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Clinically relevant and simple immune system measure is related to symptom burden in bipolar disorder

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Objective: Immunological theories, particularly the sickness syndrome theory, may explain psychopathology in mood disorders. However, no clinical trials have investigated the association between overall immune system markers with a wide range of specific symptoms including potential gender differences.

Methods: We included two similar clinical trials, the lithium treatment moderate-dose use study and clinical and health outcomes initiatives in comparative effectiveness for bipolar disorder study, enrolling 765 participants with bipolar disorder. At study entry, white blood cell (WBC) count was measured and psychopathology assessed with the Montgomery and Aasberg depression rating scale (MADRS). We performed analysis of variance and linear regression analyses to investigate the relationship between the deviation from the median WBC, and multinomial regression analysis between different WBC levels. All analyses were performed gender-specific and adjusted for age, body mass index, smoking, race, and somatic diseases.

Results: The overall MADRS score increased significantly for each 1.0×10^9/l deviation from the median WBC among 322 men (coefficient = 1.10; 95% CI = 0.32–1.89; $p = 0.006$), but not among 443 women (coefficient = 0.56; 95% CI = −0.19–1.31; $p = 0.14$). Among men, WBC deviations were associated with increased severity of sadness, inner tension, reduced sleep, reduced appetite, concentration difficulties, inability to feel, and suicidal thoughts. Among women, WBC deviations were associated with increased severity of reduced appetite, concentration difficulties, lassitude, inability to feel, and pessimistic thoughts. Both higher and lower WBC levels were associated with increased severity of several specific symptoms.

Conclusion: Immune system alterations were associated with increased severity of specific mood symptoms, particularly among men. Our results

Keywords: affective disorder; bipolar disorder; clinical trials; lithium; mood stabilisers; psychoneuroimmunology

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support the sickness syndrome theory, but furthermore emphasise the relevance to study immune suppression in bipolar disorder. Due to the explorative nature and cross-sectional design, future studies need to confirm these findings.

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Significant outcomes

- White blood cell (WBC) deviations were associated with greater Montgomery and Aasberg depression rating scale (MADRS) severity.
- Higher and lower WBC levels were associated with specific symptoms.
- The findings were most pronounced among men.

Limitations

- WBC is a very unspecific immune system marker and has a large intra-individual variation.
- We did not measure subtypes of WBCs or other inflammatory markers.
- The cross-sectional and explorative design emphasise cautious interpretations.

Introduction

An increased immune system activity has been associated with the aetiology of bipolar disorder (1–3) and higher levels of pro-inflammatory markers may be associated with increased symptom severity and specific symptom domains in bipolar disorder (4–7). The sickness syndrome theory may represent one explanation for these associations, suggesting that pro-inflammatory cytokines affect the development and severity of specific symptoms, such as decreased appetite, increased need for sleep, social withdrawal, and anhedonia (8,9).

However, despite increasing pre-clinical evidence (10), this aspect has only been investigated in a few clinical trials with small study populations (4,5,11,12). In addition, the studies did not investigate specific symptoms (4,5,13) and factors such as body mass index (BMI) may confound these findings (11). Most studies did not include BMI (4,5,12), and in a recent study among depressed individuals, adjustment for BMI neutralised the unadjusted significant associations between pro-inflammatory cytokines and increased symptom severity (11). Also, immuno-suppression may affect psychopathology (14,15), but the trials have mostly investigated pro-inflammatory markers (4,5,11,12). Finally, important effects of sex hormones have been found during immune responses (16), why possible gender differences need to be investigated.

We aimed to perform an explorative study based on two large similar clinical trials, which included WBC levels. WBC is a marker of overall immune system activity, with high levels indicating an inflammatory response (i.e. leucocytosis) and low levels indicating insufficient production of immune cells (i.e. leucopenia). We hypothesised that WBC levels are associated with specific symptoms, possibly those suggested to be affected by the sickness syndrome (8,9). We *a priori* hypothesised gender differences. We thereby expand our previous work associating WBC levels with overall increased symptom severity (13).

Materials and methods

Setting

This study represents secondary analyses based on data from two similar multi-site, randomised comparative effectiveness trials based in the United States, the lithium treatment moderate-dose use study (LiTMUS) (17) and the clinical and health outcomes initiatives in comparative effectiveness for bipolar disorder study (Bipolar CHOICE) (18). LiTMUS compared lithium treatment combined with optimised personalised treatment (OPT) to OPT alone. Bipolar CHOICE compared lithium with quetiapine. The rationale, design, and specific methods are reported in detail elsewhere (17,18). The present study examined data at study entry (i.e. at baseline) and participants could not be treated with lithium or quetiapine at baseline (>30 days without treatment), as, for example, lithium affects WBC levels (19).

Participants

For LiTMUS, 338 patients were screened and 283 (84%) were randomised. For Bipolar CHOICE, 692 were screened and 482 (70%) were randomised. In both studies, participants were aged between 18 and 62 years, and limited inclusion and exclusion criteria were defined to maximise heterogeneity of the sample and thus, generalisability of the results. The main inclusion criteria were a DSM-IV-TR bipolar I or II

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diagnosis and a clinical global impression scale for bipolar disorder ≥3 (20) (i.e. mildly symptomatic).

In both studies, the extended Mini-International Neuropsychiatric Interview (21) was used to determine psychiatric and substance use diagnoses. Mood symptom severity was measured with the MADRS in LiTMUS (22) and with the bipolar inventory of symptoms scale (BISS) in Bipolar CHOICE (23–25). In order to pool data across studies, we extracted the MADRS score from the BISS items within the Bipolar CHOICE study, which has been validated (24). Clinical interviews obtained demographic information, mental and medical history (hypertension, diabetes, and hyperlipidemia), and current medications. In both studies, baseline WBC count was assessed in each patient at study entry via a fasting blood draw from an antecubital vein and analysed immediately at the local biochemical laboratory at the hospital where the patient participated (no specific time of day, WBC is expressed in International units, i.e. $\times 10^9$/l).

Statistical analysis

We identified baseline variables possibly influencing WBC levels and symptom severity: gender, age, BMI, race, current smoking, and the medical conditions of diabetes, hypertension and hyperlipidemia. We performed all analyses on men and women separately.

In both studies, mood symptom severity was greater among patients with the highest and lowest WBC counts. Therefore, as a first step, we investigated the association between the deviation from the median WBC and symptom severity. This means that we compared participants with higher or lower WBC counts at baseline (i.e. values higher or lower than the median WBC of all participants are grouped together and expressed as a positive value) to the median baseline WBC of all participants. We grouped participants based on their deviation from the median WBC of 6.9×10^9/l into the following categories: $<1 \times 10^9$/l, 1–1.99×10^9/l, 2–2.99×10^9/l, and $\geq 3 \times 10^9$/l. Similar cut-offs have been used in previous studies (26). As the deviation from the median WBC and MADRS symptom severity fitted a linear relationship in both studies, we performed analysis of variance (ANOVA) and linear regression analysis. We set those individuals who deviated $<1 \times 10^9$/l as the reference group and report p-values. If an overall ANOVA was significant, we performed pairwise comparison between the different groups by applying Bonferroni, Scheffe, and Sidak multiple comparison tests. For the linear regression analysis, we report a coefficient including 95% confidence intervals (95% CI). The coefficient corresponds to the change in symptom severity for each 1.0×10^9/l deviation from the median WBC. We were aware that this approach grouped individuals with high or low WBC levels, but we were interested whether any deviation from the median WBC would affect psychopathology.

Second, we specifically investigated if higher or lower WBC levels were associated with symptom severity. We divided participants into the following baseline WBC categories: $<4.5 \times 10^9$/l, 4.5–5.99×10^9/l, 6–6.99×10^9/l, 7–7.99×10^9/l, 8–9.99×10^9/l, $\geq 10 \times 10^9$/l. We set individuals with a WBC of 7–7.99×10^9/l as the reference group and performed multinomial logistic regression analysis reporting relative risk ratio including 95% CI.

For all the above-mentioned analyses, we first performed basic models adjusted for age. Second, we adjusted all analyses for age, BMI, race, current smoking, and a diagnosis of diabetes, hypertension, or hyperlipidemia.

As a primary analysis, we performed all the above-mentioned analyses (i.e. ANOVA, linear regression and multinomial logistic regression analysis) on the 10 individual item scores on the MADRS among all individuals from both trials ($n = 765$). As a second step, we also performed multinomial logistic regression analysis on different WBC levels and specific symptoms on the BISS scale (i.e. the remaining 34 items not included in the MADRS scale) for the participants ($n = 482$) in the Bipolar CHOICE study. For all analyses on specific symptoms, we divided individuals depending on the severity of a single specific symptom (1–2, 3–4, or 5–6) and compared with individuals without this symptom (i.e. a score of 0).

In all the above-mentioned analyses, we corrected for multiple testing by dividing the p-value of 0.05 with the amount of tests performed.

All statistical analyses were performed using STATA version 14.

Sensitivity analyses

Carbamazepine and clozapine can lower WBC levels (27). Therefore, we performed all the above-mentioned analyses where we excluded individuals who used these compounds at baseline to explore whether this had an impact on our findings.

Results

The median WBC across both studies ($n = 765$) was 6.9×10^9/l and ranged from 3×10^9/l to 18×10^9/l, which is similar to the general US adult population with a mean of 6.9×10^9/l (range 1.5–100×10^9/l) (28). The baseline characteristics based on WBC count are shown in Table 1, and Supplementary Material Table 1 shows the baseline characteristics in both studies divided by gender. Individuals with low

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Table 1. Baseline information on 765 patients with bipolar disorder from the lithium treatment moderate-dose use study ($n=283$) and the clinical and health outcomes initiatives in comparative effectiveness for bipolar disorder study ($n=482$), depending on white blood cell counts (WBC) at study entry

	Total	WBC < 4.5	4.5 ≤ WBC < 6	6 ≤ WBC < 7	7 ≤ WBC < 8	8 ≤ WBC < 10	WBC ≥ 10
Total	765 (100)	53 (6.9)	195 (25.5)	146 (16.3)	113 (15.2)	173 (26.2)	84 (12.4)
Gender							
Female	443 (56.5)	34 (64.1)	107 (54.9)	84 (57.5)	68 (60.2)	102 (59.0)	47 (56.0)
Male	322 (43.5)	19 (35.9)	88 (45.1)	62 (42.5)	45 (39.8)	71 (41.0)	37 (44.0)
Age group							
≤30	240 (30.7)	17 (32.1)	46 (23.6)	51 (34.9)	44 (38.9)	51 (29.5)	31 (36.9)
31–45	271 (36.8)	16 (30.2)	74 (38.0)	43 (29.5)	39 (34.5)	66 (38.2)	32 (38.1)
≥45	254 (32.5)	20 (37.7)	75 (38.5)	52 (35.6)	30 (26.5)	56 (32.3)	21 (25.0)
Current smoking	399 (53.0)	16 (30.2)	95 (48.7)	64 (43.8)	52 (46.0)	107 (61.8)	64 (76.2)
BMI							
<20	33 (2.8)	6 (11.3)	10 (5.1)	4 (2.7)	6 (5.3)	4 (2.3)	3 (3.6)
20–24.99	182 (26.5)	22 (41.5)	44 (22.6)	42 (28.8)	19 (16.8)	40 (23.1)	15 (17.9)
25–29.99	225 (32.2)	8 (15.1)	73 (37.4)	43 (29.5)	34 (30.1)	49 (28.3)	18 (21.4)
≥30	315 (36.4)	17 (32.1)	66 (33.8)	56 (38.4)	54 (47.8)	75 (43.4)	46 (54.8)
Medical conditions							
Diabetes	45 (5.3)	3 (5.7)	9 (4.6)	7 (4.8)	6 (5.3)	13 (7.5)	7 (8.3)
Hypertension	138 (17.0)	8 (15.1)	36 (18.5)	21 (14.4)	17 (15.0)	32 (18.5)	24 (28.6)
Hyperlipidemia	145 (14.8)	7 (13.2)	43 (22.1)	30 (20.5)	14 (12.4)	33 (19.1)	18 (21.4)

BMI, body mass index.

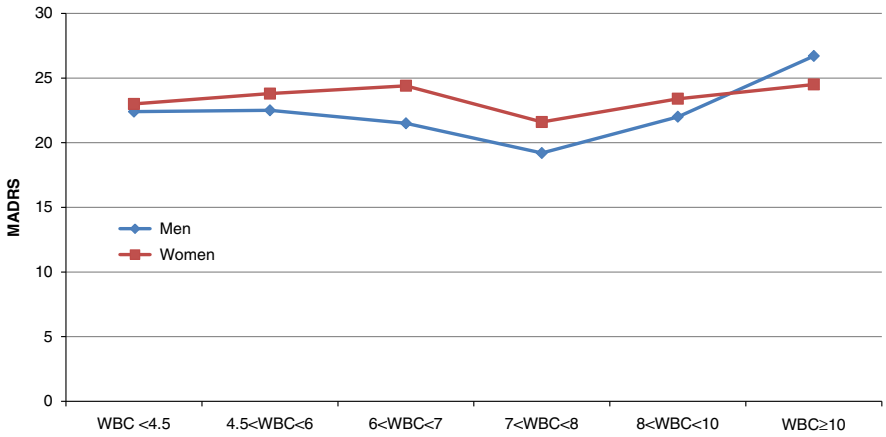

Fig. 1. White blood cell (WBC) count and Montgomery and Aasberg depression rating scale (MADRS) severity at baseline among 765 individuals with bipolar disorder. There were no significant differences between men and women for any WBC levels.

WBC levels had lower BMI and were less often smokers. Individuals with high WBC levels had higher BMI, were more often smokers, and were younger.

WBC deviations and specific MADRS symptoms

Higher and lower WBC levels were associated with higher MADRS total scores, which was more present among men despite no significant differences between men and women at any point (Fig. 1).

Tables 2 and 3 show the gender-separate association between the deviation from the median WBC and the 10 specific MADRS items. Among 322 men, WBC deviations were associated with increased severity on the following specific MADRS symptoms: reported sadness, inner tension, reduced appetite, inability to feel, and suicidal thoughts (all $p<0.05$, but only inner tension and inability to feel showed a corrected p-value <0.005). Among 443 women, WBC deviations showed a trend towards increased severity on apparent sadness ($p=0.08$), reduced appetite ($p=0.06$), and lassitude ($p=0.07$).

Higher and lower WBC levels are associated with specific MADRS symptoms

Among men, higher WBC levels (compared with men with WBC 7–7.99×10^9/l) showed a trend towards higher scores on reported sadness, apparent sadness, inner tension, reduced appetite, concentration

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Table 2. Analysis of variance* showing the correlation at study entry between the 10 specific Montgomery and Aasberg depression rating scale items and the deviation from the median white blood cell (WBC) count of 6.9×10^9/l among 322 men and 443 women with bipolar disorder from the lithium treatment moderate-dose use study and clinical and health outcomes initiatives in comparative effectiveness for bipolar disorder study

	Deviation from WBC median: 0–0.99	Deviation from WBC median: 1–1.99	Deviation from WBC median: 2–2.99	Deviation from WBC median: ≥3	Overall *p*-value†
Men ($n=322$)					
Reported sadness [mean (SD)]	2.2 (1.7)	2.3 (1.7)	2.3 (1.8)	3.0 (1.6)	0.04
Apparent sadness [mean (SD)]	2.5 (1.8)	2.5 (1.8)	2.6 (1.9)	3.2 (1.8)	0.10
Inner tension [mean (SD)]	2.0 (1.7)	2.6 (1.7)	2.3 (1.6)	2.7 (1.8)	0.02
Reduced sleep [mean (SD)]	2.6 (2.1)	3.2 (2.1)	3.2 (2.3)	3.1 (2.3)	0.14
Reduced appetite [mean (SD)]	1.2 (1.7)	1.4 (1.7)	2.0 (1.8)	1.2 (1.8)	0.03
Concentration difficulties [mean (SD)]	2.4 (1.7)	2.5 (1.8)	2.6 (1.8)	2.8 (1.8)	0.65
Lassitude [mean (SD)]	2.3 (1.9)	2.4 (1.8)	2.8 (1.8)	3.0 (2.0)	0.11
Inability to feel [mean (SD)]	2.2 (1.9)	2.2 (1.8)	2.3 (1.8)	2.9 (1.7)	0.12
Pessimistic thoughts [mean (SD)]	2.3 (1.7)	2.3 (1.8)	2.3 (1.8)	2.5 (2.0)	0.90
Suicidal thoughts [mean (SD)]	0.7 (1.0)	0.8 (1.3)	1.2 (1.6)	1.2 (1.5)	0.02
Women ($n=443$)					
Reported sadness [mean (SD)]	2.5 (1.7)	2.3 (1.6)	2.6 (1.7)	2.5 (1.7)	0.74
Apparent sadness [mean (SD)]	2.8 (1.7)	2.8 (1.7)	3.1 (1.7)	2.8 (1.8)	0.53
Inner tension [mean (SD)]	2.3 (1.6)	2.3 (1.8)	2.3 (1.8)	2.1 (1.8)	0.88
Reduced sleep [mean (SD)]	3.0 (2.2)	2.9 (2.1)	2.9 (2.1)	3.2 (2.2)	0.74
Reduced appetite [mean (SD)]	1.2 (1.5)	1.5 (1.8)	1.2 (1.6)	1.7 (1.8)	0.06
Concentration difficulties [mean (SD)]	2.8 (1.5)	2.8 (1.6)	2.7 (1.6)	3.1 (1.8)	0.53
Lassitude [mean (SD)]	2.9 (1.8)	2.8 (1.8)	3.1 (1.8)	3.0 (1.9)	0.57
Inability to feel [mean (SD)]	2.6 (1.8)	2.5 (1.8)	2.7 (1.8)	2.7 (1.7)	0.73
Pessimistic thoughts [mean (SD)]	2.5 (1.7)	2.4 (1.7)	2.7 (1.5)	2.6 (1.6)	0.53
Suicidal thoughts [mean (SD)]	0.8 (1.1)	0.8 (1.2)	0.8 (1.2)	0.9 (1.4)	0.92

* All analyses were adjusted for age, body mass index, current smoking, race, and a diagnosis of diabetes, hypertension or hyperlipidemia.
† The analyses for men and women were corrected for multiple testing by dividing the *p*-value of 0.05 with 10 (the amount of tests performed among men and women, respectively), that is, the corrected *p*-value is 0.005.

difficulties, inability to feel, pessimistic thoughts, and suicidal thoughts (most *p*-values <0.2 with several <0.05) (Supplementary Material Table 2). Lower WBC levels showed a trend towards higher scores on reported sadness, inner tension, reduced sleep, reduced appetite, concentration difficulties, and suicidal thoughts (most *p*-values <0.3 with several <0.05).

Among women, higher WBC levels (compared with women with WBC 7–7.99×10^9/l) showed a trend towards increased severity of apparent sadness, reduced appetite, concentration difficulties, lassitude, inability to feel, and pessimistic thoughts (most *p*-values <0.3 with several <0.05) (Supplementary Material Table 3). Lower WBC levels showed a trend towards increased severity of reported and apparent sadness, reduced sleep, reduced appetite, concentration difficulties, lassitude, inability to feel, and pessimistic thoughts (most *p*-values <0.2 with several <0.1). Among men and women, lower WBC levels showed a trend towards increased severity of reduced sleep, and higher WBC levels showed a trend towards lower severity for reduced sleep (most *p*-values <0.2 with several <0.05). The latter was most pronounced among men.

However, the majority of findings with $p < 0.05$ became non-significant after multiple testing.

Bipolar CHOICE: WBC levels and specific BISS symptoms

Finally, we assessed the remaining 34 BISS items among 199 men (Supplementary Material Table 4) and 283 women (Supplementary Material Table 5) from the Bipolar CHOICE study. Higher WBC levels were associated with an increment of severity on several depressive symptoms, in particular social withdrawal and anxiety symptoms among men and women. Also lower WBC levels were associated with increased severity of several depressive symptoms among men and women. Higher and lower WBC levels showed a trend towards increased severity of several manic and psychotic symptoms, which was most pronounced among women. However, due to small groups because of the detailed division, several multinomial regression analyses did not reach significance but only showed a trend. Furthermore, the majority of findings with $p < 0.05$ became non-significant after multiple testing.

Sensitivity analyses

At study entry, three individuals used clozapine and 34 used carbamazepine. Exclusion of these users did not change the results (not shown).

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Table 3. Linear regression analyses showing the association between the deviation from the median white blood cell (WBC) count and the 10 specific Montgomery and Aasberg depression rating scale (MADRS) items among 322 men and 443 women with bipolar disorder from the lithium treatment moderate-dose use study and clinical and health outcomes initiatives in comparative effectiveness for bipolar disorder study

	Model 1*		Model 2*	
	Coefficient† (95% CI)	*p*-value‡	Coefficient† (95% CI)	*p*-value‡
Men ($n=322$)				
Reported sadness	0.23 (0.05; 0.41)	0.01	0.13 (−0.01; 0.27)	0.07
Apparent sadness	0.19 (0.00; 0.38)	0.053	0.13 (−0.03; 0.28)	0.10
Inner tension	0.20 (0.02; 0.38)	0.03	0.21 (0.09; 0.34)	0.001
Reduced sleep	0.18 (−0.05; 0.41)	0.13	0.00 (−0.20; 0.20)	0.99
Reduced appetite	0.13 (−0.06; 0.32)	0.18	0.03 (−0.12; 0.19)	0.67
Concentration difficulties	0.12 (−0.07; 0.30)	0.21	0.10 (−0.03; 0.24)	0.13
Lassitude	0.23 (0.03; 0.44)	0.02	0.05 (−0.11; 0.20)	0.55
Inability to feel	0.19 (0.00; 0.38)	0.046	0.20 (0.08; 0.31)	0.001
Pessimistic thoughts	0.07 (−0.13; 0.26)	0.50	0.11 (−0.03; 0.25)	0.13
Suicidal thoughts	0.20 (0.06; 0.35)	0.006	0.14 (0.03; 0.25)	0.02
Women ($n=443$)				
Reported sadness	0.04 (−0.11; 0.19)	0.58	−0.05 (−0.18; 0.08)	0.44
Apparent sadness	0.06 (−0.09; 0.22)	0.41	0.09 (−0.01; 0.19)	0.08
Inner tension	−0.06 (−0.21; 0.09)	0.44	0.01 (−0.14; 0.13)	0.93
Reduced sleep	0.03 (−0.16; 0.22)	0.74	0.08 (−0.08; 0.24)	0.35
Reduced appetite	0.12 (−0.03; 0.27)	0.11	0.05 (−0.06; 0.17)	0.37
Concentration difficulties	0.07 (−0.07; 0.22)	0.33	0.10 (−0.05; 0.25)	0.18
Lassitude	0.08 (−0.08; 0.24)	0.33	0.12 (−0.01; 0.26)	0.07
Inability to feel	0.07 (−0.09; 0.22)	0.41	0.07 (−0.06; 0.19)	0.28
Pessimistic thoughts	0.07 (−0.07; 0.22)	0.32	0.06 (−0.08; 0.19)	0.41
Suicidal thoughts	0.03 (−0.08; 0.14)	0.61	0.04 (−0.07; 0.15)	0.49

The median WBC in this population was 6.9×10^9/l.

* Model 1 was adjusted for age; Model 2 was adjusted for age, body mass index, current smoking, race, and a diagnosis of diabetes, hypertension, or hyperlipidemia.

† The coefficient indicates the change in MADRS score for every deviation in white blood cell count, for example, a coefficient of 1.10 indicates an increase in MADRS of 1.10 for each 1.0×10^9/l deviation (both higher and lower) from the median WBC of 6.9×10^9/l.

‡ The analyses for men and women were corrected for multiple testing by dividing the *p*-value of 0.05 with 10 (the amount of tests performed among men and women, respectively), that is, the corrected *p*-value is 0.005.

Discussion

The present study found that higher WBC deviations from the median WBC were associated with greater severity of several specific bipolar disorder symptoms. Both lower and higher WBC levels were associated with an increased severity of several symptoms. In particular, the following MADRS items were affected: sadness, inner tension, reduced appetite, concentration difficulties, inability to feel, and suicidal thoughts. All the above-mentioned findings were most pronounced among men. We thereby expand our previous findings associating WBC deviations with increased overall mood symptom severity (13), and the present results give the most detailed overview to date between very specific bipolar disorder psychopathology and alterations in the immune system. As our analyses were exploratory, the design was cross-sectional, and the majority of tests did not survive multiple testing, future studies need to confirm these findings. Large, high-quality clinical trials should include a combination of markers on the overall immune system activity and a wide range of specific pro- and anti-inflammatory markers, preferably including sex hormone levels, and investigate associations with specific psychopathology over time.

Immune system alterations and symptom-specific severity in bipolar disorder

Our findings support the sickness syndrome theory, suggesting that peripheral cytokines and prostaglandins, produced during an inflammatory response, can cross the blood-brain barrier and thus, affect the CNS (8–10). Although we only had WBC counts, increased WBC levels (i.e. $>10\times10^9$) indicate leucocytosis and hence an acute inflammation. Furthermore, our results indicate that even low WBC levels affect psychopathology, representing a less expected finding. This may be explained by potential neuroprotective properties of immune cells, such as CD4+ T-cells (15), suggesting that also immune suppression may affect mood symptom severity.

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In addition, suicidal thoughts and risky behaviour showed a trend towards increased severity among men with higher or lower WBC levels. Previous studies have indicated that CNS affection during inflammatory responses may lead to self-harming and even suicidal behaviour (29). Finally, the fact that most results were most pronounced among men may be explained by the differential effects of sex hormones during immune responses, as female sex hormones may exhibit protective effects, whereas male sex hormones can act suppressive on cell-mediated immune responses (16).

WBC as a potential biomarker for more personalised treatment

In depression, studies have emphasised use of specific pro-inflammatory markers to identify subgroups of patients that may respond better to antidepressants (30) and anti-inflammatory treatment (31,32), which has been less studied in bipolar disorder (33–35). Our results suggest WBC as a valid and inexpensive biomarker, which is easy to apply and thus, relevant for everyday clinical use. Future studies should investigate whether increased and/or decreased WBC levels are associated with improved response to mood-stabilising treatment and/or anti-inflammatory drugs. These studies may explore gender differences and the combination of specific symptoms with several immune system markers.

Strengths and limitations

The LiTMUS and Bipolar CHOICE studies were very similar trials with broad inclusion and minimal exclusion criteria, thus representing populations seen in everyday clinical practice. Both studies included large sample sizes, and no patients were treated with lithium or quetiapine at the time of assessment. Furthermore, WBC is an easily available and inexpensive measure from peripheral blood, supporting the clinical relevance of our findings. In addition, we were able to adjust for important factors (i.e. BMI, smoking, race, and specific somatic diseases).

In regards to limitations, these explorative analyses were cross-sectional, performed *post-hoc* and most findings with $p < 0.05$ did not survive multiple testing. Hence, our findings have to be interpreted with caution. In addition, we did not include measures on specific subtypes of immune cells or other markers of immune activation (e.g. C-reactive protein or interleukin-6) and had only one WBC measure, with WBC being a rather non-specific marker of the immune system. In cardiovascular diseases, specific WBC subtypes seem to be associated with the risk increase associated with WBC deviations (36). Thus, it seems necessary to evaluate specific WBC lines and assess a wider range of both pro-inflammatory and anti-inflammatory markers, and several assessments over time. Furthermore, important effects of sex hormones have been found during immune responses (16), and future studies should include this aspect. In addition, although we adjusted for important somatic diseases, we had no knowledge whether participants suffered of an acute infection or an autoimmune illness, that is, the observed WBC counts may be due to other illnesses. Moreover, we did not adjust for use of other medications. As the Bipolar CHOICE and LiTMUS studies were intended to represent real-world settings, somatic diseases only resulted in exclusion if the somatic disease contraindicated treatment with the study medications. Finally, some analyses on specific symptoms were underpowered, for example, on some psychotic or manic symptoms. This was further limited as we investigated on outpatients with bipolar disorder, with most patients (>60%) being in depressed phases at study entry.

Disclosures

Dr. Nierenberg is a consultant for the Abbott Laboratories, American Psychiatric Association, Appliance Computing Inc. (Mindsite), Basliea, Brain Cells, Inc., Brandeis University, Bristol-Myers Squibb, Clintara, Corcept, Dey Pharmaceuticals, Dainippon Sumitomo (now Sunovion), Eli Lilly and Company, EpiQ, L.P./Mylan Inc., Forest, Genaissance, Genentech, GlaxoSmithKline, Hoffman LaRoche, Infomedic, Lundbeck, Janssen Pharmaceutica, Jazz Pharmaceuticals, Medavante, Merck, Methylation Sciences, Naurex, Novartis, PamLabs, Parexel, Pfizer, PGx Health, Ridge Diagnostics Shire, Schering-Plough, Somerset, Sunovion, Takeda Pharmaceuticals, Targacept, and Teva; consulted through the MGH Clinical Trials Network and Institute (CTNI) for AstraZeneca, Brain Cells, Inc, Dianippon Sumitomo/Sepracor, Johnson and Johnson, Labopharm, Merck, Methylation Science, Novartis, PGx Health, Shire, Schering-Plough, Targacept and Takeda/Lundbeck Pharmaceuticals. He receives grant/research support from American Foundation for Suicide Prevention, AHRQ, Brain and Behavior Research Foundation, Bristol-Myers Squibb, Cederroth, Cephalon, Cyberonics, Elan, Eli Lilly, Forest, GlaxoSmithKline, Janssen Pharmaceutica, Lichtwer Pharma, Marriott Foundation, Mylan, NIMH, PamLabs, PCORI, Pfizer Pharmaceuticals, Shire, Stanley Foundation, Takeda, and Wyeth-Ayerst. Honoraria include Belvoir Publishing, University of Texas Southwestern Dallas, Brandeis University, Bristol-Myers Squibb, Hillside Hospital,

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Dr. Tohen was a full time employee at Lilly (1997–2008). He has received honoraria from, or consulted for, Abbott, Actavis, AstraZeneca, Bristol-Myers Squibb, GlaxoSmithKline, Lilly, Johnson & Johnson, Otsuka, Merck, Sunovion, Forest, Gedeon Richter, Roche, Elan, Alkermes, Allergan, Lundbeck, Teva, Pamlab, Wyeth and Wiley Publishing. His spouse was a full time employee at Lilly (1998–2013).

During the last 3 years, Dr. Terence Ketter has had financial interests/arrangements or affiliations with organisations that could be perceived as real or apparent conflicts of interest. Dr. Ketter has received Grant/Research Support (through Stanford University) from the Agency for Healthcare Research and Quality, AstraZeneca Pharmaceuticals LP, Cephalon Inc. (now Teva Pharmaceuticals), Eli Lilly and Company, Pfizer, Inc., and Sunovion Pharmaceuticals; has served as a Consultant/Advisory Board Member for Allergan, Inc., Avanir Pharmaceuticals, Depotmed, Forest Pharmaceuticals, Genentech, Janssen Pharmaceuticals, Merck & Co., Inc., Myriad Genetic Laboratories, Inc., ProPhase, Sunovion Pharmaceuticals, and Teva Pharmaceuticals; has received Lecture Honoraria (NOT Speaker's Bureau payments) from Abbott Laboratories, Inc, GlaxoSmithKline, Otsuka Pharmaceuticals, Pfizer, Inc., and Sunovion Pharmaceuticals; and has received Royalties from American Psychiatric Publishing, Inc. In addition Dr. Ketter's spouse is an employee of and stockholder of Janssen Pharmaceuticals.

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Authors' Contribution: O.K.F., A.A.N., and L.G.S. planned this study and prepared the analysis plan. O.K.F. and A.A.N. had access to all data and performed the analyses. All authors contributed to the interpretation of the results. O.K.F., A.A.N., and L.G.S. wrote the first draft of the manuscript which was critically revised by all authors, and all authors have approved the final manuscript.

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Conflicts of Interest

The other authors declare no competing interests.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Supplementary materials

To view supplementary material for this article, please visit https://doi.org/10.1017/neu.2017.34

References

1. Drexhage RC, Knijff EM, Padmos RC et al. The mononuclear phagocyte system and its cytokine inflammatory networks in schizophrenia and bipolar disorder. Expert Rev Neurother 2010;**10**:59–76.
2. Bai YM, Su TP, Tsai SJ et al. Comparison of inflammatory cytokine levels among type I/type II and manic/hypomanic/euthymic/depressive states of bipolar disorder. J Affect Disord 2014;**166**:187–192.
3. Dargel AA, Godin O, Kapczinski F, Kupfer DJ, Leboyer M. C-reactive protein alterations in bipolar disorder: a meta-analysis. J Clin Psychiatry 2015;**76**:142–150.
4. Hope S, Dieset I, Agartz I et al. Affective symptoms are associated with markers of inflammation and immune

activation in bipolar disorders but not in schizophrenia. J Psychiatr Res 2011;**45**:1608–1616.
5. Lotrich FE, Butters MA, Aizenstein H, Marron MM, Reynolds CF III, Gildengers AG. The relationship between interleukin-1 receptor antagonist and cognitive function in older adults with bipolar disorder. Int J Geriatr Psychiatry 2014;**29**:635–644.
6. Hope S, Ueland T, Steen NE et al. Interleukin 1 receptor antagonist and soluble tumor necrosis factor receptor 1 are associated with general severity and psychotic symptoms in schizophrenia and bipolar disorder. Schizophr Res 2013;**145**:36–42.
7. Hope S, Hoseth E, Dieset I et al. Inflammatory markers are associated with general cognitive abilities in schizophrenia and bipolar disorder patients and healthy controls. Schizophr Res 2015;**165**:188–194.
8. Raison CL, Miller AH. Depression in cancer: new developments regarding diagnosis and treatment. Biol Psychiatry 2003;**54**:283–294.
9. Saper CB, Romanovsky AA, Scammell TE. Neural circuitry engaged by prostaglandins during the sickness syndrome. Nat Neurosci 2012;**15**:1088–1095.
10. Elenkov IJ. Neurohormonal-cytokine interactions: implications for inflammation, common human diseases and well-being. Neurochem Int 2008;**52**:40–51.
11. Krogh J, Benros ME, Jorgensen MB, Vesterager L, Elfving B, Nordentoft M. The association between depressive symptoms, cognitive function, and inflammation in major depression. Brain Behav Immun 2014;**35**:70–76.
12. Jokela M, Virtanen M, Batty GD, Kivimaki M. Inflammation and specific symptoms of depression. JAMA Psychiatry 2016;**73**:87–88.
13. Kohler O, Sylvia LG, Bowden CL et al. White blood cell count correlates with mood symptom severity and specific mood symptoms in bipolar disorder. Aust N Z J Psychiatry 2017;**51**:355–365.
14. Walsh JT, Watson N, Kipnis J. T cells in the central nervous system: messengers of destruction or purveyors of protection? Immunology 2014;**141**:340–344.
15. Brod S, Rattazzi L, Piras G, D'Acquisto F. 'As above, so below' examining the interplay between emotion and the immune system. Immunology 2014;**143**:311–318.
16. Angele MK, Pratschke S, Hubbard WJ, Chaudry IH. Gender differences in sepsis: cardiovascular and immunological aspects. Virulence 2014;**5**:12–19.
17. Nierenberg AA, Sylvia LG, Leon AC et al. Lithium treatment – moderate dose use study (LiTMUS) for bipolar disorder: rationale and design. Clin Trials 2009;**6**:637–648.
18. Nierenberg AA, Sylvia LG, Leon AC et al. Clinical and health outcomes initiative in comparative effectiveness for bipolar disorder (bipolar CHOICE): a pragmatic trial of complex treatment for a complex disorder. Clin Trials 2014;**11**:114–127.
19. Amitai M, Zivony A, Kronenberg S et al. Short-term effects of lithium on white blood cell counts and on levels of serum thyroid-stimulating hormone and creatinine in adolescent inpatients: a retrospective naturalistic study. J Child Adolesc Psychopharmacol 2014;**24**:494–500.
20. Spearing MK, Post RM, Leverich GS, Brandt D, Nolen W. Modification of the clinical global impressions (CGI) scale for use in bipolar illness (BP): the CGI-BP. Psychiatry Res 1997;**73**:159–171.
21. Sheehan DV, Lecrubier Y, Sheehan KH et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 1998;**59**(Suppl. 20):22–33; (quiz 34–57).
22. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. Br J Psychiatry 1979;**134**:382–389.
23. Bowden CL, Singh V, Thompson P et al. Development of the bipolar inventory of symptoms scale. Acta Psychiatr Scand 2007;**116**:189–194.
24. Gonzalez JM, Bowden CL, Katz MM et al. Development of the bipolar inventory of symptoms scale: concurrent validity, discriminant validity and retest reliability. Int J Methods Psychiatr Res 2008;**17**:198–209.
25. Thompson PM, Gonzalez JM, Singh V, Schoolfield JD, Katz MM, Bowden CL. Principal domains of behavioral psychopathology identified by the bipolar inventory of signs and symptoms scale (BISS). Psychiatry Res 2010;**175**:221–226.
26. Kannel WB, Anderson K, Wilson PW. White blood cell count and cardiovascular disease. Insights from the Framingham study. JAMA 1992;**267**:1253–1256.
27. Lally J, MacCabe JH. Antipsychotic medication in schizophrenia: a review. Br Med Bull 2015;**114**:169–179.
28. Liu B, Taioli E. Seasonal variations of complete blood count and inflammatory biomarkers in the US population – analysis of NHANES data. PLoS One 2015;**10**:e0142382.
29. Pandey GN, Rizavi HS, Ren X et al. Proinflammatory cytokines in the prefrontal cortex of teenage suicide victims. J Psychiatr Res 2012;**46**:57–63.
30. Uher R, Tansey KE, Dew T et al. An inflammatory biomarker as a differential predictor of outcome of depression treatment with escitalopram and nortriptyline. Am J Psychiatry 2014;**171**:1278–1286.
31. Raison CL, Rutherford RE, Woolwine BJ et al. A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory biomarkers. JAMA Psychiatry 2013;**70**:31–41.
32. Kohler O, Benros ME, Nordentoft M et al. Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse effects: a systematic review and meta-analysis of randomized clinical trials. JAMA Psychiatry 2014;**71**:1381–1391.
33. Li H, Hong W, Zhang C et al. IL-23 and TGF-beta1 levels as potential predictive biomarkers in treatment of bipolar I disorder with acute manic episode. J Affect Disord 2015;**174**:361–366.
34. Ayorech Z, Tracy DK, Baumeister D, Giaroli G. Taking the fuel out of the fire: evidence for the use of anti-inflammatory agents in the treatment of bipolar disorders. J Affect Disord 2015;**174**:467–478.
35. Arabzadeh S, Ameli N, Zeinoddini A et al. Celecoxib adjunctive therapy for acute bipolar mania: a randomized, double-blind, placebo-controlled trial. Bipolar Disord 2015;**17**:606–614.
36. Horne BD, Anderson JL, John JM et al. Which white blood cell subtypes predict increased cardiovascular risk? J Am Coll Cardiol 2005;**45**:1638–1643.

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