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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

Susana Barbosa^{1,2*}, Emanuela Martinuzzi^{1*}, Iris E. Sommer^{3,4}, Paola Dazzan⁵, Philip McGuire⁵, Celso Arango⁶, Covadonga M. Diaz-Caneja⁶, W. Wolfgang Fleischhacker⁷, Dan Rujescu⁸, Birte Glenthøj^{9,10}, Inge Winter-van Rossum¹¹, René Sylvain Kahn^{11,12}, Robert Yolken¹³, Shon Lewis¹⁴, Richard Drake¹⁴, Stefan Leucht¹⁵, Cyprien Gilet¹⁶, Olfa Khalfallah¹, Laetitia Davidovic¹, El Chérif Ibrahim¹⁷, Raoul Belzeaux^{17,18,19}, Marion Leboyer^{19,20,21}, Nicolas Glaichenhaus^{1,2,19} and the OPTiMiSE Study Group

¹ Université Côte d'Azur, Centre National de la Recherche Scientifique, Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne, France

² Clinical Research Unit, Centre Hospitalier Universitaire de Nice, France

³ Rijks Universiteit Groningen, University Medical Center Groningen, Department of Neuroscience and Department of Psychiatry, Groningen, Netherlands

⁴ University of Bergen, Department of Medical and Biological Psychology, Bergen, Norway

⁵ Department of Psychosis Studies, Institute of Psychiatry, National Institute for Health Research, Mental Health Biomedical Research Centre, King's College London, London, UK

⁶ Department of Child and Adolescent Psychiatry, Institute of Psychiatry and Mental Health, Hospital General Universitario Gregorio Marañón, IISGM, CIBERSAM, Universidad Complutense, Madrid, Spain

⁷ Department of Psychiatry, Psychotherapy and Psychosomatic Medicine, Medical University Innsbruck, Austria

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⁸ Department of Psychiatry, University of Halle, Halle, Germany

⁹ Center for Neuropsychiatric Schizophrenia Research and Center for Clinical Intervention and Neuropsychiatric Schizophrenia Research, Mental Health Center Glostrup, Denmark

¹⁰ University of Copenhagen, Glostrup, Denmark & University of Copenhagen, Faculty of Health and Medical Sciences, Dept. of Clinical Medicine, Copenhagen, Denmark

¹¹ UMC Utrecht Brain Center, Department of Psychiatry, University Medical Center Utrecht, The Netherlands

¹² Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA

¹³ John Hopkins School of Medicine, The John Hopkins Hospital, Baltimore, USA

¹⁴ Division of Psychology and Mental Health, School of Health Sciences, University of Manchester, United Kingdom

¹⁵ Technische Universität München, Klinik und Poliklinik für Psychiatrie und Psychotherapie

¹⁶ Université Côte d'Azur, Centre National de la Recherche Scientifique, Laboratoire Informatique Signaux et Systèmes de Sophia Antipolis, Sophia Antipolis, France

¹⁷ Aix-Marseille Univ, CNRS, Institut des Neurosciences de la Timone, Marseille, France

¹⁸ Assistance Publique Hôpitaux de Marseille, Sainte Marguerite Hospital, Pôle de Psychiatrie Universitaire Solaris, Marseille, France.

¹⁹ Fondation FondaMental

²⁰ Université Paris Est Créteil, INSERM, Institut Henri Mondor the Recherche Biomédicale, Laboratoire Neuro-Psychiatrie Translationnelle, Créteil, France

²¹ Assistance Publique Hôpitaux de Paris, Hôpitaux Universitaires Henri Mondor, Département Médico-Universitaire de Psychiatrie et d'Addictologie

* These authors contributed equally to this article

Corresponding author mailing address, phone and email

Nicolas Glaichenhaus, Institut de Pharmacologie Moléculaire et Cellulaire, 660 Route des Lucioles, 06560 Valbonne, France, Tel: +33 (0) 618994 461; nicolas.glaichenhaus@univ-cotedazur.fr

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Optimise Study Group

René S Kahn, Iris E Sommer, Inge Winter-van Rossum, Metten Somers, Paula C Ywema, Shitisj Kapur, Philip McGuire, Marion Leboyer, Andreas Meyer-Lindenberg, Shon W Lewis, Stefan Leucht, Celso Arango, Wolfgang W Fleischhacker, Anne Lotte Meijering, Jocelyn Petter, Resy Van de Brug, Joost Schotsman, Jildou Zwerver, Jos Peuskens, Marc De Hert, Erik Thys, Lucho G Hranov[†], Valentin Hranov, Jan Libiger, Richard Köhler, Pavel Mohr, Birte Glenthøj, Brian Broberg, Signe Düring, Lone Baandrup, Stephane Jamain, Stephan Heres, Dan Rujescu, Ina Giegling, Mark Weiser, Mor Bar Heim, Michael Davidson, Silvana Galderisi, Paola Bucci, Armida Mucci, Janusz Rybakowski, Agnieszka Remlinger Molenda, Ilan Gonen, Paul Radu, Marina Díaz-Marsá, Alberto Rodriguez, Tomas Palomo, Roberto Rodriguez-Jimenez, Paz García-Portilla, Miquel Bernardo, Julio Bobes, Christina Vilares Oliveira, Gregor Berger, Claudia Wildt, Paola Dazzan, Roccio Perez-Iglesias, Richard Drake, Sarah Gregory, Danielle Wilson, Covadonga M Díaz-Caneja.

Abstract

Background: Patients with a First-Episode of Psychosis (FEP) exhibit low-grade inflammation as demonstrated by elevated levels of C reactive protein (CRP) and pro-inflammatory cytokines.

Aims: The primary goal of this study was to investigate the association between pro-inflammatory biomarkers and clinical outcomes in unmedicated FEP patients.

Method: We used clinical data and biological samples from 289 FEP patients participating to 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 four 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.

Results: 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.

Implications: 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

Keywords: Inflammation, pro-inflammatory 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 FEP patients remain to be identified ¹. 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 ². 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 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 ³. In this latter case, the following formula was used: $(\text{baseline PANSS total score} - 30) - (\text{follow-up PANSS total score} - 30) / (\text{baseline PANSS total score} - 30) \times 100$. 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 ⁴, diagnostics tools based on biological fluids may be more cost and time efficient. Indeed 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 CRP ⁵⁻⁷, many interleukins ^{5, 6} and chemokines ⁶, TNF- α ^{5, 6}, interferon- γ (IFN- γ) ^{5, 6}, vascular endothelial growth factor (VEGF) ^{5, 6}, complement component 4 (C4) ⁸, lipids and glucometabolic biomarkers (glycated hemoglobin (HbA1c), triglycerides (TG), total cholesterol, Low Density Lipoprotein (LDL) cholesterol, fasting glucose) ⁷, redox biomarkers (glutathione (GSH), GSH peroxidase and reductase activities (GPx, GR), thioredoxin (Trx), and GSH-related metabolites), amino acids ⁹ and stress biomarkers (cortisol, sulfated dehydroepiandrosterone (DHEA-S)) ¹⁰. In a 12 weeks 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 non-responders in the early phases of

psychosis ⁵. 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 ⁷. Among the three identified PCA factors, factor 1 that accounted for hsCRP and Body Mass Index (BMI), was associated with treatment response at one year. Lastly, in a naturalistic longitudinal study on 25 FEP patients, serum levels of complement component 4 (C4) were associated with treatment response at one year after adjustment for baseline severity of symptoms and CRP levels ⁸.

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, multi-center, randomized, double-blind OPTiMiSE study in which 481 FEP patients were treated with the second-generation antipsychotic amisulpride ¹¹. We assessed serum samples collected at baseline for well-studied biomarkers of inflammation, i.e. IL-6, IL-8, TNF- α and CRP. We then used multivariable regression to study the association between these biomarkers and PANSS score and sub-scores, 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-naïve FEP and acute psychotic relapse are associated with increased serum concentrations of IL-6 ¹², TNF- α ¹² and CRP ¹³. Second, population-based longitudinal studies have shown an association between higher levels of IL-6 ¹⁴ and CRP ¹⁵ and risk for psychotic experience and a diagnosis of schizophrenia subsequently in adulthood. Third, IL-6, IL-8, and TNF- α are three of the main proinflammatory cytokines that are produced by macrophages when activated with Pathogen-Associated Molecular Patterns (PAMPs) ¹⁶. Fourth, IL-8 is a potent chemotactic factor that cause 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, body mass index (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 CDSS²⁰, social functioning using the Personal and Social Performance Scale (PSP) 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 al. ² (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 milliliters of blood were drawn into serum Vacutainer tubes and allowed to clot for 1 h before centrifugation (1500 x g, 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 standard deviations. 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 standard deviation (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 algorithm²⁴, with the aim to identify two 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 one. 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 two-tailed non-parametric 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 two-tailed non-parametric test followed by post-hoc analysis. To study associations at baseline clusters based on pro-inflammatory 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 sub-scores at baseline, age (years), sex (Female/Male), BMI (kg/m²), 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/non-remission) as the dependent variable and cluster, PANSS sub-scores at baseline, age, sex, BMI, waist circumference, use of recreational drugs, alcohol consumption and current smoking as independent variables. To study associations between PANSS sub-scores after treatment and cluster at baseline, we implemented a multivariable linear regression model using PANSS sub-scores after treatment as the dependent variables and cluster, PANSS sub-scores 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 (p) < 0.05. Statistical analyses were performed using R Stats package²⁵ 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 (ITT) 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 pro-inflammatory 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, recreative 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 (GPANSS) (**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 four 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 pro-inflammatory 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 two 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-distributed Stochastic Neighbor Embedding (t-SNE) graphical representation reflecting high intra-cluster 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 recreative 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 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 more 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 four 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 SEAs. We performed two 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 recreative 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 two 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's 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 were at increased risk for later schizophrenia²⁶. CRP is produced mainly in the liver in response to inflammation²⁷ and is commonly used to assess the presence and severity of inflammation in a wide range of diseases. However, CRP is an ambiguous pro-inflammatory marker²⁸ as it is also constitutively produced in the absence of inflammation in a pentameric form (pCRP) with anti-inflammatory properties²⁹. 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 pro-inflammatory effects²⁸. Because pro-inflammatory 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 Gangestad 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 pro-inflammatory cytokines such as TNF- α and IL-1 β could be more reliable as biomarkers of inflammation²⁸. Our results not only support this view but also suggest that combinations of pro-inflammatory 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 ⁵. In this study, 68 FEP patients – among whom 33 were sampled at baseline – were treated as usual (TAU) and assessed 12 weeks later to identify those who met remission criteria ⁵. In these 33 patients among whom 18 and 21 were responders and non-responders respectively, blood levels of IL-6 and IFN- γ were lower in responders compared to non-responders ⁵. 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, i.e. 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 recreative 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 ⁷. To this aim, they measured high sensitivity C-reactive protein (hsCRP) as a biomarker of inflammation, and BMI, lipid profile and gluco-metabolic parameters as metabolic variables. A principal component analysis (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 triglycerides (TG) 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 naïve (or almost medication naïve) 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 two noticeable exceptions^{30, 31}, we are not aware of any other study in which pro-inflammatory 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-1 α and IL-1 β 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 as 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 two 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 disappointed, we believe that publication of these negative findings is important for the field of immuno-psychiatry to move forward.

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Conflict of interest

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 the Lundbeck Foundation and Lundbeck. MJCE declares grants from the European Union. WWF declares grants and personal fees 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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