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Research Article

Effect of Cytokines and Neurotrophins in Euthymic Bipolar Patients during the Early and Late Stages of the Disease

Luiz Arthur Rangel Cyrino[1,3]*, Daniela Delwing-de Lima[1,2], Silvia Aparecida Ramos[3], Oliver Matheus Ullmann[3], Yasmin Mayer[3], Gabriela Kozuchovski Ferreira[4] and Débora Delwing-Dal Magro[5]

[1]Postgraduate Program in Health and Environment, Universidade da Região de Joinville-UNIVILLE, Rua Paulo Malschitzki, Joinville, SC CEP, Brazil

[2]Department of Medicine,Universidade da Região de Joinville-UNIVILLE, Rua Paulo Malschitzki, Zona Industrial Norte, Joinville, SC CEP, Brazil

[3]Pharmacy Department, Universidade da Região de Joinville-UNIVILLE, Rua Paulo Malschitzki, Zona Industrial Norte, Joinville, SC CEP, Brazil

[4]Department of Natural Sciences, Centro de Ciências Exatas e Naturais, Universidade Regional de Blumenau, Rua Antônio da Veiga, Blumenau, SC CEP, Brazil

[5]Postgraduate Program in Materials Science and Engineering, UNISOCIESC, Joinville, Santa Catarina, Brazil

***Corresponding Author:** Luiz Arthur Rangel Cyrino, Postgraduate Program in Health and Environment, Universidade da Região de Joinville-UNIVILLE, Rua Paulo Malschitzki, Joinville, SC CEP, Brazil.

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Abstract

Background: Bipolar Disorder (BD) is associated with neuroinflammation, and is represented by alterations in cytokines and neurotrophins, leading to neurological damage, which may reflect on cognitive functions, functionality, and disease progression.

Objective: Our aim was to analyze the effect of three cytokines (IL-6, IL-10 and TNF-α) and two neurotrophins (BDNF and GDNF) in bipolar patients and correlate them within two different groups depending on the length of the disease.

Methods: Fifty bipolar patients, type I/II, with more than six months in the euthymic phase, were studied, and then divided into two subgroups of 25 patients (≤ 3 years, and with ≥ 10 years of diagnosis, since the first episode of mania), and 25 control patients.

Results: Analyzing the levels of cytokines, there were no statistical differences between the groups evaluated, $p > 0.05$. However, BDNF levels were increased, ≤ 3 years (20.63 ± 8.21), ≥ 10 years (27.80 ± 12.50) and the control group (9.80 ± 5.94), $p < 0.001$.

Conclusion: Bipolar patients in euthymia did not show statistical differences in cytokines, but BDNF was increased, showing an improvement in neuroinflammation.

Keywords: *Bipolar Disorder; Neuroinflammation; Neuroprogression; Cytokines; Neurotrophins*

Introduction

Bipolar Disorder (BD) is a chronic and severe disease, associated with recurrent mood changes, characterized by cyclic episodes of depression and mania, which presents periods of mood stability (euthymia), with remission of symptoms. The diagnosis is based on the manifestation of at least one manic or hypomanic episode during life [1]. The aim of pharmacological treatment is the management of

acute episodes (seeking to lead a patient in mania or depression to euthymia) and to maintain the euthymic state to prevent the occurrence of new episodes, reduce subsyndromal symptoms and increase the functionality of patients. The pathophysiology of BD is not fully understood, being influenced by genetic and environmental factors, such as chronic stress. Recent studies have shown that from the first few mood episodes, neurological changes occur in the neurotransmission, neuroplasticity, growth factor signaling, metabolism, as well as oxidative stress and neuronal apoptosis, altering brain development and leading to neuroinflammation [2-4].

All these neuronal abnormalities can result in morphological changes, such as reduced prefrontal and hippocampal volumes leading to a reorganization of brain circuits, resulting in a gradual decline in behavior and cognitive functions, with impairments in functionality [4,5]. Thus, the progressive structural and biochemical changes from the first episodes of BD, or in the early-stages of the disease will evolve to more advanced stages, producing a slow evolution of the clinical process, called neuroprogression. With the progression of the disease, there is an increase in the frequency and severity of episodes of mania and depression over the years. It leads to an increase in the number of associated medical and psychiatric comorbidities, with an imbalance between pro and anti-inflammatory factors, reduction of neurotrophins, and increased oxidative stress. In addition, BD can be seen as a multisystem inflammatory disease, being represented by changes in serum biomarkers, which could function as indicators of cell toxicity in these patients [6]. In the last decade, it was observed that chronic mild inflammatory processes and immune neural interactions might be involved in the pathophysiology of major depression and BD [7,8]. One of the most intensely observed lines of research was the link between increased levels of cytokines and a decrease in serum neurotrophins in different mood states in BD. Thus, studies have shown that cytokines access the brain and interact with all pathophysiological domains relevant to BD. Furthermore, Miller., *et al*. (2009) [9] observed that peripheral cytokines reach the brain through different components, including alterations in the brain blood barrier, active transport, activation of endothelial cells, and binding to cytokine receptors. Levels of pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-2, IL-4, IL-6, tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP), as well as anti-inflammatory interleukins such as IL-10 were altered in the different phases of the disease (mania, depression, and euthymia), as well as in both the early and late-stages of the disease. A similar phenomenon was observed with neurotrophins, where, proinflammatory cytokines induce a decrease in neurotrophins, in particular, Brain-Derived Neurotrophic Factor (BDNF). It leads to decreased neuronal repair, decreased neurogenesis, and increased glutamatergic pathway that contributes to neuronal apoptosis, oxidative stress, and the induction of apoptosis in astrocytes and oligodendrocytes [10]. Therefore, it creates a perpetuating pathogenic cycle, contributing to a chronic neuroinflammation process. Thus, this research intends to corroborate with the neuroprogression and neuroinflammation hypothesis, which was developed and described by Kapczinski., *et al*. (2008) [11]; Berk (2009) [12] and Berk., *et al*. (2011) [2]. Our group focused on IL-6, TNF-α, and IL-10 and the neuroprotective substances, BDNF and Glial-Derived Neurotrophic Factor (GDNF). We seeked to help understand how a disease, which initially manifests with a relatively benign condition, can deteriorate in a few years, presenting a reduction in cognitive functions and functional recovery capacity, to the point of preventing bipolar patients from leading a normal life.

Materials and Methods

Ethics

The Research Ethics Committee of Universidade da Região de Joinville - UNIVILLE (protocol number 655.037), approved this study. Each patient underwent a clinical and psychiatric evaluation, where demographic, anthropometric, pharmacological data and clinical variables were collected. The study evaluated 50 outpatients, with BD types I/II, in their euthymic state, who were recruited from the Porto Seguro Psychiatric Hospital, located in the city of Curitiba, Brazil.

Participants

The participants were divided into three distinct groups, each one with 25 individuals: 25 euthymic BD patients in the early-stage of disease (≤ 3 years since the diagnosis of BD from the first manic episode); 25 euthymic BD patients in the late stage of disease (≥ 10 years

since the diagnosis of BD from the first manic episode), and 25 healthy controls. The psychiatric diagnosis of BD patients for types I/II was defined in the Manual Diagnosis and Statistics of Mental Disorders (DSM-V), and confirmed by Semi-Structured Clinical Interview, according to DSM-V (SCID-5-CV). Manic and depressive symptoms were assessed using the Young Mania Rating Scale (YMRS) [13] and the 17 items version of the Hamilton Depression Rating Scale (HAMD-17) [14] respectively. With the HAMD-17 scale, we evaluated depressive symptoms that had occurred within the last week, and in YMRS, manic symptoms that had presented themselves within the last 48 hrs. The cutoff scores used in the study were: YMRS > 7 as an indication of mania, and HAMD-17 > 7 as an indication of depression.

Criteria

The inclusion criteria of bipolar patients in the euthymic stage were: (a) the patients had been in euthymic phase at least six months (b) active age (18 - 60 years); (c) none of the patients had a history of addiction or substance abuse in the last year; (d) no history of neurodegenerative diseases, cancer, morbid obesity or trauma; (e) patients had no significant comorbid medical conditions, and did not receive medication in addition to those prescribed for their psychiatric condition; these should have been used for at least four weeks; (f) non-smokers (g) not pregnant or breastfeeding (h) patients were able to understand the procedures and protocol and provided written informed consent, and did not present cognitive impairment with disability or dementia, physical disabilities, e.g. visual or hearing impairing. Healthy controls were selected among hospital staff, and the subjects were matched for demographic parameters.

Demographic, clinical, and pharmacological data

Demographic variables were age, gender, marital status, education level, employment situation, and years of education. Clinical variables were age at onset, illness duration (years), hospitalization and the duration of hospitalizations, suicide attempts, relatives' antecedents of mental diseases.

In vivo studies

Plasma preparation

The blood samples were obtained between 8:00 A.M and 10:00 A.M. by the laboratory at the Porto Seguro Hospital outpatient clinic, and by the Werner laboratory in Joinville. To analyze the BDNF, GNDF, interleukin IL-6, IL-10 and TNF-α, 20 mL of peripheral blood was taken from each individual by venipuncture into an anticoagulant-free vacuum tube. Blood samples were centrifuged at 1,000-X g for 10 min. Plasma was then removed by aspiration and frozen at -80°C until the assay.

Biochemical assays and protein determination

All biochemical assays were run in duplicates. Serum levels of BDNF, GNDF, interleukin IL-6, IL-10 and TNF-α were measured through the sandwich-ELISA, using a commercial kit RAB0026, RAB0205, RAB1089, RAB0306 and RAB0244 respectively, (Sigma-Aldrich, USA). These assays employed a specific capture antibody coated on a 96-well plate. Standards and samples were pipetted into the wells and the target protein presented in the sample was attached to the wells by immobilized antibodies. The wells were washed and a specific biotinylated antibody detection was added to the target protein. After washing the unbound biotinylated antibody, streptavidin conjugated to HRP was pipetted into the wells. The wells were washed again, a TMB substrate solution was added to the wells, and the color developed in proportion to the amount of BDNF, GDNF, IL-6, IL-10 and TNF-α bound. The stop solution changed the color from blue to yellow, and the color intensity was determined (absorbance adjusted to 450nm). The specific activity was reported as ng/ml protein.

Statistical analysis

Demographic and clinical variables were analyzed using descriptive statistics, including (mean), and (standard deviation) for quantitative variables and absolute frequency (n), and relatives (%), for qualitative variables with a confidence interval of 95% in both cases.

For the qualitative nominal and ordinal data, we used the Chi-square test (χ^2) of Pearson and for two or more groups, we used Fisher's exact test. Parametric and nonparametric tests were used for the analysis of qualitative variables. The assumption of normality and homoscedasticity of each variable was analyzed with the Kolmogorov-Smirnov normality test and Levene's, respectively. For comparisons of parametric variables between two groups, the Student *t*-test was used, and for more than two groups the Tukey's test of analysis of variance (ANOVA) was used. To compare non-parametric variables between two and three independent samples, the Mann-Whitney tests and the Kruskal-Wallis tests were used, respectively. Dunn's post hoc test was performed to peer comparisons in case the main effects were significant. The most recent version of the SPSS software program (SPSS Inc., Chicago, USA) was used. To calculate the statistical power analyzes, we used the program - G* Power 3.1. Statistical significance was set at $p < 0.05$ for all tests or adopting a level of significance of 5% to reject the null hypotheses.

Results

Demographic, clinical and pharmacological characteristics

The demographic and clinical characteristics of the different groups studied were evaluated. The sample included 25 healthy controls, and 50 patients with BD divided in two groups of euthymic patients (≤ 3 and ≥ 10 years of the disease). Initially, we calculated the sample size - difference between two independent means (two tails). The analyses showed an effect size d = 0.853; α = 0.05; power (1-β err prob) = 0.80; non-centrality parameter δ =2.89; critical t = 2.01; Df = 44; sample size group 1 = 23; sample size group 2 = 23; total sample size = 46; actual power = 0.808. Thirty-six euthymic patients (72%) were female. The healthy control group had a mean age of (36.1 ± 9.87) and the euthymic patients analyzed had a mean age of (34.9 ± 10.04 years in the group of ≤ 3 years of the disease), and (47.4 ± 8.21 years in the group of ≥ 10 years of the disease). Utilizing the one-way ANOVA followed by Dunn's post hoc test, the means of healthy controls and euthymic patients differ between ages ($p < 0.01$). Utilizing the Chi-square test, there was no difference between the gender groups, occupational status, and marital status ($p > 0.05$). In addition, it was observed that after performing the Chi- square test followed by the Fisher's exact test, the groups did not significantly differ in terms of their educational level ($p > 0.05$). The mean years of education were (14.7 ± 2.18) years in the healthy control group, and the euthymic patients analyzed had a mean of (13.8 ± 2.70 years in the group of ≤ 3 years of the disease), and (12.4 ± 2.77 years in the group of ≥ 10 years of the disease). After performing the Kruskal-Wallis test, the groups significantly differed in terms of years of education ($p < 0.01$) as seen in table 1.

Healthy Controls n = 25		**Bipolar Patients ≤ 3 years n = 25**	**Bipolar Patients ≥ 10 years n = 25**	**p - Value**
Age, years[b]	35.0 (± 9.96)	34.9 (± 10.04)	47.4 (± 8.21)	p < 0 .01[c]
Gender, n				p = 0.77[a]
Male	9	7	7	
Female	16	18	18	
Education n (%)				p = 0 .13[d]
Illiterate	-	-	-	
Up to primary school	0 (0)	3 (12)	4 (16)	
Up to high school	10 (40)	10 (40)	12 (48)	
Graduate	12 (48)	12 (48)	9 (36)	
Postgraduate	3 (12)	0 (0)	0 (0)	
Years of education[b]	14.7 (±2.18)	13.8 (±2.70)	12.4 (±2.77)	p < 0.01[e]
Work situation n (%)				p = 0.17[d]
Employed	23 (92)	18 (72)	13 (52)	
Unemployed	2 (8)	6 (24)	10 (40)	
Medical benefits	0 (0)	1 (4)	0 (0)	
Invalidity	0 (0)	0 (0)	2 (8)	

Table 1: *Socio demographic characteristics.*

The bipolar patients had a mean of disease duration of ≤ 3 years (2.52 ± 0.65), and ≥ 10 years (15.64 ± 6.81), and the mean age at onset of the disease was ≤ 3 years (22.1 ± 7.01), and ≥ 10 years (25.1 ± 6.17). Twenty patients (40%) had previously been hospitalized. Among these patients, the mean duration of hospitalization was (13.2 days ± 0.967), and patients with ≤ 3 years of the disease, attempted suicide 18 times (involving 11 patients), whereas the patients with ≥ 10 years of the disease attempted suicide 40 times (involving 20 patients). The family history of BD was positive in 23 patients.

Regarding pharmacologic treatment, our results showed that 10 (20%) of the patients were on monotherapy. Among the patients on polypharmacy, 18 (36%), 16 (32%), and six (12%) of the patients received two, three, and four Psychotropic medications, respectively. The percentages of mood stabilizers, antipsychotics, antidepressants, and benzodiazepines used in patients according to their clinical symptoms, are presented in table 2. To evaluate the absence of depression or mania in the samples, the HAM-D and YMRS tests were used in the healthy control group, and the euthymic patients' group respectively. The observed results had a mean HAM-D score of (4.32 ± 2.49) for the healthy control group, and (4.10 ± 2.02) for euthymic patients with ≤ 3 years of the disease, and (3.71 ± 1.46) for euthymic patients with ≥ 10 years of the disease. After performing the ANOVA one-way test, the groups did not differ ($p > 0.05$). Regarding the YMRS score, the mean was (0.64 ± 0.90) to the healthy control group, and (0.88 ± 1.01) to euthymic patients with ≤ 3 years of the disease, and (1.28 ± 1.13) for euthymic patients with ≥ 10 years of the disease. After performing the Kruskal-Wallis test, the groups did not differ ($p > 0.05$) as seen in table 2.

Healthy Controls n = 25		**Bipolar patients ≤ 3 years n = 25**	**Bipolar patients ≥ 10 years n = 25**	**p-Value**
Illness duration (years)[a]	N/A	2.52 (± 0.65)	15.64 (± 6.81)	p < 0.001[d]
Age of onset (years)[a]	N/A	22.1 (± 7.01)	25.1 (± 6.17)	p = 0.62[d]
HAMD-17 total score[a]	4.32 (± 2.49)	4.10 (± 2.02)	3.71 (± 1.46)	p = 0.53[b]
YMRS total score[a]	0.56 (± 0.86)	0.88 (± 1.01)	1.28 (± 1.13)	p = 0.08[c]
FAST score, median (IQR)	9 (7)	22 (10)	23 (20)	p < 0.001[c]
FAB score, median (IQR)	16 (3)	14 (4.5)	14 (3,5)	p < 0.001[b]
Hospitalizations n (%)	N/A	12 (48)	8 (32)	
Treatment n (%)				
Lithium	N/A	13 (52)	15 (60)	
Other mood stabilizers	N/A	11 (44)	13 (52)	
Atypical antipsychotics	N/A	8 (32)	12 (48)	
Typical antipsychotics	N/A	2 (8)	0 (0)	
Antidepressants	N/A	7 (28)	7 (28)	
Benzodiazepines	N/A	2 (8)	7 (28)	

Table 2: *Clinical and pharmacological characteristics.*

HAMD-17 = Hamilton Depression Rating Scale; YMRS = Young Mania Rating Scale; FAST = Functioning Assessment Short Test; FAB = Frontal Assessment; Battery; N/A = Not Available; IQR = Interquartile Range; [a]: Mean (± SD); [b:] t test; [c]: Mann Whitney; [d]: Kruskal Wallis.

Evaluation of serum levels of neurotrophic factors

The serum assessment presented in table 3 and figure 1 and 2 of the neurotrophic factors GDNF and BDNF from the sample groups, are in the form of mean and standard deviation. Regarding the BDNF from bipolar patients in euthymia, it showed an improvement in the BDNF activity between the ≤ 3 years and ≥ 10 years groups, compared to the control group [$F(2,72) = 3668$; $p < 0.01$], but there were no significant changes between or within the groups regarding the GNDF [$F(2,72) = 1046$; $p = 0.25$].

Healthy Control n = 25		Bipolar patients ≤ 3 years n = 25	Bipolar patients ≥ 10 years n = 25	p-Value
Neurotrophic Factors				
GDNF (pg/mL) Means (±SD)	43.83 (± 51.25)	35.73 (±36.31)	26.44 (± 9.89)	0.25[a]
BDNF (pg/mL) Means (**± SD)**	15.61 (± 2.09)	16.94 (± 1.77)	16.72 (± 1.70)	**0.01[b]

Table 3: *Comparison of serum levels of Neurotrophic factors GDNF and BDNF in euthymic bipolar patients between the groups with ≤ 3 years and ≥ 10 years groups, since the disease onset compared to the healthy control group.*

Means ± standard deviation (SD). The normality of each variable was analyzed using theKolmogorov-Smirnov normality test.

*[a]To compare parametric variables between the three independent groups, the two-way analysis of variance (ANOVA) test followed by Tukey's test was used. [b]To compare non-parametric variables between the three independent samples, the Kruskal-Wallis test was used. Dunn's post hoc test was performed for pairwise comparisons, if the main effects were significant. Statistical significance was set at **p < 0.01 for all tests.*

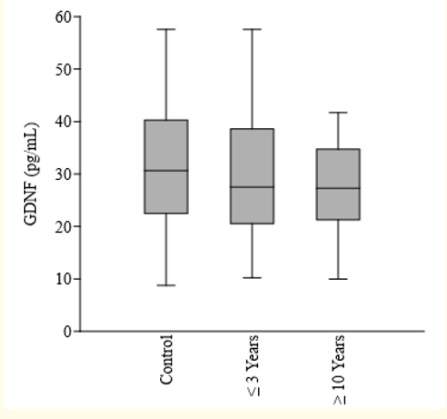

Figure 1: *Box plot - Comparison of serum levels of GDNF (pg/mL) of healthy control patients with euthymic bipolar patients with ≤ 3 and ≥ 10 years of the disease.*

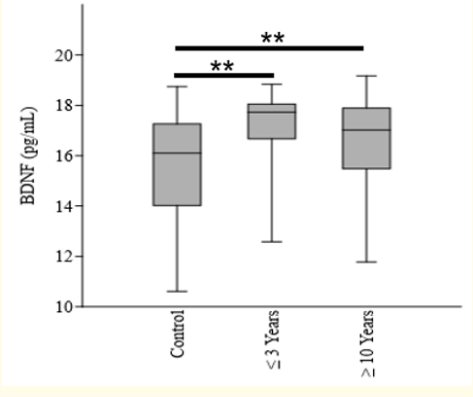

Figure 2: *Box plot - Comparison of serum levels of BDNF (pg/mL) of healthy control patients with euthymic bipolar patients with ≤ 3 and ≥ 10 years of the disease.*

Evaluation of inflammatory mediator levels

Table 4 and figure 3-5 show the serum evaluation of IL-6, IL-10 and TNF-α of the sample groups, in the form of mean and standard deviation. Regarding inflammatory parameters, bipolar patients in euthymia did not show significant changes in TNF-α, IL -6 and IL-10, between and within the groups, $p < 0.05$.

	Control Patients n = 25	**Euthymic Patients BD ≤ 3 years n= 25**	**Euthymic Patients BD ≥ 10 years n= 25**	***p* - Value**
Inflammatory Mediators				
IL-6 (pg/mL) Means (± SD)	18.83 (± 7.94)	18.75 (± 7.48)	18.10 (± 9.19)	0.89[b]
TNF-α (pg/mL) Means (± SD)	33.81 (± 31.40)	24.60 (± 21.30)	19.59 (± 20.70)	0.18[b]
IL-10 (pg/mL) Means (± SD)	11.43 (± 12.82)	9.67 (± 7.08)	7.97 (± 6.48)	0.43[b]

Table 4: *Comparison of serum levels of inflammatory mediators IL-6, IL-10, and TNF-**α** in euthymic bipolar patients between the ≤ 3 years and ≥ 10 years groups, since the disease onset compared to the healthy control group.*

*Means ± standard deviation (SD). The normality of each variable was analyzed using the Kolmogorov-Smirnov normality test. [b]To compare non-parametric variables between the three independent samples, the Kruskal-Wallis test was used. Dunn's post hoc test was performed for pairwise comparisons, if the main effects were significant. Statistical significant was set at *p < 0.05.*

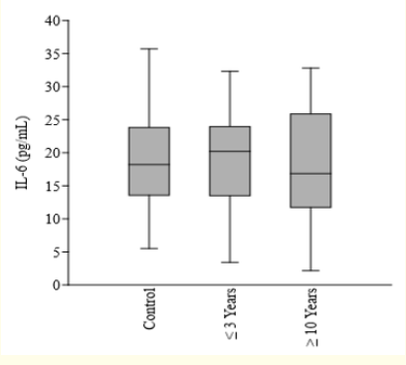

Figure 3: *Box plot - Comparison of serum levels of IL-6 (pg/mL) between the healthy control patients and the euthymic bipolar patients with ≤ 3 and ≥ 10 years of the disease.*

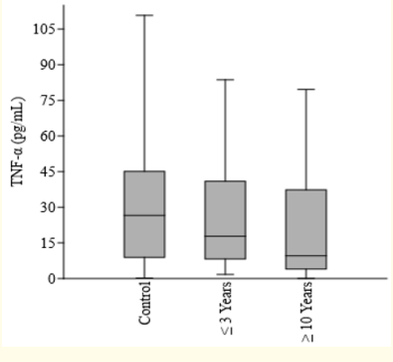

Figure 4: *Box plot - Comparison of serum levels of TNF-α (pg/mL) between the healthy control patients and the euthymic bipolar patients with ≤ 3 and ≥ 10 years of the disease.*

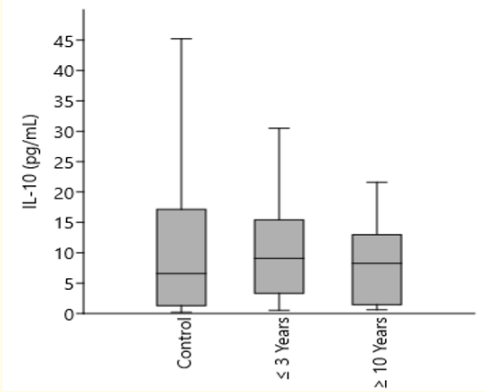

Figure 5: *Box plot - Comparison of serum levels of IL-10 (pg/mL) between the healthy control patients and the euthymic bipolar patients with ≤ 3 and ≥ 10 years of the disease.*

Discussion

As described above, both peripheral and brain cells (astrocytes, oligodendrocytes, microglial cells, and neurons) are associated with the release of elevated pro/anti-inflammatory cytokines. The imbalance between them has been implicated in neuroinflammation, causing toxicity and apoptosis of neurons and glial cells [15], leading to neuroprogression in BD, as well as in other psychiatric disorders [16]. Post-mortem studies have shown an increase or decrease in several pro/anti-inflammatory factors in the prefrontal cortex, hippocampus, and cingulate gyrus of patients with BD, and similar to those seen in schizophrenia and major depression during the acute and chronic phases of the illness [17,18]. Studies point to an increase mainly in pro-inflammatory factors such as IL-1, IL-6, and TNF-α, resulting in an increased synaptic pruning and microglial phagocytosis [19-23]. On the other hand, numerous studies have shown that anti-inflammatory cytokines (IL-4, IL-10, IL-13), increase the release of BDNF and inhibit the pro-inflammatory activity of microglia [24,25].

Recently, some studies have evaluated the circulation of inflammatory mediators during the different phases of BD as potential biomarkers for diagnosis or treatment. They showed an increase in serum, during the manic and depressive phases of IL-6, IL-1, IL-2, TNF-α, andTNFR1, when compared to controls and euthymic patients [26-28], while the concentration of anti-inflammatory IL-4 was significantly lower than in controls [3,6,29]. However, some studies were contradictory which found elevated IL-6 in mania and euthymia, but not in bipolar depression, and IL-1 levels were also significantly elevated during euthymia [29,30]. Other changes in these biomarkers were observed during the different phases after treatment. As an example, endogenous interleukin receptor antagonist (IL-1RA) levels were lower in the manic stage among chronic patients [31]. Also, the results from euthymic patients were conflicting, showing an imbalance between pro-inflammatory [32,33] and anti-inflammatory cytokines [26,32] or even showing no significant difference in the cytokine levels compared to the healthy controls [34,35].

These conflicting results are probably due to the different designs described in the various studies, where in some studies psychotropic drugs were included and not in others, different methodologies for measuring inflammatory mediators (ELISA, EIA and flow cytometry) were used, and finally, the inclusion and exclusion of certain patient's criteria were not very strict, including in the sample many patients with other inflammatory diseases or patients medicated or unmedicated for BD. In addition, research focused on different specifications of bipolar patients (Type I/II, cyclothymic, or without any distinction or specifications).

Thus, in our study, despite the control groups having higher means in the three mediators (IL-6, TNF-α, IL-10), which were measured and compared with the groups of euthymic patients with (≤ 3 years and ≥ 10 years since the onset of the disease); showed that there were no statistically significant changes between these different groups, as shown in the table 4. The lowest mean in the (≤ 3 years and ≥ 10 years) groups, about IL-6 and TNF-α, seems to agree with several studies in the research literature. A hypothesis that was raised for these results, was that all patients were euthymic for more than 6 months, and were continuously medicated, which would produce a decrease in pro-inflammatory cytokines. However, the results of IL-10 were inconclusive. The accentuated decreases found in both groups of euthymic patients related to the control group need a pathophysiological explanation, given that there should have been an increase in IL-10. One of the possibilities could be the low number of samples (n = 25) from each group, which were associated with a large standard deviation, and we might need to carry out a new study with a larger number of patients in the future.

The study of Kauer-SantÁnna., *et al*. (2009) [36] came the closest to ours. In it, the authors researched patients only with BD type I (our study included BD type I/II), which were not in an euthymic stage (in our study all patients were euthymic for more than 6 months). They studied patients with a diagnosis of < 3 years (n = 30), and > 10 years (n = 30) with the disease and compared to 60 control patients. Like our study, patients did not have significant comorbid medical conditions, they were not on medication other than those prescribed for their psychiatric condition, and all patients used psychotropic drugs. The result that they found was very different from ours. They found an increase in TNF-α, IL-6, and IL-10 mediators in the < 3 years group (n = 30) comparing with control group, and an increase in TNF-α, IL-6, but with no difference concerning IL-10 in the > 10 years group (n = 30) comparing to control group. They observed that when the levels were compared between patients in early and late-stages of the disease, there was a decrease in BDNF and IL-6 in late-stage BD compared to early-stage BD. On the other hand, TNF-α showed a significant increase in the later phase. Failure of inflammatory defenses in the late-stage of the disorder may be responsible for the reduction in BDNF and the continued elevation of cytokines; therefore, they may be related to neuroinflammation and neuroprogression in BD patients. Thus, they demonstrated a pro-inflammatory profile, which seemed to be more accentuated in individuals with longer duration of BD.

However, Berk., *et al*. (2011) [2] and Hamdani., *et al*. (2013) [8] reported that IL-10, which has an anti-inflammatory action, increased in the early stages of the disease, but decreased in the late stages. We found similar results, although they were not statistically significant. Our and their data was consistent with chronic progressive neuroinflammation, where a more significant number of previous episodes exhibit higher levels of TNF-α and IL-6 during all disease states, and when the patients are chronically treated the values of proinflammatory cytokines are controlled.

As for the neuroprotectors BDNF and GDNF, alterations in BDNF concentrations were observed in our research, but not in GDNF. Although GDNF is considered one of the most potent neurotrophic factors, and is widely found in different brain regions, its main functions are the development and maintenance of glial cells, and the nor adrenergic system in the brain [37]. However, Otsuki., *et al*. (2008) [38], quantified the serum concentrations of GDNF in 30 and 42 bipolar patients during different phases (mania, depression, and euthymia), where all of them were using psychotropic drugs. GDNF concentrations were lower in mood episodes (depression and mania) when compared to healthy controls ($p < 0.01$). However, no differences were observed between the euthymic groups of patients and the control group, which were the same results as ours.

In another study, Rosa., *et al*. (2006) [39], evaluated 44 euthymic bipolar patients and 60 unipolar depression patients, who were in remission but not medicated. They also found no significant differences when compared to healthy controls. Barbosa., *et al*. (2011) [40], studied 70 patients (35 euthymic and 35 in mania), and compared them with 50 control patients, showing no significant difference in plasma levels between the mania and control patients. However, when they compared euthymic patients with patients in the manic phase and control patients, there was a higher concentration of GDNF in the euthymic group ($p < 0.05$). In another study, Zhang., *et al*. (2015) [41], demonstrated that serum GDNF levels were reduced in patients during manic and depressive episodes, but after treatment with

mood stabilizers, they increased. Most of these studies are in line with our results, which demonstrated that during the euthymic phase, although we observed a slight decrease in the GDNF serum concentration in both the ≤ 3 years and ≥ 10 years groups, these were not significant in concerning the control group. However, when we compared the concentrations between the (≤ 3 years and ≥ 10 years) groups, despite not being significantly different, they showed that the ≥ 10 years group of the disease, had a lower concentration of GDNF serum levels than the ≤ 3 years group of the disease as shown in table 3. This raises the hypothesis that over time, even in euthymia, the body has difficulty maintaining GDNF levels close to normal levels. We must also consider that all of our patients were continuously using mood stabilizers, which could reinforce the idea that these drugs help to elevate GDNF concentrations in euthymic patients.

Regarding BDNF, as it is the most intensely distributed neurotrophin in the central nervous system [42], with the ability to regulate several biological functions such as neuroplasticity, axonal growth, neuronal apoptosis, and to be present in the local response to various neuronal and environmental stressors [43]. Serum BDNF concentrations can change due to various pathologies, such as epileptic seizures and cerebral hypoxia [44]. Regarding neuropsychiatric diseases, especially BD, we observed large variability in terms of results. Studies with patients in mania found decreases in BDNF serum levels compared to controls [45]; [46]. However, when patients reached euthymia, they showed improvement in BDNF serum levels, not differing from controls [45].

However, Palomino., *et al*. (2006) [47] and Tunçel., *et al*. (2020) [48] followed patients from their first manic episode with a psychotic condition for one year, using mood stabilizers. The researchers observed elevations in plasma levels associated with clinical improvement. Furthermore, plasma levels remained lower compared to controls. Researchers linked the improvement to the use of mood stabilizers. Also, using a model of mania in Wistar rats, through the use of amphetamine for 7 days, an increase in BDNF levels in the hippocampus was observed, but these were later treated with mood stabilizers (lithium and valproate), and the BDNF levels increased again [49,50]. Finally, Machado-Vieira., *et al*. (2007) [51], described a negative correlation between BDNF levels and the intensity of depressive and manic symptoms in non-medicated patients.

Regarding euthymic patients, Binici., *et al*. (2016) [52], observed that there were no changes in serum levels between euthymic patients and healthy control patients. Interestingly, Kauer-Sant'Anna., *et al*. (2009) [36] observed that euthymic bipolar patients with less than 3 years of the disease did not show any change in BDNF serum levels. However, the group that had the disease for more than 10 years, showed a decrease in serum levels compared to controls. Another interesting study was by Tseng., *et al*. (2008) [53], where the researchers evaluated the production of BDNF in a lymphocyte population in 12 euthymic bipolar patients during 3 years, which were on lithium monotherapy, and then compared them with 14 family members without psychiatric illnesses, as well as 13 healthy controls. The researchers observed that lymphocytes from bipolar patients had a decrease in BDNF serum levels when compared to the other groups.

In our study, all patients have been euthymic for 6 months, longer than the average time of other studies, and all patients were medicated for a longer period as well. Thus, we observed a significant increase in BDNF levels both in the ≤ 3 years and ≥ 10 years groups with the disease, compared to the control group. However, when we compared only the two groups of euthymic bipolar patients alone, we did not observe any significant changes. It corroborates with the idea that euthymic and treated patients present an improvement in clinical response and neuronal organization. When the patient is treated since the beginning of the disease, i.e. < 3 years, it is clear that the BDNF serum levels are already improving. Thus, early diagnosis and treatment seem to be advantageous for patients, and our results are in line with studies carried out by Duman and Monteggia (2006) [54], and Banasr and Duman (2008) [55].

In these studies, the authors demonstrated that psychopharmaceuticals such as lithium and valproate, used continuously in the treatment of BD, could act on neurotrophins, modulating their signaling pathways and increase their levels in the frontal cortex and hippocampus, as observed in other studies [49,56,57]. However, several studies have shown a decrease in the levels of BDNF and its receptors TrkB (tropomyosin kinase receptor), both in the blood and in the brain of bipolar patients when untreated [58-61].

If we analyze these results in light of cognitive and functional alterations, we can observe that they have been present since the beginning. Thus, studies such as Rybakowski., *et al*. (2003) [62] demonstrated a significant relationship between the performance involving the frontal cortex of bipolar patients, and neuronal damage with cognitive impairment, where the BDNF pathway played a key role in BD [60,61,63]. In summary, there are still only a few studies evaluating BDNF concentrations in bipolar patients during their different phases, and the results are still conflicting and inconclusive. This might be due to the great variability of characteristics of these patients, contributing to the incorrect conclusions of the role of BDNF and other neurotrophins in BD.

Thus, we can hypothesize that bipolar patients have a neuroinflammatory process in the brain since the first episodes of mania and depression. All these events involve a pathological reorganization in the brain, and therefore, are associated with morphological changes, such as reduced volume in the cortex and white matter, mainly in the prefrontal cortex, leading to cognitive and functional losses since the beginning, emphasizing the core of our study [64-66]. These prefrontal cortex changes are possible due to multiple episodes of mania and depression during their lifetime. In addition, the number of hospitalizations and the duration of illness in bipolar patients, can also have an impact on their neurocognitive performance, impacting their daily functionality and psychosocial aspects [67-70].

The prefrontal cortex is a heterogeneous region consisting of several specialized subregions, which communicates with the entire brain, receiving and sending projections of all kinds [65,66,71,72]. Through neuroimaging, it was possible to differentiate the areas of the prefrontal cortex, which are responsible for the different components of cognitive functions, in three main regions: the orbitofrontal, the ventromedial, and the dorsolateral. Each region has specific functions, and any neurofunctional alteration causes harm with behavioral and clinical changes [73-75]. Impairments in executive functions, impulsivity, and apathy, for example, are characteristics of dysfunctions in the frontal-subcortical circuit and neuropsychiatric disorders, such as obsessive-compulsive disorder, schizophrenia, and in BD [76-80].

Conclusion

The conclusions that we can infer, are that the results raised and analyzed so far, reinforce the hypothesis of neuroprogression and neuroinflammation developed and described by several researchers, especially during acute mood episodes. Together, these changes have been associated with systemic toxicity of the disease and damage, resulting from multiple episodes. Recurrence of mood episodes in BD usually produce worse outcomes; producing functional and cognitive impairments, and decreased responsiveness to treatment.

The pro-inflammatory state present in the mania and depression phases appears to stabilize during euthymia, in part due to the use of mood stabilizers. Among neurotrophins, consistent evidence proposes a possible role for BDNF in the pathophysiology of BD, where its levels are reduced during manic and depression episodes and the use of mood stabilizers can increase levels of neuroprotective during euthymia. Regarding neurotrophic factors, we can observe an improvement in the values in euthymic patients, especially relating to BDNF. This reinforces previous studies in which mood stability during euthymia improves dendritic formation capacity, and neuronal and glial cell viability. Ultimately, this suggests that systemic toxicity, when controlled, can improve the conditions of these cells, reinforcing the idea that the use of medication offers the patient a better quality of life.

Thus, the concepts of neuroinflammation and neuroprogression explains the clinical symptoms well, but it is still not possible to know whether all these changes are a cause or a consequence of the disease. More consistent evidence will require the monitoring of patients for several years, with laboratory tests, imaging, and neuropsychological tests from time to time, to assess the evolution of the problem. Although far from being proven, this proposal is opening the way for the search for more specific and efficient therapies, and for the development of strategies that allow the early identification of people at risk of developing this disease.

Conflict of Interest

The authors have no conflicts of interest to declare.

Authors' Contributions

LARC; DDL; SAR; OMU; YM; preparation, acquisition, study design, data collection and writing; GKF; DDM: analysis of statistical data and review; DDL; LARC; GKF: final review.

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