data whenever available: (i) first author, (ii) publication year, (iii) gender distribution of study sample (% females), (iv) mean age and BMI, (v) mean illness duration (years), (vi) treatment status (drug-free during assessment and/or treatment-naïve), (vii) percentage of the sample with atypical and/or melancholic depression, (viii) measurement of depressive symptoms, and (ix) % of current smokers.

Methodological quality of included studies

We devised a score to estimate the methodological quality of each study based on the following

parameters: (i) study sample ≥ 50 participants (1 = Yes; 0 = No); (ii) Did the study control results for potential confounders (e.g., age, BMI, gender, race)? (1 = Y; 0 = No); (iii) Were participants with MDD and HCs age- and gender-matched? (1 = Y; 0 = No); (iv) Was the time of sample collection specified? (e.g., morning vs. evening) (1 = Y; 0 = No); (v) Were participants with MDD free of antidepressant drugs during sample collection? (1 = Y; 0 = No); and (vi) Reporting of either the manufacturer of the test or its parameters (detection limit and coefficient of variation) (1 = Y; 0 = No). Thus, the score may vary from 0 to 6, with higher scores indicating better methodological quality.

Statistical analysis

Because studies used different measurement methods, we estimated a standardized mean difference and 95% CI (Hedges's g) for each immune mediator, which provides an unbiased effect size (ES) adjusted for small sample sizes (26). We assessed the heterogeneity across studies using the Cochran Q test, which provides a weighted sum of the squares of the deviations of individual study ES estimates from the overall estimate. In addition, heterogeneity across studies was quantified with the I^2 statistic, which indicates the percentage of total variation across several studies due to heterogeneity and which is considered high when $\geq 50\%$ (27). We anticipated a high degree of heterogeneity. Therefore, we pooled ES using a random-effects model according to the DerSimonian and Laird method (28). Meta-analyses were conducted only for immune mediators with at least three individual datasets.

Studies with statistically non-significant (i.e., negative) results are less likely to be published than studies with significant results (14, 29). To assess publication bias, we inspected a funnel plot graph for asymmetry and calculated the Egger's regression test for funnel plot asymmetry (30). Evidence of small-study effects (indicative of publication bias) was considered when the P -value of the Egger's test was <0.1 , and the ES of the largest study was more conservative or changed direction when compared with the overall ES estimate (funnel plots of ES estimates in which evidence of publication bias was observed are illustrated in Figs S10–S14) (14). The trim-and-fill procedure was used to estimate the ES adjusting for publication bias (31), while the fail-safe N (i.e., the file drawer statistic) was used to determine how many additional studies would be necessary to turn a significant ES non-significant (32).

We explored potential sources of heterogeneity across studies for each ES estimate, using either

subgroup (if there were at least three studies in each subgroup) or random-effects meta-regression analyses. Meta-regression analyses were conducted only when at least 10 studies provided moderator; this decision was made *a priori* because with fewer datasets, this analytic tool may provide spurious results (33). The following variables were considered in meta-regression analyses: sample size, mean age of MDD group, mean age of the HC group, differences in mean age (MDD group *minus* HC group), mean body mass index (BMI) of MDD group, mean BMI of the HC group, differences in mean BMI (MDD group *minus* HC group), % of females in the MDD group, % of females in the HC group, difference in % of females (MDD group *minus* HC group), % of current smokers, latitude of the country where the study was executed, depression severity (expressed as a percentage of the cutoff for severe depression in the rating scale), methodological quality of each included study, and mean illness duration in years. Studies were weighted in such a way that investigations with more precise parameters (indicated by sample size and 95% CI) had more influence in meta-regression analyses (34). For statistically significant ES estimates, we performed sensitivity analyses in which we excluded each study from analyses to verify whether a single study turned results non-significant or otherwise changed the direction of the ES. In addition, cumulative meta-analysis was performed for significant ES with at least 10 datasets.

All analyses were conducted in Stata MP software version 14.0 (Stata Corp, College Station, TX, USA) using the metan package. Statistical significance was considered at an alpha level of 0.05.

Results

Study selection

Following removal of duplicates, the title/abstracts of 4911 unique references were screened for eligibility. A total of 4432 references were excluded, while 479 full texts were retrieved and screened for eligibility. Of those articles, 397 were excluded (see Table S1 for reasons for exclusion). Finally, 82 original studies met inclusion criteria, which provided data from 6010 participants (3212 participants with MDD and 2798 HCs). Figure 1 provides the PRISMA flowchart for study selection.

Characteristics and methodological quality of included studies

Of the 82 studies included in our meta-analysis, in 43 studies (52.4%), participants with MDD and HCs were age- and gender-matched, while 35

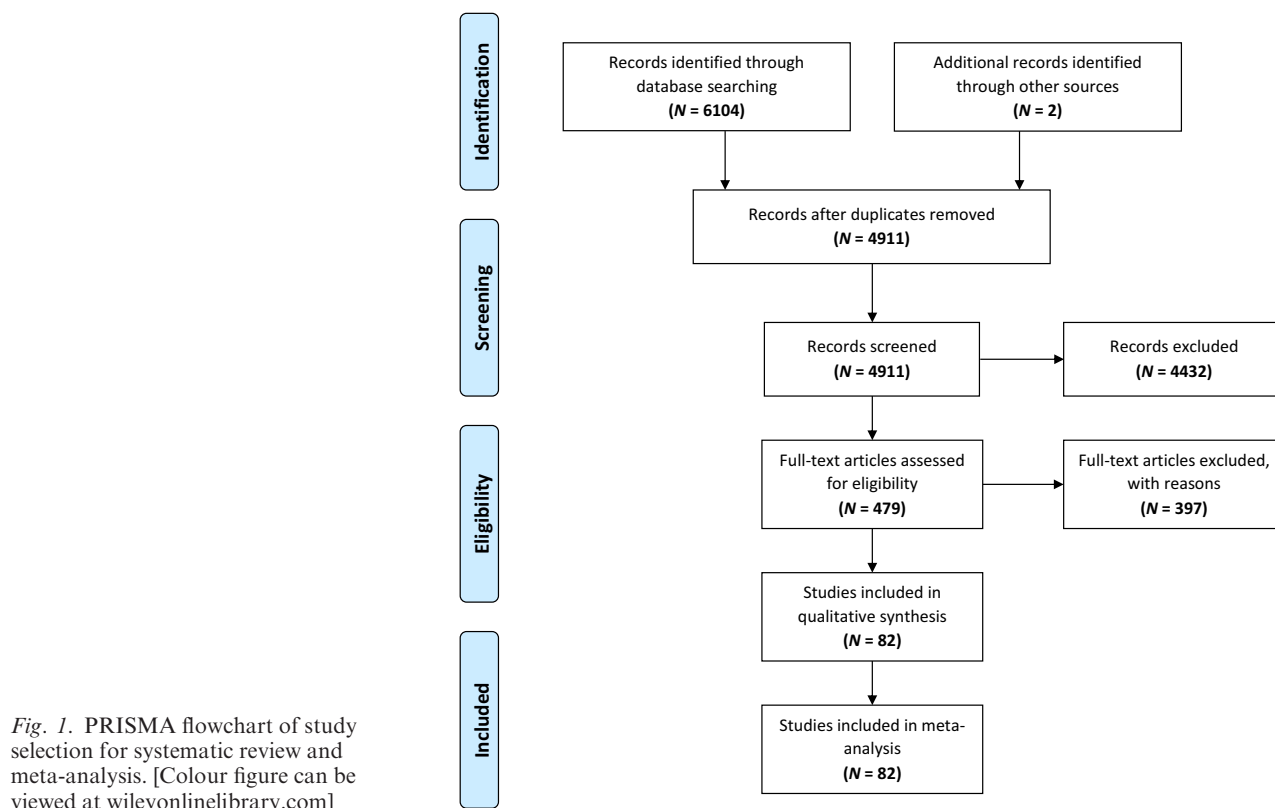


Fig. 1. PRISMA flowchart of study selection for systematic review and meta-analysis. [Colour figure can be viewed at wileyonlinelibrary.com]

studies (42.7%) adjusted results for potential confounders (e.g., age, gender distribution, depressive symptom scores, or BMI). In addition, most studies ($k = 81$; 98.8%) either reported the manufacturer of the assay or provided values of the coefficient of variation (CV) of the test. In addition, most studies ($k = 65$; 79.3%) provided data regarding medication status, whereas three (3.7%) included only treatment-naïve (i.e., never treated with antidepressants) participants with MDD. Finally, most studies did not provide information on illness duration ($k = 56$; 68.3%); the remaining studies included MDD participants with illness duration of 2.97 ± 6.15 (mean \pm SD) years. The methodological quality scores of each study varied from 1 to 6 (median: 4) (Table S2).

Studies of IL-6

IL-6 measurements were extracted from 42 studies (1587 cases and 1183 controls). Participants with MDD had higher concentrations compared to HCs ($g = 0.621$; $P < 0.001$; Table 1 and Fig. 2a). No evidence of small-study effects (which provides an indication of publication bias) was observed. Possible sources for the large heterogeneity ($I^2 = 64.9\%$) were explored using meta-regression and subgroup analyses (Tables S3 and S4). In meta-regression analyses, differences in gender

distribution (% females) in the MDD and HC groups emerged as a significant moderator ($P = 0.046$). Subgroup analyses showed that heterogeneity was smaller in studies that measured IL-6 in serum and whole blood samples compared to plasma, while results suggest that the measurement of IL-6 with ELISA is associated with higher heterogeneity compared to other types of assay.

Of 42 studies that measured IL-6, 10 provided adjusted differences in peripheral levels of this cytokine to confounders (e.g., age, gender, BMI, smoking, among other variables specific to the study) (35–44). We re-calculated this ES considering those adjusted values. Then, the overall ES of IL-6 was 0.543 (95% CI = 0.435–0.651; $P < 0.001$). The I^2 value was 49.8% ($P < 0.001$). In addition, we performed a subgroup analysis considering studies which did vs. did not adjust comparisons to confounders. The overall ES for the unadjusted studies was 0.574 (95% CI = 0.437–0.711; $P < 0.001$; $k = 32$), with an I^2 of 52.3% ($P < 0.001$). The overall ES for the adjusted studies was 0.467 (95% CI = 0.303–0.631; $P < 0.001$; $k = 10$), with a I^2 of 38.6% ($P = 0.101$). Therefore, studies that adjusted to potential confounders had a lower degree of heterogeneity.

In sensitivity analysis, the exclusion of any individual study from the analysis did not alter the direction or statistical significance of the ES

Table 1. Primary meta-analyses of studies measuring peripheral cytokines and chemokines in individuals with MDD vs. healthy controls

Mediator	<i>N</i> Studies	<i>N</i> MDD	<i>N</i> Controls	ES (95% CI)	<i>P</i> -value (overall)*	<i>I</i> ²	<i>P</i> -value (Egger)†	Small-study effects‡	Fail-safe <i>N</i>	Adjusted ES (95% CI)§
IL-6	42	1587	1183	0.621 (0.486–0.755)	<0.001	64.9	0.950	N	2497	0.621 (0.486–0.755)
TNF-α	42	1620	1457	0.675 (0.431–0.919)	<0.001	90.0	0.009	Y	2431	0.675 (0.431–0.919)
IL-1β	22	779	727	0.032 (–0.291–0.354)	0.847	89.3	0.180	N	0	–0.152 (–0.477–0.173)
IFN-γ	17	700	770	–0.477 (–0.939 to –0.015)	0.043	94.0	<0.001	Y	95	–0.477 (–0.939 to –0.015)
IL-10	17	608	675	0.375 (0.008–0.742)	0.045	89.2	0.277	N	107	0.375 (0.008–0.742)
IL-2	10	357	476	–0.108 (–0.900–0.683)	0.789	95.8	0.918	N	1	–0.108 (–0.900–0.683)
IL-4	10	350	450	–0.533 (–1.073–0.007)	0.053	91.0	0.085	Y	41	–0.533 (–1.073–0.007)
sIL-2 receptor	10	489	391	0.735 (0.418–1.052)	<0.001	77.5	0.741	N	224	0.735 (0.418–1.052)
CCL-2	8	285	287	1.718 (0.641–2.794)	0.002	96.3	0.044	Y	144	1.718 (0.641–2.794)
IL-8	7	306	217	0.032 (–0.346–0.410)	0.869	76.9	0.229	N	0	0.032 (–0.346–0.410)
sIL-6 receptor	7	344	256	0.330 (–0.008–0.667)	0.055	71.3	0.115	N	12	0.330 (–0.008–0.667)
IL-13	6	243	373	1.836 (0.812–2.861)	<0.001	96.0	0.009	Y	255	1.432 (0.445–2.418)
IL-18	5	135	143	1.720 (0.379–3.062)	0.012	95.3	0.105	N	114	1.720 (0.379–3.062)
IL-12	4	135	301	1.229 (0.275–2.182)	0.012	92.9	0.609	N	71	1.229 (0.275–2.182)
IL-1Ra	4	148	110	0.449 (0.082–0.815)	0.016	51.6	0.986	N	9	0.449 (0.082–0.815)
IL-5	4	198	322	0.396 (–0.072–0.865)	0.097	82.1	0.105	N	18	0.396 (–0.072–0.865)
CCL-3	3	110	98	1.974 (–0.231–4.179)	0.079	97.5	0.180	N	52	1.974 (–0.231–4.179)
IL-17	3	85	106	–0.121 (–0.537–0.295)	0.569	51.6	0.212	N	0	–0.121 (–0.537–0.295)
TGF-β1	3	110	68	–1.480 (–4.756–1.797)	0.376	97.7	0.367	N	5	–1.480 (–4.756–1.797)
sTNFR2	3	94	101	1.173 (0.409–1.938)	0.003	83.2	0.368	N	36	1.173 (0.409–1.938)

CI, confidence interval; ES, effect size; MDD, major depressive disorder; Y, Yes; N, No; NA, Not applicable; statistically significant results are in bold.

*In Z-test of overall effect.

†In Egger’s test of publication bias.

‡*P* < 0.1 in Egger’s test of publication bias and effect size of the largest study more conservative than the overall effect size or in the opposite direction.

§Adjusted using Duval and Tweedie’s trim-and-fill procedure.

estimate (Fig. S15). Cumulative meta-analysis indicated that ES estimates are consistent across studies since 1996 (Fig. S26).

Studies of TNF-α

TNF-α was investigated across 42 studies, and levels were significantly higher in the MDD group compared to HCs, while there was evidence of small-study effects (*g* = 0.638; *P* < 0.001; Table 1 and Fig. 2b); the ES was unaltered after adjustment for publication bias (Table 1). Heterogeneity was large (*I*² = 90.0%). The percentage of current smokers in both the MDD and HC groups moderated the ES; in both groups, a higher prevalence of smokers was associated with a higher ES estimate (Table S3). The methodological quality of included studies also emerged as a significant moderator (Table S3). The ES estimate was smaller in studies with better methodological quality. In addition, levels of TNF-α were not significantly altered in individuals with melancholic depression compared to controls (*g* = 0.141; *k* = 4; *P* = 0.418). Sensitivity analysis indicated that the exclusion of any single study (one at a time) did not alter the direction or statistical significance of the ES estimate (Fig. S16). In the cumulative meta-analysis, this ES estimate remained consistent (moderate) after

the addition of the most recent eight studies, which had similar ES estimates (Fig. S27).

Studies of IL-1β

Levels of IL-1β did not significantly differ between MDD and HC groups across 22 included studies (Hedge’s *g* = 0.032, *P* = 0.847; Table 1; Fig. S1). No evidence of small-study effects was observed (Table 1). The heterogeneity was large (*I*² = 89.3%). The mean BMI of participants with MDD emerged as a potential source of heterogeneity in meta-regression analysis; a larger BMI was associated with a higher ES estimate (Table S3). In addition, subgroup analyses suggest that heterogeneity is lower in studies that measured this immune mediator in whole blood (compared to studies that assayed IL-1β in serum or plasma) and in studies that used stimulated leukocytes (Table S4).

Studies of IFN-γ

Data for IFN-γ were extracted from 17 studies, and levels were reduced in subjects with MDD compared to the HCs (Table 1; Fig. 3a). There was evidence of small-study effects, but adjustment for publication bias did not change the ES

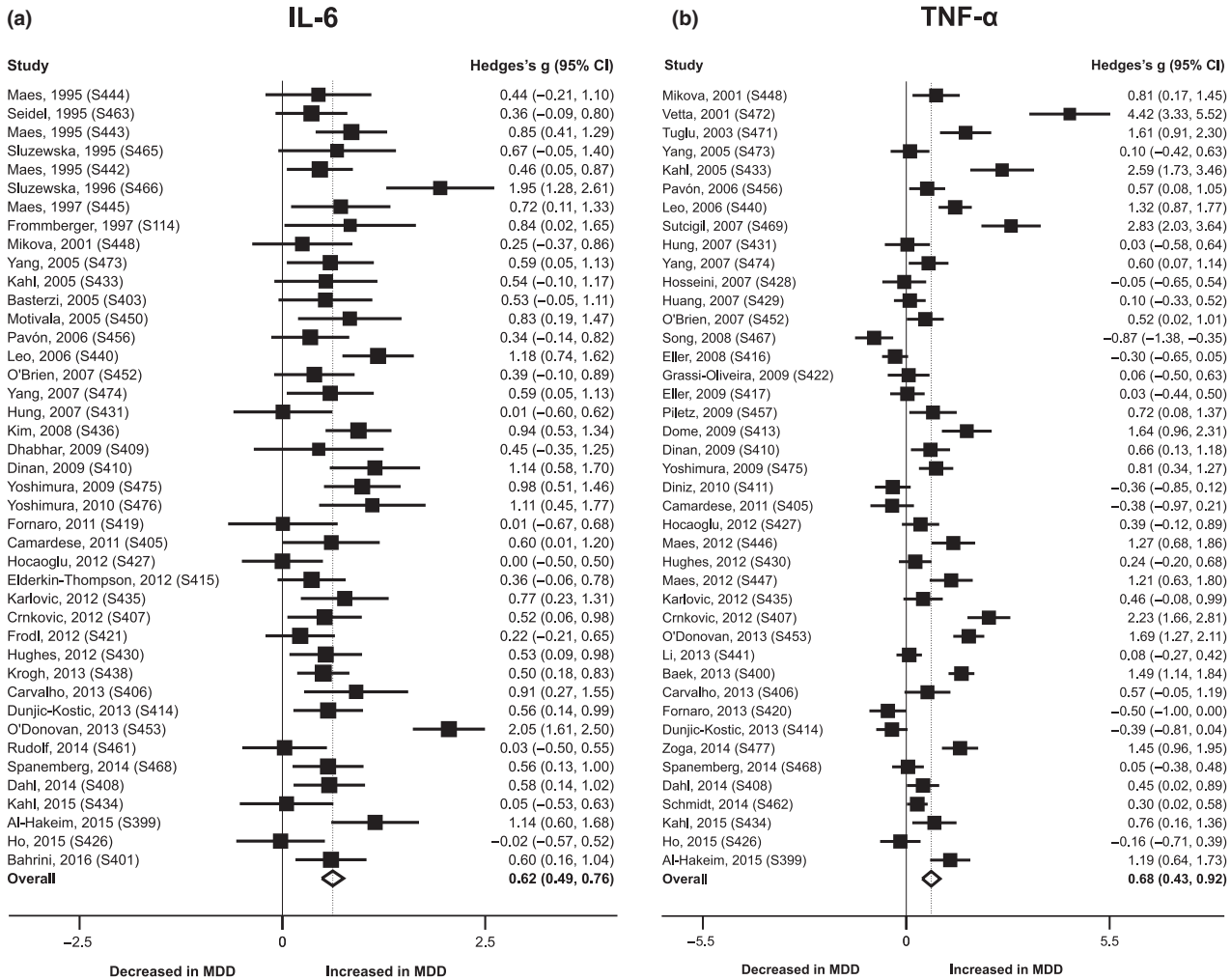


Fig. 2. Forest plots of studies which measured (a) IL-6 or (b) TNF-α or in participants with MDD compared to HCs. Effect size estimates are presented as Hedge's g with 95% confidence intervals (CIs). Square sizes are proportional to the ES of each study. References are presented in the supporting information.

($g = -0.452$; Table 1 and Fig. 3a). Heterogeneity was large ($I^2 = 94.0\%$). Mean BMI of the HC group, publication year, sample size, and mean age of the MDD and HC groups emerged as potential sources of heterogeneity in meta-regression analyses (Table S3). Sensitivity analysis revealed that the exclusion of 10 studies from analysis one by one rendered the ES estimate non-significant (Fig. S17). In addition, the cumulative meta-analysis indicates that the ES estimates for IFN-γ have not been consistent over time (Fig. S28).

Studies of IL-10

IL-10 levels were investigated in 17 studies, and levels were significantly higher in the MDD group compared to HCs, with a small ES ($g = 0.375$, $P = 0.045$) (Table 1 and Fig. 3b). No evidence of small-study effects was verified (Table 1). Heterogeneity was large ($I^2 = 89.2\%$), and subgroup

analyses suggest that heterogeneity is lower in studies that assayed IL-10 in plasma compared to serum. In addition, the ES was significant only in studies that followed a non-matched design (Table S4). Furthermore, IL-10 levels were not significantly altered in participants with MDD who were antidepressant-free when this cytokine was assayed, whereas these levels remained significantly elevated in participants with MDD who were using antidepressants (Table S4). Sensitivity analyses showed that the exclusion of 11 of 17 studies one at a time rendered the ES estimate non-significant (Fig. S18). In addition, the cumulative meta-analysis indicates that the ES has not been consistent over time (Fig. S29).

Studies of soluble IL-2 (sIL-2) receptor

We found evidence that sIL-2 receptor levels were significantly higher in the MDD group

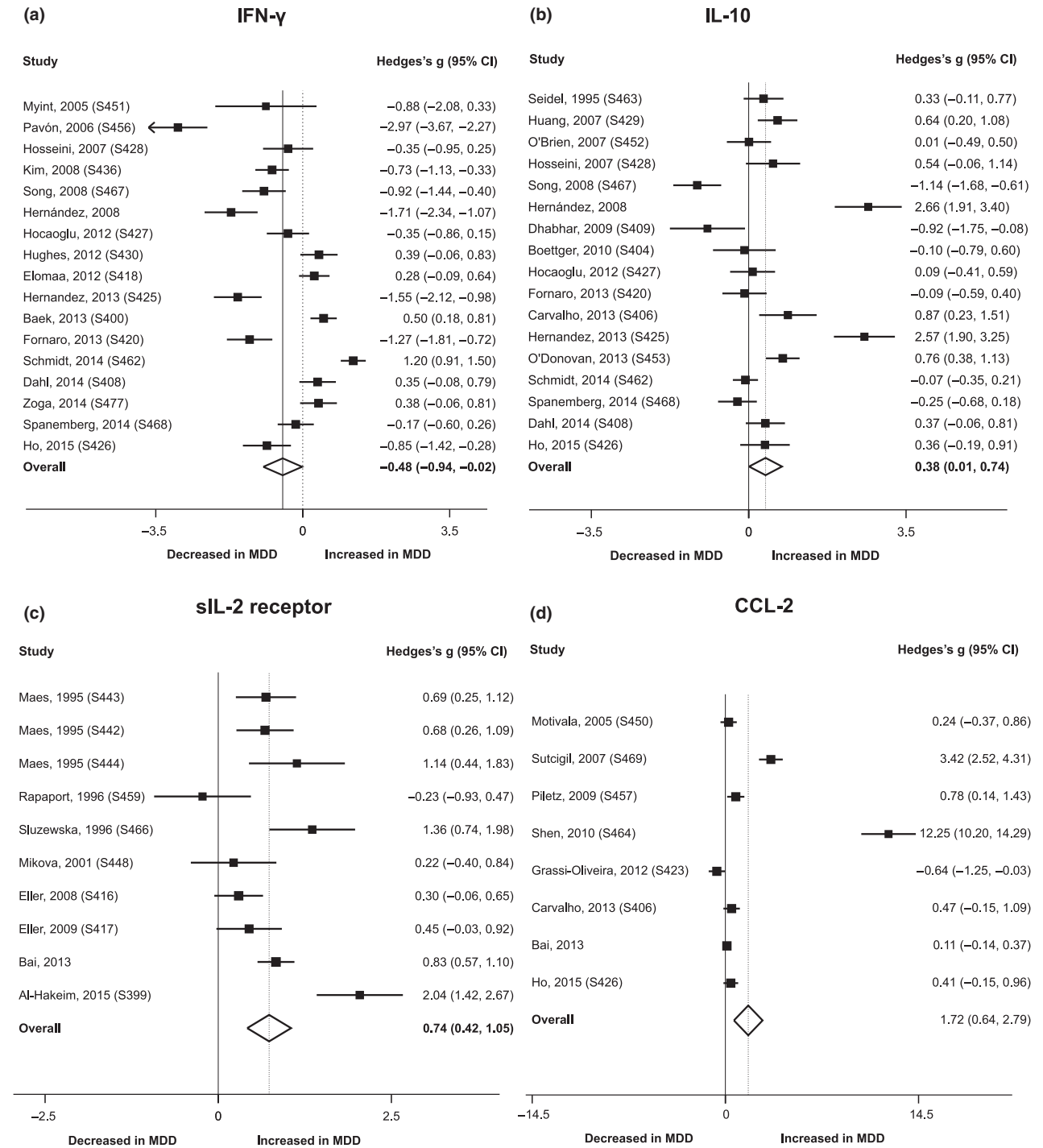


Fig. 3. Forest plots of studies which measured (a) IFN- γ or (b) IL-10 or (c) sIL-2R or (d) CCL-2 in participants with MDD compared to HCs. Effect size estimates are presented as Hedge's g with 95% confidence intervals (CIs). Square sizes are proportional to the ES of each study. References are presented in the supporting information.

compared to HCs with a moderate ES estimate (Hedge's $g = 0.735$, $P < 0.001$) (Table 1; Fig. 3c). No evidence of small-study effects was observed, and between-study heterogeneity was large ($I^2 = 77.5\%$). Subgroup analyses suggested that heterogeneity was lower in studies that

measured sIL2 in plasma (compared to serum) as well as in studies in which MDD and HC groups were not age- and gender-matched (Table S4). In sensitivity analysis, the exclusion of included studies one at a time did not alter the direction or significant of the ES estimate.

Moreover, the cumulative meta-analysis indicated that this ES estimate has been consistent over time (Fig. S30).

Studies of C-C chemokine ligand 2 (CCL-2)

Levels of CCL-2 were significantly higher in participants with MDD compared to HCs with a large ES ($g = 1.718$; $P = 0.045$) (Table 1; Fig. 3d). There was evidence of small-study effects (Table 1). However, the ES was not altered after adjustment for publication bias. Heterogeneity was large ($I^2 = 96.3\%$). The ES was not significant in studies which utilized a matched design (Table S4). In sensitivity analysis, we found that the exclusion of the study by Shen et al. (45) from the analysis turned this ES non-significant (Fig. S20).

Studies of IL-13

Levels of IL-13 were significantly higher in participants with MDD compared to HCs ($g = 1.836$; $P < 0.001$) (Table 1; Fig. 4a). The ES estimate remained large even after adjustment for publication bias ($g = 1.432$). The heterogeneity was large ($I^2 = 96.0\%$), but could not be reliably explored due to the limited number of included studies ($k = 6$).

Studies of IL-18

Levels of IL-18 were significantly higher in the MDD group compared to the HC group (Table 1; Fig. 4b). The ES estimate was high ($g = 1.720$; $P = 0.012$), although a limited number of studies were included in this meta-analysis ($k = 5$). Heterogeneity was large ($I^2 = 95.3\%$), and no evidence of small-study effects was observed (Table 1).

Studies of IL-12

Peripheral levels of IL-12 were significantly more elevated in individuals with MDD compared to HCs, with a large ES estimate ($g = 1.229$; $P = 0.012$) (Table 1; Fig. 4c). The heterogeneity was large ($I^2 = 92.9\%$), and no evidence of small-study effects was observed (Table 1). In addition, sensitivity analysis revealed that this ES could be biased by a possible outlier (46) (Fig. S22).

Studies of IL-1 receptor antagonist (IL-1Ra)

Peripheral levels of IL-1Ra were higher in the MDD group compared to HCs ($g = 0.449$; $P = 0.016$) (Table 1; Fig. 4d). Heterogeneity was

large ($I^2 = 51.6\%$), while no evidence of small-study effects was observed (Table 1). However, sensitivity analyses revealed that this ES could be biased by at least three possible outliers (42, 47, 48) (Fig. S24).

Studies of soluble TNF receptor 2 (sTNFR2)

Three studies indicate that sTNFR2 levels are higher in individuals with MDD compared to HCs with a large ES ($g = 1.173$; $P = 0.003$), but high heterogeneity ($I^2 = 83.2\%$) (Table 1; Fig. 4e). No evidence of small-study effects was observed. However, sensitivity analysis shows that removal of the study by Papakostas et al. (49) turned this ES estimate non-significant (Fig. S25).

Other mediators

Levels of IL-2, IL-4, the soluble IL-6 receptor (sIL-6R), IL-8, IL-5, CCL-3, IL-17, and transforming growth factor- β (TGF- β) were measured in at least three studies and were thus meta-analyzed. Levels of these immune mediators did not significantly differ between individuals with MDD and HCs (Table 1). Forest plots for these meta-analyses are provided in the supporting information (Figs S2–S9).

Discussion

This meta-analysis provides the largest evidence synthesis conducted to date of studies that have investigated peripheral levels of cytokines and chemokine peripheral levels in individuals with MDD compared to HCs. Our results suggest that levels of IL-6, TNF- α , IL-10, the sIL-2R, CCL-2, IL-13, IL-18, IL-12, and the sTNFR2 can be significantly elevated in individuals with MDD compared to HCs, while IFN- γ levels may be slightly reduced in the MDD group compared to HCs.

The results of our meta-analysis add significant evidence to a previous meta-analysis (7), while a recent meta-analysis was limited to studies which investigated IL-1 β , TNF- α , and IL-6 (5). The previous meta-analysis found elevated levels of TNF- α and IL-6 in depressive patients compared to HCs (7). A recent meta-analysis aimed to compare peripheral levels of cytokines among patients with schizophrenia, bipolar disorder, and also MDD (50). This recent meta-analysis also investigated the effects of antidepressant treatment on blood cytokine levels in patients with MDD (50). However, this meta-analysis included only 30 studies. In addition, a fixed-effects models were used to estimate ESs, which may be inaccurate when

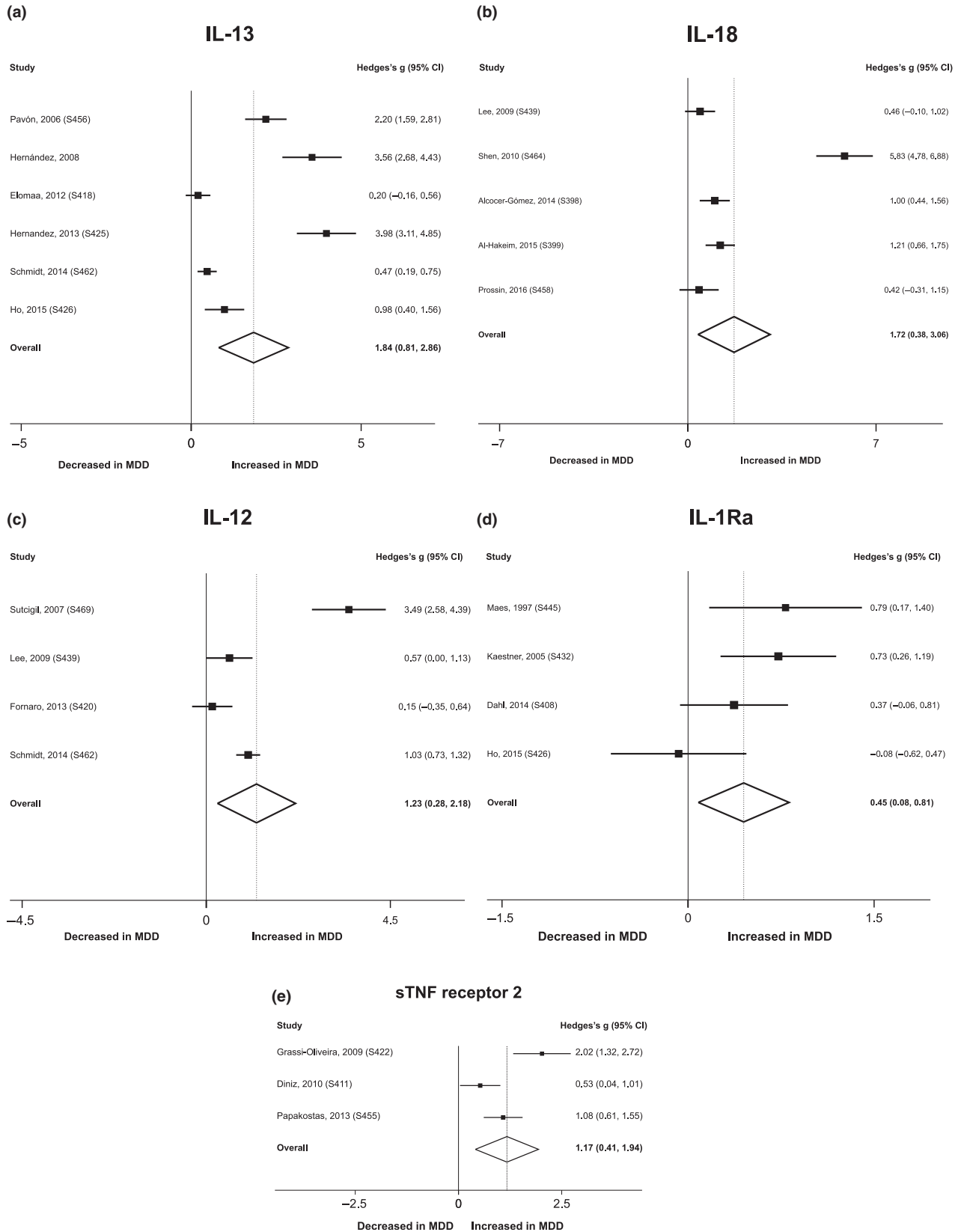


Fig. 4. Forest plots of studies which measured (a) IL-13 or (b) IL-18 or (c) IL-12 or (d) IL-1Ra or (e) sTNFR2 in participants with MDD compared to HCs. Effect size estimates are presented as Hedge's g with 95% confidence intervals (CIs). Square sizes are proportional to the ES of each study. References are presented in the supporting information.

heterogeneity is large (27). We confirmed that a high level of heterogeneity across studies characterizes this evolving field (5, 7). In addition, due to the larger number of included studies, we could more accurately explore potential sources of heterogeneity than has previously been possible.

A significant proportion of individuals with MDD exhibits a high prevalence of comorbid medical (e.g., metabolic) and psychiatric conditions (51, 52), which may contribute to immune activation in MDD. For example, it has been postulated that comorbid obesity may lead to a more pernicious outcome in MDD in part due to shared immune-inflammatory pathways (53, 54). Therefore, we *a priori* excluded studies in which participants with MDD had clearly identified comorbidities and examined the influence of other relevant confounders. This approach identified the fact that mean BMI values of the MDD group (IL-1 β and IFN- γ), mean BMI of the HC control group (IFN- γ), and current smoking (TNF- α) significantly moderated these estimates. These findings are consistent with the hypothesis that adiposity-driven inflammation may contribute to MDD-related morbidity (55, 56). In addition, it has been postulated that smoking and an unhealthy lifestyle (e.g., poor diet) could be relevant sources of immune activation in MDD (57).

Cytokines and chemokines have been classically subdivided as anti-inflammatory and proinflammatory. However, emerging evidence indicates that this subdivision may be overly simplistic. For example, IL-6 may activate a classical pathway and a trans-signaling pathway, which may have predominantly anti- and proinflammatory activities respectively (58). We found elevated IL-6 levels in participants with MDD compared to HCs. The sIL-6R was examined in relatively few studies, and its peripheral levels were more elevated in the MDD group compared to HCs at the trend level. It is worthy to note that IL-6 was cytokine more extensively investigated in this meta-analysis, with a consistent moderate ES observed in cumulative meta-analysis. In addition, IL-6 and IL-1 β may contribute to the pathophysiology of a subset of patients with MDD via excessive release of corticotrophin-releasing hormone (CRH) and by the promotion glucocorticoid receptor resistance, which may ultimately impair the negative feedback regulation of the HPA axis (59, 60).

We found evidence that IFN- γ may be reduced in participants with MDD compared to HCs. However, the ES was small and sensitivity analyses pointed to significant outliers. Furthermore, levels of IFN- γ were most often close to the limit of detection of previously available assay kits, which

may lead to analytical variability. In addition, previous studies found elevated levels of IFN- γ in stimulated peripheral blood mononuclear cells (PBMCs) of individuals with MDD compared to healthy controls, which may provide a more accurate measure of this cytokine (61, 62).

Ronald Smith was the first to propose a macrophage theory for depression in the early 1990's (63). Macrophages and their counterparts in the CNS are crucial cells of the innate immune system, which can alter and adapt their phenotypes depending on their prime activity (a M1-activated phenotype has a primary role in acute defense against pathogens, whereas a M2 phenotype is primarily involved in clearing damaged tissues and repairing activities) (64). Evidence pointing to a role of M1 cells (including microglial cells and CNS macrophages) in MDD has accumulated (2, 65). A clear limitation of this meta-analysis rests on fact that the periphery may not reflect pathophysiological events in the CNS. However, preclinical studies indicate that the blockade of the trafficking of peripheral monocytes to the brain reduced proinflammatory cytokine production and decreased depressive-like behaviours in rodent stress models (66). Thus, peripheral M1 cells could be a main source of elevated cytokines in MDD (4). This meta-analysis evidenced elevated levels of CCL-2, IL-6, IL-12, TNF- α , and IL-1 β , which are immune mediators secreted by M1 macrophages albeit not selectively (67).

The inflammatory response is tightly controlled at critical set points, and the maintenance of a healthy immune state is not a passive state, but may require an active expression of immunoregulatory genes (68). Regulatory T-cells (TRegs) are master immune regulators and play a significant role in immune tolerance (69). We found an elevation of IL-10 levels in individuals with MDD compared to HCs. This cytokine is predominantly secreted by TRegs (70). However, subgroup analyses found that this cytokine was not elevated in antidepressant-free participants with MDD, which may underscore an indirect effect of antidepressant drugs. In addition, TGF- β that is another biosignature cytokine of TRegs was not significantly altered in participants with MDD compared to HCs. Thus, a relative lack of counter-regulatory immune mechanisms may contribute to peripheral inflammation in MDD.

The main limitation of this meta-analysis is the high degree of heterogeneity of some estimates. Although we have identified some significant moderators, some possible sources of heterogeneity could not be investigated due to the lack of data across studies, while data on other potential

moderators (e.g., physical activity and diet quality) (57) were not provided by included studies and thus could not be controlled for. Notwithstanding, we could not investigate whether length of disease and number of affective episodes as possible moderators of ESs estimates due to the lack of data across studies, a previous meta-analysis, which used a different definition had found suggestive that some differences in peripheral levels of cytokines may occur in acute compared to chronic MDD relative to HCs (50). Furthermore, the current use of antidepressant drugs did not emerge as a significant moderator in our analyses. However, our exploratory meta-regressions could have limited power to detect this effect, and previous evidence indicates that antidepressant drugs may impact peripheral cytokine levels at follow-up (50). It has been postulated that melancholic depression is associated with an overactive HPA axis and possibly lower inflammation due to the modulatory effects of cortisol (15, 71). We found that few studies have categorized patients in melancholic vs. atypical depression, although we found that TNF- α levels did not differ when individuals with melancholic depression were compared to HCs. In addition, cytokines/chemokines appear to be involved in the pathophysiology of suicidal behaviour (72). Furthermore, technical challenges in the assessment of certain mediators (e.g., IL-2 and IFN- γ) (73) as well as differences in the standardization of assays across different laboratories could have contributed to the heterogeneity of some estimates. It is worthy to note that although a predefined protocol was followed, we did not publish or otherwise register it in a public database. Finally, the methodological quality of included studies has varied, and in our exploratory meta-regression analyses, this factor emerged as a significant moderator of differences in TNF- α levels between participants with MDD and HCs.

In conclusion, this meta-analysis indicates that several cytokines and CCL-2 are elevated in MDD. Our results confirm that cell-mediated immune activation may be an important pathophysiological aspect of MDD. In addition, our results provide directions for further research. For example, emerging preclinical evidence and a recent theoretical framework indicate that TH17 cells could play a significant role in the biology of depression (74, 75). However, few studies have investigated peripheral levels of IL-17, IL-17F, and IL-22, which are biosignature cytokines of TH17 cells (76). Thus, the characterization of the precise peripheral immune profile associated with MDD remains a work in progress.

Acknowledgements

CAK is supported by a postdoctoral fellowship award from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil). MM is supported by a visiting research fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Brazil). AFC is supported by a research fellowship award from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Brazil).

Declaration of interests

In the past 3 years, CLR has served on the scientific advisory board for Usona Institute. In addition, he has served on the speaker's bureau of Merck and Sunovion and on the advisory board of Otsuka and Pamlab. All other authors report no conflict of interests.

References

1. MAES M. Evidence for an immune response in major depression: a review and hypothesis. *Prog Neuropsychopharmacol Biol Psychiatry* 1995;**19**:11–38.
2. MILLER AH, RAISON CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat Rev Immunol* 2016;**16**:22–34.
3. HAROON E, FLEISCHER CC, FELGER JC et al. Conceptual convergence: increased inflammation is associated with increased basal ganglia glutamate in patients with major depression. *Mol Psychiatry* 2016;**21**:1351–1357.
4. WOHLEB ES, FRANKLIN T, IWATA M, DUMAN RS. Integrating neuroimmune systems in the neurobiology of depression. *Nat Rev Neurosci* 2016;**17**:497–511.
5. HAAPAKOSKI R, MATHIEU J, EBMEIER KP, ALENUS H, KIVIMAKI M. Cumulative meta-analysis of interleukins 6 and 1beta, tumour necrosis factor alpha and C-reactive protein in patients with major depressive disorder. *Brain Behav Immun* 2015;**49**:206–215.
6. MAES M, SCHARPE S, VAN GROOTEL L et al. Higher alpha 1-antitrypsin, haptoglobin, ceruloplasmin and lower retinol binding protein plasma levels during depression: further evidence for the existence of an inflammatory response during that illness. *J Affect Disord* 1992;**24**:183–192.
7. DOWLATI Y, HERRMANN N, SWARDFAGER W et al. A meta-analysis of cytokines in major depression. *Biol Psychiatry* 2010;**67**:446–457.
8. STUART MJ, BAUNE BT. Chemokines and chemokine receptors in mood disorders, schizophrenia, and cognitive impairment: a systematic review of biomarker studies. *Neurosci Biobehav Rev* 2014;**42**:93–115.
9. DANTZER R, O'CONNOR JC, FREUND GG, JOHNSON RW, KELLEY KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci* 2008;**9**:46–56.
10. SIMEN BB, DUMAN CH, SIMEN AA, DUMAN RS. TNFalpha signaling in depression and anxiety: behavioral consequences of individual receptor targeting. *Biol Psychiatry* 2006;**59**:775–785.
11. CHOURBAJI S, URANI A, INTA I et al. IL-6 knockout mice exhibit resistance to stress-induced development of depression-like behaviors. *Neurobiol Dis* 2006;**23**:587–594.
12. UDINA M, CASTELLVI P, MORENO-ESPANA J et al. Interferon-induced depression in chronic hepatitis C: a systematic review and meta-analysis. *J Clin Psychiatry* 2012;**73**:1128–1138.

13. THASE ME. Using biomarkers to predict treatment response in major depressive disorder: evidence from past and present studies. *Dialogues Clin Neurosci* 2014;**16**:539–544.
14. CARVALHO AF, KOHLER CA, BRUNONI AR et al. Bias in peripheral depression biomarkers. *Psychother Psychosom* 2016;**85**:81–90.
15. GOLD PW. The organization of the stress system and its dysregulation in depressive illness. *Mol Psychiatry* 2015;**20**:32–47.
16. LAMERS F, VOGELZANGS N, MERIKANGAS KR, de JONGE P, BEEKMAN AT, PENNINX BW. Evidence for a differential role of HPA-axis function, inflammation and metabolic syndrome in melancholic versus atypical depression. *Mol Psychiatry* 2013;**18**:692–699.
17. STUART MJ, SINGHAL G, BAUNE BT. Systematic review of the neurobiological relevance of chemokines to psychiatric disorders. *Frontiers Cellular Neurosci* 2015;**9**:357.
18. SOCHOCKA M, DINIZ BS, LESZEK J. Inflammatory response in the CNS: friend or foe? *Mol Neurobiol* 2016. doi: 10.1007/s12035-016-0297-1 [Epub ahead of print].
19. HOWREN MB, LAMKIN DM, SULS J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom Med* 2009;**71**:171–186.
20. HILES SA, BAKER AL, DE MALMANCHE T, ATTIA J. A meta-analysis of differences in IL-6 and IL-10 between people with and without depression: exploring the causes of heterogeneity. *Brain Behav Immun* 2012;**26**:1180–1188.
21. LIBERATI A, ALTMAN DG, TETZLAFF J et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009;**339**:b2700.
22. BAKKALBASI N, BAUER K, GLOVER J, WANG L. Three options for citation tracking: Google Scholar, Scopus and Web of Science. *Biomed Digit Libr* 2006;**3**:7.
23. ASSOCIATION AP. DSM 5. American Psychiatric Association; 2013.
24. ORGANIZATION WH. The ICD-10 classification of mental and behavioural disorders: diagnostic criteria for research; 1993.
25. HOZO SP, DJULBEGOVIC B, HOZO I. Estimating the mean and variance from the median, range, and the size of a sample. *BMC Med Res Methodol* 2005;**5**:13.
26. LAU J, IOANNIDIS JP, SCHMID CH. Quantitative synthesis in systematic reviews. *Ann Intern Med* 1997;**127**:820–826.
27. PATSOPOULOS NA, EVANGELOU E, IOANNIDIS JP. Heterogeneous views on heterogeneity. *Int J Epidemiol* 2009;**38**:1740–1742.
28. DERSIMONIAN R, LAIRD N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;**7**:177–188.
29. CARVALHO AF, KOHLER CA, FERNANDES BS et al. Bias in emerging biomarkers for bipolar disorder. *Psychol Med* 2016;**46**:2287–2297.
30. EGGER M, DAVEY SMITH G, SCHNEIDER M, MINDER C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;**315**:629–634.
31. DUVAL S, TWEEDIE R. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 2000;**56**:455–463.
32. ROSENTHAL R. The file drawer problem and tolerance for null results. *Psychol Bull* 1979;**86**:638.
33. HIGGINS JP, THOMPSON SG. Controlling the risk of spurious findings from meta-regression. *Stat Med* 2004;**23**:1663–1682.
34. THOMPSON SG, HIGGINS JP. How should meta-regression analyses be undertaken and interpreted? *Stat Med* 2002;**21**:1559–1573.
35. KROGH J, BENROS ME, JORGENSEN MB, VESTERAGER L, ELFVING B, NORDENTOFT M. The association between depressive symptoms, cognitive function, and inflammation in major depression. *Brain Behav Immun* 2014;**35**:70–76.
36. O'DONOVAN A, RUSH G, HOATAM G et al. Suicidal ideation is associated with elevated inflammation in patients with major depressive disorder. *Depress Anxiety* 2013;**30**:307–314.
37. DUNJIC-KOSTIC B, IVKOVIC M, RADONJIC NV et al. Melancholic and atypical major depression—connection between cytokines, psychopathology and treatment. *Prog Neuropsychopharmacol Biol Psychiatry* 2013;**43**:1–6.
38. ELDERKIN-THOMPSON V, IRWIN MR, HELLEMANN G, KUMAR A. Interleukin-6 and memory functions of encoding and recall in healthy and depressed elderly adults. *Am J Geriatr Psychiatry* 2012;**20**:753–763.
39. KAHL KG, BESTER M, GREGGERSEN W et al. Visceral fat deposition and insulin sensitivity in depressed women with and without comorbid borderline personality disorder. *Psychosom Med* 2005;**67**:407–412.
40. MOTIVALA SJ, SARFATTI A, OLMOS L, IRWIN MR. Inflammatory markers and sleep disturbance in major depression. *Psychosom Med* 2005;**67**:187–194.
41. MIKOVA O, YAKIMOVA R, BOSMANS E, KENIS G, MAES M. Increased serum tumor necrosis factor alpha concentrations in major depression and multiple sclerosis. *Eur Neuropsychopharmacol* 2001;**11**:203–208.
42. MAES M, BOSMANS E, DE JONGH R, KENIS G, VANDOOALAEHGE E, NEELS H. Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. *Cytokine* 1997;**9**:853–858.
43. MAES M, MELTZER HY, BOSMANS E et al. Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. *J Affect Disord* 1995;**34**:301–309.
44. LEO R, DI LORENZO G, TESAURO M et al. Association between enhanced soluble CD40 ligand and proinflammatory and prothrombotic states in major depressive disorder: pilot observations on the effects of selective serotonin reuptake inhibitor therapy. *J Clin Psychiatry* 2006;**67**:1760–1766.
45. SHEN Y, LU P, WEI L, HU X, CHEN W. Fluoxetine treatment for major depression decreases the plasma levels of cytokines. *Afr J Biotechnol* 2010;**9**:7346–7351.
46. SCHMIDT FM, LICHTBLAU N, MINKWITZ J et al. Cytokine levels in depressed and non-depressed subjects, and masking effects of obesity. *J Psychiatr Res* 2014;**55**:29–34.
47. KAESTNER F, HETTICH M, PETERS M et al. Different activation patterns of proinflammatory cytokines in melancholic and non-melancholic major depression are associated with HPA axis activity. *J Affect Disord* 2005;**87**:305–311.
48. HO PS, YEH YW, HUANG SY, LIANG CS. A shift toward T helper 2 responses and an increase in modulators of innate immunity in depressed patients treated with escitalopram. *Psychoneuroendocrinology* 2015;**53**:246–255.
49. PAPAPOSTAS GI, SHELTON RC, KINRYS G et al. Assessment of a multi-assay, serum-based biological diagnostic test for major depressive disorder: a pilot and replication study. *Mol Psychiatry* 2013;**18**:332–339.
50. GOLDSMITH DR, RAPAPORT MH, MILLER BJ. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. *Mol Psychiatry* 2016;**21**:1696–1709.
51. KESSLER RC, MERIKANGAS KR, WANG PS. Prevalence, comorbidity, and service utilization for mood disorders in the United States at the beginning of the twenty-first century. *Ann Rev Clin Psychol* 2007;**3**:137–158.

52. VANCAMPFORT D, CORRELL CU, WAMPERS M et al. Metabolic syndrome and metabolic abnormalities in patients with major depressive disorder: a meta-analysis of prevalences and moderating variables. *Psychol Med* 2014;**44**:2017–2028.
53. DU YJ, YANG CJ, LI B et al. Association of pro-inflammatory cytokines, cortisol and depression in patients with chronic obstructive pulmonary disease. *Psychoneuroendocrinology* 2014;**46**:141–152.
54. MANSUR RB, BRIETZKE E, MCINTYRE RS. Is there a “metabolic-mood syndrome”? A review of the relationship between obesity and mood disorders. *Neurosci Biobehav Rev* 2015;**52**:89–104.
55. CAPURON L, LASSELIN J, CASTANON N. Role of adiposity-driven inflammation in depressive morbidity. *Neuropsychopharmacology* 2017;**42**:115–128.
56. LIU CS, CARVALHO AF, MCINTYRE RS. Towards a “metabolic” subtype of major depressive disorder: shared pathophysiological mechanisms may contribute to cognitive dysfunction. *CNS Neurol Disord Drug Targets* 2014;**13**:1693–1707.
57. BERK M, WILLIAMS LJ, JACKA FN et al. So depression is an inflammatory disease, but where does the inflammation come from? *BMC Med* 2013;**11**:200.
58. MAES M, ANDERSON G, KUBERA M, BERK M. Targeting classical IL-6 signalling or IL-6 trans-signalling in depression? *Expert Opin Ther Targets* 2014;**18**:495–512.
59. MILLER AH, MALETIC V, RAISON CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* 2009;**65**:732–741.
60. HOROWITZ MA, ZUNSZAIN PA, ANACKER C, MUSAEYAN K, PARIANTE CM. Glucocorticoids and inflammation: a double-headed sword in depression? How do neuroendocrine and inflammatory pathways interact during stress to contribute to the pathogenesis of depression? *Mod Trends Pharmacopsychiat* 2013;**28**:127–143.
61. MAES M, SCHARPE S, MELTZER HY et al. Increased neopterin and interferon-gamma secretion and lower availability of L-tryptophan in major depression: further evidence for an immune response. *Psychiatry Res* 1994;**54**:143–160.
62. SEIDEL A, AROLT V, HUNSTIGER M, RINK L, BEHNISCH A, KIRCHNER H. Cytokine production and serum proteins in depression. *Scand J Immunol* 1995;**41**:534–538.
63. SMITH RS. The macrophage theory of depression. *Med Hypotheses* 1991;**35**:298–306.
64. KALKMAN HO, FEUERBACH D. Antidepressant therapies inhibit inflammation and microglial M1-polarization. *Pharmacol Ther* 2016;**163**:82–93.
65. YIRMIYA R, RIMMERMAN N, RESHEF R. Depression as a microglial disease. *Trends Neurosci* 2015;**38**:637–658.
66. WOHLER ES, MCKIM DB, SHERIDAN JF, GODBOUT JP. Monocyte trafficking to the brain with stress and inflammation: a novel axis of immune-to-brain communication that influences mood and behavior. *Frontiers Neurosci* 2014;**8**:447.
67. MILLS CD. Anatomy of a discovery: m1 and m2 macrophages. *Front Immunol* 2015;**6**:212.
68. NATHAN C. Points of control in inflammation. *Nature* 2002;**420**:846–852.
69. SAKAGUCHI S, YAMAGUCHI T, NOMURA T, ONO M. Regulatory T cells and immune tolerance. *Cell* 2008;**133**:775–787.
70. SAKAGUCHI S, MIYARA M, COSTANTINO CM, HAFNER DA. FOXP3+ regulatory T cells in the human immune system. *Nat Rev Immunol* 2010;**10**:490–500.
71. STETLER C, MILLER GE. Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. *Psychosom Med* 2011;**73**:114–126.
72. BLACK C, MILLER BJ. Meta-Analysis of Cytokines and Chemokines in Suicidality: distinguishing Suicidal Versus Nonsuicidal Patients. *Biol Psychiatry* 2015;**78**:28–37.
73. ELLER T, VASAR V, SHLIK J, MARON E. The role of IL-2 and soluble IL-2R in depression and antidepressant response. *Curr Opin Investig Drugs* 2009;**10**:638–643.
74. SLYEPCHENKO A, MAES M, KOHLER CA et al. T helper 17 cells may drive neuroprogression in major depressive disorder: proposal of an integrative model. *Neurosci Biobehav Rev* 2016;**64**:83–100.
75. BEUREL E, HARRINGTON LE, JOPE RS. Inflammatory T helper 17 cells promote depression-like behavior in mice. *Biol Psychiatry* 2013;**73**:622–630.
76. KORN T, BETTELLI E, OUKKA M, KUCHROO VK. IL-17 and Th17 cells. *Annu Rev Immunol* 2009;**27**:485–517.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Forest plot of studies that investigated IL-1 β .

Fig. S2. Forest plot of studies that investigated IL-2.

Fig. S3. Forest plot of studies that investigated IL-4.

Fig. S4. Forest plot of studies that investigated soluble IL-6 receptor (sIL-6 receptor).

Fig. S5. Forest plot of studies that investigated IL-8.

Fig. S6. Forest plot of studies that investigated IL-5.

Fig. S7. Forest plot of studies that investigated CCL-3.

Fig. S8. Forest plot of studies that investigated IL-17.

Fig. S9. Forest plot of studies that investigated TGF- β 1.

Fig. S10. Funnel plot of studies that investigated TNF- α .

Fig. S11. Funnel plot of studies that investigated IFN- γ .

Fig. S12. Funnel plot of studies that investigated IL-4.

Fig. S13. Funnel plot of studies that investigated CCL-2.

Fig. S14. Funnel plot of studies that investigated IL-13.

Fig. S15. Sensitivity analysis for the meta-analysis of studies that investigated IL-6.

Fig. S16. Sensitivity analysis for the meta-analysis of studies that investigated TNF- α .

Fig. S17. Sensitivity analysis for the meta-analysis of studies that investigated IFN- γ .

Fig. S18. Sensitivity analysis for the meta-analysis of studies that investigated IL-10.

Fig. S19. Sensitivity analysis for the meta-analysis of studies that investigated soluble IL-2 receptor (sIL-2 receptor).

Fig. S20. Sensitivity analysis for the meta-analysis of studies that investigated CCL-2.

Fig. S21. Sensitivity analysis for the meta-analysis of studies that investigated IL-13.

Fig. S22. Sensitivity analysis for the meta-analysis of studies that investigated IL-18.

Fig. S23. Sensitivity analysis for the meta-analysis of studies that investigated IL-12.

Fig. S24. Sensitivity analysis for the meta-analysis of studies that investigated IL-1 receptor antagonist (IL-1Ra).

Fig. S25. Sensitivity analysis for the meta-analysis of studies that investigated sTNF receptor 2.

Fig. S26. Cumulative meta-analysis of studies that investigated IL-6.

Fig. S27. Cumulative meta-analysis of studies that investigated TNF- α .

Fig. S28. Cumulative meta-analysis of studies that investigated IFN- γ .

Fig. S29. Cumulative meta-analysis of studies that investigated IL-10.

Cytokines and chemokines in depression

Fig. S30. Cummulative meta-analysis of studies that investigated soluble IL-2 receptor (sIL-2 receptor).

Fig. S31. Cummulative meta-analysis of studies that investigated CCL-2.

Fig. S32. Cummulative meta-analysis of studies that investigated IL-13.

Fig. S33. Cummulative meta-analysis of studies that investigated IL-18.

Fig. S34. Cummulative meta-analysis of studies that investigated IL-12.

Fig. S35. Cummulative meta-analysis of studies that investigated IL-1 receptor antagonist (IL-1Ra).

Fig. S36. Cummulative meta-analysis of studies that investigated soluble TNF receptor 2 (sTNF receptor 2).

Table S1. Excluded studies, with reasons.

Table S2. Characteristics of included studies.

Table S3. Meta-regressions of inflammatory markers in subjects with MDD versus healthy controls (HC).

Table S4. Subgroup analyses of cytokines and chemokines in individuals with MDD versus healthy controls (HC).