

## ORIGINAL PAPER

Ângelo B. Cunha · Ana C. Andreazza · Fabiano A. Gomes · Benicio N. Frey · Leonardo E. da Silveira · Carlos A. Gonçalves · Flávio Kapczinski

## Investigation of serum high-sensitive C-reactive protein levels across all mood states in bipolar disorder

Received: 22 August 2007 / Accepted: 6 November 2007 / Published online: 23 February 2008

**Abstract** There has been an increasing interest in the role of the immune and inflammatory systems in mood disorders. Mood episodes are associated with changes in acute phase proteins such as high-sensitivity C-reactive protein (hsCRP). The present study investigated serum hsCRP in manic, depressed, and euthymic BD patients as compared to matched healthy controls. Serum hsCRP was assessed using an ultrasensitive assay of particle-enhanced immunoturbidimetric latex agglutination. Serum hsCRP levels were increased in manic BD patients, as compared to euthymic, depressed patients and healthy controls ( $P < 0.001$ ). These findings add to the notion that changes in the inflammatory system take place during acute episodes of mania.

**Key words** bipolar disorder · high-sensitivity C-reactive protein · inflammation · Mania · pathophysiology

### Introduction

Bipolar disorder (BD) is a chronic, severe, and highly disabling illness, which is estimated to affect 1% of the population worldwide [4]. BD is associated with increased morbidity and mortality due to general medical conditions, such as cardiovascular disease, obesity and diabetes mellitus, which are not simply a result of psychiatric symptoms or personal dysfunction [22]. Recent studies have found that most bipolar patients have high rates of functional and cognitive impairment, contributing to the substantial psychosocial morbidity associated with the disorder [44]. Although there has been a considerable increase in the understanding of the pathophysiological processes of BD, the underlying biology and common mechanisms of comorbid physical and psychiatric disorders remain largely unclear.

More recently, there has been an increasing interest in the role of the immune and inflammatory systems in mood disorders. It has been demonstrated that mood episodes are associated with changes in cytokines profiles [20, 27] and acute phase proteins such as immunoglobulins, complement proteins, factor B [40], and high-sensitivity C-reactive protein (CRP) [16]. CRP is the first classic acute phase reactant to be released and its plasma values can increase 10,000-fold in response to infection and injury. It is a direct and quantitative measure of the overall acute phase response, and for this reason, its use as a marker of disease has received considerable attention [7]. CRP is produced in the liver in response to IL-6, as well as to IL-1 and tumor necrosis factor. Its protein levels begin to rise within 4–6 h after tissue injury or insult and continue to increase exponentially, doubling every 8–9 h and peaking at several hundred

Â.B. Cunha

Department of Neuropsychiatry  
Centro de Ciências da Saúde  
Universidade Federal de Santa Maria  
Faixa de Camobi Km 9  
Santa Maria, RS 97105-900, Brazil

A.C. Andreazza · C.A. Gonçalves  
Department of Biochemistry  
Instituto de Ciências Básicas da Saúde  
Universidade Federal do Rio Grande do Sul  
Rua Ramiro Barcelos, 2600/Anexo  
Porto Alegre, RS 90035-003, Brazil

F.A. Gomes · L.E. da Silveira · F. Kapczinski (✉)

A.C. Andreazza  
Bipolar Disorders Program  
Centro de Pesquisas  
Hospital de Clínicas de Porto Alegre  
Rua Ramiro Barcelos, 2350  
Porto Alegre, RS 90035-003, Brazil  
Tel.: +55-51/32227309  
Fax: +55-51/21018846  
E-Mail: kapcz@terra.com.br

B.N. Frey  
McConnell Brain Imaging Centre  
Montreal Neurological Institute  
McGill University  
Montreal, Quebec H2A 2B4, Canada

folds (depending on the stimulus) within 24–48 h. Serum CRP levels remain elevated throughout the acute phase response and only return to normal once normal tissue structure and function is restored. Therefore, it is considered a marker of systemic inflammation and several prospective studies have demonstrated a direct association between CRP levels and the risk for developing cardiovascular disease [38]. Some studies found an association of increased CRP levels with the severity of depressive symptoms [12, 37] and normalization after antidepressant treatment [26, 39]. However, these findings are not consistent across studies [21].

A few studies have reported CRP abnormalities in individuals with BD. Legros et al. [23] investigated several immunological factors such as immunoglobulins, autoantibodies and CRP in patients with psychiatric disorders including patients with bipolar depression. Although major depressive (unipolar) patients were more likely to present elevated CRP levels, there was no difference in the CRP levels between unipolar and bipolar subjects during depression. Similarly, Hornig et al. [16] compared patients with unipolar and BD and found no difference in CRP levels between the groups.

More recent studies measured the prevalence of high-sensitivity CRP (hsCRP), a more sensitive assay, in BD patients. Wade et al. [40] studied humoral immunity including hsCRP levels in manic patients and found higher hsCRP levels in the bipolar groups compared to controls, albeit the result did not reach statistical significance. Huang and Lin [17] studied bipolar patients during manic episodes and patients with unipolar depression. When compared to healthy subjects, only the bipolar group had higher levels of hsCRP. Finally, Dickerson et al. [8], studying stable outpatients with BD, found a significant correlation between manic symptoms and CRP levels.

Although there is evidence for an association of mania and elevated CRP levels, none of the studies evaluated BD patients in different phases of the illness. Thus, the present study was designed to evaluate

the involvement of inflammation in BD, as measured by serum levels of hsCRP in BD across all mood states—mania, depression and remission.

## Methods

The present study was approved by the local ethics committee (Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil) and all subjects provided written informed consent before entering in the study. Thirty euthymic, 20 depressed, and 30 manic patients were recruited from the BD s Program—Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil, and the Inpatient Psychiatric Unit—Hospital Universitário de Santa Maria, Santa Maria, Brazil. Diagnoses were carried out using the Structured Clinical Interview for DSM-IV—Axis I (SCID-I) [11]. In this study, only BD type-I patients were enrolled. Manic and depressive symptoms were assessed using the Young Mania Rating Scale (YMRS) [43] and the Hamilton Depression Rating Scale (HDRS) [14], respectively. Patients were considered euthymic if they scored <7 on both YMRS and HDRS scales. Manic and depressed patients fulfilled criteria for current manic or depressive episode, respectively, according to SCID-I. All subjects that fulfilled DSM-IV criteria for a current mixed episode were excluded from the present study. The control group consisted of 32 healthy volunteers matched by age, gender and education, who manifested interest in participating in the study. Psychiatric assessment was carried out using SCID-I, non-patient version. Control subjects did not have history of major psychiatric disorders, dementia or mental retardation. Five milliliter of blood were withdrawn from each subject by venipuncture into a free-anticoagulant vacuum tube. Measurement of CRP was performed on fasting serum with an ultrasensitive assay using particle-enhanced immunoturbidimetric latex agglutination methodology [10], using the automated analyzer Hitachi 912. The limit of detection of serum hsCRP levels in the present assay was less than 0.15 mg/l. All data are presented as mean  $\pm$  S.D. Differences between experimental groups were determined by one-way ANOVA followed by Tukey post hoc test if applicable. Pearson correlation coefficient was used to analyze the correlation between hsCRP and YMRS and HDRS scores. *P* values less than 0.05 were considered to indicate statistical significance.

## Results

The demographic characteristics of BD patients and controls are summarized in Table 1. BD patients and controls did not differ in terms of gender, age or years

**Table 1** Characteristics of BD patients and healthy controls

	Control group	Bipolar disorder patients			<i>P</i> value
		Euthymic	Manic	Depressed	
Gender (female)	65.6%	62.5%	43.8%	71.4%	0.162*
Age (years)	40.69 (12.12)	40.28 (11.99)	40.13 (12.6)	40.71 (9.25)	0.997**
Years of schooling	8.69 (3.64)	9.94 (4.80)	7.69 (3.65)	7.53 (4.77)	0.117**
Age of first mood episode	–	23.13 (11.38)	27.19 (10.87)	21.24 (13.89)	0.320**
Years of illness	–	17.34 (11.88)	12.78 (9.62)	19.50 (14.17)	0.209**
HDRS score	–	4.28 (4.16)	5.16 (3.39)	22.81 (4.36) <sup>a</sup>	0.001**
YMRS score	–	3.16 (5.44)	34.47 (7.06) <sup>b</sup>	5.10 (3.19)	0.001**

Values of variables are presented as mean ( $\pm$ S.D.)

HDRS Hamilton depression rating scale, YMRS Young Mania rating scale

\* Chi-square test

\*\* One-way ANOVA test

<sup>a</sup>Depressed > euthymic/manic

<sup>b</sup>Manic > euthymic/depressed

of schooling. BD patients were similar in age of first mood episode and length of illness. One-way ANOVA revealed that serum hsCRP levels were significantly different between groups ( $F_{(112,3)} = 64.33$ ;  $P < 0.001$ ). Serum hsCRP levels were increased in manic BD patients ( $11.81 \pm 12.29$  mg/l;  $P < 0.001$ ; Tukey post hoc), as compared with euthymic ( $2.14 \pm 2.58$  mg/l), depressed patients ( $2.30 \pm 2.23$  mg/l) and healthy controls ( $1.60 \pm 2.24$  mg/l) (Fig. 1). There were no significant differences in hsCRP levels between depressed and euthymic patients and healthy controls (all  $P > 0.05$ ). Finally, we found no correlation between hsCRP levels and YMRS or HDRS scores (all  $P > 0.05$ ).

## Discussion

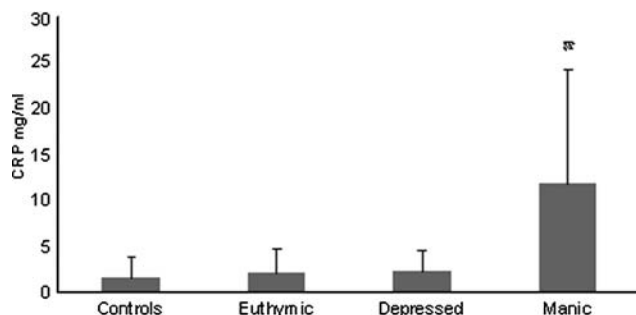
The present study demonstrated that serum levels of hsCRP were increased in bipolar patients during manic episode, as compared to euthymic, depressed patients and healthy controls. It is well established the association between BD and increased risk of cardiovascular and metabolic diseases [22, 25], but the pathophysiological mechanisms underlying this association are still under debate. It is possible that both immune and inflammatory systems play a role in these processes and that cytokines may be the one of the main mediators.

Consistent with the present study, recent studies have reported an association between inflammation, CRP and neuropsychiatric disorders, such as Alzheimer's disease and BD [20, 34, 42]. Since no clear association between depressive episodes and CRP elevation has been demonstrated to date a more intense activation of this inflammatory system may occur during manic episodes in particular. The reasons why CRP is selectively altered in manic but not in depressed or euthymic patients are unknown. While the present study is the first that investigated CRP levels across all mood states in individuals with BD, a recent study that compared other inflammatory markers between manic vs. depressed BD found higher IL-4 and lower IL-1 $\beta$  and IL-6 levels in the

manic subgroup, suggesting that manic and depressive episodes are associated with distinct patterns of cytokine production [28]. Nevertheless, our result is in accordance with Dickerson et al. [8] who found that CRP levels were higher in BD patients with YMRS  $> 6$  than in patients with YMRS  $< 6$ , and that CRP levels were positively correlated with YMRS but not HDRS scores, further suggesting that CRP may be specifically associated with the severity of manic symptoms in BD.

Inflammatory diseases may be initiated or amplified by oxidative stress [35]. The authors and others have demonstrated that BD patients have increased oxidative stress markers, such as: lipid peroxidation [1, 29, 30]; DNA damage [13]; and changes in anti-oxidants enzymes [1, 13, 30, 33]. In this context, CRP is associated with oxidative stress markers, mainly with lipid peroxidation [9]. Notably, Ratnam and Mookerjee [31] and Arcoleo et al. [2] reported that CRP can stimulate nitric oxide synthase (NOS) expression and NO production in rat and murine macrophages. More recently, Ikeda et al. [18] showed that CRP had no effect on NO synthesis, but it significantly increased NO synthesis in IL-1 $\beta$ -stimulated myocytes. Interestingly, IL-1 enhances the expression of other cytokines, such as IL-6, IL-10, TNF- $\alpha$  and S100B [19], and the latter was also shown to be increased during acute mania [1, 24]. A recent review proposed a comparative role between the peripheral biochemical markers CRP and S100B for systemic inflammation and brain injury, respectively [35], suggesting that both cytokines may induce oxidative stress or may be affected by oxidative damage.

It has been demonstrated that inflammation can release a number of neurotrophic factors upon activation, including brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) [15]. Some studies have suggested that serum levels of neurotrophic factors are altered during mood episodes in BD, including: lower BDNF [6], and higher NT-3 [41] and glial cell-derived neurotrophic factor [32]. It is unclear whether CRP is able to directly induce neurotrophin secretion, or indirectly via IL-1 and IL-6 activation [5]. It is plausible to consider that the interplay between inflammation and neurotrophic factors may contribute to some pathophysiological abnormalities seen in BD, such as cognitive decline and executive dysfunction. Very little is known about the effects of psychotropic agents on CRP levels, thus we cannot exclude that medication status may have influenced the present results. It has been demonstrated that treatment with antidepressants may reduce [26, 39] and olanzapine may increase [3] CRP levels. One study that investigated lithium augmentation to antidepressants in patients with refractory depression found that lithium augmentation may reduce CRP levels [36], while another study that assessed CRP levels in a mixed sample of bipolar I, bipolar II and unipolar patients showed that those



**Fig. 1** Serum high-sensitive C-reactive protein levels in BD patients and healthy controls. \*Different than depressed, euthymic and controls ( $p < 0.001$ ; One-way ANOVA + Tukey post hoc)

that were on lithium monotherapy were less likely to demonstrate increased levels of CRP [16]. However, the use of multiple medications and the retrospective design of these latter studies leave the question of potential effects of lithium on CRP levels unresolved. Prospective studies are clearly needed to determine the true impact of mood stabilizers on CRP levels in individuals with BD. Here it is worth mentioning that only one subject of the present study was taking anti-inflammatory agents, while 33% of the depressed, 18% of the euthymic and none of the manic patients were on antidepressants at the time that blood was collected. The vast majority of the subjects were receiving multiple combinations of mood stabilizers (lithium, valproate and/or carbamazepine) and typical and atypical antipsychotics. Thus, we believe that medication status may have had little effect (if any) on the present results.

In conclusion, we found that serum hsCRP levels are increased in BD patients during manic episodes, as compared with euthymic, depressed patients and healthy controls. This finding further supports the hypothesis of inflammatory involvement in the manic phase of BD. Longitudinal studies are necessary to better determine the clinical consequences of activation of inflammatory systems in BD.

## References

1. Andreazza AC, Cassini C, Rosa AR, Leite MC, de Almeida LM, Nardin P, Cunha AB, Cereser KM, Santin A, Gottfried C, Salvador M, Kapczinski F, Gonçalves CA (2007) Serum S100B and antioxidant enzymes in bipolar patients. *J Psychiatry Res* 41:523–529
2. Arcoleo F, Milano S, D'Agostino P, Misiano G, Cappelletti S, Gromo G, Marcucci F, Leoni F, Cillari E (1997) Effect of partially modified retro-inverso analogues derived from C-reactive protein on the induction of nitric oxide synthesis in peritoneal macrophages. *Br J Pharmacol* 120:1383–1389
3. Baptista T, Davila A, El Fakih Y, Uzcategui E, Rangel NN, Olivares Y, Galeazzi T, Vargas D, Pena R, Marquina D, Villaruel V, Teneud L, Beaulieu S (2007) Similar frequency of abnormal correlation between serum leptin levels and BMI before and after olanzapine treatment in schizophrenia. *Int Clin Psychopharmacol* 22:205–211
4. Belmaker RH (2004) Bipolar disorder. *N Engl J Med* 351:476–486
5. Carlson NG, Wieggl WA, Chen J, Bacchi A, Rogers SW, Gahring LC (1999) Inflammatory cytokines IL-1 alpha, IL-1 beta, IL-6, and TNF-alpha impart neuroprotection to an excitotoxin through distinct pathways. *J Immunol* 163:3963–3968
6. Cunha AB, Frey BN, Andreazza AC, Goi JD, Rosa AR, Gonçalves CA, Santin A, Kapczinski F (2006) Serum brain-derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes. *Neurosci Lett* 398:215–219
7. Ferranti SD de, Rifai N (2007) C-reactive protein: a nontraditional serum marker of cardiovascular risk. *Cardiovasc Pathol* 16:14–21
8. Dickerson F, Stallings C, Origoni A, Boronow J, Yolken R (2007) Elevated serum levels of C-reactive protein are associated with mania symptoms in outpatients with bipolar disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 31:952–955
9. Dohi Y, Takase H, Sato K, Ueda R (2007) Association among C-reactive protein, oxidative stress, and traditional risk factors in healthy Japanese subjects. *Int J Cardiol* 115:63–66
10. Eda S, Kaufmann J, Roos W, Pohl S (1998) Development of a new microparticle-enhanced turbidimetric assay for C-reactive protein with superior features in analytical sensitivity and dynamic range. *J Clin Lab Anal* 12:137–144
11. First MB, Spitzer RL, Gibbon M, Williams JB (1998) Structured clinical interview for DSM-IV (SCID-I). Biometrics Research Department, New York
12. Ford DE, Erlinger TP (2004) Depression and C-reactive protein in US adults: data from the Third National Health and Nutrition Examination Survey. *Arch Intern Med* 164:1010–1014
13. Frey BN, Andreazza AC, Kunz M, Gomes FA, Quevedo J, Salvador M, Gonçalves CA, Kapczinski F (2007) Increased oxidative stress and DNA damage in bipolar disorder: a twin-case report. *Prog Neuropsychopharmacol Biol Psychiatry* 31:283–285
14. Hamilton M (1960) A rating scale for depression. *J Neurol Neurosurg Psychiatry* 23:56–62
15. Hohlfeld R (2007) Does inflammation stimulate remyelination? *J Neurol* 254(Suppl 1):I47–I54
16. Hornig M, Goodman DB, Kamoun M, Amsterdam JD (1998) Positive and negative acute phase proteins in affective subtypes. *J Affect Disord* 49:9–18
17. Huang TL, Lin FC (2007) High-sensitivity C-reactive protein levels in patients with major depressive disorder and bipolar mania. *Prog Neuropsychopharmacol Biol Psychiatry* 31:370–372
18. Ikeda U, Maeda Y, Yamamoto K, Shimada K (2002) C-reactive protein augments inducible nitric oxide synthase expression in cytokine-stimulated cardiac myocytes. *Cardiovasc Res* 56:86–92
19. Kim SH, Smith CJ, Van Eldik LJ (2004) Importance of MAPK pathways for microglial pro-inflammatory cytokine IL-1 beta production. *Neurobiol Aging* 25:431–439
20. Kim YK, Jung HG, Myint AM, Kim H, Park SH (2007) Imbalance between pro-inflammatory and anti-inflammatory cytokines in bipolar disorder. *J. Affect Disord* (in press)
21. Kuo HK, Yen CJ, Chang CH, Kuo CK, Chen JH, Sorond F (2005) Relation of C-reactive protein to stroke, cognitive disorders, and depression in the general population: systematic review and meta-analysis. *Lancet Neurol* 4:371–380
22. Kupfer DJ (2005) The increasing medical burden in bipolar disorder. *JAMA* 293:2528–2530
23. Legros S, Mendlewicz J, Wybran J (1985) Immunoglobulins, autoantibodies, other serum protein fractions in psychiatric disorders. *Eur Arch Psychiatry Neurol Sci* 235:9–11
24. Machado-Vieira R, Lara DR, Portela LV, Gonçalves CA, Soares JC, Kapczinski F, Souza DO (2002) Elevated serum S100B protein in drug-free bipolar patients during first manic episode: a pilot study. *Eur Neuropsychopharmacol* 12:269–272
25. Newcomer JW (2006) Medical risk in patients with bipolar disorder and schizophrenia. *J Clin Psychiatry* 67(Suppl 9):25–30
26. O'Brien SM, Scott LV, Dinan TG (2006) Antidepressant therapy and C-reactive protein levels. *Br J Psychiatry* 188:449–452
27. O'Brien SM, Scully P, Scott LV, Dinan TG (2006) Cytokine profiles in bipolar affective disorder: focus on acutely ill patients. *J Affect Disord* 90:263–267
28. Ortiz-Dominguez A, Hernandez ME, Berlanga C, Gutierrez-Mora D, Moreno J, Heinze G, Pavon L (2007) Immune variations in bipolar disorder: phasic differences. *Bipolar Disord* 9:596–602
29. Ozcan ME, Gulec M, Ozerol E, Polat R, Akyol O (2004) Antioxidant enzyme activities and oxidative stress in affective disorders. *Int Clin Psychopharmacol* 19:89–95
30. Ranjekar PK, Hinge A, Hegde MV, Ghatge M, Kale A, Sitasawad S, Wagh UV, Debsikdar VB, Mahadik SP (2003) Decreased antioxidant enzymes and membrane essential polyunsaturated fatty acids in schizophrenic and bipolar mood disorder patients. *Psychiatry Res* 121:109–122

31. Ratnam S, Mookerjee S (1998) The regulation of superoxide generation and nitric oxide synthesis by C-reactive protein. *Immunology* 94:560–568
32. Rosa AR, Frey BN, Andreazza AC, Cereser KM, Cunha AB, Quevedo J, Santin A, Gottfried C, Goncalves CA, Vieta E, Kapczinski F (2006) Increased serum glial cell line-derived neurotrophic factor immunocontent during manic and depressive episodes in individuals with bipolar disorder. *Neurosci Lett* 407:146–150
33. Savas HA, Gergerlioglu HS, Armutcu F, Herken H, Yilmaz HR, Kocoglu E, Selek S, Tutkun H, Zoroglu SS, Akyol O (2006) Elevated serum nitric oxide and superoxide dismutase in euthymic bipolar patients: impact of past episodes. *World J Biol Psychiatry* 7:51–55
34. Schmidt R, Schmidt H, Curb JD, Masaki K, White LR, Launer LJ (2002) Early inflammation and dementia: a 25-year follow-up of the Honolulu Asia Aging Study. *Ann Neurol* 52:168–174
35. Sen J, Belli A (2007) S100B in neuropathologic states: the CRP of the brain? *J Neurosci Res* 85:1373–1380
36. Sluzewska A, Sobieska M, Rybakowski JK (1997) Changes in acute-phase proteins during lithium potentiation of antidepressants in refractory depression. *Neuropsychobiology* 35:123–127
37. Suarez EC (2004) C-reactive protein is associated with psychological risk factors of cardiovascular disease in apparently healthy adults. *Psychosom Med* 66:684–691
38. Sugano Y, Anzai T, Yoshikawa T, Satoh T, Iwanaga S, Hayashi T, Maekawa Y, Shimizu H, Yozu R, Ogawa S (2005) Serum C-reactive protein elevation predicts poor clinical outcome in patients with distal type acute aortic dissection: association with the occurrence of oxygenation impairment. *Int J Cardiol* 102:39–45
39. Tuglu C, Kara SH, Caliyurt O, Vardar E, Abay E (2003) Increased serum tumor necrosis factor- $\alpha$  levels and treatment response in major depressive disorder. *Psychopharmacology (Berl)* 170:429–433
40. Wadee AA, Kuschke RH, Wood LA, Berk M, Ichim L, Maes M (2002) Serological observations in patients suffering from acute manic episodes. *Hum Psychopharmacol* 17:175–179
41. Walz JC, Andreazza AC, Frey BN, Cacilhas AA, Cereser KM, Cunha AB, Weyne F, Stertz L, Santin A, Goncalves CA, Kapczinski F (2007) Serum neurotrophin-3 is increased during manic and depressive episodes in bipolar disorder. *Neurosci Lett* 415:87–89
42. Yasojima K, Schwab C, McGeer EG, McGeer PL (2000) Human neurons generate C-reactive protein and amyloid P: upregulation in Alzheimer's disease. *Brain Res* 887:80–89
43. Young RC, Biggs JT, Ziegler VE, Meyer DA (1978) A rating scale for mania: reliability, validity and sensitivity. *Br J Psychiatry* 133:429–435
44. Zarate CA Jr, Tohen M, Land M, Cavanagh S (2000) Functional impairment and cognition in bipolar disorder. *Psychiatr Q* 71:309–329