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Regulation of inflammatory pathways in schizophrenia: A comparative study with bipolar disorder and healthy controls

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ABSTRACT

Background: Immune-inflammatory processes have been implicated in schizophrenia (SCH), but their specificity is not clear.
Main aim: To identify potential differential intra-/intercellular biochemical pathways controlling immune-inflammatory response and their oxidative-nitrosative impact on SCH patients, compared with bipolar disorder (BD) patients and healthy controls (HC).
Methods: Cross-sectional, naturalistic study of a cohort of SCH patients ($n = 123$) and their controls [BD ($n = 102$) and HC ($n = 80$)].
Statistical analysis: ANCOVA (or Quade test) controlling for age and gender when comparing the three groups, and controlling for age, gender, length of illness, cigarettes per day, and body mass index (BMI) when comparing SCH and BD.
Results: Pro-inflammatory biomarkers: Expression of COX-1 was statistically higher in SCH and BD than HC ($P < 0.0001$; $P < 0.0001$); NFκB and PGE2 were statistically higher in SCH compared with BD ($P = 0.001$; $P < 0.0001$) and HC ($P = 0.003$; $P < 0.0001$); NLRP3 was higher in BD than HC ($P = 0.005$); and CPR showed a gradient among the three groups. Anti-inflammatory biomarkers: BD patients had lower PPARγ and higher 15d-PGJ2 levels than SCH ($P = 0.005$; $P = 0.008$) and HC ($P = 0.001$; $P = 0.001$). Differences between SCH and BD: previous markers of SCH (NFκB and PGE2) and BD (PPARγ and 15d-PGJ2) remained statistically significant and, interestingly, iNOS and COX-2 (pro-inflammatory biomarkers) levels were statistically higher in SCH than BD ($P = 0.019$; $P = 0.040$).
Conclusions: This study suggests a specific immune-inflammatory biomarker pattern for established SCH (NFκB, PGE2, iNOS, and COX-2) that differentiates it from BD and HC. In future, their pharmacological modulation may constitute a promising therapeutic target.

1. Introduction

Schizophrenia (SCH) is a severe, complex, multifactorial disorder that affects approximately 0.7% of the world population [1,2]. In recent years, there have been changes in the approach to SCH, with a focus on the search for biological markers [3–7]. In this sense, there is renewed interest in immune-inflammatory changes and their associated oxidative-nitrosative consequences as key pathophysiological mechanisms of the neuroprogressive pathways of this disorder [8].

Several hypotheses involving inflammatory processes caused both by external and endogenous factors have been implicated in SCH [8–11]. Inflammation is a complex biological protective mechanism, but when excessive in intensity or time, it becomes harmful. Intracellular events, such as cytoplasmic/-nuclear tran-

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scription factors, mainly kappaB (NFκB), control the expression of several oxidative and nitrosative mediators through activation of inducible enzymes, as key factors in this regulation. Furthermore, intercellular elements such as cytokines and chemokines are crucial elements of proper inflammatory response. Such a complex defense mechanism is finely regulated by compensatory anti-inflammatory pathways [12]. One of these mechanisms involves cyclopentenone prostaglandins (PGs) such as 15-deoxy-Δ12,14-PGJ2 (15d-PGJ_2) [13], one of the proposed endogenous ligands for the gamma isoform of peroxisome proliferator-activated nuclear receptors, PPARγ. The PPARγ is a transcription factor that mitigates inflammation by repressing the expression of pro-inflammatory cytokines and the inducible isoforms of COX and NOS: COX-2 and iNOS [14,15].

Early studies in SCH described elevations in plasma levels of pro-inflammatory cytokines [16,17] and decreases in anti-inflammatory cytokines [18]. However, recent studies focus on intra- and intercellular biochemical pathways controlling inflammatory response and found a systemic imbalance in some pro-/anti-inflammatory mediators in these patients [11]. Most of the imbalance studies have been carried out in early stages of the disease: subtle alterations in immune-inflammatory mediators and oxidative-nitrosative stress have already been found at disease onset [19,20]. In particular, there was an increase in levels of pro-inflammatory NFκB, iNOS and COX-2 in patients with a first episode of psychosis (FEP) compared with healthy controls (HC) [21] and of PGE2 in patients with established SCH [22]. Furthermore, the inhibitory subunit of NFκB, 15d-PGJ_2, and PPARγ expression and transcriptional activity were lower in FEP patients [21] along with anti-inflammatory PGs in peripheral monocytes in patients with established SCH [23]. In addition, the systemic pro-/anti-inflammatory deregulation found in FEP became more severe after a 1-year follow-up [24].

C-reactive protein (CRP) is a widely used biomarker of systemic inflammation, and higher CRP levels have been reported in SCH compared with HC patients [25–29], even in patients without antipsychotic treatment [30]. For homocysteine (Hcy), an intermediate amino acid containing a sulfhydryl radical that can act as an oxidant, the results are controversial. While some studies found higher levels of this oxidative stress biomarker in several subgroups of SCH [31–33] compared with HC patients [31,34], others did not [35,36].

A review of the literature suggests that there could be an overlap in peripheral immune-inflammatory mechanisms across severe mental disorders (SMD), what justify our research [37]. For example, Goldsmith et al. (2016) describe similarities in the pattern of cytokine alterations in SCH, bipolar disorder (BD), and major depressive disorder (MDD) during the acute (significant increases of IL-6, TNF-α, sIL-2R, and IL-1RA) and chronic (significant increases of IL-6, sIL-2R, and IL-1β) phases of illness that may suggest the existence of common underlying pathways for immune dysfunction [38]. Thus, it is necessary to evaluate markers of inflammation and immune activation across the whole psychosis continuum. In this sense, it was recently found that there is a strong increase in the levels of inflammatory activity in SCH and a relatively lesser increase in schizoaffective and affective disorders respectively [39]. In line with the proposed psychosis continuum model, the aim of our study was to identify the potential differential intra- and intercellular biochemical pathways controlling inflammatory response and their oxidative-nitrosative consequences in SCH, compared with BD and healthy controls (HC). An additional aim was to identify whether there are differential pathways of the psychopathological (positive, negative, and depressive), cognitive, and functional dimensions of SCH.

2. Methods

2.1. Study design

Cross-sectional, naturalistic study of a cohort of SCH patients in outpatient treatment at two mental health centers in Oviedo (Corredoria and Ería) in northern Spain, and their controls (BD patients and HC). The Clinical Research Ethics Committee of Hospital Universitario Central de Asturias in Oviedo approved the study protocol. All participants gave written informed consent prior to their enrollment.

2.2. Participants

Of the 325 participants recruited, a total of 305 individuals were included in the analysis after removing outliers. Of these, 123 were SCH patients (mean age 40.75, 67.5% males), 102 BD patients (mean age 48.37, 37.3% males), and 80 HC (mean age 35.81, 38.8% males). If an alfa error of 5% with a power of 90% is considered for the ANCOVA tests performed in this work, an effect size value of f = 0.2634 is obtained. This value, according to Cohen (1988) can be considered as a medium effect size and suitable for our research purposes [40].

Outpatients attending their regular appointments with their clinicians were offered to participate in the study and healthy controls were recruited by snowball sampling. There were statistically significant differences among the groups in age and gender ($F = 27.668$, $P < 0.0001$; $Chi^2 = 25.707$, $P < 0.0001$).

Inclusion criteria for SCH and BD were: (1) DSM-IV-TR diagnosis of SCH or BD; (2) age > 17 years; and (3) written informed consent. Inclusion criteria for HC: (1) no past or current mental disorder (DSM-IV-TR diagnosis) and (2) written informed consent. Exclusion criteria for patients and controls were: (1) no written informed consent; (2) physical comorbidity that could interfere with immune-inflammatory biomarkers (acute infection, fever, acute allergies, cancer, or autoimmune diseases) was determined by directly asking patients about them; (3) treatment with immunosuppressive drugs or vaccines within the 6 months prior to enrollment in the study, or treatment with anti-inflammatory drugs within the two days prior to blood collection.

2.3. Assessments

2.3.1. Psychometric instruments

Psychopathology in SCH patients was evaluated using the Spanish versions of the Clinical Global Impression (CGI) [41], which assesses the severity in global psychopathology, and the Positive and Negative Syndrome Scale (PANSS) [42], which measures the severity of positive, negative, and general psychopathology symptoms. In addition, the Negative Symptom Assessment-16 (NSA-16) [43] and the Hamilton Depression Rating Scale (HDRS) [44] were employed to assess the severity of negative and depressive symptoms, respectively. The Screen for Cognitive Impairment in Psychiatry (SCIP) [45] was used to assess cognition. Finally, to assess patient functioning, we used the Personal and Social Performance (PSP) [46].

2.3.2. Specimen collection and preparation

Venous blood samples (10 mL) were collected at 8:00 am after fasting overnight.

2.3.3. Biochemical analyses of PBMC samples

To perform all biochemical analyses, PBMC samples were first fractionated into cytosolic and nuclear extracts:

- preparation of cytosolic and nuclear extracts: to obtain a high purity nuclear fraction, practically without cytosolic contami-

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nation [24] in order to ensure isolation of active components of transcription factors a widely utilized method was employed;
- western blot analysis: the protein levels of inducible nitric oxide synthase (iNOS), cyclooxygenases 1 and 2 (COX-1, COX-2), NLRP3 inflammasome, and the inhibitory subunit of NFκB, IκBα, in the cytosolic extracts and the protein levels of NFκB, the gamma isoform of peroxisome proliferator-activated nuclear receptors, PPARγ, and the Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) in the nuclear extracts from PBMC samples were quantified by western blot (WB) analysis.

2.3.4. Biochemical analyses in plasma and serum

Prostaglandin levels: plasma levels of COX byproducts PGE2 and 15d-PGJ_2 were measured by enzyme immunoassay (EIA) using reagents in kit form (Prostaglandin E2 EIA Kit-Monoclonal, Cayman Chemical Europe, Tallinn, Estonia; and 15-deoxy-Δ12,14- Prostaglandin J2 ELISA Kit, DRG Diagnostics, Marburg, Germany, respectively) following manufacturer's instructions.

Lipid peroxidation: this was assayed by thiobarbituric acid reactive substances (TBARS) assay (Cayman Chemical Europe), based on the reaction of malondialdehyde (MDA) and thiobarbituric acid (TBA) at high temperature (95 °C) and acidic conditions. The MDA-TBA adduct formed was measured colorimetrically at 530–540 nm (Synergy 2).

Hcy and CRP: both were determined at the Hospital Universitario Central de Asturias (HUCA) laboratory. Plasma levels of homocysteine (Hcy) were obtained by inmunochemiluminescence (Inmulite 2000 system, SIEMENS) and serum levels of CRP by immunoturbidimetric analysis (CRPLX, Roche/Hitachi Cobas c501).

2.4. Statistical analyses

Demographic variables are described as means (SD) and percentages. The outliers of the immune-inflammatory markers were identified by SPSS in the box plots by deleting the individual data points and removed before any statistical analysis. The number of outliers was different for each inflammatory and oxidative-nitrosative marker. The most frequently marker removed was 15d-$PGJ_2$for SCH and HC while for BD disorder was PGE2. Differences between SCH patients and both control groups (BD patients and HC) were assessed using an analysis of covariance (ANCOVA) when the assumptions were met; otherwise, a Quade test [47] — a non-parametric alternative — was used. As there were statistically significant differences among the three groups in age and gender, we controlled for these variables when comparing them. In addition, when comparing SCH and BD patients, we controlled for other potential confounders (length of illness in years, number of cigarettes per day, and body mass index [BMI]). A post hoc analysis (Bonferroni) was done to determine which groups had significant differences.

Finally, to determine the potential differential biomarkers for the psychopathological, cognitive, and functional dimensions of SCH, partial correlations with Bonferroni correction were used in which we controlled for potential confounders (age, length of illness in years, number of cigarettes per day, BMI, and number of antipsychotics). In addition, to control for gender, these same partial correlations were done for men and women separately.

3. Results

3.1. Demographic and clinical characteristics of the sample of SCH patients

The majority were male, never married, living with their family of origin, had a primary or secondary level of education level and a work status of "not working" (Table 1). The mean length of illness was 13.85 (10.9) years, and 56.1% of the patients had a more than 10-year history.

3.2. Pharmacological treatment of SCH and BD patients

The pharmacological treatment of SCH patients is described in Table 1. Regarding BD patients, 100% were under mood stabilizers (42.6% lithium, 38.2% quetiapine, 27.7% valproic acid, 5% lamotrigine and 1% oxcarbamacepine), 35.3% were under antipsychotics (11.8% olanzapine, 6.9% aripiprazole, 5.9% paliperidone, 2% paliperidone one-month long-acting injectable, 5.9% risperidone and 3% others), 49% antidepressants (28.6% SSRI, 8.8% SNRI, 3.9% tricyclics and 8.8% others) and 61.8% benzodiazepines.

3.3. Biomarkers of inflammation in SCH patients compared with BD patients and HC

Table 2 and Figs. 1 and 2 show the comparisons among the three groups after controlling age and gender. Regarding the pro-inflammatory parameters, expression of NFκB, a crucial inflammatory transcription factor, was higher in SCH than in BC and in HC ($P < 0.001$, $P = 0.003$, respectively), although no difference was found between BD and HC. COX-1 expression was statistically higher in both patient groups than in HC ($P < 0.0001$), although no differences were found between SCH and BD. Similarly, one of its soluble products, PGE2, was higher in SCH when compared with BC and with HC ($P < 0.001$). Plasma levels of CPR described a gradient among the three groups, that was statistically higher in SCH and BD patients than in HC ($P < 0.0001$, $P = 0.048$), and levels were higher in SCH than in BD patients ($P = 0.031$).

Regarding anti-inflammatory parameters (nuclear expression of PPARγ, a transcription factor that mitigates inflammation, and plasma levels of 15d-PGJ_2, an anti-inflammatory PG and one of the endogenous PPARγ ligands), no differences were found between SCH patients and HC. However, BD patients showed changes in these anti-inflammatory pathways: lower PPARγ and higher 15d-PGJ_2 levels than SCH patients ($P = 0.005$; $P = 0.008$) and HC ($P = 0.001$; $P = 0.001$).

No between-group differences were found in other oxidative/antioxidative parameters.

3.4. Biomarkers of inflammation in SCH compared with BD

The results of the comparison between SCH and BD after controlling for age, gender, length of illness, number of cigarettes per day, and BMI are shown in Table 3. As can be observed, the changes in inflammatory markers in SCH (NFκB, $P = 0.001$, PGE2, $P < 0.0001$) and anti-inflammatory markers in BD (PPARγ, $P = 0.041$, 15d-PGJ_2, $P = 0.028$) remain statistically significant. Interestingly, after controlling for these new confounders, the inducible isoforms of the specific inflammatory enzymes iNOS and COX-2 show statistically higher levels in SCH than BD ($P = 0.019$; $P = 0.040$). However, the differences in expression of constitutive COX-1 and plasma CRP levels between SCH and BD disappear.

3.5. Potential differential inflammatory biomarkers of the psychopathological, cognitive, and functional dimensions of SCH

In males, there was a slight positive correlation of the positive dimension with PPARγ ($r = 0.27$, $P < 0.05$) and the four measures of the negative dimension with Hcy (PANSS negative $r = 0.34$, $P < 0.01$; PANSS Marder Negative Factor $r = 0.38$, $P < 0.01$; NSA global $r = 0.27$, $P < 0.05$; NSA total $r = 0.38$, $P < 0.01$). Furthermore, the cognitive dimension negatively correlated with PPARγ

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Table 1
Demographic and clinical characteristics of the sample of patients with schizophrenia.

Mean age (sd)	40.75 (10.37)	Suicide attempts	
Gender, males [*n* (%)]	83 (67.5)	Yes [n (%)]	35 (28.5)
Civil status [*n* (%)]		Mean number (sd)	3.17 (2.90)
Never married	82 (66.7)	CGI-S [mean (sd)]	4.20 (1.03)
Married or cohabiting	29 (23.6)	PANSS [mean (sd)]	
Widowed or separated/divorced	12 (9.8)	Positive	13.50 (5.42)
Living arrangement [*n* (%)]		Negative	18.93 (5.07)
Alone	19 (15.4)	Marder Negative Factor	17.78 (5.85)
Family of origin	69 (56.1)	General psychopathology	32.96 (8.56)
Own family	31 (25.2)	Total	65.56 (15.66)
Institutionalized	2 (1.6)	NSA-16 [mean (sd)]	42.96 (12.16)
Other	2 (1.6)	HDRS [mean (sd)]	9.30 (7.15)
Educational level [*n* (%)]		Cognition	
Primary school	45 (36.6)	SCIP [mean (sd)]	2.87 (1.78)
Secondary school	54 (43.9)	Functioning	
Higher education	24 (19.5)	PSP [mean (sd)]	49.13 (16.23)
Years of education [mean (sd)]	13.02 (4.81)	BMI [mean (sd)][a]	29.39 (6.03)
Work status [*n* (%)]		Psychotropic medication	
Working (full / part-time)	9 (7.3)	Mean number (sd)	2.77 (1.46)
Not working	104 (84.6)	Antipsychotic medication	
Homemaker or student	10 (8.1)	Mean number (sd)	1.65 (0.84)
Alcohol		Yes [n (%)]	121 (98.4)
Consumption, yes [n (%)]	31 (25.2)	Paliperidone	58 (47.2)
SAUs / week [mean (sd)]	7.61 (10.48)	Risperidone	39 (31.7)
Tobacco[a]		Olanzapine	31 (25.2)
Consumption, yes [n (%)]	65 (52.8)	Aripiprazole	19 (15.5)
Cigarettes / day [mean (sd)]	22.68 (12.67)	Typical	17 (13.8)
Marihuana		Quetiapine	15 (12.2)
Consumption, yes [n (%)]	5 (4.1)	Clozapine	11 (8.9)
Days / last month [mean (sd)]	19 (15.10)	Ziprasidone	6 (4.9)
Months / last year [mean (sd)]	10.60 (3.13)	Amisulpride	6 (4.9)
Length of illness, years [mean (sd)][a]	13.85 (10.88)	Antidepressant medication	29 (23.6)
Hospitalizations		Yes [*n* (%)]	60 (48.8)
Yes [*n* (%)]	90 (73.8)	Benzodiazepine medication	65.56 (15.66)
Mean number (sd)	3.87 (4.98)	Yes [*n* (%)]	42.96 (12.16)

BMI: Body Mass Index; CGI-S: Clinical Global Impression-Severity; HDRS: Hamilton Depression Rating Scale; NSA-16: Negative Symptom Assessment-16; PANSS: Positive and Negative Syndrome Scale; PSP: Personal and Social Performance; SCIP: Screen for Cognitive Impairment in Psychiatry; sd: standard deviation; SAUs: standard alcohol units.

[a] Patients with BD: 45.1% smokers [mean of cigarettes per day among smokers: 17.17 (10.49)], mean length of illness 20.58 (11.85) years and BMI 28.98 (4.95).

Table 2
Comparisons of inflammatory and oxidative-nitrosative markers among groups after controlling for age and gender.

	SCH Mean, SE	BD Mean, SE	HC Mean, SE	Statistical test, *P*	η^2	SCH-BD Statistical test, p	SCH-HC Statistical test, p	BD-HC Statistical test, *P*
Pro-inflammatory								
NFkB	130.44, 5.72	93.37, 5.92	98.33, 6.25	7.850 (2)[b], 0.001		0.001	0.003	0.444
iNOS	113.54, 4.94	92.37, 5.28	93.96, 5.76	2.164 (2)[b], 0.117				
COX-1	129.56, 3.36	116.35, 3.99	97.54, 4.18	14.941 (2)[b], < 0.0001		0.130	< 0.0001	< 0.0001
COX-2	113.02, 4.66	94.90, 4.99	91.98, 5.69	2.502 (2)[b], 0.084				
PGE2	620.23, 33.12	278.20, 40.66	249.71, 42.72	21.514 (2)[b], < 0.0001		< 0.0001	< 0.0001	0.467
NLRP3	121.55, 5.41	127.50, 6.13	98.37, 6.74	3.370 (2)[b], 0.036		0.229	0.135	0.005
CRP	0.47, 0.05	0.30, 0.05	0.20, 0.06	8.855 (2)[b], < 0.0001		4.705 (1), 0.031	18.396 (1), < 0.0001	3.984 (1), 0.048
Anti-inflammatory								
PPARγ	109.68, 4.97	79.29, 5.56	100.15, 5.75	6.156 (2)[b], 0.002		0.005	0.916	0.001
15d-PGJ_2	48.58, 14.67	171.85, 16.27	6.07, 19.43	6.781 (2)[b], 0.001		0.008	0.289	0.001
IkBα	95.33, 4.67	96.82, 5.25	95.24, 5.45	0.140 (2)[b], 0.869				
Oxidants								
MDA	93.65, 6.82	91.62, 8.19	87.51, 8.44	0.158[a], 0.854	0.001			
Hcy	13.47, 0.44	13.08, 0.49	12.65, 0.55	0.693[a], 0.501	0.005			
Ant-ioxidants								
NRF2	135.62, 6.64	106.29, 7.18	97.99, 7.49	2.641 (2)[b], 0.074				

BD: bipolar disorder; COX-1 and COX-2: isoforms 1 and 2 of the enzyme cyclooxygenase; CRP: C-reactive protein; HC: healthy controls; Hcy: homocysteine; IkBα: inhibitory subunit of NFκB; iNOS: inducible nitric oxide synthase; MDA: malondialdehyde; NFκB: nuclear factor kappaB; NLRP3: NLRP3 inflammasome; NRf2: transcription factor NRf2; PGE2: prostaglandin E2; 15d-PGJ_2: prostaglandin J2; PPARγ: peroxisome proliferator-activated receptor gamma; SCH: schizophrenia; SE: standard error.

[a] ANCOVA.

[b] Quade test. Covariables: age, gender. See Figs. 1 and 2 for units of each parameter.

($r = -0.30$, $P < 0.05$) (Table 4). After Bonferroni correction only the correlations between Hcy and PANSS Negative, PANSS Marder Negative Factor and NSA total remained significant. None of the immune-inflammatory biomarkers correlated with the depressive dimension or functioning.

In females, the positive dimension positively correlated with Hcy ($r = 0.44$, $P < 0.05$) and, as in males, the cognitive dimension negatively correlated with PPARγ ($r = -0.44$, $P < 0.05$) (Table 5). However, after Bonferroni correction none of the correlations were significant.

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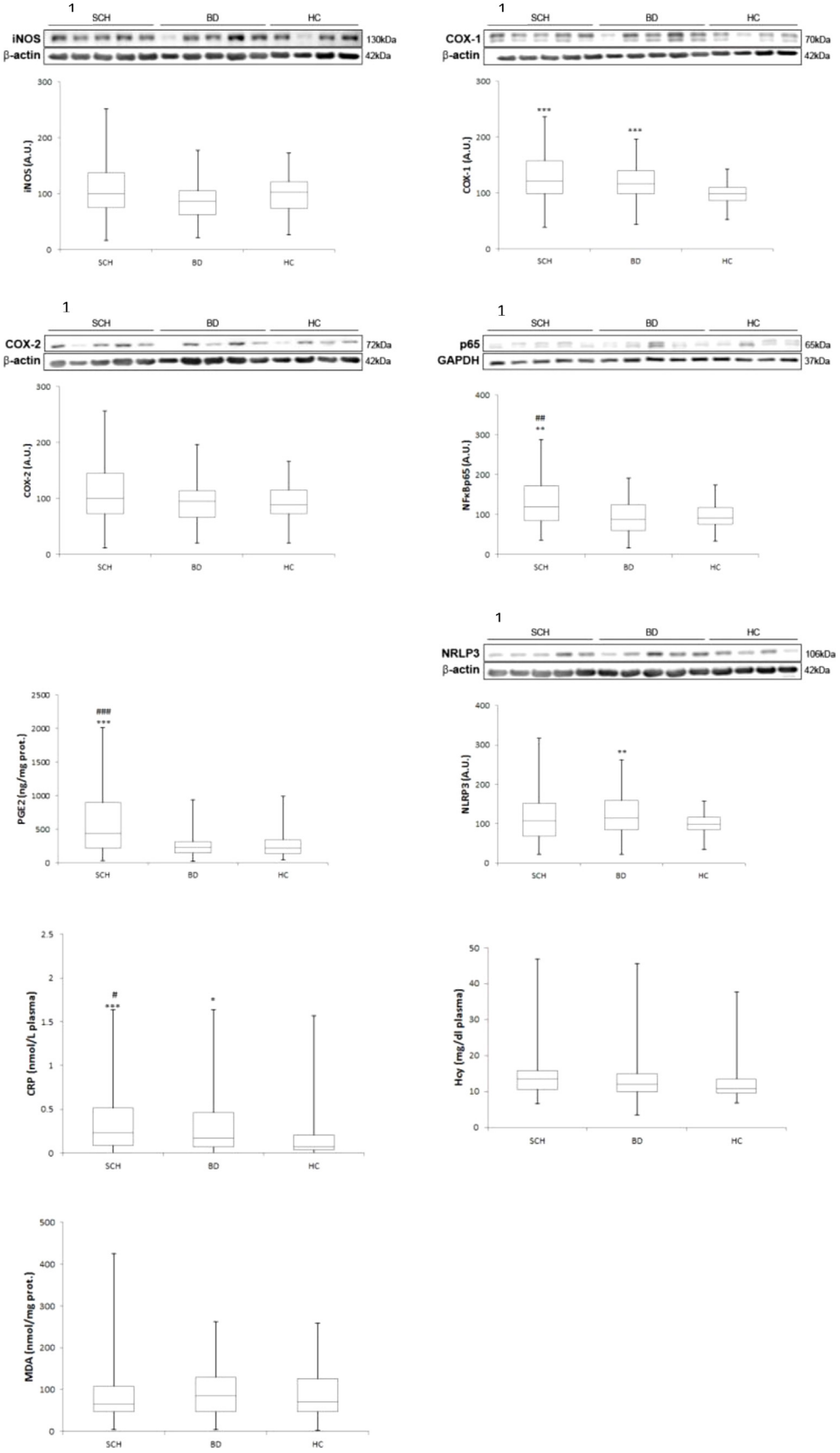

Fig. 1. Mean differences (SD) on inflammatory / oxidative components in plasma and PBMC in patients with schizophrenia compared with bipolar disorder and healthy controls. Western blot analysis of iNOS, COX-1, COX-2, NFkB p65 and NLRP3. Plasma levels of CRP, HCy and MDA. AU, arbitrary units. ANCOVA and Quade test were used. [1]Densitometric analysis of the proteins studied and with the corresponding housekeeping proteins used as loading control (in cytosolic and nuclear extracts). *: compared with HC; * $P \leq 0.05$; ** $P \leq 0.005$; *** $P \leq 0.0005$. $^{\#}$: compared with BD; $^{\#}$ $P \leq 0.05$; $^{\#\#}$ $P \leq 0.005$; $^{\#\#\#}$ $P \leq 0.0005$.

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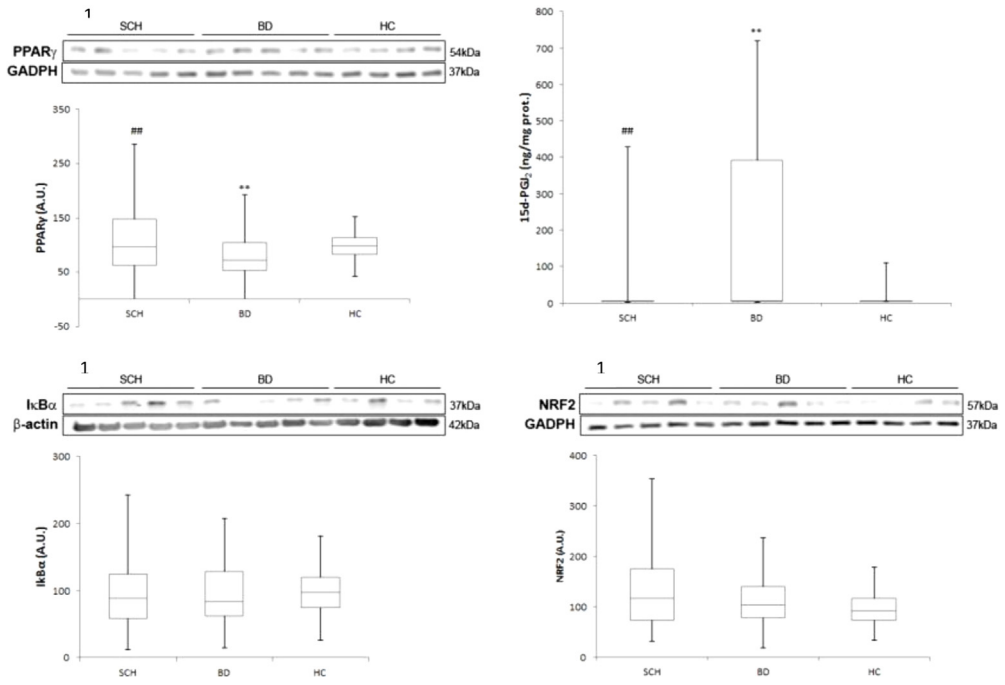

Fig. 2. Mean differences (SD) on antiinflammatory / antioxidant components in plasma and PBMC in patients with schizophrenia compared with bipolar disorder and healthy controls. Western blot analysis of PPARg, IkBa and NRF2. Plasma levels of 15d-PGJ_2. AU, arbitrary units. ANCOVA and Quade test were used. [1]Densitometric analysis of the proteins studied and with the corresponding housekeeping proteins used as loading control (in cytosolic and nuclear extracts). *: compared with HC; * $P \leq 0.05$; ** $P \leq 0.005$; *** $P \leq 0.0005$. #: compared with BD; # $P \leq 0.05$; ## $P \leq 0.005$; ### $P \leq 0.0005$.

Table 3
Comparisons of inflammatory and oxidative-nitrosative markers between SCH and BD after controlling for age, gender, length of illness, cigarettes/day, and BMI.

	SCH Mean, SE	BD Mean, SE	Statistical test, *P*	η^2
Pro-inflammatory				
NFkB	129.65, 6.54	94.24, 6.92	12.660[a], 0.001	0.089
iNOS	114.71, 5.48	87.49, 6.20	5.579 (1)[b], 0.019	
COX-1	131.25, 3.96	112.87, 4.91	3.432 (1)[b], 0.065	
COX-2	115.06, 5.05	89.74, 5.76	4.306 (1)[b], 0.040	
PGE2	615.51, 39.33	267.14, 50.83	18.754 (1)[b], < 0.0001	
NLRP3	122.39, 6.35	125.43, 7.47	0.088[a], 0.767	0.000
PCR	0.46, 0.05	0.32, 0.06	2.611\0.108	0.013
Anti-inflammatory				
PPARγ	108.42, 5.85	83.58, 6.85	4.262 (1)[b], 0.041	
15d-PGJ_2	43.07, 17.06	154.16, 19.35	4.894 (1)[b], 0.028	
IkBα	94.81, 5.17	93.52, 6.06	0.024[a], 0.877	0.000
Oxidants				
MDA	93.14, 7.35	90.19, 9.10	0.057[a], 0.811	0.000
Hcy	13.61, 0.46	13.30, 0.54	0.171\0.679	0.001
Anti-oxidants				
NRF2	136.18, 7.88	107.77, 8.59	2.182 (1)[b], 0.142	

BD: Bipolar Disorder; COX-1 and COX-2: Isoforms 1 and 2 of the enzyme cyclooxygenase; CRP: C-reactive protein; Hcy: Homocysteine; IkBα: inhibitory subunit of NFκB; iNOS: Inducible Nitric Oxide Synthase; MDA: Malondialdehyde; NFκB: Nuclear Factor KappaB; NLRP3: NLRP3 inflammasome; NRf2: Transcription Factor NRf2; PGE2: Prostaglandin E2; 15d-PGJ_2: Prostaglandin J2; PPARγ: Peroxisome Proliferator-Activated Receptor Gamma; SCH: Schizophrenia; SE: standard error.

[a] ANCOVA.

[b] Quade test. Covariables: age, gender, length of illness, cigarettes/day, and BMI.

4. Discussion

Our study adds support to the hypotheses of an inflammatory process underlying the pathophysiology of SCH, as we found a significant increase in the levels of crucial intra- and intercellular inflammatory response components: NFκB, iNOS, COX-2, and PGE2 in SCH patients compared with BD and HC. Furthermore, unlike the previous literature on the subject, our results allow us to suggest that these alterations may be considered a specific pro-inflammatory biomarker pattern for SCH, since we included patients with another SMD (BD) as a control group.

Regarding pro-inflammatory parameters, after controlling for age and gender, we found significantly higher levels of COX-1 in SCH and BD patients compared with HC. Therefore, COX-1 may be considered a biomarker of SMD. However, the little literature there is on this constitutive form of cyclooxygenase did not find significant differences in its levels in the post-mortem frontal cortex of SCH patients vs. HC [48]. We also identified a significant increase in other pro-inflammatory mediators, NFκB and PGE2, in SCH patients compared with BD patients and HC, suggesting that these mediators may be specific markers of SCH. The activity of these two markers has also been found to be significantly increased in FEP [21,24]. In patients with established SCH, PGE2 levels were significantly higher than in HC [22,23], while another study did not detect statistically significant differences between SCH and BD patients [49].

Previous studies on the widely used and nonspecific marker of CRP levels have also found higher levels in SCH than HC [25–30] independent of antipsychotic treatment [30] and disease progression [30]. The novelty of the present study is that this marker shows a differential gradient among the three groups, with significantly higher levels in patients with SCH than BD and HC, and also significantly higher levels in patients with BD than HC.

The comparison between patients with SCH and BD allowed us to control for some additional relevant confounding variables. In this case, the previous markers of SCH, NFκB and PGE2, remained statistically significantly increased, and two new inducible pro-inflammatory and pro-oxidant enzymes, iNOS and COX-2, reached statistical significance. On the contrary, the difference in CRP levels between SCH and BD disappeared, suggesting that it may be related to other associated inflammatory conditions, such as smoking, obesity, or chronicity. In general, our results indicate that

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Table 4
Partial correlation coefficients between psychopathological, cognitive, and functional dimensions of SCH and inflammatory and oxidative-nitrosative biomarkers after controlling for age, length of illness, cigarettes/day, and BMI (males).

	PANSS positive	PANSS negative	PANSS Marder Negative Factor	NSA global	NSA total	HDRS total	SCIP total	PSP total
Pro-inflammatory								
NFkB	0.07	−0.07	−0.12	−0.05	−0.15	0.05	−0.06	−0.10
iNOS	−0.10	0.19	0.12	0.26	0.25	0.03	0.07	−0.27
COX-1	−0.09	0.04	0.04	0.05	0.01	−0.08	0.07	0.09
COX-2	−0.16	0.04	0.03	0.09	0.00	0.05	0.14	0.03
PGE2	0.01	0.08	0.13	0.07	0.06	0.02	0.18	−0.07
NLRP3	−0.07	0.17	0.11	0.10	0.05	0.18	0.19	−0.17
CRP	0.01	−0.13	−0.17	0.04	0.00	−0.03	0.04	−0.02
Anti-inflammatory								
PPARγ	0.27*	−0.23	−0.20	−0.24	−0.17	−0.09	−0.30*	0.08
15d-PGJ_2	−0.15	−0.06	−0.07	−0.22	−0.14	0.00	−0.18	0.10
IkBα	0.11	0.06	−0.00	0.01	0.02	0.16	0.04	−0.13
Oxidants								
MDA	0.01	0.02	−0.00	0.05	0.05	0.11	0.10	−0.07
Hcy	−0.09	0.34**	0.38**	0.27*	0.38**	−0.07	0.09	−0.19
Anti-oxidants								
NRf2	0.13	−0.14	−0.17	−0.07	−0.09	−0.13	−0.26	−0.02

COX-1 and COX-2: isoforms 1 and 2 of the enzyme cyclooxygenase; CRP: C-reactive protein; HDRS: Hamilton Depression Rating Scale; Hcy: homocysteine; IkBα: inhibitory subunit of NFκB; iNOS: inducible nitric oxide synthase; MDA: malondialdehyde; NFκB: nuclear factor kappaB; NLRP3: NLRP3 inflammasome; NRf2: transcription factor NRf2; NSA-16: Negative Symptom Assessment-16; PANSS: Positive and Negative Syndrome Scale; SCIP: screen for cognitive impairment in psychiatry; PGE2: prostaglandin E2; 15d-PGJ_2: prostaglandin J2; PPARγ: peroxisome proliferator-activated receptor gamma; PSP: personal and social performance.
* < 0.05.
** < 0.01.

Table 5
Partial correlation coefficients between psychopathological, cognitive, and functional dimensions of SCH and inflammatory and oxidative-nitrosative biomarkers after controlling for age, length of illness, cigarettes/day, and BMI (females).

	PANSS positive	PANSS negative	PANSS Marder Negative Factor	NSA global	NSA total	HDRS total	SCIP total	PSP Total
Pro-inflammatory								
NFkB	0.41	0.07	0.17	0.03	0.01	0.20	−0.42	−0.14
iNOS	−0.00	0.03	0.13	−0.12	−0.08	0.21	0.17	−0.06
COX-1	0.07	−0.25	−0.20	−0.29	−0.34	−0.06	0.13	−0.04
COX-2	0.39	−0.25	−0.18	−0.08	−0.14	−0.10	0.07	−0.20
PGE2	−0.08	0.22	0.17	0.31	0.31	−0.02	0.33	−0.30
NLRP3	0.16	0.07	0.01	0.12	0.08	0.27	0.12	−0.29
CRP	0.15	−0.05	−0.00	0.13	0.10	0.06	0.04	−0.05
Anti-inflammatory								
PPARγ	0.06	−0.12	−0.13	−0.08	−0.14	−0.34	−0.44*,**	0.23
15d-PGJ_2	−0.05	−0.09	−0.10	−0.14	−0.17	0.05	0.21	0.08
IkBα	0.16	−0.03	−0.12	−0.18	−0.18	−0.07	0.05	−0.02
Oxidants								
MDA	0.27	−0.08	0.03	0.10	0.06	−0.01	−0.01	−0.06
Hcy	0.44*,**	−0.03	0.03	−0.07	−0.02	0.14	0.12	−0.12
Anti-oxidants								
NRf2	0.46	−0.09	0.02	−0.02	−0.04	0.12	−0.03	−0.04

COX-1 and COX-2: isoforms 1 and 2 of the enzyme cyclooxygenase; CRP: C-reactive protein; HDRS: Hamilton Depression Rating Scale; Hcy: homocysteine; IkBα: inhibitory subunit of NFκB; iNOS: inducible nitric oxide synthase; MDA: malondialdehyde; NFκB: nuclear factor kappaB; NLRP3: NLRP3 inflammasome; NRf2: transcription factor NRf2; NSA-16: Negative Symptom Assessment-16; PANSS: Positive and Negative Syndrome Scale; SCIP: screen for cognitive impairment in psychiatry; PGE2: prostaglandin E2; 15d-PGJ_2: prostaglandin J2; PPARγ: peroxisome proliferator-activated receptor gamma; PSP: personal and social performance.
* < 0.05.
** < 0.01.

the inflammatory component is more pronounced in SCH than in BD, as patients with SCH showed significantly higher NFκB, PGE2, iNOS, and COX-2 activity. It could be argued that our BD patients using lithium may have decreased levels of these biomarkers through the inhibition of GSK-3 [50]. However, when we compared them between BD patients with and without lithium we did not find any statistical significance (data not shown). Interestingly, after controlling for relevant confounding variables, specific intra- and intercellular inflammatory parameters remain significant in SCH, whereas other more nonspecific parameters disappear, supporting their possible role as trait biomarkers.

Regarding anti-inflammatory mediators, although previous studies in acutely decompensated chronic SCH [23] and FEP [21,24] found significantly lower levels of 15d-PGJ_2 and PPARγ, we found no significant differences between SCH patients and HC. A possible explanation could be a difference in functioning of the pro-/anti-inflammatory system over the stages/phases of SCH, i.e., while in the early and acute phases of the disorder, the anti-inflammatory system is able to fight against activation of the pro-inflammatory system, in the late stages when negative symptoms predominate this ability is lost. Another explanation could be BMI. While in the studies of Martínez-Gras et al. (2011) and García-

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Bueno et al. (2013; 2014) mean BMI was normal (24.9 kg/m^2), the cohort studied here had a mean BMI of 29.4, at the upper limit of overweight. Although more studies are needed, the results presented here point toward a possible role of negative symptoms and increased BMI in the lack of anti-inflammatory system response in chronic SCH. Interestingly, such a response is still present in BD patients, with lower levels of PPARγ and higher levels of 15d-PGJ$_2$ than the other two groups, which may represent compensatory mechanism.

With respect to Hcy, although it has been suggested that high levels are a risk factor for SCH [33,51–53], some studies, including our own research, find no differences between SCH patients and HC [35,36].

Regarding our aim of determining the potential differential pathways of the psychopathological (positive, negative, and depressive), cognitive, and functional dimensions of SCH, we found PPARγ was associated with the positive and cognitive dimensions. Higher levels of this anti-inflammatory parameter were related to lower cognitive impairment in both genders. Given the lack of pharmacological approaches to treating this dimension, this finding is of special importance and to our knowledge, this is the first report to show this relationship in chronic SCH patients. These results are in line with studies that have found a relationship between inflammation and poor cognitive performance [54]. Levels of inflammatory cytokines and higher levels of CRP have been related to cognitive impairment in SCH [55–60]. In SCH patients, PPARγ-dependent endogenous counterbalancing mechanisms seem to be active, compensating the inflammation and probably exerting the pro-energetic, neuroprotective, and antiexcitotoxic profile previously described in in vivo experimental models [61]. In FEP patients, after controlling for the possible effects of confounding factors, better performance on sustained attention tasks is associated with higher levels of anti-inflammatory signaling, which may suggest that this is also a protective factor for cognition [62]. In addition, a negative association has been reported between cognitive impairment and oxidative stress [20].

With regard to Hcy, high levels have been related to the severity of psychopathology [63,64], PANSS total score [65], and the acute phase of the disorder [66]. In our case, Hcy levels were related to the positive dimension in females and the negative dimension in males, so these data are difficult to interpret, and more studies will be necessary. In the case of women, these data seem to be in line with studies finding a relationship between higher levels of immune-inflammatory parameters and positive symptoms [25,67,68] or acute stages of the disorder [69]. Contrary, some studies have found a positive correlation between Hcy levels and the severity of negative symptoms [34,63,66].

Some limitations of this study should be noted. First, there are some issues concerning the information obtained from the sample. In this sense, the lack of clinical information (cigarettes/day and BMI) of the HC group could overestimate the biomarkers differences found between this group and the others. Furthermore, physical comorbidities were asked directly to patients, so this information could be not as accurate as expected. Secondly, it is a cross-sectional study, so we cannot make a causal argument for the observed associations. Thirdly, as SCH and BD differ with respect to age (younger age of onset and thus younger age for a similar length of illness in SCH versus BD) and gender (a greater proportion of females with BD than SCH), it was not possible to match the three groups for these variables. Thus, we had to control for these confounding variables as covariates. Moreover, the sample included stable SCH patients on outpatient maintenance treatment, so it is possible that these results are specific to this subgroup of patients, jeopardizing the generalizability of our results to other subgroups with SCH, such us unstable patients. Another potential weakness of the study is antipsychotics as a confounding variable because they have an anti-inflammatory effect and they can induce changes in the weight and metabolism. To that end, we controlled the number of antipsychotics that each patient to mitigate this effect, because the study design did not allow us to control either for type of antipsychotic or equivalent dose of haloperidol, given the different effects of the antipsychotics on endocrine-metabolic and immune-inflammatory biomarkers. Finally, assessment of the SCH dimensions was not as precise as we would have liked, especially with regard to the cognitive and depressive dimensions. Cognition was assessed with a screening test, the SCIP, instead of a more powerful cognitive battery, and the depressive dimension with the HDRS, which is a scale that also includes anxiety symptoms, making it possible to overestimate depressive symptoms.

Key strengths deserve mention. First, we used two control groups, healthy subjects and another SMD with the aim of controlling and thereby increasing the specificity of our results. Secondly, we included a broad spectrum of inflammatory markers in both PBMC and plasma samples, allowing in-depth insights into relationships between multiple components of the pro- and anti-inflammatory signaling pathways. Thirdly, for assessing the negative dimension of the SCH, in addition to the PANSS negative subscale, we used its Marder Negative Factor and one more specific scale, the NSA. Finally, we excluded people with physical comorbidities or treatment with immunosuppressive or anti-inflammatory drugs, which could interfere with the status of the study's immune-inflammatory biomarkers.

With regard to implications for clinical practice, although more scientific evidence is needed, this study provides further evidence of the inflammatory hypothesis of SMD. It proposes a specific cluster of inflammatory biomarkers for established SCH (NFκB, iNOS, COX-2, and PGE2) that differentiates it from BD. Moreover, some biomarkers may be related to the positive, negative, and cognitive dimensions of SCH. In future, their pharmacological modulation may constitute a promising therapeutic target.

Authors' contributions

LG-A, MPG-P, PSM and JB designed the study. LG-A, MPG-P, LF-T, LG-B, and PASM acquired the data. JCL and JRC perform biochemical analyses. LG-A and MPG-P conducted statistical analyses. LG-A and MPG-P wrote the 1st draft of the manuscript. The rest reviewed it and gave the final approval.

Disclosure of interest

Julio Bobes has received research grants and served as consultant, advisor or speaker for the companies: AB-Biotics, Adamed, Almirall, AstraZeneca, Bristol-Myers Squibb, Ferrer, Glaxo- Smith-Kline, Hoffman La Roche, Janssen-Cilag, Lilly, Lundbeck, Merck, Novartis, Organon, Otsuka, Pfizer, Pierre-Fabre, Sanofi-Aventis, Servier, Shering-Plough and Shire, research funding from the Spanish Ministry of Economy and Competiveness–Centro de Investigación Biomedica en Red area de Salud Mental (CIBERSAM) and Instituto de Salud Carlos III-, Spanish Ministry of Health, Social Services and Equality – Plan Nacional sobre Drogas- and the 7th Framework Program of the European Union.

Leticia Garcia-Alvarez has received honoraria from the 7th Framework Program European Union and has served as speaker for Pfizer.

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All other researchers declare that they have no competing interest.

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