



King's Research Portal

Document Version
Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Nettis, M. A., Lombardo, G., Hastings, C., Zajkowska, Z., Mariani, N., Nikkheslat, N., Worrell, C., Enache, D., McLaughlin, A., Kose, M., Sforzini, L., Anna, B., Cleare, A., Young, A., Pariante, C., & Mondelli, V. (2021). Augmentation therapy with Minocycline in treatment-resistant depression patients with low-grade peripheral inflammation: results from a double-blind randomized clinical trial. *Neuropsychopharmacology*.

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

1 **Augmentation therapy with Minocycline in treatment-resistant**
2 **depression patients with low-grade peripheral inflammation: results**
3 **from a double-blind randomized clinical trial**

4
5
6 Maria Antonietta Nettis^{1,2} M.D., Giulia Lombardo¹ M.A., Caitlin Hastings¹ M.A, Zuzanna
7 Zajkowska¹ Ph.D., Nicole Mariani¹ B.A., Naghmeh Nikkheslat¹ Ph.D, Courtney Worrell¹
8 M.A., Daniela Enache³ Ph.D., Anna McLaughlin¹ M.A., Melisa Kose¹ B.A., Luca Sforzini¹
9 M.D., Anna Bogdanova¹ M.A., Anthony Cleare^{1,2} Ph.D., Allan H. Young^{1,2} Ph.D., Carmine
10 M. Pariante^{1,2} Ph.D., Valeria Mondelli^{1,2} Ph.D.

11
12 ¹King's College London, Institute of Psychiatry, Psychology and Neuroscience, Department
13 of Psychological Medicine, London, UK.

14
15 ² National Institute for Health Research Mental Health Biomedical Research Centre, South
16 London and Maudsley NHS Foundation Trust and King's College London, London, UK.

17
18 ³ Karolinska Institute, Department of Neurobiology, Care Sciences and Society, Division
19 of Neurogeriatrics, Stockholm, Sweden.

20
21 Running Title: minocycline in treatment-resistant depression with low grade inflammation

22
23 **Corresponding author:**

24 Dr Valeria Mondelli

25 The Maurice Wohl Clinical Neuroscience Institute

26 Institute of Psychiatry, Psychology and Neuroscience, King's College London

27 Cutcombe Road

28 London, SE5 9RT

29 United Kingdom

Telephone: +44(0)2078480353 Email: valeria.mondelli@kcl.ac.uk

30 **Abstract**

31
32 This study aimed to investigate the role of baseline levels of peripheral inflammation
33 when testing the efficacy of antidepressant augmentation with minocycline in patients
34 with treatment-resistant depression.

35 We conducted a 4-week, placebo-controlled, randomized clinical trial of minocycline
36 (200 mg/day) added to antidepressant treatment in 39 patients selected for elevated
37 levels of serum C-reactive protein ($CRP \geq 1\text{mg/L}$), $n=18$ randomised to minocycline (M)
38 and $n=21$ to placebo (P). The main outcome was the change in Hamilton Depression
39 Rating Scale (HAM-D-17) score from baseline to week 4, expressed both as mean and
40 as full or partial response, in the overall sample and after further stratification for
41 baseline $CRP \geq 3\text{mg/L}$. Secondary outcomes included changes in other clinical and
42 inflammatory measures.

43 Changes in HAM-D-17 scores and the proportion of partial responders did not differ
44 between study arms. However, we found a greater decrease in CGI severity score in the
45 minocycline versus the placebo group ($p=0.03$). After stratification for CRP levels
46 $<3\text{mg/L}$ (CRP^-) or $\geq 3\text{mg/L}$ (CRP^+), CRP^+/M patients showed the largest changes in
47 HAM-D-17 scores (mean \pm SD= 12.00 ± 6.45) compared with CRP^-/M (2.42 ± 3.20 ,
48 $p < 0.001$), CRP^+/P (3.50 ± 4.34 , $p=0.003$) and CRP^-/P (2.11 ± 3.26 , $p=0.006$) patients, and
49 the largest proportion (**83.3%**, **$p=0.04$**) of partial treatment response at week 4. The
50 threshold point for baseline CRP to distinguish responders from non-responders to
51 minocycline was 2.8mg/L . Responders to minocycline had higher baseline IL-6
52 concentrations than non-responders ($p=0.015$); $IFN\gamma$ was significantly reduced after
53 treatment with minocycline compared with placebo ($p=0.03$).

54 Our data show some evidence of efficacy of add-on treatment with minocycline in MDD
55 patients but only in those with low-grade inflammation defined as $CRP \geq 3\text{mg/L}$.

56

57 **Introduction**
58

59 Emerging evidence of the role of the immune system in Major Depressive Disorder
60 (MDD) has stimulated a growing interest in exploring the antidepressant properties of
61 anti-inflammatory agents, either as monotherapy or as add-on treatment to
62 antidepressants (1, 2). Targeting inflammation has been proposed as a potential new
63 strategy to treat MDD patients, in particular those who exhibit increased peripheral blood
64 concentrations of inflammatory biomarkers and do not benefit from standard
65 antidepressants (3).

66
67 Meta-analytical findings support a beneficial effects of anti-inflammatory-treatment in
68 depression (1), although studies so far only include Non-Steroidal Anti-Inflammatory
69 Drugs (NSAIDs), such as COX-2 inhibitors, and cytokine inhibitors, which have direct
70 anti-inflammatory effects, and the clinical application of these drugs in depression
71 remains controversial for both safety and efficacy reasons. For example, NSAIDs and
72 cytokine inhibitors increase the risk of cardiovascular adverse events (4) and the risk of
73 infections (5), respectively, and so their safety in combination with antidepressants is still
74 unclear. Furthermore, evidence suggests that the concurrent use of NSAIDs and
75 antidepressants increases the risk of haemorrhage (6). Finally, efficacy results are
76 inconsistent, particularly for NSAIDs like COX-2 inhibitors, which, at least in some
77 studies, showed only a modest and non-sustained antidepressant efficacy (7), or may
78 even have an antagonistic effect on the antidepressant actions of selective serotonin
79 reuptake inhibitors (SSRIs) (8). One of the reasons for such inconsistent results is that
80 the inflammatory cascade leading to depression probably involves multiple pathways
81 connecting the peripheral immune system to the Central Nervous System (CNS), and
82 these may not be specifically targeted by classic anti-inflammatory treatments (1, 9).

83
84 Minocycline is a tetracycline antibiotic minocycline with broad anti-inflammatory
85 properties and, importantly, a good penetration into the CNS through the blood-brain
86 barrier, which accounts for its neuroprotective ability (10). Indeed, this drug has
87 inhibitory actions on mechanisms relevant to 'inflammation-induced depression', such

88 as the kynurenine and the p-38 pathways: through the kynurenine pathway,
89 inflammation leads to the activation of indoleamine 2,3-dioxygenase (IDO), a key
90 enzyme in the metabolism of the serotonin precursor, tryptophan, resulting in a
91 reduction of serotonin levels and an increase in neurotoxic metabolites (11); and
92 through the p-38 pathway, inflammation leads to an increase in the expression and
93 function of the serotonin transporter, resulting in a reduction of serotonin in the synaptic
94 space (12-14). Moreover, evidence suggests that minocycline is also anti-oxidant and
95 anti-apoptotic, and modulates glutamate and monoamine neurotransmission (10, 15).

96
97 Because of these unique properties of minocycline, and their relevance in the
98 pathogenesis of depression, research has been conducted on the antidepressant
99 efficacy of this drug, but results are not conclusive, due to the paucity and heterogeneity
100 of studies. An initial open-label clinical trial testing the effects of adjunctive minocycline
101 in MDD patients reported a significant improvement in depressive symptoms (16). After
102 that, two placebo-controlled randomized trials (RCTs) have assessed the augmentation
103 therapy with minocycline 200 mg/day in MDD: one study found that minocycline was
104 superior to placebo in improving Clinical Global Impression scores, quality of life and
105 functioning, but not depressive symptoms (17), while the second, which specifically
106 included treatment-resistant patients, found a clear effect on depressive symptoms, with
107 a larger decrease in Hamilton Depression Rating Scale (HAM-D) scores after
108 minocycline compared with placebo (18). A third RCT has tested the antidepressant
109 properties of minocycline in HIV patients with mild-to-moderate depression, and
110 administered as monotherapy rather than add-on treatment: the study found that
111 minocycline was superior to placebo in improving depressive symptoms measured with
112 the HAM-D (19). In conclusion, as a recent meta-analysis has pointed out (20), a
113 potential antidepressant effect has been observed for minocycline compared with
114 placebo, but conclusions are limited by the heterogeneity of the studies. Furthermore,
115 there is a lack of trials aiming to identify clinical subgroups that are more likely to benefit
116 from minocycline treatment.

117 Of note, no study so far has considered prospectively the baseline inflammatory state of
118 patients as a key factor moderating response to minocycline. This could be particularly

119 relevant in view of the secondary results from an RCT with add-on treatment with
120 Infliximab, a tumour necrosis factor (TNF)-alpha-antagonist, in patients with treatment
121 resistant depression; in the exploratory “post-hoc” stratification analyses of this study,
122 the authors found that only patients with higher levels of C-reactive protein (CRP>5
123 mg/L) showed improvement with Infliximab, while placebo was superior to Infliximab in
124 improving depressive symptoms in those with CRP levels equal/below the identified
125 threshold of 5 mg/L (21).

126 Here we present the results from our clinical trial MINDEP (MINocycline in DEPression),
127 in which we aimed to test the role of baseline levels of peripheral inflammation in the
128 efficacy of 4-week add-on treatment with minocycline in MDD patients not responding to
129 antidepressant treatment. Specifically patients were all selected for elevated levels of
130 peripheral inflammation, measured as CRP levels ≥ 1 mg/L, a threshold that, as
131 discussed in a recent meta-analysis, defines “elevated levels of CRP” that are present in
132 around 60% of depressed patients (22). In subsequent secondary analyses, we
133 compared the clinical outcomes of patients with CRP levels < 3 mg/L or ≥ 3 mg/L, also
134 based on the evidence that values above such threshold are associated with no-
135 response to standard antidepressants (3).

136 We hypothesized that adjunct minocycline would be associated with greater
137 improvement in depressive symptoms, measured at week 4 (end of treatment) when
138 compared with placebo, and that this would be associated with normalization of
139 peripheral inflammatory abnormalities at week 4.

140

141

142 **Methods**

143 Overview

144 This was a single centre, randomised (1:1 minocycline/placebo) placebo controlled,
145 parallel group trial of adjunctive minocycline (200 mg/day) added to ongoing treatment
146 in patients who had failed to respond adequately to at least one antidepressant in the

147 current depressive episode and had elevated peripheral inflammation as shown by CRP
148 levels $\geq 1\text{mg/L}$. All visits took place at the Clinical Research Facility of King's College
149 Hospital, London.

150 Patients were recruited, between August 2016 and September 2019, from new referrals
151 to primary and secondary care services linked to the South London and Maudsley NHS
152 Foundation Trust (SLaM) and via public advertisement. All patients provided written
153 consent after reading the information provided.

154 Besides antidepressants (selective serotonin reuptake inhibitors, tricyclics, monoamine
155 oxidase inhibitors, noradrenergic and specific serotonin antagonists and serotonin
156 noradrenaline reuptake inhibitors), current allowed medications included mood
157 stabilizers (with the exception of valproic acid) and antipsychotics, as long as patients
158 were on stable treatment for at least 6 weeks at the time they entered the study.
159 Participants undertaking psychotherapy and other psychosocial interventions were also
160 included.

161 This study was reviewed and approved by the London - Brighton & Sussex Research
162 Ethics Committee Research Ethics Committee (REC) and by the Medicines and
163 Healthcare products Regulatory Agency (MHRA) for Clinical Trial Authorisation. Trial
164 registration: EudraCT Number 2015-003413-26. The trial ended when all participants
165 were recruited.

166

167 Study Sample size

168 A previous study testing the antidepressant effect of adjunctive treatment with
169 minocycline in 41 patients reported an improvement in HAM-D score in the minocycline
170 group with an effect size of $d=1.2$ (95% CI 0.39, 1.84)(18). Assuming a similar response
171 rate in our sample, with ~ 20 patients in each arm we would have more than 95% power
172 to detect a similar reduction in HAM-D scores.

173 Inclusion and Exclusion criteria

174

175 Participants with MDD were selected according to the following selection criteria: 1)
176 aged 25-60, with a current DSM 5 diagnosis of nonpsychotic major depressive disorder,
177 confirmed by the Mini International Neuropsychiatric Interview (MINI); 2) non-responders
178 to the current antidepressant taken at therapeutic doses, as defined in the Maudsley
179 Prescribing guidelines , for at least 6 weeks, as indicated by a current score of at least
180 14 on the 17- item Hamilton Depression Rating Scale (HAM-D-17); 3) tolerant to the
181 current antidepressant and accepting augmentation with minocycline; 4) having the
182 ability to understand and sign a written informed consent form prior to participation in
183 any screening procedures; 5) having CRP levels ≥ 1 mg/L at the screening visit; and 6)
184 [having no planned changes in their current therapy for the duration of the study.](#)

185

186 The exclusion criteria were: 1) active suicidal ideation of significant concern to require
187 intensive monitoring by secondary psychiatry services; 2) primary diagnosis of bipolar
188 disorder, obsessive-compulsive disorder, eating disorder, post-traumatic stress
189 disorder, or substance/alcohol misuse disorder; 3) taking warfarin; 4) having received
190 tetracycline within the previous 2 months, or having a history of sensitivity or intolerance
191 to this class of drugs; 5) having an acute infection or an autoimmune or inflammatory
192 disorder; 6) having hepatic or renal failure; and 7) taking any other psychotropic
193 medications other than their current antidepressant that has not been approved by a
194 study investigator prior to enrolment. All female participants did a pregnancy test before
195 starting the study and pregnant participants and those unwilling to use an acceptable
196 form of contraceptive throughout the study period (e.g., condoms, IUD/IUS, injection,
197 patch, ring) were also excluded.

198

199 Study procedure

200 Recruitment. All interested patients, either identified by clinical teams or expressing
201 direct interest, were sent a patient information sheet which they were given time to read
202 (at least 24 hours). If they agreed to take part, they went through a pre-screening phone

203 call to check eligibility. Then, a screening visit was set up in order to obtain signed
204 informed consent for the study and also signed consent for the research team to have
205 access to their medical notes.

206 Screening visit. Participants recruited to the trial underwent structured diagnostic
207 interviews using the Mini International Neuropsychiatric Interview (MINI) to confirm a
208 diagnosis of DSM-5 major depressive disorder (23) (MDD). The HAM-D-17 (24) was
209 used to measure symptom severity and treatment response. Blood samples were also
210 collected to test full blood count, liver and kidney function panel, and CRP levels. Vital
211 signs, temperature, height and weight were measured as well, together with a
212 pregnancy test for female participants.

213 Baseline visit. Within 1 month from the screening visit, eligible patients came back for
214 the baseline visit. They were randomised to treatment with either minocycline (200 mg
215 daily) or placebo and underwent a blood sample for measurement of biological markers
216 and a clinical assessment including the HAM-D-17 (25), the Beck Depression Inventory
217 II (BDI-II) (26), the Snaith–Hamilton Pleasure Scale (SHAPS) (27), the Spielberger
218 State-Trait Anxiety Rating Scale (STAI) (28), the Clinical Global Impression (CGI) scale
219 (29), the Brief Life Events (BLE) questionnaire (30) and the Perceived Stress Scale
220 (PSS) (31). Participants were also given a diary to assess their study drug compliance.

221 Randomisation. Patients were randomised (1:1 minocycline/placebo) by the method of
222 block randomization, stratified by gender, via a web-based randomization system at the
223 Clinical Trials Unit (CTU) at the IoPPN. Patients and clinicians remained blind to
224 treatment allocation. Placebo and minocycline were manufactured by Guy's and St
225 Thomas' NHS Foundation Trust; minocycline was also manufactured by Guy's and St
226 Thomas' NHS Foundation Trust by encapsulating the Dexcel®-Pharma brand
227 (Acnamino™) 100mg capsules. During the study, patients were instructed to take two
228 capsules of the experimental medication (placebo or minocycline 100 mg) once a day.
229 The dose was based on evidence from a previous clinical trial demonstrating a
230 significant effect in reducing severity of depressive symptoms following treatment with
231 minocycline with the dose of 200mg/day in MDD (18).

232

233 Week 4 visit. After completion of the minocycline/placebo course, participants were
234 assessed within 14 days of course completion. Participants underwent blood sampling
235 for measurement of inflammatory markers, pregnancy test (female participants) and a
236 clinical assessment with the same measures used at the baseline visit.

237 Day-to-day care of patients during the trial remained the responsibility of their usual
238 consultant psychiatrist or other mental health professional. Adverse events (AEs) and
239 concomitant medications were also monitored during the entire trial.

240 Although data on inter-rater reliability was not formally collected, all assessments were
241 carried out by two psychiatrists (MAN and LS) and by research assistants who are
242 experienced Masters' level clinical psychologists and who were trained in clinical
243 assessments and diagnostic interviews by two authors (VM and CMP).

244 Overall, we screened 124 patients, out of which 49 met the inclusion criteria. From
245 these 49, 5 patients decided not to take part in the trial; the final number of randomized
246 patients was 44 (22:22). Five patients withdrew for different reasons (2 patients
247 experienced side effects, 1 was lost in follow-up and 1 withdrew for unknown reasons in
248 the minocycline group; 1 left for family issues in the placebo group); the final sample
249 consisted of 39 patients, 18 in the minocycline group and 21 in the placebo group (Fig.
250 S1 in the supplemental material shows the Consort Flow diagram).

251

252 Table 1 shows the descriptive results for patients at the baseline, including the clinical
253 outcome measure HAM-D-17 and the high sensitivity (hs)CRP. Patients in the two study
254 arms were comparable for socio-demographic variables, illness duration and medication
255 use.

256

257 **Table 1 around here**

258 Outcome measures

259 The primary clinical outcome was the mean change from baseline to week 4 on the
260 HAM-D-17, including the percentage of patients who showed treatment response,
261 defined as 50% reduction in the baseline scores (32, 33), or partial response, defined as
262 25% reduction in the baseline scores (34). Secondary outcomes included changes from
263 baseline to week 4 in inflammatory biomarkers, Beck Depression Inventory, State and
264 Trait Anxiety Inventory, Clinical Global Impression scale, Snaith–Hamilton Pleasure
265 Scale and Perceived Stress Scale.

266

267 Biomarkers

268 From baseline and follow-up samples, we analysed serum high sensitivity (hs)CRP
269 using a Roche Cobas 8000 (35). Serum pro-inflammatory and anti-inflammatory
270 cytokines, including interferon (IFN)- γ , interleukin (IL)-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-
271 12p70, IL-13, and tumour necrosis factor (TNF)- α were measured using Meso Scale
272 Discovery (MSD) V-PLEX sandwich immunoassays, MSD Pro-inflammatory Panel 1
273 (human) kit (36, 37), and plates read on an MSD QuickPlex SQ 120, as previously
274 published (38, 39). The inter-assay coefficient of variations was <10%. The results were
275 analysed using MSD DISCOVERY WORKBENCH analysis software. Of note, levels of
276 IL-1 β , IL-4 and IL-12p70 were below the minimum detectable value for most of the
277 subjects, so these cytokines were not included in the statistical analyses.

278

279

280 Side effects

281

282 We calculated side effects frequency as the percentage of patients experiencing a given
283 side effect among those randomized in each study arm. As both study arms originally
284 counted 22 patients randomized, we used the formula $(n*100)/22$. This allowed us to
285 account for patients who dropped out from the study because of side effects.

286

287 Statistical analysis

288 The primary analyses included a Pearson's Chi-square test to examine the difference in
289 percentage of treatment response or partial response (defined as 50% or 25% reduction
290 from baseline in the HAM-D-17 score, respectively) between the two study arms, and
291 an independent t-test to test differences in changes in HAM-D-17 scores between the
292 two study arms groups. Finally, we further examined differences in changes in HAM-D-
293 17 scores between patients with hsCRP above or below the cut-off 3 mg/L at baseline
294 (3); for this purpose, we divided the sample by patients with $hsCRP \geq 3$ mg/L ($hsCRP^+$)
295 and patients with $hsCRP < 3$ mg/L ($hsCRP^-$), and by treatment group, generating 4 final
296 groups: $hsCRP^+/M$ (n=6), $hsCRP^+/P$ (n=12), $hsCRP^-/M$ (n=12) and $hsCRP^-/P$ (n=9) (see
297 Table 2). Then, we performed a one-way ANOVA, to investigate differences among
298 these 4 groups of patients in the HAM-D-17 change.

299
300 All of the aforementioned analyses were conducted in both the complete dataset and
301 using intention-to-treat approach. Specifically, we used multiple imputation to handle
302 missing data (40, 41), generating HAM-D-17 scores at week 4 (end of treatment) for the
303 5 withdrawn participants. The procedure involved a linear regression model (automatic
304 method set in SPSS) and generated 12 imputations, that is, equivalent to the
305 percentage of incomplete cases, which in our study was 11.4% (42). The imputation
306 model included variables used in the analysis model and associated with the imputed
307 variable, like the Study Arm, baseline CRP ($r=0.341$, $p=0.034$), baseline HAM-D-17
308 ($r=0.341$, $p=0.034$) and baseline STAI-S scores ($r=0.45$, $p=0.005$) (42). We compared
309 the observed and the imputed variables by tabulating the summary statistics (Table S2
310 supplementary materials) and with both parametric and non-parametric tests (42).

311
312
313 Finally, we conducted a Receiver Operating Characteristic (ROC) curve analysis, with
314 both parametric and non-parametric methods, to test the ability of baseline hsCRP
315 levels to correctly differentiate treatment response and to identify/confirm the exact
316 threshold point at which hsCRP would correctly identify treatment response. As CRP
317 showed a non-normal distribution (Shapiro-Wilk test=0.001), in the parametric method

318 we applied the natural logarithmic transformation, which was able to normalize the CRP
319 variable (Shapiro-Wilk test=0.892). The baseline CRP levels (i.e., measured on the day
320 patients were randomized) were used to identify threshold in the analysis and for all
321 statistical purposes. It should be noted that the screening CRP, used to include patients
322 in the study, and the baseline CRP were markedly correlated, as shown by a correlation
323 analysis (Spearman's $\rho=0.749$, $p<0.001$).

324
325 For additional analyses, bootstrapped paired t-test was used to examine within-group
326 changes, and independent t-test was used to examine differences in changes between
327 the two study arms. Spearman's correlations were used to investigate correlation
328 between changes in blood biomarkers and changes in depressive symptoms. We
329 performed Wilcoxon Signed-Rank and Mann-Whitney U tests to investigate differences
330 within and between study arm in blood biomarkers raw values from baseline to week 4.

331
332 In terms of potential covariates, the 2 study arms did not differ in in age, BMI, gender,
333 ethnicity, tobacco and alcohol consumption. Moreover, even if, as expected, BMI was
334 correlated with baseline CRP in the whole sample, (Spearman's $\rho=0.498$, $p=0.001$),
335 having a BMI higher ($n=22$) or lower ($n=17$) than 30 (validated threshold for obesity) did
336 not affect HAM-D-17 change in the whole sample ($t=0.829$, $p=0.413$).

337 All the statistical analyses were performed using SPSS V 26.0.

338

339

340 **Results**

341

342 Clinical outcome

343

344 Both the minocycline and placebo group showed significant improvement in HAM-D-17
345 scores (bootstrapped $t=3.74$, $p=0.008$; $t=3.43$ $p=0.003$, respectively, Table 2A) and we
346 found no significant difference between study arms in the HAM-D-17 change ($t=1.57$,
347 $p=0.13$).

348

349 We could not divide our sample in treatment responders and non-responders by using
350 the 50% improvement cut-off for the HAM-D-17, because in all our sample, only 3
351 patients showed such improvement. Thus, we considered the percentage of patients
352 who showed at least a partial response, defined as 25% reduction in the baseline
353 scores according to the Canadian Network for mood and anxiety treatment (34). In the
354 overall sample, 8 out of 18 patients (44.4%) in the minocycline group showed a partial
355 improvement, compared with 9 patients out of 21 (42.9%) in the placebo group
356 (Pearson χ^2 test $\chi^2=0.01$, $p=0.92$).

357

358 **Table 2 A & B around here**

359

360 When we explored differences after further stratification based on CRP levels above or
361 below 3 mg/L, we found some evidence of efficacy for minocycline in the high
362 inflammation group. Specifically, the one-way ANOVA showed a significant difference
363 among the four groups of patients (CRP \geq 3 mg/L + minocycline (CRP⁺/M) $n=6$, CRP $<$ 3
364 mg/L + minocycline (CRP⁻/M) $n=12$, CRP \geq 3 mg/L + placebo (CRP⁺/P) $n=12$, CRP $<$ 3
365 mg/L + placebo (CRP⁻/P) $n=9$ ($F_{3,35}=8.53$, $p<0.001$). In particular, CRP⁺/M patients had
366 the largest HAM-D-17 change from baseline to week 4 (mean \pm SD=12.00 \pm 6.45)
367 compared with CRP⁻/M (2.42 \pm 3.20, $p<0.001$, Cohen $d=1.9$), CRP⁺/P (3.50 \pm 4.34,
368 $p=0.002$, Cohen $d=1.5$) and CRP⁻/P (2.11 \pm 3.26, $p<0.001$, Cohen $d=1.9$) patients
369 (Bonferroni corrected, see Fig. 1).

370 Furthermore, the hsCRP⁺/M group had the highest proportion (83.3%, 5 out of 6) of
371 partial responders (Table 2B) ($\chi^2=8.27$, $p=0.04$).

372
373 We repeated these analyses using an intention-to-treat approach and multiple
374 imputation. There were no differences between the observed and imputed HAM-D-17
375 mean values at week 4, as confirmed by both parametric and non-parametric tests
376 across all 12 imputations (see Table S2, all $p>0.05$). After adding the imputed values for
377 the 5 drop-out subjects, the two study arms (with $n=22$ each) still showed no difference
378 in all baseline demographics. Moreover, we found very similar results compared with the
379 complete dataset. Specifically, the independent t-test again found no statistically
380 significant difference in the HAM-D-17 change between the placebo and minocycline
381 group, although in the intention-to-treat analyses actually reached trend-level
382 significance, suggesting a greater reduction in HAM-D-17 in the minocycline than in the
383 placebo group (pooled $t=1.75$, $p=0.08$). Adding the 5 imputed data, the 4 subgroups
384 stratified by baseline hsCRP included $n=8$ CRP⁺/M; $n=14$ CRP⁻/M; $n=12$ CRP⁺/P; $n=10$
385 CRP⁻/P. Multiple ANOVAs comparing the 4 subgroups were conducted using the 12
386 different imputation sets, and all confirmed the significant results of the complete
387 dataset analysis (F ranging 4.15-10.04, p-values ranging $p<0.001-0.012$), and all
388 confirming that the CRP⁺/M group had a higher HAM-D-17 change (pooled mean SD
389 10.63 ± 6.54) compared with the other 3 groups (CRP⁻/M $=2.82\pm 3.71$; CRP⁻/P $=1.9\pm$
390 3.55 ; CRP⁺ P $=3.50\pm 4.34$).

391 Finally, the Chi-square test confirmed that CRP⁺/M patients made up the larger
392 proportion (pooled=78.7%) of those with partial response, with a χ^2 range =12.42-4.85
393 and a p value range=0.006-0.18.

394
395 The ROC analysis with non-parametric methods revealed that the threshold point for
396 hsCRP that best distinguishes responders from non-responders in the minocycline
397 group was 2.8 mg/L, with an area under the ROC curve = 0.792. The same threshold
398 was found when using parametric methods and logarithmic CRP.

399
400

401 **Fig 1 around here**

402

403 In the minocycline group, patients who were partially responders had higher baseline IL-
404 6 (Mann-Whitney $U=66.0$, $p=0.01$) and hsCRP levels ($U=13.0$, $p=0.02$) compared with
405 no-responders. No such difference was found in the placebo group.

406

407 When we analysed the other clinical measures, we found a significant improvement in
408 BDI-II, SHAPS, and STAI-T scores both in the minocycline and the placebo study arm,
409 with no significant differences between groups. However, analysis of changes in the
410 CGI severity score showed that patients receiving minocycline improved more than
411 those receiving placebo, as reflected by a significantly greater decrease in CGI severity
412 score in the minocycline versus the placebo group ($t=2.24$, $p=0.03$) (Table 3).

413

414

415 **Biological outcomes**

416

417 hsCRP and inflammatory biomarkers showed no significant changes from baseline to
418 week 4 (Tables 2A, Table 4), except for the changes in IFN- γ levels that were
419 significantly different between groups (Mann Whitney $U=105.5$ $p=0.03$), with patients
420 taking minocycline showing a decrease in IFN- γ , but not those taking placebo (Table 4).
421 We found no significant results when the 4 subgroups based on baseline CRP were
422 compared for changes in inflammatory markers.

423

424 **Table 3 and 4 around here**

425

426

427 **Side effects**

428

429 There was no significant difference in the frequency of reported adverse effects
430 between groups. The most common reported side effects were dizziness, dyspepsia,

431 diarrhoea, headache and nausea. Table S1 summarises all side effects reported by
432 participants (See supplemental material).

433

434

435 **Discussion**

436

437 In our sample of patients selected for elevated CRP (≥ 1 mg/L) we found no clear
438 difference between minocycline and placebo in improving depressive symptoms at
439 week 4 (even if the intention-to-treat analysis found trend levels of significant difference,
440 suggesting that the minocycline group shows a greater reduction in depressive
441 symptoms than the placebo group, possibly indicating that a significant effect could
442 have been found in a larger sample or with a longer treatment). However, we do
443 demonstrate that, across different analysis approaches, there is an association between
444 baseline levels of hsCRP indicating low-grade inflammation (hsCRP levels ≥ 3 mg/L)
445 and response to minocycline, such that an increased response to minocycline was
446 found in these patients. In particular, we found that patients with baseline hsCRP levels
447 ≥ 3 mg/L have an average change of 12 points in HAM-D-17 scores from baseline to
448 week 4, with a minimum standardized effect size of 1.5 (range: 1.5-1.9) when compared
449 with the other groups (18). Moreover, responders to minocycline (showing at least 25%
450 symptoms reduction) not only have higher levels of baseline hsCRP, but also of
451 baseline IL-6. We also found that the effect of minocycline on depressive symptoms by
452 week 4 is mirrored by a reduction in IFN- γ levels, but not in the levels of hsCRP or other
453 cytokines.

454

455 Overall, our results corroborate the accumulating evidence that anti-inflammatory
456 strategies, and in particular minocycline, can have an antidepressant effect only when
457 depression is associated with increased inflammation. Our primary hypothesis that CRP
458 $= 1$ mg/L could serve as inflammatory threshold to identify response to minocycline is
459 not strongly supported by our data, while we find robust evidence in favour of using
460 CRP ≈ 3 mg/L. This has been considered the cut-off for “low-grade inflammation” which
461 characterizes over a quarter of patients with depression and can predict not only

462 treatment-resistance to antidepressants, but also comorbid, immune related physical
463 illnesses (22). CRP levels ≥ 3 mg/L have also been associated with reduced
464 connectivity within reward related circuits (measured with fMRI) and with alterations of
465 glutamate metabolism (43). This is particularly relevant considering minocycline
466 modulation of the glutamatergic neurotransmission (10).

467
468 Our findings that levels of CRP and IL-6 are predictive of minocycline response in
469 depression are consistent with existing evidence. For example, high baseline CRP
470 before treatment has previously been associated with better response in MDD patients
471 to the cytokine inhibitor Infliximab (21). Similar to our findings, high basal levels of IL-6
472 predicted antidepressant efficacy of anti-inflammatory agents, including celecoxib (44)
473 and minocycline itself, as showed in a 6-week trial in bipolar depression (45).

474
475 In contrast with both these studies, we did not find a reduction in IL-6 following
476 minocycline administration. In particular, in the study by Savitz and colleagues,
477 participants with bipolar depression who responded to minocycline had significantly
478 greater decreases of IL-6 over 6 weeks of treatment when compared with non-
479 responders. By contrast, we found no reduction in inflammatory biomarkers following
480 minocycline administration in our sample of patients. Only changes in IFN- γ levels were
481 significantly different in the two study arms, indicating a modest reduction in IFN- γ levels
482 in the minocycline group compared with placebo. However, such change did not
483 correlate with changes in any clinical measure. The reason for such discrepancy
484 between our findings and those by Savitz and colleagues might be the shorter exposure
485 to minocycline in our study (4 weeks vs 6 weeks) or the characteristics of the clinical
486 sample, which was different in terms of diagnosis and degree of treatment-resistance.
487 Indeed, these features can affect the immune profile in terms of both peripheral and
488 central inflammation (46, 47). Nevertheless, our data suggest that minocycline exerts an
489 antidepressant effect that is already detectable at 4 weeks and that such effect is
490 associated with baseline inflammatory status and possibly with some reduction of
491 inflammation over time, with stronger biological changes that might have been visible
492 with longer treatment.

493

494 Of course, the lack of clear changes in immune biomarkers even in the CRP⁺/M group,
495 that shows a significant clinical improvement, may imply the mechanism behind this
496 effect is not related to a reduction of peripheral inflammation (at least not after 4 weeks),
497 and that other pharmacological mechanisms activated by minocycline might be
498 involved. Indeed, as mentioned above, due to its ability to cross the blood-brain barrier,
499 minocycline might act on several inflammatory pathways primarily localised in the CNS
500 and involved in the development of depressive symptoms. In addition to its described
501 effects as anti-oxidant and modulator of several neurotransmitters, minocycline is an
502 inhibitor of microglia activation (10), a possible component of brain neuroinflammatory
503 processes that have been reported in patients with depression (9). Indeed, a number of
504 preclinical studies have shown the ability of minocycline to ameliorate depressive-like
505 symptoms via suppression of microglia activation (48, 49). It is therefore possible that
506 minocycline could exert its antidepressant properties through a more direct effect on
507 CNS inflammation, preceding that on peripheral inflammation. So far, a correlation
508 between neuroinflammatory processes and peripheral inflammatory biomarkers has not
509 been found in patients with MDD (9, 50), suggesting possibly the presence of complex
510 and not linear interaction between central and peripheral inflammation, with potentially
511 different timings and dynamics involved in development and regression of central and
512 peripheral inflammatory processes.

513

514 Minocycline has also been suggested to inhibit metabolic pathways such as the
515 kynurenine pathway, which is activated during inflammation (13). Relevant for our study
516 is the well-known activating effect that inflammatory cytokines, in particular IFN- γ , exert
517 on the transcription of indoleamine 2,3-dioxygenase (IDO), the rate-limiting enzyme of
518 kynurenine (KYN) pathway of tryptophan (TRY) metabolism. Indeed, previous data
519 suggest that upregulated production of IFN- γ in the periphery and in the brain can trigger
520 the kynurenine pathway as part of the inflammatory cascade involved in aging and in
521 psychiatric disorders (51). Therefore, in addition to the well-known effect of minocycline
522 on the inhibition of IDO, our data suggest that minocycline could also inhibit IDO via
523 reduction in the IFN- γ levels, as indicated by the decrease in IFN- γ levels in the

524 minocycline group compared with the placebo group in our study. This is also supported
525 by previous preclinical studies showing that minocycline can reduce the expression of
526 IFN- γ (10)".

527
528

529 Our results should be discussed in light of a previous 12-week RCT, by Husain and
530 colleagues, in patients with treatment-resistant depression (18). In line with this study,
531 we confirmed the efficacy of minocycline in treatment resistant depression, but we
532 added that the basal inflammatory status is also relevant to predict response to
533 minocycline. In the study by Husain and colleagues, the superiority of minocycline over
534 placebo in improving depressive symptoms was found without considering patients'
535 basal peripheral inflammatory levels. This discrepancy might be due to the fact that the
536 aforementioned study did not find an overall response to placebo and also to the
537 different length of the trial (12 weeks) compared to ours (4 weeks). Interestingly, in the
538 study by Husain and colleagues, treatment differences started to appear at week 4 and
539 became evident by week 8. We hypothesize that patients with lower levels of peripheral
540 inflammation (in our sample those with hsCRP<3 mg/L) might have a delayed response
541 to minocycline and that a clearer difference between minocycline and placebo could
542 appear with a longer duration of treatment.

543

544 The two studies also differ for the severity of baseline depressive symptoms, with
545 patients in the study by Husain et al. showing more severe depressive symptoms than
546 our sample (average baseline HAM-D total score > 30 as opposed to values < 20 in our
547 sample). As the authors explain, placebo response might decline with increasing
548 severity of baseline depression scores (52). This could also explain why they found
549 minocycline response without taking into account patients' basal inflammation. Finally, it
550 must be considered that the aforementioned study was conducted in Pakistan while
551 ours had place in London. Thus, the different settings, as well as patients'
552 heterogeneity might contribute to explain different results.

553 In line with the same study, we found that minocycline was well-tolerated compared with
554 placebo in terms of side effects, and there was no significant difference in the frequency
555 of adverse events between the 2 groups.

556

557 Finally, our exploratory analyses with secondary outcome clinical measures found no
558 particular difference between minocycline and placebo in the other clinical scales, with
559 the exception of CGI, which showed a larger improvement in the minocycline group, in
560 line with previous studies (53).

561

562 Overall, data from our study suggest that minocycline could be a relatively safe and
563 well-tolerated augmentation strategy for MDD, in particular for patients with
564 inflammation-related depression who do not benefit sufficiently from antidepressants
565 alone. Moreover, integrating the measurement of biological markers such as CRP
566 (which is relatively inexpensive) in patients' first assessments could help identifying
567 potential responders to minocycline.

568 It is also worth noting that this is the third RCT with positive results on minocycline in
569 unipolar depression. Such evidence suggests that minocycline antidepressant effect
570 might be diagnosis-specific, considering that results in bipolar depression are more
571 conflicting. Indeed, a recent work pointed out that minocycline was not superior to
572 placebo for the acute management of bipolar depression (54). [However, our study also
573 indicates that conventional diagnosis should be complemented with the assessment of
574 biological factors, like the immune markers, in order to identify effective treatments for
575 depression, including anti-inflammatories.](#)

576

577 The main strengths of our study were 1) the a priori recruitment of patients with elevated
578 inflammation and 2) the measurement of several inflammatory biomarkers, which had
579 not been performed in previous studies. This enabled us to add knowledge on the
580 relationship between clinical and biological outcomes in immune-related depression
581 treated with minocycline. [Moreover, the comparison between CC and ITT analysis
582 increased the robustness of the data.](#)

583 Our results should also be interpreted in light of some limitations, such as the small
584 sample size. Indeed, although our sample size was similar to that of previous RCTs with
585 minocycline, the further division of the sample in 4 groups led to even smaller sizes
586 (ranging from 6 to 12 patients [and from 8 to 14 patients per subgroup in the CC and ITT](#)

587 [analysis, respectively](#)). Moreover, we could not identify enough patients with treatment
588 response as defined by a 50% reduction in the HAM-D-17 score and we had to consider
589 partial response, instead. This is probably because of the shorter trial duration, i.e., 4
590 weeks compared with longer RCTs. Another limitation is the lack of follow-up data after
591 the 4 weeks assessment, so that we cannot comment on the long-term efficacy of both
592 minocycline and placebo. [Finally, we could not add more clinical information such as the](#)
593 [number of failed treatments in patients' lifetime and in the current episode and the](#)
594 [duration of the current episode of depression. This information would have helped to](#)
595 [better understand the low response rate in the present study, in terms of 50% reduction](#)
596 [in the HAM-D-17 scores.](#)

597

598 **Conclusions**

599

600 In conclusion, we found suggestive evidence that minocycline was a beneficial add-on
601 therapy in a subgroup of MDD patients with levels of hsCRP \geq 3 mg/L. Such
602 antidepressant effect was independent from changes in peripheral biomarkers and
603 suggests the involvement of other mechanisms, possibly related to central inflammation.
604 Although replications in larger samples are needed, we believe our study has a
605 potentially important clinical impact, as we moved a step towards the identification of
606 personalized treatments for depression.

607

608

609 **Funding and disclosure**

610

611 This research was funded by the National Institute for Health Research (NIHR)
612 Biomedical Research Centre at South London and Maudsley NHS Foundation Trust
613 and King's College London. The views expressed are those of the authors and not
614 necessarily those of the NHS, the NIHR, or the Department of Health and Social Care. Dr
615 Valeria Mondelli is supported by MQ: Transforming Mental Health (Grant: MQBF1) and
616 by the Medical Research Foundation (grant number MRF-160-0005-ELP-MONDE).

617
618 Prof Pariante and Dr Mondelli have received research funding from Johnson & Johnson
619 as part of a research program on depression and inflammation. Prof Pariante has
620 received research funding from the Medical Research Council (UK) and the Wellcome
621 Trust for research on depression and inflammation as part of two large consortia that
622 also include Johnson & Johnson, GSK and Lundbeck. Prof Cleare has in the last three
623 years received honoraria for speaking from Lundbeck; honoraria for consulting from
624 Livanova, Lundbeck, Allergan and Janssen; sponsorship for conference attendance from
625 Janssen; and research grant support for work that includes inflammation and depression
626 from the Medical Research Council (UK), Wellcome Trust (UK), the National Institute for
627 Health Research (UK) and Protexin Probiotics International Ltd. The remaining authors
628 have nothing to disclose.

629

630

631 **Acknowledgments**

632

633 We thank the National Institute for Health Research (NIHR) Biomedical Research Centre
634 at South London and Maudsley NHS Foundation Trust and King's College London and
635 the National Institute for Health Research NIHR / Wellcome King's Clinical Research
636 Facility. All visits took place at the Clinical Research Facility of King's College Hospital.
637 The team of nurses has to be thanked for providing their valuable expertise to the study.

638

639

640 **Authors' contribution**

641

642 A Cleare, A Young, C M Pariante & V. Mondelli contributed to the conception and design
643 of the work, to critically revisiting the work and to provide final approval of the version to
644 be published.

645 MA Nettis, CM Pariante & Valeria Mondelli took care of data interpretation.

646 MA Nettis performed statistical analysis and drafted the manuscript.

647 M A Nettis, Giulia Lombardo, Caitlin Hastings, Zuzanna Zajkowska, Courtney Worrell,
648 Daniela Enache, Anna McLaughlin, Melisa Kose, Luca Sforzini and Anna Bogdanova
649 collected all data and completed patients recruitment.
650 Nicole Mariani and Naghmeh Nikkheslat processed blood samples and analysed peripheral
651 inflammatory biomarkers.
652
653

654 **References**

655

656 **Uncategorized References**

- 657 1. Kohler O, Krogh J, Mors O, Benros ME. Inflammation in Depression and the
658 Potential for Anti-Inflammatory Treatment. *Curr Neuropharmacol*. 2016;14(7):732-42.
- 659 2. Miller AH, Haroon E, Felger JC. Therapeutic Implications of Brain-Immune
660 Interactions: Treatment in Translation. *Neuropsychopharmacology*. 2017;42(1):334-59.
- 661 3. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary
662 imperative to modern treatment target. *Nat Rev Immunol*. 2016;16(1):22-34.
- 663 4. Schjerning Olsen A-M, Fosbøl EL, Lindhardsen J, Folke F, Charlot M, Selmer C,
664 et al. Duration of treatment with nonsteroidal anti-inflammatory drugs and impact on risk
665 of death and recurrent myocardial infarction in patients with prior myocardial infarction: a
666 nationwide cohort study. *Circulation*. 2011;123(20):2226-35.
- 667 5. Toussi SS, Pan N, Walters HM, Walsh TJ. Infections in children and adolescents
668 with juvenile idiopathic arthritis and inflammatory bowel disease treated with tumor
669 necrosis factor- α inhibitors: systematic review of the literature. *Clinical infectious
670 diseases*. 2013;57(9):1318-30.
- 671 6. Shin JY, Park MJ, Lee SH, Choi SH, Kim MH, Choi NK, et al. Risk of intracranial
672 haemorrhage in antidepressant users with concurrent use of non-steroidal anti-
673 inflammatory drugs: nationwide propensity score matched study. *BMJ*. 2015;351:h3517.
- 674 7. Kohler O, Benros ME, Nordentoft M, Farkouh ME, Iyengar RL, Mors O, et al.
675 Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse
676 effects: a systematic review and meta-analysis of randomized clinical trials. *JAMA
677 Psychiatry*. 2014;71(12):1381-91.
- 678 8. Warner-Schmidt JL, Vanover KE, Chen EY, Marshall JJ, Greengard P.
679 Antidepressant effects of selective serotonin reuptake inhibitors (SSRIs) are attenuated

680 by antiinflammatory drugs in mice and humans. *Proc Natl Acad Sci U S A*.
681 2011;108(22):9262-7.

682 9. Enache D, Pariante CM, Mondelli V. Markers of central inflammation in major
683 depressive disorder: A systematic review and meta-analysis of studies examining
684 cerebrospinal fluid, positron emission tomography and post-mortem brain tissue. *Brain*
685 *Behav Immun*. 2019;81:24-40.

686 10. Soczynska JK, Mansur RB, Brietzke E, Swardfager W, Kennedy SH,
687 Woldeyohannes HO, et al. Novel therapeutic targets in depression: minocycline as a
688 candidate treatment. *Behav Brain Res*. 2012;235(2):302-17.

689 11. Roman M, Irwin MR. Novel neuroimmunologic therapeutics in depression: A
690 clinical perspective on what we know so far. *Brain Behav Immun*. 2020;83:7-21.

691 12. Miller AH, Raison CL. Cytokines, p38 MAP kinase and the pathophysiology of
692 depression. *Neuropsychopharmacology*. 2006;31(10):2089-90.

693 13. Rojewska E, Ciapala K, Piotrowska A, Makuch W, Mika J. Pharmacological
694 Inhibition of Indoleamine 2,3-Dioxygenase-2 and Kynurenine 3-Monooxygenase,
695 Enzymes of the Kynurenine Pathway, Significantly Diminishes Neuropathic Pain in a Rat
696 Model. *Front Pharmacol*. 2018;9:724.

697 14. Pae CU, Marks DM, Han C, Patkar AA. Does minocycline have antidepressant
698 effect? *Biomed Pharmacother*. 2008;62(5):308-11.

699 15. Hashimoto K, Ishima T. A novel target of action of minocycline in NGF-induced
700 neurite outgrowth in PC12 cells: translation initiation [corrected] factor eIF4A1. *PLoS*
701 *One*. 2010;5(11):e15430.

702 16. Miyaoka T, Wake R, Furuya M, Liaury K, Ieda M, Kawakami K, et al. Minocycline
703 as adjunctive therapy for patients with unipolar psychotic depression: an open-label
704 study. *Prog Neuropsychopharmacol Biol Psychiatry*. 2012;37(2):222-6.

- 705 17. Dean OM, Kanchanatawan B, Ashton M, Mohebbi M, Ng CH, Maes M, et al.
706 Adjunctive minocycline treatment for major depressive disorder: A proof of concept trial.
707 Aust N Z J Psychiatry. 2017;51(8):829-40.
- 708 18. Husain MI, Chaudhry IB, Husain N, Khoso AB, Rahman RR, Hamirani MM, et al.
709 Minocycline as an adjunct for treatment-resistant depressive symptoms: A pilot
710 randomised placebo-controlled trial. J Psychopharmacol. 2017;31(9):1166-75.
- 711 19. Emadi-Kouchak H, Mohammadinejad P, Asadollahi-Amin A, Rasoulinejad M,
712 Zeinoddini A, Yalda A, et al. Therapeutic effects of minocycline on mild-to-moderate
713 depression in HIV patients: a double-blind, placebo-controlled, randomized trial. Int Clin
714 Psychopharmacol. 2016;31(1):20-6.
- 715 20. Rosenblat JD, McIntyre RS. Efficacy and tolerability of minocycline for
716 depression: A systematic review and meta-analysis of clinical trials. J Affect Disord.
717 2018;227:219-25.
- 718 21. Raison CL, Rutherford RE, Woolwine BJ, Shuo C, Schettler P, Drake DF, et al. A
719 randomized controlled trial of the tumor necrosis factor antagonist infliximab for
720 treatment-resistant depression: the role of baseline inflammatory biomarkers. JAMA
721 Psychiatry. 2013;70(1):31-41.
- 722 22. Osimo EF, Baxter LJ, Lewis G, Jones PB, Khandaker GM. Prevalence of low-
723 grade inflammation in depression: a systematic review and meta-analysis of CRP levels.
724 Psychol Med. 2019;49(12):1958-70.
- 725 23. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al.
726 The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and
727 validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin
728 Psychiatry. 1998;59 Suppl 20:22-33;quiz 4-57.
- 729 24. Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry.
730 1960;23:56-62.

- 731 25. Rohan KJ, Rough JN, Evans M, Ho SY, Meyerhoff J, Roberts LM, et al. A
732 protocol for the Hamilton Rating Scale for Depression: Item scoring rules, Rater training,
733 and outcome accuracy with data on its application in a clinical trial. *J Affect Disord.*
734 2016;200:111-8.
- 735 26. Kneipp SM, Kairalla JA, Stacciarini JM, Pereira D, Miller MD. Comparison of
736 depressive symptom severity scores in low-income women. *Nurs Res.* 2010;59(6):380-
737 8.
- 738 27. Nakonezny PA, Carmody TJ, Morris DW, Kurian BT, Trivedi MH. Psychometric
739 evaluation of the Snaith-Hamilton pleasure scale in adult outpatients with major
740 depressive disorder. *Int Clin Psychopharmacol.* 2010;25(6):328-33.
- 741 28. Spielberger CD, DeNike LD. Descriptive behaviorism versus cognitive theory in
742 verbal operant conditioning. *Psychol Rev.* 1966;73(4):306-26.
- 743 29. Busner J, Targum SD. The clinical global impressions scale: applying a research
744 tool in clinical practice. *Psychiatry (Edgmont).* 2007;4(7):28-37.
- 745 30. Brugha TS, Cragg D. The List of Threatening Experiences: the reliability and
746 validity of a brief life events questionnaire. *Acta Psychiatr Scand.* 1990;82(1):77-81.
- 747 31. Roberti JW, Harrington LN, Storch EA. Further psychometric support for the 10-
748 item version of the perceived stress scale. *Journal of College Counseling.*
749 2006;9(2):135-47.
- 750 32. MacQueen G, Santaguida P, Keshavarz H, Jaworska N, Levine M, Beyene J, et
751 al. Systematic Review of Clinical Practice Guidelines for Failed Antidepressant
752 Treatment Response in Major Depressive Disorder, Dysthymia, and Subthreshold
753 Depression in Adults. *Can J Psychiatry.* 2017;62(1):11-23.
- 754 33. Frank E, Prien RF, Jarrett RB, Keller MB, Kupfer DJ, Lavori PW, et al.
755 Conceptualization and rationale for consensus definitions of terms in major depressive
756 disorder. Remission, recovery, relapse, and recurrence. *Arch Gen Psychiatry.*
757 1991;48(9):851-5.

- 758 34. Kennedy SH, Lam RW, McIntyre RS, Tourjman SV, Bhat V, Blier P, et al.
759 Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 Clinical
760 Guidelines for the Management of Adults with Major Depressive Disorder: Section 3.
761 Pharmacological Treatments. *Can J Psychiatry*. 2016;61(9):540-60.
- 762 35. von Eckardstein A, Roth HJ, Jones G, Preston S, Szekeres T, Imdahl R, et al.
763 cobas 8000 Modular analyzer series evaluated under routine-like conditions at 14 sites
764 in Australia, Europe, and the United States. *J Lab Autom*. 2013;18(4):306-27.
- 765 36. Dabito D, Margolick JB, Lopez J, Bream JH. Multiplex measurement of
766 proinflammatory cytokines in human serum: comparison of the Meso Scale Discovery
767 electrochemiluminescence assay and the Cytometric Bead Array. *J Immunol Methods*.
768 2011;372(1-2):71-7.
- 769 37. King E, O'Brien J, Donaghy P, Williams-Gray CH, Lawson RA, Morris CM, et al.
770 Inflammation in mild cognitive impairment due to Parkinson's disease, Lewy body
771 disease, and Alzheimer's disease. *Int J Geriatr Psychiatry*. 2019;34(8):1244-50.
- 772 38. Hepgul N, Pariante CM, Dipasquale S, DiForti M, Taylor H, Marques TR, et al.
773 Childhood maltreatment is associated with increased body mass index and increased C-
774 reactive protein levels in first-episode psychosis patients. *Psychol Med*.
775 2012;42(9):1893-901.
- 776 39. Russell A, Hepgul N, Nikkheslat N, Borsini A, Zajkowska Z, Moll N, et al.
777 Persistent fatigue induced by interferon-alpha: a novel, inflammation-based, proxy model
778 of chronic fatigue syndrome. *Psychoneuroendocrinology*. 2019;100:276-85.
- 779 40. Thabane L, Mbuagbaw L, Zhang S, Samaan Z, Marcucci M, Ye C, et al. A tutorial
780 on sensitivity analyses in clinical trials: the what, why, when and how. *BMC Med Res*
781 *Methodol*. 2013;13:92.
- 782 41. Jakobsen JC, Gluud C, Wetterslev J, Winkel P. When and how should multiple
783 imputation be used for handling missing data in randomised clinical trials - a practical
784 guide with flowcharts. *BMC Med Res Methodol*. 2017;17(1):162.

- 785 42. Nguyen CD, Carlin JB, Lee KJ. Model checking in multiple imputation: an
786 overview and case study. *Emerg Themes Epidemiol.* 2017;14:8.
- 787 43. Haroon E, Fleischer CC, Felger JC, Chen X, Woolwine BJ, Patel T, et al.
788 Conceptual convergence: increased inflammation is associated with increased basal
789 ganglia glutamate in patients with major depression. *Mol Psychiatry.* 2016;21(10):1351-
790 7.
- 791 44. Abbasi SH, Hosseini F, Modabbernia A, Ashrafi M, Akhondzadeh S. Effect of
792 celecoxib add-on treatment on symptoms and serum IL-6 concentrations in patients with
793 major depressive disorder: randomized double-blind placebo-controlled study. *J Affect*
794 *Disord.* 2012;141(2-3):308-14.
- 795 45. Savitz JB, Teague TK, Misaki M, Macaluso M, Wurfel BE, Meyer M, et al.
796 Treatment of bipolar depression with minocycline and/or aspirin: an adaptive, 2x2
797 double-blind, randomized, placebo-controlled, phase IIA clinical trial. *Transl Psychiatry.*
798 2018;8(1):27.
- 799 46. Himmerich H, Patsalos O, Lichtblau N, Ibrahim MAA, Dalton B. Cytokine
800 Research in Depression: Principles, Challenges, and Open Questions. *Front Psychiatry.*
801 2019;10:30.
- 802 47. Kiraly DD, Horn SR, Van Dam NT, Costi S, Schwartz J, Kim-Schulze S, et al.
803 Altered peripheral immune profiles in treatment-resistant depression: response to
804 ketamine and prediction of treatment outcome. *Transl Psychiatry.* 2017;7(3):e1065.
- 805 48. Zheng LS, Kaneko N, Sawamoto K. Minocycline treatment ameliorates interferon-
806 alpha- induced neurogenic defects and depression-like behaviors in mice. *Front Cell*
807 *Neurosci.* 2015;9:5.
- 808 49. Han Y, Zhang L, Wang Q, Zhang D, Zhao Q, Zhang J, et al. Minocycline inhibits
809 microglial activation and alleviates depressive-like behaviors in male adolescent mice
810 subjected to maternal separation. *Psychoneuroendocrinology.* 2019;107:37-45.

811 50. Nettis MA, Veronese M, Nikkheslat N, Mariani N, Lombardo G, Sforzini L, et al.
812 PET imaging shows no changes in TSPO brain density after IFN-alpha immune
813 challenge in healthy human volunteers. *Transl Psychiatry*. 2020;10(1):89.

814 51. Oxenkrug GF. Interferon-gamma-inducible kynurenines/pteridines inflammation
815 cascade: implications for aging and aging-associated psychiatric and medical disorders.
816 *J Neural Transm (Vienna)*. 2011;118(1):75-85.

817 52. Fournier JC, DeRubeis RJ, Hollon SD, Dimidjian S, Amsterdam JD, Shelton RC,
818 et al. Antidepressant drug effects and depression severity: a patient-level meta-analysis.
819 *JAMA*. 2010;303(1):47-53.

820 53. Zheng W, Zhu XM, Zhang QE, Cheng G, Cai DB, He J, et al. Adjunctive
821 minocycline for major mental disorders: A systematic review. *J Psychopharmacol*.
822 2019;33(10):1215-26.

823 54. Husain MI, Chaudhry IB, Khoso AB, Husain MO, Hodsoll J, Ansari MA, et al.
824 Minocycline and celecoxib as adjunctive treatments for bipolar depression: a multicentre,
825 factorial design randomised controlled trial. *Lancet Psychiatry*. 2020;7(6):515-27.

826

827

828

829 **Figure legends**

830

831 Fig. 1 Difference in HAM-D mean change, calculated as baseline scores minus week 4
832 scores, between patients divided by Study Arm X baseline hsCRP. Patients with hsCRP
833 levels ≥ 3 mg/L and taking minocycline (CRP⁺/M) showed a significantly larger
834 improvement compared with all other patients.

835 HAM-D= Hamilton Depression Rating Scale

836 CRP⁺= baseline hsCRP levels ≥ 3 mg/L

837 CRP⁻= baseline hsCRP levels < 3 mg/L

838 M= Minocycline

839 P= Placebo

840

841

Table 1 Socio-demographic variables

	Minocycline n=18	Placebo n=21	Statistics
Age, mean (SD)	47.0(10.0)	43.7(10.7)	t=0.98 p=0.33
Gender, F (%)	55.6	57.1	X ² =0.01 p=0.92
Ethnicity, White(%)	72.2	75.5	X ² =0.01 p=0.92
BMI, mean (SD)	31.0(6.8)	31.6(6.2)	t=-0.28 p=0.78
Current Smoker, yes(%)	22.2(n=4)	19.0(n=4)	X ² =0.06 p=0.80
Alcohol units per week, mean (SD)	7.2(10.3)	9.7(9.9)	t=-0.69 p=0.49
Current Medication*			
1) SSRI (%)	61.1	47.6	X ² =4.0 p=0.26
2) OTHER AD (%)	27.8	14.3	
3) AD+AP (%)	5.6	14.3	
4) > 2 AD (%)	5.6	23.8	
5) AD+BENZODIAZEPINES (%)	11.1	4.8	
Months on current medication			
1) ≤ 6 months (%)	35.3	20.0	X ² =-2.53 p=0.13
2) 6 to 12 months (%)	0.0	10.0	
3) ≥ 12 months (%)	64.7	70.0	
Depression duration from onset (years, mean(SD))	21.30 (10.92)	18.05 (12.39)	t=0.89 p=0.38
Baseline CTQ total score, mean (SD)	52.94(20.22)	45.86(11.45)	t=1.36 p=0.18
Baseline BLE			
1) Stressful events, yes (%)	44.4	76.2	X ² =4.12 p=0.04 X ² =0.39 p=0.82
2) Number of severe events			
• None (%)	25	37.5	
• One (%)	50	43.8	
• 2 or more	25	18.8	
Baseline PSS total score, mean (SD)	19.16(2.41)	21.14(3.18)	t=-2.15 p=0.04
Baseline HAM-D-17 score, mean (SD)	19.06 (3.45)	17.00 (3.26)	t=1.9 p=0.06
Baseline hsCRP, mean (SD)	3.13 (2.52)	4.49 (5.20)	t=-0.98. p=0.33

*AD= antidepressant; AP= anti-psychotic medication

CTQ= Childhood Trauma Questionnaire
 BLE= Brief Life Events scale
 PSS= Perceived Stress Scale
 HAM-D-17= Hamilton Depression Rating Scale
 hsCRP= high sensitivity C-reactive protein

Table 2 A) HAM-D-17 and CRP descriptive statistics; B) proportions of responders and non-responders by groups

A		Baseline	n	Week 4	n	Baseline vs Week4 Statistics (bootstrapped)	
HAM-D-17, mean (SD)	Minocycline	19.06 (3.45)	18	13.44 (5.17)	18	t=3.74 p=0.008	
	Placebo	17.00 (3.26)	21	14.10 (5.59)	21	t=3.43 p=0.003	
	CRP+/M	21.50 (2.59)	6	9.5 (5.32)	6	t=4.55 p=0.02	
	CRP+/P	16.08 (2.91)	12	12.58 (5.45)	12	t=2.79 p=0.03	
	CRP-/M	17.83 (3.24)	12	15.42 (3.36)	12	t=2.61 p=0.03	
	CRP-/P	18.22 (4.36)	9	16.11 (5.42)	9	t=1.94 p=0.11	
	hsCRP, mean (SD)	Minocycline	3.13 (2.52)	18	3.30 (3.24)	17	t=0.41 p=0.70
		Placebo	4.49 (5.20)	21	4.03 (3.53)	21	t=0.52 p=0.61
		CRP+/M	5.68 (2.95)	6	5.13 (4.84)	6	All p > 0.05
		CRP+/P	6.62 (6.11)	12	5.86 (3.72)	12	
CRP-/M		1.85 (0.72)	12	2.30 (1.39)	11		
CRP-/P		1.75 (0.62)	9	1.59 (0.58)	9		

B		HAM-D-17 improvement <25%	n	HAM-D-17 improvement ≥25%	n	Statistics
	CRP+/M	16.7%	1	83.3%	5	X²=8.27 p=0.04
	CRP+/P	41.7%	5	58.3%	7	
	CRP-/M	75.0%	9	25.0%	3	
	CRP-/P	77.8%	7	22.2%	2	

HAM-D-17= Hamilton Depression Rating Scale

hsCRP= high sensitivity C-reactive protein (analysis conducted with logarithmic CRP)

CRP+= baseline hsCRP levels ≥ 3 mg/L

CRP-= baseline hsCRP levels < 3 mg/L

M= Minocycline P= Placebo

Table 3 Within and between groups analyses in other clinical scales

		<i>n</i>	<i>Minocycline</i>	<i>n</i>	<i>Placebo</i>	<i>Between-groups statistics</i>
BDI-II mean (SD)	Baseline	18	24.27 (9.75)	21	26.71 (9.20)	t=0.24 p=0.81
	Week4	18	17.33 (20.75)	21	20.38 (17.11)	
	Mean Change	18	6.94 (8.46)**	21	6.33 (7.17)**	
CGI mean (SD)	Baseline	18	4.44 (0.86)	19	4.26 (0.65)	t=2.24 p=0.03
	Week4	18	3.39 (1.04)	21	3.85 (1.01)	
	Mean Change	18	1.05 (1.21)**	19	0.32 (0.75)	
PSS mean (SD)	Baseline	18	19.16 (2.41)	21	21.14 (3.18)	t=-0.95, p=0.35
	Week4	18	20.05 (2.92)	21	20.71 (4.23)	
	Mean Change	18	-0.89 (4.40)	21	-0.43 (4.24)	
SHAPS mean (SD)	Baseline	17	7.18 (3.69)	18	5.60 (3.50)	t=0.88 p=0.38
	Week4	18	4.61 (4.92)	19	4.20 (4.21)	
	Mean Change	17	3.00 (4.00)*	18	2.00 (2.66)**	
STAI-S mean (SD)	Baseline	17	51.18 (11.68)	21	54.09 (8.56)	t=-0.42 p=0.67
	Week4	17	47.33 (13.77)	21	48.67 (11.19)	
	Mean Change	17	4.05 (11.40)	21	5.43 (8.62)**	
STAI-T mean (SD)	Baseline	16	57.75 (8.15)	19	59.48 (6.37)	t=0.002 p=0.99
	Week4	16	49.69 (13.14)	21	54.31 (8.97)	
	Mean Change	14	5.57 (9.47)*	19	5.58 (10.18)*	

BDI-II= Beck Depression Inventory II

CGI= Clinical Global Impression scale

PSS= Perceived stress scale

SHAPS= Snaith–Hamilton Pleasure Scale

STAI-S= Spielberger State-Trait Anxiety Rating Scale-State

STAI-T= Spielberger State-Trait Anxiety Rating Scale-Trait

** within-group paired t-test, p<0.01

* within-group paired t-test, p<0.05

Table 4 Within and between group analyses on inflammatory biomarkers

		<i>Minocycline</i> Baseline n=18 Week4 n=17 Mean Change n=17	<i>Placebo</i> Baseline n=21 Week4 n=21 Mean Change n=21	<i>Between-arms</i> <i>statistics</i>
IL2 mean (SD) (pg/ml)	Baseline	0.18 (0.14)	0.14 (0.12)	U= 131.5 p=0.17
	Week4	0.22 (0.15)	0.14 (0.11)	
	Mean Change	-0.035 (0.12)	0.00 (0.06)	
IL6 mean (SD) (pg/ml)	Baseline	0.87 (0.32)	0.84 (0.44)	U= 173.0 p=0.88
	Week4	1.25 (1.7)	0.76 (0.38)	
	Mean Change	-0.36 (1.59)	0.07 (0.33)	
IL8 mean (SD) (pg/ml)	Baseline	9.2 (2.64)	10.77 (3.44)	U= 131.0 p=0.16
	Week 4	11.14 (4.21)	10.57 (3.62)	
	Mean Change	1.76 (3.38)*	-0.19 (3.24)	
IL10 mean (SD) (pg/ml)	Baseline	0.30 (0.25)	0.39 (0.32)	U= 153.0 p=0.45
	Week4	0.26 (0.21)	0.43 (0.48)	
	Mean Change	0.04 (0.33)	-0.04 (0.19)	
IL13 mean (SD) (pg/ml)	Baseline	0.63 (0.49)	0.63 (0.49)	U= 143.0 p=0.31
	Week4	0.49 (0.46)	0.58 (0.53)	
	Mean Change	0.08 (0.31)	-0.12 (0.52)	
TNF α mean (SD) (pg/ml)	Baseline	3.29 (0.75)	3.18 (0.65)	U= 135.0 p=0.21
	Week4	3.51 (0.78)	3.30 (0.73)	
	Mean Change	-0.29 (0.54)	-0.12 (0.31)	
IFN γ mean (SD) (pg/ml)	Baseline	2.97 (2.03)	2.51 (2.15)	U=105.5 p=0.03
	Week4	2.21 (1.61)	2.76 (1.79)	
	Mean Change	0.48 (0.93)	-0.24 (1.67)	

IL=interleukin

TNF=tumour necrosis factor

IFN=interferon

*within group Wilcoxon Signed Ranks test, p<0.05

HAM-D -17 CHANGE ACROSS STUDY ARMS AND BASELINE INFLAMMATION GROUPS

