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RESEARCH

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RNA editing-based biomarker blood test for the diagnosis of bipolar disorder: protocol of the EDIT-B study

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Abstract

Introduction Misdiagnosis of bipolar disorder (BD) can lead to ineffective treatment, increased risk of manic episodes, and increased severity. Objective diagnostic tests or precise tools to diagnose BD and distinguish it from major depressive disorder (MDD) in depressed patients are lacking.

Aim To assess the external diagnostic validity of a blood-based test using an RNA epigenetic signature for the differential diagnosis of BD versus MDD in patients with depression.

Methods and analysis Multicentre cross-sectional study including an adult sample of inpatients or outpatients diagnosed with BD or MDD, currently treated for a major depressive episode. A structured diagnostic interview based on validated scales will be conducted. Sociodemographic variables, clinical history, toxic consumption, current treatment and quality of life will be assessed. Blood samples will be obtained and stored at −80 °C until RNA sequencing analysis. The EDIT-B is a blood-based test that combines RNA editing biomarkers and individual data (e.g., age, sex, and tobacco consumption). The clinical validation performance of the EDIT-B will be evaluated using the area under the curve, sensitivity, specificity, positive and negative predictive values, and likelihood ratios.

Ethics and dissemination The principles of the Declaration of Helsinki 2013, precision psychiatry research and good clinical practice will be followed. The Research Ethics Committees of the participating centres approved the study. Participants will receive an information sheet and must sign the informed consent before the interview. Participants' data will be pseudonymized at the research sites. Any publication will use fully anonymized data. Publications with the final study results will be disseminated in international peer-reviewed journals and presented at international conferences.

Study registration: This study has been registered on clinicaltrials.gov (NCT05603819). Registration date: 28-10-2022.

Keywords Bipolar disorder, RNA editing, Epigenetics, Depression & mood disorders, Diagnosis, Machine learning

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Introduction

Bipolar disorder (BD) is a common and disabling condition characterized by episodes of either mania or hypomania and depression. Depressive episodes are frequently the initial manifestation of BD because patients are more likely to seek professional help during these episodes than during manic, hypomanic, or mixed episodes. This tendency is often attributed to a lack of insight during the latter types of episodes [1], as well as a higher frequency of depression as the initial polarity [2], particularly among women [3]. BD leads to significant impairments in personal, social, and occupational areas, placing substantial burdens on individuals and healthcare systems [4, 5].

The clinical presentation of depressive episodes in individuals with BD closely resembles that of individuals with major depressive disorder (MDD). Currently, the diagnosis of a depressive episode relies on clinical interviews and the use of psychometric instruments. The overlap in clinical presentation between BD and MDD, combined with the higher prevalence of MDD, often leads to inadequate screening and, consequently to misdiagnosis of BD [1, 6]. The estimated mean delay between the first depressive episode and BD diagnosis is approximately 9 years [7], which is likely greater for individuals with depressive polarity [8]. Nonetheless, certain clinical features—such as an age of onset between 15 and 25 years, subthreshold mania, frequent insomnia or hypersomnia, cyclothymic traits, recurrent suicidal ideation, and having a first-degree relative with BD—have shown promise in predicting the onset of BD, particularly among young adults. Early differentiation is important as pharmacological interventions seem to be more effective at an early illness course [9]. Although these features do not provide a definitive solution, they are valuable clinical indicators that can help identify high-risk cases and improve early detection efforts.

However, early differentiation between BD and MDD remain challenging often leading to a wrong diagnosis. Misdiagnosis can lead to detrimental outcomes, such as reduced treatment efficacy, clinical progression of the illness [10], heightened risk of manic episodes, and increased severity. Both inaccurate diagnosis and inadequate treatment of BD may contribute to elevated rates of hospitalization, suicide attempts, and suicide deaths. Furthermore, it can worsen the risk of manic episodes and lead to more frequent rapid cycling [11, 12]. The economic burden associated with misdiagnosis or inadequate treatment of BD encompasses not only the direct expenses of treatment but also the significantly greater indirect costs, such as decreased productivity, elevated unemployment, and increased mortality [13].

A critical factor that substantially adds to the burden linked with BD is the absence of objective diagnostic tests and of dependable and precise tools to aid in the differential diagnosis between BD and MDD, enabling the implementation of suitable treatments for these disorders. In this respect, although there is evidence that immune biomarkers and lipid peroxidation are associated with certain major depression phenotypes [14] and literature have showed possible distinct immune biomarkers profiles for MDD and BD [15], characterization is still unclear. Thus, when assessing specifically the differences among activated immune and oxidative pathways between MDD and BD no biomarker differences have been showed [14]. This is an emerging field of "precision psychiatry", which may radically change psychiatric practices worldwide [11]. Even so, the comprehensive analysis of immune, neurochemical, neurobiological, and metabolic biomarkers influencing mood disorders remains highly complex and will require time. Moreover, the absence of validated biological markers (biomarkers) to delineate boundaries between various subtypes of depression poses a significant challenge. In this context, blood biomarkers stand out as a promising tool, being reliable, minimally invasive, and easy to implement, potentially paving the way toward precision medicine in psychiatry.

Epigenetics regulates gene expression [16]. Various epigenetic modifications, such as deoxyribonucleic acid (DNA) methylation, histone modifications, ribonucleic acid (RNA) editing, and miRNA dysregulation, have been linked to psychiatric disorders [17]. Specifically, RNA editing enables the generation of multiple protein variants within cells and tissues by modifying ribonucleotides according to the corresponding DNA sequence, which involves substitutions, deletions, or insertions [18]. Alterations in neurotransmitter receptors caused by RNA editing have been found in postmortem studies of suicide victims [19, 20], patients with schizophrenia [21], patients with BD [22], and patients with psychotic disorders [20, 23]. RNA editing has also been associated with autism and postpartum psychosis [18]. Furthermore, prior findings have demonstrated the potential of RNA editing in identifying depression in postmortem sections [19] and in blood after interferon treatment [24].

A recent study showed that a blood-based signature comprising specific RNA editing biomarkers exhibits high accuracy in the biological detection of BD [25]. This signature encompasses biomarkers from eight genes implicated in various mood and inflammation regulation pathways, alongside individual data such as age, which is analysed by an artificial intelligence algorithm. This signature effectively distinguishes between BD and MDD within a sample of individuals experiencing moderate to severe depression ($n = 245$). The differential diagnosis of

BD from MDD was replicated in an external independent clinical validation sample ($n=143$), which showed high sensitivity (86.4%) and specificity (80.8%) (area under the receiver operating characteristic curve (AUC-ROC) 0.904) [25]. This biomarker signature, as well as two supplementary signatures showing high accuracy, have been selected to be validated in a new clinical sample.

This protocol outlines the rationale, methodology and expected outcomes of the EU-supported EDIT-B project (Grant number 220628/230125), which aims to identify among three previously tested and selected blood-based RNA epigenetic signatures the best signature in terms of external diagnostic validity (sensitivity, specificity, AUC, etc.) for the differential diagnosis of BD versus MDD in individuals with acute major depressive episodes.

Methods and analysis

Study design and setting

The present trial is a multicentre cross-sectional study including an adult sample diagnosed with bipolar disorder (BD) or major depressive disorder (MDD). Participants are recruited from four clinical sites: two in Barcelona, Spain; one in Paris, France; and one in Copenhagen, Denmark (Fig. 1). Different centres around Europe have been selected to include populations with different characteristics to ensure further applicability and generalizability of the expected results. This study has been registered on clinicaltrials.gov under the number NCT05603819 (Table 1). The SPIRIT reporting guidelines were used for reporting the study and the checklist has been included [26] (Table 2).

Eligibility criteria

Eligible participants are between 18 and 80 years old diagnosed with BD or MDD and currently treated for a major depressive episode (MDE), both of which were verified using the Mini-International Neuropsychiatric Interview (MINI) [27] for the Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5). Both inpatients and outpatients are eligible for recruitment. The Montgomery–Åsberg Depression Rating Scale (MADRS) [28] total score should be equal to or greater than 20 at the time of assessment. The BD participants must have experienced at least one manic or hypomanic episode, while the MDD participants must have had at least one MDE. All participants must provide signed informed consent following oral and written study information.

The exclusion criteria for MDD participants are having first-degree family history of BD (e.g., parents, siblings or children), a total score on the Young Mania Rating Scale (YMRS) [29] equal to or greater than 12 at the time of assessment, pregnancy, BD or MDD secondary to major central nervous system affect or a diagnosis of schizoaffective disorder (Fig. 1).

EDIT-B assay test

The EDIT-B assay is a blood-based test system that combines sequencing data related to RNA editing biomarkers and individual data, including age, sex, tobacco consumption, alcohol abuse and treatment (antiepileptic, antipsychotic, anxiolytic, hypnotic/sedative and antidepressant data). The biomarkers originate from different genes: GAB2 (growth factor receptor bound protein 2-associated protein 2); IFNAR1 (interferon alpha/beta receptor 1); LYN (tyrosine-protein kinase Lyn); MDM2 (E3 ubiquitin-protein ligase Mdm2); PRKCB (protein kinase C beta type); IL17RA (interleukin 17 receptor A), PTPRC (protein tyrosine phosphatase receptor type C, also called CD45 antigen) and ZNF267 (zinc finger protein 267) [25]. These biomarkers have been described in previous work [25, 30] and have been selected using multiple criteria. First, an editome analysis was performed in a discovery cohort ($n=57$ with 31 controls and 26 patients with depression) identifying several hundred potential targets. Then, stringent quality inclusion criteria (no location in intergenic regions or near SNPs; editing present in at least 25% of samples; median/mean coverage $\geq 30\times$; RNA Editing $0.8 \geq$ Fold Change ≥ 1.20; p value < 0.05 and AUC ROC > 0.7) were applied following biological process analyses (with Gene Ontology and Reactome Pathways) and gene disease association analysis (DisGeNET) leading to a total of 8 genes. The biomarkers were recently validated in two independent clinical studies showing that they can be used to significantly differentiate patients with BD from patients with MDD [25].

As described in this previous work, an ensemble machine learning method, called ExtraTrees method—is derived from randomForest—was used for unipolar and bipolar depression differentiation, including RNA editing biomarkers, psychiatric treatment classes, and demographic variables (age, sex, substance use) as features. The ExtraTrees model learned effectively the complex relationships among these factors and their combined impact on diagnostic outcomes.

To execute EDIT-B assay test, blood samples are extracted using routine methods, and biological analyses are carried out by laboratories accredited for RNA sequencing (EN UN ISO 15189:2023). RNA is extracted (QIAsymphony, QIAGEN) and reverse transcribed (PrimeScript, TAKARA), and cDNA is subsequently amplified with specific primers (Q5 Hot Start High Fidelity enzyme, New England Biolabs), purified with magnetic beads (SPRIselect, Beckman Coulter) and indexed (Nextera XT, Illumina). After sample pooling and purification (SPRIselect, Beckman

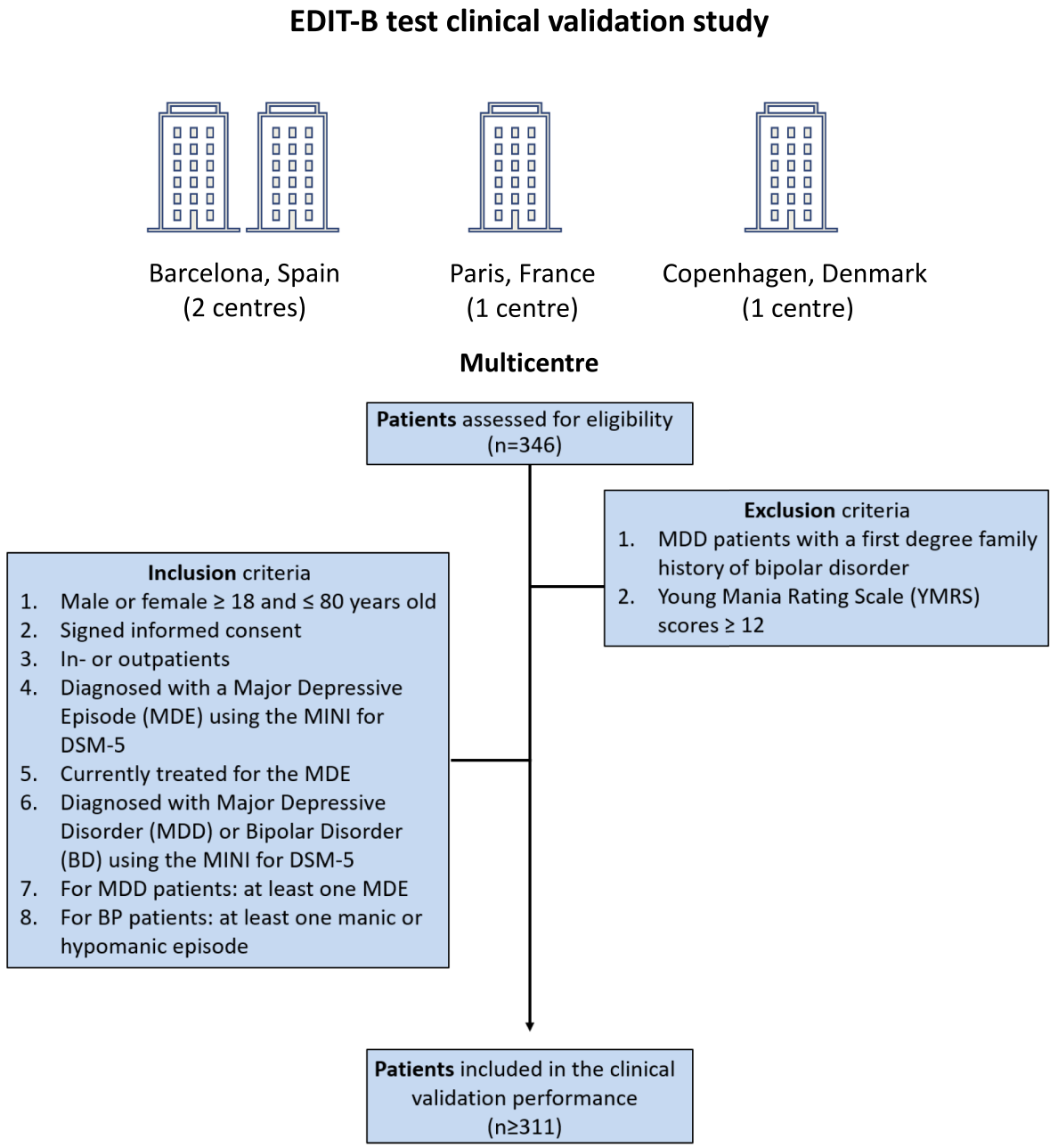

Fig. 1 EDIT-B study design. The EDIT-B study is a multicentre study recruiting participants in four different centres in Spain, France and Denmark, respectively. Participants are assessed for eligibility with several inclusion and exclusion criteria during the inclusion visit

Coulter), the resulting library is sequenced (NextSeq 500/550 Mid-Output, Illumina). Detailed information about the biological and sequencing protocols used has been published elsewhere [25]. The EDIT-B assay is intended to assist physicians in diagnosing BD and differentiating between BD and MDD.

Enrolment

Eligible participants are invited to participate by psychiatrists or psychologists from the clinical centres. The information sheet is provided during the inclusion visit, and all participants' questions regarding their study participation are answered by the professional. Before

Table 1 EDIT-B study summary

Study title	Clinical validation study for EDIT-B test: an aid for differential diagnosis of bipolar disorder, based on RNA editing blood biomarkers
Short title	EDIT-B
Study participants	In- and out adult patients between 18 and 80 years old diagnosed with bipolar disorder or major depressive disorder, and currently treated for an acute major depressive episode
Study centres	4 centres in 3 countries (Denmark, France, Spain)
Start date	June 2022
Planned end date	December 2024
Ethics approval and registration number	France/BRC ID: 2021-A03062-39 Denmark/Journal-nr.: H-22010899 Spain (Hospital Clinic): Reg. HCB/2022/0042 Spain (Parc Sanitari Sant Joan de Déu): C.I. PS-05-22 ClinicalTrials.gov ID: NCT05603819
Study objectives and methods	The objective of this study is to estimate three EDIT-B signatures in term of their external validity. For this purpose, performance of the test will be estimated by calculating for each signature its sensitivity, specificity and its accuracy to predict the diagnosis of bipolar disorder. Area under the ROC curve of each signature will be calculated. Additionally, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio and odds ratio will be calculated

the interview, the participants must sign the written informed consent. If they are unable to act on their own behalf, a witness or guardian will sign the consent on their behalf. Once the consent is signed, the interview will be conducted, including: (a) sociodemographic variables (age, sex, ethnicity, race, civil status, coexistence, academic level, years of education, labour qualifications, current occupation); (b) clinical history (type of BD (I vs. II), duration of the illness, age of onset of the first depressive, manic or hypomanic episode, number of depressive, manic, hypomanic or mixed episodes); (c) personal psychiatric history (history of psychotic symptoms, delusions, hallucinations, catatonia, seasonal pattern, rapid cycling, melancholy, atypical symptoms, psychotic depression, post-partum depression); (d) family psychiatric history (any history of affective disorders, history of suicide, and any other mental disorders for relatives of first, second, and/or third degree of consanguinity); (e) current and previous interventions received (e.g., medical procedures, psychological therapy, alternative treatments); (f) current toxic consumption (e.g., tobacco, nicotine, caffeine, cannabis); and (g) current medication for the diagnosed disorder and any other concomitant medications, start date, dose and frequency are registered. Moreover, MADRS [28], YMRS [29], MINI scale [27], and the European Quality of Life 5 Dimensions Questionnaire (EQ-5D) [31] (before and after diagnosis) will be completed. The first participant was recruited in the summer of 2022, and the last site initiation occurred at the beginning of 2023. The enrolment has been completed up to 80%. We plan to complete the study at the end of 2024.

All clinical and biological data is collected during a unique visit. Thus, participation ends after completing the interview and blood sample collection. Blood samples are collected in two PAXgene® RNA collection tubes (2.5 mL each), shipped to a central laboratory and stored at −80 °C until analysis. Sample analysis will be performed in batches. The extracted RNA from the blood and remaining blood samples will be stored at −80 °C for 5 years and may be used for additional studies if the patient agrees to participate in the consent form. Otherwise, the samples will be destroyed. Neither global nor individual results of the signatures will be communicated to the investigators before the end of the study. Figure 2 summarizes the participant flow from inclusion with blood sampling until the final analysis.

The sample size was fixed to provide sufficient precision (±5%) in the estimates of the diagnostic performance of the EDIT-B test in terms of sensitivity, specificity, accuracy and area under the receiver operating characteristic curve (AUC-ROC). Based on the literature [25], the estimated values of sensitivity, specificity and AUC-ROC are expected to be close to 86%, 80%, and 90%, respectively. A total sample size of 436 participants with MDE with a 1:1: ratio of MDD ($n=218$) vs. BD ($n=218$) will be included. A maximal 10% attrition rate was considered. The Buderer and Jones methods [32, 33] were used for these calculations.

Patient and public involvement

Patients were not involved when designing the study, but the study investigators actively participated in the study design and setting of the research question. We carefully

Table 2 Reporting checklist for the EDIT-B study protocol

		Reporting item	Page number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3	Date and version identifier	n/a
Funding	#4	Sources and types of financial, material, and other support	11
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	11–12
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	n/a
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	n/a
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	5&9
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	2–3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3
Objectives	#7	Specific objectives or hypotheses	3
Trial design	#8	Description of trial design including type of trial (e.g., parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g., superiority, equivalence, noninferiority, exploratory)	3
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	3
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (e.g., surgeons, psychotherapists)	3
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	3–5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (e.g., drug dose change in response to harms, participant request, or improving/worsening disease)	n/a
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (e.g., drug tablet return; laboratory tests)	n/a
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (e.g., systolic blood pressure), analysis metric (e.g., change from baseline, final value, time to event), method of aggregation (e.g., median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8–9
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see figure)	3–5
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	5

Table 2 (continued)

		Reporting item	Page number
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	4–5
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (e.g., computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g., blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	n/a
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	n/a
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	n/a
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how	n/a
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (e.g., duplicate measurements, training of assessors) and a description of study instruments (e.g., questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	n/a
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (e.g., double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9–10
Statistics: additional analyses	#20b	Methods for any additional analyses (e.g., subgroup and adjusted analyses)	9–10
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (e.g., as randomized analysis), and any statistical methods to handle missing data (e.g., multiple imputation)	9
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	9–10
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	9–10
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	n/a
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	10

Table 2 (continued)

		Reporting item	Page number
Protocol amendments	#25	Plans for communicating important protocol modifications (e.g., changes to eligibility criteria, outcomes, analyses) to relevant parties (e.g., investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	10
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorized surrogates, and how (see Item 32)	5&10
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	10
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	12
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	9
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (e.g., via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	5&8
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorized surrogates	-
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	5

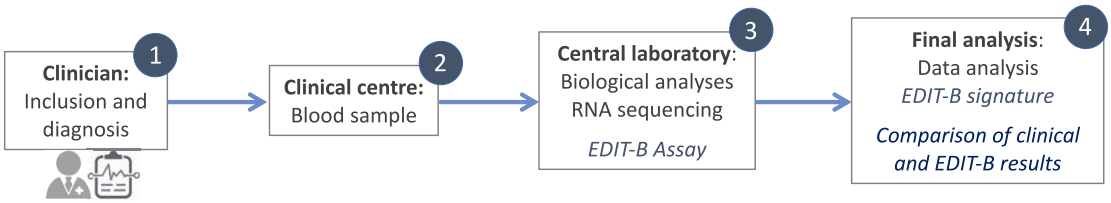

Fig. 2 Summary of the participant flow in the EDIT-B study. (1) The clinician includes the participant in EDIT-B study and evaluate the diagnosis; (2) After inclusion, a blood sample is taken from the participant at the respective centre; (3) Blood sample is sent to the central laboratory that performs the biological analysis; (4) Data analysis is done using EDIT-B signature before clinical and EDIT-B results are compared for final analysis

assessed the burden of the clinical study intervention on participants. Before and throughout the study, the general public was informed in press releases about the study advancement. At the end of the study, the study investigators will be informed of the global and individual results of their participants. Study participants will be informed of both upon their request to their investigator. Study results are intended to be disseminated in scientific articles, at conferences and to the general public through press releases and social media. For dissemination plans and methods, patients are not involved directly, but dissemination is discussed with the investigators of the study and partially performed by them.

Outcomes

The primary aim of this study is to identify among three RNA biomarker signatures the best in terms of external validation of the diagnostic performance (sensitivity, specificity, accuracy and AUC-ROC) of BD versus MDD differentiation in participants with current MDE. The

endpoint is the categorization of the patient according to the signatures as patient with MDD or BD compared to the diagnosis made by the treating psychiatrist using the MINI. Secondary analyses will be performed depending on the available information and statistical power of the collected data.

Data management plan

The data is collected via paper and later translated into an electronic CRF (e-CRF) by the investigator and/or study coordinator and the online operation of the data management checks. A clinical research assistant (CRA) monitors and validates the data continuously during the study period. During the recording process, the investigator or the study coordinator will also respond to online controls in case of inconsistencies or to queries raised by the e-CRF data manager/CRAs. At the end of the study, the investigator must sign the e-CRFs. Four validation batches are planned to check for discrepancies in the database. Validation batches were conducted at the beginning of the study.

Data checks will be performed to identify any errors, inconsistencies or missing data within the e-CRF by predefined computerized and manual checks. All systematic edit checks (manual, automatic, reconciliations) will be applied to the database to detect any discrepancies. The data manager reviews each discrepancy to validate the relevance of the issue and possibly raises a query. Manual queries can be generated according to the data manager evaluation, upon medical coder request, or following review of the medical listings.

After the recruitment of the last participant and after all queries have been solved, the data manager will proceed to the database lock of the e-CRF. A final extraction will be performed, and the final datasets will be produced and locked with a database lock certificate. A second database, consisting of the EDIT-B result (BD profile/MDD profile), will be locked when the last participant is analysed. Unlock and relock databases can be constructed by the data manager at the study coordinator's request; in the case of unlock, the data manager provides a list of data modifications made on the unlocked database.

Only personal data needed for the objective of the study will be collected. To protect personal data, participants' data will be pseudonymized at the research sites. Only the principal investigator and study coordinator at each site have access to the encryption table. During and after the study, appropriate technical and organizational measures will be implemented to ensure the processing of personal data in accordance with the European General Data Protection Regulation. Pseudonymized participants' data will be archived for a maximum of 25 years after the end of the study on health data hosting servers by Euris and located in France. The final study results will be archived, and any publication will use fully anonymized data.

Data analysis plan

Data from all included participants who provided signed informed consent and who did not withdraw from the study will be analysed. Descriptive analysis of the samples will be performed.

The clinical validation performance of the three signatures will be evaluated using the following matrices: AUC-ROC, sensitivity, and specificity. Additionally, the positive predictive value, negative predictive value, positive and negative likelihood ratios, and odds ratios will be calculated. The categorization of disorder type (BD vs. MDD) by experts relies on the MINI, which is considered the gold standard for diagnosis.

To determine the best signature among the three, the performances of the tested signatures will be compared on a two-by-two basis by assessing sensitivity and specificity utilizing methods based on the McNemar test, as proposed by Hawass [34]. Furthermore, AUC-ROC values of the three signatures will be compared using the DeLong method, which is akin to accuracy assessment through the no informative rate test [34, 35].

ExtraTrees models (as used for EDIT-B assay) are capable to handle complex and non-linear interactions frequently encountered in clinical studies. Additionally, their capacity to assess feature importance enhances transparency by providing clinicians with insights into variables influencing the most significantly predictions. In this study, the importance of features will be evaluated using randomForestExplainer package [36]. This tool makes it possible to explore deeply various importance metrics of the artificial intelligence model, such as total number of trees and nodes, mean minimal depth, node impurity and frequency as root variables. To further dissect interactions, relationships between RNA editing biomarkers and psychiatric treatment ATC classes (e.g., antipsychotics, antidepressants, and antiepileptics) will be investigated. Specifically, the occurrence of splits involving RNA editing biomarkers within maximal subtrees corresponding to each treatment class will be examined. This analysis will provide deeper insights into how these treatment categories influence RNA editing biomarkers and contribute to the model's decision process. Finally, conditional minimal depth will be calculated, a metric that quantifies the strength of interactions between features, to rank and evaluate the interaction dynamics between RNA editing biomarkers and other variables. This systematic approach will shed light on the interplay of treatment effects and RNA editing biomarkers within the

predictive framework, enhancing the understanding of their combined diagnostic power.

Considering the nature of the study, all calculations will be conducted on valid data without missing value imputation. However, the percentage of participants for whom the test has been unfeasible will be calculated since it is also an additional important parameter of test performance under real-life conditions.

The analysis will be conducted using SAS version Viya 3.05.

Ethics and dissemination

This study is conducted in accordance with the ethical principles set out in the latest version of the World Medical Association's Declaration of Helsinki 2013, attending to all the nuances involved in precision psychiatry research [37] and following the requirements of good clinical practice.

Prior to starting the study, approval was obtained from several research ethics committees, as the study is performed in different countries. For French approval, the documents were reviewed, and the study was approved under ID 2021-A-03194-37 by the research ethics committee Ile-de-France X. In Spain, the research ethics committees of the Hospital Clinic of Barcelona and the Sant Joan de Déu Foundation reviewed and approved the study under ID HCB/2022/0042 and PS-05-22, respectively. For Danish approval, the documents were addressed to De Videnskabsetiske Komiteer for Region Hovedstaden (the Scientific Ethics Committees for the Capital Region of Denmark) and approved under project ID H-22010899. Any amendments to the study will be resubmitted to the ethics committee.

A copy of the consent form, signed and dated by the participant and the principal investigator or the physician representing the investigator, will be given to the participant prior to the screening visit. One copy will be retained at the clinical site. At the end of the study, another copy will be sent, in a temper-proof sealed envelope, to the coordinator of the study. In addition, the investigator will specify in the research participants' medical files all the procedures conducted for the study.

A publication with the final study results will be disseminated in an international peer-reviewed journal and presented at international conferences.

Discussion

The current diagnostic assessment of bipolar disorder (BD) includes a subjective component that complicates timely diagnosis. General practitioners are the first to see patients who present with a major depressive episode (MDE), and they find it challenging to suspect a diagnosis of BD and therefore to refer patients to a specialist. The training and expertise of psychiatrists are essential for accurate evaluation. However, trials for the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) demonstrated an intraclass kappa value of 0.28 [38]. This represents a minimal agreement and means that, under the study conditions, highly trained psychiatrists agreed on the diagnosis between 4 and 15% of the time. Healthcare systems often lack the resources and tools to ensure responsiveness across all levels of care. Moreover, the existence of various subtypes of the disorder, each exhibiting distinct dysfunctions, further complicates the process of achieving a precise diagnosis. In this context, standardized screening tools become crucial to complement clinical evaluations. For instance, the Mood Disorder Questionnaire (MDQ) has demonstrated a sensitivity slightly above 70% in outpatient psychiatric clinics [39], as well as good accuracy for screening BD in primary care [40]. However, some studies indicate that in patients with bipolar spectrum illness, the sensitivity reaches 70% only for BD type I [41]. Meanwhile, scales such as the 32-item Hypomania symptom check-list, first revision (HCI-32-R1), have shown utility in detecting hypomanic symptoms [42], which can further aid in identifying at-risk patients. Although diagnostic scales are useful tools to support screening and diagnosis, their results are often highly heterogeneous and frequently based on small sample sizes. Therefore, their use should be approached with an awareness of their limitations. On the other hand, the diversity inherent in BD diagnoses, as classified by existing systems, may have impeded progress in psychiatric treatment, despite contributing to diagnostic consistency in the field. Furthermore, once patients are diagnosed, few receive evidence-based care, and those who do receive such care have a low probability of recovery [43].

Studies suggest that biomarkers could aid in diagnosing BD. Metabolomics biomarker signatures may improve diagnostic accuracy [44], and biochemical biomarkers combined with decision trees might differentiate BD from MDD [45]. However, further validation or improvement of their performance is necessary. Thus, there remains a need to identify and validate reliable biomarkers with strong performance that can effectively distinguish between BD and MDD [13, 46].

Advances in biomarker development hold promise for revolutionizing treatment guidance and identifying new targets for early intervention and recurrent episode prevention [47]. Recognizing early signs of recurrence may be indicative of noncompliance [48], warning healthcare providers and enlightening patients. The ongoing progress in biomarker measurement technologies represents an innovative leap that could forecast and prevent adverse clinical outcomes by furnishing real-time updates on the

patient's current clinical condition, along with predictive insights suggestive of future relapses, ultimately enhancing patient well-being. Furthermore, it will empower patients to supervise their own health conditions, aiding them in making well-informed choices regarding treatment alternatives. The ultimate objective is to enhance patient outcomes through accessible technology.

Groups of patients with different BD subtypes or dysfunctions may show divergent responses to treatment [49, 50]. This diversity suggests several underlying causes and physiological processes linked to the same disorder, which could result in varying illness courses and treatment outcomes even among individuals with identical diagnoses [51]. Therefore, reducing the significant variability in psychiatric conditions through a deeper comprehension of their biological basis might enable progress in treatment by identifying accurate biomarkers for diagnosis, prognosis, and predicting treatment responses. On the other side, psychiatric disorders remain a complex interplay of biological, psychological and social factors, needing a global evaluation that considers all dimensions.

Future approaches should therefore combine clinical evaluation with biomarkers permitting to establish a diagnosis that is based on different criteria. This helps to find a balance between variation and one-sided input that could lead to misdiagnosis. Introducing biomarkers into clinical routine is crucial keeping them as a complementary diagnostic tool and not as a standalone method. This opens a new era advancing precision psychiatry and improving patient outcomes.

Longitudinal studies should be conducted to examine the dynamics of fluctuating episodes, particularly in BD patients, and to assess biomarkers over time, possibly establishing specific biomarker patterns for diseases onset, relapse, or treatment responses. This might help to understand the progression of the diseases.

BD and MDD are heterogeneous disorders, encompassing bipolar disorder types I and II, melancholic depression psychotic depression and various comorbidities such as personality disorders and Attention-Deficit/Hyperactivity Disorder (ADHD). To include populations with diverse characteristics, aiming to ensure applicability and generalizability of the expected results, the study employs only a few exclusion criteria. Consequently, the findings will reflect the biological variability present in real-life heterogenous populations. However, this extensive heterogeneity in the study population may reduce the discriminatory power of the biomarker analyses and their results.

Risk and benefit

Participants benefit from a thorough diagnostic evaluation, including a MINI interview, by participating in the study. Since participants' diagnoses are established according to the highest clinical standards before any procedures related to the clinical study, the foreseeable risk associated with participating in the research is negligible. Moreover, sample extraction is akin to a routine blood test, and no adverse effects are anticipated.

From a society perspective, the EDIT-B clinical study will contribute to the development of innovative solutions that lead to earlier and personalized management of patients with mood disorders, including BD and MDD, by improving the diagnostic accuracy and differential diagnosis of BD. Based on a blood test complementary to clinical evaluation, the treatment will be more effective, and the risk of antidepressant-induced mania [52] may be diminished. This may also improve the definition of mixed states with MDE 53, reducing hospitalizations and costs. Given the urgent need for accurate diagnostic methods for the earlier management of patients, this study will contribute to the development of innovative solutions for timely diagnosis and appropriate treatment, reducing avoidable side effects and hospitalization rates and completely improving patient outcomes. Moreover, the use of EDIT-B will reduce the economic burden and optimize the use of medical and financial resources.

Abbreviations

BD	Bipolar disorder
MDD	Major depressive disorder
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
AUC-ROC	Area under the receiver operating characteristic curve
MDE	Major depressive episode
MINI	Mini-International Neuropsychiatric Interview
DSM-5	Diagnostic and Statistical Manual of Mental Disorders 5
MADRS	Montgomery–Åsberg Depression Rating Scale
YMRS	Young Mania Rating Scale
EQ-5D	European Quality of Life 5 Dimensions Questionnaire
MDQ	Mood Disorder Questionnaire
HCI-32-R1	32-Item Hypomania symptom check-list, first revision
CRA	Clinical research assistant
ADHD	Attention-Deficit/Hyperactivity Disorder

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Author contributions

AM-M and DV conceptualized the paper and prepared and reviewed the original draft and the final version. JZ, JZP, VC, AG-P, MG-C, MV, LWB, SS, MF, DW, CH, JMH, LVK and EV provided information for the Introduction and Discussion sections and provided suggestions for changes and improvements throughout the manuscript. All authors approved the submitted version.

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Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Ethical approval has been obtained in France by the research ethics committee Ile-de-France X (ID 2021-A-03194-37), in Spain by the research ethics committees of the Hospital Clinic of Barcelona (ID HCB/2022/0042) and the Sant Joan de Déu Foundation (PS-05-22) and in Denmark by the De Videnskabsetiske Komiteer for Region Hovedstaden (the Scientific Ethics Committees for the Capital Region of Denmark) (ID H-22010899).

Consent for publication
Not applicable.

Competing interests
Alcediag is an innovative diagnostic company that focuses on mental health, developing and commercializing innovative in vitro diagnostic tests. Alcediag has developed the EDIT-B test, which is based on proprietary RNA editing biomarkers. DW is the CSO of Alcediag. EV has received grants and served as consultant, advisor or CME speaker for the following entities outside the submitted work: AB-Biotics, AbbVie, Adamed, Angelini, Biogen, Beckley-Psytech, Biohaven, Boehringer-Ingelheim, Celon Pharma, Compass, Dainippon Sumitomo Pharma, Ethypharm, Ferrer, Gedeon Richter, GH Research, Glaxo-Smith Kline, HMNC, Idorsia, Johnson & Johnson, Lundbeck, Luye Pharma, Medincell, Merck, Newron, Novartis, Orion Corporation, Organon, Otsuka, Roche, Rovi, Sage, Sanofi-Aventis, Sunovion, Takeda, Teva, and Viatris. AG-P has received CME-related honoraria or consulting fees from Janssen-Cilag, Lundbeck, Casen Recordati, the LCN, Rovi and Angelini, outside the submitted work. JZP has received honoraria from Lundbeck Pharma A/S within the last three years, outside the submitted work. LVK has received honoraria from Lundbeck Pharma and Teva within the last three years, outside the submitted work.

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References

1. Hirschfeld RM. Differential diagnosis of bipolar disorder and major depressive disorder. J Affect Disord. 2014;169(S1):S12–6.
2. Vieta E, Salagre E, Grande I, Carvalho AF, Fernandes BS, Berk M, et al. Early intervention in bipolar disorder. Am J Psychiatry. 2018;175(5):411–26.
3. Bhardwaj S, Sinha D, Pawar A, Mane A. Predominant polarity in bipolar affective disorder and its impact on cognition and quality of life. Indian J Psychiatry. 2023;65(6):641–6.
4. Benacek J, Martin-Key NA, Spadaro B, Tomasik J, Bahn S. Using decision-analysis modelling to estimate the economic impact of the identification of unrecognised bipolar disorder in primary care: the untapped potential of screening. Int J Bipolar Disord. 2022;10:15.
5. Sletved KSO, Ziersen SC, Andersen PK, Vinberg M, Kessing LV. Socio-economic functioning in patients with bipolar disorder and their unaffected siblings—results from a nation-wide population-based longitudinal study. Psychol Med. 2023;53(3):706–13.
6. Kessing LV. Diagnostic stability in bipolar disorder in clinical practise as according to ICD-10. J Affect Disord. 2005;85(3):293–9.
7. Fritz K, Russell AMT, Allwang C, Kuiper S, Lampe L, Malhi GS. Is a delay in the diagnosis of bipolar disorder inevitable? Bipolar Disord. 2017;19(5):396–400.
8. Rosa AR, Andreazza AC, Kunz M, Gomes F, Santin A, Sanchez-Moreno J, et al. Predominant polarity in bipolar disorder: diagnostic implications. J Affect Disord. 2008;107(1–3):45–51.
9. Ratheesh A, Hammond D, Watson M, Betts J, Siegel E, McGorry P, et al. Bipolar at-risk criteria and risk of bipolar disorder over 10 or more years. JAMA Netw Open. 2023;6(9): e2334078.
10. Kessing L, Andersen P. Evidence for clinical progression of unipolar and bipolar disorders. Acta Psychiatr Scand. 2017;135(1):51–64.
11. Altamura AC, Buoli M, Caldiroli A, Caron L, Cumerlato Melter C, Dobrea C, et al. Misdiagnosis, duration of untreated illness (DUI) and outcome in bipolar patients with psychotic symptoms: a naturalistic study. J Affect Disord. 2015;182:70–5.
12. Altamura AC, Dell'Osso B, Berlin HA, Buoli M, Bassetti R, Mundo E. Duration of untreated illness and suicide in bipolar disorder: a naturalistic study. Eur Arch Psychiatry Clin Neurosci. 2010;260(5):385–91.
13. Bessonova L, Ogden K, Doane MJ, O'sullivan AK, Tohen M. The economic burden of bipolar disorder in the United States: a systematic literature review. Clinicecon Outcomes Res. 2020;12:481–97.
14. Sowa-Kućma M, Styczeń K, Siwek M, Misztak P, Nowak RJ, Dudek D, et al. Lipid peroxidation and immune biomarkers are associated with major depression and its phenotypes, including treatment-resistant depression and melancholia. Neurotox Res. 2018;33(2):448–60.
15. Poletti S, Mazza MG, Benedetti F. Inflammatory mediators in major depression and bipolar disorder. Transl Psychiatry. 2024;14:247.
16. Wu YL, Lin ZJ, Li CC, Lin X, Shan SK, Guo B, et al. Epigenetic regulation in metabolic diseases: mechanisms and advances in clinical study. Signal Transduct Target Ther. 2023;8(1):98.
17. Abdolmaleky HM, Zhou JR, Thiagalingam S. Cataloging recent advances in epigenetic alterations in major mental disorders and autism. Epigenomics. 2021;13(15):1231–45.
18. Abdolmaleky HM, Zhou J-R, Thiagalingam S. An update on the epigenetics of psychotic diseases and autism. Epigenomics. 2015;7(3):427–49.
19. Weissmann D, van der Laan S, Underwood MD, Salvetat N, Cavarec L, Vincent L, et al. Region-specific alterations of A-to-I RNA editing of serotonin 2c receptor in the cortex of suicides with major depression. Transl Psychiatry. 2016;6(8):e878–9.
20. Chimienti F, Cavarec L, Vincent L, Salvetat N, Arango V, Underwood MD, et al. Brain region-specific alterations of RNA editing in PDE8A mRNA in suicide decedents. Transl Psychiatry. 2019;9(1):91.
21. Ansell BRE, Thomas SN, Bonelli R, Munro JE, Freytag S, Bahlo M. A survey of RNA editing at single-cell resolution links interneurons to schizophrenia and autism. RNA. 2021;27(12):1482–96.
22. Hayashi MAF, Salvetat N, Cayzac C, Checa-Robles FJ, Dubuc B, Mereuze S, et al. Euthymic and depressed bipolar patients are characterized by different RNA editing patterns in blood. Psychiatry Res. 2023;328:3–6.
23. Barbon A, Magri C. RNA editing and modifications in mood disorders. Genes. 2020;11(8):872.
24. Salvetat N, Van der Laan S, Vire B, Chimienti F, Cleophax S, Bronowicki JP, et al. RNA editing blood biomarkers for predicting mood alterations in HCV patients. J Neurovirol. 2019;25(6):825–36.
25. Salvetat N, Checa-Robles FJ, Delacrétaz A, Cayzac C, Dubuc B, Vetter D, et al. AI algorithm combined with RNA editing-based blood biomarkers to discriminate bipolar from major depressive disorders in an external validation multicentric cohort. J Affect Disord. 2024;356:385–93.

26. Chan AW, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin JA, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. BMJ. 2013;346:1–42.
27. Sheehan D, Lecrubier Y, Sheehan K, Amorim P, Janavs J, Weiller E, 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.
28. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. Br J Psychiatry. 1979;134(4):382–9.
29. Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: reliability, validity and sensitivity. Br J Psychiatry. 1978;133(11):429–35.
30. Salvetat N, Checa-Robles FJ, Patel V, Cayzac C, Dubuc B, Chimienti F, et al. A game changer for bipolar disorder diagnosis using RNA editing-based biomarkers. Transl Psychiatry. 2022;12(1):1–10.
31. Janssen MF, Pickard AS, Golicki D, Gudex C, Niewada M, Scalone L, et al. Measurement properties of the EQ-5D-5L compared to the EQ-5D-3L across eight patient groups: a multi-country study. Qual Life Res. 2013;22(7):1717–27.
32. Fenn Buderer NM. Statistical methodology: I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. Acad Emerg Med. 1996;3(9):895–900.
33. Jones SR, Carley S, Harrison M. An introduction to power and sample size estimation. Emerg Med J. 2003;20(5):453–8.
34. Hawass NED. Comparing the sensitivities and specificities of two diagnostic procedures performed on the same group of patients. Br J Radiol. 1997;70:360–6.
35. Bicego M, Mensi A. Null/No Information Rate (NIR): a statistical test to assess if a classification accuracy is significant for a given problem. arXiv preprint arXiv:2306.06140. 2023.
36. Paluszynska A, Biecek P, Jiang Y. Explaining and visualizing random forests in terms of variable importance. R package version 0.10.1. 2022. https://CRAN.R-project.org/package=randomForestExplainer.
37. Fusar-Poli P, Manchia M, Koutsouleris N, Leslie D, Woopen C, Calkins ME, et al. Ethical considerations for precision psychiatry: a roadmap for research and clinical practice. Eur Neuropsychopharmacol. 2022;63:17–34.
38. Freedman R, Lewis DA, Michels R, Pine DS, Schultz SK, Tamminga CA, et al. The initial field trials of DSM-5: new blooms and old thorns. Am J Psychiatry. 2013;170(1):1–5.
39. Hirschfeld RMA, Williams JBW, Spitzer R, Calabrese J, Flynn L, Keck P, et al. Development and validation of a screening instrument for bipolar spectrum disorder: the Mood Disorder Questionnaire. Am J Psychiatry. 2000;157:1873–5.
40. Sasdelli A, Lia L, Luciano CC, Nespeca C, Berardi D, Menchetti M. Screening for bipolar disorder symptoms in depressed primary care attenders: comparison between Mood Disorder Questionnaire and Hypomania Checklist (HCL-32). Psychiatry J. 2013;2013:1–9.
41. Miller CJ, Klugman J, Berv DA, Rosenquist KJ, Ghaemi SN. Sensitivity and specificity of the Mood Disorder Questionnaire for detecting bipolar disorder. J Affect Disord. 2004;81(2):167–71.
42. Leão IAT, Del Porto JA. Cross validation with the mood disorder questionnaire (MDQ) of an instrument for the detection of hypomania in Brazil: the 32 item hypomania symptom check-list, first revision (HCI-32-R1). J Affect Disord. 2012;140(3):215–21.
43. Hirschfeld RM. Bipolar spectrum disorder: improving its recognition and diagnosis. J Clin Psychiatry. 2001;62(Suppl 1):5–9.
44. Tomasik J, Harrison SJ, Rustogi N, Olmert T, Barton-Owen G, Han SYS, et al. Metabolomic biomarker signatures for bipolar and unipolar depression. JAMA Psychiat. 2024;81(1):101–6.
45. Zhu Y, Wu X, Liu H, Niu Z, Zhao J, Wang F, et al. Employing biochemical biomarkers for building decision tree models to predict bipolar disorder from major depressive disorder. J Affect Disord. 2022;308:190–8.
46. Vismara M, Girone N, Cirnigliaro G, Fasciana F, Vanzetto S, Ferrara L, et al. Peripheral biomarkers in DSM-5 anxiety disorders: an updated overview. Brain Sci. 2020;10(8):1–37.
47. Kessing LV, Munkholm K, Faurholt-Jepsen M, Miskowiak KW, Nielsen LB, Frikke-Schmidt R, et al. The bipolar illness onset study: research protocol for the BIO cohort study. BMJ Open. 2017;7(6): e015462.
48. Samalin L, Belzeaux R. Why does non-adherence to treatment remain a leading cause of relapse in patients with bipolar disorder? Eur Neuropsychopharmacol. 2023;73:16–8.
49. Cuthbert BN, Insel TR. Toward the future of psychiatric diagnosis: the seven pillars of RDoC. BMC Med. 2013;11:126.
50. Decker K, Murata S, Baig N, Hasan S, Halaris A. Utilizing the systemic immune-inflammation index and blood-based biomarkers in association with treatment responsiveness amongst patients with treatment-resistant bipolar depression. J Pers Med. 2023;13(8):1–23.
51. Feczko E, Miranda-Dominguez O, Marr M, Graham AM, Nigg JT, Fair DA. The heterogeneity problem: approaches to identify psychiatric subtypes. Trends Cogn Sci. 2019;23(7):584–601.
52. Hsu J-W, Chen L-C, Tsai S-J, Huang K-L, Bai Y-M, Su T-P, et al. Disease progression to bipolar disorder among adolescents and young adults with antidepressant-resistant and antidepressant-responsive depression: does antidepressant class matter? Eur Neuropsychopharmacol. 2023;74:22–9.
53. Fagiolini A, Cuomo A. Treating major depressive disorder with mixed features. Eur Neuropsychopharmacol. 2023;69:58–9.

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