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Original Article

Multivariate Hematological Dispersion in Bipolar Disorder: A Mahalanobis Distance-Based Approach to Routine Blood Cell Profiles

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Abstract

Background: Complete blood count (CBC)-derived inflammatory indices have been widely investigated in bipolar disorder, but they provide limited information about the joint organization of correlated blood cell populations. We examined whether covariance-aware multivariate characterization of routine hematological data captures information beyond conventional inflammatory ratios. **Methods:** We conducted a retrospective secondary analysis of 92 female psychiatric inpatients (35 with bipolar disorder and 57 with non-bipolar psychiatric disorders). Multivariate hematological dispersion was quantified using cross-fitted squared Mahalanobis distance based on neutrophil, lymphocyte, monocyte, and platelet counts. Between-group differences were assessed using Welch's *t*-test and sensitivity analyses, with false discovery rate correction for multiple hematological comparisons. Exploratory receiver operating characteristic analysis and logistic regression were also performed. **Results:** Conventional hematological indices showed no statistically significant between-group differences. With reference-comparable scoring, mean squared Mahalanobis distance (MHD^2) was 7.583 in bipolar patients and 4.445 in non-bipolar patients (Welch $p = 0.0473$; Mann–Whitney $p = 0.2246$). The nominal difference did not survive adjustment for eight hematological comparisons (false discovery rate [FDR] q = 0.3781). Discrimination was limited (area under the curve (AUC) approximately 0.576). An exploratory age-adjusted model yielded an odds ratio (OR) of 1.87 per 1-standard deviation (SD) increase (95% confidence interval [CI] 1.03–3.37; $p = 0.038$); this association was sensitive to additional covariate adjustment. **Conclusions:** Covariance-aware analysis suggested an exploratory difference in multivariate hematological configuration between bipolar and other psychiatric inpatients. The evidence was sensitive to analytic assumptions, did not survive multiplicity adjustment, and does not establish a clinically useful diagnostic biomarker. Independent validation is required.

Keywords: bipolar disorder; Mahalanobis distance; hematology; inflammation; biomarkers

Main Points

1. Conventional blood cell counts and complete blood count (CBC)-derived inflammatory ratios did not significantly differentiate bipolar from non-bipolar psychiatric inpatients in this female cohort.

2. In the corrected reference-comparable analysis, mean squared Mahalanobis distance (MHD^2) was nominally higher in bipolar disorder, but the difference was not supported by the Mann–Whitney test or multiple-testing correction.

3. Cross-fitted MHD^2 demonstrated limited diagnostic discrimination, with an area under the curve (AUC) of approximately 0.576; the framework remains exploratory.

4. An exploratory age-adjusted association was observed, but sensitivity to clinical covariates precludes claims of independent prediction.

5. MHD^2 is an exploratory analytical measure and requires independent external validation with a prespecified reference structure.

1. Introduction

Bipolar disorder is increasingly recognized as a multisystem disorder in which immune, metabolic, and neurobiological alterations interact across mood states rather than representing isolated consequences of affective episodes. Accumulating evidence indicates that bipolar disorder is associated with chronic low-grade inflammation, altered cytokine signaling, an increased burden of inflammation-related medical comorbidities, and state-dependent immune activation during mood episodes [1]. More recent work has emphasized that immune-inflammatory abnormalities in mood disorders are highly heterogeneous, thereby limiting the diagnostic utility of any single inflammatory marker [2,3,4,5].

Complete blood count (CBC) parameters are inexpensive, routinely available, and therefore attractive candidates for investigating peripheral inflammatory processes in mood disorders. Consequently, CBC-derived inflammatory ratios, including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR), have been widely investigated.

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Meta-analytic evidence suggests that NLR and PLR may reflect inflammatory activation in mood disorders; however, reported effect sizes are generally modest, and substantial between-study heterogeneity remains [6,7].

Despite extensive investigation of CBC-derived inflammatory ratios, no consensus hematological biomarker has emerged for bipolar disorder. Their discriminative performance has generally remained modest, and different studies have identified inconsistent inflammatory signatures across patient populations. These observations suggest that the principal limitation may reside not only in the biomarkers themselves but also in the analytical framework through which hematological information is interpreted. Rather than producing isolated abnormalities in individual blood cell populations, bipolar disorder may be characterized by alterations in the coordinated organization of multiple hematological components. Consequently, clinically relevant disease-related information may be better captured by multivariate patterns of hematological organization than by any individual biomarker or pairwise inflammatory ratio [8,9].

Conventional hematological indices are inherently reductionist because they summarize immune-inflammatory biology using isolated cell counts or pairwise ratios. This approach implicitly assumes that disease-related immune alterations are primarily expressed as mean-level shifts in individual parameters. However, neutrophils, lymphocytes, monocytes, and platelets are not independent biological entities; they operate within a coordinated hematopoietic and inflammatory network. Accordingly, analytical approaches capable of quantifying multivariate hematological organization may capture biologically meaningful information that is not accessible through conventional univariate or ratio-based biomarkers [10].

This conceptual shift is particularly relevant to bipolar disorder, which is characterized by marked biological and clinical heterogeneity. Emerging research has begun to move beyond single biomarkers toward immune profiles, inflammatory biotypes, and multivariate biological signatures. For example, peripheral inflammatory biotypes have been used to identify biologically meaningful subgroups of bipolar depression and to explore heterogeneity in response to adjunctive anti-inflammatory treatment strategies [11]. Collectively, these developments support a transition from isolated biomarker analyses toward systems-oriented biological characterization in psychiatry [12,13].

Multivariate distance-based approaches provide a statistical framework for quantifying such biological organization. Mahalanobis distance measures how far an individual's multivariate profile deviates from a reference profile while accounting for the covariance structure among variables. By capturing coordinated deviations across multiple biological dimensions rather than isolated abnormalities, it is particularly well suited to biologically heterogeneous disorders such as bipolar disorder. In medical biomarker research, Mahalanobis distance has been proposed as a statistical proxy for physiological dysregulation and loss of homeostatic organization across circulating biomarkers [14]. This systems-oriented framework also aligns with the broader goals of precision psychiatry by evaluating whether multivariate biological organization provides clinically relevant information beyond conventional hematological biomarkers [15,16,17,18].

The present study was conducted as a secondary analysis of a psychiatric inpatient cohort originally established to investigate sleep-wake disturbances and inflammatory biomarkers. The cohort comprised 92 psychiatric inpatients who underwent standardized assessments of sleep disturbances together with measurement of CBC-derived inflammatory indices, including NLR, PLR, and MLR. In the present analysis, we used four routinely available hematological parameters—neutrophil, lymphocyte, monocyte, and platelet counts—to test whether bipolar disorder is associated with increased multivariate hematological dispersion rather than isolated alterations in conventional inflammatory ratios [19,20].

We hypothesized that bipolar disorder might be associated with greater mean covariance-adjusted hematological deviation from a psychiatric comparison reference, rather than necessarily greater within-group dispersion.

2. Materials and Methods

2.1 Study Design and Participants

This retrospective secondary analysis used data from an existing psychiatric inpatient cohort originally established to investigate sleep–wake disturbances and hematological inflammatory markers. The present analysis focused on the multivariate configuration of routinely measured blood cell counts.

Because the study center maintains a dedicated female psychiatric inpatient ward, whereas male patients requiring hospitalization are routinely referred to neighboring hospitals, all participants were women. The female-only sample therefore reflects the routine organization of psychiatric inpatient services during the study period rather than an intentional sex restriction.

Eligible participants were aged ≥18 years, hospitalized in the psychiatric inpatient unit, underwent routine complete blood count testing within 72–96 hours after admission, and completed the standardized clinical assessments performed during hospitalization. Patients with acute infection, autoimmune or inflammatory disease, hematological disorders, active malignancy, pregnancy, or incomplete laboratory data were excluded.

The final cohort comprised 92 female psychiatric inpatients: 35 with bipolar disorder and 57 with other psychiatric disorders (27 with schizophrenia, 16 with schizoaffective disorder, and 14 with major depressive disorder). Patients underwent inpatient clinical assessment and differential diagnostic evaluation; diagnostic classification was

based on the final clinical diagnosis established according to Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria rather than the provisional admission diagnosis.

2.2 Clinical Assessments

All participants underwent standardized psychiatric and sleep evaluations between 72 and 96 hours after admission as part of the original study protocol. The assessment battery included the Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), Insomnia Severity Index (ISI), Biological Rhythms Interview of Assessment in Neuropsychiatry (BRIAN), and Iowa Sleep Experiences Survey (ISES). PSQI, ESS, and ISI were repeated immediately before discharge to assess clinical change.

Because the present investigation represents a secondary analysis, sleep-related variables were not considered primary outcomes and were used only to characterize the original cohort.

2.3 Hematological Measurements

Peripheral venous blood samples were obtained as part of routine clinical care within 72–96 hours after hospital admission. Medication exposure and other clinical factors may have influenced hematological measurements; the sampling interval alone cannot exclude treatment-related effects.

Complete blood count analysis included: neutrophil count, lymphocyte count, monocyte count, platelet count.

Conventional inflammatory indices including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR) were available from the original dataset and were used for descriptive comparison with the proposed multivariate framework.

2.4 Multivariate Hematological Dispersion Framework

Each participant was represented by a four-dimensional hematological vector comprising neutrophil, lymphocyte, monocyte, and platelet counts. Multivariate hematological dispersion was operationalized using squared Mahalanobis distance (MHD^2; D^2) from the non-bipolar psychiatric reference distribution. To avoid comparing reference-group participants scored in sample with bipolar participants scored out of sample, the primary corrected analysis used leave-one-out reference estimation for each non-bipolar participant: the centroid and covariance matrix were calculated from the other 56 non-bipolar participants. Bipolar participants were evaluated against the reference fitted using all 57 non-bipolar participants. The original full-reference calculation was retained only as an apparent descriptive analysis.

For each participant, $D^2 = (x - \mu)^T\Sigma^{-1}(x - \mu)$, where x is the four-dimensional hematological vector and μ and Σ are the applicable non-bipolar reference centroid and covariance matrix. The reference is psychiatric and sample-derived, not a healthy or normative distribution. MHD^2 therefore represents individual covariance-adjusted distance from a specified reference profile; it does not directly estimate within-group dispersion.

Higher MHD^2 values indicate greater covariance-adjusted multivariate distance from the hematological reference profile of the non-bipolar psychiatric comparison group. Importantly, this reference is group-derived and is neither a healthy nor a normative reference. Accordingly, MHD^2 is interpreted here as an exploratory measure of multivariate hematological configuration relative to the covariance structure observed in the non-bipolar psychiatric comparison group, rather than as a measure of physiological abnormality or a stand-alone diagnostic score. This distinction is central to interpretation of the present proof-of-concept analysis. A conceptual overview is presented in Fig. 1.

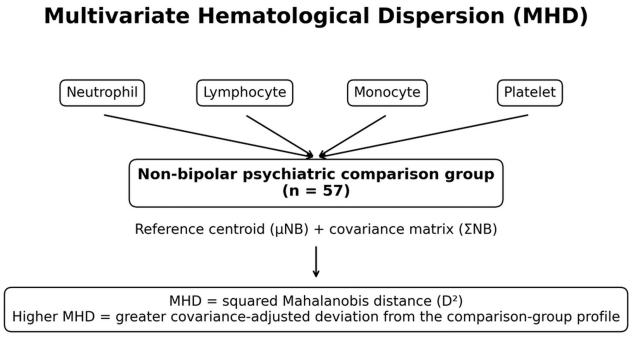

Fig. 1. Conceptual framework of multivariate hematological dispersion. Squared Mahalanobis distance (MHD^2) is calculated from neutrophil, lymphocyte, monocyte, and platelet counts. In the corrected primary analysis, the non-bipolar psychiatric comparison group (n = 57) defines the reference centroid and covariance structure. Each participant's MHD^2 represents the squared Mahalanobis distance (D^2) from this comparison-group-derived multivariate hematological profile. Higher MHD^2 values indicate greater covariance-adjusted multivariate deviation. The reference is psychiatric and sample-derived, not a healthy or normative reference.

2.5 Statistical Analysis

All 92 participants had complete data for the four cell counts used to construct MHD^2 and for bipolar-group assignment. Analyses of additional clinical covariates were conducted on available observations; no imputation was performed.

Continuous variables were summarized as mean ± standard deviation (SD) or median (interquartile range [IQR]), whereas categorical variables are presented as frequencies and percentages. Normality was assessed using

the Shapiro–Wilk test together with visual inspection of histograms and Q–Q plots. Between-group comparisons were performed using independent-samples *t* test or Welch *t* test for normally distributed variables and the Mann–Whitney U test for non-normally distributed variables. Categorical variables were compared using Pearson's χ^2 test or Fisher's exact test, as appropriate.

The primary estimand was the between-group mean difference in cross-fitted squared Mahalanobis distance (MHD^2). The primary nominal comparison used Welch's *t* test; Mann–Whitney, median/IQR, Levene, robust MCD and outlier checks were sensitivity analyses. Eight exploratory hematological comparisons were adjusted using Benjamini–Hochberg false discovery rate (FDR); none survived correction. All analyses were post hoc secondary analyses rather than prespecified diagnostic validation.

Exploratory discrimination was summarized using same-cohort receiver operating characteristic (ROC) AUCs and stratified nonparametric bootstrap confidence intervals (3000 resamples; random seed 20261023), conditional on the estimated reference scores. These intervals do not account for external reference-estimation uncertainty and are not external validation.

MHD^2 was compared descriptively with NLR, PLR, and MLR; paired DeLong comparisons were used to assess correlated AUC differences. Because the reference profile was derived from the same cohort, apparent AUCs are exploratory and may be optimistic. No independent external validation was available.

Exploratory logistic regression evaluated the association between cross-fitted MHD^2 (per one-standard-deviation increase) and bipolar-group membership, with age adjustment and additional clinical covariates considered in separate sensitivity models. Associations are not interpreted as independent diagnostic prediction.

Sensitivity analyses used a Minimum Covariance Determinant (MCD) estimator (scikit-learn) with the default support fraction and an alternative exploratory support fraction of 0.75; each non-bipolar patient was scored against an MCD reference excluding that individual, and bipolar patients against the full non-bipolar MCD reference (random_state = 20261023). Results were additionally assessed with Mann–Whitney, a 20% trimmed-mean comparison, and an outlier-deletion analysis. These were exploratory checks rather than independent confirmation.

Exploratory Spearman correlations assessed MHD^2 associations with the four constituent cell counts and three derived ratios. Such correlations are mathematically dependent on the inputs used to construct MHD^2 and do not provide independent biological validation.

3. Results

3.1 Participant Characteristics

The analytic cohort comprised 92 female psychiatric inpatients, including 35 with bipolar disorder and 57 with other psychiatric disorders. The comparison group was reported as schizophrenia (n = 27), schizoaffective disorder (n = 16), and major depressive disorder (n = 14). Diagnoses reflected final inpatient clinical assessment according to DSM-5 criteria following provisional International Classification of Diseases (ICD)-coded admission diagnoses. Demographic characteristics are summarized in Table 1.

3.2 Conventional Hematological Markers

No individual blood-cell count or conventional ratio showed a nominally significant between-group difference (all Welch $p > 0.15$). The eight exploratory hematological comparisons, including MHD^2, did not remain significant after Benjamini–Hochberg correction (minimum q = 0.378). The between-group hematological comparisons are summarized in Table 2.

3.3 Multivariate Hematological Dispersion

Mean cross-fitted MHD^2 was 7.58 ± 8.75 in the bipolar group and 4.44 ± 3.02 in psychiatric comparators; medians (IQRs) were 4.33 (2.61–9.38) and 4.01 (2.55–5.31), respectively. The mean difference was 3.14 (conditional bootstrap 95% confidence interval (CI) 0.41–6.35); Welch $p = 0.0473$, Mann–Whitney $p = 0.2246$, and median-centered Levene $p = 0.0054$. The nominal Welch association did not survive eight-test FDR correction (q = 0.378). Robust MCD results varied by support choice (**Supplementary Table 1**). The distributions of cross-fitted MHD^2 scores in the two diagnostic groups are shown in Fig. 2.

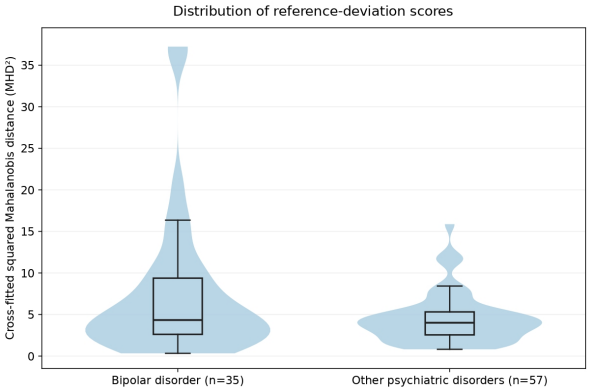

Fig. 2. Distribution of cross-fitted squared Mahalanobis distance (MHD^2) in bipolar disorder (n = 35) and other psychiatric inpatients (n = 57). Reference-group observations were scored using leave-one-out covariance estimates. Group means were 7.58 and 4.44; Welch $p = 0.0473$ and Mann–Whitney $p = 0.2246$. The distribution is right-skewed and heteroscedastic.

Median-centered Levene testing indicated heterogeneity of variance ($p = 0.0054$). Sensitivity to extreme observations and covariance estimation limits interpretation of the nominal mean difference.

Table 1. Demographic and clinical characteristics of the study sample.

Characteristic	Bipolar (n = 35)	Non-bipolar (n = 57)	Statistic	*p*
Age, years	41.6 ± 12.0	40.7 ± 12.4	Welch t = 0.32	0.750
Hospitalization duration, days	23 (20–28)	18 (12–30)	U = 1269.0	0.029
Diagnostic composition of the non-bipolar cohort				
Schizophrenia, n (%)	—	27 (47.4)	—	—
Schizoaffective disorder, n (%)	—	16 (28.1)	—	—
Major depressive disorder, n (%)	—	14 (24.6)	—	—

Note. Continuous values are presented as mean ± SD or median (IQR), as appropriate. Diagnostic subgroup counts reflect the reported final clinical DSM-5 classification. SD, standard deviation; IQR, interquartile range; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition.

Table 2. Conventional hematological biomarkers and multivariate hematological dispersion according to diagnosis.

Marker	Bipolar (n = 35), mean ± SD	Non-bipolar (n = 57), mean ± SD	Cohen's d	Welch's *p*	Mann–Whitney *p*	AUC
Neutrophil ($\times 10^9$/L)	4.733 ± 2.041	4.174 ± 1.390	0.336	0.158 (q = 0.416)	—	0.567
Lymphocyte ($\times 10^9$/L)	2.325 ± 0.886	2.105 ± 0.667	0.290	0.212 (q = 0.416)	—	0.563
Monocyte ($\times 10^9$/L)	0.473 ± 0.170	0.435 ± 0.134	0.259	0.260 (q = 0.416)	—	0.545
Platelet ($\times 10^9$/L)	265.429 ± 72.131	269.246 ± 56.799	–0.061	0.791 (q = 0.865)	—	0.483
NLR	2.248 ± 1.395	2.152 ± 0.958	0.084	0.721 (q = 0.865)	—	0.480
PLR	124.523 ± 49.400	138.095 ± 43.181	–0.297	0.185 (q = 0.416)	—	0.399
MLR	0.218 ± 0.082	0.221 ± 0.080	–0.037	0.865 (q = 0.865)	—	0.488
MHD^2	7.583 ± 8.749	4.445 ± 3.023	0.534	0.047 (q = 0.378)	0.225	0.576

Note. Values are mean ± SD. MHD^2 is the cross-fitted squared Mahalanobis distance relative to the non-bipolar psychiatric reference (leave-one-out reference for non-bipolar participants). Welch *p* values are nominal; eight-test Benjamini–Hochberg q values are shown. The MHD^2 Mann–Whitney *p* value is a sensitivity analysis. AUCs are same-cohort exploratory estimates and not externally validated diagnostic accuracy. NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; AUC, area under the ROC curve; ROC, Receiver operating characteristic.

Exploratory Spearman correlation analyses showed positive associations of cross-fitted MHD^2 with neutrophil ($\rho = 0.256$, $p = 0.0139$), lymphocyte ($\rho = 0.210$, $p = 0.0449$), and monocyte counts ($\rho = 0.354$, $p = 0.0005$), and a negative association with PLR ($\rho = -0.280$, $p = 0.0068$). No nominally significant correlations were observed with platelet count, NLR, or MLR (**Supplementary Table 2**). These correlations do not constitute independent validation because the MHD^2 calculation incorporates the underlying blood cell counts.

3.4 Discriminative Performance and Internal Stability

Cross-fitted MHD^2 yielded a same-cohort ROC AUC of 0.576 (conditional stratified bootstrap 95% CI 0.448–0.702); NLR, PLR, and MLR AUCs were 0.480, 0.399, and 0.488, respectively. Paired DeLong tests did not show significant superiority of MHD^2 over NLR, PLR, or MLR ($p = 0.263$, 0.077, and 0.265, respectively). The direction of PLR was not optimized post hoc. These results do not establish clinically useful discrimination. The corresponding exploratory ROC curves are presented in Fig. 3.

3.5 Multivariable Logistic Regression

In exploratory age-adjusted logistic regression, a one-standard-deviation increase in cross-fitted MHD^2 was associated with bipolar-group membership (OR = 1.87, 95% CI 1.03–3.37; $p = 0.038$). This association was not consistently statistically significant across additional clinical sensitivity models and should not be interpreted as an independently validated predictor. The age-adjusted logistic regression results are summarized in Table 3.

3.6 Effect Size

Standardized mean differences are displayed in Fig. 4. The cross-fitted MHD^2 effect size was Cohen's d = 0.534; all effect-size confidence intervals were obtained from 3000 stratified bootstrap resamples conditional on the calculated MHD^2 scores.

4. Discussion

The principal finding was a higher mean cross-fitted squared Mahalanobis distance (MHD^2) in women with bipolar disorder than in other psychiatric inpatients (Welch $p = 0.047$). This nominal mean difference was not supported by the Mann–Whitney test ($p = 0.225$) or by correction for the eight exploratory hematological comparisons (Benjamini–Hochberg q = 0.378). The distinction between a difference in mean reference deviation and a consistently shifted distribution is important: the findings suggest an exploratory group association rather than a robust diagnostic

Table 3. Multivariable logistic regression analysis for bipolar disorder.

Variable	Odds Ratio (OR)	95% CI	*p* value
Cross-fitted MHD^2 (per 1 SD)	1.867	1.035–3.368	0.038
Age (per 1-year increase)	1.004	0.969–1.041	0.818
Sample size	92		
Model purpose	Exploratory association		

Note. Bipolar disorder was the dependent variable (n = 92). MHD^2 was standardized across the analytic sample; the odds ratio corresponds to a one-SD increase in cross-fitted MHD^2. The age odds ratio is per one-year increase. Models are exploratory associations rather than diagnostic prediction. OR, odds ratio; CI, confidence interval.

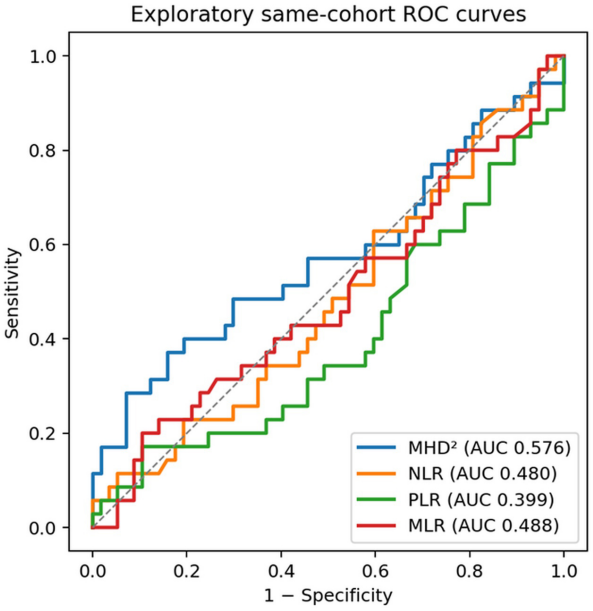

Fig. 3. Same-cohort exploratory ROC curves for cross-fitted MHD^2, NLR, PLR, and MLR (AUCs 0.576, 0.480, 0.399, and 0.488, respectively). PLR direction was retained as observed; reversing it would yield an AUC of 0.601 but would not constitute prespecified diagnostic performance. No external validation was performed.

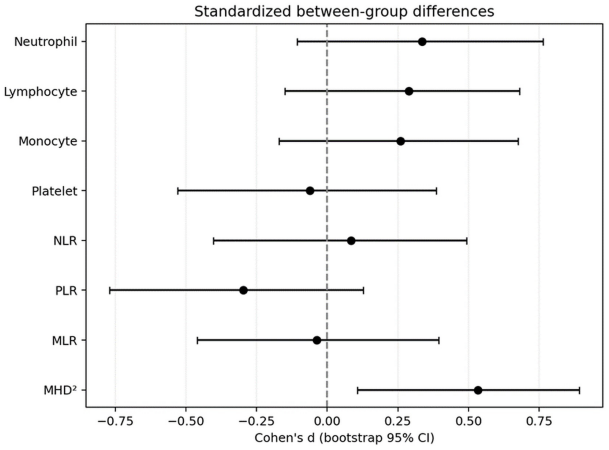

Fig. 4. Cohen's d standardized mean differences for four cell counts, three CBC-derived ratios, and cross-fitted MHD^2. Error bars show conditional stratified bootstrap 95% confidence intervals (3000 resamples; seed 20261023). Positive values indicate higher means in bipolar disorder. CBC, complete blood count.

signature. Discriminatory performance was limited (AUC = 0.576), and the age-adjusted association should be interpreted cautiously in this small sample.

This pattern may be compatible with biological heterogeneity in bipolar disorder, although it does not demonstrate a disorder-specific inflammatory mechanism. Unlike pairwise ratios, squared Mahalanobis distance summarizes the joint configuration of neutrophil, lymphocyte, monocyte, and platelet counts relative to a specified reference distribution. An elevated distance describes greater individual deviation from that reference; it does not, by itself, establish increased within-group dispersion or identify which biological pathway is responsible.

The findings do not establish that MHD^2 is superior to conventional inflammatory indices. Paired ROC comparisons with NLR, PLR, and MLR did not demonstrate statistically significant AUC differences (DeLong $p = 0.263$, 0.077, and 0.265, respectively). These measures answer related but distinct analytical questions, and any incremental clinical value of covariance-aware profiling remains unproven. CBC-derived indices have shown heterogeneous results across bipolar disorder studies, potentially reflecting mood state, medication exposure, medical comorbidity, age, sex, and comparator selection [7,21].

A systems-oriented interpretation is biologically plausible because circulating leukocyte and platelet populations are regulated through interconnected hematopoietic, cytokine, neuroendocrine, and inflammatory pathways [22,23]. Bipolar disorder itself has been conceptualized as a multisystem condition involving interactions among immune activation, oxidative stress, mitochondrial function, metabolism, and circadian regulation [24,25,26]. MHD^2 provides a statistical representation of joint hematological configuration; it does not establish the biological mechanism underlying that configuration.

The transdiagnostic comparison group (27 patients with schizophrenia, 16 with schizoaffective disorder, and 14 with major depressive disorder, as reported in the study cohort) provides a clinically relevant but heterogeneous reference. Shared inflammatory abnormalities across psychiatric disorders may attenuate contrasts with bipolar disorder, while diagnostic composition and differences in covariance structure may also affect distance-based comparisons. Subgroup estimates should therefore not be interpreted as validated disorder-specific effects in this modest sample.

From a translational perspective, the framework uses measurements already available in routine complete blood counts. Nevertheless, the weak apparent discrimination, lack of demonstrated superiority over conventional ratios, and sensitivity to analytical assumptions preclude individual-level diagnostic use. The current analysis is exploratory and hypothesis-generating rather than a diagnostic validation study.

The clinical relevance of a candidate biomarker framework depends on reproducibility, accessibility, and incremental information beyond existing measures. Although molecular, genetic, metabolomic, and neuroimaging approaches have advanced biological characterization in bipolar disorder, translation to routine practice remains limited by cost, availability, methodological complexity, and incomplete external validation [27,28].

Complete blood count testing is inexpensive and routinely obtained in psychiatric inpatient care. A covariance-aware approach to these existing measurements may therefore offer a pragmatic research framework for studying multivariate peripheral biology without additional laboratory procedures.

Accordingly, MHD^2 should be viewed as a complementary research measure rather than a replacement for conventional CBC-derived inflammatory indices. Its reproducibility should be tested with a prespecified external reference, prospectively collected clinical covariates, and independent patient cohorts before claims of clinical utility are considered.

4.1 Strengths

A strength of this study is the use of a transdiagnostic psychiatric inpatient comparison group rather than healthy controls. This permits an exploratory assessment of hematological differences against a clinically relevant psychiatric reference, although the comparator's heterogeneity also complicates interpretation and limits generalizability.

A further methodological contribution is the examination of four correlated blood cell counts in a covariance-aware framework, alongside conventional CBC-derived ratios. The revised primary analysis used leave-one-out reference scoring for non-bipolar participants, while bipolar participants were scored against the non-bipolar reference, reducing the asymmetric in-sample scoring bias identified in the original analysis. This correction improves internal comparability but does not constitute independent external validation.

Finally, the framework relies on routinely collected complete blood count measurements, which makes further investigation feasible without specialized assays. Feasibility, however, should not be confused with demonstrated diagnostic accuracy, clinical benefit, or cost-effectiveness.

4.2 Limitations

Several limitations should be considered when interpreting the present findings.

First, the retrospective and cross-sectional design precludes conclusions regarding causality or temporal relationships between multivariate hematological organization and bipolar disorder. Longitudinal studies are required to determine whether MHD^2 varies across different mood states, predicts relapse, or changes in response to treatment.

Second, this single-center study included 92 patients (35 with bipolar disorder and 57 psychiatric comparators). The modest sample size limits precision, particularly for sensitivity analyses, subgroup contrasts, and multivariable adjustment. No prospective sample-size calculation was undertaken for the present retrospective exploratory analysis; consequently, nonsignificant findings should not be interpreted as evidence of equivalence.

Third, the study included only female psychiatric inpatients because of the organization of inpatient services at the study center. This substantially limits generalizability to male patients and mixed-sex populations, and sex-specific replication is required.

Fourth, hematological measures may be influenced by obesity, smoking, metabolic disorders, infection, menstrual phase, and psychotropic treatment. Available covariates permitted only limited exploratory adjustments, and some associations were attenuated after inclusion of medication or admission-history variables. Detailed mood-episode characterization, lithium-specific exposure, medication dose and duration, and menstrual-phase information were not sufficiently available for comprehensive control of confounding. Residual confounding therefore remains a substantial concern.

Fifth, because this study relied exclusively on routinely available CBC parameters, inflammatory cytokines such as Interleukin-6 (IL-6), tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and C-reactive protein (CRP) were not simultaneously evaluated. Consequently, direct comparisons between MHD^2 and established molecular inflammatory biomarkers could not be performed.

Finally, although leave-one-out scoring removed the direct reference self-scoring asymmetry, all reference estimates originated from this single cohort. The results were sensitive to skewness, outliers, and MCD support specifications, and the Welch finding did not survive multiplicity correction. The MHD^2 AUC of 0.576 was imprecise; the conditional bootstrap interval included 0.50. The analysis

was not externally validated. Larger, prospectively characterized samples with prespecified reference structures are needed.

4.3 Future Directions

Future research should prospectively validate the MHD^2 calculation using an independent, prespecified reference cohort and assess its stability across diagnostic subgroups, sex, mood episodes, psychotropic regimens, and relevant somatic or menstrual factors. Larger samples should support robust covariance estimation, outlier-resistant analyses, and adequately powered assessment of incremental discrimination over conventional inflammatory ratios.

Future investigations should also examine MHD^2 alongside established inflammatory biomarkers, including C-reactive protein, interleukin-6, tumor necrosis factor-alpha, and other cytokines. Integration with clinical, neurocognitive, metabolic, imaging, genetic, or digital phenotyping measures may clarify whether covariance-aware hematological information contributes incremental value within multimodal psychiatric biomarker models.

5. Conclusions

In this exploratory sample of female psychiatric inpatients, cross-fitted MHD^2 was nominally higher on average in bipolar disorder than in other psychiatric diagnoses. However, the difference was not consistent across distributional and robust analyses, did not survive multiplicity adjustment, and showed limited discriminatory performance without demonstrated superiority over conventional CBC-derived ratios. Covariance-aware hematological profiling remains a research approach warranting independent replication, not a validated diagnostic biomarker.

Availability of Data and Materials

The data underlying this study are not publicly available because they contain potentially sensitive clinical information. De-identified data may be made available from the corresponding author upon reasonable request, subject to applicable ethical and institutional approvals.

Author Contributions

KSK: conceptualization, methodology, formal analysis, visualization, writing - original draft, writing - review and editing, supervision. ISG: investigation, data curation, writing - review and editing. BB: investigation, data curation, writing - review and editing. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The original study protocol was approved by the Recep Tayyip Erdoğan University Non-Interventional Clinical Research Ethics Committee on December 24, 2020 (Approval No. 2020/03). The present study represents a retrospective secondary analysis of anonymized data collected under the approved protocol. No additional informed consent was required for the present secondary analysis. The study was conducted in accordance with the Declaration of Helsinki.

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Conflicts of Interest

The authors declare no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During manuscript preparation, the authors used the ChatGPT to assist with language editing and manuscript formatting. The authors reviewed and edited the output and take full responsibility for the content of the manuscript.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/AP58110.

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