Sex differences in a double-blind randomized clinical trial with minocycline in treatment-resistant depressed patients: CRP and IL-6 as sex-specific predictors of treatment response

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ABSTRACT

Background: Inflammation is a well-known risk factor for depression. Specifically, patients who do not respond to antidepressant treatment show higher levels of inflammatory biomarkers compared with responders. Thus, several studies have investigated the efficacy of anti-inflammatory add-on treatment in this population. However, major depressive disorder is more prevalent in females than in males, with sex differences present in antidepressant treatment response and in immune system regulation. To explore sex differences in inflammatory profiles and treatment responses, we investigated a cohort of patients with treatment resistant depression (TRD), for which they received an adjunctive, anti-inflammatory treatment with minocycline – the Minocycline in Depression (MINDEP) study.

Methods: The MINDEP study is a 4-week double-blind, randomised, placebo-controlled clinical trial (stratified by sex) with 39 TRD participants, which demonstrated the efficacy of minocycline, an antibiotic with anti-inflammatory properties, in TRD patients with major depressive disorder (MDD) and evidence of low-grade inflammation measured with C-reactive protein (CRP) ≥ 3 mg/L. In these secondary analyses, we investigated the differential effects of minocycline in females (N = 22, 10 randomised to minocycline and 12 randomised to placebo) and in males (N = 17, 8 randomised to minocycline and 9 randomised to placebo) on changes in depressive symptoms (Δ-Hamilton Rating Scale for Depression (HAM-D)-17), taking also into consideration CRP levels (CRP ≥ 3 mg/L vs. CRP < 3 mg/L). Additionally, we investigated the role of serum IL-6 in predicting treatment response to minocycline, using sex-specific medians of IL-6, in novel exploratory analyses.

Results: Sex differences in Δ-HAM-D-17 indicate that only females (F = 10.49, p = 0.005), but not males (F = 1.64, p = 0.22), presented an effect of CRP levels on the response to minocycline. Also, we detected sex differences in the relationship between serum CRP and IL-6 levels: CRP was strongly correlated with IL-6 in females (Spearman’s ρ = 0.658, P < 0.001) but not in males (p = 0.007, p = 0.979). Exploratory analyses found that IL-6 was indeed a better predictor of response than minocycline than CRP, as we found an interaction between study arms and IL-6 groups (above and below the IL-6 sex-specific median) in females (F = 4.435 p = 0.050) and, at trend statistical level, in males (F = 4.258 p = 0.060). Moreover, Δ-HAM-D-17 was numerically comparable in the two high-IL-6 group taking minocycline (females, mean 9.20 ± SD 7.80; males, mean 8.80 ± SD 5.97), confirming that high IL-6, differently from high CRP, identified responders to minocycline both in males and females.

Conclusion: Our findings highlight the need of sex-specific inflammatory biomarkers in predicting antidepressant response to anti-inflammatories in TRD patients, with the possibility of CRP being a relevant predictor of treatment response only for females, and IL-6 being relevant for both sexes.

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1. Introduction

Major depressive disorder (MDD) shows sex differences, not only in incidence but also in symptomatology and antidepressant response, but whether such differences can be exploited for sex-specific pharmacological interventions is still unclear. Women are almost twice as likely as men to develop depression (Kuehner 2017), with female patients having younger age of MDD onset and more frequent atypical depression in comparison with men (Schuch et al., 2014). Furthermore, female patients tend to respond better to selective serotonin reuptake inhibitors (SSRIs), whereas male patients respond better to tricyclic antidepressants (TCAs) (Young et al., 2009; Keers and Aitchison 2010). Notably, men have increased risk to attempted and completed suicides, even though suicidal ideation and suicidal behaviour are more associated with female sex (Barrigon and Gega-Schwartzman, 2020; Cantor 2000; Oquendo et al., 2001; Skogman et al., 2004).

One of the most relevant findings in depression is the key role of inflammation. Specifically, depressed patients show higher levels of inflammatory biomarkers in comparison with controls (Osimó et al., 2020; Pittarouli et al., 2021), with patients who do not respond to the common antidepressant treatments having even higher levels of inflammatory biomarkers in comparison with responders (Cattaneo et al., 2013, 2015, 2020). Interestingly, the immune system differs between males and females. For instance, in the general population, females show a stronger immune response in comparison with males (Klein and Flanagan 2016), with higher rates of auto-immune diseases (Quintero et al., 2012). The immune system might also play a relevant role in women with depression. For instance, in one of the few studies testing sex-differences in this context, depressed females show increased interleukin (IL)-8, interferon (INF)-γ, and leptin, and decreased IL-5 and adiponectin, compared with female controls, while there are no significant differences in inflammatory markers between depressed males and male controls (Birur et al., 2017). However, our recent study from the UK Biobank finds that CRP is elevated in both males and females (Kuehner et al., 2020; Nettis et al., 2021). Briefly, the MINDEP study was a 4-week single centre, double-blind, randomised (1:1 minocycline/placebo), placebo-controlled, parallel group trial with add-on minocycline treatment (200 mg/day), investigating the efficacy of minocycline in TRD patients with major depressive disorder and increased peripheral inflammation detected by CRP levels equal to or more than 1 mg/L (Nettis et al., 2021). Patients were randomised (1:1 minocycline/placebo) by the method of block randomisation, stratified by sex, via a web-based randomisation system at the Clinical Trials Unit at the IoPPN. Treatment resistant depression was defined as non-response to the current antidepressant treatment taken for 6 weeks at therapeutic dose and Hamilton Depression Rating Scale (HAMD)-17 score ≥14.

2. Methods

The main methods and results are described in the recently published paper (Nettis et al., 2021). Briefly, the MINDEP study was a 4-week single centre, double-blind, randomised (1:1 minocycline/placebo), placebo-controlled, parallel group trial with add-on minocycline treatment (200 mg/day), investigating the efficacy of minocycline in TRD patients with major depressive disorder and increased peripheral inflammation detected by CRP levels equal to or more than 1 mg/L (Nettis et al., 2021). Patients were randomised (1:1 minocycline/placebo) by the method of block randomisation, stratified by sex, via a web-based randomisation system at the Clinical Trials Unit at the IoPPN. Treatment resistant depression was defined as non-response to the current antidepressant treatment taken for 6 ≥ weeks at therapeutic dose and Hamilton Depression Rating Scale (HAMD)-17 score ≥14.

2.1. Depressive symptoms

We used HAMD-17 and Beck Depression Inventory-II (BDI-II) clinical assessments. We calculated two total sub-scores based on the BDI-II items identified by Thoms et al. (2010). The following BDI-II items evaluated cognitive/affective and somatic symptoms, respectively: 1) sadness, pessimism, past failure, loss of pleasure, guilty feelings, punishment feelings, self-dislike, self-criticism, suicidal ideation, crying, agitation, loss of interest, indecisiveness, worthlessness (items 1-14); 2) loss of energy, sleep problems, irritability, appetite problems, concentration, fatigue, loss of interest in sex (items 15-21).

2.2. Clinical outcome

We investigated the clinical improvement using the same criterion and by calculating Δ-HAMD-17 score (baseline HAMD-17 – week-4 HAMD-17) between baseline and end of treatment.

2.3. CRP groups

Nettis et al. (2021), highlighted that the presence of low-grade inflammation at the baseline (high-sensitivity (hs-) CRP ≥3 mg/L) in the minocycline study arm was associated with greater clinical improvement. This finding was also corroborated by the hypothesis-free ROC analysis which identified the cut-off ≥2.8 mg/L as best discriminating responders vs. non-responders to minocycline. Of course, the authors performed the analysis without considering differences between males and females. Therefore, we investigated sex differences in treatment response by focussing on the original cut-off equal to 3 mg/L, that is by...
analysing male (n = 17) and female (n = 22) patients, separately with hs-CRP ≥ 3 mg/L (CRP >3 group, 8 males and 10 females) and hs-CRP <3 mg/L (CRP <3 group, 9 males and 12 females; all had CRP >1 mg/L).

2.4. Inflammatory biomarkers

As Nettis et al. (2021) described, the whole inflammatory panel included the following serum pro- and anti-inflammatory biomarkers: hs-CRP and IL-1β, IL-2, IL-4, IL-6, IL-10, IL12p70, IL-13, TNF-α, INF-γ, at baseline and week-4, using MSD V-PLEX sandwich immunoassays, MSD Pro-inflammatory Panel 1 (human) kit (Dabiao et al., 2011; King et al., 2019) and plates read on an MSD QuickPlex SQ 120, as we had extensively used before (Heppgl et al., 2012; Russell et al., 2019). The inter-assay coefficient of variations was <10%. A large number of samples for IL-1β (35%), IL-4 (82%) and IL12p70 (25%) levels were non detected or below the lowest detectable values for baseline and week 4 time points, and we excluded these cytokines from the statistical analyses. Most of the investigated inflammatory biomarkers exhibited a non-normal distribution; thus, we applied the natural logarithmic transformation.

2.5. Statistical analyses

Sex differences at the baseline: The analyses included One-Way ANOVA, independent samples t-test, Chi-square test and bivariate Spearman’s correlation to investigate differences in demographic data (age, BMI, ethnicity, self-report medications, current smoker users and alcohol units per week), inflammatory profile, childhood trauma questionnaire (CTQ) and depressive symptoms between male and female TRD patients at the baseline. We performed Spearman’s bivariate correlation analyses for evaluations of the association between two continuous variables within sexes.

Treatment response: Univarite general linear model (GLM) analyses and independent samples t-tests were used to investigate the effects of study arms and CRP groups on Δ-HAMD-17. We performed Spearman’s bivariate correlational analyses between baseline hs-CRP and Δ-HAMD-17, and between baseline IL-6 and Δ-HAMD-17, to investigate their potential role as predictors of treatment response to minocycline, separately in males and females. We evaluated the difference between pair of correlations using Fisher z statistics in the cocor package in R http://comparingcorrelations.org/ (Diedenhofen and Musch, 2015).

As exploratory analyses, we performed GLM analyses separately in males (N = 17) and females (N = 22) to further clarify sex specific role of study arms and CRP groups. The GLM analysed three models for CRP stratification: 1) the first model had as fixed factors study arms and sex (overall sample); 2) the second model had as fixed factors study arms, sex and CRP groups (overall sample); and 3) the third model had as fixed factors study arms and CRP groups (within sex analyses).

We calculated sex-specific medians of IL-6 (females: 0.640 pg/ml; males: IL-6 0.960 pg/ml) to split the female and male samples as above and below the respective IL-6 medians, and investigate the role of baseline IL-6 levels in treatment response. Therefore, similarly to the previous analyses, we performed the GLM analyses investigating sex-specific IL-6 groups and study arms as fixed factors, in the overall sample and separately in females and males.

All statistical analyses were performed using IBM SPSS Statistics Version 27.0 (IBM Ltd, UK).

3. Results

3.1. There are minimal sex differences at baseline

Males were older than females (males, 48.3 ± 7.0 years; females, 39.90 ± 10.60 years, t 2.98 p = 0.005), and they exhibited higher consumption of alcohol than women (males 14.28 ± 12.65; females 3.43 ± 4.21, t = 3.425 p = 0.003). There was no significant difference in BMI between the groups (means were around 31–32 kg/m² for both sexes t = 0.26 p = 0.80); see Table 1 for all results. Note, 10 out of 22 female patients (45%) self-reported the use of oral contraceptive/hormonal therapy.

We did not detect significant differences between sexes in HAMD-17 total score (males, mean 18.71 ± SD 4.51; females, mean 17.36 ± SD 2.32; t = 1.12 p = 0.27) or BDI total score (males, mean 25.59 ± SD 9.82; females, mean 25.59 ± SD 9.32; t = 0.07 p = 0.95), or the subscores (somatic and cognitive/afffective sub-scales) at the baseline (see Table 1).

Chi-square test did not detect significant difference CRP groups distribution between sexes, with 9 males and 12 females in CRP <3 group, and 8 males and 10 females in the CRP >3 group (p = 0.010 p = 0.92). However, on average females exhibited numerically higher hs-CRP levels in comparison with males (males, mean 2.994 ± SD 1.60 mg/L; females, mean 4.53 ± SD 5.36 mg/L) even though the difference was not significant (t = 0.524 p = 0.60). Interestingly, male patients had numerically higher IL-6 levels than females (males 0.956 ± 0.344 pg/ml; females 0.777 ± 0.410 pg/ml), but again the difference was not significant (t = 1.561 p = 0.15). There were no significant differences in other immune biomarkers between males and females at baseline (see Table 2).

### Table 1

**Demographics characteristics of the MINDEP sample.**

| Demographics | Males (n = 17) | Females (n = 22) | Statistics |
|--------------|---------------|-----------------|------------|
| BMI (kg/m2) mean (SD) | 31.65 (5.69) | 31.10 (7.05) | t = 0.262 p = 0.795 |
| Age (years) mean (SD) | 48.35 (7.05) | 39.90 (10.60) | t = 2.98 p = 0.005* |
| Current smoker users (yes) N (%) within sex | 5 (29.4%) | 6 (27.3%) | χ² = 0.88 |
| Alcohol units per week, mean (SD) | 14.28 (12.65) | 3.43 (4.21) | t = 3.425 p = 0.003 |
| Class of current antidepressant medication (N, % within sex) | 1) SSRI (n = 9, 53.0%) | 1) SSRI (n = 11, 50%) | χ² = 0.70 |
| 2) Other AD (n = 2, 11.8%) | 2) Other AD (n = 6, 27.3%) | χ² = 1.43 p = 0.262 |
| 3) AD + AP (n = 2, 11.8%) | 3) AD + AP (n = 2, 9.1%) | χ² = 0.344 pg/ml |
| 4) two or more AD (n = 3, 17.6%) | 4) two or more AD (n = 3, 13.6%) | χ² = 0.264 |
| Months on current medication (N, % within sex) | 1) ≤ 6 months (n = 4, 26.7%) | 1) ≤ 6 months (n = 6, 27.3%) | χ² = 1.49 p = 0.47 |
| 2) 6 < > 12 months (n = 11, 73.3%) | 2) 6 < > 12 months (n = 10, 27.3%) | χ² = 0.005 |
| Oral contraceptive/ hormonal therapy (N, %) | n/a | n/a | n/a |
| HAMD-17, mean (SD) | 18.71 (4.51) | 17.36 (2.32) | t = 1.12 p = 0.275 |
| HAMD-17, mean (SD) | 18.71 (4.51) | 17.36 (2.32) | t = 1.12 p = 0.275 |
| BDI-II total score, mean (SD) | 25.59 (9.82) | 25.59 (9.32) | t = 0.076 p = 0.947 |
| BDI-C/A, mean (SD) | 17.59 (6.65) | 19.14 (7.66) | t = 0.662 p = 0.512 |
| BDI-S, mean (SD) | 8.00 (4.09) | 6.45 (2.30) | t = 1.395 p = 0.176 |

Note: AD (antidepressants); AP (antipsychotics); BMI (body mass index); N (number). BDI (Beck’s Depression Inventory scale); BDI-C/A (BDI cognitive affective subscale); BDI-S (BDI somatic subscale); HAMD-17 (Hamilton Depression Rating Scale 17 items).

*Bold means that the results are statistically significant.

Italicized text that the results are different at statistical trend.

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3.2. Sex differences in Δ-HAMD-17 indicate that only females, but not males, show an effect of CRP levels on the response to minocycline

The GLM analyses revealed sex differences in the interaction between treatment response and baseline inflammatory status (CRP groups) (see Table 3). The GLM model 1 is the same as published by Nettis et al. (2021) and is presented here as background information. This model shows an effect at statistical trend for study arms (placebo vs. minocycline) on Δ-HAMD-17 in the overall samples (F = 3.10, p = 0.087), describing numerical superiority in the improvement following minocycline when compared with placebo in the overall samples. GLM model 2 shows a significant effect of study arms (F = 12.17, p = 0.001), and of their interaction with CRP groups (CRP < 3 and CRP ≥ 3 mg/L) (F = 9.18, p = 0.005), again describing the previously reported, and statistically significant, improvement in depressive symptoms only in those taking minocycline and with CRP ≥ 3 mg/L (Nettis et al., 2021). Interestingly for the specific hypotheses of the present report, we also found an interaction, at statistical-trend level, between sex and CRP groups (F = 3.80, p = 0.060) on Δ-HAMD-17, suggesting that the effects of the CRP groups on the response to minocycline was different in males and females.

The GLM univariate model 3 further clarifies this (trend-level) interaction between sex and CRP groups (see Table 3). Specifically, in females we detected a significant effect of study arms (F = 5.53, p = 0.030), and of their interaction with CRP groups (F = 10.49, p = 0.005), that is, the same results as the main sample: females with CRP ≥ 3 mg/L show a greater improvement with minocycline compared with females with CRP < 3 mg/L. Specifically, t-test and post-hoc analyses with Bonferroni’s correction show that female patients overall did not show a difference in Δ-HAMD-17 comparing minocycline (mean 5.10 ± SD 6.95) with placebo (mean 4.08 ± SD 4.12; t = 0.426 p = 0.675), but CRP ≥ 3 females receiving minocycline showed a greater clinical improvement (Δ-HAMD-17, N = 3, mean 14.33 ± SD 4.72) than CRP < 3 females receiving minocycline (N = 7, mean 11.14 ± SD 2.03, p < 0.001), CRP ≥ 3 females receiving placebo (N = 7, mean 5.14 ± SD 4.37, p < 0.05) and CRP < 3 females receiving placebo (N = 5, mean 2.60 ± SD 3.64; p < 0.05) (see Table 4).

In contrast, in males we detected a significant effect of study arms (F = 6.17, p = 0.027) but no interaction between study arms and CRP groups (F = 1.64, p = 0.22) (see Table 3). This suggests that all male subjects, both CRP ≥ 3 and CRP < 3, improve with minocycline. Specifically, in the whole sample of males, patients taking minocycline exhibited greater clinical improvement (Δ-HAMD-17, mean 6.25 ± SD 5.95) than patients taking placebo (Δ-HAMD-17, mean 1.33 ± SD 3.08; t = 2.179 p = 0.046). Additionally, we did not detect significant differences in Δ-HAMD-17 between the four sub-groups based on study arm and CRP (CRP ≥ 3 minocycline, CRP ≥ 3 placebo, CRP < 3 minocycline and CRP < 3 placebo) (F = 2.572 p = 0.99) in the male sample.

3.3. Serum CRP correlates with IL-6 only in females

The finding that only female patients showed an interaction between CRP groups and study arms, while male patients did not, might be indicative of a different type of immunological profile in females vs. males with CRP below and above 3 mg/L. To test this hypothesis, we conducted explorative Spearman’s correlations between CRP and the cytokines, separately in males and females within sexes. Results are displayed in Table 5.

Interestingly, we found that CRP was strongly correlated with IL-6 in females (Spearman’s ρ = 0.658, p < 0.001) but was not correlated in males (Spearman’s ρ = 0.007, p = 0.979), and the two pairs of correlations were significantly different (z = −2.2210, p = 0.0264). Consistent with these findings, CRP ≥ 3 females had higher IL-6 levels than CRP < 3 females, even if only at statistical trend (t = 2.065 p = 0.063), whereas there was not difference in IL-6 levels between CRP ≥ 3 and CRP < 3 males (t = 0.264 p = 0.79). This sex difference in the association between CRP and IL-6 was also consistent with our aforementioned finding that females had numerically higher CRP than males, and males had numerically higher IL-6 than females (see Table 2).

3.4. Serum IL-6 is a better predictor of response to minocycline than CRP

Our findings so far suggested that high IL-6 could be a better predictor of response to minocycline than high CRP: all males responded to minocycline regardless of CRP levels, and as a group males had (numerically) higher IL-6 than females, while only CRP ≥ 3 female, who also had higher IL-6 levels, responded to minocycline. It is also of note that Nettis et al. (2021) had already shown, in this same sample, that partial responders to minocycline (showing at least 25% symptoms
reduction) not only have higher levels of baseline hs-CRP, but also higher levels of baseline IL-6 (Nettis et al., 2021). We thus decided to test if IL-6 at the baseline is a better predictor of treatment response to minocycline than increased CRP, and if, differently from CRP, is equally predictive both in males and females.

To investigate this hypothesis, we split both males and females in those above and below the IL-6 levels sex-specific medians (females = 0.640 pg/ml; males = 0.960 pg/ml), and then we repeated the GLM analysis on the response (Δ-HAMD-17) in the whole sample, and separately in males and females. See Table 6.

In the whole sample, we found an interaction between study arms and IL-6 groups (F = 3.189 p = 0.037), which was also present as statistically significant in females (F = 4.435 p = 0.050) and as statistical trend level in males (F = 4.258 p = 0.060). Most importantly, Δ-HAMD-17 was numerically comparable in the two high-IL-6 group taking minocycline (females, mean 9.20 ± 3.64; males, mean 8.80 ± 3.57), indicating that high IL-6, differently from high CRP, identifies responders to minocycline both in males and females.

To validate that these findings were due to using IL-6 as a biomarker rather than a sex-specific threshold, we replicated the GLM analyses considering sex-specific CRP medians (CRP = 2.90 mg/L for males; CRP = 2.50 mg/L for females). We found, again, that only females presented an interaction between CRP levels and study arm (F = 10.487 p = 0.005), but not males (F = 1.645 p = 0.22).

4. Discussion

To our knowledge, this is the first report investigating sex differences in the efficacy of add-on anti-inflammatory in TRD patients with increased inflammation in the context of a clinical trial. We conducted secondary analyses in our MINDEP study, comparing minocycline and placebo in TRD patients selected for hs-CRP ≥ 1 mg/L. Our published findings show that those with high inflammatory level (CRP ≥ 3 mg/L) at the baseline have a greater clinical improvement after minocycline add-on treatment (Nettis et al., 2021). The present paper, based on secondary and exploratory analyses in undoubtedly a small sample, indicates that these effects are different in males and females. Specifically, TRD male patients benefit from minocycline treatment independently of their baseline CRP levels, while TRD female patients benefit from minocycline only if they have CRP ≥ 3 mg/L at baseline. We finally tested if high IL-6 could be a better predictor of response than CRP and found that both male and female patients with increased baseline IL-6 levels showed similar improvement. If replicated in larger samples, this evidence indicates that there is the need to consider sex-specific biomarkers (and biomarkers levels) in stratifying TRD patients to access adjuvant anti-inflammatory strategy.

4.1. Is there a sex-specific relationship between CRP and IL-6?

Our preferred interpretation of these findings is that they suggest that the relationship between CRP levels and the overall immune profile is different in males and females, as indicated also by the numerical evidence of higher CRP in females (vs. males) and higher IL-6 in males (vs. females). This suggestive evidence contradicts the commonly-held assumption that CRP is primarily the end-product (and a direct representation) of IL-6 signal in the blood (Kim and Bajaj, 2014; El Ayadi et al., 2018) and thus usually considered as reflecting IL-6 levels. Indeed, we found a clear correlation between CRP and IL-6 levels only in females, but not in males. This is interesting in the view of the evidence that low-grade inflammation detected by CRP above 3 mg/L mirrors peripheral and central inflammation measured through other cytokines in
both plasma and cerebrospinal fluid (CSF) (Felger et al., 2020); however, the authors of this study also found some important sex-specific differences: for example, high anhedonia was predicted by CSF CRP only in females.

Previous studies also indicate the presence of sex-differences in the relationship between CRP and IL-6. Lockwood et al. (2016) found that females produce more IL-6 response than males in response to acute laboratory stress, and Ye et al. (2021) found, in the UK Biobank Cohort, that females showed a stronger association than males between CRP and depressive symptoms (Ye et al., 2021). This suggests that females are more sensitive to stress-induced inflammation. However, Lockwood et al. also found that only males, and not females, show a correlation between baseline CRP levels and the IL-6 increase, that is, the opposite correlational pattern that we found (Lockwood et al., 2016). This suggests that sex-specific effect may vary based on the functional status of the immune system and the stress response. Indeed, Kudielka and Kirschbaum propose that the higher immune sensitivity to stress in females might be due to males being able to mount a stronger hypothalamic-pituitary-adrenal (HPA) axis response than females, leading to suppression of the immune response (Kudielka and Kirschbaum, 2005). We have extensively discussed before the effects of sex hormones on the immune system (Lombardo et al., 2021); while the picture is complex, male sex hormones (androgens) have mainly anti-inflammatory properties (Gilliver, 2010), whereas female sex hormones have both pro- and anti-inflammatory properties (Bereshchenko et al., 2018). We can speculate that the anti-inflammatory action of androgens might limit the increase of baseline CRP in response to baseline IL-6 in males, thus leading to the lack of correlation we found – a model that is similar to that proposed by Lockwood et al. in females to explain the lack of correlation between baseline CRP and stress-evoked IL-6. Additionally, there is the need to consider female-specific factors, as both CRP and IL-6 vary in their levels throughout the menstrual cycle (Gaskins et al., 2012; Eagan et al., 2021). Moreover, the use of oral contraceptives (in 10 out of the 22 females in our study) can influence both CRP and IL-6 levels (Pradhan et al., 2002), and indeed in one study IL-6 shows a significant association with CRP levels only in females using oral contraceptives but not in those using vaginal contraceptives (Divani et al., 2015).

4.2. Is IL-6 a better predictor of response to minocycline than CRP?

We found that subjects with high IL-6 levels (measured as above the sex-specific median of levels) show better response to minocycline in both females and males, with similar Δ-HAMD-17 in males and females (Δ-HAMD-17 mean 8.8 vs 9.2). The ability of increased levels of IL-6 at the baseline to predict the response to minocycline could be due the fact that minocycline shows inhibitory properties on IL-6 levels. In fact, minocycline can lower IL-6 concentrations in macrophage and microglial cells in animal model (male rats) (Moini-Zanjani et al., 2016), and patients with schizophrenia and taking minocycline treatment exhibit a reduction in IL-6 (and IL-1p) levels, and these decreases correlate with improvement in cognitive symptoms (Zhang et al., 2019). In our sample, males have numerically higher mean IL-6 than females, and this could explain why, as a group, they respond better to minocycline. It is possible that the better response previously described in male patients with stroke (Amiri-Nikpour et al., 2015) could also be described by higher levels of IL-6, but the authors did not test this.

4.3. Limitations

Three major limitations need to be considered for our study. Firstly, while the main GLM and correlational analyses are in the complete sample (n = 39), the division in CRP/IL-6- and sex-based groups meant that some of the secondary and exploratory analyses compared very small groups, some single-figured. There is no doubt that these findings should be considered as a pilot data, useful for guiding new directions in research, and not as established findings. Secondly, we did not have information of the menstrual phase of female patients, and approximately half of women were on oral contraceptives. This is important as higher number of follicular waves are associated with higher inflammatory levels (Clancy et al., 2013), and hormonal changes during menstrual cycle are associated with variations in CRP levels (Wander et al., 2008; Gaskins et al., 2012).

5. Conclusion

In conclusion, our pilot findings confirms that TRD patients of both sexes with evidence of increased peripheral inflammation – above sex-specific thresholds for serum IL-6 – show greater clinical improvement after minocycline treatment. In this study, IL-6 performs better than CRP as predictor of response, as high CRP only predicts response in females. We thus want to draw attention to the need of sex-specific inflammatory biomarkers in predicting response to anti-inflammatory medications in TRD patients, in order to exploit the full potential for developing sex-specific tailored medicine.

Declaration of competing interest

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Data availability

Data will be made available on request.

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