

# A Randomized Controlled Trial of the Tumor Necrosis Factor Antagonist Infliximab for Treatment-Resistant Depression

## *The Role of Baseline Inflammatory Biomarkers*

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**Context:** Increased concentrations of inflammatory biomarkers predict antidepressant nonresponse, and inflammatory cytokines can sabotage and circumvent the mechanisms of action of conventional antidepressants.

**Objectives:** To determine whether inhibition of the inflammatory cytokine tumor necrosis factor (TNF) reduces depressive symptoms in patients with treatment-resistant depression and whether an increase in baseline plasma inflammatory biomarkers, including high-sensitivity C-reactive protein (hs-CRP), TNF, and its soluble receptors, predicts treatment response.

**Design:** Double-blind, placebo-controlled, randomized clinical trial.

**Setting:** Outpatient infusion center at Emory University in Atlanta, Georgia.

**Participants:** A total of 60 medically stable outpatients with major depression who were either on a consistent antidepressant regimen ( $n=37$ ) or medication-free ( $n=23$ ) for 4 weeks or more and who were moderately resistant to treatment as determined by the Massachusetts General Hospital Staging method.

**Interventions:** Three infusions of the TNF antagonist infliximab (5 mg/kg) ( $n=30$ ) or placebo ( $n=30$ ) at baseline and weeks 2 and 6 of a 12-week trial.

**Main Outcome Measures:** The 17-item Hamilton Scale for Depression (HAM-D) scores.

**Results:** No overall difference in change of HAM-D scores

between treatment groups across time was found. However, there was a significant interaction between treatment, time, and log baseline hs-CRP concentration ( $P=.01$ ), with change in HAM-D scores (baseline to week 12) favoring infliximab-treated patients at a baseline hs-CRP concentration greater than 5 mg/L and favoring placebo-treated patients at a baseline hs-CRP concentration of 5 mg/L or less. Exploratory analyses focusing on patients with a baseline hs-CRP concentration greater than 5 mg/L revealed a treatment response ( $\geq 50\%$  reduction in HAM-D score at any point during treatment) of 62% (8 of 13 patients) in infliximab-treated patients vs 33% (3 of 9 patients) in placebo-treated patients ( $P=.19$ ). Baseline concentrations of TNF and its soluble receptors were significantly higher in infliximab-treated responders vs nonresponders ( $P<.05$ ), and infliximab-treated responders exhibited significantly greater decreases in hs-CRP from baseline to week 12 compared with placebo-treated responders ( $P<.01$ ). Dropouts and adverse events were limited and did not differ between groups.

**Conclusions:** This proof-of-concept study suggests that TNF antagonism does not have generalized efficacy in treatment-resistant depression but may improve depressive symptoms in patients with high baseline inflammatory biomarkers.

**Trial Registration:** clinicaltrials.gov Identifier: NCT00463580.

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**D**ESPITE ADVANCES IN THE treatment of major depression, one-third of depressed patients fail to respond to conventional antidepressant medication.<sup>1</sup> One pathophysiologic mechanism hypothesized to contribute to treatment resistance in depression is inflammation. A number of in-

flammatory biomarkers (including inflammatory cytokines, acute phase proteins, chemokines, and adhesion molecules) have been found to be reliably elevated in depressed patients and have been associated with a decreased likelihood of response to conventional antidepressants.<sup>2-4</sup> Moreover, factors linked to a poor antidepressant treatment response, includ-

ing early life stress, anxiety disorders, and neuroticism, have been associated with increased inflammation.<sup>5-11</sup> Data also indicate that inflammatory cytokines can sabotage and circumvent the mechanisms of action of conventional antidepressant medications.<sup>2</sup> For example, inflammatory cytokines can increase the expression and activity of monoamine transporters, the primary antidepressant target for monoamine reuptake inhibition.<sup>12,13</sup> In addition, inflammatory cytokines can reduce the concentration of monoamine precursors through the activation of enzymes such as indoleamine 2,3-dioxygenase, which breaks down tryptophan, the primary amino acid precursor for serotonin, into kynurenine.<sup>14</sup> Inflammation can also reduce the availability of the enzyme cofactor, tetrahydrobiopterin, which is essential for activities of tryptophan hydroxylase and tyrosine hydroxylase, which are rate-limiting enzymes for synthesis of serotonin, norepinephrine, and dopamine.<sup>15,16</sup> Inflammatory cytokines have also been shown to inhibit neurogenesis through the activation of nuclear factor  $\kappa$ B.<sup>17</sup> Neurogenesis is an important component of the salutary effects of conventional antidepressants in several depressive-like behaviors in animal models of depression, including anhedonia.<sup>18-20</sup> Finally, inflammatory cytokines can reduce the number of glutamate transporters that are expressed and increase the amount of glutamate that is released from astrocytes, thereby activating pathophysiologic mechanisms (eg, glutamate excitotoxicity) that are not primary targets of conventional antidepressant medications.<sup>2,15,21,22</sup>

Given the association of inflammatory cytokines with treatment resistance, there has been interest in testing whether inhibiting inflammatory cytokines might be used as therapy for treatment-resistant depression (TRD). One inflammatory cytokine, tumor necrosis factor (TNF), may be especially relevant in this regard. Tumor necrosis factor has been reliably shown to be elevated in depressed patients.<sup>23</sup> Moreover, increases in TNF have been associated with depressive symptoms during chronic exposure to interferon alpha.<sup>24</sup> In addition, peripheral administration of a TNF antagonist has been shown to improve depressed mood in patients with psoriasis.<sup>25</sup> Tumor necrosis factor antagonism has also been found to resolve major depression in patients with Crohn disease<sup>26</sup> and to reduce fatigue in patients with advanced cancer.<sup>27</sup> Moreover, gene-targeted deletion of TNF receptors in mice leads to an antidepressant-like phenotype and reduced anxiety-like behavior during immune activation.<sup>28,29</sup> Nevertheless, to our knowledge, no previous study has tested whether the administration of a peripherally active cytokine antagonist to otherwise healthy patients with TRD might reverse depressive symptoms.

Therefore, we endeavored to determine whether repeated intravenous administration of a monoclonal antibody directed at TNF (ie, infliximab) would improve the mood of depressed patients with TRD. Such a targeted biologic therapy was chosen not only to directly test the cytokine hypothesis of depression<sup>30,31</sup> but also to obviate nonimmunologic effects that may potentially confound interpretation of the mechanism of action of other readily available medications with anti-inflammatory properties, including acetylsalicylic acid, cyclo-oxygenase in-

hibitors, and minocycline, all of which have relevant off-target effects.<sup>32-35</sup> Based on the hypothesis that an anticytokine strategy might only be effective in patients with a high level of inflammation prior to treatment, we also measured the concentration of the acute phase protein, high-sensitivity C-reactive protein (hs-CRP), and the concentrations of TNF and its soluble receptors I and II at baseline and throughout the study as biomarkers of inflammation. Like the concentration of TNF, the concentration of CRP has been shown to be reliably elevated in depressed patients and has been associated with the development of depression and with antidepressant nonresponse.<sup>36-38</sup> In addition, the baseline CRP concentration has been found to be a potent predictor of response of patients treated with infliximab for inflammatory disorders, including Crohn disease.<sup>39,40</sup> The TNF soluble receptors I and II have also been associated with depression, anxiety, and fatigue in laboratory animals and humans and are believed to reflect TNF activity.<sup>4,28,29,41-43</sup>

## METHODS

### STUDY OVERVIEW AND ELIGIBILITY CRITERIA

Our study was a single-site, parallel-group, randomized, double-blind trial of infliximab vs placebo for antidepressant nonresponders with major depression according to the *DSM-IV*, as assessed by the Structured Clinical Interview for *DSM-IV*.<sup>44</sup> Owing to the exploratory nature of our study, participants with bipolar disorder, depressed phase, were also included.

Eligible participants, recruited from television, radio, and newspaper advertisements, were men and women between the ages of 25 to 60 years who were on a consistent antidepressant regimen or off antidepressant therapy for at least 4 weeks prior to baseline. No changes in antidepressant treatment were allowed during the study. All participants were required to have experienced moderate resistance to treatment in the current episode, as determined by a score of 2 or higher using the Massachusetts General Hospital Staging method for treatment resistance,<sup>45</sup> and to exhibit moderate severity of depression, as determined at screening by a score 14 or higher using the Quick Inventory of Depressive Symptomatology, Self-Report.<sup>46</sup> Exclusion criteria included the presence of any autoimmune disorder (confirmed by laboratory testing); a history of tuberculosis (confirmed by chest radiography, a skin test, and a blood test) or being at high risk of tuberculosis exposure; the presence of hepatitis B or C or human immunodeficiency virus infection (confirmed by laboratory testing); evidence of active fungal infection; a history of recurrent viral or bacterial infections; a history of cancer, excluding basal cell or squamous cell carcinoma of the skin (fully excised with no recurrence); the presence of an unstable cardiovascular, endocrinologic, hematologic, hepatic, renal, or neurologic disease (determined by physical examination and laboratory testing); a history of schizophrenia (determined by use of the Structured Clinical Interview for *DSM-IV*); active psychotic symptoms of any type; substance abuse and/or dependence within the past 6 months (determined by use of the Structured Clinical Interview for *DSM-IV*); active suicidal ideation determined by a score of 3 or higher on item 3 of the 17-item Hamilton Scale for Depression (HAM-D)<sup>47</sup>; and/or a score of less than 28 on the Mini-Mental State Examination.<sup>48</sup> All participants provided written informed consent, and all procedures were approved a priori by

the institutional review board of Emory University in Atlanta, Georgia. Our study was registered at [clinicaltrials.gov](http://clinicaltrials.gov) (NCT00463580) in April 2007.

## STUDY PROCEDURES

Participants were enrolled at Emory University between December 2008 and March 2011. To achieve similar representation of baseline inflammatory status in each group, group assignment, determined at screening, was stratified based on either an hs-CRP concentration of 2 mg/L or greater or an hs-CRP concentration of less than 2 mg/L (to convert to nanomoles per liter, multiply by 9.524). An hs-CRP concentration of 2 mg/L was chosen because it is the central value in the "medium" relative risk category of inflammation (1-3 mg/L) recommended by the American Heart Association and the Centers for Disease Control and Prevention.<sup>49</sup> Group assignment was also stratified by sex. Following screening for inclusion and exclusion criteria, all participants reported to the infusion center in the Emory Division of Digestive Diseases on 3 separate occasions (baseline, 2 weeks, and 6 weeks) to receive an infusion of either infliximab (5 mg/kg) or placebo over 120 minutes through an indwelling catheter. The baseline visit was scheduled no later than 1 month after screening. The dosing protocol and scheduling of infliximab infusions were matched to the standard induction regimen for treatment of inflammatory bowel disease.<sup>50</sup> Independent pharmacists dispensed infliximab or placebo in a 250-mL saline bag according to a computer-generated randomization list, blocked in units of 4, provided by a study statistician. The placebo was matched to infliximab on the basis of color and consistency when dissolved in saline. Infliximab and placebo were provided free of charge by Centocor Ortho-Biotech Services (Horsham, Pennsylvania). Assessments of clinical (using the HAM-D and the Clinical Global Impression–Severity scale)<sup>51</sup> and inflammatory status (hs-CRP and TNF and its soluble receptors I and II) were conducted at baseline and weeks 1, 2, 3, 4, 6, 8, 10, and 12. For any participant who showed evidence of infection, infusions were delayed until symptoms resolved and/or until appropriate treatment (eg, antibiotics) was initiated. During the trial, HAM-D assessments on week 3 were discontinued owing to participant/staff burden (11 participants had week 3 assessments). The HAM-D assessments on week 10 were discontinued for the same reason but were resumed for a total of 32 participants. Patients were not allowed to take nonsteroidal or steroidal anti-inflammatory medications during the study, except for 81 mg of aspirin if medically indicated (n=3). Medications for hypertension, diabetes, hypothyroidism, allergies, infections, or other medical conditions were allowed as dictated by the patients' treating physicians. All study staff were blinded to treatment assignment until the entire trial was completed.

## OUTCOMES

As indicated in the registered trial, the primary end point was change in depression severity as measured by the HAM-D. Additional end points included treatment response, which was defined as a 50% or more reduction in HAM-D score at any point during the study, and remission, which was considered a HAM-D score of 7 or less at the end of treatment (week 12). Secondary end points included the Clinical Global Impression–Severity scale score. Analyses were also conducted on interactive effects of baseline inflammatory status as assessed by plasma hs-CRP concentration with change in depression severity over time, as well as change in inflammatory status from baseline to week 12. Tumor necrosis factor and its soluble receptors were similarly considered in exploratory analyses.

## LABORATORY ASSESSMENTS

To limit the effect of circadian rhythms and stress on inflammatory measures, blood samples were obtained using EDTA-coated tubes through an indwelling catheter between 8 and 10 AM after the participant had at least 30 minutes of rest. Blood samples were immediately centrifuged at 1000g for 15 minutes at 4°C, and plasma was removed and stored at –80°C. The CRP concentration was measured by the immunoturbidometric method, using the Beckman AU 480 chemistry analyzer and the Ultra WR CRP reagent kit (Sekisui Diagnostics). Inter-assay and intra-assay coefficients of variation were reliably less than 3%. The concentrations of TNF and its soluble receptors I and II were assessed as previously described in duplicate using a sandwich enzyme-linked immunosorbent assay (R&D Systems).<sup>24</sup> The mean inter-assay and intra-assay coefficients of variation were reliably 10% or less.

## SAMPLE SIZE

Power calculations were based on standard deviations derived from published literature of HAM-D scores in patients with TRD.<sup>52</sup> Given a standard deviation of 7 and the other parameters adopted (60 participants, 80% power, and a 2-sided  $\alpha$  level of .05), the trial was powered to detect differences in change scores between groups of 5 points on the HAM-D.

## STATISTICAL ANALYSIS

The *t* test and  $\chi^2$  analyses were used to compare sociodemographic and clinical variables between groups, as well as number/percentage of participants who achieved treatment response/remission or experienced an adverse event. Nonparametric tests were used for cases in which data were not normally distributed. An intent-to-treat analysis using mixed-effects model for repeated measures was used to analyze change from baseline of HAM-D scores as a function of treatment, time, and their interaction. To evaluate the effect of baseline inflammatory status on treatment assignment and response, the baseline hs-CRP concentration (log-transformed linear and quadratic terms) was considered in the model. Quadratic terms were used because each treatment group exhibited a unique convex (upside-down U-shaped) trend of primary end points with increasing concentrations of baseline hs-CRP. Study week was entered into mixed-effects models for repeated measures as a categorical variable for purposes of illustrating group least squares mean values over time. For determining significance and effect sizes for treatment group differences by time, study week was analyzed as a continuous variable. An autoregressive covariance structure (AR-1) best fit the data and, thus, was used for final analyses of the mixed-effects model for repeated measures. Tumor necrosis factor and its soluble receptors were also tested in a similar fashion to hs-CRP in exploratory analyses. Fixed-effects parameters were tested with the Wald test (*t* test). The independent effect of age, sex, body mass index, Massachusetts General Hospital Staging method score, diagnosis of bipolar disorder, concomitant psychotropic medications, and comorbid medical conditions also were examined in analyses of mixed-effects models for repeated measures, to evaluate possible interactions with treatment and time. In addition, these analyses were applied to examine the effect of treatment on longitudinal assessments of hs-CRP concentrations. Where indicated, *t* tests were used in exploratory analyses of differences between inflammatory biomarkers at baseline in responders vs nonresponders and in changes from baseline to week 12. Statistical significance was based on a 2-sided  $\alpha$  of .05. No interim analysis was conducted.

**Table 1. Sociodemographic and Clinical Characteristics of the Study Sample**

Characteristic	Infliximab-Treated Patients (n = 30)	Placebo-Treated Patients (n = 30)
Age, mean (SD), y	42.5 (8.2)	44.3 (9.4)
Female sex, No. (%)	20 (67)	20 (67)
Ethnicity, No. (%)		
White	23 (77)	23 (77)
Black	6 (20)	5 (17)
Other	1 (3)	2 (7)
Education (highest degree), No. (%)		
Graduate degree	8 (27)	7 (23)
College graduate	13 (43)	13 (43)
Partial college	8 (27)	9 (30)
High school graduate	1 (3)	1 (3)
Unemployed (looking for work or disabled), No. (%)	12 (41) <sup>a</sup>	12 (40)
Age of onset of MDD, mean (SD), y	19.1 (8.3)	18.9 (10.7)
Lifetime episodes of MDD, mean (SD), No.	8.7 (24.8)	7.8 (24.8)
Duration of current MDD episode, mean (SD), mo	184.4 (148.8)	238.7 (165.3)
Antidepressant trials in current episode, mean (SD), No.	4.6 (3.2)	3.7 (2.1)
MGH-S score, mean (SD)	7.7 (6.6)	6.1 (3.5)
Family history of MDD, No. (%)	27 (90)	23 (79) <sup>a</sup>
Mood-relevant psychotropic medication, No. (%)	16 (53)	21 (70)
Comorbid medical illness, No. (%)	13 (43)	19 (63)
Bipolar disorder, No. (%)	3 (10)	6 (20)
BMI, mean (SD)	31.2 (6.9)	32.7 (8.0)
Baseline hs-CRP concentration, mean (SD), mg/L	6.3 (8.9)	5.4 (8.2)
Baseline HAM-D score, mean (SD)	24.1 (4.0)	23.6 (3.8)
Baseline CGI-S scale score, mean (SD)	4.8 (0.6)	4.8 (0.8)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CGI-S, Clinical Global Impression–Severity; HAM-D, 17-item Hamilton Scale for Depression; hs-CRP, high-sensitivity C-reactive protein; MDD, major depressive disorder; MGH-S, Massachusetts General Hospital Staging method.

SI conversion factor: To convert hs-CRP to nanomoles per liter, multiply by 9.524.

<sup>a</sup>Data were missing for 1 participant.

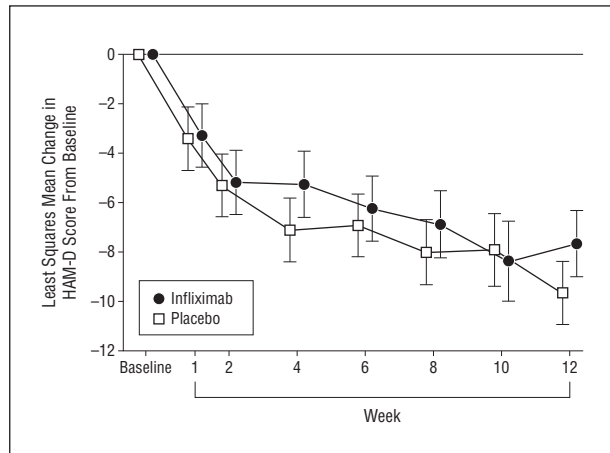
## RESULTS

Baseline sociodemographic and clinical data for the 60 patients randomly assigned to infliximab or placebo are shown in **Table 1**. No statistically significant differences between groups in any of the variables listed were observed. It should be noted that both groups exhibited mean hs-CRP concentrations above 5 mg/L and that 27 of 60 patients (45%) had a hs-CRP concentration greater than 3 mg/L. Of the 60 patients randomly assigned, 27 in the infliximab group and 28 in the placebo group completed all 3 infusions. Three participants in the infliximab group received only 1 infusion and dropped out (2 owing to scheduling conflicts and 1 lost to follow-up). Two participants in the placebo group received only 2 infusions (both owing to medical/psychiatric complications), and 1 of these participants dropped out at that time. Two participants in the infliximab group dropped out during the follow-up phase (1 owing to scheduling conflicts and 1 lost to follow-up). In total, 90% of the participants completed all 12 weeks of the trial (29 of 30 participants in the placebo group [97%] and 25 of 30 participants in the infliximab group [83%];  $P = .19$ ) (eFigure 1, <http://www.jamapsych.com>).

No differences in change in HAM-D scores over time were found between treatment groups ( $t_{58} = 0.10$ ,  $P = .92$ ), nor was there a significant interaction between treatment and time ( $t_{305} = 0.86$ ,  $P = .39$ ) (**Figure 1**). How-

ever, there was a significant effect of time, with HAM-D scores significantly decreasing from baseline to end of treatment ( $t_{305} = 2.53$ ,  $P = .01$ ) in both infliximab- and placebo-treated groups ( $P < .05$ ). Of note, no significant interactive effects of the noted sociodemographic or clinical covariates were observed with treatment group and time. Interestingly, when the baseline hs-CRP concentration (log-linear and quadratic terms) was entered into the model, there was a significant interaction among treatment, time, and log hs-CRP concentration ( $t_{302} = 2.65$ ,  $P = .01$ ). To identify the critical threshold for the influence of baseline hs-CRP concentration, we examined change in HAM-D from baseline to week 12 (infliximab minus placebo) in relation to log hs-CRP concentration. A baseline hs-CRP concentration greater than 5 mg/L was found to be the point at which infliximab-treated patients began to exhibit a greater decrease in HAM-D scores than placebo-treated patients (**Figure 2**). Similar results were found for the Clinical Global Impression–Severity scale (eFigure 2). **Figure 3** illustrates the difference between infliximab and placebo based on analysis of subgroups of patients with successively higher baseline hs-CRP concentrations (>1, >3, and >5 mg/L), with infliximab being superior to placebo by more than 3 points in participants with a baseline hs-CRP concentration greater than 5 mg/L.

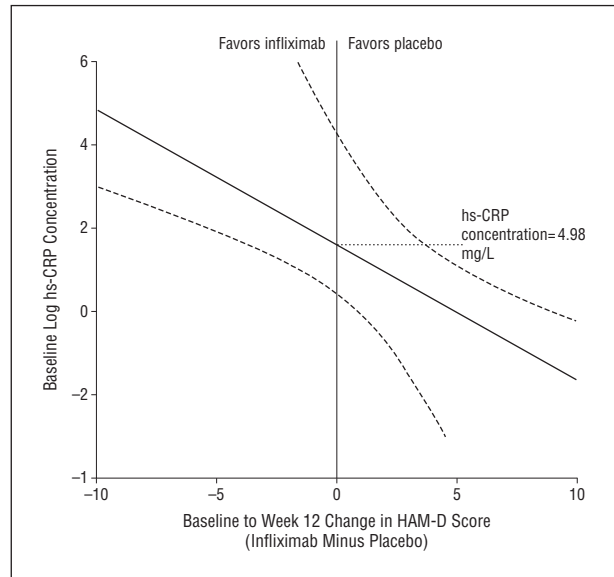
Based on these results, exploratory analyses were focused on patients with TRD who had a baseline hs-CRP concentration of either greater than 5 mg/L or 5 mg/L or



**Figure 1.** Change in scores on the 17-item Hamilton Scale for Depression (HAM-D) for patients with treatment-resistant depression (TRD) randomly assigned to infliximab or placebo. Using a double-blind, placebo-controlled, randomized design, we examined the change in HAM-D scores in an intent-to-treat mixed-effects model for repeated measures analysis of 60 patients with TRD who were administered 3 infusions of the tumor necrosis factor antagonist infliximab (n = 30) or placebo (n = 30) at baseline and at 2 and 6 weeks of a 12-week trial. There was no main effect of treatment assignment and no treatment  $\times$  time interaction. However, there was a significant effect of time, with both groups exhibiting significant decreases in HAM-D scores across treatment weeks ( $P = .01$ ). Depicted is the least squares mean change in HAM-D score from baseline to the indicated week using an unstructured covariance matrix with time as a categorical variable. The error bars indicate SEM.

less to maximize potential differences between groups. Of note, no significant differences in sociodemographic or clinical variables were found between infliximab- and placebo-treated participants with TRD who had a baseline hs-CRP concentration greater than 5 mg/L, including the distribution of bipolar depressed participants, which was 2 of 13 participants (15%) in the infliximab group and 1 of 9 participants (11%) in the placebo group ( $P = .77$ ) (eTable). Among participants with a baseline hs-CRP concentration of 5 mg/L or less, placebo-treated patients were more likely than infliximab-treated patients to have a comorbid medical illness ( $P = .02$ ) and also were more likely to be on concurrent psychotropic medications for their mood disorder ( $P = .03$ ). Including these variables in the mixed-effects models for repeated measures did not substantially change the treatment  $\times$  time interaction.

Although the treatment  $\times$  time interactions were not statistically significant in exploratory analyses of these relatively small groups, separate analyses of the mixed-effects models for repeated measures highlighted the opposite effect of infliximab vs placebo for groups with a baseline hs-CRP concentration greater than 5 mg/L (n = 22) and those with 5 mg/L or less (n = 38) (**Figure 4**). Infliximab was superior to placebo in improving HAM-D scores for participants with a baseline hs-CRP concentration greater than 5 mg/L (Figure 4A), but placebo was superior for participants with a baseline hs-CRP concentration of 5 mg/L or less (Figure 4B). Consistent with these findings, the standardized effect size difference at 12 weeks (from mixed-effects models for repeated measures) was moderate to large, in opposite directions: 0.41 favoring infliximab in the group with a baseline hs-CRP concentration greater than 5 mg/L com-

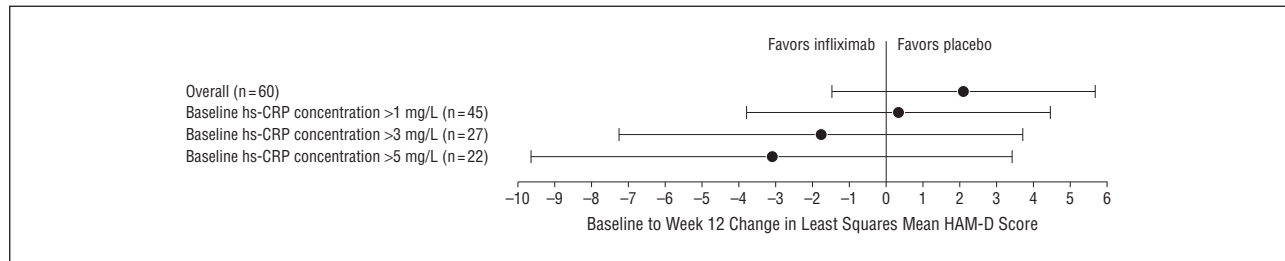


**Figure 2.** Relationship between change in score on the 17-item Hamilton Scale for Depression (HAM-D) from baseline to week 12 (infliximab minus placebo) and log baseline plasma high-sensitivity C-reactive protein (hs-CRP) concentration in patients with treatment-resistant depression (TRD) who were treated with infliximab or placebo. Based on an interaction between treatment, time, and baseline log hs-CRP concentration ( $P = .01$ ), the relationship between baseline log plasma hs-CRP concentration and change in HAM-D score from baseline to week 12 (infliximab minus placebo) was examined in 60 patients with TRD who were administered 3 infusions of either the tumor necrosis factor antagonist infliximab (n = 30) or placebo (n = 30) at baseline and at 2 and 6 weeks of a 12-week trial. The solid line represents the adjusted means of the change in HAM-D score from baseline to week 12 (infliximab minus placebo) at each log baseline hs-CRP value based on the mixed-effects model. The dotted lines represent the 95% CIs. At plasma hs-CRP concentrations greater than 4.98 mg/L, the change in HAM-D scores favored infliximab-treated patients, whereas at plasma hs-CRP concentrations below 4.98 mg/L, the change in HAM-D scores favored placebo-treated patients.

pared with  $-0.82$  favoring placebo in the group with a baseline hs-CRP concentration of 5 mg/L or less.

To explore which specific symptoms were improving in infliximab-treated patients vs placebo-treated patients with a hs-CRP concentration greater than 5 mg/L, the median change in individual items on the HAM-D from baseline to end of treatment was examined. The symptoms that were more responsive in infliximab-treated patients vs placebo-treated patients included anhedonia, psychomotor retardation, depressed mood, psychic anxiety, and suicidal ideation (**Figure 5**). Examination of symptoms that improved in participants with a baseline hs-CRP concentration of 5 mg/L or less also provided evidence of reduced severity of anhedonia, psychomotor retardation, and psychic anxiety in infliximab-treated patients (eFigure 2). In contradistinction, placebo-treated patients with a baseline hs-CRP concentration of 5 mg/L or less showed reduced severity in HAM-D items of hypochondriasis, somatic general, and feelings of guilt (eFigure 3).

Treatment response rates did not differ between groups in the entire sample and were 50% for each group ( $\chi^2 = 0.00$ ,  $P = .99$ ). For participants with a baseline hs-CRP concentration greater than 5 mg/L, however, the treatment response rate was 62% (8 of 13 participants) in the infliximab group and 33% (3 of 9 participants) in



**Figure 3.** Change in score on the 17-item Hamilton Scale for Depression (HAM-D) from baseline to week 12 (infliximab minus placebo) in patients with treatment-resistant depression (TRD) subgrouped by baseline plasma high-sensitivity C-reactive protein (hs-CRP) concentration. To examine the effect of varying concentrations of baseline plasma hs-CRP on the least squares mean change in the HAM-D score from baseline to week 12 (infliximab minus placebo), the sample of patients with TRD were subgrouped into all participants (overall), all participants with a baseline hs-CRP concentration greater than 1 mg/L (n = 45), all participants with a baseline hs-CRP concentration greater than 3 mg/L (n = 27), or all participants with a baseline hs-CRP concentration greater than 5 mg/L (n = 22). When only participants with a hs-CRP concentration greater than 5 mg/L were considered, a 3.1 difference in the HAM-D score favoring infliximab was found between infliximab- and placebo-treated patients. The error bars indicate 95% CI.

the placebo group ( $\chi^2 = 1.69, P = .19$ ) (Figure 6). Based on these percentages, the number of patients needed to be treated with infliximab in order to have one more responder (number needed to treat) for participants with a hs-CRP concentration greater than 5 was 3.45 (as opposed to 8-10 for conventional antidepressants).<sup>53</sup> In contrast, for participants with a hs-CRP concentration of 5 mg/L or less, the treatment response rate was 41% (7 of 17 participants) for the infliximab group and 57% (12 of 21 participants) for the placebo group ( $\chi^2 = 0.96, P = .33$ ). In this case, the disadvantage to infliximab (number needed to harm) was calculated as 6.25. In the sample as a whole, remission rates favored placebo at 26% vs 9% for infliximab, although the difference did not reach significance ( $\chi^2 = 1.7, P = .19$ ).

Regarding the effect of infliximab and placebo on hs-CRP concentrations across treatment, there was a significant effect of treatment ( $t_{57} = 2.25, P = .03$ ), but no effect of time ( $t_{433} = 0.10, P = .92$ ) and no treatment  $\times$  time interaction ( $t_{433} = 0.27, P = .78$ ) (Figure 7). Infliximab was found to lead to a significant reduction in hs-CRP concentrations compared with placebo at all visits beyond baseline ( $P < .05$ ). Placebo nonresponders exhibited somewhat higher baseline hs-CRP concentrations than placebo responders (mean [SD] concentration, 7.8 [11.0] mg/L vs 3.1 [2.6] mg/L;  $P = .12$ ), whereas infliximab responders exhibited similar plasma hs-CRP concentrations compared with infliximab nonresponders (mean [SD] concentration, 6.9 [7.4] mg/L vs 5.8 [10.5] mg/L;  $P = .76$ ). Of note, in treatment responders, the mean change in hs-CRP concentration from baseline to week 12 in infliximab-treated patients (n = 13) was significantly greater than that in placebo-treated patients (n = 15) (mean [SD] change, -4.7 [11.6] mg/L vs 1.3 [1.7] mg/L;  $P < .01$ ).

Exploratory analyses of TNF and its receptors revealed no significant interactions between treatment, time, and the log of any of the TNF markers. However, all 3 TNF markers were significantly higher in infliximab responders vs infliximab nonresponders (all  $P < .05$ ) (eFigure 4). No differences in baseline TNF markers were found between placebo responders and nonresponders ( $P > .36$ ).

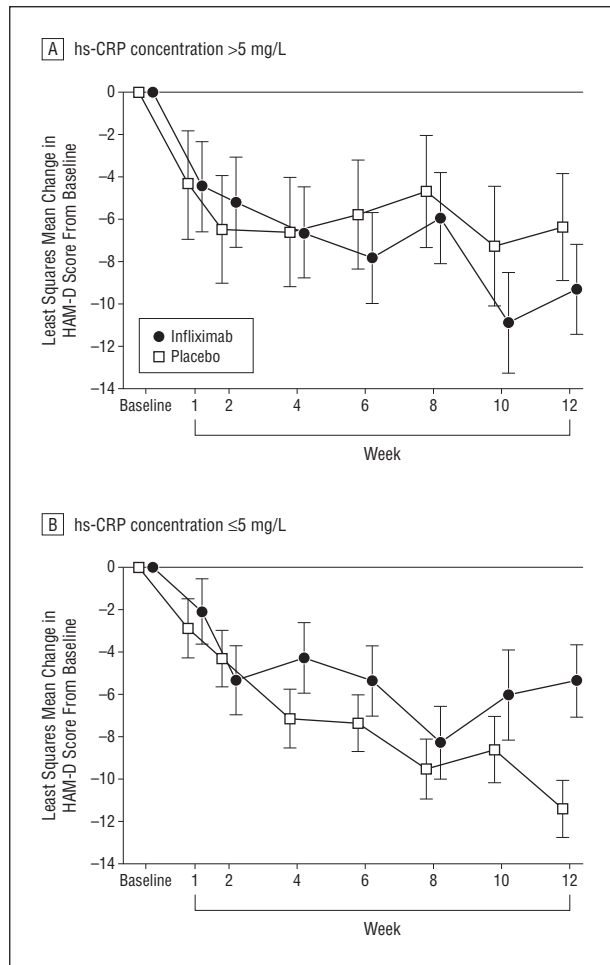
Adverse events reported in more than 5% of the total patient population are shown in Table 2. Except for an increased number of participants positive for elevated uri-

nary leukocyte esterase in the placebo group, no statistically significant differences between groups were found. No serious adverse events were reported for either group.

## COMMENT

Although well tolerated, infliximab did not show overall superiority to placebo on depressive symptom outcome, suggesting that blockade of peripheral TNF is not effective as a generalized strategy for TRD. However, an association was observed between baseline concentrations of the inflammatory biomarker hs-CRP and subsequent response to infliximab, such that an increasing advantage for infliximab on depression-related outcome became apparent as baseline values of hs-CRP increased. For participants with pretreatment hs-CRP concentrations greater than 5 mg/L, randomization to infliximab resulted in a 3.1 point greater reduction in HAM-D scores from baseline to week 12 compared with placebo. This between-group difference in the study's primary outcome measure is similar in magnitude to differences typically seen between antidepressant medications and placebo in randomized studies and is considered a clinically meaningful difference according to the National Institute for Health and Clinical Excellence.<sup>54</sup>

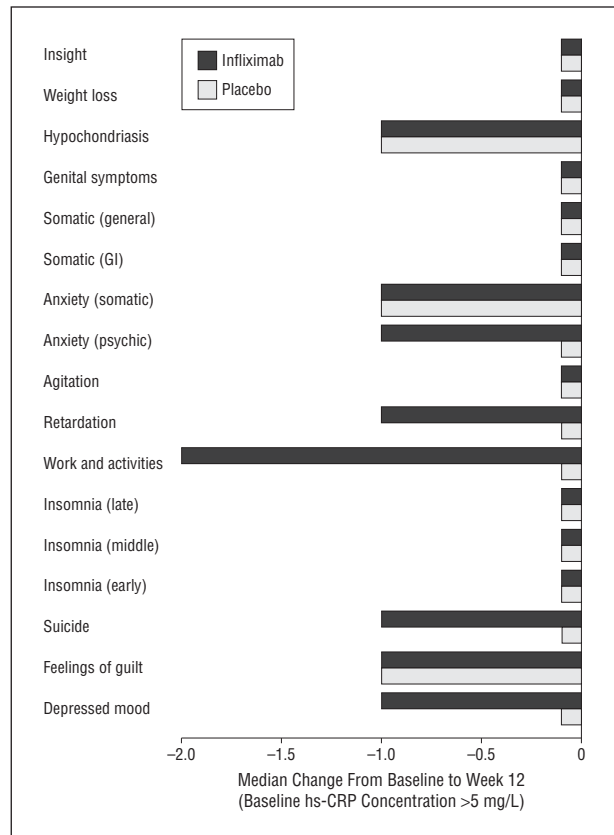
For participants with a baseline hs-CRP concentration greater than 5 mg/L, treatment with infliximab benefitted a wide range of depressive symptoms, including depressed mood, psychomotor retardation, and performance of work and other activities (anhedonia/fatigue), as well as psychic anxiety and suicidal ideation. This pattern of symptom change is intriguing given recent studies showing that these symptoms correspond to brain regions that are targeted by inflammatory cytokines. For example, peripheral cytokine administration or induction of inflammatory cytokines using typhoid vaccination or endotoxin administration have been shown to induce psychomotor slowing and anhedonia in association with reduced neural activity in the basal ganglia, an area of central importance to psychomotor speed and hedonic tone.<sup>55-57</sup> In addition, administration of inflammatory stimuli have been found to activate subgenual and dorsal areas of the anterior cingulate cortex, brain regions implicated in anxiety disorders and depression.<sup>58-61</sup> Finally, a reduction in suicidal ideation may rep-



**Figure 4.** Change in score on the 17-item Hamilton Scale for Depression (HAM-D) from baseline to week 12 for infliximab- vs placebo-treated patients with treatment-resistant depression (TRD) who had a baseline high-sensitivity C-reactive protein (hs-CRP) concentration greater than 5 mg/L and in those who had a baseline hs-CRP concentration of 5 mg/L or less. An intent-to-treat mixed-effects model for repeated measures analysis of patients with TRD who were administered the tumor necrosis factor antagonist infliximab or placebo was conducted separately for patients with a baseline hs-CRP concentration greater than 5 mg/L ( $n = 22$ ) (A) and those with a baseline hs-CRP concentration of 5 mg/L or less ( $n = 48$ ) (B). In general, opposite effects of infliximab were found depending on baseline hs-CRP concentration. Infliximab was superior to placebo in improving HAM-D scores for participants with a baseline hs-CRP concentration greater than 5 mg/L, but placebo was superior for participants with a baseline hs-CRP concentration of 5 mg/L or less. Although there was a main effect of time on change in HAM-D scores for patients with a baseline hs-CRP concentration greater than 5 mg/L, there were no effects of treatment assignment and no treatment  $\times$  time interaction. In addition, no main effects of treatment assignment, time, or their interaction were found in participants with a baseline hs-CRP concentration of 5 mg/L or less. Depicted is the least squares mean change in HAM-D score from baseline to the indicated week using an unstructured covariance matrix with time as a categorical variable. The error bars indicate SEM.

represent a potential advantage of cytokine blockade that warrants further exploration and is consistent with data showing increased density of microglia and astrocytes in the anterior cingulate cortex of suicide victims.<sup>62,63</sup>

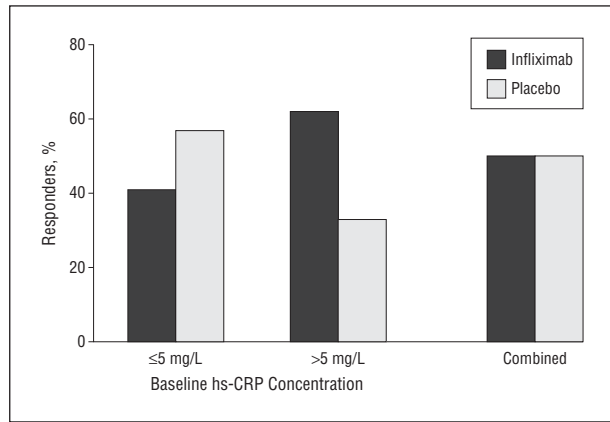
The search for biomarkers that predict antidepressant response has proven remarkably difficult, and the literature is littered with conflicting findings regarding an array of putative genetic and physiological predictor variables. Nevertheless, the specificity of the associa-



**Figure 5.** Median change in individual items on the 17-item Hamilton Scale for Depression from baseline to week 12 for infliximab- vs placebo-treated patients with treatment-resistant depression (TRD) who had a baseline high-sensitivity C-reactive protein (hs-CRP) concentration greater than 5 mg/L. To determine which symptoms were differentially improved as a function of infliximab treatment, the median change in the 17 items of the Hamilton Scale for Depression was plotted. Infliximab- vs placebo-treated patients with TRD showed greater improvement in symptoms of anxiety (psychic), retardation, work and activities, suicide, and depressed mood from baseline to week 12 if their baseline plasma hs-CRP concentration was greater than 5 mg/L.

tion between TNF and CRP and infliximab's mechanism of action may have decreased the "signal-to-noise" ratio sufficiently to allow TNF and its soluble receptors, as well as CRP, to function as predictor variables in a way not possible for biomarkers less directly linked to the mechanism of action of more complex pharmacologic or somatic interventions.

Although the results did not reach statistical significance, elevated concentrations of hs-CRP prior to treatment were associated with a reduced response to placebo. These data are consistent with previous findings associating an increase in the concentration of peripheral inflammatory biomarkers with resistance to antidepressants.<sup>2</sup> The mechanisms by which inflammation might impair the placebo response are unknown. However, the therapeutic relationship has been suggested as a core component of the placebo response,<sup>64</sup> and social isolation is a core behavior induced by cytokines.<sup>65</sup> These findings raise the possibility that chronic inflammatory activity may impair an individual's ability to access social perceptions and emotions that are essential for placebo response. Given that approximately 37% of patients who respond to antidepressants result from placebo effects,<sup>33</sup>

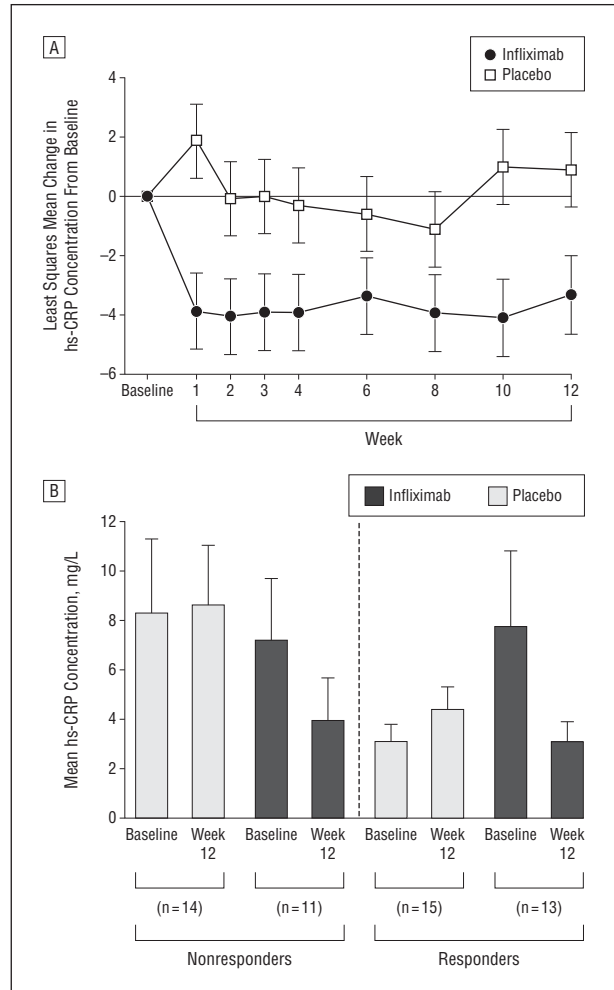


**Figure 6.** Percentage of treatment responders among infliximab- vs placebo-treated patients with treatment-resistant depression (TRD) and a baseline high-sensitivity C-reactive protein (hs-CRP) concentration of 5 mg/L or less or of greater than 5 mg/L. The percentage of treatment responders, which was defined by a 50% or more drop in the 17-item Hamilton Scale for Depression at any time during treatment, was compared between infliximab- and placebo-treated patients with TRD and a baseline plasma hs-CRP concentration of 5 mg/L or less or of greater than 5 mg/L, as well as combined. Infliximab-treated patients exhibited a higher response rate than placebo-treated patients when the hs-CRP concentration was greater than 5 mg/L, but infliximab-treated patients exhibited a lower response rate than placebo-treated patients when the hs-CRP concentration was 5 mg/L or less. These results did not reach statistical significance ( $P = .19$ ). No difference was found between the groups when combined ( $P = .99$ ).

these data may provide a novel explanation for the association between increased inflammation and antidepressant nonresponsiveness.

An unexpected observation was that placebo outperformed infliximab in participants with low levels of baseline inflammation. These results suggest that some minimal level of peripheral inflammatory activity may be required to exhibit an antidepressant response. It is becoming increasingly apparent that low, physiologic levels of cytokine activity are essential for a number of brain processes associated with protection from depression, including neuroplasticity and neurogenesis.<sup>66</sup> Moreover, recent data indicate that the behavioral effects of serotonergic antidepressants are dependent on TNF and IFN- $\gamma$ -mediated upregulation of p11, a biochemical marker of antidepressant response, in the central nervous system (CNS).<sup>67</sup> In keeping with this finding, studies in animals and observational data in humans suggest that nonsteroidal anti-inflammatory drugs may interfere with the behavioral effects of serotonergic antidepressants through their disruption of antidepressant-induced cytokine effects on p11.<sup>67</sup> Taken together, these data suggest that caution may be warranted in the use of anti-inflammatory strategies for patients with depression without evidence of increased inflammation, and they provide a counterbalance to prevailing views of inflammatory processes as primarily depressogenic.

Because infliximab is too large to cross the blood-brain barrier and enter the CNS at appreciable levels, our findings suggest that direct CNS action may not be necessary for a pharmacologic agent to possess antidepressant activity. If confirmed in subsequent studies, these findings suggest that body-based approaches to treatment of depression may be developed that would avoid the high rate of somatic and behavioral adverse effects



**Figure 7.** Change in plasma high-sensitivity C-reactive protein (hs-CRP) concentrations across treatment and as a function of treatment response in infliximab- vs placebo-treated patients with treatment-resistant depression (TRD). The plasma hs-CRP concentration was measured across the study in all patients with TRD. Infliximab treatment had a significant main effect on plasma hs-CRP concentration ( $P < .05$ ), leading to significant decreases in hs-CRP concentrations compared with placebo at all time points. Depicted is the least squares mean change in hs-CRP concentration from baseline to the indicated week using an unstructured covariance matrix with time as a categorical variable (A). Comparing placebo responders vs nonresponders, placebo responders had lower hs-CRP concentrations. Infliximab responders exhibited higher baseline hs-CRP concentrations and lower week-12 hs-CRP concentrations than did infliximab nonresponders, but these results also did not reach statistical significance. There was a main effect of treatment on change in hs-CRP concentration from baseline to week 12 ( $P < .05$ ). Moreover, infliximab responders exhibited a significantly greater decrease in hs-CRP concentrations from baseline to week 12 than did placebo responders ( $P < .01$ ) (B). The error bars indicate SEM.

observed with current antidepressants as a result of their indiscriminant action on receptors throughout the CNS. Relevant in this regard is the relatively low rate of adverse effects observed in participants who received infliximab. Nevertheless, it should be noted that the response in participants with a high level of inflammation may also be the result of increased permeability of infliximab through the blood-brain barrier. Indeed, a high level of peripheral inflammation has been associated with a reduced level of blood-brain barrier integrity.<sup>68</sup>

Several limitations of the present study warrant comment. Our study was underpowered to adequately test



the possibility that infliximab would only show superiority to placebo in participants with an elevated baseline hs-CRP concentration. Moreover, the low power may have increased the likelihood of false positives, including the interaction between treatment, time, and log hs-CRP concentration. In addition, the focus of the analyses on participants with a baseline hs-CRP concentration greater than 5 mg/L maximized the apparent efficacy of infliximab vs placebo, although it should be noted that baseline inflammatory status was proposed as a potential moderator of treatment response in the statistical analysis plan for our study. Nevertheless, future studies focused on participants with a hs-CRP concentration greater than 5 mg/L (which represented 37% [22 of 60 participants] of our sample) and/or elevated markers of TNF activation seem warranted. It should also be noted that no guidelines currently exist for either the dose or the schedule of administration for infliximab or other cytokine antagonists for depression. Indeed, the finding that patients with lower levels of baseline inflammation actually did worse while being treated with infliximab might suggest that the medication was overdosed for TRD, a condition associated with far lower levels of inflammation (ie, decreased concentrations of inflammatory biomarkers) than typically seen in inflammatory diseases such as ulcerative colitis and Crohn disease. Finally, the relatively short follow-up period may have obviated the capacity to detect interactive or synergistic effects between conventional antidepressants and TNF antagonism that may appear later in treatment, after the potential negative effects of cytokines on antidepressant action have been removed.

The high rate of placebo response observed in the present study reduced the power to test the antidepressant efficacy of infliximab. This high rate of placebo response was unexpected, given the far lower rates of placebo response typically reported for TRD. Nevertheless, given the long duration of the current major depressive disorder episode (a mean duration of more than 15 years for each group) and the fact that some participants were without any medication or other treatment, the sample may have represented a highly motivated and thereby responsive sample for study participation. Although a limitation, the high rate of placebo response serves as a cautionary note for interpretation of studies with highly invasive treatments in highly motivated patients that are not accompanied by adequate placebo control.

A final limitation is that no direct measures of the effect of either infliximab or placebo on the CNS were obtained. Nevertheless, studies in laboratory animals and humans suggest that, when activated, peripheral cytokines access the CNS and produce changes in the brain similar to major depression.<sup>69</sup> Moreover, data suggest that antagonism of peripheral cytokines can have activity in the brain. For example, in an animal model of traumatic brain injury, peripheral administration of the TNF antagonist etanercept reduced CNS levels of proinflammatory cytokines and attenuated inflammatory damage to brain tissue.<sup>70</sup> Similarly, in humans with progressive neuro-Behçet syndrome, infliximab has been reported to improve behavioral symptoms in concert with reductions in cerebrospinal fluid concentrations of IL-6.<sup>71</sup> Fi-

**Table 2. Summary of Adverse Events**

Adverse Event	No. (%)	
	Infliximab-Treated Patients (n = 30)	Placebo-Treated Patients (n = 30)
Headaches	20 (67)	18 (60)
Coughing	5 (17)	5 (17)
Sore throat	4 (13)	6 (20)
Insomnia	5 (17)	4 (13)
Diarrhea	3 (10)	6 (20)
Upper respiratory infection	4 (13)	2 (7)
Nasal congestion	4 (13)	2 (7)
Myalgia	4 (13)	1 (3)
Panic attacks	2 (7)	3 (10)
Rash	4 (13)	1 (3)
Fever	1 (3)	1 (3)
Increased presence of urinary leukocyte esterase	0 (0)	10 (33) <sup>a</sup>
Sinus congestion	4 (13)	0 (0)
Yeast infection	3 (10)	1 (3)
Increased urinary white blood cells	0 (0)	3 (10)

<sup>a</sup>  $P < .005$ .

nally, etanercept was found to reduce the percentage and amount of rapid eye movement sleep compared with placebo in abstinent men with a history of alcohol dependence.<sup>72</sup>

In summary, this proof-of-concept study suggests that TNF antagonism does not exhibit generalized efficacy in TRD. However, interesting yet complex interrelationships between inflammation and treatment response to both TNF antagonism and placebo were revealed. Although participants with a high level of inflammation responded preferentially to infliximab, infliximab-treated participants with a low level of inflammation appeared to do worse than placebo-treated participants. In contrast, increased inflammation predicted a poor response to placebo. Taken together, the data suggest that there is a subgroup of patients with TRD who have increased inflammation and respond to cytokine antagonism but not to placebo. The data represent an important first step in the personalization of antidepressant therapy and provide promise for future development and elaboration of inflammatory biomarkers that identify patients who may be uniquely responsive to immune-targeted therapy.

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