Baseline Levels of C-Reactive Protein and Proinflammatory Cytokines Are Not Associated With Early Response to Amisulpride in Patients With First Episode Psychosis: The OPTiMiSE Cohort Study

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Patients with a First-Episode of Psychosis (FEP) exhibit low-grade inflammation as demonstrated by elevated levels of C-reactive protein (CRP) and proinflammatory cytokines. The primary goal of this study was to investigate the association between proinflammatory biomarkers and clinical outcomes in unmedicated FEP patients. We used clinical data and biological samples from 289 FEP patients participating in the Optimization of Treatment and Management of Schizophrenia in Europe (OPTIMISE) clinical trial. Patients were assessed at baseline and 4–5 weeks after treatment with amisulpride. Baseline serum levels of interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-α, and CRP were measured. We first used multivariable regression to investigate the association between each of the 4 tested biomarkers and the following clinical outcomes: Positive and Negative Syndrome Scale (PANSS), Calgary Depression Score for Schizophrenia (CDSS), remission according to Andreasen’s criteria, and Serious Adverse Events (SAEs). As a complementary approach, we used an unsupervised clustering method to stratify patients into an “inflamed” or a “non-inflamed” biotype based on baseline levels of IL-6, IL-8, and TNF-α. We then used linear and
logistic regressions to investigate the association between the patient biotype and clinical outcomes. After adjusting for covariates and confounders, we did not find any association between IL-6, IL-8, TNF-α, CRP, or the patient biotype and clinical outcomes. Our results do not support the existence of an association between baseline levels of CRP and proinflammatory cytokines and early response to amisulpride in unmedicated FEP patients. ClinicalTrials.gov Identifier: NCT01248195.

Key words: inflammation/proinflammatory cytokines/remission/psychosis/longitudinal study

Introduction

Early treatment response is an excellent predictor of long-term symptomatic and functional outcome in psychosis. Unfortunately, and despite extensive research, reliable biomarkers of early treatment response and clinical outcome in First-Episode of Psychosis (FEP) patients remain to be identified. In a typical study aimed at identifying biomarkers of treatment response, FEP patients are sampled at baseline for blood or neuroimaging biomarkers and assessed at inclusion as well as several weeks or months later for clinical outcomes. In most cases, response to treatment is defined as a reduction in symptom severity to the levels required by the remission criteria of the Schizophrenia Working Group Consensus. This consensus established a set of criteria that provide an absolute threshold in severity of symptoms that should be reached for clinical improvement. Alternatively, some authors have used a continuous measure of treatment response which is defined as change in Positive and Negative Syndrome Scale (PANSS) total scores from baseline to follow-up, considering baseline PANSS total score and subtracting a score of 30, as even individuals without any mental health problem could score 30 in the PANSS. In this latter case, the following formula was used: (baseline PANSS total score − 30)(baseline PANSS total score − 30) × 100. Other clinical outcomes such as changes in the Clinical Global Impression Scale (CGI), the Brief Psychiatric Rating Scale (BPRS), the Calgary Depression Scale for Schizophrenia (CDSS), and occurrence of Serious Adverse Events (SAEs) may also be assessed.

While several studies have demonstrated the feasibility of using neuroimaging biomarkers for predicting clinical outcome in FEP patients, diagnostics tools based on biological fluids may be more cost and time efficient. In these studies, several authors have aimed at identifying soluble biomarkers in blood or saliva which, when measured at baseline in FEP patients, were associated with clinical outcomes several weeks, months, or years later. A wide range of biomarkers have been tested including the acute phase protein (C-reactive protein [CRP]), many interleukins (ILs) and chemokines, tumor necrosis factor (TNF-α), interferon-γ (IFN-γ), vascular endothelial growth factor, complement component 4 (C4), lipids and glucometabolic biomarkers (glycated hemoglobin [HbA1c], triglycerides [TGs], total cholesterol, low-density lipoprotein cholesterol, fasting glucose), redox biomarkers (glutathione [GSH], GSH peroxidase and reductase activities [GPx, GR], thioredoxin [Trx], and GSH-related metabolites), amino acids, and stress biomarkers (cortisol, sulfated dehydroepiandrosterone [DHEA-S]). In a 12-week follow-up study on 68 FEP patients, salivary levels of cortisol measured shortly after awakening as well as blood levels of IFN-γ and IL-6 were lower in responders compared to nonresponders in the early phases of psychosis. In a longitudinal study on 42 FEP patients, the authors used a principal component analysis (PCA) to reduce the dimensionality of the dataset accounting for both inflammation and metabolic status. Among the 3 identified PCA factors, factor 1 that accounted for high sensitivity C-reactive protein (hsCRP) and body mass index (BMI), were associated with treatment response at 1 year. Lastly, in a naturalistic longitudinal study on 25 FEP patients, serum levels of complement C4 were associated with treatment response at 1 year after adjustment for baseline severity of symptoms and CRP levels.

While several studies have allowed for the identification of candidate neuroimaging and soluble biomarkers that could be used for predicting clinical outcome in FEP patients, many of them have suffered from several shortcomings including small sample size and treatment heterogeneity. As an attempt to overcome these shortcomings, we used clinical data and biological samples from the multinational, multicenter, randomized, double-blind OPTiMiSE study in which 481 FEP patients were treated with the second-generation antipsychotic amisulpride. We assessed serum samples collected at baseline for well-studied biomarkers of inflammation, ie, IL-6, IL-8, TNF-α, and CRP. We then used multivariable regression to study the association between these biomarkers and PANSS score and subscores, CDSS, remission, and SAEs.

While inflammation is an intricate process involving dozens of molecules, we have chosen to focus on IL-6, IL-8, TNF-α, and CRP for the following reasons. First, antipsychotic-naive FEP and acute psychotic relapse are associated with increased serum concentrations of IL-6, IL-8, TNF-α, and CRP. Second, population-based longitudinal studies have shown an association between higher levels of IL-6 and CRP and risk for psychotic experience and a diagnosis of schizophrenia subsequently in adulthood. Third, IL-6, IL-8, and TNF-α are 3 of the main proinflammatory cytokines that are produced by macrophages when activated with Pathogen-Associated Molecular Patterns. Four, IL-8 is a potent chemotactic factor that causes neutrophils and other granulocytes
that promote inflammation to migrate toward the site of infection.17

Methods

Study Subjects

The OPTiMiSE study was conducted in 27 academic hospitals and clinics in 14 European countries and Israel (Clinicaltrials.gov identifier is NCT01248195).11 FEP patients were recruited between May 2011 and April 2016 at the participating centers from nearby healthcare facilities. Eligible patients were aged 18–40 years and met the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM IVth edition) for schizophrenia, schizophreniform disorder, or schizoaffective disorder. A total of 481 patients were enrolled and signed informed consent. Diagnosis was confirmed by the Mini International Neuropsychiatric Interview plus.18 Patients were excluded if: more than 2 years had passed since the start of the FEP; any antipsychotic drug had been used for more than 2 weeks in the previous year and/or for a total of 6 weeks during lifetime; patients had a known intolerance or met any of the contraindications for at least one of the study drugs; patients were coercively treated and/or represented by a legal guardian or under legal custody; or patients were pregnant or breast feeding. All participants to the study signed a written informed consent form.

Clinical Assessment

A screening visit was conducted during which eligibility was assessed. Baseline data were obtained regarding sex, age, diagnoses, BMI, waist circumference, recreational drug use; alcohol consumption, caffeine consumption, smoking, and current treatments. Patients were clinically assessed at baseline for positive, negative, and general psychopathology symptoms using the PANSS scale,19 depression using the CDSS,20 social functioning using the Personal and Social Performance (PSP) Scale,21 wellbeing using the Subjective Wellbeing under Neuroleptics (SWN) and overall severity of symptoms with the CGI scale.22 Patients were treated with up to 800 mg/day amisulpride in an open-label design and clinically assessed 4–5 weeks later for psychopathology using the PANSS scale, symptomatic remission according to the criteria of Andreasen et al2 (a score ≤3 simultaneously on 8 PANSS items: P1, P2, P3, N1, N4, N6, G5, and G9), percentage of weight gain and the occurrence of SAE.

Healthy Individuals

Healthy individuals were recruited as part of a study registered in ClinicalTrials.gov (ID: NCT02209142). An extensive clinical and psychological examination, using both Structured Clinical Interview for DSM Disorders23 and a self-rating questionnaire, as well as clinical and biological examinations (including body temperature, blood cell count) were used to determine the absence of any medical conditions or any psychiatric disorders. None of the subjects had received any vaccinations within a month before inclusion. All subjects gave informed written consent. The study was conducted in accordance with the latest version of the Declaration of Helsinki and was approved by the appropriate French ethical and medical authorities.

Blood Samples

Venous blood was obtained from fasting subjects between 7:00 AM and 9:00 AM. Five millimeters of blood were drawn into serum Vacutainer tubes and allowed to clot for 1 h before centrifugation (1500g, 10 min). Sera were stored in 0.5 ml aliquots at −80°C for 3–4 years. Samples were thawed once and immediately assessed for cytokine and CRP levels by immunoassay.

Immunoassay

Serum levels of IL-6, IL-8, TNF-α, and CRP, were measured using the Pro-inflammatory Panel 1 and Vascular Injury Panel 2 v-PLEX kits (MSD) at a single site at the Centre National de la Recherche Scientifique (CNRS). All assays were performed according to the manufacturer's instructions. Data were acquired on the V-PLEX Sector Imager 2400 plate reader and analyzed using the Discovery Workbench 3.0 software (MSD). Standard curves for each cytokine were generated using standards provided in the kits. Serial 4-fold dilutions of the standards were run to generate a 7-standard concentration set, and the diluent alone was used as a blank. Cytokine concentrations were determined from the standard curve using a 4-parameter logistic curve fit to transform the mean luminescence intensities into concentrations. As the lower limit of detection (LLOD), we used the median LLOD compiled over multiple plates and corresponding to the concentration calculated based on the signal recorded for the blank plus 2.5 SDs. For each cytokine and the CRP, samples that fell below the LLOD were given a substituted value equal to half the LLOD. Supplementary table 1 shows, for each biomarker and each cohort the LLOD, the number and percentage of samples that fall below the LLOD, the lower (min), upper (max), median, and mean values, as well as the SD.

Data Analysis

Data Preprocessing. Our dataset did not contain any missing data. For each cytokine and the CRP, samples that fell below the LLOD were given a substituted value equal to half the LLOD. As cytokines and CRP are
believed to be stable when stored at −80°C and since the samples were thawed only once, biomarker levels were not adjusted for storage duration.

**Unsupervised Clustering Analysis.** We stratified patients based on serum cytokine levels at baseline using the k-spectral clustering algorithm,\(^{24}\) with the aim to identify 2 homogeneous clusters within our study sample. We first normalized each feature (serum levels of each cytokine) independently so that the norm of each feature becomes equal to 1. The k-spectral clustering method uses the spectrum (eigenvalues) of the similarity matrix of the data to perform dimensionality reduction before clustering in fewer dimensions. The similarity matrix is provided as an input and consists of a quantitative assessment of the relative similarity of each pair of points in the dataset. Silhouette values were computed as a measure of clustering appropriateness. We used the t-distributed Stochastic Neighbor Embedding (t-SNE) method for graphical representation of the clusters. Codes will be available under request.

**Statistical Analyses.** Comparison analyses between clusters were performed using the 2-tailed nonparametric Mann-Whitney U test or the Chi-square test (as indicated). Comparison analyses of cytokine and CRP levels between cluster 1 and 2 patients, and healthy individuals were performed using the Kruskal-Wallis 2-tailed nonparametric test followed by post hoc analysis. To study associations at baseline clusters based on proinflammatory cytokines at baseline and clinical scores, we implemented a multivariable linear regression model using the cluster as the dependent variable and baseline PANSS total score and subscores, age (years), sex (female/male), BMI (kg/m\(^2\)), waist circumference (cm), use of recreational drugs (yes/no), coffee consumption (number of cups/day), alcohol consumption (yes/no), and tobacco smoking (yes/no) as independent variables. To study associations between remission after treatment and cluster at baseline, we implemented a multivariable logistic regression model using the outcome (remission/nonremission) as the dependent variable and cluster, PANSS subscores at baseline, age, sex, BMI, waist circumference, use of recreational drugs, alcohol consumption, and current smoking as independent variables. To study associations between remission after treatment and cluster at baseline, we implemented a multivariable linear regression model using PANSS subscores after treatment as the dependent variables and cluster, PANSS subscores at baseline, age, sex, BMI, waist circumference, use of recreational drugs, and alcohol consumption and current smoking as independent variables. Statistical significance was set according to a \(P\) value <.05. Statistical analyses were performed using R Stats package\(^{25}\) and graphs were generated using GraphPad Prism version 6.00 for iOS (GraphPad Software, USA).

**Results**

A total of 481 FEP patients were included in the OPTiMiSE clinical trial.\(^{11}\) Out of the 446 patients in the intention-to-treat sample, 348 had serum samples collected at baseline before the study treatment was initiated. From these patients, 289 were included in this study and assessed clinically at baseline and after 4–5 weeks of treatment with the antipsychotic amisulpride (table 1). Serum samples at baseline were assessed for the proinflammatory cytokines IL-6, IL-8, and TNF-α, and for the acute phase protein CRP (Supplementary table 1). Low to medium positive correlations were observed between IL-6, IL-8, and TNF-α, but not CRP, suggestive of coordinated but not similar variations in the levels of these biomarkers in FEP patients (Supplementary figure 1).

We first investigated the association between IL-6, IL-8, TNF-α, and CRP considered as continuous variables and PANSS total score and subscores, CDSS, remission, and SAEs. After adjusting for age, gender, BMI, waist circumference, recreational drugs use, consumption of coffee and alcohol, and tobacco smoking, no association was found between any of these biomarkers and total PANSS score (table 2), CDSS (table 3), remission (table 4), SAEs (table 5), positive PANSS (PPANSS) (Supplementary table 2), negative PANSS (NPANSS) (Supplementary table 3), or general psychopathology PANSS (GPPANSS) (Supplementary table 4) subscores.

Because inflammatory biomarkers may be associated with a clinical outcome only when above a specific threshold, we dichotomized each biomarker at the 15th/85th upper/lower percentile before studying its association and clinical outcomes. None of the 4 tested inflammatory biomarkers was associated with total PANSS score (Supplementary table 5), PPANSS (Supplementary table 6), NPANSS (Supplementary table 7), GPPANSS (Supplementary table 8) subscores, CDSS (Supplementary table 9), remission (Supplementary table 10), or SAEs (Supplementary table 11).

Because an individual’s inflammatory status may be better captured by a combination of proinflammatory cytokines than by any individual one, we used an unsupervised classification approach to stratify patients into an “inflamed” and a “non-inflamed” cluster based on serum levels of IL-6, IL-8, and TNF-α. These 2 clusters, accounted for 66% and 34% of the total sample respectively (table 1). In agreement with the relatively high mean silhouette value of the clustering solution, cluster 1 and cluster 2 patients projected in distinct regions of t-SNE graphical representation reflecting high intracluster consistency (Supplementary figure 2). While cluster 1 patients exhibited a lower BMI compared to those of cluster 2, they were similar with respect to sex ratio, age, waist circumference, used of recreational drugs, and consumption of coffee and alcohol (table 1). Cluster 2 patients had higher serum levels of IL-6, IL-8, and TNF-α at baseline.
Baseline Levels of C-Reactive Protein and Proinflammatory Cytokines when compared to cluster 1 patients or healthy controls (Supplementary figure 3). In contrast, serum levels of IL-8 and TNF-α were similar in both cluster 1 patients and healthy controls, while IL-6 was more abundant in cluster 1 patients. Of note, CRP levels were similar in cluster 1 patients, cluster 2 patients and healthy controls. We next studied the association between the patient cluster and clinical outcomes. After adjustment for covariates and confounders, we did not find any association between patient cluster and total PANSS score (table 2), CDSS (table 3), remission (table 4), SAEs (table 5), PPANSS (Supplementary table 2), NPANSS (Supplementary table 3), or GPPANSS (Supplementary table 4) subscores.

Discussion

Main Findings

Here, we have investigated the association between baseline levels of 4 inflammatory biomarkers and clinical outcomes in 289 FEP patients 4–5 weeks after treatment with amisulpride was initiated. We first analyzed each biomarker independently and assessed their association with PANSS total score and subscores, CDSS, remission, and SAEs. We performed 2 types of analyses: one in which we considered each biomarker as a continuous variable, and another in which we dichotomized them at the 15th upper/85th lower percentile. After adjustment for age, sex, BMI, waist circumference, use of recreational drug, coffee and alcohol consumption, and tobacco smoking, neither IL-6 nor IL-8, TNF-α, and CRP was associated with any of the tested clinical outcomes. We next used an unsupervised clustering method to stratify FEP patients into 2 clusters based on serum levels of IL-6, IL-8, and TNF-α. While these cytokines were present at normal levels in cluster 1 patients, their levels were abnormally elevated in cluster 2 patients, indicative of an inflamed profile. The patient cluster was neither associated with total PANSS score at baseline, nor with PPANSS, NPANSS, or GPPANSS subscores, CDSS, remission, or SAEs. To summarize, we could not identify any association between the patient inflammatory profile and any of the clinical outcome that we have assessed.

Other Findings

An unexpected finding of this study is that cluster 1 and cluster 2 patients had comparable blood levels of CRP, and that these levels were comparable to those found in healthy controls. This is an important result as previous studies attempting to use CRP to identify people at risk of later schizophrenia have been mixed, with some, but not others, finding that patients with increased levels of CRP and higher BMI have been found to have increased risk for later schizophrenia. CRP is produced mainly in the liver in response to inflammation and is commonly used to assess the presence and severity of inflammation in a wide range of diseases. However, we found that patient's inflammatory profile and any of the clinical outcome that we have assessed, were not associated with CRP levels in our study.
## Table 2. Association Between Total PANSS Score After Treatment and IL-6, IL-8, TNF-α, CRP and patient cluster

| Total PANSS After Treatment | β (95% CI) | P Value | β (95% CI) | P Value | β (95% CI) | P Value | β (95% CI) | P Value | β (95% CI) | P Value |
|-----------------------------|------------|---------|------------|---------|------------|---------|------------|---------|------------|---------|
| IL-6                        | −3.14      | .4      | (−10.513, 4.228) |         |            |         |            |         |            |         |
| IL-8                        | 0.07       | .58     | (−0.178, 0.317) |         |            |         |            |         |            |         |
| TNF-α                       | 0.77       | .583    | (−1.981, 3.515) |         |            |         |            |         |            |         |
| CRP                         | 0 (0, 0)  | .32     |            |         |            |         |            |         |            |         |
| Cluster                     | 1.43       | .6      | (−3.907, 6.773) |         |            |         |            |         |            |         |
| Age                         | −0.48      | <.01    | (−0.78, −0.19) |         |            |         |            |         |            |         |
| Sex (male)                  | −2.89      | .2      | (−7.338, 1.559) |         |            |         |            |         |            |         |
| BMI                         | 0.16       | .65     | (−7.466, 1.392) |         |            |         |            |         |            |         |
| Waist circumference         | −0.56      | .05     | (−5.046, 0.874) |         |            |         |            |         |            |         |
| Recreational drugs          | 1.14       | .63     | (−0.204, 0.304) |         |            |         |            |         |            |         |
| Coffee                      | 0.45       | .16     | (−3.457, 5.743) |         |            |         |            |         |            |         |
| Alcohol                     | −2.09      | .3      | (−0.175, 1.069) |         |            |         |            |         |            |         |
| Tobacco                     | −0.38      | .86     | (−6.091, 1.905) |         |            |         |            |         |            |         |
| Total PANSS at baseline     | 0.52       | <.0001  | (−4.713, 3.948) |         |            |         |            |         |            |         |

Note: Linear regressions were fitted using total PANSS score as the dependent variable and IL-6, IL-8, TNF-α, CRP, or patient’s cluster as the independent variables separately (with adjustments for demographic and clinical variables). Estimates (b), 95% confidence intervals (CI) and P values are shown. An association was considered to be statistically significant when P value < .05. Significant associations highlighted in bold. BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; IL, interleukin; PANSS, Positive and Negative Syndrome Scale; TNF-α, tumor necrosis factor alpha.
Table 3. Association Between CDSS After Treatment and IL-6, IL-8, TNF-α, CRP and Patient Cluster

| CDSS After Treatment | β (95% CI) | P Value | β (95% CI) | P Value | β (95% CI) | P Value | β (95% CI) | P Value | β (95% CI) | P Value |
|----------------------|-----------|---------|-----------|---------|-----------|---------|-----------|---------|-----------|---------|
| IL-6                 | -1.01     | .22     | 0 (−0.05, 0.054) | .94     | 0.1 (−0.487, 0.694) | .73     | 0 (0, 0) | .15     | −0.1     | .82     |
| IL-8                 | 0 (−0.064, 0.064) | .88     | 0 (−0.068, 0.06) | .91     | 0 (0.0, 0) | .15     | 0 (−0.069, 0.059) | .88 |
| TNF-α                | 0 (−1.502, 0.502) | .33     | 0 (−1.546, 0.461) | .29     | 0 (−1.542, 0.463) | .29     | 0.4 (−1.543, 0.457) | .29 |
| CRP                  | 0.02      | .81     | 0 (−0.149, 0.152) | .98     | 0 (−0.15, 0.151) | .99     | 0 (0.01, 0.94) | .96 |
| Cluster              | 0.16      | .72     | 0 (−0.087, 0.022) | .76     | 0 (−0.087, 0.022) | .77     | 0 (−0.08, 0.022) | .76 |
| Age                  | 0.05      | .49     | 0 (−0.123, 0.152) | .45     | 0 (−0.127, 1.121) | .45     | 0 (−0.126, 1.127) | .45 |
| Sex (male)           | −0.4      | .24     | 0 (−0.087, 0.022) | .25     | 0 (−0.087, 0.022) | .24     | 0 (−0.08, 0.022) | .24 |
| BMI                  | −0.02     | .81     | 0 (−0.0149, 0.152) | .98     | 0 (−0.015, 0.151) | .99     | 0 (0.01, 0.94) | .96 |
| Waist circumference  | −0.03     | .24     | 0 (−0.0149, 0.152) | .98     | 0 (−0.015, 0.151) | .99     | 0 (0.01, 0.94) | .96 |
| Recreational drugs   | −0.4      | .58     | 0 (−0.087, 0.022) | .76     | 0 (−0.087, 0.022) | .77     | 0 (−0.08, 0.022) | .76 |
| Coffee               | −0.5      | .33     | 0 (−0.123, 0.152) | .45     | 0 (−0.127, 1.121) | .45     | 0 (−0.126, 1.127) | .45 |
| Alcohol              | 0.46      | .3      | 0 (−0.0149, 0.152) | .98     | 0 (−0.015, 0.151) | .99     | 0 (0.01, 0.94) | .96 |
| Tobacco              | −0.4      | .39     | 0 (−0.0149, 0.152) | .98     | 0 (−0.015, 0.151) | .99     | 0 (0.01, 0.94) | .96 |
| CDSS at baseline     | 0.51      | <.0001  | 0 (−1.361, 0.484) | <.0001  | 0 (−1.361, 0.483) | <.0001  | 0 (−1.315, 0.526) | <.0001 |

Note: Linear regressions were fitted using CDSS as the dependent variable and IL-6, IL-8, TNF-α, CRP, or patient’s cluster as the independent variables separately (with adjustments for demographic and clinical variables). Estimates (b), 95% confidence intervals (CI) and P values are shown. An association was considered to be statistically significant when P value < 0.05. Significant associations highlighted in bold. BMI, body mass index; CDSS, Calgary Depression Score for Schizophrenia; CI, confidence interval; CRP, C-reactive protein; IL, interleukin; TNF-α, tumor necrosis factor alpha.
Table 4. Association Between Remission After Treatment and IL-6, IL-8, TNF-α, CRP and Patient Cluster

| Remission          | OR (95% CI)   | P Value | OR (95% CI)   | P Value | OR (95% CI)   | P Value | OR (95% CI)   | P Value | OR (95% CI)   | P Value |
|--------------------|---------------|---------|---------------|---------|---------------|---------|---------------|---------|---------------|---------|
| IL-6               | 0.71 (0.222, 2.111) | .54     | 1.01 (0.968, 1.044) | .73     | 1.33 (0.873, 2.039) | .19     | 1 (1, 1)      | .87     | 0.803 (0.399, 1.582) | .53     |
| IL-8               |               |         |               |         |               |         |               |         |               |         |
| TNF-α              |               |         |               |         |               |         |               |         |               |         |
| CRP                |               |         |               |         |               |         |               |         |               |         |
| Cluster            |               |         |               |         |               |         |               |         |               |         |
| Age (years)        | 0.94 (0.894, 0.984) | <.01    | 0.94 (0.893, 0.983) | <.01    | 0.94 (0.895, 0.986) | <.05    | 0.94 (0.893, 0.982) | <.01    | 0.914 (0.863, 0.971) | <.01    |
| Sex (male)         | 1.14 (0.571, 2.255) | .71     | 1.13 (0.564, 2.233) | .74     | 1.14 (0.568, 2.259) | .72     | 1.12 (0.561, 2.222) | .75     | 0.741 (0.336, 1.533) | .41     |
| BMI                | 1 (0.906, 1.105) | .96     | 0.99 (0.902, 1.091) | .9      | 0.99 (0.895, 1.084) | .78     | 0.99 (0.902, 1.092) | .91     | 1.01 (0.934, 1.099) | .75     |
| Waist circumference | 1.01 (0.98, 1.052) | .43     | 1.02 (0.981, 1.053) | .4      | 1.02 (0.981, 1.053) | .4      | 1.01 (0.98, 1.052) | .41     | 1.02 (0.981, 1.053) | .4      |
| Recreational drugs | 0.79 (0.386, 1.581) | .5      | 0.77 (0.38, 1.543) | .46     | 0.74 (0.362, 1.487) | .4      | 0.77 (0.379, 1.543) | .46     | 0.771 (0.336, 1.73)  | .53     |
| Coffee             | 1.04 (0.95, 1.148) | .36     | 1.04 (0.951, 1.149) | .36     | 1.05 (0.951, 1.151) | .35     | 1.04 (0.949, 1.147) | .37     | 1.073 (1.073, 1.198) | .22     |
| Alcohol            | 1.24 (0.674, 2.282) | .49     | 1.26 (0.691, 2.32) | .45     | 1.27 (0.693, 2.33) | .44     | 1.26 (0.689, 2.318) | .45     | 1.35 (0.673, 2.737)  | .39     |
| Tobacco            | 1.63 (0.849, 3.189) | .14     | 1.62 (0.842, 3.159) | .15     | 1.63 (0.847, 3.19) | .15     | 1.63 (0.845, 3.18) | .15     | 1.916 (0.896, 4.221) | .1      |
| PPANSS at baseline | 1.03 (0.973, 1.102) | .28     | 1.03 (0.972, 1.101) | .29     | 1.03 (0.969, 1.097) | .34     | 1.03 (0.972, 1.099) | .3      | 0.99 (0.92, 1.069)  | .83     |
| NPANSS at baseline | 1.1 (1.049, 1.164) | <.001   | 1.11 (1.05, 1.166) | <.001   | 1.11 (1.052, 1.169) | <.0001  | 1.1 (1.05, 1.165)  | <.001   | 1.094 (1.031, 1.168) | <.01    |
| GPPANSS at baseline| 0.99 (0.947, 1.031) | .58     | 0.99 (0.945, 1.03) | .56     | 0.98 (0.941, 1.027) | .46     | 0.99 (0.946, 1.03) | .57     | 0.97 (0.92, 1.018)  | .22     |

Note: Logistic regressions were fitted using remission as the dependent variable and IL-6, IL-8, TNF-α, CRP, or patient's cluster as the independent variables separately (with adjustments for demographic and clinical variables). Estimates (b), 95% confidence intervals (CI) and P values are shown. An association was considered to be statistically significant when P value < .05. Significant associations highlighted in bold. BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; GPPANSS, general psychopathology PANSS; IL, interleukin; NPANSS, negative PANSS; OR, odds ratio; PPANSS, positive PANSS; TNF-α, tumor necrosis factor alpha.
Table 5. Association Between SAEs and IL-6, IL-8, TNF-α, CRP and Patient Cluster

| SAEs                | OR (95% CI)   | P Value | OR (95% CI)   | P Value | OR (95% CI)   | P Value | OR (95% CI)   | P Value | OR (95% CI)   | P Value |
|---------------------|---------------|---------|---------------|---------|---------------|---------|---------------|---------|---------------|---------|
| IL-6                | 1.1 (0.382, 3.315) | .86     |               |         |               |         |               |         |               |         |
| IL-8                | 0.98 (0.947, 1.016) | .27     |               |         |               |         |               |         |               |         |
| TNF-α               |               |         |               |         |               |         |               |         |               |         |
| CRP                 |               |         |               |         |               |         |               |         |               |         |
| Cluster             |               |         |               |         |               |         |               |         |               |         |
| Age                 |               |         |               |         |               |         |               |         |               |         |
| Sex (male)          | 0.52 (0.271, 0.993) | <.05   | 0.51 (0.265, 0.969) | <.05   | 0.53 (0.276, 1.009) | <.05   | 0.56 (0.292, 1.082) | <.05   | 0.96 (0.928, 0.998) | <.05   |
| BMI                 | 1.08 (0.979, 1.195) | .14     | 1.08 (0.985, 1.192) | .11     | 1.08 (0.98, 1.191) | .13     | 1.09 (0.989, 1.204) | .09     | 1.0926 (1.089) | .94     |
| Waist circumference | 0.96 (0.928, 0.998) | <.05   | 0.96 (0.928, 0.997) | <.05   | 0.96 (0.929, 0.998) | <.05   | 0.96 (0.928, 0.999) | <.05   | 0.96 (0.928, 0.998) | <.05   |
| Recreational drugs  | 1.4 (0.715, 2.778) | .33     | 1.41 (0.717, 2.789) | .32     | 1.39 (0.709, 2.755) | .34     | 1.53 (0.768, 3.064) | .23     | 1.405 (0.625, 3.245) | .41     |
| Coffee              | 1.1 (0.998, 1.232) | .07     | 1.1 (0.996, 1.227) | .08     | 1.1 (0.999, 1.234) | .07     | 1.09 (0.991, 1.224) | .1     | 1.073 (0.957, 1.218) | .26     |
| Alcohol consumption | 1.05 (0.583, 1.87) | .88     | 1.04 (0.581, 1.851) | .9     | 1.04 (0.585, 1.862) | .88     | 0.99 (0.547, 1.777) | .97     | 1.105 (0.551, 2.226) | .77     |
| Tobacco             | 0.67 (0.349, 1.269) | .22     | 0.68 (0.354, 1.288) | .24     | 0.67 (0.35, 1.273) | .23     | 0.7 (0.363, 1.34) | .29     | 0.607 (0.267, 1.27) | .18     |
| PPANSS at baseline  | 1.03 (0.972, 1.099) | .3     | 1.03 (0.968, 1.095) | .36     | 1.03 (0.971, 1.098) | .31     | 1.04 (0.975, 1.103) | .25     | 1.062 (0.987, 1.15) | .11     |
| NPANSS at baseline  | 1.04 (0.989, 1.088) | .14     | 1.03 (0.986, 1.086) | .14     | 1.04 (0.99, 1.089) | .13     | 1.04 (0.995, 1.096) | .09     | 1.03 (0.972, 1.092) | .32     |
| GPPANSS at baseline | 0.96 (0.917, 0.997) | <.05   | 0.96 (0.918, 0.998) | <.05   | 0.96 (0.915, 0.995) | <.05   | 0.95 (0.91, 0.99) | <.05   | 0.961 (0.912, 1.006) | .09     |

Note: Logistic regressions were fitted using SAEs as the dependent variable and IL-6, IL-8, TNF-α, CRP, or patient’s cluster as the independent variables separately (with adjustments for demographic and clinical variables). Estimates (b), 95% confidence intervals (CI) and P values are shown. An association was considered to be statistically significant when P value < .05. Significant associations highlighted in bold. BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; GPPANSS, general psychopathology PANSS; IL, interleukin; NPANSS, negative PANSS; OR, odds ratio; PPANSS, positive PANSS; SAEs, Serious Adverse Events; TNF-α, tumor necrosis factor alpha.
CRP is an ambiguous proinflammatory marker as it is also constitutively produced in the absence of inflammation in a pentameric form (pCRP) with anti-inflammatory properties. In contrast, the monomeric CRP (mCRP) is produced locally by dissociation of the pCRP, in response to inflammatory cues in inflamed or damaged tissues and has potent proinflammatory effects. Because proinflammatory mCRP is mostly retained in tissues, the CRP measured in serum by immunoassays exclusively corresponds to the anti-inflammatory pCRP form, in particular in the case of low-grade inflammation. It is only in the case of acute inflammation that the overload of CRP in tissues is reflected in the serum. Based on these data, Del Giudice and Gangstad have pointed out that measuring serum CRP levels is insufficient to establish the existence of a low-grade inflammatory state, and have proposed that other proinflammatory cytokines such as TNF-α and IL-1β could be more reliable as biomarkers of inflammation. Our results not only support this view but also suggest that combinations of proinflammatory cytokines, rather than individual ones, should be used to identify “inflamed” patients. This may have implications for the design of prospective placebo-controlled clinical trials aimed at comparing disease outcome in “inflamed” and “non-inflamed” patients, or for selecting “inflamed” patients for add-on anti-inflammatory treatment.

While the primary objective of this study was to investigate association between inflammatory biomarkers and clinical outcome after treatment with amisulpride, our results provided insight into the association between clinical variables at baseline and clinical outcome. As for PANSS total score and subscores after treatment, they were negatively associated with age in all models, and— as expected—positively associated with their respective score and subscores at baseline. Remission was negatively and positively associated with age and NPANSS at baseline respectively. As for SAEs, it was associated with sex, waist circumference and GPPANSS at baseline in models in which IL-6, IL-8, and TNF-α were used as covariates. Also, lower levels of CRP decreased the odds of SAEs but the effect size was extremely low.

Discrepancies With Other Studies
An association between inflammatory biomarkers at baseline and response treatment was first reported by Mondelli et al in a highly cited paper. In this study, 68 FEP patients—among whom 33 were sampled at baseline—were treated as usual and assessed 12 weeks later to identify those who met remission criteria. In these 33 patients among whom 18 and 21 were responders and nonresponders respectively, blood levels of IL-6 and IFN-γ were lower in responders compared to nonresponders. These authors concluded that “Inflammatory biomarkers predict poor response in FEP.” The discrepancy between our results and this previous finding may be explained by several reasons. First, while Mondelli et al studied response to clinician-led antipsychotic treatment after 12 weeks, we searched for biomarkers that were associated with response to a single antipsychotic, ie, amisulpride, 4–5 weeks after the treatment was initiated. Second, our study sample consisted of 289 FEP patients compared to 33 in Mondelli’s paper. Third, our results were adjusted for age, gender, BMI, waist circumference, use of recreational drugs, consumption of coffee and alcohol, tobacco smoking, and clinical symptoms’ severity at baseline while Mondelli et al used univariate statistical methods to analyze their data. In another longitudinal study on 42 FEP patients, the authors aimed at identifying biomarkers of metabolism and inflammation that were associated with clinical outcomes. To this aim, they measured hsCRP as a biomarker of inflammation, and BMI, lipid profile, and glucometabolic parameters as metabolic variables. A PCA was then used to reduce the dimensionality of the dataset accounting for both inflammation and metabolic status. Results showed that a PCA factor that accounted for hsCRP, BMI, and TGs measured at baseline was associated with total PANSS score, PPANSS and NPANSS subscores, and treatment response rate at 1-year follow-up. While these latter results did show an effect of abnormal metabolic and inflammatory status on the brain, the use of PCA did not allow for assessing the relative contribution of metabolic and inflammatory biomarkers to the outcome.

Strengths
One important strength of this study is that it takes advantage of clinical and biological samples collected within the frame of one of the largest longitudinal clinical studies in FEP patients. Patients were not only medication naive (or almost medication naive) at baseline, but also treated with the same antipsychotic and assessed 4–5 weeks after the treatment was initiated, therefore precluding biases due to different medications or assessment at different time points. Second, the longitudinal design of the OPTiMiSE clinical trial allowed for assessing the association of inflammatory biomarkers collected at baseline and clinical outcomes 4–5 weeks later. Third, and with at least 2 noticeable exceptions, we are not aware of any other study in which proinflammatory cytokines, and more generally inflammatory biomarkers, were considered altogether—rather than individually—to determine the “inflamed” or “non-inflamed” status of an individual.

Limitations
Our results should be considered and interpreted in light of the following limitations. First, we did not study the association between IL-1α and IL-1β because the immunoassay platform that we used was not sensitive.
enough to detect these cytokines in the majority of serum samples. Second, our results were adjusted to age, gender, BMI, consumption of alcohol and tobacco, and use of recreational drugs, but not to other variables that may be associated with either cytokine levels or clinical outcome such as socioeconomical status, physical exercise, diet. Third, patients with an autoimmune or an allergic disease, with an infectious disease and/or who have been treated anti-inflammatory drugs, corticosteroids or antibiotics within the prior months were not excluded. Third, we only assessed the association between inflammation at baseline and clinical outcome in FEP patients after 4–5 weeks of treatment with amisulpride. It remains to be established whether similar results would be obtained when patients are treated with another antipsychotic and for a longer period. Fourth, the serum samples from healthy individuals that we have used in this study were not collected as part of the OPTiMiSE clinical trial but as part of another clinical study. However, because the Standard Operating Procedures (SOP) for blood collection and serum preparation were the same in the 2 studies, and all serum samples were assessed in the same laboratory using the same procedure, we believe that biomarker levels could readily be compared across samples.

Implications and Future Directions

The present study does not support an association between baseline levels of biomarkers of inflammation and early clinical outcomes in FEP patients. However, our data do not rule out the possibility that inflammatory biomarkers could predict long-term clinical outcomes such as relapse in chronic schizophrenia. Testing this hypothesis in a prospective study is warranted. While the results presented here could be considered as disappointing, we believe that publication of these negative findings is important for the field of immunopsychiatry to move forward.

Supplementary Material

Supplementary data are available at Schizophrenia Bulletin Open online.

Funding

We thank all the patients who participated in the OPTiMiSE study. The OPTiMiSE study was funded by the European Commission within the 7th Program (HEALTH-F2-2010-242114). This work was also supported by the Investissements d’Avenir program managed by the Agence Nationale pour la Recherche (ANR) under reference ANR-11-IDEX-0004-02 (Labex BioPsy) (to WBHA). Additional financial support was obtained from the Fondation de France (NG) and the Fondation FondaMental (NG), the LABEX SIGNALIFE (#ANR-11-LABX-0028-01), and the FHU OncoAge. ML’s team is affiliated with the Paris School of Neuroscience (ENP) and the Bio-Psy Laboratory of Excellence.

Acknowledgments

RSK declares personal fees for consultancy from Alkermes, Minerva Neuroscience, Gedeon Richter, and Otsuka; and personal (speaker) fees from Otsuka/Lundbeck. SL declares fees for consulting from LB Pharma, Lundbeck, Otsuka, Teva, and Gedeon Richter; and fees for lectures from Janssen, Eli Lilly, Lundbeck, Otsuka, Sanofi, and Servier. SWL declares personal fees from Otsuka. CA declares grants, personal fees, and other fees from Janssen-Cilag, Lundbeck, Otsuka, Acadia, Abbott, Amgen, AstraZeneca, Bristol-Myers Squibb, Caja Navarra, Cibersam, Fundación Alicia Koplowitz, Forum, Instituto de Salud Carlos III, Gedeon Richter, Merck, Ministerio de Ciencia e Innovación, Ministerio de Sanidad, Ministerio de Economía y Competitividad, Mutua Madrileña, Pfizer, Roche, Servier, Shire, Schering Plough, Sumitomo Dainippon Pharma, Sunovio, and Takeda. SH declares personal fees from Janssen, Eli Lilly, Otsuka/Lundbeck, and Teva. CMD-C declares grants from the European Commission Seventh Framework Programme, Instituto de Salud Carlos III, Spanish Ministry of Economy and Competitiveness, and Fundación Alicia Koplowitz. SG declares grants from the European Union; personal fees from Janssen, Janssen-Cilag Polska, Gedeon Richter, Recordati, Pierre Fabre, Angelini-Acraf, and Forum; and grants and personal fees from Otsuka. BG declares grants from Janssen, Lundbeck, Boeringer-Ingelheim, and Otsuka, and personal fees from Teva, Dainippon-Sumitomo, Allergan, Gedeon Richter, and Recordati. IES declares consultancy fee from Gabater and a grant from Janssen. All remaining authors declare no competing interests.

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