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Clinical staging and serum cytokines in bipolar patients during euthymia

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ABSTRACT

Aims: Changes in serum cytokines and altered neutrophin concentration have been associated with bipolar disorder (BD). Our aim here was to analyze peripheral blood biomarkers according to the clinical stages of BD.
Method: Euthymic BD-I patients were grouped according to their level of functioning in early-stage (n = 25) and late-stage (n = 23), and compared to healthy siblings (n = 23) and genetically unrelated healthy controls (n = 21). Neurotrophin (neurotrophin-3 and BDNF) concentration and biomarkers of inflammation, including cytokines (IL-6, IL-10 and TNF-α), leukocytes count and acute phase proteins, were measured.
Results: IL-10 concentration was significantly increased in early-stage patients compared to late-stage patients, healthy siblings and controls whereas TNF-α concentration was significantly increased in late-stage patients compared to controls. Total leukocytes, neutrophil and monocyte count were significantly increased in late-stage patients compared to healthy siblings and controls. The concentration of IL-6, neurotrophin-3 and BDNF was unchanged in euthymia. Healthy siblings did not show significant changes in any biomarker.
Conclusions: The concentration of IL-10, TNF-α, neutrophil and monocytes subtype count in blood is altered in patients with BD during euthymic state. The link between peripheral inflammation and different stages in BD deserves further studies.

1. Introduction

Bipolar disorder (BD) is a severe, chronic mood disorder characterized by recurrent episodes of depression and (hypo)mania, interspersed with periods of clinical remission or euthymia. BD is associated with an important global disability as well as increased morbidity and mortality (Soreca et al., 2009; Vieta et al., 2013). Several clinical staging systems have been proposed to classify BD patients into different stages of the disease, taking into account clinical, neurocognitive and functional variables as well as peripheral biomarkers (Berk et al., 2007; Kapczinski et al., 2009, 2014).

It has been postulated that the pathophysiology of BD could be understood as a progressive process in which the action of certain exogenous agents can produce small changes in the structures of the central nervous system (CNS) that could alter the course of the disease (Post, 1992, 2007b). The molecular mediators associated with these changes include genes, neurotrophic factors, hormones, the inflammatory/immune system and oxidative/nitrosidative stress (Vieta et al., 1999; McEwen and Wingfield, 2003; Caspi and Moffitt, 2006; Berk et al., 2007, 2011; Schloesser et al., 2008; Leboyer et al., 2012; Gama et al., 2013; Goldstein and Young, 2013; Rege and Hodgkinson, 2013; Barbosa et al., 2014a).

Notably, most of the studies on peripheral biomarkers in BD have focused on depression and/or mania and have consistently shown increased concentrations of pro-inflammatory cytokines, such as TNF-α (O'Brien et al., 2006; Brietzke et al., 2009; Kauer-Sant'Anna et al., 2009; Hope et al., 2011; Ortiz-Dominguez et al., 2007; Barbosa et al., 2011, 2012b; Munkholm et al., 2013; Modabbernia et al., 2013) and IL-6 (O'Brien et al., 2006; Kauer-Sant'Anna et al., 2009; Brietzke et al., 2009; Hope et al., 2011, Modabbernia et al., 2013) in BD patients. Moreover, several reports have shown that cytokine concentrations return to normal values after resolution of acute episodes (Munkholm

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et al., 2013; Modabbernia et al., 2013).

Although serum cytokines have been extensively assessed during periods of clinical stability or euthymia (Kapczinski et al., 2011; Drexhage et al., 2011; Barbosa et al., 2012a; Gubert et al., 2013; Versace et al., 2014; Hsu et al., 2014; Wieck et al., 2014; Barbosa et al., 2014c; Isgren et al., 2015; Bengesser et al., 2015), little is known regarding changes in cytokines as BD progresses, as there very few studies have compared the early and late stages of BD (Andreazza et al., 2009; Kauer-Sant'Anna et al., 2009; Magalhães et al., 2012; Pfaffenseller et al., 2014; Fries et al., 2014).

Other inflammatory markers have been shown to be increased during the acute phases of BD, includingleukocytes count (Ballin et al., 1998; Barbosa et al., 2014b; Çakır et al., 2015) and reactant acute phase proteins, such as fibrinogen and erythrocyte sedimentation rate (ESR) (Maes et al., 1997; Vargas et al., 2013).

Neurotrophins, including brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3), play a crucial role in synaptic plasticity processes and neuronal survival and differentiation (Post, 2007a). Compared to healthy controls, peripheral concentrations of BDNF are decreased in BD patients during depressive and manic/mixed episodes (Fernandes et al., 2011, 2014; Piccinni et al., 2015; Polyakova et al., 2014) and seem to return to normal values during euthymia (Fernandes et al., 2014; Polyakova et al., 2014). However, some studies found decreased (Monteleone et al., 2008) and still higher (Barbosa et al., 2013) concentrations of BDNF during euthymia. Moreover, multi-episode patients had lower BDNF concentrations than patients with a first episode (Kauer-Sant'Anna et al., 2009), suggesting that successive relapses can hinder restoration of neurotrophin levels. Although increases in peripheral NT-3 concentrations have been reported during the acute episodes (Walz et al., 2007; Fernandes et al., 2010), no data have been published related to euthymiaor the different stages of BD.

Finally, the comparison of these biomarkers in BD patients with subjects genetically at-risk for BD might allow an in-depth examination of the molecular pathways underlying the pathophysiology of BD. It is noteworthy that very few studies have examined these biomarkers in healthy relatives of BD patients.

The main objective of this study was to analyze peripheral cytokines (IL-6, IL-10 and TNF-α), leukocytes blood count, reactant acute phase proteins (fibrinogen and ESR) and neurotrophins (NT-3 and BDNF) concentrations in euthymic BD-I patients and healthy siblings, according to Kapczinski's clinical staging model based on clinical, neurocognitive and functional variables in the interepisodic period (Kapczinski et al., 2009, 2014).

2. Method

2.1. Sample

An observational, cross-sectional study was carried out to compare peripheral blood biomarkers in four different groups: euthymic BD patients in early stages of the disease, without functional impairment; euthymic BD patients in late stages of the disease, with functional impairment; healthy subjects but with an increased genetic risk for developing the disease, in this case siblings of patients diagnosed with BD ('latent stage') and genetically unrelated healthy controls without personal or family history of BD. The criterion employed to classify patients in early- and late-stages of BD was the median of the Functioning Assessment Short Test (FAST), as used recently (Rosa et al., 2014). The cut-off value to classify patients between early- and late stage of BD was 32 since it corresponds to the median value of the FAST scale in our sample.

The sample was recruited in 2013 at the University Hospital Doctor Peset in Valencia, Spain. Inclusion criteria were: adults under 60 years old; diagnosed with DSM-IV-TR BD type I; outpatient or living in a residence; clinically euthymic confirmed with psychometric criteria (Hamilton Rating Scale for Depression, HRSD < 8 and Young Mania Rating Scale, YMRS < 7) for at least two months; receiving a stable regimen of medication for at least 4 weeks; and able to understand the study procedures and to provide written informed consent. Exclusion criteria were: current hospitalization; cognitive impairment (intellectual disability or dementia); physical, visual or hearing disabilities that would prevent from understanding the protocol; and inability to read or understand Spanish.

Inclusion criteria for relatives and healthy controls were: adults under 60 years old; no diagnosis of Axis I disorders confirmed by the SCID-I interview; able to understand the procedures of the study and to provide written informed consent. In addition, relatives should have a brother or sister diagnosed with BD type I, and healthy controls should have no family history of severe mental illness such as schizophrenia, psychotic disorder, BD or major depressive disorder in first or second-degree relatives. The same exclusion criteria used for patients applied to the other groups.

2.2. Assessment

Each subject underwent a complete clinical, neuropsychological and functional assessment. Demographic, anthropometric, pharmacological and clinical variables (age of onset, illness duration, number of episodes, number of admissions, time since last relapse and admission, comorbid drug use, history of suicide attempts, history of psychotic symptoms, history of rapid cycling, and family psychiatric history) were coded. In order to properly assess the role of biomarkers of interest, subjects were asked about any infection occured during the two weeks prior to evaluation. Only one subject from the group of siblings reported having a pharyngitis during the previous week, which did not require treatment at all.

2.3. Measurements of cytokines, neurotrophic factors, haemogram and biochemical blood analysis

Blood samples were obtained from each subject between 7:30 a.m. and 9 a.m. 10 ml blood was collected was collected into two BD Vacutainer tubes containing EDTA for plasma or without it for serum samples. After extraction, the blood samples were allowed to stand for 15 min and were centrifuged at 1500 rpm for 10 min at room temperature. Subsequently the plasma and serum supernatants were aliquoted and stored at − 80 °C until analysis. The plasma concentration of BDNF and serum concentration of TNF-α, IL-6 e IL-10, NT-3 were measured using commercial enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (BDNF (ab99978), TNF-α (ab100654), IL-6 (ab46042), IL-10 (ab46059), and NT-3 (ab100615) Human ELISA Kit, Abcam®). To minimize assay variance all the measurements were conducted in duplicates and on the same day (Navarro-Martínez et al., 2015). The results were expressed as the concentration of BDNF (pg/ml).

In order to better characterize the analysis of biomarkers of inflammation, leukocyte series and reactant acute phase proteins such as fibrinogen and ESR were analyzed. These haematological and biochemical parameters were measured at the hospital clinical laboratory.

2.4. Statistical analysis

Demographic and clinical variables were analyzed using descriptive statistics, including measures of central tendency (mean) and dispersion (standard deviation) for quantitative variables and absolute frequency (n) and relatives (%) for qualitative variables, with a confidence interval of 95% to two tails in both cases. Parametric and non-parametric tests were used for the analysis of continuous variables depending on the restrictions of applicability (normal) and the nature of the variable. The association between categorical variables was analyzed with the chi-square test (χ^2) of Pearson or, where necessary,