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Preliminary communication

Increased serum levels of eotaxin/CCL11 in late-stage patients with bipolar disorder: An accelerated aging biomarker?

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ABSTRACT

Background: Bipolar disorder (BD) is commonly comorbid with many medical disorders including atopy, and appears characterized by progressive social, neurobiological, and functional impairment associated with increasing number of episodes and illness duration. Early and late stages of BD may present different biological features and may therefore require different treatment strategies. Consequently, the aim of this study was to evaluate serum levels of eotaxin/CCL11, eotaxin-2/CCL24, IL-2, IL-4, IL-6, IL-10, IL-17, TNF-α, IFNγ, BDNF, TBARS, carbonyl, and GPx in a sample of euthymic patients with BD at early and late stages compared to controls.

Methods: Early-stage BD patients, 12 late-stage patients, and 25 controls matched for sex and age were selected. 10 mL of peripheral blood was drawn from all subjects by venipuncture. Serum levels of BDNF, TBARS, carbonyl content, glutathione-peroxidase activity (GPx), cytokines (IL-2, IL-4, IL-6, IL-10, IL-17, TNF-α and IFNγ), and chemokines (eotaxin/CCL11 and eotaxin-2/CCL24) were measured.

Results: There were no demographic differences between patients and controls. No significant differences were found for any of the biomarkers, except chemokine eotaxin/CCL11, whose serum levels were higher in late-stage patients with BD when compared to controls ($p=0.022$; Mann–Whitney *U* test).

Limitations: Small number of subjects and use of medication may have influenced in our results.

Conclusion: The present study suggests a link between biomarkers of atopy and eosinophil function and bipolar disorder. These findings are also in line with progressive biological changes partially mediated by inflammatory imbalance, a process referred to as neuroprogression.

1. Introduction

Bipolar disorder (BD) is a chronic psychiatric illness characterized by recurrent episodes of mania and depression (Barnett and Smoller, 2009). It affects about 2.4% of the world population (Merikangas et al., 2011) and often has a negative impact on the lives of patients (Barnett and Smoller, 2009).

Bipolar disorder is also linked with medical comorbidity, particularly auto-immune and atopic disorders including asthma and atopic dermatitis (Perugi et al., 2014). A large population based study showed a significant association between bipolar disorder and asthma (Lin et al., 2014). Similar associations with atopy are found in depression (Sanna et al., 2014). This association implicates the drivers of eosinophil function such as CCL11, a cytokine that recruits eosinophils. It stimulates eosinophil chemotaxis, driving allergic responses. The first papers suggesting enhanced eosinophil responses in bipolar disorder were published over half a century ago (Lehmann et al., 1950).

Evidence suggests that the progressive social, neurobiological, and functional impairment observed in BD is associated with the

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number of episodes and illness duration (Fries et al., 2012; Gama et al., 2013). Most patients at early disease stages have more favorable clinical outcomes when compared to patients at late stages (Kauer-Sant'Anna et al., 2009). Illness progression is often associated with shorter inter-episode intervals, poorer adherence to pharmacological treatment, and an increased risk of hospitalization (Kapczinski et al., 2009a; Schuepbach et al., 2008; Swann et al., 1999; Tohen et al., 1990). Furthermore, individuals with a long-term course of illness present significant impairment in cognitive tasks and functioning when compared to both controls and early-stage patients (Berk et al., 2011a; Rosa et al., 2014). Finally, considering that the resilience of patients becomes impaired, their vulnerability to stressor events may contribute to the triggering of new episodes (Kapczinski et al., 2008; Kessing et al., 1998).

Despite a growing body of research, there are no robust biological tests currently available to differentiate between different BD states, or different stages indicating neuroprogression. Some factors have been prioritized in the search for biomarkers, such as neurotrophic factors, especially brain-derived neurotrophic factor (BDNF), cytokines – usually focusing on pro-inflammatory but also anti-inflammatory ones, and oxidative stress markers (Berk et al., 2011b; Gubert et al., 2013; Kunz et al., 2011; Kunz et al., 2008; Munkholm et al., 2013; Pfaffenseller et al., 2013). However, most studies report differences only in acute states (i.e. mania or depression BD), and there seems to be little overall consistency in the data reported.

Evidence points towards an interaction between chemokines and neurotransmitters systems in the brain, playing crucial roles in brain development and function (Capuron and Miller, 2011). Chemokines are a special type of cytokines involved in the attraction of cells to inflammatory sites, being promising in the study of the role of inflammation in BD (Miller et al., 2011; Potvin et al., 2008). High levels of eotaxin/CCL11 and eotaxin-2/CCL24 have been found in patients with schizophrenia and in BD in comparison with healthy subjects (Barbosa et al., 2013; Brietzke et al., 2009; Magalhaes et al., 2014; Teixeira et al., 2008). In addition, high levels of eotaxin/CCL11 have been associated with decreased neurogenesis in the hippocampus, and with impaired learning and memory in mice (Villeda et al., 2011). Interestingly increased levels of eotaxin/CCL11 have been strongly related to the age-related cognitive decline in healthy subjects (Villeda et al., 2011) and in subjects with schizophrenia (SZ) (Asevedo et al., 2013). Eotaxin/CCL11 is implicated in the relationship between severe neuropsychiatric diseases, such as BD and SZ and accelerated aging (Pedrini et al., 2014; Rizzo et al., 2013).

Considering the possible role of atopic pathways, and that early and late stages of BD are clinically different in many aspects and may present different biological features, requiring specific treatment strategies, the aim of this study was to evaluate whether neurotrophic factor, cytokines and oxidative stress parameters are different between them. For this purpose, serum levels of eotaxin/CCL11, eotaxin-2/CCL24, IL-2, IL-4, IL-6, IL-10, IL-17, TNF-α, IFNγ, BDNF, TBARS, carbonyl, and GPx were determined in a sample of euthymic patients with BD at early and late stages of the disease compared to healthy controls.

2. Methodology

This study was approved by the Research Ethics Committee of Hospital de Clínicas de Porto Alegre (HCPA) (protocol no. 110144). Thirty-one patients with a diagnosis of type I BD in euthymic phase were recruited at the Bipolar Disorder Program of Hospital de Clínicas de Porto Alegre, Brazil. Diagnosis was established following a structured clinical interview (SCID-I) and was based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). Among these patients, 17 were in an early stage of the disease, and 14 at a late stage. The criteria for classifying patients into early vs. late stage were based on the clinical staging model proposed by Kapczinski et al. (2009b), taking into account the number of previous episodes, functioning (evaluated using the Functioning Assessment Short Test—FAST), comorbidities, and cognitive parameters. The Young Mania Rating Scale (YMRS) assessed the severity of symptoms of mania, and the Hamilton Depression Rating Scale (HAM-D), assessed depressive symptoms.

The control group comprised 27 healthy subjects matched for sex and age; 14 were matched to early-stage patients, and 13 to late-stage patients. Exclusion criteria were having a history of autoimmune diseases or chronic infections/inflammatory diseases, having any severe systemic disease, or having received immunosuppressive therapy. Controls with a first-degree family history of psychiatric disorders were also excluded.

Following psychiatric evaluation, 10 mL of peripheral blood was drawn from all subjects by venipuncture into tubes without anticoagulant. Immediately after withdrawal, the blood was centrifuged at $4000 \times g$ for 10 min, and the serum was aliquoted and stored at $-80\ ^{\circ}C$ until the experiment.

BDNF serum levels were measured by sandwich-ELISA using a commercial kit according to manufacturer instructions (Millipore, USA). Lipid peroxidation levels were measured using the TBARS adapted method described by Wills (1966). Oxidative damage to proteins was analyzed by determining the content of carbonyl groups, as previously described by Levine et al. (1990). Selenium-dependent GPx activity was measured by taking tert-butyl-hydroperoxide as the substrate at 340 nm, as described by Wendel (1981).

Serum cytokines (IL-2, IL-4, IL-6, IL-10, IL-17 A, IFN-γ, and TNF-α) were simultaneously measured by flow cytometry using the Cytometric Bead Array Human Th1/Th2/Th17 Kit (BD Biosciences, San Jose, CA). Acquisition was performed with a FACSCanto II flow cytometer (BD Biosciences, San Jose, CA). Measurement of eotaxin/CCL11 and eotaxin-2/CCL24 chemokines in the serum of patients and controls was performed by sandwich-ELISA according to manufacturer instructions (DuoSet, R & D Systems, Minneapolis, MN, USA).

Analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0. Demographic and clinical characteristics were analyzed using the chi-square, Mann–Whitney's or Student's t test, as appropriate. Descriptive data were expressed as mean and standard deviation or as median and interquartile range. p-Values < 0.05 were considered significant. A multivariate linear model was used to control for the effect of body mass index (BMI) on peripheral measures.

3. Results

Clinical and demographic characteristics of the sample are shown in Table 1. All comparisons were performed between patients and their respective control groups. As shown in Table 1, patients and the control group presented a similar age and gender distribution ($p=0.985$ and $p=0.901$ respectively). There was a statistically significant difference in BMI between late-stage BD patients and their respective controls, but no other demographic differences were found. The general linear model did not show a significant influence of BMI on peripheral blood measures (Pillai's trace test, $p=0.115$, $F=1.551$).

Biomarker results are described in Table 2. No significant differences were found for any of the stress oxidative, neurotrophic or inflammatory biomarkers, except for serum levels of