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ORIGINAL ARTICLE

Association of interleukin-10 levels with age of onset and duration of illness in patients with major depressive disorder

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Objective: To investigate peripheral levels of interleukin-10 (IL-10) in patients with major depressive disorder (MDD) and bipolar disorder (BD) and evaluate the relationship between IL-10, age of disease onset, and duration of illness.
Methods: Case-control study nested in a population-based cohort of 231 individuals (age 18-24 years) living in Pelotas, state of Rio Grande do Sul, Brazil. Participants were screened for psychopathology using the Mini-International Neuropsychiatric Interview (MINI) and the Structured Clinical Interview for DSM-IV (SCID-I). Serum IL-10 was measured using commercially available immunoassay kits.
Results: Peripheral levels of IL-10 were not significantly different in individuals with MDD or BD as compared to controls. However, higher IL-10 levels were found in MDD patients with a later disease onset as compared with controls or early-onset patients. In addition, IL-10 levels correlated negatively with illness duration in the MDD group. In the BD group, age of onset and duration of illness did not correlate with IL-10 levels.
Conclusion: Higher levels of IL-10 are correlated with late onset of MDD symptoms. Moreover, levels of this cytokine might decrease with disease progression, suggesting that an anti-inflammatory balance may be involved in the onset of depressive symptoms and disease progression in susceptible individuals.

Keywords: Mood disorders; bipolar; mood disorders; unipolar; neurochemistry; neuroimmunology; biological markers

Introduction

Mood disorders, such as major depressive disorder (MDD) and bipolar disorder (BD), are expected to become the second most prevalent group of illnesses by the year 2020.[1,2] These chronic conditions can lead to severe impairments of social and physical functioning and are associated with high medical costs, disability, morbidity, and mortality.[3] Major gaps remain in our understanding of the neuropathological changes associated with these conditions, and the efficacy of therapeutic approaches for their treatment is still far from optimal.[4] However, it is becoming evident that morphological and biochemical changes that occur in the brain of psychiatric patients are associated with episode-related deterioration patterns, starting at the onset of illness and worsening with disease progression.[5]

In the early 1990s, it was hypothesized that cytokines and related secretory products released from immune cells communicate with the endocrine and central nervous systems to collectively modulate their functions.[6] Since these initial reports of neural-immune interactions, the action of pro- and anti-inflammatory cytokines on brain cells has proven to be an important response associated with psychiatric symptoms.[7-9] The role of the immune system in the etiology and progression of psychiatric disorders is predominately supported by studies in MDD. Depressive patients have activated inflammatory pathways, with increased levels of proinflammatory cytokines and acute-phase proteins, and increased expression of chemokines and adhesion molecules.[10,11] However, activation of the immune system has also been demonstrated in BD, and much evidence suggests a link between altered cytokine levels and BD.[5,12]

Cytokines can be produced by immune cells in the blood, cross the blood-brain barrier, and induce malfunctioning of

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several neurotransmitter and hormonal systems.[13,14] Indeed, some studies have demonstrated abnormalities in cytokine production from peripheral monocytes in depressed individuals.[15] Interleukin-10 (IL-10), traditionally classified as a T-helper lymphocyte type-2 cytokine, is one of the key cytokines involved in the downregulation of inflammatory responses. IL-10 has the ability to suppress the production of proinflammatory cytokines and plays an important role in the regulation of overactive responses that would otherwise result in autoinflammatory diseases.[16,17]

Findings suggesting a relationship between psychiatric disorders and IL-10 production are inconsistent, with reports describing increased,[18,19] unchanged,[20] and even decreased IL-10 levels in patients with MDD and BD.[21] Antidepressants have been shown to stimulate production of IL-10 and a reduction of the general proinflammatory/anti-inflammatory cytokine ratio.[22] Furthermore, in vitro studies with lymphocytes and monocytes cultured in human whole blood reported that the mood stabilizer lithium also caused an increase in IL-10 levels.[23]

Despite these important discoveries, there is no clear understanding of whether a general proinflammatory/anti-inflammatory state could be involved in the onset of psychiatric symptoms. Thus, the aim of this study was to investigate whether peripheral levels of IL-10 might be associated with diagnosis, age of onset, or disease duration in patients with MDD or BD. We hypothesized that increased levels of anti-inflammatory cytokines might have beneficial effects, potentially contributing to a delay in onset of psychiatric symptoms.

Methods

Participants

This was a case-control study nested in a population-based cohort of 1,560 individuals aged 18 to 24 years and living in the urban area of Pelotas, state of Rio Grande do Sul, Brazil. Details on the larger population-based study have been published elsewhere.[24] Briefly, sample selection was performed by clusters, between August 2007 and December 2008, in the 515 census sectors of the municipality of Pelotas (as defined by the Brazilian Institute of Geography and Statistics, IBGE), and considering 39,667 individuals in this age range.[25] To ensure the necessary sample size was achieved, 89 census-based sectors were systematically selected. The study was approved by the institutional Ethics Committee. All participants provided written informed consent and completed a questionnaire designed to collect socio-demographic data.

As an initial screening for psychopathology, the Mini-International Neuropsychiatric Interview (MINI) was administered to the whole cohort.[26] We attempted to recruit every person with a past or current history of mania/hypomania from the population-based study. Two additional groups were recruited: individuals without a history of mood disorders (healthy controls) and those with current depression but no past history of mania/hypomania. Each group comprised 93 participants.

Importantly, we did not exclude individuals due to presence of other mental disorders. Therefore, our sample contained participants with anxiety disorders. The most prevalent was generalized anxiety disorder (GAD, 53 individuals), followed by obsessive-compulsive disorder (OCD, 22 individuals) and posttraumatic stress disorder (PTSD, 19 patients). Anxiety disorders are frequently comorbid with mood disorders, and the frequency of GAD, OCD, and PTSD was significantly higher in participants with MDD and BD than in controls. To improve the reliability of diagnosis, we used the Structured Clinical Interview for DSM-IV (SCID).[27] Some participants were reclassified after this interview, which was used as the group-defining criterion for the present study. After this strategy, our final sample consisted of 94 controls, 82 participants with MDD, and 55 participants with BD (33 type I and 22 type II) (Figure 1).

Instruments

As noted above, the MINI was used as an initial screening test to select MDD, BD, and control subjects, and the SCID was used to enhance the reliability of diagnosis. The SCID interviews were conducted by two psychologists who had received intensive training at the specialist outpatient facilities of Hospital de Clínicas de Porto Alegre, under the supervision of one of the senior investigators (FK). Additionally, the Young Mania Rating Scale (YMRS) and the Hamilton Depression Rating Scale (HDRS) were used to assess (hypo)manic and depressive symptoms.[28,29] We used a cutoff age of 19 years to define groups with early onset of mood disorders (MDD or BD). This age was chosen on the basis of the World Health Organization criteria, which define adolescence as the period of life between 10 and 19 years of age.[30] Information on drug misuse was obtained with the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST), an instrument which has been cross-culturally adapted to Brazilian Portuguese and was shown to have good psychometric validity for the assessment of substance use patterns in a sample of Brazilian participants.[31]

Quantitation of IL-10 in serum

After the SCID interview, at 8:00-11:00 a.m., each participant underwent collection of a 10-mL sample of peripheral blood by venipuncture into an anticoagulant-free vacuum tube. The blood was immediately centrifuged at 4,000 $\times$ *g* for 15 min and the serum stored at -80 °C until analysis. Serum samples were assayed by laboratory technicians blinded to the clinical characteristics of participants. At the end of the study, IL-10 levels were measured using a commercially available immunoassay kit (DuoSet, R&D Systems, Minneapolis, MN, USA). All samples and standards were measured in duplicate, and the coefficient of variation was $< 5\%$.

Statistical analysis

Descriptive analyses are presented as percentage or mean $\pm$ standard deviation. The sociodemographic and

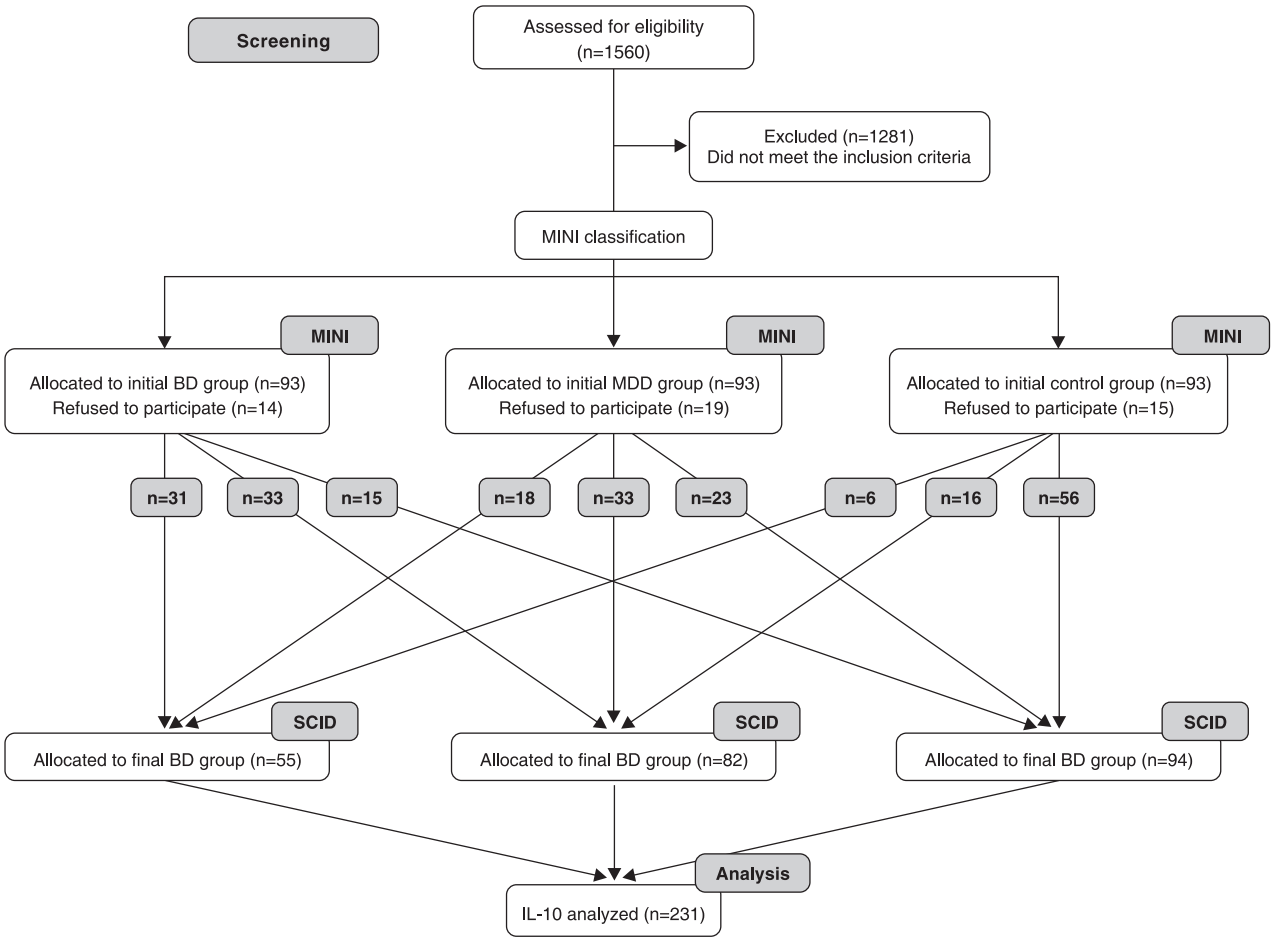

Figure 1 Flowchart of participant processing, from enrollment to biological sample collection. BD = bipolar disorder; MDD = major depressive disorder; MINI = Mini-International Neuropsychiatric Interview; SCID = Structured Clinical Interview for DSM-IV.

clinical characteristics of the sample were compared using an unpaired Student's *t*-test or the chi-square test, as appropriate. IL-10 levels had a non-Gaussian distribution, and values were logarithmically transformed before comparisons between diagnosis (MDD, BD, and control group), by analysis of variance (ANOVA) followed by Duncan post-hoc tests when appropriate, or age of onset, by Student's *t*-test. Relationships between variables were assessed with the Spearman rank correlation coefficient, a nonparametric measure of correlation. Endpoint differences between groups for age of onset and IL-10 levels were expressed as effect sizes using G*Power. Additionally, Poisson regression analysis with robust variances was performed to evaluate associations between the variables of interest and IL-10 levels. Statistical analyses were performed using SPSS version 16.0. P-values < 0.05 were considered statistically significant.

Results

The sociodemographic and clinical profile of the sample, stratified by diagnosis, is summarized in Table 1. The three groups were not significantly different in terms of age ($F_{2,228} = 2.25$, $p = 0.108$). We found a significant ($p = 0.012$) association between diagnosis and gender in our sample: MDD and BD patients were predominantly female (76.8% and 74.5%, respectively), while the control group had a lower frequency of female participants (57.4%). The other sociodemographic variables analyzed (ethnicity and socioeconomic class) did not differ between the MDD and control or BD and control groups ($p = 0.320$ and $p = 0.623$, respectively). The average age of disease onset was lower in patients with BD (15.98±4.31 years) than in patients with MDD (17.54±3.84 years) ($t_{135} = 2.158$, $p < 0.05$). Accordingly, the duration of illness was significantly higher in BD patients (5.84±3.80 years) than in MDD patients (4.23±4.01 years) ($t_{135} = -2.36$, $p = 0.033$). As expected, HDRS scores were higher in the MDD (12.8±7.7) and BD (14.6±8.6) groups as compared with the control group (1.55±3.4) ($F_{2,228} = 92.68$, $p = 0.001$). YMRS scores were higher in the BD group (4.0±6.9) than in MDD (0.6±1.8) or control (0.3±1.0) ($F_{2,228} = 31.252$, $p = 0.001$). No association was found between IL-10 levels and the sociodemographic variables of interest (age, $p = 0.99$; gender, $p = 0.99$; ethnicity, $p = 0.09$; socioeconomic level, $p = 0.06$).

Current use of psychiatric medication was very low in this sample, with only 20 participants (8.6% of the total sample) reporting use of mood stabilizers, antipsychotics, or antidepressants. Most participants who reported use

Table 1 Distribution of sociodemographic and clinical parameters of the sample according to diagnosis

Variable	Control (n=94)	Major depressive disorder (n=82)	Bipolar disorder (n=55)	p-value
Age (years)	22.4±2.5	21.7±2.0	21.8±2.2	0.108
Gender				
Female	54 (57.4)	63 (76.8)	41 (74.5)	0.012
Male	40 (42.6)	19 (23.2)	14 (25.5)	
Socioeconomic class*				
High (A + B)	35 (37.2)	24 (29.3)	14 (25.5)	0.320
Middle (C)	46 (48.9)	40 (48.8)	33 (60.0)	
Low (D + E)	13 (13.8)	18 (22.0)	8 (14.5)	
Ethnicity				
White	65 (69.1)	51 (62.2)	36 (65.5)	0.623
Nonwhite	29 (30.9)	31 (37.8)	19 (34.5)	
Age of onset (years)	-	17.54±3.84	15.98±4.31	0.033
Disease duration (years)	-	4.23±4.01	5.84±3.80	0.020
HDRS score	1.55±3.40	12.80±7.70	14.60±8.60	0.001
YMRS score	0.30±1.00	0.60±1.80	4.00±6.90	0.001
IL-10 (pg/mL)	5.04±6.45	5.68±7.65	5.74±6.83	0.768

Data presented as n (%) or mean ± standard deviation.
Age: $F_{2,228}$ = 2.25; age of onset: t_{135} = 2.158; disease duration: t_{135} = -2.36; HDRS score: $F_{2,228}$ = 92.68; YMRS score: $F_{2,228}$ = 31.252; IL-10: $F_{2,228}$ = 0,264.
* Socioeconomic class defined according to criteria set forth by the Brazilian Institute of Geography and Statistics (IBGE).

of psychiatric medication were BD patients in a current depressive episode (31.8%) and MDD patients in a current depressive episode (also 31.8%), followed by euthymic MDD patients (13.6%). However, it is important to highlight that use of psychiatric medication was not associated with changes in peripheral IL-10 levels (p = 0.664, data not shown).

Overall, we found that IL-10 levels were not significantly different across the MDD, BD, and control groups (5.68±7.65, 5.04±6.45, and 5.74±6.83 pg/mL, respectively; $F_{2,228}$ = 0.264, p = 0.903). However, we found a significant increase in peripheral IL-10 levels in patients with a later onset (age ⩾ 20 years) of MDD symptoms (p = 0.006, effect size = 0.240; Figure 2A), but not for age of BD onset (p = 0.399; effect size = 0.110; Figure 2B). According to the Poisson regression model adjusted for age and sex, MDD patients with late disease onset (age ⩾ 20 years) had higher levels of IL-10 as compared to the control group (PR = 1.82; 95%CI 1.04-3.18). Disease duration was not used as a factor for adjustment, because this variable was highly correlated with age of disease onset (Pearson correlation coefficient = -0.87; p ⩽ 0.05).

In addition, serum levels of IL-10 correlated negatively with duration of illness in patients with MDD (r = -0.258, p = 0.021, Figure 3A), but not in patients with BD (r = -0.164, p = 0.235, Figure 3B).

Discussion

The present study showed that higher levels of IL-10 are associated with later onset of psychiatric symptoms and negatively correlated with disease duration in patients with MDD, but not in those with BD. Interestingly, IL-10 levels in early-onset MDD patients were similar to those of control participants, but significantly increased in MDD patients with a later onset of illness. Changes in the anti-inflammatory/proinflammatory balance have been widely

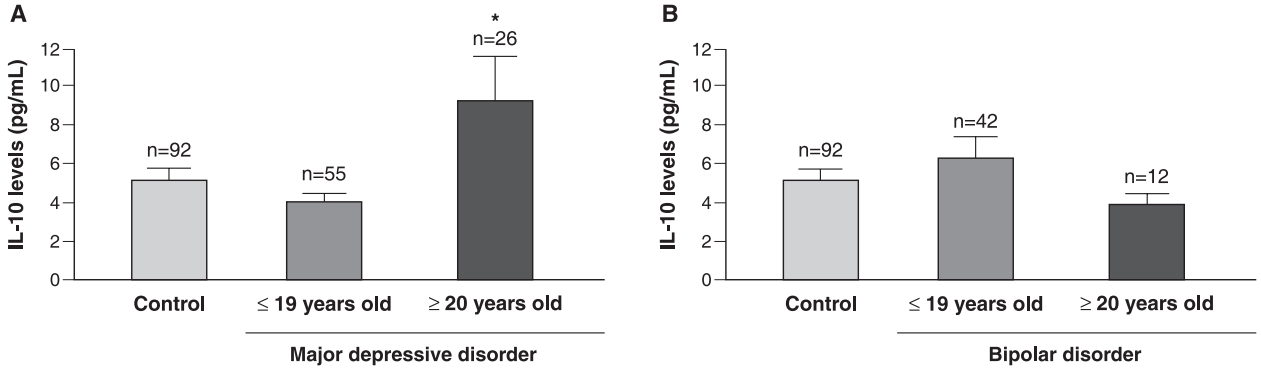

Figure 2 Serum IL-10 levels stratified by age of onset (years) of psychiatric symptoms in patients with A) major depressive disorder (p = 0.006) or B) bipolar disorder (p = 0.399). Values are expressed as mean ± standard error of mean. Analysis of variance (ANOVA) followed by Duncan post-hoc test performed using log-transformed values. *p < 0.01 when compared to control group and early onset of illness.

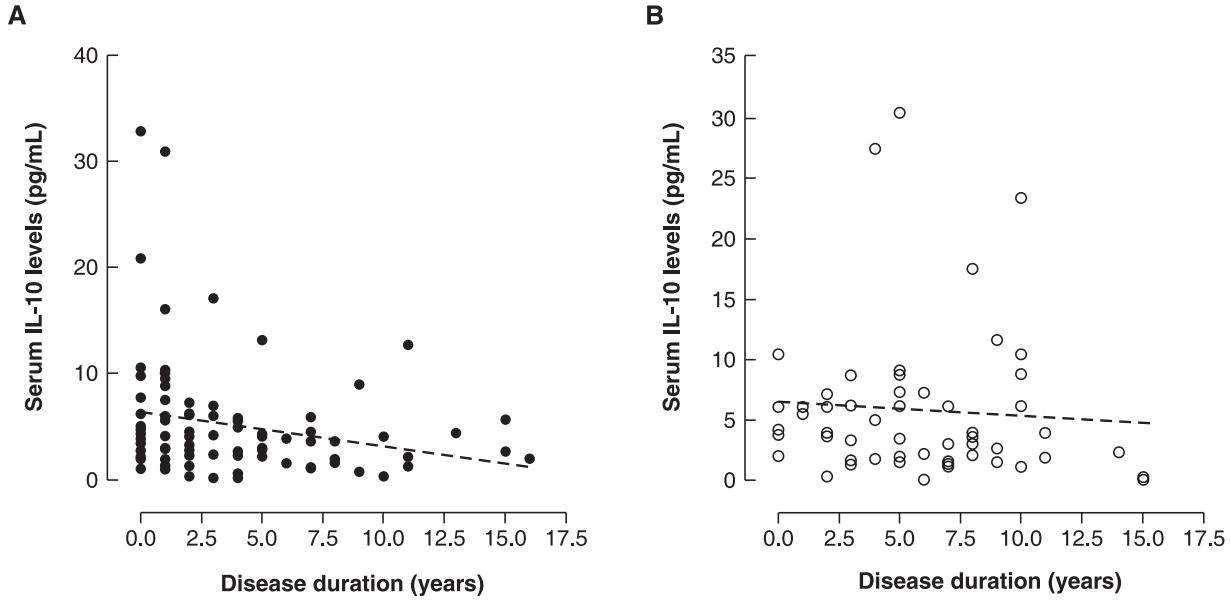

Figure 3 Correlation between serum IL-10 levels and disease duration (in years) in patients with A) major depressive disorder ($r = -0.258$, $p = 0.021$) or B) bipolar disorder ($r = -0.164$, $p = 0.235$).

implicated in the symptomatology and progression of mood disorders. Our findings suggest that IL-10 levels alone cannot predict the diagnosis of MDD; however, in vulnerable individuals, this cytokine might be associated with age of onset and disease progression.

Controversy remains as to the relationship between psychiatric disorders and IL-10 levels.[18,19,21] These conflicting results are probably a reflection of sample sizes, lack of control for confounding factors, and heterogeneity in clinical presentation, including, possibly, in disease duration and severity.[10] In the present study, peripheral levels of IL-10 in patients with MDD and BD were not significantly different from those of control participants. Our results corroborate two recent meta-analyses that found no significant differences of IL-10 levels in patients with MDD and BD.[9,12]

There has been substantial discrepancy in the definition of early-onset mood disorders, with different studies employing a variety of different cutoffs. Here, we used a cutoff age of 19 years, which corresponds to the WHO definition of the end of adolescence, a period of life characterized by dramatic developmental and psychological changes.[30] According to this cutoff, patients classified as having late disease onset (age $\geqslant$ 20 years) had higher levels of IL-10 when compared to patients who experienced their first depressive symptoms during or before adolescence. These changes in IL-10 levels according to disease onset were not observed in BD patients. In our study, the mean age at onset of psychiatric symptoms was 17.54 ± 3.84 years in the MDD group and 15.98 ± 4.31 years in the BD group. This is consistent with previous epidemiological studies, which suggest that patients with BD generally have an earlier age of onset than those with MDD, with an estimated mean difference of 6 years.[32-34] Although the etiological factors that contribute to the onset of psychiatric symptoms are still poorly understood, early onset generally indicates greater overall severity, and could predispose the patient to other features of illness that contribute to poor outcomes.[35-37] Early onset of MDD is associated with severe symptoms, increased medical and psychiatric comorbidities, more depressive episodes, and suicidal attempts.[38,39] In BD, early onset is also associated with higher rates of depression and suicidal ideation.[40]

It is well accepted that the neuropathological and behavioral changes that occur in the brain of patients with psychiatric disorders are associated with cumulative deterioration patterns.[6] In the present study, we also found a negative correlation between IL-10 levels and disease duration for MDD, but not for BD. Bipolar patients had an early onset of psychiatric symptoms when compared to MDD patients, as well as increased disease duration (5.84 ± 3.80 vs. 4.23 ± 4.01 years). We did not observe an association between IL-10 levels and disease duration in BD patients. Kauer-Sant'Anna et al. reported that the IL-10 levels or patients with BD decline with disease progression. However, these discrepancies might be related to differences in the number of episodes, which were not evaluated in the present study.[21] In addition, antidepressants and mood stabilizers have been shown to stimulate production of IL-10 and reduction of the general proinflammatory/anti-inflammatory cytokine ratio.[25,26]

It is worth noting that our sample consisted mostly of young participants, and that psychiatric medication use was practically absent. Only 8.6% of participants reported the use of any psychiatric medication, and IL-10 levels were not different in these patients. Thus, the identification of physiological alterations that could precede symptom onset might lead to initiation of interventions early in the course of the disorder, which is generally more effective.

The present study should be interpreted in the context of its limitations. Some clinical variables, including age

at illness onset and illness duration, were assessed retrospectively, and their measurements might be influenced by participant recall or reporting bias. Moreover, the narrow age range of the sample may have been a limiting factor for the correlation between IL-10 and disease timing in patients with BD, and may be implicated in the weak correlation observed with progression of MDD. However, especially when dealing with biological markers of complex disorders, the impact of each individual factor is expected to be small. Longitudinal studies are needed to further examine these issues. Despite these limitations, the present data show that higher levels of the anti-inflammatory cytokine IL-10 are associated with later onset of psychiatric symptoms and negatively correlated with disease duration in MDD, but not in BD. As healthy immune regulation is accomplished through counter-balancing of the effects of pro- and anti-inflammatory cytokines and IL-10 normally inhibits the actions of proinflammatory cytokines and reduces inflammation, we speculate that, in susceptible patients, lower levels of IL-10 might contribute to an adverse immunological profile and precipitation of psychiatric symptoms. However, the involvement of this cytokine in the early diagnosis and prognosis of this disorder still needs to be further explored, as do the mechanisms that could be involved in regulation of the immune system and manifestation of psychiatric symptoms.

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Disclosure

The authors report no conflicts of interest.

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