Are Serum Levels of Inflammatory Markers Associated With the Severity of Symptoms of Bipolar Disorder?

Xiuhua Wu
Third Affiliated Hospital of Sun Yat-sen University
Zhongcheng Chen
Third Affiliated Hospital of Sun Yat-sen University
Yingtao Liao
Third Affiliated Hospital of Sun Yat-sen University
Zhihua Yang
Third Affiliated Hospital of Sun Yat-sen University
Xiaolin Liang
Third Affiliated Hospital of Sun Yat-sen University
Nianhong Guan
Third Affiliated Hospital of Sun Yat-sen University
Zhaoyu Gan (ganzhy@mail.sysu.edu.cn)
Third Affiliated Hospital of Sun Yat-sen University

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Abstract

Background: To explore the relationship between serum levels of inflammatory markers and symptomatic severity of bipolar disorder (BD).

Material and Methods: A cross-sectional study was conducted on 126 BD patients with current depressive episode (BDD), 102 BD patient with current mixed or (hypo)manic episode (BDM) and 94 healthy controls (HC). Fasting serum levels of CRP, leptin (LEP), adiponectin (ADP), visfatin (VIS), TNF-α, IL-2, IL-6, IL-10, IL-17), and monocyte chemoattractant protein-1 (MCP-1) were measured with enzyme-linked immunosorbent assay (ELISA). Symptomatic severity of BD was assessed with HAMD-17 and YMRS. Generalized linear model was used to determine the association between the serum levels of inflammatory markers and symptomatic severity of BD.

Results: The serum levels of IL-6, IL-10 and IL-17, and the IL-6/IL-10 ratio were significantly lower in mild BDD than in HC. In moderate BDD, the serum levels of MCP, IL-6 and IL-17 were significantly lower than in HC. In severe BDD, the serum level of ADP, MCP-1, IL-10 and IL-17and the IL-17/IL-10 ratio were significantly lower than in HC. The serum levels of TNF-α and the IL-6/IL-10 ratio were significantly higher in mild BDM than in HC. In moderate BDM, the serum level of VIS, IL-2, and IL-17 were significantly higher than in HC, but the IL-6/IL-10 ratio was significantly lower than in control. In severe BDM, the serum levels of IL-6 and IL-17 and the ratios of IL-6/IL-10 and IL-17/IL-10 were significantly lower than in HC, but the neutrophil/lymphocyte ratio was significantly higher than in HC.

Conclusions: In BDD, immune-inhibition is persistently predominant, while in BDM, immune system is first activated then inhibited with the worsening of the symptoms. The dynamic change of serum inflammatory markers might help stage BD and guide the future immune treatment of BD.

Background

Since Kraepelin first noted that bipolar disorder (BD) had an accelerating and progressive course in 1921[1], several clinical staging systems have been put forward to stage BD with peripheral biomarkers.[2], and one of the staging systems is immune system [3]. Over the past decade, growing and converging evidence has demonstrated that immune dysfunction is involved in the pathophysiology of BD [4]. Therefore, BD is viewed as a multi-system inflammatory disease[5] and anti-inflammatory agents are thought to be a potential treatment option of BD [6]. However, inflammatory markers, as biomarkers to stage BD, have been seldom studied. As far as we know, only one systematic review [3] has ever summarize the available literature of studies about the clinical variables related with staging BD patients. According to this review, the most frequently evaluated clinical variables related to BD staging were onset age, duration of illness course, number of affective episodes and cognitive function, but few studies have ever used symptomatic severity as a clinical variable to stage BD and associate it with peripheral inflammatory markers. This paucity of data is further problematic since symptoms are not only the target of treatment but also reflect the severity of illness, and the latter is an important clinical variable associated with the stage of BD[7].

Elevated pro-inflammatory cytokines had been reported to be associated with greater symptom burden in BD [8, 9]. However, this way that treats the relationship between serum levels of inflammatory markers and symptomatic severity as a linear one might simplify their association. Supposing BD was a multi-system inflammatory disease as someone claimed [5], the serum levels of inflammatory markers would first increase with the worsening of symptoms and then decrease because of exhaustion with the evolvement of illness like other inflammatory diseases [10]. Additionally, peripheral inflammatory biomarkers are variable and heterogeneous. Lots of factors might affect the serum level of inflammatory biomarkers, including gender[11], age[12], marital status[13], body mass index (BMI) [14], psychoactive substance abuse[11], atypical features[15], duration of illness[16], pharmaceutical treatment[17], and so on. Finally, among the peripheral inflammatory biomarkers, some are associated with mood states (state markers) and some are related to specific features of the long-term course of illness (trait markers)[18]. This further complicates the relationship between serum levels of inflammatory biomarkers and the stage of BD and poses a big challenge in studies of this area.

In this study, we are going to assess the relationship between serum levels of inflammatory cytokines and symptomatic severity of bipolar disorder in a large, drug-naive BD population, aiming to explore the possibility of serum levels of inflammatory cytokines as biomarkers to stage BD and guide the future immune treatment of BD if possible.

Materials And Methods

Subjects

The sample consisted of 228 BD patients and 94 healthy controls. The cases came from the inpatients or outpatients who sought medical help in the psychiatric department of the Third Affiliated Hospital of Sun Yat-sen University between August 8, 2012 and January 6, 2018. The healthy controls were volunteers recruited from the local community during the same period. All the participants were Han Chinese, aged between 16 and 65, had no current active physical illness associated with inflammatory response confirmed by reviewing their previous medical records and routine clinical examination, had no history of psychoactive substance abuse in the past six months, and provided written informed consent. Participants who aged under 18 were required to provide written informed consent from their guardians. The cases had to meet the following criteria: a) fulfill the diagnostic criteria of bipolar disorder of any kind according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM- -TR);b)had not received any psychopharmaceutical treatment within 3 months prior to recruitment; c) had no comorbid organic mental disorder. The healthy controls were screened for mental disorders using the Chinese version of the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) Axis 1 Disorders (SCID-I), none patient version, and those with a current or history of major psychiatric disorders, dementia, mental retardation, would be excluded. In addition, participants who were pregnant or postpartum, under steroid or non-steroid anti-inflammatory drugs or other immune-inhibitors treatment were also excluded. This study was reviewed and approved by the Clinical Research Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University.

Measurement

Diagnosis of BD

The diagnosis of BD was based on the structural interview with the Chinese version of the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) Axis 1 Disorders (SCID-I), patient version.

Assessment of the symptomatic severity of BD

The symptomatic severity of BD was evaluated with the 17-item Hamilton Depression Scale (HAMD-17) [19] and the Young Mania Rating Scale (YMRS)[20].

Biochemical measurement

Ten milliliters of fasting blood were withdrawn between 7:00am and 9:00 am from each subject by venipuncture into two free-anticoagulant vacuum tubes. One tube of blood was immediately sent to the library of our hospital for routine blood test and measurement of C reactive protein (CRP). Routine blood test was performed in a automated blood cell counter (Hiesen Mikang co., Ltd, Kōbe, Japan). CRP were measured by immune transmission turbidimetry with a biochemical analyzer (Sichuan Mike Biological Co., Ltd and Nippon ihua co., Ltd, Tokyo, Japan). The other tube of blood was immediately centrifuged at 3000g for 5 min, and the serum was kept frozen at −80°C until assayed. The concentrations of leptin (LEP) adiponectin (ADP) visfatin (VIS), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-17 (IL-17), and monocyte chemoattractant protein-1 (MCP-1) were assessed with enzyme-linked immunosorbent assay (ELISA) using assay from Shanghai Caiyou Industrial Co. Ltd.

Statistic analysis

Participants were categorized into BD group and HC group according to the inclusion criteria, and the BD group was further divided into two subgroups——depressive group and (hypo)manic or mixed group based on their current episode. Patients with current mixed episode was classified as (hypo) manic group, considering the number of patients with current (hypo)manic episode was small in this study and (hypo)manic and mixed episode shared similar treatment strategy[21]. The scores of HAMD-17 and YMRS were treated as a categorized variables, for HAMD-17: 0 (no depression): <7; 1 mild depression : ≥7 and 17 2 (moderate depression): ≥17 and <24 ; 3 (severe depression): ≥24 for YMRS: 0 (no mania): <6; 1 (mild mania): ≥6 and <13 2 moderate mania :≥13 and <19; 3 (server mania): ≥19. For normally distributed data, independent-sample t-test was used to test the difference between groups, while for non-normally distributed variable, comparison between groups was analyzed with Wilcoxon rank sum test. Difference in categorical parameters between groups was tested using Chi-square test. Generalized linear model was built to assess the impact of severity of symptoms on the serum levels of inflammatory markers. Odds ratios (OR) and 95% confidence intervals (95% CI) were used to quantify the strength of associations. The Bonferroni adjusted significance test was used for multiple comparisons. All data were analyzed using commercial statistical package SPSS 24.0 (SPSS, Inc., Chicago, IL).

Results

Demographic and clinical characteristics of participants

As seen in Table1, 126 BD patients with current depressive episode (BDD), 102 BD patients with current (hypo)manic or mixed episode (BDM) and 94 healthy controls (HC) were recruited in this study. The level of education and the proportion of female participants among BDD were significantly lower than those among HC (P<0.001), but no significant difference was found in age, marital status, and BMI between BDD and HC. Compared to HC, BDM was younger (P<0.001) and had a lower level of education (P<0.001), but did not significantly differ in gender proportion, marital status and BMI.

Table 1
Demographic and clinical characteristics of participants

	Bipolar disorder		Control	P^1	P^2
	Depressive	(hypo)manic	N=94		
	N=126	N=102			
Age (mean±SD)(year)	27.8±10.7	25.4±8.1	29.6±11.0	0.241	**0.002**
Female n(%)	65(51.6)	66 (64.7)	68(72.3)	**0.002**	0.284
Education (mean±SD)(year)	13.0±3.4	12.8±3.0	16.2±3,5	**<0.001**	**<0.001**
Married n (%)	42(33.3)	29(28.4)	28(29.8)	0.661	0.876
BMI (mean±SD)	21.7±2.9	21.5±3.6	21.5±3.8	0.674	0.997
Severity of symptoms					
1 (mild)	17	35	NA		
2(moderate)	35	33	NA		
3(severe)	74	33	NA		
Note:					
1. Bipolar depression compared with Control;					
2. Bipolar disorder with current (hypo)manic or mixed episode compared with Control.					
3. BMI: body mass index					
4. Bold font denotes p < 0.05					

The association between severity of depressive symptoms and serum level of inflammatory markers

Table 2 demonstrated that compared to HC, most of the observed inflammatory markers, including pro-inflammatory and anti-inflammatory markers, showed a trend of decrease in BDD of any degree. In mild BDD, the serum level of IL-6(P=0.003, OR=$e^{-1.087}$=0.337, 95%CI=$e^{-1.807}$-$e^{-0.366}$=0.164-0.693), IL-10(P=0.002, OR=$e^{-1.069}$=0.343, 95%CI=$e^{-1.746}$-$e^{-0.393}$=0.174-0.675) and IL-17(P<0.001, OR=$e^{-1.203}$=0.300, 95%CI=$e^{-1.81}$-$e^{-0.595}$=0.164-0.551) were significantly lower than those in HC, the IL-6/IL-10 ratio was significantly lower (P =0.008, OR=$e^{-0.892}$=0.410, 95%CI=$e^{-1.544}$-$e^{-0.23}$=0.311-0.990) in mild BDD than in health control, but after Bonferroni correction, the difference did not reach significance. In moderate BDD, the serum level of MCP (P=0.002, OR=e^{-733}=0.480, 95%CI=$e^{-1.194}$-$e^{-0.272}$=0.303-0.762), IL-6 (P<0.001, OR=$e^{-0.928}$=0.395, 95%CI=$e^{-1.41}$-$e^{-0.446}$=0.244-0.640) and IL-17 (P<0.001, OR=$e^{-1.035}$=0.355, 95%CI =$e^{-1.478}$-$e^{-0.593}$=0.228-0.553) were significantly lower than those in HC, but no significant difference was found in the ratios of IL-2/IL-10, IL-6/IL-10, IL-17/IL-10 and neutrophil/lymphocyte (N/L) between BDD and HC. In severe BDD, the serum level of ADP (P=0.002, OR=$e^{-0.81}$=0.445, 95%CI=$e^{-0.915}$-$e^{-0.199}$=0.400-0.820), MCP-1(P<0.001, OR=$e^{-0.557}$=0.573, 95%CI=$e^{-1.179}$-$e^{-0.44}$=0.308-0.644), IL-10(P=0.001, OR=$e^{-0.616}$=0.540, 95%CI=$e^{-0.979}$-$e^{-0.253}$=0.376-0.776, and IL-17(P<0.001, OR=$e^{-1.235}$=0.291, 95%CI=$e^{-1.607}$-$e^{-0.863}$=0.200-0.422) were significantly lower than those in HC, and the IL-17/IL-10 ratio was aslo lower (P<0.001, OR=$e^{-0.752}$=0.471, 95%CI=$e^{-1.091}$-$e^{-0.412}$=0.335-0.662).

Table 2
The association between severity of depressive symptoms and serum level of inflammatory markers

Inflammatory markers	N	Goodness of fit	Omnibus Test	BDD[1]										
		(value/df)	(*P*)	mild				moderate				severe		
				β[2]	95%CI		*P*[3]	β[2]	95%CI		*P*[3]	β[2]	95%CI	
WBC[4]	156	3.312	0.463	-0.409	-1.606	0.789	0.504	0.037	-0.793	0.868	0.930	0.400	-0.241	1.042
CRP[5]	172	0.951	0.535	-0.121	-0.691	0.449	0.677	-0.175	-0.593	0.243	0.412	-0.225	-0.535	0.085
ADP[5]	210	1.612	0.013	-0.482	-1.149	0.185	0.157	-0.481	-0.928	-0.034	0.035	-0.557	-0.915	-0.199
LEP[5]	210	1.591	0.113	-0.295	-.0959	0.368	0.383	0.188	-0.257	0.632	0.407	-0.330	-0.686	0.026
VIS[5]	210	1.322	0.512	-0.255	-.866	0.357	0.414	-0.218	-0.627	0.191	0.297	-0.217	-0.545	0.111
MCP-1[5]	210	1.730	<0.001	-0.904	-1.592	-0.215	0.010	-0.733	-1.194	-0.272	**0.002**	-0.810	-1.179	-0.440
IL-2[5]	156	0.794	0.392	-0.048	-0.552	0.457	0.853	-0.162	-0.530	0.205	0.386	0.153	-0.156	0.462
IL-6[5]	210	1.914	<0.001	-1.087	-1.807	-0.366	**0.003**	-0.928	-1.410	-0.446	**<0.001**	-0.544	-0.930	-0.158
IL-10[5]	210	1.665	0.001	-1.069	-1.746	-0.393	**0.002**	-0.581	-1.034	-0.128	0.012	-0.616	-.979	-0.253
IL-17[5]	156	1.197	<0.001	-1.203	-1.810	-0.595	**<0.001**	-1.035	-1,.478	-0.593	**<0.001**	-1.235	-1.607	-0.863
TNF-α[5]	210	1.215	0.405	-0.106	-0.694	0.483	0.725	-0.319	-0.714	0.075	0.112	0.027	-0.288	0.343
N/L[6]	154	0.206	0.328	-0.182	-0.476	0.113	0.226	0.003	-0.201	0.208	0.975	0.087	-0.071	0.246
IL-2/IL-10[5]	156	0.921	0.403	0.382	-0.157	0.922	0.165	-0.024	-0.417	0.369	0.904	0.162	-0.169	0.493
IL-6/IL-10[5]	210	1.584	0.032	-0.892	-1.554	-0.230	0.008	-0.361	-0.805	0.082	0.110	-0.368	-0.724	-0.013
IL-17/IL-10[5]	156	0.978	0.001	-0.319	-0.874	0.235	0.257	-0.296	-0.699	0.108	0.151	-0.752	-1.091	-0.412

Note:

1. Adjustment for age, gender, marital status, BMI, education, and severity of manic symptoms.

2. Taking the healthy control group as reference, if the β is positive, the level of inflammatory marker is higher than that of healthy control group, and if the β i negative, the level of inflammatory marker is lower than that of healthy control group

3. Bold font denotes p < 0.05/15=0.0033

4. According to the scatter plot and statistical test, these data are approximately normally distributed, so the linear model is used in modeling;

5. According to the scatter plot and statistical test, these data are skewed distributed, but approximately normally distributed after log conversion, so gamma link model is chosen for modeling.

6. N/L: Granulocyte/lymphocyte ratio

The association between severity of manic symptoms and serum level of inflammatory markers

Table 3 showed a different change model of serum level of inflammatory markers across manic symptoms of varying degree. With the worsening of manic symptoms, most of the observed pro-inflammatory markers were seen to elevate first then decrease, but the anti-inflammatory marker, IL-10, decreased first then increased and finally decreased, though the change did not reach significance. In mild BDM, only the serum level of TNF-α(P<0.001, OR=$e^{1.000}$=2.718, 95%CI= $e^{0.490}$-$e^{1.511}$=1.632-4.531) was significantly higher than that in HC, but the IL-6/IL-10 ratio was significantly lower (P<0.001, OR=$e^{1.141}$=3.130, 95%CI= $e^{0.586}$-$e^{1.696}$=1.797-5.452) in case than in control. In moderate BDM, the serum levels of VIS(P<0.001, OR=$e^{0.862}$=2.368, 95%CI= $e^{0.419}$-$e^{1.305}$=1.520-3.688), IL-2(P<0.001, OR=$e^{0.983}$=2.672, 95%CI= $e^{0.606}$-$e^{1.361}$=1.833-3.900), and IL-17(P<0.001, OR=$e^{1.375}$=3.955, 95%CI= $e^{0.951}$-$e^{1.798}$=2.588-6.038), were seen to be significantly higher than in HC, but the IL-6/IL-10 ratio was also significantly lower (P=0.003, OR=$e^{-0.685}$=0.504, 95%CI= $e^{-1.141}$-e^{-0230}=0.319-0.794) in the case group. However, when the manic symptoms further worsen to the severe degree, most of the observed pro-inflammatory markers decreased. Of them, IL-6 (P=0.001, OR=$e^{-0.813}$=0.443, 95%CI= $e^{-1.281}$-$e^{-0.346}$=0.278-0.708) and IL-17 (P<0.001, OR=$e^{-1.110}$=0.330, 95%CI= $e^{-1.716}$-$e^{-0.504}$=0.180-0.604) reached significance. In addition, the ratios of IL-6/IL-10 (P<0.001, OR=$e^{-1.011}$=0.364, 95%CI= $e^{-1.442}$-$e^{-0.580}$=0.236-0.560) and IL-17/IL-10 (P=0.001, OR=$e^{-0.761}$=0.467, 95%CI= $e^{-1.209}$-$e^{-0.314}$=0.298-0.730) were significantly lower in the case group than in the control group. Finally, the N/L ratio was found to be significantly higher in severe BDM than in HC (P=0.001, OR=$e^{0.499}$=1.647, 95%CI= $e^{0.212}$-$e^{0.787}$=1.236-2.198).

Table 3
The association between severity of manic symptoms and serum level of inflammatory markers

Inflammatory markers	N	Goodness of fit (value/df)	Omnibus Test (*P*)	BDM[1]											
				mild				moderate				severe			
				β[2]	95%CI		*P*[3]	β[2]	95%CI		*P*[3]	β[2]	95%CI		
WBC[4]	146[1]	3.807	0.345	0.462	-0.685	1.609	0.430	0.761	-0.074	1.595	0.074	0.228	-0.538	0.994	
CRP[5]	161[6]	1.452	0.065	-0.238	-0.800	0.323	0.406	0.472	0.074	0.869	0.020	0.166	-0.199	0.531	
ADP[5]	189	2.196	0.022	0.604	-0.018	1.227	0.057	0.401	-0.116	0.918	0.128	-0.319	-0.802	0.164	
LEP[5]	190	1.6741	0.010	0.309	-0.244	0.862	0.273	0.458	0.004	0.912	0.048	-0.412	-0.814	0.017	
VIS[5]	190	1.584	<0.001	0.068	-0.472	0.608	0.805	0.862	0.419	1.305	**<0.001**	-0.281	-0.700	0.138	
MCP-1[5]	191	2.029	0.082	-0.355	-0.957	0.246	0.247	0.023	-0.471	0.517	0.929	-0.584	-1.047	-0.121	
IL-2[6]	144	52.166	<0.001	0.499	0.004	0.995	0.048	0.983	0.606	1.361	**<0.001**	0.134	-0.286	0.554	
IL-6[5]	190	2.035	0.007	-0.355	-0.958	0.248	0.248	0.106	-0.388	0.601	0.674	-0.813	-1.281	-0.346	
IL-10[5]	191	1.854	0.22	-0.588	-1.167	-0.010	0.046	0.086	-0.389	0.561	0.723	-0.177	-0.622	0.268	
IL-17[6]	144	26.162	<0.001	-0.395	-1.083	0.293	0.260	1.375	0.951	1.798	**<0.001**	-1.110*	-1.716	-0.504	
TNF-α[6]	190	1.396	NA[7]	1.000	0.490	1.511	**<0.001**	0.440	0.021	0.860	0.039	0.014	-0.382	0.410	
N/L[8,5]	144	0.573	0.009	0.163	-0.266	0.592	0.456	0.217	-0.095	0.5303	0.173	0.499	0.212	0.787	
IL-2/IL-10[5]	144	0.928	0.077	0.450	-0.027	0.926	0.064	0.219	-0.161	0.598	0.259	-0.150	-0.526	-0.226	
IL-6/IL-10[5]	190	1.687	<0.001	1.141	0.586	1.696	**<0.001**	-0.685	-1.141	-0.230	**0.003**	-1.011	-1.442	-0.580	
IL-17/IL-10[5]	144	1.368	0.001	-0.028	-0.595	0.539	0.922	0.318	-0.133	0.769	0.167	-0.761	-1.209	-0.314	

Note:

1. Adjustment for age, gender, marital status, BMI, education, and severity of depressive symptoms

2. Taking the healthy control group as reference, if the β is positive, the level of inflammatory marker is higher than that of healthy control group, and if the β i negative, the level of inflammatory marker is lower than that of healthy control group

3. Bold font denotes p < 0.05/15=0.0033

4. According to the scatter plot and statistical test, these data are approximately normally distributed, so the linear model is used in modeling;

5. According to the scatter plot and statistical test, these data are skewed distributed, but approximately normally distributed after log conversion, so gamma link model is chosen for modeling.

6. The data dispersion is too high to be modeled when linear or gamma with log link mode is used, so tweedie with log link mode is used instead.

7. Unable to compute the initial model log likelihood due to numerical problems

8. N/L: Granulocyte/lymphocyte ratio

Discussion

We investigated the relationship between serum levels of inflammatory markers and symptomatic severity of bipolar disorder among 228 drug-naïve patients with bipolar disorder. Our study has three major findings. First, in BDD, both pro-inflammatory markers and anti-inflammatory markers are lower than those in HC in spite of the symptomatic severity. Second, in BDM, the serum levels of pro-inflammatory markers first elevate then decrease with the worsening of the manic symptoms. Third, the imbalance of pro-inflammatory markers and anti-inflammatory markers is seen in both BDD and BDM, which is shown by an alteration (decrease in BDD and increase in BDM) in the IL-6/IL-10 ratio when the symptoms are mild and a decrease in the IL-17/IL-10 ratio when the symptoms are severe.

Contrary to the view from studies in major depressive episode (MDD) [22] [23]that serum levels of both pro-inflammatory cytokines and ant-inflammatory cytokines slightly increase in depressive episode, our study finds that both pro-inflammatory cytokines and anti-inflammatory cytokines decrease in bipolar depression. In addition, the decrease of the ratios of IL-6/IL-10 and IL-17/IL-10 found in this study also suggests that immune-inhibition is prominent in bipolar depression, especially in severe BD. Different from studies in MDD, few studies have ever assessed the relationship between the serum levels of inflammatory markers and depressive symptoms from studies in BDD. Several studies with small sample size have found that bipolar depression is associated with a pro-inflammatory state [24, 25]. However, in two review studies [26, 27] which meta-analyzed the cytokines alteration in BD, no consistent conclusion about the relationship between cytokines and bipolar depression was reached since few studies on bipolar depression were included and the sample sizes of the

included studies were all small. In addition, the heterogeneity between studies also played a part in this inconsistency[27]. A recent comparison study[28] base on a machine learning approach found that compared to healthy control, BDD had higher levels of C-C Motif Chemokine Ligand (CCL)3, CCL4, CCL5, CCL11, CCL25, CCL27, CXCL11, IL-9 and TNF-α. However, in this study, all the participants were medicated, potential confounders including age, gender, BMI, and tobacco or alcohol use were not controlled. In addition, the average age of the participants were bigger than those in our study (46.59±10.8 VS. 27.8±10.7), which might be another factor that makes their conclusion different from ours. In addition, the foregoing conclusion could not be duplicated by other studies either. For example, a study [29]from German showed that both pro-inflammatory and anti-inflammatory markers, but not CRP were inversely correlated with the severity and symptoms of major depression. A recent study [30]from China found that the serum levels of IL-13 and TNF-αwere significantly lower in BDD than in MDD, and the serum levels of IL-4 and TNF-αincreased in the treatment response subgroup of BDD. In recent years, anti-inflammatory agents were expected to be a promising treatment target of MDD [31]and BDD[32]. However, negative results from large clinical trials [33, 34]not only overturns the conclusion from small studies that the adjunctive use of anti-inflammatory drugs might help improve depressive symptoms in MDD or BDD, but also make us rethink the relationship between serum levels of inflammatory cytokines and depressive symptoms. Moreover, pro-inflammatory cytokines are not always neurotoxic. Instead, low-dose of pro-inflammatory cytokines such as IL-6, TNF-a, and IFN-γ have been proved to have a neuroprotective role[35].

The relationship between serum levels of inflammatory markers and BDM has been widely studied[36]. Most of previous studies find that both pro-inflammatory cytokines (IL-1RA[37], IL-1, Il-2[38], sIL-2R[39, 40], IL-6[38, 41], sIL-6R[39], TNF-α[41], CXCL10[42], CXCL11[42], CRP[39], IL-17[43], IFN-γ[25] and IL-18[44]) and anti-inflammatory markers (IL-4[38] and IL-10[45]) elevate in the manic episode. However, our study only partially supports this view. In our study, the elevation of pro-inflammatory markers was seen only in mild and moderate BDM, while in severe BDM the serum levels of pro-inflammatory were found to decrease. This finding is consistent with our previous hypothesis about the dynamic change of inflammatory markers over the course of a multi-system inflammatory disease. As far as we know, this has rarely been explored before. Although serum levels of some inflammatory cytokines like sIL-2R[40], IL-17[43], neural cell adhesion molecule 1(NCAM-1)[45], carcinoembryonic antigen (CEA)[46] and IFN-γ[25] have been reported to be positively correlated with severity of manic symptoms, the relationship between serum levels of inflammatory markers, according to our study, seems to be more complicated than a positive correlation.

Inflammatory ratios except N/L, which are less affected by exercise, BMI, and other confounding factors than other commonly used markers of inflammation[47], have been rarely studied in BD before. Partially in line with previous reports [48]that N/L ratio is higher in BDM than in BDD or MDD, our study finds that N/L ratio is significantly higher in severe BDM than in HC, suggesting an imbalance in favor of innate immunity [49]. In addition, we also find that the ratios of IL-6/IL-10 and IL-17/IL-10 vary with the clinical phase of BD and severity of symptoms: in BDD, the IL-6/IL-10 ratio first decrease in mild BDD and then the IL-17/IL-10 ratio decrease in severe BDD; while in BDM, the IL-6/IL-10 ratio first elevate in mild BDM and then decrease with the IL-17/IL-10 ratio in severe BDM. That is to say, the IL-6/IL-10 imbalance is an early immune response to BD, while IL-17/IL-10 imbalance is an indicator of deterioration of BD. To our knowledge, these have not been reported before. Although the pathophysiological meaning of IL-6/IL-10 and IL-17/IL-10 balance is far from being interpreted clearly, it is possible to suppose that they play a role in homing of inflammatory cells and therefore in the outcome of inflammation[50].

This study has several strengths. First of all, it comprises of one of the largest sample of BD patients ever examined. Second, all the subjects were Han Chinese and drug-naïve, most of them were young, and active physical diseases and recent substance abuse were excluded, which provided us a good sample with great homogeneity. Third, generalized linear model was used to statistically analyze the data, whereas as many potential confounding factors as possible were adjusted for, so the power of test greatly improved. Fourth, as many inflammatory cytokines as possible were measured at the same time, which makes the assessment of the immune state of the subjects more multidimensional. Finally, all the blood samples were treated in the same way and in the same laboratory, thus minimized the effect of measurement deviation. However, several limitations should be addressed when interpreting the foregoing conclusion. First, its cross-sectional design limits causal conclusion about the relationship between serum levels of inflammatory markers and BD. Second, symptoms of BD were treated as a binary variable—depressive or manic symptoms, while other domains of symptoms like psychotic symptoms, or cognitive symptoms were not concerned, which had been proved to be associated with the levels of inflammation. Third, although we have the largest sample of BD ever examined, the sample might be still not big enough to detect some small but clinically meaningful effect of inflammatory marker on the symptomatic severity of BD, especially when the sample is divided into several subgroups. Fourth, some but fortunately not much inflammatory markers' data was too dispersed to build a good generalized linear model, therefore caution should be exercised when interpretating the corresponding results.

Conclusion

The serum level of inflammatory markers and the balance between pro-inflammatory markers and anti-inflammatory markers not only vary with the types of affective episode but also with the severity of affective symptoms. In BDD, immune-inhibition is persistent predominant, while in BDM, immune system is first activated then inhibited with the worsening of the symptoms. The dynamic change of serum inflammatory markers might not only help stage BD but also guide the future immune treatment of BD.

Declarations

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Ethics approval and consent to participate

This study were reviewed and approved by the Clinical Research Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University. The research had been performed in accordance with the Declaration of Helsinki. Written informed consents had been attained from all the participants based on the principle

of self determination.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article and and its supplementary information file.

Competing interests

All authors report no biomedical financial interests or potential conflicts of interest.

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Authors' contributions

Zhaoyu Gan and Nianhong Guan conceived and designed the study and Revised it for intellectual content. Zhaoyu Gan, Xiuhua Wu, Yingtao Liao, Zhihua Yang and Nianhong Guan took recruitment and management of the case. Zhongcheng Chen was in charge of biochemical measurement. Xiuhua Wu Zhongcheng Chen and Xiaolin Liang contributed to data analysis and interpretation. Xiuhua Wu and Zhongcheng Chen Drafted the Article. All authors read and approved the final manuscript.

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