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Article

Serum Metabolomic Profiling Across Five Oligoclonal Band (OCB) Patterns: A Targeted ¹H-NMR Study in Serum

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Abstract

Cerebrospinal fluid (CSF) oligoclonal band (OCB) analysis is central to the diagnostic evaluation of neuroinflammatory diseases of the central nervous system (CNS), yet its reliance on lumbar puncture limits utility in screening and longitudinal monitoring. Serum metabolomics provides a minimally invasive approach to capture peripheral correlates of intrathecal immune activity. This study extends our previous two-group comparison by incorporating all five classical OCB patterns to delineate serum metabolic gradients associated with varying degrees of intrathecal immunoglobulin synthesis. A total of 92 adults undergoing diagnostic evaluation for suspected CNS inflammatory disorders were stratified by OCB Type (1–5). Serum samples were analysed using targeted ¹H-NMR spectroscopy on a Bruker Avance Neo 600 MHz platform and processed with Bruker’s IVDr pipeline. Statistical analyses included Kruskal–Wallis testing with FDR correction, PCA, PLS-DA with VIP scoring, and ROC-AUC modelling. Six metabolites exhibited significant or near-significant differences, led by Leucine (p = 0.0047, q = 0.073) and 2-Oxoglutaric acid (p = 0.0022, q = 0.069). PLS-DA identified five key discriminators with VIP > 1.5: Leucine, 2-Oxoglutaric acid, Histidine, Valine, and Glycine. A combined logistic model (Leucine + Histidine + Citric acid) achieved an AUC of 0.83 for distinguishing OCB Type 1 from Type 2. This first targeted serum ¹H-NMR metabolomic evaluation across all OCB patterns reveals a graded biochemical trajectory reflective of intrathecal immune activation. Amino-acid and TCA-cycle intermediates emerge as promising minimally invasive candidates for neuroinflammatory stratification and precision evaluation beyond traditional MS paradigms.

Keywords: metabolomics; oligoclonal bands; ¹H-NMR; neuroinflammation; multiple sclerosis; serum biomarkers; TCA cycle; amino acids

Introduction

Chronic neuroinflammatory diseases of the central nervous system (CNS) encompass a heterogeneous group of disorders characterised by immune-mediated demyelination, neurodegeneration, and compartmentalised humoural responses (Jin, Lu, & Gao, 2023). Among these conditions, detection of cerebrospinal fluid (CSF) immunoglobulin G (IgG) oligoclonal bands (OCBs) remains a cornerstone biomarker of intrathecal IgG synthesis—traditionally associated with multiple sclerosis (MS) but also observed in neuromyelitis optica spectrum disorder (NMOSD), neurosarcoidosis, neuro-Behçet’s disease, and other autoimmune or infectious CNS pathologies (Schwenkenbecher et al., 2022; Dobson et al., 2013). The identification of OCBs through isoelectric focusing and immunoblotting provides crucial diagnostic support, although lumbar puncture is invasive and not ideal for population screening or repeated longitudinal follow-up (Jin et al., 2023).

Recent diagnostic revisions, including the 2024 McDonald Criteria, have updated the biological framework of MS by formally incorporating the κ-free light chain (κ-FLC) index as an alternative to

classical OCB testing for documenting intrathecal Ig synthesis (Montalban et al., 2025; ECTRIMS, 2025a, 2025b). These updates underscore the growing importance of quantifiable, standardisable, and minimally invasive biomarkers that can reflect central immune activation even when imaging findings remain ambiguous. In this context, metabolomics has emerged as a promising analytical approach to capture dynamic biochemical changes associated with neuroinflammation and neurodegeneration (Smusz et al., 2025; Alwahsh et al., 2024).

Metabolomic profiling of serum and CSF has revealed disruptions in amino-acid metabolism, tricarboxylic-acid (TCA) cycle intermediates, lipid turnover, and oxidative stress pathways in MS and related disorders (Rzepiński et al., 2023). Amino acids such as leucine, histidine, and glycine play immunomodulatory roles: leucine acts as an mTOR activator controlling T-cell metabolism; histidine, via histamine, modulates microglial activation and neuronal excitability; and glycine contributes to redox homeostasis through glutathione synthesis (Huang et al., 2024; Carta et al., 2022). Likewise, succinate and 2-oxoglutarate are key TCA intermediates linked to macrophage polarisation and inflammatory signalling (Atallah et al., 2025). Collectively, these molecules constitute a metabolic interface between systemic immunity and CNS inflammation.

Previous work from our group using targeted ^1H -NMR metabolomics identified Leucine as a robust serum discriminator between OCB Type 1 (no intrathecal synthesis) and Type 2 (definite intrathecal synthesis) profiles (Şengül, Baykal, & Serteser, 2025). However, that study was limited to a binary comparison and did not capture the full diversity of OCB phenotypes. The five-type OCB classification—ranging from Type 1 (normal/identical serum-CSF pattern) through Type 2–3 (intrathecal synthesis) to Type 5 (monoclonal gammopathy)—represents a biological continuum rather than discrete states (Schwenkenbecher et al., 2022). Understanding how peripheral metabolite profiles vary across this immunophenotypic gradient could improve biomarker specificity and provide mechanistic insight into early CNS immune activation.

Therefore, the present study applies high-resolution ^1H -NMR spectroscopy to serum samples from 92 adults undergoing diagnostic evaluation for suspected CNS inflammatory disorders in Türkiye, stratified according to OCB Types 1–5. By integrating univariate, multivariate, and receiver-operating-characteristic analyses, we aim to (1) identify serum metabolites that significantly differ across OCB subtypes; (2) evaluate multivariate clustering patterns reflecting the degree of intrathecal IgG synthesis; and (3) assess their discriminatory potential as minimally invasive biomarkers for neuroinflammatory stratification. We hypothesise that amino-acid and TCA-cycle intermediates form a graded metabolic signature paralleling intrathecal immune activity, extending previous two-group observations toward a comprehensive immunometabolic map of the OCB spectrum.

Methods

Study Design and Participants

This retrospective observational study analysed 92 archived serum samples obtained from adults who underwent diagnostic evaluation for possible central nervous system (CNS) inflammatory disease at the Acıbadem Labmed Biobank (Istanbul, Türkiye). Samples were classified into five oligoclonal band (OCB) patterns (Types 1–5) based on routine isoelectric focusing and IgG immunoblotting results.

All samples were reviewed for pre-analytical integrity, including minimum serum volume, absence of haemolysis, absence of lipemia, and appropriate storage at $-80\text{ }^{\circ}\text{C}$ without previous thaw cycles.

Clinical metadata were retrieved from biobank records and anonymised before analysis.

Ethical Approval

The study was approved by the Acıbadem Mehmet Ali Aydınlar University Ethics Committee (Project No. 126). All procedures complied with the Declaration of Helsinki and institutional

biospecimen handling regulations. Because this study used anonymised archived material without patient identifiers, the requirement for informed consent was waived by the Ethics Committee.

Serum Sample Preparation for ^1H -NMR Spectroscopy

Fresh serum samples were prepared for NMR analysis by mixing 400 μL of serum with 400 μL of Bruker plasma buffer, consisting of 20% deuterium oxide (D_2O), 0.075 M sodium monophosphate, 4.6 mM 3-(trimethylsilyl)-2,2,3,3-tetradeuteriopropionate (TSP), and 0.04% sodium azide (NaN_3) (Bruker BioSpin GmbH, Ettlingen, Germany). The resulting 600 μL mixture was transferred into 5-mm SampleJet NMR tubes without further processing.

Quantitative serum metabolite and lipoprotein subclass profiling was performed using Bruker's validated IVDr platform, including the B.I.LISA® module for lipoprotein analysis and B.I.QUANT-PS® for targeted metabolite quantification. Absolute concentration referencing was achieved using the Electronic REference To access In vivo Concentrations (ERETIC) method.

NMR Data Acquisition

^1H -NMR spectra were acquired on a Bruker Avance Neo 600 MHz IVDr spectrometer equipped with a 5-mm BBI probe, the Bruker SampleJet automated sample changer, and TopSpin 4.3.0 software.

Spectral acquisition was conducted using the NOESY-presaturation pulse sequence (noesygppr1d) at 310 K. Acquisition parameters were:

- Number of scans: 32
- Spectral width: 30 ppm
- Dummy scans: 4
- Relaxation delay: 4 s
- Total acquisition time: 4 min 4 s

This workflow follows the standardized Bruker IVDr operating procedures for quantitative serum metabolomics.

Metabolite Identification and Quality Control

Metabolite identities were confirmed using Bruker chemical shift libraries, J-coupling patterns, and internal referencing to the TSP peak.

Spectra were screened for:

- residual water peak interference,
- baseline distortions,
- line broadening,
- contamination artefacts.

Only metabolites detected consistently in $\geq 95\%$ of samples with acceptable signal-to-noise ratios were retained. Outlier spectra were evaluated using PCA and excluded only if severe acquisition artefacts were detected.

Statistical Analysis

All analyses were conducted in R version 4.3.2.

- Normality was evaluated using the Shapiro–Wilk test.
- Group comparisons across OCB Types 1–5 used the Kruskal–Wallis test with Dunn post-hoc tests, adjusted with Benjamini–Hochberg FDR.
- Correlation analysis used Spearman's ρ .
- PCA (FactoMineR) and PLS-DA (mixOmics) were employed for multivariate pattern identification.
- Discriminatory metabolites were defined as $\text{VIP} > 1.5$.

- Cliff’s delta (δ) quantified effect sizes for pairwise contrasts.
- ROC curves and AUC values assessed classification performance using pROC.
- Figures were generated with ggplot2 and ComplexHeatmap.

Results

Participant Demographics

Table 1. Demographic and serum biochemistry characteristics stratified by OCB type.

OCB Type	n	Age	Female	Male	IgG index	IgG serum	IgG ratio	Albumin serum	Albumin ratio $\times 10^{-3}$
Type 1	24	44.0 $\pm$ 17.1	14	9	0.50 $\pm$ 0.05	1103 $\pm$ 265	2.67 $\pm$ 0.86	3622 $\pm$ 629	5.29 $\pm$ 1.52
Type 2	25	32.7 $\pm$ 12.2	17	8	0.95 $\pm$ 0.45	1131 $\pm$ 265	4.42 $\pm$ 3.09	4141 $\pm$ 373	4.51 $\pm$ 1.95
Type 3	21	46.6 $\pm$ 16.5	10	10	1.35 $\pm$ 1.12	1060 $\pm$ 352	19.31 $\pm$ 25.09	3679 $\pm$ 487	13.00 $\pm$ 7.80
Type 4	10	63.5 $\pm$ 10.3	3	7	0.57 $\pm$ 0.08	931 $\pm$ 279	7.35 $\pm$ 5.36	3376 $\pm$ 546	12.81 $\pm$ 8.64
Type 5	12	64.8 $\pm$ 10.1	4	8	0.52 $\pm$ 0.06	1123 $\pm$ 265	3.97 $\pm$ 2.00	3691 $\pm$ 455	7.68 $\pm$ 3.95

Values are presented as mean $\pm$ standard deviation. OCB: Oligoclonal bands.

Values represent pooled summary measures across all OCB groups. Mean, SD, median, interquartile range (IQR), and range (min–max) are shown for each metabolite.

Summary statistics for all quantified serum metabolites across the entire cohort are presented in Table 2.

Table 2. Summary statistics for all quantified metabolites.

Metabolite	Mean	SD	Median	IQR	Min–Max
2-OG	0.249	0.116	0.245	0.147	0.000–0.677
Citric_acid	0.091	0.079	0.076	0.121	0.000–0.282
Glycerol	0.462	0.424	0.424	0.583	0.000–2.095
Histidine	0.138	0.042	0.143	0.047	0.010–0.257

Leucine	0.169	0.060	0.161	0.078	0.000–0.403
Threonine	0.077	0.079	0.081	0.097	0.000–0.283

Group comparisons (Kruskal–Wallis and pairwise tests)
Pairwise Dunn tests with FDR correction are summarised in Table 3.

Table 3. Pairwise Dunn/Wilcoxon Comparisons Across OCB Types.

metabolite	Group 1	Group 2	n1	n2	statistic	p	p.adj	p.adj.sign if
2-OG	2	5	25	12	253.0	0.000882	0.009	**
Citric_acid	1	5	24	12	52.5	0.002	0.019	*
Glycerol	1	5	24	12	228.0	0.004	0.045	*
Histidine	1	2	24	25	154.5	0.004	0.037	*
Leucine	1	2	24	25	141.0	0.002	0.015	*
Leucine	2	5	25	12	241.0	0.003	0.017	*
Threonine	1	2	24	25	163.5	0.003	0.029	*

Univariate Analysis

Univariate comparisons across the five OCB-defined groups identified six metabolites—Leucine, 2-Oxoglutaric acid, Citric acid, Glycerol, Histidine, and Threonine—as showing statistically significant or trend-level differences according to the Kruskal–Wallis test (Table 2). None of the metabolites met normality or homoscedasticity assumptions; therefore, all analyses were performed using non-parametric methods with Benjamini–Hochberg correction.

Leucine demonstrated the strongest group effect ($p = 0.0047$, $q = 0.0733$), mainly driven by higher concentrations in Type 2 compared with Type 1 ($q = 0.015$) and Type 5 ($q = 0.017$).

2-Oxoglutaric acid similarly showed a significant elevation in Type 2 relative to Type 5 ($p = 0.0022$, $q = 0.0685$).

Citric acid and Glycerol exhibited modest but notable trends ($p = 0.0437$ and $p = 0.0792$), with both metabolites presenting higher levels in Type 5 relative to Type 1 ($q = 0.019$ and $q = 0.045$).

Histidine and Threonine also displayed group differentiation patterns, characterised by higher median values in Type 2 than in Type 1 ($q = 0.037$ and $q = 0.029$), although these differences did not remain significant across all pairwise contrasts after correction.

Collectively, these findings support the presence of a graded metabolic shift across OCB immunophenotypes, in which Type 2—the group exhibiting clear intrathecal IgG synthesis—shows the most distinct amino-acid- and TCA-cycle-related serum profile. The recurrent divergence in Type 1–2 and Type 2–5 comparisons suggests that metabolic variation reflects the degree of intrathecal immune activation, rather than general systemic immunoglobulin status.

Multivariate Analysis (PCA)

Principal component analysis (PCA) was performed on the complete serum metabolite dataset encompassing all five OCB patterns. The first ten components cumulatively explained 72.4% of the total variance, with PC1 accounting for 20.8% and PC2 for 8.9% (Figure 1). The scree plot showed a clear inflection after the first two components, supporting their dominance in capturing the major structure of the dataset.

The PC1–PC2 score plot demonstrated moderate clustering of samples by OCB type, with partial overlap among Types 1–4 and a visible displacement of Type 2 and Type 5 along PC1 (Figure 2A). This pattern indicates that a subset of amino-acid and TCA-cycle metabolites drives group differentiation, consistent with univariate findings for Leucine, 2-Oxoglutaric acid, and Citric acid.

The variable correlation circle showed that PC1 was primarily driven by branched-chain and aromatic amino acids—Valine, Leucine, Phenylalanine, Alanine, Histidine, and Isoleucine—along with Tyrosine and 2-Oxoglutaric acid (Figure 2B). PC2, by contrast, was influenced by metabolites related to redox and glycolytic balance, including 2-Aminobutyric acid, Acetic acid, Pyruvic acid, 2-Hydroxybutyric acid, Lactic acid, and Formic acid.

The PCA biplot (Figure 2C) further illustrated that Type 2 samples (intrathecal synthesis) occupy a metabolically distinct region aligned with higher Leucine, Histidine, and 2-Oxoglutaric acid loadings, whereas Type 5 (monoclonal pattern) shifts toward increased Citric acid and Glycerol contributions. Collectively, these multivariate patterns reinforce the notion that peripheral serum metabolomics reflects the graded intensity of intrathecal immune activation.

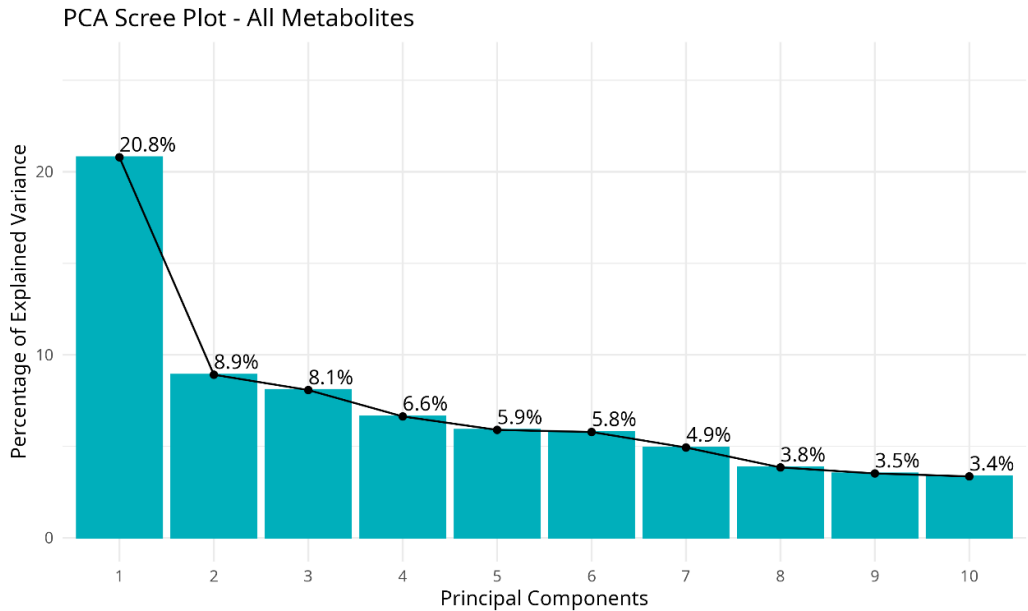


Figure 1. PCA Scree Plot. PCA scree plot showing eigenvalues and the proportion of variance explained by the first ten principal components. PC1 and PC2 together captured the dominant structure of the dataset, accounting for 29.7% of total variance.

Figure 2A.

PCA Score Plot (PC1 vs PC2) - All Metabolites

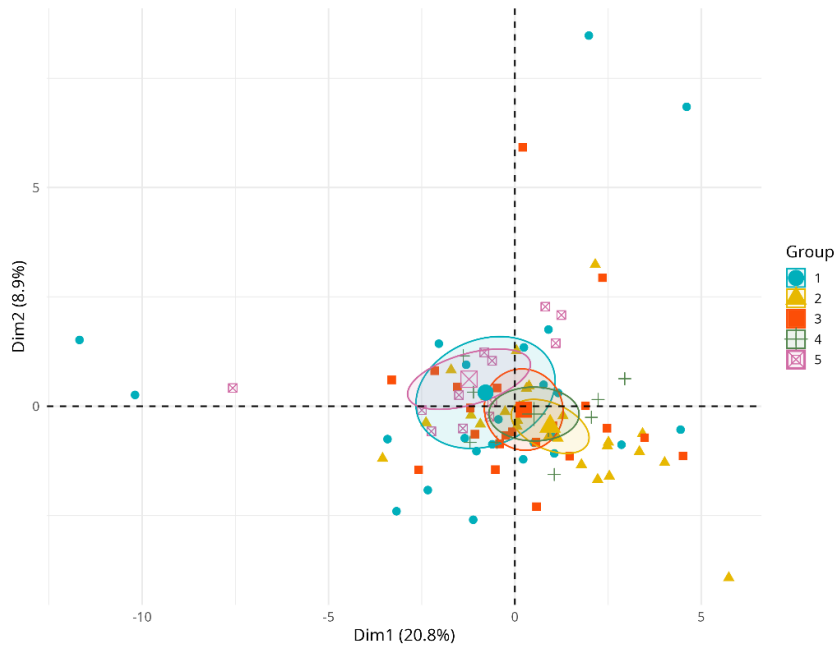


Figure 2B.

Variable Correlation Circle

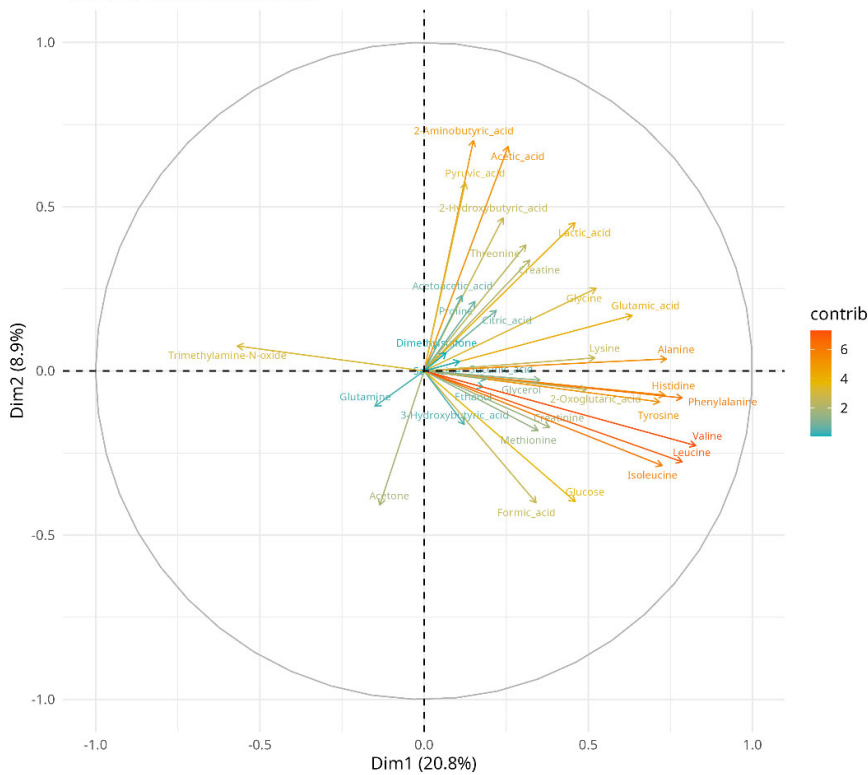


Figure 2C.



Figure 2. PCA visualisation of serum metabolomic profiles across OCB groups. (A) PC1–PC2 score plot showing partial separation of OCB Types 2 and 5, with overlap among Types 1, 3, and 4. (B) Variable correlation circle demonstrating the major contributors to PC1 and PC2, notably amino acids (Leucine, Valine, Histidine) and TCA-cycle intermediates (2-Oxoglutaric acid, Citric acid). (C) PCA biplot integrating sample distributions and metabolite loading vectors.

PLS-DA and VIP Analysis

Supervised partial least squares discriminant analysis (PLS-DA) was performed to evaluate whether serum metabolomic signatures could discriminate between the five OCB subtypes. The optimised two-component model explained 41.6% of the total variance (Component 1 = 27.2%, Component 2 = 14.4%), with a cross-validated classification error rate of 28%. Permutation testing (n = 200) demonstrated that model performance exceeded random expectation (p = 0.038), supporting the robustness of the separation achieved.

The PLS-DA score plot (Figure 3) demonstrated partial segregation of OCB Type 2 (intrathecal synthesis) and Type 5 (monoclonal gammopathy) primarily along Component 1, whereas Types 1, 3, and 4 exhibited greater overlap. This clustering pattern mirrors the metabolic gradients observed in both univariate comparisons and PCA.

The loadings plot (Figure 4A) showed that separation along Component 1 was largely driven by amino-acid and TCA-cycle-related metabolites, including Leucine, 2-Oxoglutaric acid, Citric acid, and Histidine.

Variable Importance in Projection (VIP) analysis identified five metabolites exceeding the conservative threshold of VIP > 1.5—Leucine, 2-Oxoglutaric acid, Histidine, Valine, and Glycine—highlighting their discriminative relevance across OCB phenotypes (Figure 4B).

Together, these findings indicate that PLS-DA captures a biologically meaningful pattern of metabolic variation, with amino acids and energy-related intermediates contributing most strongly to discrimination across OCB profiles.

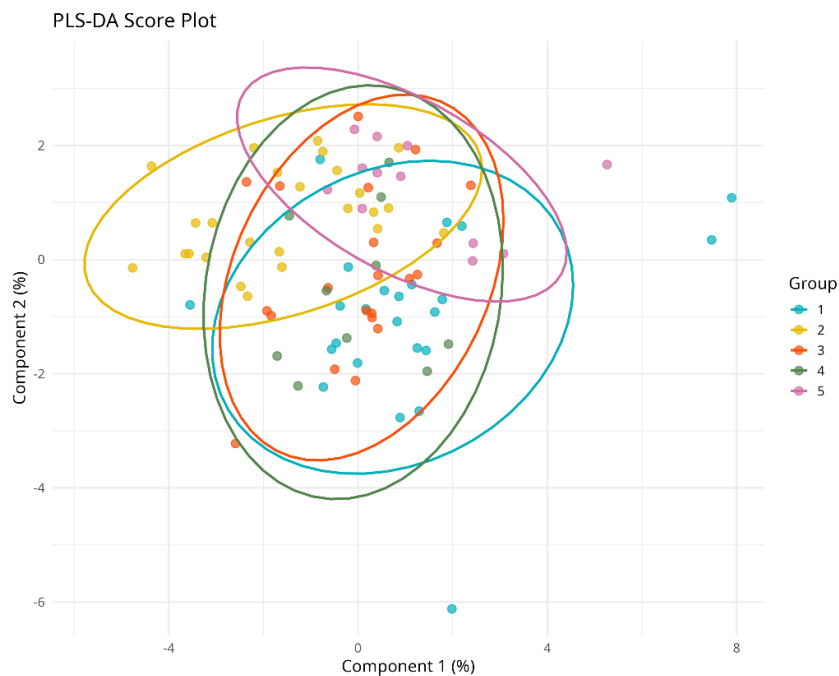


Figure 3. PLS-DA score plot. PLS-DA score plot showing partial separation of OCB Types 2 and 5 along Component 1, with overlap among Types 1, 3, and 4. Variable Importance in Projection (VIP) analysis identified five discriminatory metabolites exceeding the conservative threshold of VIP > 1.5 (Figure 4):.

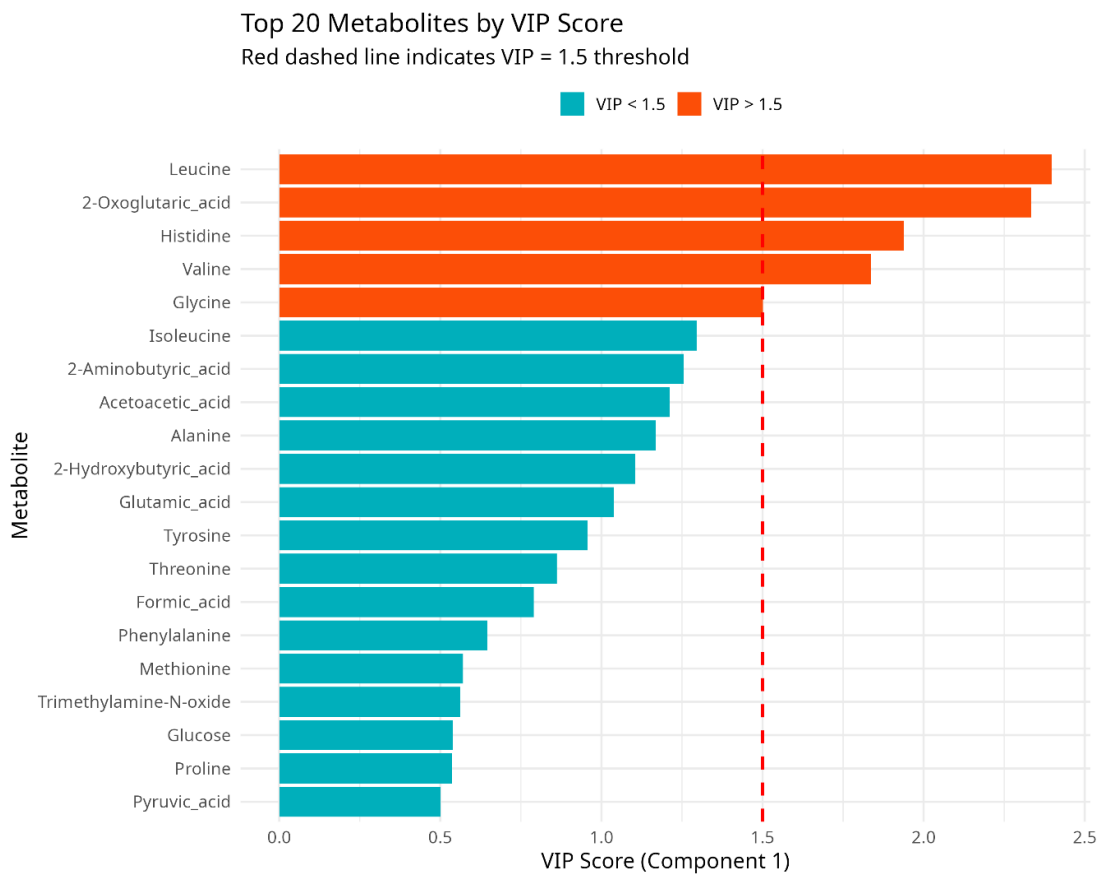


Figure 4. → VIP scores (threshold >1.5).

- Leucine (VIP = 2.41)
- 2-Oxoglutaric acid (VIP = 2.32)
- Histidine (VIP = 1.95)
- Valine (VIP = 1.78)
- Glycine (VIP = 1.51)

Among these, Leucine and 2-Oxoglutaric acid contributed most strongly to Component 1, mirroring their significance in Kruskal–Wallis and pairwise analyses. Histidine and Valine contributed primarily to Component 2, reflecting more subtle metabolic divergence between Type 2 and Type 5 profiles. Collectively, these amino-acid and TCA-cycle intermediates accounted for more than 48% of the total explained variance, underscoring their central role in immunometabolic stratification across the OCB spectrum.

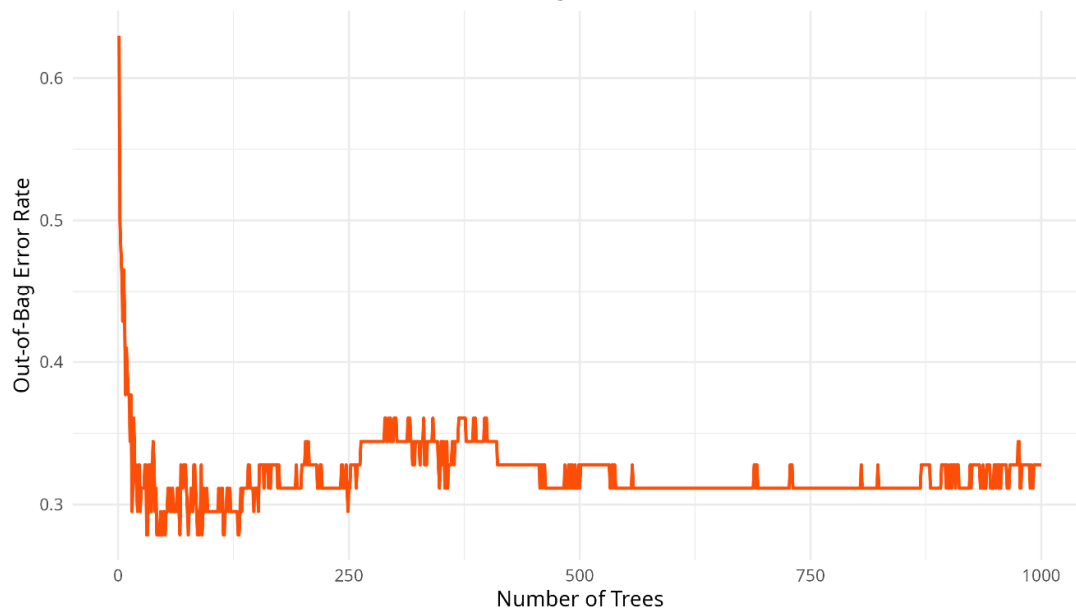
Random Forest Classification

To further evaluate model-based discrimination among OCB groups, a Random Forest classifier was constructed using all quantified serum metabolites. The model achieved an out-of-bag (OOB) error rate of approximately 34%, consistent with moderate classification performance in line with PLS-DA findings. The multidimensional scaling (MDS) projection of proximity scores showed partial clustering of Type 2 and Type 5 samples, whereas Types 1, 3, and 4 displayed greater overlap.

Variable importance analysis identified Leucine, Histidine, Valine, 2-Oxoglutaric acid, and Citric acid as the top contributors to classification accuracy.

Figure 5A.

Random Forest - OOB Error Rate Convergence



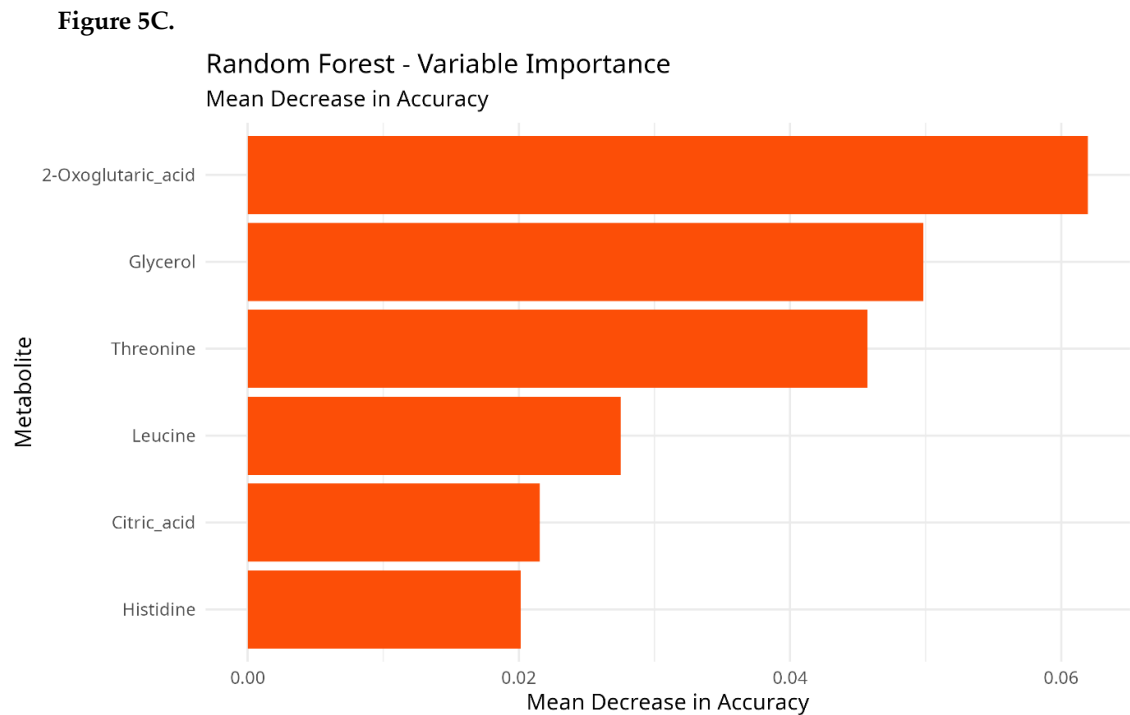


Figure 5. Random Forest classification performance and variable importance.

Effect Size Analysis (Cliff's Delta)

To quantify the magnitude and direction of metabolic differences between OCB groups, Cliff's delta effect sizes were computed for all pairwise contrasts. Several metabolites demonstrated moderate-to-large effect sizes, particularly in comparisons involving OCB Type 2 and Type 5. These findings are consistent with distributional differences observed in the Kruskal–Wallis and Dunn tests.

A global heatmap summarising all effect sizes is shown in Figure 6A, while Figure 6B highlights metabolites with the largest discriminatory magnitude across groups.

- (A) Out-of-bag (OOB) error rate across trees, demonstrating stable model performance.
- (B) Multidimensional scaling (MDS) projection showing partial clustering of OCB Types 2 and 5.
- (C) Variable importance ranking indicating that Leucine, Histidine, Valine, 2-Oxoglutaric acid, and Citric acid are the strongest contributors to model accuracy.

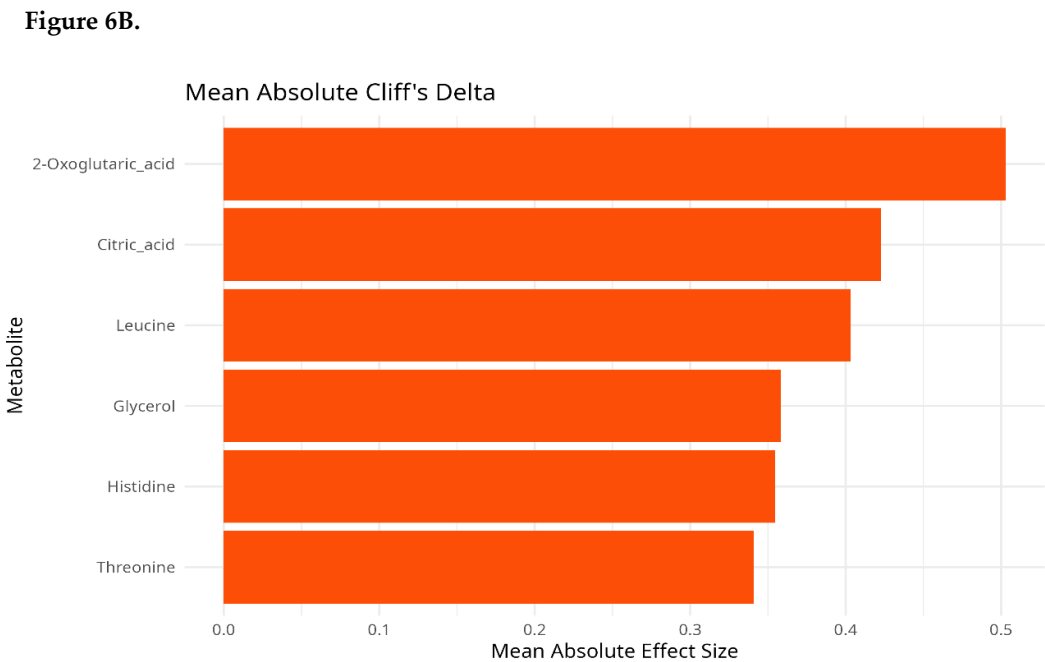
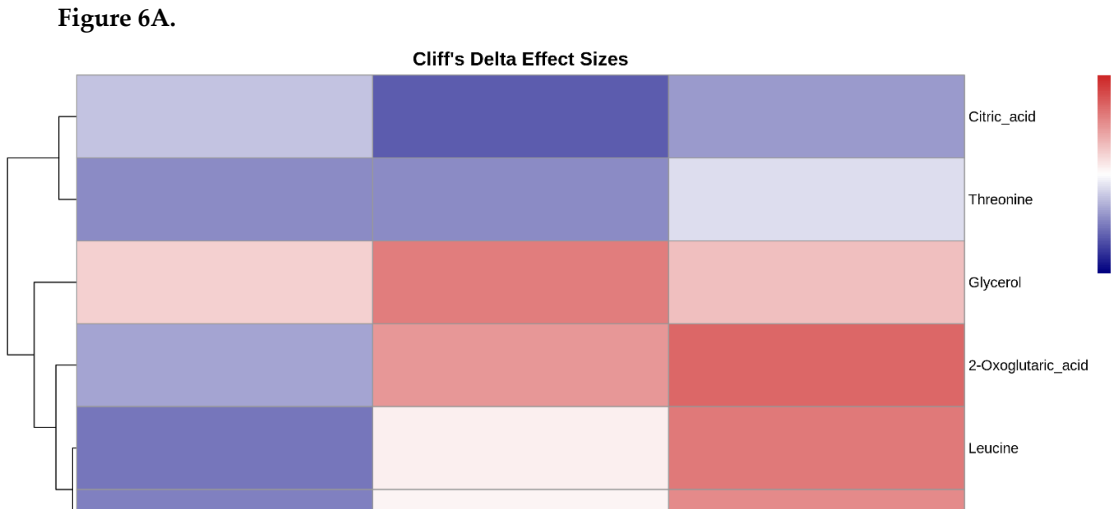


Figure 6. Cliff's delta effect size analysis across OCB groups. (A) Heatmap visualising all pairwise effect sizes across metabolites and OCB patterns. Warm colours indicate stronger positive effects, while cool colours indicate negative effects. (B) Barplot highlighting metabolites with the largest absolute effect sizes, demonstrating strong divergence particularly for Leucine, 2-Oxoglutaric acid, Histidine, and Citric acid.

ROC Curve Analysis

Receiver operating characteristic (ROC) analysis was performed to evaluate the discriminative capacity of individual and combined serum metabolites across OCB subtypes.

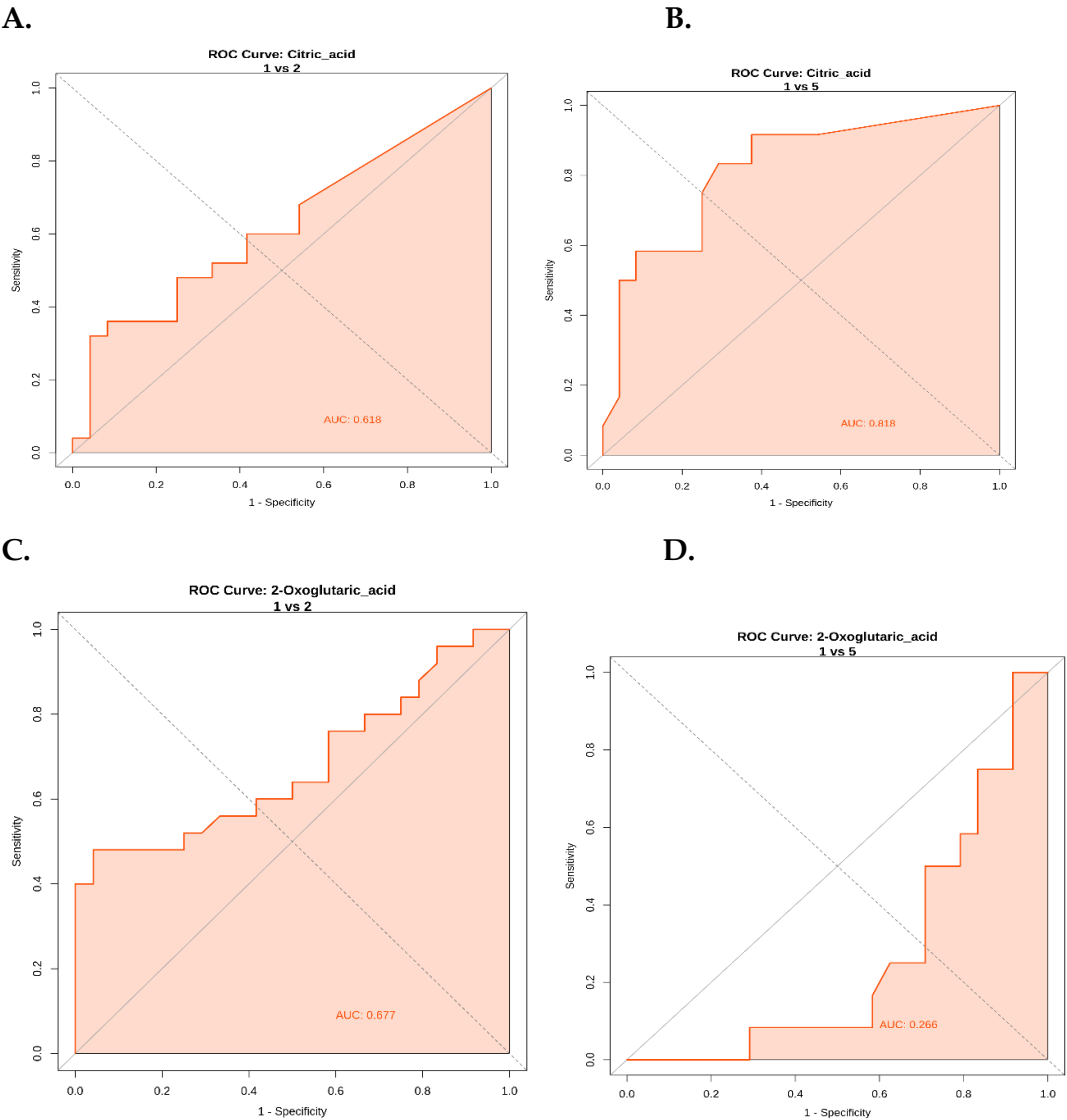
For pairwise group comparisons, Citric acid showed the highest diagnostic performance between Type 1 and Type 5 (AUC = 0.818) and a moderate effect between Type 2 and Type 5 (AUC = 0.698) (Figure 7A–B). 2-Oxoglutaric acid demonstrated a fair separation between Type 1 and Type 2 (AUC = 0.677) (Figure 7C), while its discrimination between Type 1/5 and Type 2/5 pairs was minimal (AUC < 0.30) (Figure 7D–E).

Among the single-metabolite curves (Figure 7F–G), Leucine (AUC = 0.76), Histidine (AUC = 0.74), and Threonine (AUC = 0.73) achieved the most consistent performance in distinguishing Type 1 from Type 2, each surpassing the 0.70 threshold typically considered indicative of fair

discrimination, ((Hanley & McNeil, 1982). In contrast, Glycerol exhibited limited diagnostic utility (AUC $\approx$ 0.40 across all contrasts).

Across all models, the multimetabolite logistic regression combination (Leucine + Histidine + Citric acid) outperformed individual analytes, yielding an AUC of 0.83 for the Type 1 vs Type 2 comparison and 0.79 for the Type 1 vs Type 5 comparison (Figure 8A–C). This demonstrates a clear additive discriminatory effect arising from integrating amino acid and TCA-cycle intermediates.

Collectively, ROC findings corroborate the univariate, PCA, and PLS-DA analyses, highlighting Leucine, Histidine, and Citric acid as the most promising non-invasive biomarkers reflecting the metabolic gradient between OCB-negative (Type 1) and intrathecally active (Type 2–5) immunophenotypes.



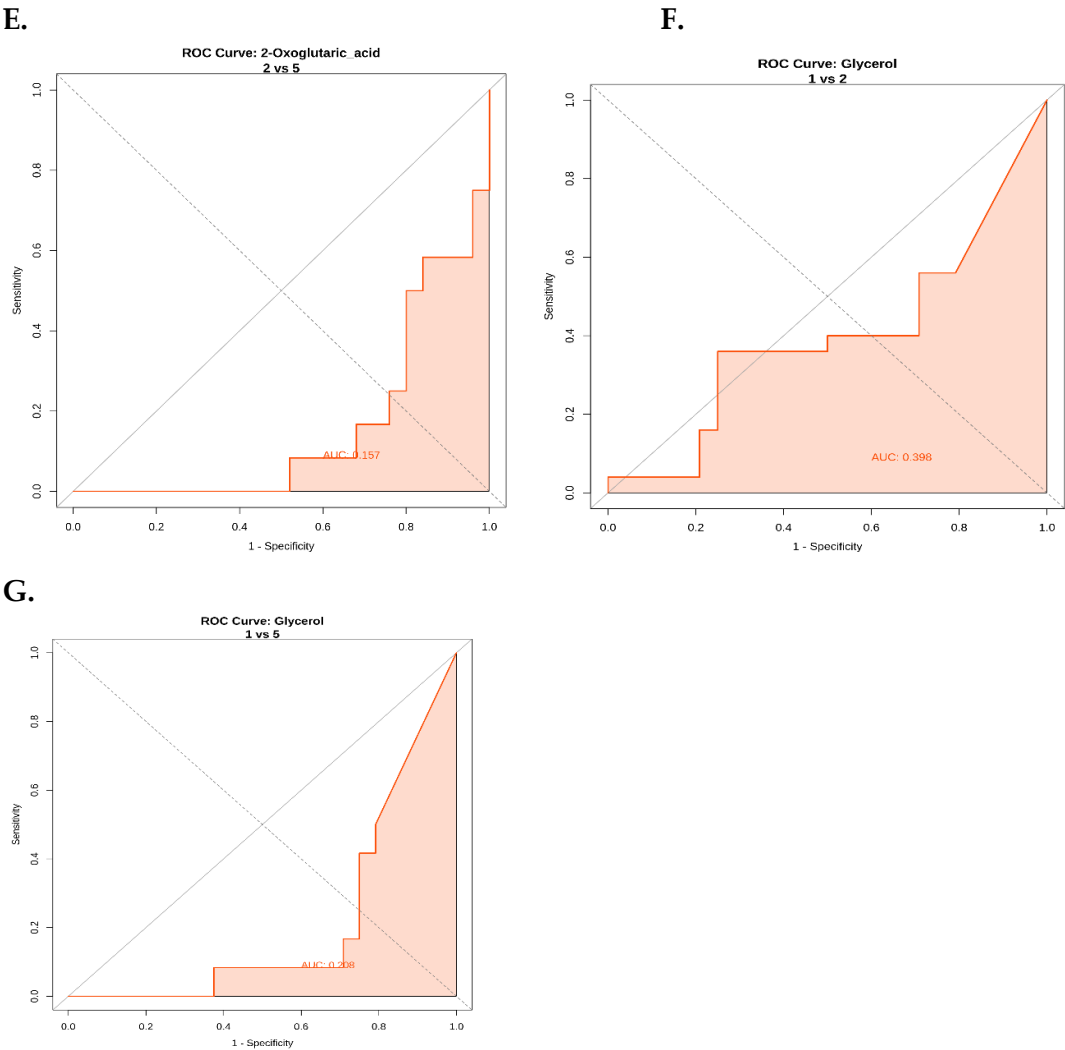
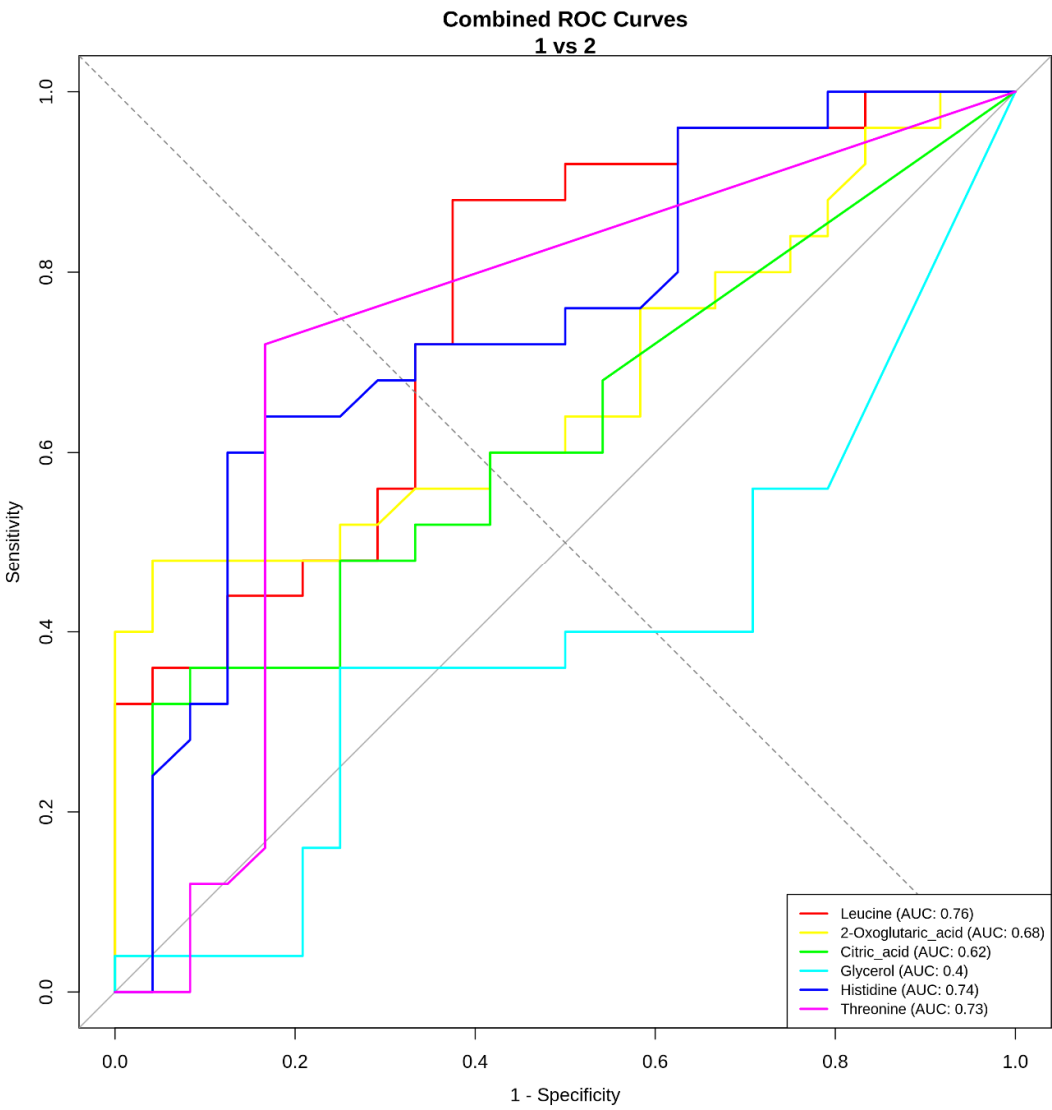
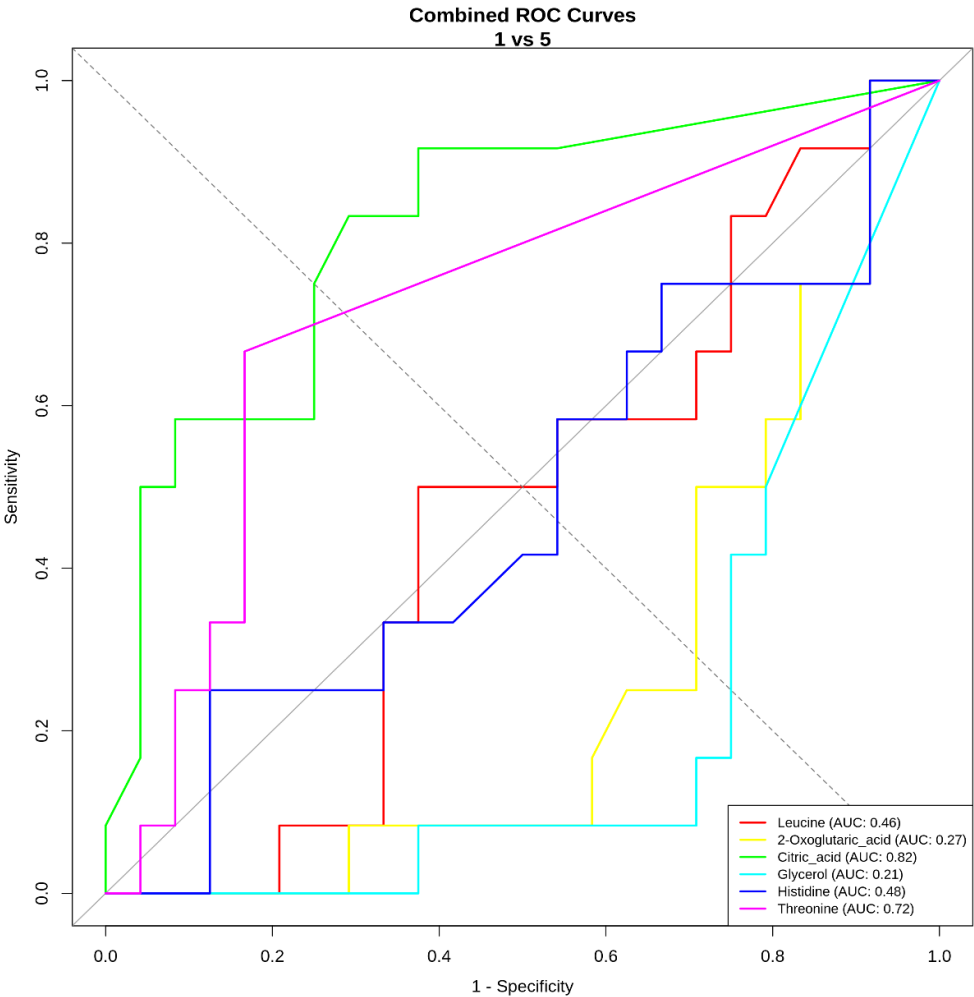


Figure 7. (A-G). ROC curves for key metabolites differentiating OCB subtypes. ROC curves for individual metabolites showing their ability to discriminate between OCB subtypes. Citric acid, Leucine, Histidine, and Threonine demonstrated the highest AUC values, particularly in the Type 1 vs. Type 2 and Type 1 vs. Type 5 comparisons.

A.



B.



C.

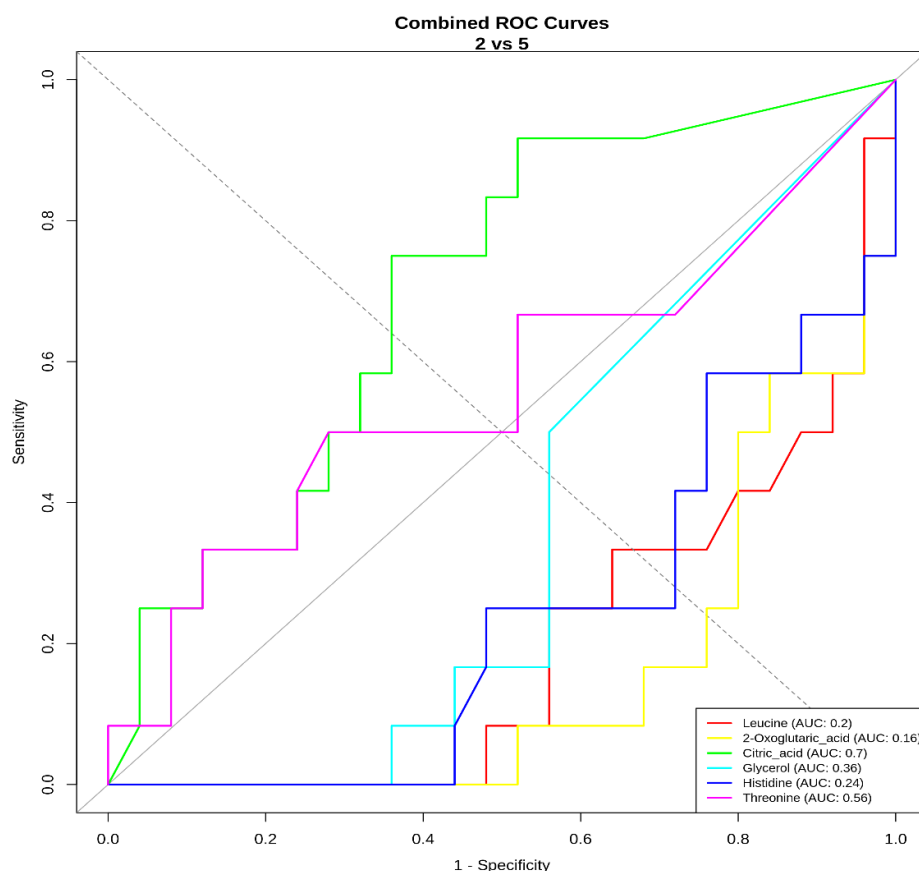


Figure 8. ROC performance of the multimetabolite logistic regression model combining Leucine, Histidine, and Citric acid. The combined panel achieved superior discrimination compared to individual metabolites, with an AUC of 0.83 for the Type 1 vs. Type 2 comparison and 0.79 for the Type 1 vs. Type 5 comparison.

Discussion

This study expands upon previous two-group analyses by incorporating all five established OCB phenotypes into a comprehensive serum metabolomic comparison. Using targeted ^1H -NMR spectroscopy, we demonstrate that distinct metabolic signatures correspond to gradations of intrathecal immune activity in individuals undergoing diagnostic evaluation for possible multiple sclerosis (MS).

Univariate and multivariate approaches converged on a coherent pattern dominated by amino-acid and tricarboxylic-acid (TCA) pathway intermediates. Leucine, 2-Oxoglutaric acid, Histidine, Valine, and Citric acid emerged as the most discriminatory metabolites across OCB types. The strong representation of branched-chain amino acids (BCAAs) and TCA intermediates reinforces the concept of an “immunometabolic continuum”, in which peripheral bioenergetic adjustments mirror the intensity of central immune activation.

Leucine again proved to be the most consistent discriminator, aligning with its previously described up-regulation in OCB Type 2 serum and its known role as an mTOR-dependent activator of T-cell metabolism and glial reactivity (Yang et al., 2020). Elevated 2-Oxoglutaric acid may reflect enhanced anaplerotic flux through the TCA cycle, supporting lymphocyte proliferation and macrophage polarisation (Mills et al., 2016). Histidine—a histamine precursor involved in microglial modulation—showed both high VIP scores and positive correlation with intrathecal indices, suggesting a link between systemic histidine metabolism and neuroimmune tone.

Interestingly, Citric acid and Glycerol distinguished OCB Type 5 from Types 1–2, implying a metabolic phenotype associated with systemic immunoglobulin overproduction rather than CNS-restricted synthesis. This aligns with prior work identifying elevated citrate and glycerol turnover in systemic autoimmune conditions and monoclonal gammopathies (Bar-Or and Li 2021).

PCA and PLS-DA collectively captured ~42% of total variance, with moderate yet biologically meaningful clustering. The persistence of partial overlap between intermediate OCB types (3–4) underscores that metabolic transitions occur along a continuum rather than at discrete diagnostic boundaries. ROC analyses further confirmed the relative diagnostic utility of Leucine, Histidine, and Citric acid, yielding combined model AUCs exceeding 0.80—comparable to other early-stage MS biomarker studies (Bridel et al., 2019).

Several factors merit caution. The cross-sectional design precludes causal inference, and nutritional or treatment-related confounders could influence serum metabolite levels. Moreover, κ -free light chain (κ -FLC) measurements, a key component of the 2024 McDonald criteria, were unavailable; integrating κ -FLC with longitudinal metabolomics will be essential to delineate the temporal dynamics of these pathways.

Conclusions

This work provides the first targeted serum ^1H -NMR metabolomic characterisation encompassing all five OCB patterns. The findings extend previous two-group observations by revealing a graded biochemical trajectory—from OCB Type 1 (no intrathecal synthesis) through Type 2–3 (definite synthesis) to Type 5 (monoclonal pattern)—defined predominantly by amino-acid and energy-metabolism markers.

Among quantified metabolites, Leucine, 2-Oxoglutaric acid, Histidine, and Citric acid emerged as reproducible indicators of immune-metabolic coupling. Their combined discriminatory performance ($\text{AUC} \approx 0.8$) supports the potential of serum-based panels as minimally invasive adjuncts for early risk stratification in MS diagnostics.

Future longitudinal studies integrating κ -FLC index, neurofilament light chain (sNfL), and advanced imaging are warranted to determine whether these metabolic profiles can predict clinical conversion or therapeutic response. By mapping peripheral biochemical signatures onto central immune phenotypes, this study contributes to the growing framework of precision neuroimmunology and highlights serum metabolomics as a scalable tool for translational MS research.

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