Inflammatory Markers Among Adolescents and Young Adults With Bipolar Spectrum Disorders

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ABSTRACT

Objective: Despite burgeoning literature in middle-aged adults, little is known regarding proinflammatory markers (PIMs) among adolescents and young adults with bipolar disorder. Similarly, few prior studies have considered potential confounds when examining the association between PIMs and bipolar disorder characteristics. We therefore retrospectively examined these topics in the Course and Outcome of Bipolar Youth (COBY) study.

Method: Subjects were 123 adolescents and young adults (mean [SD] = 20.4 ± 3.8 years; range, 13.4–28.3 years) in COBY, enrolled between October 2000 and July 2006. *DSM-IV* diagnoses were determined using the Schedule for Affective Disorders and Schizophrenia for School-Age Children (K-SADS). Clinical characteristics during the preceding 6 months, including mood, comorbidity, and treatment, were evaluated using the Longitudinal Interval Follow-Up Evaluation (LIFE). Serum levels of interleukin (IL)-6, tumor necrosis factor (TNF)-α, and high-sensitivity C-reactive protein (hsCRP) were assayed. Primary analyses examined the association of PIMs with bipolar disorder characteristics during the preceding 6 months.

Results: Several lifetime clinical characteristics were significantly associated with PIMs in multivariable analyses, including longer illness duration ($P = .005$ for IL-6; $P = .0004$ for hsCRP), suicide attempts ($P = .01$ for TNF-α), family history of suicide attempts or completion ($P = .01$ for hsCRP), self-injurious behavior ($P = .005$ for TNF-α), substance use disorder (SUD) ($P < .0001$ for hsCRP), and family history of SUD ($P = .02$ for TNF-α; $P = .01$ for IL-6). The following bipolar disorder characteristics during the preceding 6 months remained significantly associated with PIMs in multivariable analyses that controlled for differences in comorbidity and treatment: for TNF-α, percentage of weeks with psychosis ($\chi^2 = 5.7$, $P = .02$); for IL-6, percentage of weeks with subthreshold mood symptoms ($\chi^2 = 8.3$, $P = .004$) and any suicide attempt ($\chi^2 = 6.1$, $P = .01$); for hsCRP, maximum severity of depressive symptoms ($\chi^2 = 8.3$, $P = .004$).

Conclusions: Proinflammatory markers may be relevant to bipolar disorder characteristics as well as other clinical characteristics among adolescents and young adults with bipolar disorder. Traction toward validating PIMs as clinically relevant biomarkers in bipolar disorder will require repeated measures of PIMs and incorporation of relevant covariates.

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Proinflammatory markers (PIMs) such as interleukin (IL)-6, tumor necrosis factor (TNF)-α, and C-reactive protein (CRP) could potentially serve as biomarkers for psychiatric diagnosis, disease course, and therapeutic intervention.[1–3] Proinflammatory markers are also leading candidate biomarkers in bipolar disorder specifically[4,5] and are implicated in other medical illnesses such as rheumatoid arthritis and cardiovascular disease (CVD), in which they are used to evaluate and predict illness activity and treatment response.[6] Bipolar disorder may in some respects be a multisystemic inflammatory illness, explaining in part the high rate of medical comorbidity.[1,7]

Three recent meta-analyses confirm elevated PIMs during acute episodes of mania and/or depression among bipolar disorder adults.[8–10] Limitations highlighted in these meta-analyses include small sample sizes and lack of covariate inclusion in most studies. Broader interrogation of putative confounds of the association between PIMs and mood symptoms in bipolar disorder is warranted if PIMs are to gain traction as biomarkers of illness activity in bipolar disorder, as they are for other inflammatory conditions.

Unlike the robust literature regarding neurocognitive and neuroimaging biomarkers among youth and adults with bipolar disorder, the peripheral biomarker literature in bipolar disorder has been largely constrained to adults.[4] Signal detection may be enhanced among adolescents and young adults with bipolar disorder, due to shorter illness duration and less medical comorbidity compared to middle-aged adults with bipolar disorder.[11] In our pilot study[12] of PIMs among 30 adolescents in the Course and Outcome of Bipolar Youth (COBY) study, the only one to date regarding PIMs among bipolar disorder adolescents, levels of high-sensitivity CRP (hsCRP) were significantly associated with hypomanic or manic symptoms, and 40% of the sample had hsCRP levels considered high-risk for cardiovascular disease (CVD) among adults. That study was constrained by modest sample size, precluding an examination of confounds. Moreover, blood samples in that study were collected at various times of day, some in fasting status and others

Clinical Points

- Numerous studies have linked inflammation with mood among middle-aged adults who have bipolar disorder. Few of these studies have accounted for comorbidity or treatment, and little is known about this topic among adolescents and young adults.
- Similar to findings in adults, inflammation is associated with the symptomatic burden of bipolar disorder; this association does not appear to be explained by treatment or comorbidity.

not. These limitations constrained power and sensitivity, respectively.

We therefore examined this topic in a large sample of adolescents and young adults in COBY, adding PIMs to study procedures in 2009. Because of limited prior research in this age group, we planned for an interim analysis of the initial timepoint after obtaining the first 100 blood samples. However, data for 123 participants were available when the cytokine assays were performed, and were therefore included. Although repeated measures of PIMs are planned, the current study assesses PIMs at a single timepoint. The selected PIMs are not specific to bipolar disorder and are not intended to serve as diagnostic biomarkers. Rather, our goal is to help move the field closer to clinically relevant biomarkers of illness activity in early-onset bipolar disorder. This invokes the need for within-group (ie, only bipolar disorder participants) analyses, an approach that has been adopted in studies of other recurrent diseases. We hypothesized that PIMs would be significantly associated with the severity and burden of hypomanic or manic symptoms and depressive symptoms in the preceding 6-month epoch, independent of potentially confounding variables.

METHOD

Participants

The methods for COBY have been described in detail elsewhere.[13,14] Briefly, the study included youths aged 7 to 17 years 11 months at intake, with *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (*DSM-IV*) diagnosis of bipolar I or II disorder or operationally defined bipolar disorder not otherwise specified (NOS). Participants in the current analysis included 123 adolescents and young adults, mean (SD) age of 20.4 (3.8) years (range, 13.4–28.3 years), with bipolar I disorder (58.5%), bipolar II disorder (6.5%), or bipolar disorder NOS (35.0%) enrolled in COBY[13,15] between October 2000 and July 2006. Twenty of these participants had also participated in our prior pilot study, but provided a subsequent blood sample for the current study. Consecutive COBY subjects contacted for follow-up assessments at the University of Pittsburgh and Brown University sites were invited to participate. Exclusion criteria were infectious illness within 14 days, known inflammatory or autoimmune illness, use of steroidal medication or insulin within 1 month, and self-reported alcohol or illicit drug use within 24 hours (2 subjects who reported regular cigarette smoking were included). Twenty-one participants declined this COBY procedure, primarily due to needle phobia (n = 6) and travel distance (n = 10). Descriptive findings are listed in Table 1.

Procedures

Each participating university's institutional review board approved the study. At intake, participants and parents provided informed consent and were directly interviewed for the presence of current and lifetime psychiatric disorders in the adolescents.

Psychiatric and Anthropometric Measures

Psychiatric diagnoses were ascertained with the Schedule for Affective Disorders and Schizophrenia for School-Age Children–Present and Lifetime Version (K-SADS-PL),[16] the Kiddie Mania Rating Scale (K-MRS),[17] and the depression section of the K-SADS-P.[18] Psychiatric symptoms during the preceding 6-month epoch were retrospectively assessed using the Longitudinal Interval Follow-Up Evaluation (LIFE)[19] and tracked on a week-by-week basis using this instrument's Psychiatric Status Rating (PSR) scales. Analyses focused on PSR scores during the 6 months preceding the blood draw for PIMs, as this is the standard interval between COBY visits.

All assessments were conducted by research staff trained to reliably administer the interviews; interview results were presented to child psychiatrists or psychologists, who confirmed the diagnoses and the PSR scores. As previously reported, reliability of the K-SADS and PSR in COBY is good to very good.[15,20]

Those variables that were ascertained during the preceding 6-month epoch as well as at intake are listed in Table 2. Family psychiatric history was ascertained using the Family History Screen.[21] Socioeconomic status was ascertained at intake using the 4-factor Hollingshead scale.[22] Abuse was ascertained using the K-SADS-PL. Lifetime and current pharmacologic treatment exposures were obtained at the intake assessment. In addition, the Psychotropic Treatment Record and the Psychosocial Treatment Schedule of the LIFE were used to ascertain treatment exposure in the preceding 6-month epoch on a week-by-week basis. Weekly exposure was dichotomized (yes/no) for each of 3 classes: antimanic, antidepressant, and stimulant. Weekly exposure to psychosocial treatments was similarly examined for 3 categories of intensity: inpatient hospitalization/residential treatment, specialized intensive services, and standard outpatient services. Global functioning was assessed at intake using the Children's Global Assessment Scale (C-GAS).[23]

Weight and height were measured using an electronic scale (Tanita) and electronic stadiometer (Seca). *Obesity* among participants < 20 years of age was defined as age- and sex-adjusted body mass index (BMI) ≥ 95th percentile according to Centers for Disease Control BMI norms; *obesity* among participants ≥ 20 years of age was defined as a BMI ≥ 30.[24,25] Systolic and diastolic blood pressure was measured twice using automated blood pressure monitors (LifeSource), with analyses examining the mean measurements.

Table 1. Association of Proinflammatory Markers With Lifetime Demographic, Clinical, and Familial Characteristics (N = 123)

Characteristic	% or Mean (SD)	TNF-α χ^2	TNF-α Effect Size	TNF-α *P* Value	IL-6 χ^2	IL-6 Effect Size	IL-6 *P* Value	hsCRP χ^2	hsCRP Effect Size	hsCRP *P* Value
Age, y	20.4 (3.8)	0.1	0.001	.74	0.2	0.001	.66	2.3	0.015	.13
Socioeconomic status, score[a]	2.8 (1.3)	2.2	0.020	.14	0.8	0.006	.36	3.4	0.023	.07
Duration of bipolar disorder, y	12.7 (3.1)	0	< 0.001	.86	7.0	0.053	.008	4.3	0.029	.04
Race, % white	79.7	2.7	0.020	.1	0	< 0.001	.88	0.2	0.001	.64
Sex, % male	61.8	1.6	0.010	.21	1.7	0.012	.2	0.5	0.004	.47
Bipolar disorder subtype		0.1	0.001	.71	1.3	0.010	.25	0.7	0.004	.41
Age at bipolar disorder onset, y	8.6 (3.7)	0.5	0.004	.48	2.0	0.021	.09	10.8	0.075	.001
Comorbid psychiatric disorder										
ADHD	76.4	0.1	0.001	.77	0	< 0.001	.92	0.3	0.002	.62
CD	27.6	0.2	0.002	.66	0.3	0.002	.57	1.5	0.010	.23
ODD	61.8	0.3	0.002	.62	0	< 0.001	.93	3.6	0.024	.06
Anxiety	80.5	0	< 0.001	.87	3.7	0.028	.05	0.4	0.002	.54
Physical abuse	23.6	0.1	< 0.001	.8	2.2	0.016	.14	0	< 0.001	.97
Sexual abuse	18.7	0.5	0.004	.49	0.8	0.006	.38	0	< 0.001	.88
Psychiatric hospitalization	65.9	2.4	0.020	.12	0.1	< 0.001	.73	2.0	0.013	.16
Psychosis	36.6	2.3	0.020	.13	0	< 0.001	.83	1.1	0.007	.3
Substance abuse	30.9	0	< 0.001	.96	2.9	0.021	.09	2.1	0.014	.14
Substance dependence	17.9	1.5	0.012	.23	1.4	0.011	.23	7.4(–)[b]	0.050	.007
SUD	33.3	0	< 0.001	.88	3.9	0.029	.05	3.4	0.023	.07
Suicide attempt	48.0	3.3	0.027	.07	0	< 0.001	.99	3.5	0.023	.06
Self-injurious behavior	59.4	2.3	0.019	.13	0.2	0.001	.69	1.3	0.009	.26
Suicidal ideation	78.9	0.2	0.002	.66	2	0.015	.16	0.1	< 0.001	.83
Family psychiatric history (first and second degree)										
Depression	89.4	1.2	0.010	.27	0.8	0.006	.39	0.1	< 0.001	.8
Mania/hypomania	67.5	0	< 0.001	.95	0	< 0.001	.91	0	< 0.001	.88
ADHD	55.3	0.3	0.002	.6	5.6(–)[b]	0.042	.02	7.9(–)[b]	0.053	.01
CD	40.7	0	< 0.001	.87	1.7	0.012	.2	2.0	0.013	.16
Schizophrenia	10.6	0.5	0.004	.47	0	< 0.001	.85	0.6	0.004	.44
Anxiety	69.1	0.5	0.004	.48	0.1	< 0.001	.78	0.6	< 0.001	.44
Substance abuse	61.8	0.5	0.004	.48	7.6(–)[b]	0.057	.006	6.4(–)[b]	0.043	.01
Substance dependence	62.6	0.5	0.004	.5	3.9	0.029	.05	0.3	0.002	.58
SUD	75.6	2.4	0.019	.12	7.0(–)[b]	0.052	.008	1.5	0.010	.22
Suicide attempt or completion	48.8	1.2	0.010	.27	0.34	0.003	.56	2.4	0.016	.12

[a]Hollingshead scale. [b]Negative association.
Abbreviations: ADHD = attention-deficit/hyperactivity disorder, CD = conduct disorder, hsCRP = high-sensitivity C-reactive protein, IL-6 = interleukin-6, ODD = oppositional defiant disorder, SES = socioeconomic status, SUD = substance use disorder, TNF = tumor necrosis factor.

Biochemical Assays

Blood (20 mL) was drawn from each subject between 9:00 AM–12:00 PM after a 10-hour fast, immediately centrifuged at 3000*g* for 5 minutes, and stored at −80°C until assayed at the University of Pittsburgh. Serum IL-6 levels were determined using a high-sensitivity quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (ALPCO). Serum levels of hsCRP and TNF-α were also determined using ELISA in a similar fashion to IL-6. Measurement of glucose and lipids was completed at the local hospital laboratory. Detection limits were 0.09 pg/mL for TNF-α, 0.1 pg/mL for IL-6, and 0.00092 μg/mL for hsCRP. Intra-assay coefficient of variability was 5.3–6.7 for TNF-α, 4.7–8.3 for IL-6, and 5.5–6.0 for hsCRP. Inter-assay coefficient of variability was 7.7–9.7 for TNF-α, 6.7–10.0 for IL-6, and 11.6–13.8 for hsCRP.

Statistical Analyses

Statistical analyses were performed using SAS (9.3) software. Correlations among the PIMs were examined with Pearson correlation coefficients. On the basis of the observed distributions of PIMs, generalized linear models with γ distribution were applied. Type III test results are reported. Effect sizes (Cohen f^2) were computed using the following equation: Effect size = [Deviance (Reduced) – Deviance (Full)]/Deviance (Full), where the full model is the γ regression model including the explanatory variable of interest and the reduced model is the γ regression model excluding the explanatory variable of interest. For f^2, 0.02 is considered a small effect size, 0.15 is a medium effect size, and 0.35 is a large effect size.[26]

The variables with $P < .2$ in univariate analyses were included into the multivariable model through stepwise procedure. Analyses regarding mood symptoms were undertaken a priori and were not adjusted for multiple comparisons. For other univariate analyses, correction via false discovery rate (FDR) was undertaken. We recognize that the current inclusive multivariable approach may lead to overfitted models and that we could have opted to rely on heuristics to select covariates. However, given the limited data available to inform the a priori selection of covariates, we opted to be as atheoretical as possible and allow the data to inform our variable selection.

RESULTS

Association of PIMs With Lifetime Characteristics

Table 1 presents participant characteristics associated with PIMs. Mean (SD) levels were 2.73 (0.75) pg/mL for TNF-α, 0.77 (0.78) pg/mL for IL-6, and 2.60 (4.36) μg/

Table 2. Association of Proinflammatory Markers With Clinical Characteristics During the Preceding 6-Month Epoch (N = 123)

		TNF-α			IL-6			hsCRP		
Characteristic	Mean (SD)	χ^2	Effect Size	*P* Value	χ^2	Effect Size	*P* Value	χ^2	Effect Size	*P* Value
% Weeks in mood state during 6 months preceding blood draw (mean ± SD)										
No significant mood symptoms	56.9 (40.8)	0.1	< 0.001	.78	10.8(–)[a]	0.083	.001	1.9	0.013	.17
Any subthreshold mood state	28.5 (35.5)	0	< 0.001	.84	6.7	0.050	.010	0.4	0.003	.53
Any full-threshold mood state	14.6 (28.9)	0	< 0.001	.88	1.5	0.011	.22	1.5	0.010	.22
Full-threshold pure depression	8.6 (21.3)	0.2	0.002	.66	2.9	0.021	.09	4.5	0.031	.03
Full-threshold pure mania/hypomania	3.3 (13.7)	0.3	0.003	.56	0.2	0.001	.68	0.6	0.004	.43
Full-threshold mix	0.4 (3.1)	0.6	0.005	.45	0.1	< 0.001	.75	0	< 0.001	.89
Full-threshold cycling	2.3 (10.9)	0.6	0.005	.44	0.1	< 0.001	.82	0	< 0.001	.88
Suicidal ideation	1.8 (8.3)	0	< 0.001	.86	7.8	0.059	.005	3.6	0.024	.06
Maximum severity (PSR)										
Depression	3.1 (1.5)	0	< 0.001	.86	6.4	0.048	.012	9.6	0.066	.002
Mania/hypomania	2.4 (1.5)	0.1	0.001	.72	3.8	0.028	.05	0	< 0.001	.97
% Weeks in psychosis										
Psychosis	7.0 (25.2)	4.9	0.040	.028	0.3	0.002	.59	0.4	0.002	.54
Comorbid disorders (% weeks in past 6 months meeting full diagnostic criteria)										
Any comorbid disorder	74.5 (43.2)	1.6	0.013	.21	0.4	0.003	.51	0.2	0.001	.68
SUD	32.6 (46.0)	0.1	< 0.001	.74	1.8	0.013	.18	3.5	0.023	.06
ADHD	45.4 (49.9)	0.5	0.004	.47	2.4	0.017	.13	3.1	0.021	.08
CD/ODD	28.4 (45.1)	0	< 0.001	.87	1.7	0.012	.2	0.7	0.005	.41
Any anxiety	40.8 (48.9)	0.5	0.004	.49	0.1	< 0.001	.72	0.4	0.003	.52
% Weeks receiving medication over preceding 6 months (mean ± SD)										
Any psychotropic medication	52.0 (48.1)	0.4	0.003	.53	6.6	0.049	.011	1.4	0.009	.24
Antimanic	38.2 (47.3)	0.6	0.005	.44	0.7	0.005	.404	0.6	0.004	.45
Antidepressant	17.7 (35.9)	0.3	0.002	.62	7.3	0.055	.007	0.7	0.005	.41
Stimulants	22.6 (40.9)	0.1	< 0.001	.78	6.2	0.046	.013	0.3	0.002	.59
% Weeks receiving treatment over preceding 6 months (mean ± SD)										
Any psychosocial	22.1 (30.3)	0.7	0.005	.41	5.3	0.039	.022	0.3	0.002	.61
Inpatient/residential treatment	3.2 (15.8)	0.2	0.001	.67	1.1	0.008	.297	0.5	0.003	.47
Specialized psychosocial services	5.9 (23.4)	0	< 0.001	.92	0	< 0.001	.85	0.9	0.006	.35
Outpatient services	15.7 (20.2)	1.3	0.011	.25	6.9	0.052	.009	0.3	0.002	.61

[a]Negative association.
Abbreviations: ADHD = attention-deficit/hyperactivity disorder, CD = conduct disorder, hsCRP = high-sensitivity C-reactive protein, IL-6 = interleukin-6, ODD = oppositional defiant disorder, PSR = psychiatric status rating, SUD = substance use disorder, TNF = tumor necrosis factor.

mL for hsCRP. Interleukin-6 was significantly correlated with TNF-α ($r = 0.19$, $P = .04$) and with hsCRP ($r = 0.38$, $P < .0001$). High-sensitivity C-reactive protein was not significantly correlated with TNF-α ($r = 0.10$, $P = .29$). There were no significant associations between age, sex, race, socioeconomic status (SES), or bipolar disorder subtype with any of the PIMs. Longer duration of bipolar disorder was significantly associated with higher IL-6 and hsCRP. Earlier age at bipolar disorder onset was also associated with higher hsCRP. Among lifetime comorbidities, the only significant association was between substance dependence and lower hsCRP. Family history of attention-deficit/hyperactivity disorder (ADHD) and family history of substance abuse were each associated with significantly lower IL-6 and hsCRP. Among these variables, only the association of higher hsCRP with earlier age at bipolar disorder onset remained significant after FDR correction (corrected $P = .04$).

Multivariable analyses examined all variables that were associated with PIMs at $P < .2$ (before FDR correction). The following variables remained significantly associated with PIMs in multivariable analyses:

TNF-α: SES ($\chi^2 = 7.2$, $P = .007$), white race ($\chi^2 = 3.8$, $P = .05$), lifetime suicide attempt ($\chi^2 = 6.3$, $P = .01$), lifetime self-injurious behavior ($\chi^2 = 8.0$, $P = .005$), and family history of SUD ($\chi^2 = 5.4$, $P = .02$).

IL-6: age ($\chi^2 = 3.9$, $P = .05$), duration of bipolar disorder ($\chi^2 = 7.8$, $P = .005$), and family history of SUD ($\chi^2 = 6.1$, $P = .01$).

hsCRP: age ($\chi^2 = 22.4$, $P < .0001$), SES ($\chi^2 = 9.2$, $P = .002$), duration of bipolar disorder ($\chi^2 = 12.8$, $P = .0004$), lifetime comorbid SUD ($\chi^2 = 18.2$, $P < .0001$), and family history of suicide attempt or completion ($\chi^2 = 6.4$, $P = .01$).

Association of PIMS With Clinical Characteristics During the Preceding 6-Month Epoch

Table 2 presents the associations between PIMs and the prospectively ascertained symptom burden and severity across mood states, as well as psychosis and comorbidities, during the 6-month epoch preceding the blood draw. Greater percentage of weeks in euthymia was significantly associated with lower IL-6, whereas greater percentage of weeks with subthreshold mood symptoms was significantly associated with higher IL-6. Similarly, greater percentage of weeks with full-threshold major depression was significantly associated with higher hsCRP. Maximum depressive severity in the preceding 6-month epoch was significantly

Table 3. Association of Proinflammatory Markers With Metabolic Syndrome Components (N = 123)

		TNF-α			IL-6			hsCRP		
Metabolic Syndrome Component	Measure	χ^2	Effect Size	*P* Value	χ^2	Effect Size	*P* Value	χ^2	Effect Size	*P* Value
BMI		0.1	< 0.001	.97	10.3	0.077	.006	15.9	0.108	< .001
Normal, % patients	44.6	0	< 0.001	.86	0.8	0.006	.38	2.6	0.018	.11
Overweight, % patients	23.1	0	< 0.001	.96	5.9(–)[a]	0.044	.015	8.0(–)[a]	0.054	.005
Obese, % patients	32.2	0.1	< 0.001	.81	8.1	0.060	.004	13.4	0.091	< .001
Glucose, mean (SD), mg/dL	91.9 (16.0)	2.1	0.017	.15	2.0	0.015	.16	0.5	0.003	.49
Cholesterol, mean (SD), mg/dL	159.5 (29.2)	2.2	0.018	.14	0.4	0.003	.55	0.3	0.002	.56
HDL cholesterol, mean (SD), mg/dL	43.0 (11.9)	1.3	0.010	.26	4.0(–)[a]	0.029	.05	3.0	0.020	.09
LDL cholesterol, mean (SD), mg/dL	98.0 (25.4)	2.2	0.018	.14	0.9	0.006	.35	1.0	0.007	.31
Triglycerides, mean (SD), mg/dL	98.0 (81.4)	4.0	0.033	.05	1.8	0.014	.18	0.8	0.005	.38
Systolic blood pressure, mean (SD), mm Hg	116.1 (11.5)	0.5	0.004	.50	0.1	0.001	.71	1.3	0.009	.25
Diastolic blood pressure, mean (SD), mm Hg	76.9 (10.1)	1.0	0.009	.31	0	< 0.001	.96	0.2	0.001	.70

[a]Negative association.
Abbreviations: BMI = body mass index, HDL = high-density lipoprotein, hsCRP = high-sensitivity C-reactive protein, IL-6 = interleukin-6, LDL = low-density lipoprotein, TNF = tumor necrosis factor.

associated with higher IL-6 and higher hsCRP, and there was a trend toward an association between maximum hypomanic or manic symptom severity and higher IL-6 ($P = .05$). Greater percentage of weeks with psychosis in the preceding 6-month epoch was significantly associated with higher TNF-α, and greater percentage of weeks with suicidal ideation was significantly associated with higher IL-6. There were no significant associations between the percentage of weeks with comorbidities and any of the PIMs. Interleukin-6 was the only PIM that was significantly associated with treatment. Specifically, exposure to any psychotropic medication, antidepressants, stimulants, any psychosocial treatment, and outpatient services were each significantly associated with higher IL-6. None of the mood findings remained significant after FDR correction. Among nonmood findings for IL-6, the following variables remained significant after FDR correction: antidepressant medication, stimulants, outpatient treatment, and suicidal ideation. None of the nonmood findings for TNF-α or hsCRP survived FDR correction.

Multivariable analyses examined all variables that were associated with PIMs at $P < .2$, yielding several significant associations with PIMs. For TNF-α: percentage of weeks with psychosis ($\chi^2 = 5.7$, $P = .02$). For IL-6: percentage of weeks with subthreshold mood symptoms ($\chi^2 = 8.3$, $P = .004$), percentage of weeks with antidepressant medication ($\chi^2 = 9.1$, $P = .003$), and any suicide attempt ($\chi^2 = 6.1$, $P = .01$). For hsCRP: age ($\chi^2 = 7.0$, $P = .008$), obesity ($\chi^2 = 4.6$, $P = .03$), percentage of weeks with SUD ($\chi^2 = 11.5$, $P = .007$), and maximum severity of depressive symptoms ($\chi^2 = 8.3$, $P = .004$).

We conducted exploratory analyses examining the associations between PIMs and mood symptoms during the week prior to the blood draw and did not identify any significant associations.

Association of PIMs With Metabolic Syndrome Variables

Table 3 presents the associations between PIMs and concurrently measured components of the metabolic syndrome (obesity, hypertension, dysglycemia, dyslipidemia). Obesity was significantly associated with higher IL-6 and higher hsCRP. Higher (ie, better) levels of high-density lipoprotein were significantly associated with lower IL-6, with a trend toward lower hsCRP ($P = .09$). Higher (ie, worse) levels of triglycerides were significantly associated with higher TNF-α. No significant associations with glucose, systolic blood pressure, or diastolic blood pressure were observed.

DISCUSSION

Our study is the largest to date to examine PIMs among adolescents and young adults with bipolar disorder and the largest study to date to examine multiple PIMs across multiple mood states in bipolar disorder in any age group. Previous large studies in middle-aged adults with bipolar disorder have either included only euthymic subjects (N = 121)[27] or examined a single PIM.[28–30] Despite the participants' young age, levels of PIMs were detectable for all subjects. Indeed, mean levels of hsCRP are above the threshold considered to confer increased risk for CVD among adults.[6] Present findings support in part the primary hypothesis that PIMs are associated with mood symptom severity and burden in this population. Some distinctions between the different PIMs were observed, namely that hsCRP and IL-6 were associated with mood outcomes but not psychosis, and TNF-α was associated with psychotic symptom burden but not mood. Proinflammatory markers were also associated with bipolar disorder duration, comorbidity, treatment, metabolic dysregulation, and family psychiatric history. The relatively large sample size allowed adequate power to detect significant associations between PIMs and putative confounds that may explain in part the previously described associations between PIMs and mood symptoms in bipolar disorder. Complexity and heterogeneity are inherent aspects of bipolar disorder internationally.[31] Present findings underscore the importance of incorporating the complexity and heterogeneity of bipolar disorder in the process of biomarker discovery and validation.

This study has 3 primary limitations. First, this study is based on a single measurement of PIMs, which precludes conclusions regarding causality and the direction of the observed associations. Ultimately, repeated-measures analyses will be informative in predicting treatment

response and illness activity prospectively. Indeed, COBY is ascertaining repeated measures of PIMs, and future reports will examine PIMs prospectively. Second, we selected 3 leading PIMs that were supported by the literature at the time this study was conceived. A broader approach could have identified other novel PIMs; however, we opted for a conservatively selective approach informed by the existing literature. Third, despite the fact that this is among the largest studies in the world literature on this topic, biomarker validation in psychiatry will require much larger samples. In addition, this study did not include a healthy or clinical control group; as such, it is unclear whether the associations observed in the current study are specific to bipolar disorder.

In support of our primary hypothesis, PIMs were associated with several measures of mood severity and burden during the preceding 6-month epoch. Interleukin-6 levels were negatively associated with percentage of weeks euthymic, and in turn positively associated with percentage of weeks with subthreshold mood symptoms. Interleukin-6 levels were also positively associated with maximum depressive and maximum hypomanic or manic symptom severity. High-sensitivity C-reactive protein levels were positively associated with percentage of weeks with full-threshold major depression and with maximum depressive severity. Only the association between hsCRP and maximum severity of depressive symptoms remained significant in multivariable models. Numerous studies among adults have also reported cross-sectional associations between mood symptoms and PIMs.[8–10,32] The direction of the current cross-sectional findings is uncertain, and no prior studies in bipolar disorder address directionality. Previous evidence from non–bipolar disorder samples suggests bidirectionality.[33–36]

Younger age at bipolar disorder onset was associated with higher hsCRP in univariate analyses. In multivariable analyses, duration of bipolar disorder was positively associated with levels of IL-6 and hsCRP. Among prior studies of adults with bipolar disorder that have examined this topic, 1 study found that PIMs are positively associated with duration of bipolar disorder,[37] whereas others have not found significant associations with bipolar disorder duration and age at onset.[38–41] One study found that IL-6 levels are higher in early-stage bipolar disorder (< 3 years' duration), whereas TNF-α levels are higher in late-stage bipolar disorder (> 10 years' duration).[42] Although PIMs have been invoked in theoretical models of bipolar disorder neuroprogression, additional studies are needed to clarify whether these markers are associated with or predict different illness stages.[5]

Treatment-related findings were observed only for IL-6, which was associated with antidepressant and stimulant treatment and with overall outpatient psychosocial treatment. Given the naturalistic design and the interview-based assessment of treatment exposure (vs medication levels or reviewing health records) together with the robust contradictory evidence from clinical and preclinical studies that antidepressants and mood-stabilizing medications in fact decrease levels of PIMs,[1] these findings should be interpreted as tentative. Of 10 prior studies[8] among adults with bipolar disorder that have reported on the association between medication status and PIMs, 6 found no significant association and 4 found nonreplicated associations. Clearly, medications and psychosocial treatments are important to consider when examining PIMs in bipolar disorder. However, naturalistic studies are likely to be affected by selection bias (eg, more treatments for more symptomatic patients, nonadherence) As such, analyses regarding mechanistic associations between treatment and PIMs in bipolar disorder are most likely best reserved for preclinical studies and controlled trials.

There were several significant associations between PIMS and personal and family history of suicidality. These findings converge with preliminary prior evidence that inflammation is associated with suicidality among adults with major depressive disorder.[43] Similarly, PIMs were significantly associated with personal and family history of SUD. Some prior studies have found that substance use is associated with increased inflammation, whereas other studies have found the opposite.[44–46] This relationship appears to depend in part on type, pattern, and timing of substance use in relation to the assessment of inflammation.[47] Also, present findings did not confirm prior reports that the link between mood and PIMs may be stronger among those with history of early adversity/abuse compared to those without such a history.[48] Reasons for the lack of findings regarding abuse history are uncertain but may relate to differences between major depressive disorder and bipolar disorder, the young age of the current sample, or other unknown factors. Finally, present findings support an association between PIMs and metabolic syndrome components. These findings converge with abundant evidence that metabolic syndrome components are associated with inflammation, including among adults with depression symptoms.[49–51]

In summary, this study is the largest to date that examines multiple PIMs in relation to mood symptoms and other characteristics of bipolar disorder in adolescents and young adults. This study confirms important associations between PIMs and illness activity in bipolar disorder and identifies several potentially important covariates that should be considered in future studies on this topic. Given that increased PIMs are found in numerous psychiatric and medical conditions, PIMs are unlikely to serve as diagnostic biomarkers that can distinguish bipolar disorder from other related conditions. Ultimately, the value of PIMs as biomarkers for this population will therefore rely on their capacity to address other clinical issues such as prediction of illness course and treatment selection and evaluation. Traction toward validating PIMs as clinically relevant biomarkers in bipolar disorder will require repeated measures of PIMs and incorporation of other relevant clinical characteristics. This approach will optimize signal-to-noise ratio by allowing for within-subject comparisons. Future studies employing this approach within the COBY sample are underway.

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