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RESEARCH ARTICLE

Validation of a diagnostic screening blood test for bipolar disorder

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BACKGROUND: Bipolar disorder is difficult to diagnose, and probably is both overdiagnosed and underdiagnosed. Misdiagnosis has deleterious consequences for the prognosis of the disorder. In a previous study (n = 134), measurement of blood cellular membrane potential (expressed as membrane potential ratio [MPR™]) diagnosed bipolar illness with a sensitivity of .78 and a specificity of .88. The current study was performed as a validation of the initial study.

METHODS: We recruited consecutive outpatients with DSM-IV-diagnosed bipolar I disorder (BD I) and those without bipolar illness (n = 153) and measured their MPR™.

RESULTS: BD I patients were relatively hyperpolarized, with an MPR™ of .601 (± standard deviation .0179), compared with non-bipolar patients (MPR™ .675 ± .0174). The sensitivity and specificity for BD I are .93 and .90, respectively.

CONCLUSIONS: Before clinical application, future studies must utilize consensus diagnosis as the "gold standard" diagnosis and examine the effect of mood-stabilizing drugs on MPR™.

KEYWORDS: diagnosis, screening, blood test, bipolar disorder, validation

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INTRODUCTION

Proper diagnosis of mental illness is the foundation for effective therapy and appropriate prognosis. Diagnosis of mental disorders is currently based on the presence of a range of specific signs and symptoms. Frequently, many diagnostic criteria are subjective. However, many men-

TABLE
Demographics of patient population (ages of children 8.9 ± SD 2.56; adolescents 16.3 ± 1.85; adults 41.8 ± 12.57 years)

Age group	Ethnicity/sex	Bipolar I disorder	Negatives
Adults	AF	1	3
	AM	1	3
	BF	8	9
	BM	1	6
	HF	0	2
	HM	0	3
	WF	20	32
	WM	9	31
Children	AF	0	0
	AM	0	0
	BF	0	1
	BM	0	0
	HF	0	0
	HM	0	0
	WF	2	2
	WM	1	3
Adolescents	AF	0	0
	AM	0	0
	BF	0	1
	BM	0	0
	HF	1	1
	HM	0	0
	WF	3	2
	WM	4	3
Total		**51**	**102**

AF: Asian female; AM: Asian male: BF: Black female: BM: Black male; HF: Hispanic female; HM: Hispanic male; SD: standard deviation; WF: White female; WM: White male.

tal illnesses have a biologic basis that might allow for a more objective diagnosis. This is clearly true for bipolar illness, where a multitude of biologic abnormalities offer opportunities for laboratory diagnosis.

Ion deregulation has been demonstrated to be an important pathophysiologic correlate of bipolar disorder. Abnormalities in sodium (Na^+), potassium (K^+), and hydrogen transport across the membrane have been the most reproducible biologic finding in bipolar disorder patients.[1] Nearly 75% of the susceptibility loci that have been linked to bipolar illness include genes that are involved in ion regulation.[2] Ankyrin 3, a scaffolding protein for the voltage-gated sodium channel, and other genes that regulate ion channels, appear to be of particular importance.[3-5] It has been previously reported that the ion dysregulation is associated with hyperpolarization of transmembrane potential in manic bipolar disorder patients.[6] Subsequently, this phenomenon was found to be sufficiently general to predictably distinguish bipolar from non-bipolar disorder patients.[7] This finding has been operationalized into a blood test for the diagnosis of bipolar I disorder (BD I).[7]

Thiruvengadam and Chandrasekaran[7] reported the results of the initial clinical trial for evaluating the validity of using membrane potential of blood cells, expressed as MPR™, as a diagnostic tool for bipolar disorder. This initial trial was limited to hospitalized patients and included neither children nor adolescents. To better characterize this blood test in a broader range of patients, a second clinical trial was conducted with the participation of several community clinical psychiatrists. The results of this trial are presented in this paper.

METHODS

MPR™ measurement

The methods employed in the current study previously have been reported.[7] Briefly, whole blood cells were incubated separately in a reference buffer and a test buffer. The reference buffer contained all ions including Na^+, K^+, and calcium (Ca^{2+}) along with glucose and HEPES (4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid). The test buffer contained ethacrynate in addition to these salts, but not K^+. A lipid-soluble fluorescent dye, dihexyloxacarbocyanine iodide (DiOC6[3]), was used to measure the membrane potentials in a plate reader. The ratio of membrane potential in K^+-free buffer to that in the reference buffer was called MPR™, and was used as the diagnostic parameter. The analyses were performed in a blinded fashion. This procedure can be completed in 1 day, and costs approximately $500.

Participants

All the patients in this study came from the private practices of clinical psychiatrists with many years of clinical experience, located in the Baltimore metro area. The patient population included consecutive appropriate adult participants (**TABLE**). All participants provided informed consent, which stated that the blood tests were

for experimental purposes and would not be used to determine their treatment or prognosis.

Diagnostic procedure

Clinical diagnoses were assigned after reviewing all available information, including a combination of data from medical records, assessment tools, clinical interviews with the patient and relatives, and treatment response. The clinical diagnosis was used for our analyses of specificity and sensitivity. Diagnoses strictly followed DSM-IV diagnostic criteria, but did not involve the more reliable structured diagnostic interview procedure. Collection of ancillary clinical data, such as medications, current mood state, comorbid diagnoses, presence of rapid cycling, or thyroid status, was limited by inadequate funds. Consequently, many questions regarding the effect of clinical variable on MPR™ remain unanswered.

Statistical analysis

The *t* test was used to examine the differences in MPR™ among bipolar and non-bipolar disorder patients. Logistic regression was used to assess the predictive power of the MPR™ for the diagnosis.[8]

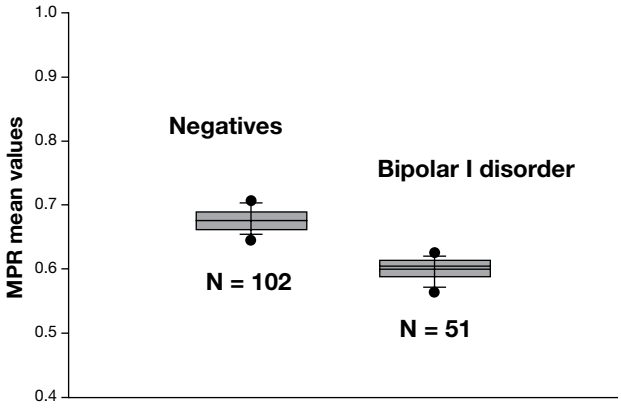
RESULTS

The study included 51 BD I participants and 102 non-bipolar disorder participants. The ethnicity and sex covered a range prevalent in North America (**TABLE**). BD I patients were relatively hyperpolarized with an MPR™ of .601 ± SD .0179 compared with non-bipolar disorder patients (.684 ± .0174). Participants without BD I had diagnoses of schizophrenia, various types of depression and anxiety, or no psychiatric illnesses. In this replication study, the sensitivity and specificity for BD I are .93 and .90, respectively. In the previous study the sensitivity was .78 and the specificity was .88.[7] It is important to note that the study population included 8 children and 14 adolescents. Although the subsample of these youths is too small to perform a statistical analysis, the values of these patients were different from controls and were not different than adult values.

DISCUSSION

Bipolar disorder is difficult to diagnose and is both under-[9,10] and over-diagnosed.[11,12] This results in prolonged delays in patients receiving the correct diagnosis.[9] Misdiagnosis frequently is associated with inappropriate treatment, which can worsen the course of the illness or be ineffective.[10] Moreover, making a diagnosis may be complicated by comorbid conditions.[13,14]

FIGURE
MPR™ of bipolar I disorder (BD I) and non-bipolar disorder patients



A total of 153 patient samples are shown. There were 102 negatives (including patients with schizophrenia, unipolar depression, obsessive-compulsive disorder, and other anxiety disorders) and 51 BD I samples. Statistical analysis using the ANOVA test shows that the 2 groups are significantly different from each other ($P < .001$). The data are presented in box plots. The bottom line of the rectangular box represents the 25th percentile of the data population while the top line represents the 75th percentile. The lines inside the box represent the mean values. Similarly, the bottom cross line represents the 5th percentile and the top cross line represents the 95th percentile.

ANOVA: analysis of variance; MPR: membrane potential ratio.

The current study utilized a mixed outpatient clinical sample to replicate a previous study which was performed with acutely ill inpatients. In this outpatient sample, the MPR™ accurately diagnosed BD I with a high level of specificity (.90) and sensitivity (.93).

The current state of clinical diagnosis in psychiatry is in some disarray. Diagnostic stability over time generally is low,[15-17] but inpatient diagnoses and severe psychotic diagnoses tend to be more stable.[15,16] Bipolar diagnoses remain consistent over a 12-year period in only approximately 49% of patients.[15] Clinical diagnoses may resemble the outcome of structured diagnostic interviews, but show variability across geographic location[18] and are less concordant in non-psychotic individuals.[19] The

"gold standard" of consensus diagnosis in the setting of a structured interview[20] is too cumbersome to be used in any settings other than well-funded research studies. Improving diagnosis is imperative in psychiatry.

The Mood Disorders Questionnaire (MDQ) was a response to the diagnostic problems in bipolar illness.[9] While initially designed as a screening tool, the MDQ was quickly utilized as a diagnostic tool.[21,22] The sensitivity of this questionnaire varies greatly from .28 to .73 with different study populations, while the specificity stays relatively stable at .9 to .97.[9,23] The MPR™ test appears stable between inpatient[7] and outpatient populations (current study).

There are clear limitations to the current study. The first is the use of clinical diagnoses rather than a standardized research diagnosis. As previously noted, clinical diagnoses can be problematic with over- and underdiagnoses, and inconsistency of diagnosis over time.[9-12,15] However, the consistency of the MPR™ test among 2 separate studies, using different clinical populations and clinicians, suggests that the blood test may be reasonably accurate. Nonetheless, prior to widespread application, the MPR™ must be tested in populations defined by the "gold standard" consensus diagnosis.[20] In addition, many of the participants were treated with medications. Because the clinicians believed these patients had BD I, it is likely that most were on mood-stabilizing medications. Most effective mood stabilizers interfere with sodium entry in an activity-dependent manner[24] and can alter the membrane potential of cells. However, although lithium has the potential to depolarize cells,[25] the MPR™ found that bipolar disorder patients' cells were hyperpolarized (**FIGURE**), suggesting that the results are not simply a measure of drug effect. Nonetheless, the effect of mood-stabilizing drugs on MPR™ must be determined. Finally, the MPR™ has been studied only in BD I and not other types of bipolar illness. Many of these limitations in design are related to inadequate funding.

Despite these limitations, the MPR™ test offers significant promise as a potential diagnostic blood test for bipolar disorder. Sensitivity and specificity of .93 and .90, respectively, compare favorably with other tests used to diagnose complex diseases such as antinuclear antigen for systemic lupus erythematosus or prostate specific antigen for prostate carcinoma.[7] Performance of needed additional validation, with specific studies utilizing a "gold standard" diagnosis, and subanalyses that clarify the role of mood state, medications, age, and illness course, are required prior to clinical utilization.

CONCLUSIONS

The current study replicates the high sensitivity and specificity of the MPR™ test for the diagnosis of BD I. While additional work is required prior to clinical application, the results to date are quite promising. In addition to its clinical utility, this test will also help further illuminate the pathophysiology of this disease. ■

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