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Adjunctive minocycline for major depressive disorder: A sub-study exploring peripheral immune-inflammatory markers and associated treatment response

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ABSTRACT

Background: Adjunctive minocycline shows promise in treating affective and psychotic disorders; however, the therapeutic mechanism remains unclear. Identifying relevant biomarkers may enhance the efficacy of novel adjunctive treatment candidates. We thus investigated the peripheral immune-inflammatory profile in a randomized controlled trial (RCT) of minocycline in major depressive disorder (MDD).

Methods: This sub-study investigated serum samples from a RCT evaluating minocycline (200 mg/day, 12 weeks) in addition to treatment as usual for MDD (ACTRN12612000283875). Of the original sample ($N = 71$), serum assays were conducted in 47 participants (placebo $n = 24$; minocycline $n = 23$) targeting an array of 46 immune-inflammatory analytes including cytokines, chemokines, and acute-phase reactants. General estimating equations (GEE) were used to assess whether analyte concentration at baseline (effect modification) and change in analytes (change association) influenced change in Montgomery-Åsberg Depression Rating Scale (MADRS) score over time. The Benjamini–Hochberg approach was applied when adjusting for false discovery rates (FDR).

Results: GEE models revealed several interaction effects. After adjusting for FDR several change association-models survived correction. However, no such models remained significant for effect modification. Three-way GROUP × TIME × MARKER interactions were significant for complement C3 ($B = -10.46$, 95%CI [-16.832, −4.095], $q = 0.019$) and IL-1Ra ($B = -9.008$, 95%CI [-15.26, −2.751], $q = 0.036$). Two-way GROUP × BIOMARKER interactions were significant for ICAM-1/CD54 ($B = -0.387$, 95%CI [-0.513, −0.26], $q < 0.001$) and IL-8/CXCL8 ($B = -4.586$, 95%CI [-7.698, −1.475], $q = 0.036$) indicating that increases in the serum concentration of these analytes were associated with an improvement in MADRS scores in the minocycline group (compared with placebo).

Conclusions: Change in complement C3, IL-1Ra, IL-8/CXCL8, and ICAM-1 may be associated with greater change in depressive scores following adjunctive minocycline treatment in MDD. Further investigations are needed to assess the utility of these biomarkers.

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1. Introduction

1.1. Overview

Consistent clinical and preclinical evidence points to aberrations of immune-inflammatory pathways playing a role in major depressive disorder (MDD) for at least some individuals (Maes and Carvalho, 2018), reflecting the heterogeneity of the disorder and pointing to potential subtypes (Milaneschi et al., 2020). Notably, MDD is highly comorbid with inflammatory diseases including cardiovascular diseases, diabetes, metabolic disorders, and autoimmune conditions (Menard et al., 2017). However, treatment innovation and biomarker identification remain significant challenges for the field because the underlying pathophysiology of MDD is still to be determined.

1.2. Inflammation and depression

MDD has been associated with both up- and down-regulation of several immune-inflammatory markers, including cytokines, chemokines, and acute phase reactants (Kohler et al., 2017; Leighton et al., 2018). For example, a recent meta-analysis (17 studies, $n = 7761$ depressed, $n = 155{,}728$ controls) found that the prevalence of low-grade inflammation (peripheral C-reactive protein (CRP) concentration >3 mg/L) in depressed patients was 24%, 95%CI [17, 34], versus 16%, 95% CI [11, 23] in non-depressed matched controls $OR = 1.46$, 95%CI [1.22, 1.75] (Osimo et al., 2019).

Another meta-analysis of 82 studies ($N = 6010$) found elevated levels of interleukins (IL)-6, IL-10, IL-12, IL-13, IL-18, the soluble IL-2 receptor (sIL-2R), IL-1 receptor antagonist (IL-1Ra), tumor necrosis factor (TNF)-α, and soluble TNF receptor 2 (TNFR2), in addition to reduced levels of interferon gamma (IFN-γ) in individuals with MDD relative to healthy controls (Kohler et al., 2017). However, outside of a handful of markers (e.g., IL-6, TNF-α, IL-10, IFN- γ and sIL-2R), fewer than 10 studies included in the meta-analysis had assessed these other analytes. Further, given the likelihood of heterogeneity associated with meta-analysis due to important differences in study design, follow-up duration, participants characteristics and other known issues relating to estimating a causal effect through pooled aggregate measures, these results should be interpreted cautiously (Kohler et al., 2017).

While much research has focused on prototypical interleukins and interferons as biomarker candidates, preclinical work has shown that chemokines (chemotactic cytokines) may play a role in depressive behaviors. Moreover, chemokines are linked with peripheral–central crosstalk (Leighton et al., 2018). A meta-analysis of 73 studies presented evidence linking abnormalities in blood chemokine levels with depression in humans (Leighton et al., 2018). Individuals with depression had lower blood levels of the C–C motif ligand macrophage inflammatory protein (MIP)-1β/CCL4 and higher levels of C-X-C motif ligands CXCL4 and CXCL7. Sensitivity analysis of studies with only physically healthy participants identified higher blood levels of monocyte chemoattractant protein (MCP)-1/CCL2, MIP-1α/CCL3, eotaxin-1/CCL11, CXCL7 and IL-8/CXCL8 and lower blood levels of MIP-1β/CCL4. Thus, other markers such as chemokines may be useful for the classification of immune-inflammatory biomarker profiles of depressed individuals (Leighton et al., 2018).

However, these findings are confounded by the treatments that have been administered, and this should be considered in the context of understanding the pathophysiology of MDD. A meta-analysis (45 studies, $N = 1517$) found that treatment with antidepressants was associated with decreases in a handful of markers of inflammation, including IL-6 (Hedges' $g = -0.45$, 95%CI [−0.66, −0.25]), TNF-α ($g = -0.20$, 95% CI [−0.37, −0.04]), IL-10 ($g = -0.57$, 95%CI [−1.01, −0.12]), and MCP-1/CCL2 ($g = -1.50$, 95%CI [−2.58, −0.42]) (Kohler et al., 2018). These results could be a consequence of direct medication effects or have been mediated via symptom reduction, where acute illness is associated with higher inflammation. Limited by the number of eligible studies, this meta-analysis was unable to provide evidence for a link between peripheral inflammation and treatment response (Kohler et al., 2018).

More recently, Liu and colleagues conducted a meta-analysis of 44 studies specifically investigating peripheral inflammation and antidepressant treatment response (Liu et al., 2020). This study found that antidepressant treatment decreased levels of TNF-α in treatment responders ($g = 0.60$, 95%CI [0.26,0.94]). Further, those MDD patients who responded to treatment also had lower baseline levels of IL-8/CXCL8 than the non-responders ($g = -0.28$, 95%CI [−0.43, −0.13]) (Liu et al., 2020). These findings collectively suggest that peripheral inflammatory effects may be implicated in treatment response.

1.3. Anti-inflammatory treatments in depression

Evidence of inflammatory aberrations in MDD has led to trials of several agents with anti-inflammatory effects. A recent systematic review and meta-analysis of thirty randomized control trials (RCTs) investigating adjunctive anti-inflammatory agents ($N = 1610$) reported reduced depressive symptoms ($SMD = -0.55$, 95%CI [−0.75, −0.35]), improved response rates ($RR = 1.52$, 95%CI [1.30, 1.79]) and remission rates ($RR = 1.79$, 95%CI [1.29, 2.49]) in participants who received anti-inflammatory agents (e.g., statins, celecoxib) versus placebo (Bai et al., 2020). In that study, subgroup analyses indicated that anti-inflammatory agents were generally effective as adjunctive treatments (18 trials, $N = 1{,}041$, SMD -0.70, 95%CI [−0.97, −0.43]). Specifically, minocycline was relatively safe, and showed significant antidepressant effects in MDD (3 trials, $N = 151$, SMD -0.79, 95%CI [−1.29, −0.28]) (Bai et al., 2020).

Minocycline, a broad-spectrum tetracycline antibiotic has a range of pleiotropic properties, including anti-inflammatory, immunomodulatory and neuroprotective effects; for review see (Dean et al., 2012; Garrido-Mesa et al., 2013). The potential use of minocycline for the treatment of depression is supported by the pooled re-analysis of two harmonized RCTs (2 studies, $N = 112$, Cohen's $d = 0.71$, 95%CI [0.29, 1.14]) (Zazula et al., 2021), as well as other recent systematic reviews and meta-analyses that have evaluated effects of minocycline on depressive symptoms in clinical trials (Cai et al., 2020; Rosenblat and McIntyre, 2018), and preclinical antidepressant effects in anhedonia- and immobility-based animal models (22 studies, $N = 816$, $SMD = -1.07$, 95%CI [−1.41, −0.74]) (Reis et al., 2019).

Most recently, Nettis et al. (2021) conducted a short 4-week RCT evaluating minocycline (200 mg/d) versus placebo in patients with treatment-resistant depression and serum levels of CRP ≥1 mg/L. While the main findings were not significant at this low setpoint, sub-analyses involving further stratification of the groups based on CRP levels (< or ≥ 3 mg/L) indicated that participants treated with minocycline were most likely to exhibit a partial response if they had CRP levels ≥ 3 mg/L. The study found a significant reduction in IFN-γ for the minocycline treated group and higher baseline levels of IL-6 in minocycline responders. Within-groups mean increase in IL-8/CXCL8 was also significant for the minocycline treated group (Nettis et al., 2021).

Minocycline has also been investigated in bipolar disorder. In contrast to MDD, no clear beneficial effect of minocycline (200 mg/d) has been reported in bipolar depression following 6-weeks (Savitz et al., 2018) or 12-weeks of treatment (Husain et al., 2020), although study design, smaller sample size, and outcome measures have been identified as limiting factors (Miller and Pariante, 2020). Interestingly, participants who had higher baseline IL-6 levels, and greater reductions in IL-6 levels showed greater improvement over the course of the 6-week trial (Savitz et al., 2018).

In sum, the evaluation of the therapeutic potential of adjunctive anti-inflammatory agents, like minocycline, for the treatment of mood disorders is warranted. However, both heterogeneity across studies and variability within studies remain a significant challenge, and it is not known which biomarkers (if any) may predict, or impact response to adjunctive minocycline. In this sub-study, we take a logical next step,

leveraging a subset of samples from one of the few RCTs conducted to date that robustly assessed the effects of adjunctive minocycline in MDD (Dean et al., 2017). The aim was to examine the immune-inflammatory profile of participants and apply general estimating equation (GEE) models to investigate causal relationships between these markers, adjunctive minocycline treatment and clinical trial outcome.

2. Materials and methods

2.1. Participants

The original RCT (ACTRN12612000283875) investigated the efficacy of adjunctive minocycline (200 mg/day) compared to placebo (in addition to any usual treatment) as a treatment for MDD. The trial was conducted over 12 weeks using the Montgomery-Åsberg Depression Rating Scale (MADRS) as the primary outcome. The details of participants and outcomes from the overarching trial ($N = 71$) have been reported previously; see (Dean et al., 2017). Briefly, the original trial reported a 4-point difference in MADRS scores between treatment groups at endpoint, but the primary outcome was not statistically significant overall. Several of the secondary clinical measures (quality of life, global experience, and functioning) were significantly improved following minocycline treatment.

The current study included serum from $N = 47$ participants (placebo $n = 24$; minocycline $n = 23$) who provided at least one blood sample at baseline or endpoint (week 12). Due to sample availability, no participants from the Bangkok, Thailand site were included in these analyses. Of the remaining two Australian sites (Geelong, Victoria and Melbourne, Victoria), Geelong accounted for 72% of participants ($n = 34$), divided evenly between placebo and minocycline ($n = 17$ each). All participants had a current diagnosis of MDD, corroborated by the Mini International Neuropsychiatric Interview (MINI) plus v5.0 (Sheehan et al., 1998). Further descriptive details are provided in Table 1.

Comparing the characteristics of included participants to those excluded due to sample availability, no statistically significant differences were observed at baseline for total MADRS score, illness duration, sex, or treatment allocation. Statistically significant differences were however noted for age at baseline, and body mass index. See Table S1.

2.2. Biological specimen collection and handling

BD Vacutainer® 8.5 mL serum-separating tubes (SST™) containing gel and clot activator (Becton Dickinson, Franklin Lakes, NJ, USA) were used for blood collection and processing. Samples were centrifuged at 1006 × *g*, serum was then aliquoted and stored below $-70^{\circ C}$ until testing.

2.3. Peripheral immune-inflammatory marker quantification

The samples were shipped to a commercial laboratory for immune-inflammatory profile measurements (Rules-Based Medicine (RBM), Austin, TX, USA). A total of $N = 82$ samples underwent molecular analyses with the multi analyte profile (MAP) Human InflammationMAP® v1.0 panel (Luminex Corp, Austin, TX, USA).

2.4. Statistical analysis

2.4.1. Descriptive statistics and data inspection

For the assessment of continuous and categorical group demographics comparisons, one-way analysis of variance (ANOVA), t-tests, Chi-square tests, Fisher's Exact tests or Fisher-Freeman-Halton Exact tests were used respectively. Pearson's correlations were used for initial assessment of the relationship between change in MADRS score and biological analytes. To mitigate the influence of outliers in statistical modelling, log natural (ln) transformation was considered for all analytes, and applied where appropriate after visual inspection of box plots, as indicated in table notes.

2.4.2. Generalized estimating equation (GEE) models

Effect modification was investigated using GEEs to assess the relationship between baseline markers and change in MADRS score. Effect modification in this instance refers to a phenomenon wherein the effect of treatment exposure (GROUP) on the outcome (MADRS score) over the duration of the trial (TIME) differs depending on a third variable (MARKER) at baseline. In the context of this study, our GROUP variable refers to treatment allocation in the original clinical trial (minocycline 200 mg/d or placebo), the TIME variable refers to visits over the 12-week intervention (baseline, weeks 2, 4, 8, 12), while the MARKER variable refers to the immune-inflammatory analyte used in the model (CRP, IL-1Ra etc.). Additional GEE models were also employed to investigate co-occurring associations between change in markers (baseline, week 12) and change in MADRS score (baseline, weeks 2, 4, 8, 12).

For both marker effect modification on MADRS, and association between change in marker and change in MADRS over time we employed GEE models to estimate the effects. The GEE models included GROUP and TIME as nominal factors, MARKER as a continuous variable, all two-way interactions, and the three-way interaction. The three-way interactions between GROUP × TIME × MARKER were explored primarily. In this setting, we followed the guidelines of Kraemer and colleagues, and as such the three-way interaction measured effect modification (Kraemer et al., 2002). In models with time-updating MARKER, the three-way interaction estimates group-specific differential change from baseline association between the analytes and total MADRS score across follow-ups. In instances where three-way interactions were not statistically significant ($p < 0.05$), two-way GROUP × MARKER and TIME × MARKER interactions were investigated. Example syntax for models can be found in supplementary materials section (see Table S2).

The Benjamini–Hochberg approach was applied to GROUP × TIME × MARKER and GROUP × MARKER outcomes to adjust for false discovery rates (FDR) to keep overall type I error across above mentioned comparisons at $\alpha = 0.05$. In these cases, the resultant adjusted *p*-values (*q*) were also reported. In the case of additional findings considering partial TIME × MARKER interactions, significance was set at $\alpha = 0.05$ as these outcomes were not related to treatment associated clinical outcomes. Co-authors who carried out the statistical analysis were blinded to treatment until the initial GEE analysis was complete.

2.4.3. Software

IBM SPSS Statistics v 26.0 and 27.0 were used for all statistical analyses. Benjamini–Hochberg corrections for FDR were applied using the SPSS extension stats_padjust v 1.0.4. Microsoft Excel 365 (v 2104) was used for data formatting and table preparation. GraphPad Prism version 8.0 was used for generating figures.

3. Results

3.1. Demographics

Means, standard deviations, frequencies and percentages are shown in Table 1. Overall, the mean age of participants was 54.2 years, and 55% were female. The mean illness duration was 17.42 years, and the mean MADRS score at baseline was 31.21 ± 4.35. No significant differences between groups were observed for measures of age, sex, research site, body mass index (BMI), illness duration, report of one or more psychiatric comorbidity, number of comorbid anxiety disorders, comorbid substance disorders, current suicide risk or suicidality risk rating, number of psychotropic medications, or medications overall. There were no significant differences observed between groups in the reported rates of nervous system, cardiovascular system, endocrine system, gastrointestinal system, musculoskeletal system, or other medical comorbidities; however, a significant difference in cases of respiratory system (placebo: 16.7%; minocycline: 43.5%) and genitourinary

Table 1
Subset demographics.

	total		placebo		minocycline		
	N =	47	*n* =	24	*n* =	23	
Age	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	
Years	54.19	±12.81	53.17	±12.32	55.26	±13.49	
[range]	[26.42–78.50]		[26.42–71.42]		[26.75–78.50]		
Sex	*n*	%	*n*	%	*n*	%	
Female	26	55.3%	13	54.2%	13	56.5%	
Illness duration - MDD	mean	sd	mean	sd	mean	sd	
formal diagnosis (years)	17.42	±12.17	16.21	±11.53	18.68	±12.93	
[range]	[0.67–42]		[3–42]		[0.67–41]		
Psychiatric comorbidity - current	*n*	%	*n*	%	*n*	%	
psychiatric disorders (1+)	40	85.1%	21	87.5%	19	82.6%	
anxiety disorders							
0	*9*	19.1%	*3*	12.5%	*6*	26.1%	
1	17	36.2%	7	29.2%	10	43.5%	
2	12	25.5%	7	29.2%	5	21.7%	
3 +	9	19.1%	7	29.2%	2	8.7%	
substance use disorders							
0	*41*	87.2%	*19*	79.2%	*22*	95.7%	
1	5	10.6%	4	16.7%	1	4.3%	
2	1	2.1%	1	4.2%	0	0.0%	
3 +	0	0.0%	0	0.0%	0	0.0%	
Suicidality - risk	*n*	%	*n*	%	*n*	%	
current risk	33	70.2%	16	66.7%	17	73.9%	
risk classification							
None	14	29.8%	8	33.3%	6	26.1%	
Low	14	29.8%	6	25.0%	8	34.8%	
Moderate	7	14.9%	3	12.5%	4	17.4%	
High	12	25.5%	7	29.2%	5	21.7%	
Body mass index (BMI)	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	
index score	28.74	±5.21	27.62	±5.39	29.91	±4.87	
[range]	[19.09–39.80]		[20.38–38.78]		[19.09–39.80]		
BMI classification	*n*	%	*n*	%	*n*	%	
under (<18.5)	0	0	0	0	0	0	
normal (18.5–24.9)	14	29.8	10	41.7	4	17.4	
over (25.0–29.9)	14	29.8	6	25	8	34.8	
obese (>29.9)	19	40.4	8	33.3	11	47.8	
Medications - current	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	
Medications (*n*)	6.17	±3.52	6.04	±3.26	6.30	±3.83	
Psychotropic medications (*n*)	1.68	±0.93	1.67	±1.01	1.70	±0.88	
Medications - class	*n*	%	*n*	%	*n*	%	
antidepressant(s)	41	87.2%	21	87.5%	20	87.0%	
Benzodiazepines	8	17.0%	3	12.5%	5	21.7%	
Antipsychotic	11	23.4%	5	20.8%	6	26.1%	
mood stabiliser	5	10.6%	4	16.7%	1	4.3%	
pain medication	20	42.6%	10	41.7%	10	43.5%	
complimentary/vitamins	32	68.1%	16	66.7%	16	69.6%	
other medications	37	78.7%	20	83.3%	17	73.9%	
Medical comorbidities - current	*n*	%	*n*	%	*n*	%	
nervous system	15	31.9%	8	33.3%	7	30.4%	
respiratory system	14	29.8%	4	16.7%	10	43.5%	*
cardiovascular system	14	29.8%	7	29.2%	7	30.4%	
endocrine system	12	25.5%	6	25.0%	6	26.1%	
gastrointestinal system	16	34.0%	9	37.5%	7	30.4%	
genitourinary system	8	17.0%	1	4.2%	7	30.4%	*
musculoskeletal system	29	61.7%	15	62.5%	14	60.9%	
other illnesses	15	31.9%	7	29.2%	8	34.8%	

Notes. Demographics and outcomes for original cohort published by Dean et al. (2017).
All data presented in this table collected during baseline visit (week 0). *$p < 0.05$.

system (placebo: 4.2%; minocycline: 30.4%) comorbidity were observed.

3.2. Detection rates and descriptive statistics

For the purposes of our study, only analytes that were above the lower limit of quantitation (LLOQ) with coefficient of variance (CV) ≤30% were deemed reliably 'detectable'. Of the 46 analytes assessed for immune-inflammation profiling, 27 were detectable in over 90% of the samples evaluated, and 16 analytes were detectable in <60% of the samples, see Table S3. As such, thirty analytes were included in the final analysis. A total of 35 participants had serum samples with viable makers at both baseline and endpoint. Means and SDs for biological analytes are provided in supplementary materials, see Table S4a (baseline and endpoint) and Table S4b (change, per protocol).

3.3. Within-groups comparisons for change in immune-inflammatory markers

Unadjusted paired *t*-tests (two-tailed) were conducted to assess within-groups comparisons for change in immune-inflammatory markers between baseline and endpoint. IL-18 significantly increased at endpoint (versus baseline), both in the total cohort, ($t_{(34)} = -2.180$, $p = 0.036$, Cohen's $d = 66.45$) and for the minocycline cohort ($t_{(15)} = -2.213$, $p = 0.043$, $d = 61.35$). Significant reductions in haptoglobin (Hp) and matrix metalloproteinase (MMP)-9 were also observed at endpoint for the minocycline group, ($t_{(14)} = 2.316$, $p = 0.036$, $d = 0.513$; and $t_{(13)} = 2.887$, $p = 0.013$, $d = 10.37$, respectively). No other statistically significant comparisons were observed. Full details of comparisons have been provided in Table S5.

3.4. Correlations

Pearson's correlation analyses were conducted initially to examine the general relationship between markers and MADRS score. Given these initial analyses were used chiefly for data inspection prior to GEE, they were not corrected for FDR. Results are summarized in Fig. 1. See Tables S6a and S6b for tabulated correlation coefficients for all markers versus MADRS relationships examined.

3.5. Marker effect modification models (GEE)

Three-way GROUP × TIME × MARKER interactions were observed for BDNF ($B = -0.436$, 95%CI [−0.74, −0.132], $p = 0.005$), MIP-1β/CCL4 ($B = -6.889$, 95%CI [−11.705, −2.072], $p = 0.005$), complement C3 ($B = -11.35$, 95%CI [−19.734, −2.971], $p = 0.008$), IL-1Ra ($B = -0.041$, 95%CI [−0.073, −0.009], $p = 0.092$) and IL-8/CXCL8 ($B = -2.520$, 95% CI [−0.073, −0.009], $p = 0.092$). After correcting for FDR, none of these three-way interactions remained significant. Plots for notable markers are shown in Fig. 2. Full and partial effect modification model outcomes shown in Table 2.

In this study, two-way TIME × MARKER interactions were treated as additional findings, and as such were not corrected for FDR. Significant TIME × MARKER interactions were observed for stem cell factor (SCF) ($B = 0.013$, 95%CI [0.005, 0.020], $p = 0.001$) and factor VII ($B = -0.010$, 95%CI [−0.020, −0.001], $p = 0.027$). This suggests that these markers at baseline, irrespective of treatment group were predictive of change in the MADRS score post-baseline.

3.6. Association between marker change and MADRS change (GEE)

Three-way GROUP × TIME × MARKER interactions were significant for complement C3 ($B = -10.46$, 95%CI [−16.832, −4.095], $p = 0.001$), IL-1Ra ($B = -9.008$, 95%CI [−15.26, −2.751], $p = 0.005$). Two-way GROUP × BIOMARKER interactions were significant for ICAM-1/CD54 ($B = -0.387$, 95%CI [−0.513, −0.26], $p < 0.001$), IL-8/CXCL8 ($B = -4.586$, 95%CI [−7.698, −1.475], $p = 0.004$), MMP-9 ($B = -0.53$, 95%CI [−0.976, −0.085], $p = 0.020$), RANTES/CCL5 ($B = 0.514$, 95%CI [0.079, 0.95], $p = 0.021$), and ferritin ($B = -6.016$, 95%CI [−11.864, −0.168], $p = 0.044$).

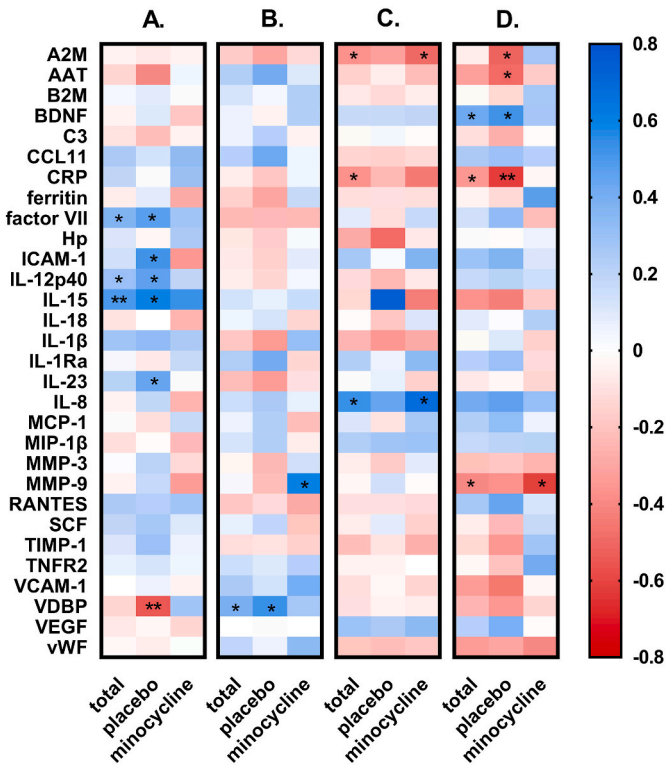

Fig. 1. *Pearson's correlations heatmap.* Negative correlation coefficients represented by red shading; positive coefficients represented by blue shading. **(A)** *baseline analyte vs. baseline MADRS score* - A positive coefficient suggests higher marker concentration at baseline correlated with higher initial depression score; a negative coefficient suggests lower marker concentration at baseline correlated with higher depression score. **(B)** *baseline analyte vs. change in MADRS score* - A positive coefficient suggests a lower concentration of analyte at baseline correlated with improvement in MADRS between baseline and week 12; a negative coefficient suggests higher baseline marker correlated with improvement in MADRS. **(C)** *change in marker vs. endpoint MADRS score* - A positive coefficient suggests an increase in marker concentration (change between baseline and week 12) correlated with worse depression score at end; a negative correlation suggests that an increase in marker concentration between baseline and week 12 correlated with better depression score at endpoint. **(D)** *Change in marker vs. change in MADRS score* - A positive correlation coefficient indicates that a reduction in marker concentration correlated with improvement in depression score between baseline and week 12; a negative coefficient indicates that an increase in marker concentration (change) correlated with improvement in depression score. *$p < 0.05$; **$p < 0.01$, unadjusted. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

After correcting for FDR, the three-way interactions for C3, IL-1Ra, and two-way interactions for ICAM-1/CD54 and IL-8/CXCL8 remained significant. As these *B*-values were negative, these interactions indicate that every 1-unit increase in the analytes between baseline and endpoint was associated with an additional *B*-unit improvement in MADRS in the minocycline group compared to the placebo group post-baseline. Full and partial change association GEE model outcomes are shown in Table 3.

Significant two-way TIME × MARKER interactions were observed for IL-23 ($B = -2.25$, 95%CI [−3.95, −0.55], $p = 0.009$), MIP-1β/CCL4 ($B = 4.8$, 95%CI [1.119, 8.48], $p = 0.011$), MCP-1 ($B = 0.01$, 95%CI [0.001, 0.019], $p = 0.034$), MMP-3 ($B = 0.349$, 95%CI [0.019, 0.679], $p = 0.038$) and again SCF ($B = 0.011$, 95%CI [0.000, 0.021], $p = 0.047$) and

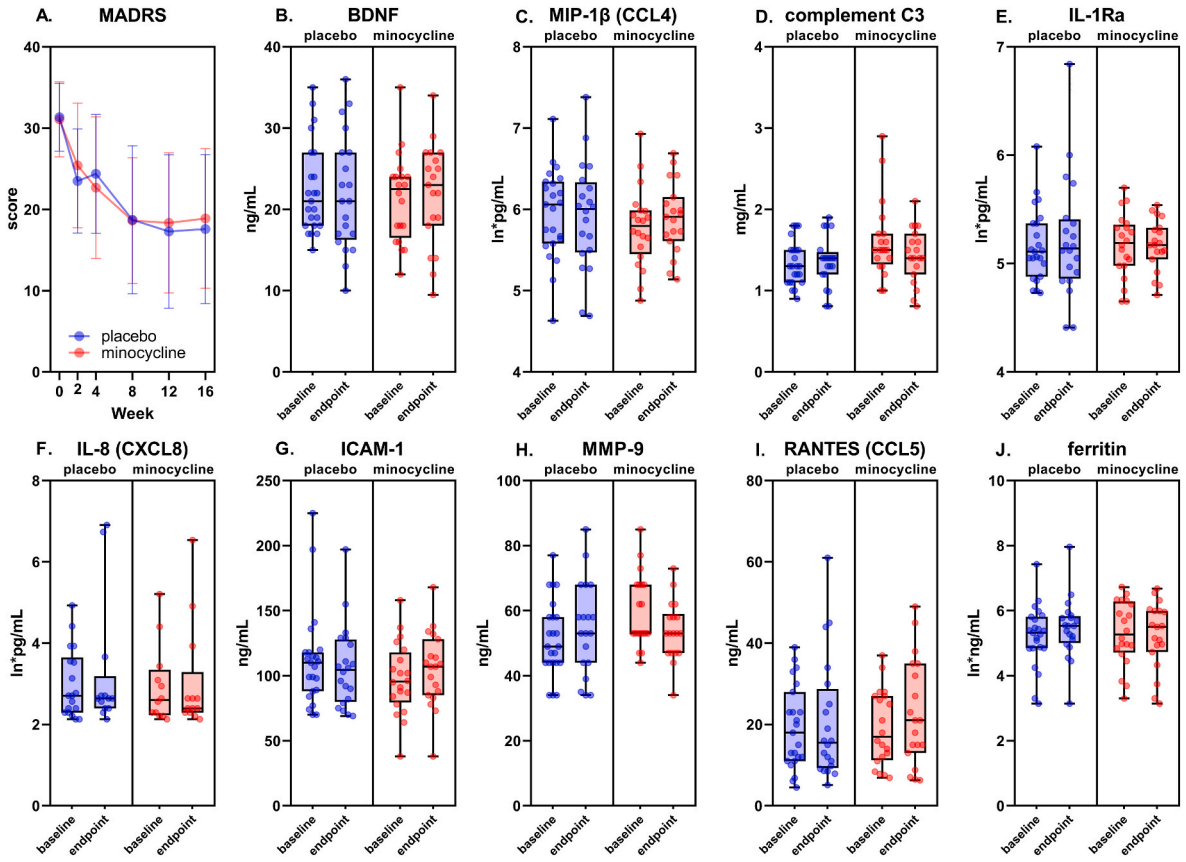

Fig. 2. **(A)** Mean MADRS total scores ± standard deviation over time for this cohort subset. **(B–J).** Box and whiskers plots (min to max) for notable markers following analyses using GEE models. In all graphs the placebo group represented by blue shading, and minocycline group represented in red shading. Full descriptive statistics provided in Table S4. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

factor VII ($B = -0.014$, 95%CI [−0.022, −0.006], $p = 0.001$). This suggests that change in these markers between baseline and endpoint, irrespective of treatment group, was associated with change in MADRS.

4. Discussion

The underlying pathophysiology and biological treatment response pathways of both conventional pharmacotherapy and novel anti-inflammatory agents need further investigation. These agents may be more efficacious if we have corresponding biomarker profiles guiding treatment application (Felger et al., 2020). A subset of people with MDD appear to exhibit elevated inflammatory cytokines, and higher baseline inflammation is associated with resistance to antidepressant treatment (Leighton et al., 2018). The pleiotropic properties of minocycline include immunomodulatory and anti-inflammatory effects. Therefore, these pathways are an intuitive starting point for biomarker profiling. In this study we attempted to quantify 46 immune-inflammatory markers in peripheral serum samples collected during an RCT of adjunctive minocycline (200 mg/d) for MDD. Of these analytes, 30 were detectable in a sufficient number of samples for analysis using GEE models.

4.1. Markers of treatment response: minocycline specific observations

4.1.1. Complement C3

Despite being a common marker included in routine blood tests, relatively few studies have investigated the relationship between complement C3 and MDD, which remains unclear. One early study reported no significant differences between the mean levels of C3 detected between depressed patients and controls (Berk et al., 1997). Another, examining an acute phase protein response across psychiatric disorders (schizophrenia ($n = 27$), mania ($n = 23$), MDD ($n = 29$) and healthy control ($n = 21$) participants) found that C3 was significantly higher in MDD patients than controls, and in non-medicated patients compared to those taking antidepressants (Maes et al., 1997b). A more recent study assessing plasma C3 levels in medication-free MDD patients ($n = 49$) compared to healthy controls ($n = 45$), found results consistent with this (Luo et al., 2022). In contrast, a large cross-sectional cross-disorder study reported significantly lower levels of C3 in MDD ($n = 1521$), than in schizophrenia ($n = 1659$) or bipolar disorder ($n = 1901$) (Lyu et al., 2021). In a smaller study ($n = 95$), serum C3 levels were significantly lower in *de novo* depression than in recurrent depression and control groups (Tao et al., 2020). Emerging evidence supports a role for C3 as a biomarker for insulin resistance and cardiometabolic diseases (Ursini and Abenavoli, 2018). Thus, C3 may show potential in the case of patients presenting with immunometabolic features, which have been argued to constitute a distinct phenotype of depression (Milaneschi et al., 2020); this line of inquiry is deserving of further investigation.

Notably, serum C3 at baseline was reported to be negatively associated with response to the tricyclic antidepressant imipramine ($n = 26$), and conversely was positively associated with mixed antidepressant treatments ($n = 38$) (Chan et al., 2016). In the current study GEE models indicated a significant association between change in MADRS and C3 ($B = -10.46$, $q = 0.019$), suggesting that increase in C3 over time was associated with greater therapeutic response to minocycline post-baseline. There was also some indication that higher C3 levels at baseline might predict response to minocycline over time ($B = -11.35$, $p = 0.008$), however, this model did not survive correction for FDR ($q = 0.079$).

Table 2
Generalized estimating equations - Effect modification.

marker		*B*	95% CI		*P*	*q*	sig.
			lower	upper			
full model (GROUP × TIME × MARKER)							
BDNF		−0.436	−0.740	−0.132	**0.005**	0.076	†
MIP-1β	a	−6.889	−11.71	−2.072	**0.005**	0.076	†
C3		−11.35	−19.73	−2.971	**0.008**	0.079	†
IL-1Ra		−0.041	−0.073	−0.009	**0.012**	0.092	ns
IL-8	a,b	−2.520	−4.601	−0.440	**0.018**	0.105	ns
partial model (GROUP × MARKER)							
CRP	a,b	1.862	−0.142	3.866	0.069	0.318	ns
MMP-9		−0.464	−0.975	0.047	0.075	0.318	ns
VEGF	b	−0.015	−0.033	0.002	0.085	0.318	ns
VDBP	b	0.021	−0.012	0.054	0.221	0.673	ns
factor VII		−0.042	−0.111	0.026	0.224	0.673	ns
IL-15		−10.05	−32.20	12.11	0.374	0.970	ns
Hp		4.556	−5.934	15.05	0.395	0.970	ns
CCL11	a	4.999	−7.423	17.42	0.430	0.970	ns
IL-12p40	b	−6.686	−25.00	11.62	0.474	0.970	ns
TNFR2	a	−8.352	−39.31	22.61	0.597	0.970	ns
MCP-1	b	0.005	−0.016	0.026	0.629	0.970	ns
MMP-3		−0.570	−3.017	1.878	0.648	0.970	ns
ferritin	a,b	−1.943	−10.35	6.460	0.650	0.970	ns
IL-18	b	−0.006	−0.037	0.025	0.693	0.970	ns
RANTES		0.170	−0.813	1.154	0.735	0.970	ns
vWF	b	0.008	−0.045	0.062	0.757	0.970	ns
IL-23		1.135	−9.326	11.60	0.832	0.970	ns
IL-1β	b	0.229	−1.951	2.409	0.837	0.970	ns
ICAM-1		0.030	−0.254	0.314	0.838	0.970	ns
AAT		−1.593	−18.02	14.84	0.849	0.970	ns
VCAM-1		−0.004	−0.054	0.046	0.870	0.970	ns
TIMP-1	b	0.003	−0.035	0.041	0.882	0.970	ns
B2M		1.606	−24.79	28.00	0.905	0.970	ns
SCF		−0.001	−0.046	0.044	0.968	0.999	ns
A2M		−0.006	−13.63	13.61	0.999	0.999	ns

Notes. Three-way GROUP × TIME × MARKER interactions first explored; where not statistically significant, two-way GROUP × MARKER interactions were investigated. MARKER variable was time-invariant. Benjamini-Hochberg adjusted *p*-values (*q*) used to adjust for false discovery rates (FDR) and maintain an $\alpha = 0.05$; †$q < 0.08$. [a] log natural (ln) transformation applied to analyte; [b] exchangeable correlation matrix used.

Table 3
Generalized estimating equations – Association of change co-occurring in markers and MADRS outcomes.

marker		*B*	95% CI		*P*	*q*	sig.
			lower	upper			
full model (GROUP × TIME × MARKER)							
C3		−10.46	−16.83	−4.095	**0.001**	**0.019**	*
IL-1Ra	a	−9.008	−15.26	−2.751	**0.005**	**0.036**	*
partial model (GROUP × MARKER)							
ICAM-1		−0.387	−0.513	−0.260	**0.000**	**0.000**	*
IL-8	a	−4.586	−7.698	−1.475	**0.004**	**0.036**	*
MMP-9		−0.530	−0.976	−0.085	**0.020**	0.103	ns
RANTES		0.514	0.079	0.950	**0.021**	0.103	ns
ferritin	a	−6.016	−11.86	−0.168	**0.044**	0.188	ns
MMP-3		−1.330	−2.752	0.092	0.067	0.250	ns
MIP-1β	a,b	−5.717	−12.11	0.679	0.080	0.266	ns
factor VII		−0.025	−0.056	0.005	0.103	0.288	ns
TNFR2	a	−17.25	−38.14	3.639	0.106	0.288	ns
VCAM-1		−0.023	−0.061	0.014	0.220	0.549	ns
VDBP	b	0.018	−0.014	0.051	0.264	0.570	ns
IL-1β	b	−0.702	−1.939	0.535	0.266	0.570	ns
Hp		3.293	−3.456	10.04	0.339	0.644	ns
AAT		−6.496	−19.93	6.940	0.343	0.644	ns
B2M		−11.79	−37.39	13.82	0.367	0.647	ns
A2M		−4.233	−14.26	5.798	0.408	0.680	ns
IL-12p40	b	−5.876	−21.99	10.24	0.475	0.750	ns
IL-18	b	−0.008	−0.034	0.018	0.546	0.819	ns
SCF		−0.010	−0.049	0.029	0.612	0.875	ns
TIMP-1		0.034	−0.113	0.181	0.651	0.884	ns
CCL11	a	−1.614	−9.915	6.687	0.703	0.884	ns
vWF		−0.019	−0.132	0.093	0.734	0.884	ns
VEGF	b	−0.004	−0.025	0.018	0.737	0.884	ns
IL-15		2.663	−18.46	23.78	0.805	0.922	ns
IL-23		0.308	−2.890	3.506	0.850	0.922	ns
BDNF		−0.062	−0.747	0.624	0.860	0.922	ns
MCP-1		0.002	−0.038	0.042	0.917	0.949	ns
CRP	a	0.157	−5.382	5.696	0.956	0.956	ns

Notes. Three-way GROUP × TIME × MARKER interactions first explored; where not statistically significant, two-way GROUP × MARKER interactions were investigated. MARKER variable was time-updating. Benjamini-Hochberg adjusted *p*-values (*q*) used to adjust for false discovery rates (FDR) and maintain an $\alpha = 0.05$; [a] log natural transformation applied to marker; [b] exchangeable correlation matrix used. *$q < 0.05$.

4.1.2. *Interleukin 1 receptor antagonist (IL-1Ra)*

IL-1Ra is a naturally occurring antagonist for IL-1 receptors (the primary receptor of IL-1β), inhibiting proinflammatory signaling via competitive binding. Thus, IL-1Ra exerts 'anti-inflammatory' effects via its involvement in regulatory feedback (Fields et al., 2019). An early study reported significantly higher serum IL-1Ra in subjects with MDD and treatment-resistant depression, wherein sub-chronic antidepressant treatment had no significant effects on serum level (Maes et al., 1997a). Meta-analyses are congruent with this finding, having reported elevated levels of peripheral IL-1Ra in neuropsychiatric disorders, including depression (Goldsmith et al., 2016; Kohler et al., 2018). Baseline serum IL-1Ra was also positively associated with improved response in a mixed treatment cohort ($n = 38$) (Chan et al., 2016). In the current study, there was some indication that IL-1Ra levels at baseline might predict minocycline response over time ($B = -0.041$, $p = 0.012$) however this interaction effect did not survive adjustment for FDR ($q = 0.092$). However, increase in IL-1Ra was found to be associated with reduction in MADRS post-baseline in minocycline group, compared to the placebo group ($B = -9.008$, $p = 0.005$, $q = 0.036$).

4.1.3. *Soluble intercellular adhesion molecule-1 (ICAM-1/CD54)*

ICAM-1 is an immunoglobulin (Ig)-like transmembrane glycoprotein that plays an important regulatory role in proinflammatory immune responses. Prior studies indicate it is differentially expressed across psychiatric disorders (Muller, 2019). Adhesion and transmigration of neutrophils across the blood brain barrier (BBB) is in part dependent on ICAM-1 and its upregulation. This process in response to chemokine synthesis and proinflammatory secretion might play a significant role in the progression of BBB dysfunction. A 'leaky' BBB may play a role in the onset and/or progression of neuropsychiatric disorders, including MDD (Morris et al., 2018a). Increased BBB permeability and dysfunction of the neurovascular unit can be induced by peripheral inflammation. Moreover, increased BBB permeability may also have effects on peripheral immune and inflammatory pathways, although this is an emerging area of research (Morris et al., 2018a).

Soluble ICAM-1 is found in the serum; it arises from the proteolytic cleavage of membrane-bound ICAM-1. Levels of soluble ICAM-1 are generally low in healthy individuals, and increases are observed in illness with functional implications (Muller, 2019). A large meta-analysis investigating markers of microvascular dysfunction in late-life depression reported that higher levels of soluble ICAM-1 in the plasma were associated with depression (*OR* = 1.58, 95%CI [1.28, 1.96]) (van Agtmaal et al., 2017). Soluble ICAM-1 in serum was also reported to be positively associated with treatment-response in two mixed antidepressant cohorts ($n = 38$, $n = 21$), and negatively associated with venlafaxine treatment response ($n = 30$) (Chan et al., 2016). In our study, the GEE models indicated a significant partial association between change in MADRS and soluble ICAM-1 ($B = -0.387$, $q < 0.001$), suggesting that an increase in soluble ICAM-1 was associated with greater reductions in post-baseline MADRS scores for minocycline-treated participants.

4.1.4. Interleukin-8 (IL-8/CXCL8)

IL-8/CXCL8 is an α-chemokine with a range of proinflammatory effects, predominantly involving chemotaxis, and the activation and adhesion of neutrophils (Tsai, 2021). A recent meta-analysis has indicated that MDD patients who responded to treatment had lower baseline levels of IL-8/CXCL8 than the non-responders (Liu et al., 2020). Earlier meta-analyses have drawn varied conclusions on IL-8/CXCL8; this inconsistency has been attributed to potential treatment-specific and sex-specific differences (Tsai, 2021). A 4-week RCT reported a significant within-groups mean increase in IL-8/CXCL8 for the minocycline treated group (Nettis et al., 2021). In this study, there was some indication that IL-8/CXCL8 levels at baseline may predict improvement in MADRS score over time in the minocycline group, however the interaction did not survive correction for FDR ($B = -2.52$, $p = 0.018$, $q = 0.105$). Change in IL-8/CXCL8 was however, found to be associated with change in MADRS over time in the minocycline group, compared to the placebo group ($B = -4.586$, $p = 0.004$, $q = 0.036$).

Although they did not survive correction for FDR, several other analytes may warrant further investigation as putative biomarkers. These are discussed in brief below.

4.2. Other possible predictive markers of minocycline response

4.2.1. Brain derived neurotrophic factor (BDNF)

BDNF plays an important role in neuronal survival and synaptogenesis. Due to its implication in the pathophysiology of MDD and the antidepressant response, BDNF has been widely investigated as a possible biomarker and therapeutic target; for review, see (Castren and Monteggia, 2021). Most studies report low BDNF levels in MDD, with increases associated with antidepressant response. Notably, a meta-analysis reported a significant, albeit modest overall effect of treatment-resistant depression treatments on peripheral BDNF levels (21 studies, $g = 0.34$) (Meshkat et al., 2022). In the current study, there was some evidence that higher levels of baseline BDNF predicted greater improvement over time in the minocycline group, but the interaction did not survive correction for FDR, ($B = -0.436$, $p = 0.005$, $q = 0.076$). It should be noted, BDNF was measured in these samples previously ($n = 47$) using enzyme-linked immunosorbent assays (ELISA); in that study no significant moderation effects were observed (Hasebe et al., 2022). Differences between these outcomes could be due to sample availability (a difference of $n = 4$), assay sensitivity, or minor differences in statistical methodology.

4.2.2. Macrophage inflammatory protein 1β (MIP-1β/CCL4)

A previous meta-analysis found blood MIP-1β/CCL4 levels were significantly lower in depressed individuals, with no evidence of heterogeneity (5 studies, $N = 507$; $SMD = -0.31$) (Leighton et al., 2018). MIP-1β/CCL4 is chemoattractant for monocytes, microglia and $CD4^+$ T cells, and plays a role in natural killer (NK) cell activation. Blunted NK cell activity was previously reported in inpatients with MDD ($n = 36$) compared to healthy controls ($n = 13$) (Maes et al., 1994), and reduced NK cell percentages have been reported in MDD (with melancholic features) predicting antidepressant non-response (Grosse et al., 2016). Plasma MIP-1β/CCL4 levels were positively correlated with depressive symptoms ($coef. = 0.94$, $OR = 2.55$), and negatively correlated with TLR4 (toll-like receptor 4 gene) CpG site cg05429895 methylation in women ($N = 92$, $coef. = -0.78$) (Rasmusson et al., 2021). In our study, though there was some evidence of MIP-1β effect modification effects in minocycline treated-participants, this model did not survive correction for FDR ($B = -6.889$, $p = 0.005$, $q = 0.076$).

4.3. Other possible associations between change in markers and minocycline response

Although they did not survive correction for FDR, several other analytes were observed to change across time in association with change in MADRS after treatment with minocycline.

4.3.1. Matrix metalloproteinase 9 (MMP-9)

Though failing to survive corrections for FDR ($B = -0.530$, $p = 0.020$, $q = 0.103$), a significant reduction in MMP-9 was observed at endpoint for the minocycline group, ($p = 0.036$, $d = 0.513$). This marker is implicated in the mechanisms of minocycline, and its putative neuroprotective actions (Garrido-Mesa et al., 2013). Metalloproteinases play an important role in modulating inflammation and have been investigated in several inflammatory diseases including MDD (Bobinska et al., 2016). They break down the extracellular matrix and promote tissue remodeling via the activation of cytokines and chemokines (Yabluchanskiy et al., 2013). Upregulation of MMP-9 may be involved in IL-1β-induced disturbances in the BBB (Morris et al., 2018a). A study investigating gene expression of metalloproteinase polymorphisms in individuals with MDD ($n = 142$) versus controls ($n = 100$) reported increased gene expression of metalloproteinases at the mRNA level, in addition to increased protein concentration and activity of MMP-9 (and proMMP-9), possibly reflecting a common factor for somatic disease and MDD (Bobinska et al., 2016). Incidentally, medications used for the treatment of cardiovascular disease (which is highly comorbid with MDD) tend to inhibit the production and activity of MMP-9 (Yabluchanskiy et al., 2013).

4.3.2. Regulated on activation, normal T cell expressed and secreted (RANTES/CCL5)

In the periphery RANTES/CCL5 is involved in T lymphocyte, basophil, and eosinophil chemotaxis and activation. In addition to its important role in inflammation, it also plays a role in angiogenesis. Within the CNS it affects microglial chemotaxis and HPA axis modulation (Singhal and Baune, 2018). An observational study in 60 MDD patients who underwent 5 weeks of antidepressant treatment reported that, relative to non-responders, RANTES/CCL5 and CCR levels were significantly lower in responders, even prior to treatment (Bauer et al., 2020). In our study, the partial interaction for RANTES/CCL5 did not survive correction for FDR in the change association models ($B = 0.514$, $p = 0.021$, $q = 0.103$).

4.3.3. Ferritin

Ferritin, which plays an important role in iron sequestration and homeostasis, is considered an acute-phase protein. A study in 38 MDD patients and 15 healthy volunteers found higher levels of ferritin MDD patients with melancholia than in those with non-melancholic major depression and normal controls. The study also found significantly increased serum ferritin in treatment-resistant patients (Maes et al., 1996). This is consistent with research reporting elevated serum ferritin in post-stroke depression versus controls (Zhu et al., 2016). In this study, interaction effects for change in ferritin was not significant after FDR adjustment ($B = -6.016$, $p = 0.044$, $q = 0.188$).

4.4. Additional findings not associated with treatment group

While not a part of our primary investigations, we observed several significant TIME × MARKER interactions in our GEE models independent of treatment group. Significant partial interactions for effect modification were observed for SCF ($B = 0.013$, $p = 0.001$), and coagulation factor VII (f., proconvertin) ($B = -0.010$, $p = 0.027$), suggesting that levels of these markers at baseline were predictive of change in MADRS score. Significant partial interactions for change association were observed for IL-23 ($B = -2.25$, $p = 0.009$), MIP-1β/CCL4 ($B = 4.8$, $p = 0.011$), MCP-1 ($B = 0.01$, $p = 0.034$), MMP-3 ($B = 0.349$, $p = 0.038$) and again SCF ($B = 0.011$, $p = 0.047$) and factor VII ($B = -0.014$, $p = 0.001$), which suggests that change in these markers between baseline and endpoint, irrespective of treatment group, was associated with additional change in MADRS. Given their association with treatment-response independent of treatment, these analytes may be worthy of further consideration in

the future.

4.5. Conclusions

While much research to date has investigated inflammatory markers, few have attempted to identify predictive biomarkers to identify possible causal relationships. While we found some evidence that concentration of baseline C3, IL-1Ra, IL-8/CXCL8, BDNF, and MIP-1β/CCL4 may be useful as predictive biomarkers of minocycline treatment response, none of these findings survived correction for FDR. Importantly, FDR correction may be less imperative for analytes in interdependent protein-protein networks, such as cytokines and neurotrophic factors (Mehterov et al., 2022), and so these results should not be fully discounted. We also found evidence suggesting that change in complement C3, IL-1Ra, ICAM-1/CD54, and IL-8/CXCL8, from baseline to endpoint was associated with change in overall depressive score over 12-weeks following minocycline treatment, suggesting putative therapeutic pathways. Additionally, there was some indication for change in MMP-9, RANTES/CCL5, and ferritin, although these did not survive FDR correction. Interestingly, Chan and colleagues previously found nine proteins were significantly associated with antidepressant response, amongst these were soluble ICAM-1 and C3, supporting a possible role for these biomarkers in antidepressant response (Chan et al., 2016).

The current study assessed a substantial number of analytes and, therefore, we have attempted to follow a general principle of parsimony. While not shown, as a matter of course, in cases where the base model returned a target interaction that was statistically significant, iterative models were employed to examine age, sex, illness duration and BMI as potential covariate predictors over time. Though for some markers these covariates were statistically significant, in all cases the target interactions were found to remain statistically significant, with marginal improvements to standard error, confidence intervals and/or *B*-values. Collectively this suggests that the biomarker related change association observed was not better explained by these covariates. For this reason, only base GEE models were presented.

Minocycline may exert its antidepressant-like effects act via the suppression of the NLRP3 inflammasome (Wong et al., 2016). Minocycline can inhibit caspase-1 signalling pathways, attenuating the subsequent maturation and secretion of IL-1β (Wong et al., 2016). IL-1β is reported to increase the expression of a host of immune-inflammatory factors, including cytokines (e.g., IL-1Ra), hepatic acute phase reactants (CRP, Hp), complement components (including C3) and adhesion molecules (including ICAM-1) (Dinarello, 1996). IL-1β also reportedly upregulates a range of neutrophil-active chemokines, including e.g., IL-8/CXCL8, RANTES/CCL5, MIP-1β,/CCL4 (Chou et al., 2010; Maes and Carvalho, 2018; Tsai, 2021). While we did not observe significant effects for IL-1β in this study, this is an important line of inquiry for future research. It is possible that the changes in immune-inflammatory analytes included in this study could reflect this. The NLRP3 inflammasome has been implicated in the pathophysiology of MDD and may be a useful treatment target in neuropsychiatric disorders (Morris et al., 2018b). Given that minocycline is an antibiotic, the possibility that observed antidepressant effects may be in part mediated by gut microbiota also cannot be discounted and is an important consideration for future studies (Wong et al., 2016).

4.5.1. Limitations and future directions

The need for a larger cohort, and replication of these findings notwithstanding, several next steps are suggested. Investigation of other relevant immunomodulatory interactants, for example the kynurenine pathway is of particular interest; for comprehensive meta-analysis, see (Marx et al., 2021). Recent evidence suggest that specific symptom clusters or subtypes may be reflected in immunophenotypes, which may be helpful when considering treatment. For example, anti-cytokine treatments have been shown to be more effective in treating anhedonia – a core feature of MDD (Felger and Miller, 2020). Further, the stratification of patients based on immunometabolic disturbances has been proposed to aid in treatment selection (Milaneschi et al., 2020).

Although the current sub-study was part of planned secondary analysis, samples were only available for a proportion of participants in the original trial. To minimize participant burden during the original trial, venous blood collected was non-fasted and consequently, any confounding influences of food consumption on inflammatory profile could not be discounted (Kiecolt-Glaser, 2010). The effects of having a greater number of medical comorbidities in the minocycline group on the response to an adjunctive anti-inflammatory treatment are unknown. Biological samples were also only collected at two timepoints (at baseline and week 12), meaning true mediation effects could not be assessed using GEE; as a result, change association models were used.

A limitation of the current study is that several markers were not detectable in sufficient samples/consistent enough concentrations to be included in these analyses (IL-6, IL-10, IL-17, MIP-1α/CCL3, and TNF-α among others). Nuanced perspectives have proposed a conceptual shift to a systems approach to immune biomarker investigations, framing analytes in the context of an immune-inflammatory response system (IRS) and compensatory immune-regulatory reflex system (CIRS) (Maes and Carvalho, 2018). This approach involves the calculation of *z*-unit weighted composite scores, allowing for broader assessment of immunoregulatory profiles (e.g., Th1, Th2, Th17, Treg, M1) which may play an important role in affective disorders (Maes and Carvalho, 2018). In the case of this study, as aforementioned several analytes important in these profiles were consistently below the LLOQ or simply not detected. Here, GEE was used to investigate the relationship between treatment, individual analytes and time using a working correlation matrix. This longitudinal approach is less reductive but does limit the ability to explain analytes in concert. Future studies could consider principal components analysis, partial least squares, or network analysis approaches to explore these markers further.

To date, while investigations into immune-inflammatory markers have provided interesting insights into the pathophysiology of MDD, efforts to use these analytes as pragmatic biomarkers have largely been *a priori*, and limited in their clinical use (Berk et al., 2019). Indeed, biomarker tools cannot be adopted before being empirically validated for translational utility. Evidence currently suggests minocycline is a promising candidate for adjunctive treatment in MDD. However, it is evident from recent studies e.g., (Nettis et al., 2021), that not all patients will benefit from such a treatment. The current study has detected certain novel theragnostic serum markers which could be potentially used as therapeutic biomarkers for adjunctive minocycline treatment but was unable to identify any predictive markers after correcting for FDR. Regardless, the outcomes of this study underscore a need to look more broadly than at common markers (e.g., CRP), especially when exploring novel therapies. Developing complex algorithms based on multiple simultaneous markers as well as salient clinical features may be a logical next step towards precision approaches for MDD and related disorders (Dean and Walker, 2022).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Bai, S., Guo, W., Feng, Y., Deng, H., Li, G., Nie, H., Guo, G., Yu, H., Ma, Y., Wang, J., Chen, S., Jing, J., Yang, J., Tang, Y., Tang, Z., 2020. Efficacy and safety of anti-inflammatory agents for the treatment of major depressive disorder: a systematic review and meta-analysis of randomised controlled trials. J. Neurol. Neurosurg. Psychiatry 91, 21–32.

Bauer, O., Milenkovic, V.M., Hilbert, S., Sarubin, N., Weigl, J., Bahr, L.M., Wetter, T.C., Heckel, B., Wetzel, C.H., Rupprecht, R., Nothdurfter, C., 2020. Association of chemokine (C-C motif) receptor 5 and ligand 5 with recovery from major depressive disorder and related neurocognitive impairment. Neuroimmunomodulation 27, 152–162.

Berk, M., Wadee, A.A., Kuschke, R.H., O'Neill-Kerr, A., 1997. Acute phase proteins in major depression. J. Psychosom. Res. 43, 529–534.

Berk, M., Walker, A.J., Nierenberg, A.A., 2019. Biomarker-guided anti-inflammatory therapies: from promise to reality check. JAMA Psychiatr. 76, 779–780.

Bobinska, K., Szemraj, J., Czarny, P., Galecki, P., 2016. Expression and activity of metalloproteinases in depression. Med. Sci. Mon. Int. Med. J. Exp. Clin. Res. 22, 1334–1341.

Cai, D.B., Zheng, W., Zhang, Q.E., Ng, C.H., Ungvari, G.S., Huang, X., Xiang, Y.T., 2020. Minocycline for depressive symptoms: a meta-analysis of randomized, double-blinded, placebo-controlled trials. Psychiatr. Q. 91, 451–461.

Castren, E., Monteggia, L.M., 2021. Brain-Derived neurotrophic factor signaling in depression and antidepressant action. Biol. Psychiatr. 90, 128–136.

Chan, M.K., Cooper, J.D., Bot, M., Birkenhager, T.K., Bergink, V., Drexhage, H.A., Steiner, J., Rothermundt, M., Penninx, B.W., Bahn, S., 2016. Blood-based immune-endocrine biomarkers of treatment response in depression. J. Psychiatr. Res. 83, 249–259.

Chou, R.C., Kim, N.D., Sadik, C.D., Seung, E., Lan, Y., Byrne, M.H., Haribabu, B., Iwakura, Y., Luster, A.D., 2010. Lipid-cytokine-chemokine cascade drives neutrophil recruitment in a murine model of inflammatory arthritis. Immunity 33, 266–278.

Dean, O.M., Data-Franco, J., Giorlando, F., Berk, M., 2012. Minocycline: therapeutic potential in psychiatry. CNS Drugs 26, 391–401.

Dean, O.M., Kanchanatawan, B., Ashton, M., Mohebbi, M., Ng, C.H., Maes, M., Berk, L., Sughondhabirom, A., Tangwongchai, S., Singh, A.B., McKenzie, H., Smith, D.J., Malhi, G.S., Dowling, N., Berk, M., 2017. Adjunctive minocycline treatment for major depressive disorder: a proof of concept trial. Aust. N. Z. J. Psychiatr. 51, 829–840.

Dean, O.M., Walker, A.J., 2022. Current approaches to precision medicine in psychiatry: are we just spinning our wheels? Eur. Neuropsychopharmacol 66, 11–13.

Dinarello, C.A., 1996. Biologic basis for interleukin-1 in disease. Blood 87, 2095–2147.

Felger, J.C., Haroon, E., Patel, T.A., Goldsmith, D.R., Wommack, E.C., Woolwine, B.J., Le, N.A., Feinberg, R., Tansey, M.G., Miller, A.H., 2020. What does plasma CRP tell us about peripheral and central inflammation in depression? Mol. Psychiatr. 25, 1301–1311.

Felger, J.C., Miller, A.H., 2020. Identifying immunophenotypes of inflammation in depression: dismantling the monolith. Biol. Psychiatr. 88, 136–138.

Fields, J.K., Gunther, S., Sundberg, E.J., 2019. Structural basis of IL-1 family cytokine signaling. Front. Immunol. 10, 1412.

Garrido-Mesa, N., Zarzuelo, A., Galvez, J., 2013. Minocycline: far beyond an antibiotic. Br. J. Pharmacol. 169, 337–352.

Goldsmith, D.R., Rapaport, M.H., Miller, B.J., 2016. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. Mol. Psychiatr. 21, 1696–1709.

Grosse, L., Carvalho, L.A., Birkenhager, T.K., Hoogendijk, W.J., Kushner, S.A., Drexhage, H.A., Bergink, V., 2016. Circulating cytotoxic T cells and natural killer cells as potential predictors for antidepressant response in melancholic depression. Restoration of T regulatory cell populations after antidepressant therapy. Psychopharmacology (Berl) 233, 1679–1688.

Hasebe, K., Mohebbi, M., Gray, L., Walker, A.J., Bortolasci, C.C., Turner, A., Berk, M., Walder, K., Maes, M., Kanchanatawan, B., Ashton, M.M., Berk, L., Ng, C.H., Malhi, G. S., Singh, A.B., Dean, O.M., 2022. Exploring interleukin-6, lipopolysaccharide-binding protein and brain-derived neurotrophic factor following 12 weeks of adjunctive minocycline treatment for depression. Acta Neuropsychiatr. 34, 220–227.

Husain, M.I., Chaudhry, I.B., Khoso, A.B., Husain, M.O., Hodsoll, J., Ansari, M.A., Naqvi, H.A., Minhas, F.A., Carvalho, A.F., Meyer, J.H., Deakin, B., Mulsant, B.H., Husain, N., Young, A.H., 2020. Minocycline and celecoxib as adjunctive treatments for bipolar depression: a multicentre, factorial design randomised controlled trial. Lancet Psychiatr. 7, 515–527.

Kiecolt-Glaser, J.K., 2010. Stress, food, and inflammation: psychoneuroimmunology and nutrition at the cutting edge. Psychosom. Med. 72, 365–369.

Kohler, C.A., Freitas, T.H., Maes, M., de Andrade, N.Q., Liu, C.S., Fernandes, B.S., Stubbs, B., Solmi, M., Veronese, N., Herrmann, N., Raison, C.L., Miller, B.J., Lanctot, K.L., Carvalho, A.F., 2017. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. Acta Psychiatr. Scand. 135, 373–387.

Kohler, C.A., Freitas, T.H., Stubbs, B., Maes, M., Solmi, M., Veronese, N., de Andrade, N. Q., Morris, G., Fernandes, B.S., Brunoni, A.R., Herrmann, N., Raison, C.L., Miller, B. J., Lanctot, K.L., Carvalho, A.F., 2018. Peripheral alterations in cytokine and chemokine levels after antidepressant drug treatment for major depressive disorder: systematic review and meta-analysis. Mol. Neurobiol. 55, 4195–4206.

Kraemer, H.C., Wilson, G.T., Fairburn, C.G., Agras, W.S., 2002. Mediators and moderators of treatment effects in randomized clinical trials. Arch. Gen. Psychiatr. 59, 877–883.

Leighton, S.P., Nerurkar, L., Krishnadas, R., Johnman, C., Graham, G.J., Cavanagh, J., 2018. Chemokines in depression in health and in inflammatory illness: a systematic review and meta-analysis. Mol. Psychiatr. 23, 48–58.

Liu, J.J., Wei, Y.B., Strawbridge, R., Bao, Y., Chang, S., Shi, L., Que, J., Gadad, B.S., Trivedi, M.H., Kelsoe, J.R., Lu, L., 2020. Peripheral cytokine levels and response to antidepressant treatment in depression: a systematic review and meta-analysis. Mol. Psychiatr. 25, 339–350.

Luo, X., Fang, Z., Lin, L., Xu, H., Huang, Q., Zhang, H., 2022. Plasma complement C3 and C3a are increased in major depressive disorder independent of childhood trauma. BMC Psychiatr. 22, 741.

Lyu, N., Xing, G., Yang, J., Zhu, X., Zhao, X., Zhang, L., Wang, G., 2021. Comparison of inflammatory, nutrient, and neurohormonal indicators in patients with schizophrenia, bipolar disorder and major depressive disorder. J. Psychiatr. Res. 137, 401–408.

Maes, M., Bosmans, E., De Jongh, R., Kenis, G., Vandoolaeghe, E., Neels, H., 1997a. Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. Cytokine 9, 853–858.

Maes, M., Carvalho, A.F., 2018. The compensatory immune-regulatory reflex system (CIRS) in depression and bipolar disorder. Mol. Neurobiol. 55, 8885–8903.

Maes, M., Delange, J., Ranjan, R., Meltzer, H.Y., Desnyder, R., Cooremans, W., Scharpe, S., 1997b. Acute phase proteins in schizophrenia, mania and major depression: modulation by psychotropic drugs. Psychiatr. Res. 66, 1–11.

Maes, M., Meltzer, H.Y., Stevens, W., Calabrese, J., Cosyns, P., 1994. Natural killer cell activity in major depression: relation to circulating natural killer cells, cellular indices of the immune response, and depressive phenomenology. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 18, 717–730.

Maes, M., Van de Vyvere, J., Vandoolaeghe, E., Bril, T., Demedts, P., Wauters, A., Neels, H., 1996. Alterations in iron metabolism and the erythron in major depression: further evidence for a chronic inflammatory process. J. Affect. Disord. 40, 23–33.

Marx, W., McGuinness, A.J., Rocks, T., Ruusunen, A., Cleminson, J., Walker, A.J., Gomes-da-Costa, S., Lane, M., Sanches, M., Diaz, A.P., Tseng, P.T., Lin, P.Y., Berk, M., Clarke, G., O'Neil, A., Jacka, F., Stubbs, B., Carvalho, A.F., Quevedo, J., Soares, J.C., Fernandes, B.S., 2021. The kynurenine pathway in major depressive disorder, bipolar disorder, and schizophrenia: a meta-analysis of 101 studies. Mol. Psychiatr. 26, 4158–4178.

Mehterov, N., Minchev, D., Gevezova, M., Sarafian, V., Maes, M., 2022. Interactions among brain-derived neurotrophic factor and neuroimmune pathways are key components of the major psychiatric disorders. Mol. Neurobiol. 59, 4926–4952.

Menard, C., Pfau, M.L., Hodes, G.E., Russo, S.J., 2017. Immune and neuroendocrine mechanisms of stress vulnerability and resilience. Neuropsychopharmacology 42, 62–80.

Meshkat, S., Alnefeesi, Y., Jawad, M.Y., J, D.D.V., N, B.R., Ceban, F., Mw Lui, L., McIntyre, R.S., Rosenblat, J.D., 2022. Brain-Derived Neurotrophic Factor (BDNF) as a biomarker of treatment response in patients with Treatment Resistant Depression (TRD): a systematic review & meta-analysis. Psychiatr. Res. 317, 114857.

Milaneschi, Y., Lamers, F., Berk, M., Penninx, B., 2020. Depression heterogeneity and its biological underpinnings: toward immunometabolic depression. Biol. Psychiatr. 88, 369–380.

Miller, A.H., Pariante, C.M., 2020. Trial failures of anti-inflammatory drugs in depression. Lancet Psychiatr. 7, 837.

Morris, G., Fernandes, B.S., Puri, B.K., Walker, A.J., Carvalho, A.F., Berk, M., 2018a. Leaky brain in neurological and psychiatric disorders: drivers and consequences. Aust. N. Z. J. Psychiatr. 52, 924–948.

Morris, G., Walker, A.J., Berk, M., Maes, M., Puri, B.K., 2018b. Cell death pathways: a novel therapeutic approach for neuroscientists. Mol. Neurobiol. 55, 5767–5786.

Muller, N., 2019. The role of intercellular adhesion molecule-1 in the pathogenesis of psychiatric disorders. Front. Pharmacol. 10, 1251.

Nettis, M.A., Lombardo, G., Hastings, C., Zajkowska, Z., Mariani, N., Nikkheslat, N., Worrell, C., Enache, D., McLaughlin, A., Kose, M., Sforzini, L., Bogdanova, A., Cleare, A., Young, A.H., Pariante, C.M., Mondelli, V., 2021. Augmentation therapy with minocycline in treatment-resistant depression patients with low-grade peripheral inflammation: results from a double-blind randomised clinical trial. Neuropsychopharmacology 46, 939–948.

Osimo, E.F., Baxter, L.J., Lewis, G., Jones, P.B., Khandaker, G.M., 2019. Prevalence of low-grade inflammation in depression: a systematic review and meta-analysis of CRP levels. Psychol. Med. 49, 1958–1970.

Rasmusson, A.J., Gallwitz, M., Soltanabadi, B., Ciuculete, D.M., Mengel-From, J., Christensen, K., Nygaard, M., Soerensen, M., Bostrom, A.E., Fredriksson, R., Freyhult, E., Mwinyi, J., Czamara, D., Binder, E.B., Schioth, H.B., Cunningham, J.L., 2021. Toll-like receptor 4 methylation grade is linked to depressive symptom severity. Transl. Psychiatry 11, 371.

Reis, D.J., Casteen, E.J., Ilardi, S.S., 2019. The antidepressant impact of minocycline in rodents: a systematic review and meta-analysis. Sci. Rep. 9, 261.

Rosenblat, J.D., McIntyre, R.S., 2018. Efficacy and tolerability of minocycline for depression: a systematic review and meta-analysis of clinical trials. J. Affect. Disord. 227, 219–225.

Savitz, J.B., Teague, T.K., Misaki, M., Macaluso, M., Wurfel, B.E., Meyer, M., Drevets, D., Yates, W., Gleason, O., Drevets, W.C., Preskorn, S.H., 2018. Treatment of bipolar depression with minocycline and/or aspirin: an adaptive, 2x2 double-blind, randomized, placebo-controlled, phase IIA clinical trial. Transl. Psychiatry 8, 27.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatr. 59 (Suppl. 20), 22–33 quiz 34-57.

Singhal, G., Baune, B.T., 2018. Inflammasomes Action as an Important Mechanism in Experimental and Clinical Depression. Inflammation and Immunity in Depression. Elsevier, pp. 161–171.

Tao, H., Chen, X., Zhou, H., Fu, J., Yu, Q., Liu, Y., 2020. Changes of serum melatonin, interleukin-6, homocysteine, and complement C3 and C4 levels in patients with depression. Front. Psychol. 11, 1271.

Tsai, S.J., 2021. Role of interleukin 8 in depression and other psychiatric disorders. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 106, 110173.

Ursini, F., Abenavoli, L., 2018. The emerging role of complement C3 as A biomarker of insulin resistance and cardiometabolic diseases: preclinical and clinical evidence. Rev. Recent Clin. Trials 13, 61–68.

van Agtmaal, M.J.M., Houben, A., Pouwer, F., Stehouwer, C.D.A., Schram, M.T., 2017. Association of microvascular dysfunction with late-life depression: a systematic review and meta-analysis. JAMA Psychiatr. 74, 729–739.

Wong, M.L., Inserra, A., Lewis, M.D., Mastronardi, C.A., Leong, L., Choo, J., Kentish, S., Xie, P., Morrison, M., Wesselingh, S.L., Rogers, G.B., Licinio, J., 2016. Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition. Mol. Psychiatr. 21, 797–805.

Yabluchanskiy, A., Ma, Y., Iyer, R.P., Hall, M.E., Lindsey, M.L., 2013. Matrix metalloproteinase-9: many shades of function in cardiovascular disease. Physiology 28, 391–403.

Zazula, R., Husain, M.I., Mohebbi, M., Walker, A.J., Chaudhry, I.B., Khoso, A.B., Ashton, M.M., Agustini, B., Husain, N., Deakin, J., Young, A.H., Berk, M., Kanchanatawan, B., Ng, C.H., Maes, M., Berk, L., Singh, A.B., Malhi, G.S., Dean, O. M., 2021. Minocycline as adjunctive treatment for major depressive disorder: pooled data from two randomized controlled trials. Aust. N. Z. J. Psychiatr. 55, 784–798.

Zhu, L., Han, B., Wang, L., Chang, Y., Ren, W., Gu, Y., Yan, M., Wu, C., Zhang, X.Y., He, J., 2016. The association between serum ferritin levels and post-stroke depression. J. Affect. Disord. 190, 98–102.