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A multisystem composite biomarker as a preliminary diagnostic test in bipolar disorder

Munkholm K, Vinberg M, Pedersen BK, Poulsen HE, Ekstrøm CT, Kessing LV. A multisystem composite biomarker as a preliminary diagnostic test in bipolar disorder.

Objective: Diagnosis and management of bipolar disorder (BD) are limited by the absence of available laboratory tests. We aimed to combine data from different molecular levels and tissues into a composite diagnostic and state biomarker.
Methods: Expression levels of 19 candidate genes in peripheral blood, plasma levels of BDNF, NT-3, IL-6 and IL-18, leukocyte counts, and urinary markers of oxidative damage to DNA and RNA were measured in 37 adult rapid-cycling patients with BD in different affective states during a 6- to 12-month period and in 40 age- and gender-matched healthy individuals in a longitudinal, repeated measures design comprising a total of 211 samples. A composite biomarker was constructed using data-driven variable selection.
Results: The composite biomarker discriminated between patients with BD and healthy control individuals with an area under the receiver operating characteristic curve (AUC) of 0.83 and a sensitivity of 73% and specificity of 71% corresponding with a moderately accurate test. Discrimination between manic and depressive states had a moderate accuracy, with an AUC of 0.82 and a sensitivity of 92% and a specificity of 40%.
Conclusion: Combining individual biomarkers across tissues and molecular systems could be a promising avenue for research in biomarker models in BD.

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Key words: bipolar disorder; biomarkers; blood; urine; gene expression

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Significant outcomes

- For the first time, we explored the potential of combining data from different molecular levels and tissues into a composite marker of diagnosis and state.
- The composite biomarker discriminated moderately well between patients with bipolar disorder and healthy control individuals and between a manic and a depressed state.

Limitations

- The sample size was modest considering the use of a split-sample design in developing and testing the composite marker.
- Medication and differences in behaviour might have influenced the measured analytes.
- Our study population consisted of patients with rapid-cycling bipolar disorder and results may therefore not be generalizable to all patients with bipolar disorder.

Introduction

A major limitation in the diagnosis and management of bipolar disorder is the exclusive reliance on subjective clinical information in the absence of available laboratory tests (1). Diagnostic criteria are arbitrary, often leading to wrong and delayed diagnoses, and it is difficult to assess whether affective symptoms are indicative of emerging affective episodes.

Identification of peripheral blood biomarkers of disease and disease activity (2) has the potential to both advance the understanding of pathophysiological processes and to improve clinical treatment of bipolar disorder (3). Although our understanding of its biological background is inadequate, growing evidence indicates that inflammatory disturbances (4), altered neuroplasticity (5), oxidative stress, and disturbances related to mitochondrial function (6) are associated with bipolar disorder. Candidate biomarkers in bipolar disorder could therefore potentially be identified within these interrelated biological processes (7). Thus, disturbances in the immuno-inflammatory system have been implicated in the etiology, pathophysiology, and phenomenology of bipolar disorder (8), and alterations of inflammatory markers in peripheral blood have been found across affective states and in euthymia in bipolar disorder (9, 10). Findings include alterations of the cytokines tumor necrosis factor-alpha (TNF-α), IL-6, and IL-1β, among others (9). Impairment of neuroplasticity may also be involved in the pathophysiology of bipolar disorder and potentially contribute to neuroprogressive changes during the course of the illness (11, 12). Brain-derived neurotrophic factor (BDNF) is an important regulatory mediator of cellular plasticity (13), and levels of BDNF in peripheral blood appear altered in bipolar disorder (14) along with other neurotrophins such as neurotrophin-3 (NT-3) (15). Mitochondrial dysfunction and oxidative stress have increasingly been speculated to be involved in the pathophysiology of bipolar disorder (6) supported by findings of alterations of oxidative stress marker levels in peripheral blood, particularly involving oxidative stress to DNA and RNA (16). Oxidative damage to RNA has been proposed as a novel disease mechanism contributing to medical diseases (17), and urinary levels of markers of RNA and DNA damage, which are considered more reliable than plasma levels (17), have been found altered in patients with bipolar disorder (18, 19). Other lines of evidence implicate disturbances within the arachidonic acid cascade in bipolar disorder, supported by findings of altered gene expression in peripheral blood (20, 21). A range of candidate genes for gene expression analysis have further been proposed, some of which were based on findings from genome-wide association studies, such as Ankyrin-3 (*ANK3*) and calcium channel, voltage-dependent, L type, alpha 1C subunit (*CACNA1C*) (22), and through using convergent functional genomics, such as krueppel-like factor 12 (*KLF12*) and *BDNF* (23) but also based on other lines of evidence such as gene expression in peripheral blood (24) and proteomic analysis (25).

While there is potential for the identification of biomarkers related to these pathways, one single biomarker is unlikely to provide a useful diagnostic laboratory test given the likely complex biological nature of bipolar disorder. Previous efforts of combining individual biomarkers have used a focused approach related to either certain pathways or specific biological tissues. Thus, studies have either investigated panels of genes related to either specific pathways (26), selected candidate genes (24), or panels of proteins and done so exclusively in peripheral blood (27–29).

We previously investigated the potential for a composite gene expression marker to function as a clinically relevant biomarker and found promising results discriminating between patients with bipolar disorder and healthy control individuals (30). Here, we extend on our previous work by exploring the potential of combining individual biomarker data related to the abovementioned pathways from different molecular levels and tissues into a composite marker of diagnosis and state in bipolar disorder.

Aims of the study

The aim of the study was to explore the potential of constructing a composite biomarker of diagnosis and state based on individual candidate protein and gene expression markers in peripheral blood and urine from patients with bipolar disorder and healthy control individuals using a data-driven approach.

Material and methods

The study was conducted at the Psychiatric Center Copenhagen, Copenhagen, Denmark, during the period of June 2010 to May 2012. Detailed analyses of each individual biomarker and study methods have been reported elsewhere (19, 21, 30–32). For the current report, only study participants with a full set of values of individual biomarker analytes were included.

Participants

Patients with bipolar disorder. Inclusion criteria were a DSM-IV diagnosis of rapid-cycling bipolar disorder and age between 18 and 70 years. Exclusion criteria were current drug abuse, insufficient Danish language skills, pregnancy, and significant physical illness (i.e., chronic heart disease, chronic pulmonary disease, inflammatory disease, chronic infectious disease, and neurodegenerative disease), determined by available case material, patients' self-report, and routine blood chemistry tests. Patients were recruited through referral by psychiatrists at hospitals or out-patient facilities throughout the region of Zealand, Denmark. The patients were on stable medication in the month preceding study entry and received treatment as usual without influence from study investigators during the study period. In order to minimize medication change during the study, inclusion in the study was postponed in case of a planned medication change. During the study period, the patients remained in contact with the primary study investigator, with planned visits and clinical assessment every month. The patients performed daily self-evaluation of mood on a Likert scale from −3 to +3 (depressive–euthymic–manic) if possible, and they were instructed to contact the study investigator in case of alterations of mood state. Patients were evaluated with clinical assessments collection of blood and urine samples upon signs of new affective episodes. When possible, an evaluation was performed at return to a subsequent euthymic state or change to an affective episode of opposite polarity.

Of the initial study sample, 33 patients with bipolar disorder had a full set of analyte values and were included, while two bipolar patients declined further examination after 1-month and 3-month follow-up, respectively. The remaining bipolar patients were followed for a minimum of 6 months with a mean (SD) follow-up period of 11.9 (3.0) months.

Healthy control individuals. Inclusion criteria were no history of psychiatric disorder in the subjects or their 1st-degree relatives and age between 18 and 70 years. Exclusion criteria were identical to those applied to patients with bipolar disorder. Healthy control individuals were recruited among blood donors affiliated with the Blood Bank at Rigshospitalet, Copenhagen, by approaching them on random days in the waiting room. Of the initial study sample, 35 age- and gender-matched healthy individuals had a full set of analyte values and were included. Healthy individuals were evaluated with clinical assessments and collection of blood and urine samples on two occasions approximately 3 months apart with a mean (SD) follow-up period of 2.9 (0.9) months.

Assessment and biochemical analysis were postponed in case of clinical signs of acute infection, allergic symptoms, or other acute medical condition.

All participants provided written informed consent. The study protocols were approved by the Committee on Health Research Ethics of the Capital Region of Denmark (protocol no. H-4-2010-006). The study complied with the Declaration of Helsinki.

Study procedure

Clinical assessments. All participants were assessed by a specialist in psychiatry (KM), using standardized semistructured interviews. The Schedules for Clinical Assessment in Neuropsychiatry (SCAN) interview (33) was used for diagnostic purposes and was based on available case material, referral reports, the interview with the participant, and the Hypomania Checklist (HCL-32) (34), completed by the participant. A DSM-IV diagnosis of rapid-cycling bipolar disorder was established for the patients and absence of lifetime psychiatric morbidity was confirmed for healthy control individuals.

A clinical diagnosis according to DSM-IV was established at each study visit concurrently with the collection of samples for laboratory analysis. Severity of depressive symptoms was assessed using the 17-item Hamilton Depression Rating Scale (HAMD-17) (35), and manic symptoms were assessed using the Young Mania Rating Scale (YMRS) (36), with a time period of 3 days applied.

Categorization of affective states was based on clinical evaluation according to the SCAN interview combined with the HAMD-17 and YMRS rating scales without applying duration criteria: euthymic (HAMD-17 and YMRS < 8), depressive (HAMD-17 > 7 and YMRS < 8), manic/hypomanic (YMRS >7 and HAMD-17 < 8), and mixed state (HAMD-17 > 7 and YMRS > 7).

Laboratory methods. *Blood and urine sample collection and preparation*. Fasting blood and urine samples were collected between 8.30 AM and 10.30 AM, after a minimum period of 15-min rest.

For RNA analysis, nine milliliters of blood was drawn by venipuncture into a citrate phosphate dextrose adenine-containing vacuum tube (Vacuette), which was kept at room temperature before and after the blood draw. Peripheral blood

mononuclear cells (PBMCs) were collected applying the standard Ficoll-Paque PLUS isolation procedure (GE Healthcare Life Sciences), within 1 h of blood draw. PBMCs were aliquoted into 1.5-ml Eppendorf tubes and kept frozen at −80°C until assayed.

For protein analysis, five milliliters of blood was drawn into a vacuum tube containing EDTA (Vacuette®), which was kept on ice before and after blood draw, and within 30 min centrifuged at 1590 × g and 4°C for 15 min. Plasma was aliquoted into Eppendorf® tubes and kept frozen at −80°C until assayed.

For nucleoside oxidation analysis, a freshly voided spot urine sample was obtained using a standard sampling kit without any additives (In Vitro as, Fredensborg, Denmark). The sample was kept on ice and centrifuged at 4°C and 1590 g for 15 min, after which aliquots of 1.5 ml were transferred to Eppendorf tubes and stored at 80°C until analysis.

In addition, standard clinical chemistry parameters were analyzed, including total leukocytes which were entered in subsequent models.

Laboratory personnel performing all described analyses were blinded to the category and clinical state of participants.

Peripheral blood mononuclear gene expression. Candidate genes were selected for mRNA analysis based on previous findings and current hypothesis regarding the pathophysiology of bipolar disorder, focusing on evidence from peripheral blood (Appendix S1).

The following genes were assessed: *ANK3, CACNA1C, RSGRP1, KLF12, BDNF, POLG, PDE4B, PGAM1, ADARB1, NUDT1, NDUFV2, GPER1, ESR1, ESR2, SP1, SP4, ACTB, ABL1, GAPDH, SDHA, GSK3B and OGG1, PTGDS, AKR1C3, and TBP.*

Further details regarding the mRNA quantification are available in the Appendix S1 and have previously been described (21, 30).

Plasma proteins. Plasma concentrations of BDNF, NT-3, and the cytokines IL-6, IL-10, IL-18, IL-1β, and TNF-α were measured using commercially available ELISA kits according to the procedure provided by the manufacturers. Further details regarding the quantification of plasma BDNF, NT-3, and cytokine levels are available in the Appendix S1 and have previously been described (31, 32).

Urinary 8-oxoGuo and 8-oxodG. The urinary content of the oxidized nucleosides 8-oxodG and 8-oxoGuo was quantified using a modified ultraperformance liquid chromatography and mass spectrometry (UPLC-MS/ MS) assay, described in detail elsewhere (26) and in the Appendix S1.

Statistical analysis

Baseline characteristics were compared using Mann–Whitney *U*-test for continuous variables and chi-squared test for categorical variables. For the calculation of a composite biomarker score, a split-sample design was employed (30), with the total sample randomly split into two equal sized samples, a training set and a test set, with equal distributions of patients with bipolar disorder and healthy control individuals. All values of individual markers were standardized and used in further calculations, in order to assign equal prior weight to all predictors and to make models across samples easily comparable (37).

Variable selection was undertaken using penalized regression by the least absolute shrinkage and selection operator (Lasso) method (38) in a generalized linear mixed model in the R package glmmLasso (39). Lasso combines sparse variable selection and parameter shrinkage through the tuning parameter, lambda, and in such a model, *P*-values for individual parameters are not relevant and are not reported. As we will only use the lasso model for prediction, we are not interested in particular regression coefficients. Lambda was determined based on Bayes Information Criteria (BIC) run on the training set. Using the selected lambda value, penalized generalized mixed model analyses were run on the training set entering all individual markers along with age and gender as independent variables in four separate models depending on the outcome of interest. In these four models, the binary variables 'patient with BD vs. healthy control individual', 'depression vs. euthymia', 'mania vs. euthymia' and 'mania vs. depression', respectively, were entered as the dependent variable. The variables selected in these models were used in subsequent generalized mixed models on the training set as independent variables along with the dependent variables used in the variable selection step, and the model coefficient for each predictor was used in construction of the composite score. The composite biomarker score (p) was constructed using the formula:

$$p(\text{group}) = \exp(B_0 + B_1 * x_1 + \ldots. + B_k * x_k) / (1 + \exp(B_0 + B_1 * x_1 + \ldots. + B_k * x_k)),$$

where B_0 is the constant and $B_1 \ldots B_k$ represent model coefficients and $x_1 \ldots x_k$ are individual values of the predictor variables entered into the generalized linear mixed model. The composite

Table 1. Demographic and clinical characteristics of study participants

A. Characteristics at inclusion	Patients with bipolar disorder	Healthy control individuals	*P*-value
N	33	35	
Age (years)	37.4 (20.3)	31.4 (21.9)	0.09
Gender (female-male)	22–11	21–14	0.8
Education (years total)	16.5 (4.0)	16.5 (3.0)	0.3
Body Mass Index	24.0 (5.2)	24.3 (3.2)	0.6
Duration of illness (years)	18.0 (18.0)		
Bipolar I (%)	20 (60.6)		
Bipolar II (%)	13 (39.4)		
Number of hospitalizations	2 (2.25)		
Lithium treatment (%)	14 (42.4)		
Anticonvulsant treatment (%)	24 (72.7)		
Antipsychotic treatment (%)	24 (72.7)		
Antidepressant treatment (%)	12 (36.4)		

B. Number of samples and symptom severity of participants at time of assessment

		Samples from patients with bipolar disorder $N = 140$			
	Samples from healthy control individuals $N = 71$	Euthymic $N = 78$	Depressive $N = 44$	Manic/hypomanic* $N = 11$	Mixed state $N = 7$
HAMD-17	0 (1)	4 (3)	17 (6.25)	4 (4)	7 (2)
YMRS	0 (0)	0 (2)	0 (0.5)	14 (5.5)	17 (4.0)

A: Data are expressed as median (Interquartile Range) or *n* (%).

B: Data are expressed as median (Interquartile Range). *N* represents number of samples. Values are presented as raw values, unadjusted for repeated measures. HAMD-17: Hamilton rating scale, 17 items.

YMRS, Young mania rating scale.

*Manic patients, $n = 19$/Hypomanic patients, $n = 5$.

biomarker score has a value between 0 and 1 and indicates the probability of belonging to either of the two groups tested in the model (e.g., patient with BD or healthy control individual). The composite biomarker score was tested on the test dataset by receiver operating characteristics (ROC) analysis (40) calculating the area under the curve (AUC) of the ROC curve. In these analyses, it was investigated whether the AUC of the ROC curves was different from the null hypothesis, AUC = 0.5. Assigning a cutoff of 0.5 on the constructed composite score, sensitivity and specificity was along with positive and negative predictive values were calculated. Finally, accuracy of the composite score as a diagnostic test was assessed by calculating the likelihood ratios. These represent the probability of the test result in patients with a given disease to the probability of the same test result in patients without the disease (41) and are stable to the prevalence of the disease. The positive likelihood ratio (LR [+]) was calculated as (sensitivity/1−specificity), and the negative likelihood ratio (LR [−]) was calculated as (1−sensitivity/specificity).

Because we explored four different discriminant outcomes, we applied Bonferroni correction, yielding a corrected level of statistical significance of $P < 0.0125$. All statistical analyses were conducted using the freely available statistical software R.

Results

Study sample description

Characteristics of the study population are described in Table 1A. There were no overall statistically significant differences between patients with bipolar disorder and healthy control individuals with regard to age, gender distribution, educational level, or body mass index. All the participants were out-patients at the time of inclusion. The number of samples obtained with valid levels of all available individual biomarkers along with symptom severity at the time of assessment is presented in Table 1B. Further information on the number of study visits is available in the Appendix S1.

Discrimination between patients with bipolar disorder and healthy control individuals

Results are presented in Table 2. Individual variables selected for the composite markers were

Table 2. Test characteristics of composite biomarker scores in all comparisons

Comparison	ROC AUC	95% CI		*P*-value	Sensitivity	Specificity	PLR	NLR	PPV	NPV
		Min	Max							
BD vs. HC	0.826	0.749	0.904	0.0016	0.73	0.71	2.53	0.38	0.85	0.54
Dep vs. Eu	0.535	0.379	0.691	0.0063	0.21	0.78	0.94	1.02	0.38	0.60
Man vs. Eu	0.594	0.384	0.805	0.0115	0.20	0.83	1.2	0.96	0.14	0.88
Dep vs. Man	0.817	0.630	1	0.0091	0.92	0.40	1.53	0.21	0.88	0.50

AUC, Area under the curve; BD, patients with bipolar disorder; CI, confidence interval; Dep, depressed; Eu, euthymic; HC, healthy control individuals; Man, manic; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio;PPV, positive predictive value; ROC, receiver operating characteristic.

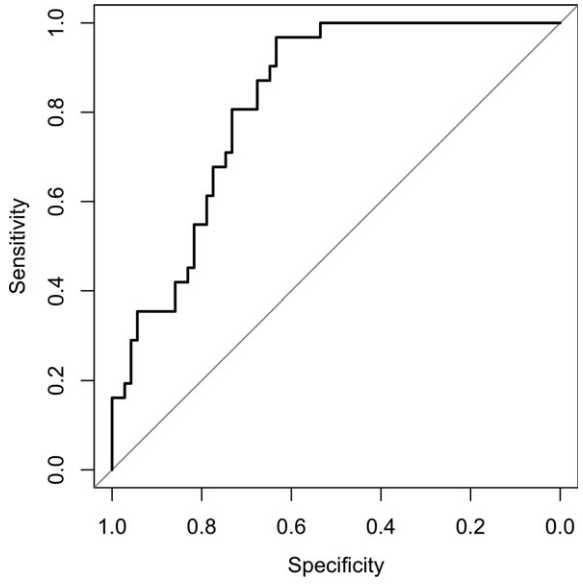

Fig. 1. Receiver operating characteristics (ROC) curve of discrimination between patients with bipolar disorder and healthy control individuals.

POLG, *ADARB1*, *OGG*, 8-oxoGuo, leukocytes, and age. Applying the composite biomarker on the test set the AUC of the ROC curve was 0.83 (95% CI: 0.75 to 0.90, $P = 0.002$) (Fig. 1). Patients with bipolar disorder and healthy control individual were discriminated with a sensitivity of 73% and a specificity of 71%, corresponding with a positive predictive value of 0.85 and a negative predictive value of 0.54. Positive and negative likelihood ratios of 2.5 and 0.4, respectively, indicated small to moderate shifts in probability of a correct diagnosis using the composite score.

Discrimination between acute affective states and euthymia in patients with bipolar disorder

Individual variables selected for the composite marker discriminating between euthymia and depression were *PDE4B*, *MAPK6*, 8-oxodG, age and sex, and IL-18 for discrimination between euthymia and mania. AUCs of the ROC curve were low for discrimination between euthymia and both depression and mania, with values of 0.54 and 0.60 respectively (Table 2).

Discrimination between manic and depressed states in patients with bipolar disorder

Individual variables selected for the composite marker were IL-6 and IL-18. The composite score discriminated depression from mania with an AUC of the ROC curve of 0.82 (95% CI: 0.63 to 1.00, $P = 0.01$), corresponding with a sensitivity of 0.92 and a specificity of 0.40. Positive and negative likelihood ratios of 1.53 and 0.40, respectively, indicated smaller shifts in probability of a correct diagnosis using the composite marker (Table 2).

Discussion

This report was undertaken to determine the potential for a multisystem composite biomarker as a test to discriminate between patients with bipolar disorder and healthy control individuals and between affective states within patients with bipolar disorder. To that end, we combined individual proteomic and gene expression biomarkers from both peripheral blood and urine into a composite biomarker in a data-driven approach in a training dataset and tested the marker in an independent test dataset. We found that the composite biomarker discriminated moderately well between patients with bipolar disorder and healthy control individuals and between a manic and depressed state, but not between a euthymic state and acute affective states. In the present analysis, the composite biomarker achieved an AUC of 0.83 in discriminating between patients with BD and healthy control individuals compared with an AUC of 0.73 of our previous strictly gene expression composite marker, although we were not able to perform formal tests to compare the two.

The individual biomarker selected in a data-driven manner may point to putative pathways

potentially involved in the pathophysiology of bipolar disorder. The individual components of each of the composite biomarkers were involved in different interrelated molecular pathways, which primarily are related to oxidative stress, mitochondrial function, RNA editing and DNA repair, and inflammation. Thus, for discriminating between patients with bipolar disorder and healthy control individuals, the variables selected were peripheral blood mRNA expression of the genes *POLG*, *ADARB1* and *OGG*, urinary 8-oxoGuo, and peripheral blood leukocytes, and for discrimination between a manic and a depressed state, the variables selected were the cytokines IL-6 and IL-18 in peripheral blood. An in-depth discussion of the potential underlying neurobiological mechanisms of the individual components of the composite biomarkers is provided in the Appendix S1.

The identification of pathways related to oxidative stress, mitochondrial function, RNA editing and DNA repair, and inflammation as part of our multisystem composite marker partially overlapped with the components of the multianalyte panel identified in a recent study of a composite diagnostic biomarker in bipolar disorder by Haenisch et al. (29). In the study by Haenisch et al. (29), seven of 20 analytes in the panel were inflammation related, while the remaining components were mainly clustered into lipid transport-related proteins and proteins with metalloendopeptidase activity. The analyte panel used in the discovery stage by Haenisch et al. was strictly protein based, while our approach allowed for the final selection of markers on both a proteomic and a transcriptomic level and involved components in both blood and urine. Our composite multisystem biomarker did not achieve the discriminatory capacity obtained in the validation stage by Haenisch et al. (29). Using similar data-driven feature selection, Haenisch et al. found a high AUC of the ROC curve of 0.92 when comparing patients with bipolar disorder and healthy control individuals. In the study by Haenisch et al., a larger total range of individual markers was available allowing for a composite marker in the validation stage consisting of 16 individual serum proteins selected from a total of 115 analytes in a previous discovery stage. Thus, even though the study by Haenisch et al. did not analyze other tissues or body fluids than blood and focused on proteins only, one explanation for the high discriminatory power in the study may be the screening of a large quantity of potential biomarkers and the subsequent inclusion of a relatively large number of individual markers. Another study, however, analyzed a total of 166 potential individual markers and used a different data-driven approach, arriving at a lower discriminatory property of a composite biomarker with an AUC of the ROC curve of 0.77 (27).

The current findings with an apparent improved diagnostic accuracy compared with our strict gene expression composite biomarker in peripheral blood may suggest that the use of a data-driven approach involving both gene expression and proteomic markers from not only peripheral blood but also urine has potential to arrive at more sensitive and specific composite biomarkers. While the diagnostic accuracy using our composite marker was moderate, the obtainment of even more analytes for feature selection could potentially have generated a more accurate final biomarker. It is also possible, that other studies, which, up to now, have considered only proteins or gene expression markers separately and only in peripheral blood (27, 29, 42), could have achieved better results using a multisystem, multi-tissue approach as we attempted in the current report.

Our multisystem biomarker discriminated moderately well between a manic state and a depressed state, while it was not able to discriminate between a euthymic state and current affective states. This appears to contrast findings of individual biomarkers in patients with bipolar disorder of differences in biomarker levels between euthymic patients and symptomatic states, rather than between mania and depression (14, 43) and thus not necessarily related to the distinct polarity of the affective state. However, the evidence base largely consists of indirect comparisons from case–control studies of patients with bipolar disorder and healthy control individuals, with relatively few studies directly comparing groups of patients with bipolar disorder in various affective states (9, 14, 16, 43). The current study is one of very few biomarker studies that include intra-individual alterations of biomarker levels between affective states, a design that may be more sensitive to elucidate potentially subtle biomarker differences between mania and depression. This may underlie our ability to identify a composite biomarker with discriminatory capacity to differentiate a depressed state from a manic state and could potentially indicate that the individual components of the multisystem biomarker were related to processes that are qualitatively different between a manic and a depressed state. In contrast, the relatively low symptom severity in the current study may have contributed to the lack of discriminatory power between euthymia and current affective states of our composite biomarker.

There are several limitations in the current study. While our study sample was well characterized, it was relatively small, especially when using a

split-sample design in developing and testing our composite marker and our results should therefore be regarded as preliminary. The total number of individual markers was relatively low and we speculate that the inclusion of a larger range of individual potential biomarkers could have increased the accuracy of our final composite biomarkers. The two groups were matched for age, gender, education, and BMI, but we cannot exclude that medication and differences in behaviour; for example, physical activity pattern might have influenced the measured analytes. Further, our study population which consisted of patients with rapid-cycling bipolar disorder and healthy control individuals recruited in a blood bank may not be representative of a broad range of patients with bipolar disorder and healthy individuals without the disorder. Importantly, while our comparison between patients with bipolar disorder and healthy control individuals is useful in establishing proof of concept for a multisystem composite biomarker, the clinical relevance of such a marker involves discrimination from patients with unipolar depression or the identification of individuals at high risk of developing bipolar disorder. In that regard, it is a limitation that we only included healthy individuals as a control group and that the composite biomarker was not tested in a clinical setting. Further efforts in evaluating novel biomarkers involve replication in independent samples, prospective validation in prospective cohorts, evaluating of incremental added and clinical utility, among others (44). Building on our current efforts, we are presently undertaking a large cohort study, including relatives of patients with bipolar disorder, where we will investigate the potential for a composite biomarker as a marker of illness risk (45).

While the specific composite multisystem biomarker in the current study is not useful for application into current practice, we suggest that the methods are used in future studies investigating biomarkers in bipolar disorder. This involves using a data-driven feature selection across individual potential markers from blood, urine, and other readily available tissues as well as analyzing proteomic, gene expression, and other markers. Further, for a composite biomarker to have clinical applicability, the use of tissues and laboratory methods that have potential to be integrated into a clinical setting should be considered. Along that line, in an ongoing cohort study involving patients with bipolar disorder, their healthy first-generation relatives and healthy control individuals we are planning to combine markers from multiple and readily available tissues (45). In the current study, variable selection was entirely data driven and we investigated composite markers that would be sensitive to trait and state separately. This allowed for selecting markers that may better distinguish state in a separate composite marker and selection of markers that may better distinguish trait in another composite marker, since the evidence indicates that certain potential individual biomarkers may better reflect state alterations while others reflect trait alterations (14, 43, 46). From a clinical perspective, however, the identification of one single composite marker would be more optimal and this should be one of the focuses for future efforts in the area.

In conclusion, we found promise for at multisystem composite biomarker as a diagnostic and state marker in bipolar disorder developed using a data-driven approach. Findings are limited by a small sample size. Future studies are recommended to investigate the potential for combining individual biomarkers from multiple tissues and on different molecular levels.

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Declaration of interest

KM, BKP, HEP, and CTE report no conflict of interests. LVK has within the latest 3 years been a consultant for Sunovion. MV has within the last 3 years been a consultant for Lundbeck.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:
Appendix S1. A multisystem composite biomarker as a preliminary diagnostic test in bipolar disorder.