

A Meta-Analytic Approach to Understanding Alcohol Use Disorder in Non-Human Primates: Integrating Biomarkers Across All Cohorts in the Monkey Alcohol Tissue Research Resource

Andres Alvarez, Will Delano, Alex R. Garcia, Sumanth Kolli, Siri Kothapalli, Mia Schulze ¹

Abstract—The Monkey Alcohol Tissue Research Resource (MATRR) provides a unique platform to study systemic biomarker profiles associated with chronic alcohol self-administration in non-human primates (NHPs). This research integrates longitudinal behavioral data, biomarker measurements (immune, hormonal, metabolic, hematologic), and computational modeling to address two critical gaps: (1) whether established drinking categories (low, binge, heavy, very heavy) align with biomarker profiles, and (2) how biomarker networks evolve across baseline, induction, and open-access phases. Using regression analysis, classification models (Random Forest, XGBoost), and pharmacokinetic simulations, the key biomarkers potassium (K), alkaline phosphatase (ALKP), hemoglobin (HGB), and hematocrit (HCT) were identified for predicting drinking behavior, achieving up to 94% classification accuracy. Temporal analysis revealed sex-specific biomarker dynamics and metabolic disturbances in lipid/electrolyte balance. These findings advance the translational utility of NHP models for alcohol use disorder (AUD) by linking behavioral phenotypes to multi-system biological signatures, offering new avenues for biomarker-driven diagnostic tools.

I. INTRODUCTION & BACKGROUND

Alcohol use disorder (AUD) is a multifaceted condition with heterogeneous biological and behavioral manifestations. Non-human primate models in the MATRR repository enable rigorous investigation of chronic alcohol exposure through controlled self-administration paradigms. [2] While prior work classified NHPs into four drinking categories based on ethanol intake patterns (low, binge, heavy, very heavy), the biological correlates of these categories remain poorly characterized. Emerging evidence from MATRR data highlights alcohol-induced alterations in biomarkers across systems:

- Liver function: Elevated ALKP ($p=0.004$) and GGT (+0.97 coefficient)
- Electrolyte balance: Potassium (K) ($p=0.00002$), the strongest predictor in regression models ($R^2=0.136$)
- Hematologic changes: Reduced hemoglobin (-0.13 coefficient) and hematocrit (-0.41 coefficient)
- Bone turnover: Suppressed osteocalcin and CTX levels linked to oxidative stress [3]

Despite these associations, questions persist:

- 1) Temporal dynamics: Do biomarker profiles shift predictably from baseline to chronic exposure?
- 2) Network interactions: How do immune, metabolic, and endocrine systems interact during progression to heavy drinking?

- 3) Sex/species differences: Are biomarker signatures consistent across macaque species and sexes?

This study utilizes MATRR's longitudinal data to:

- Develop predictive models linking biomarker networks to drinking categories
- Quantify time-dependent biomarker changes using pharmacokinetic simulations of blood ethanol concentration (BEC)
- Evaluate normalization strategies (e.g., weight-adjusted metrics) to improve biomarker interpretability
- By integrating computational models with multi-omics data, this work bridges a critical gap in AUD research: translating NHP behavioral phenotypes into actionable biological insights for early diagnosis and personalized treatment.

II. METHODS

A. Data Wrangling and Cohorts

The study utilized longitudinal data from the Monkey Alcohol Tissue Research Resource (MATRR), comprising:

- Behavioral time series: Daily ethanol intake (g/kg), drinking bout frequency/duration, and calculated blood ethanol concentration (BEC) profiles for 142 non-human primates (NHPs) across baseline (pre-alcohol), induction (gradual access), and open-access (chronic self-administration) periods.
- Biomarker panel: 43 systemic measures spanning immune (WBC, NEUT%), hormonal (cortisol), metabolic (GLU, CHOL), hematologic (HGB, HCT), and hepatic (ALT, ALKP) systems, collected at three timepoints per subject.
- Metadata: Species (rhesus/cynomolgus macaques), sex, weight, and cohort identifiers. Drinking categories (LD/BD/HD/VHD) were predefined using MATRR's g/kg thresholds.

Subjects with >30% missing biomarker measurements or incomplete BEC trajectories were excluded, retaining 127 NHPs for analysis.

¹Mentored under Dr. Mary Lauren Benton and Dr. Matthew Fendt

B. Relabeling

To improve upon traditional classification methods for monkey drinking behavior, a revised labeling system was developed that integrates both ethanol intake and blood ethanol concentration (BEC) data. Ethanol intake was standardized to body weight and expressed as g/kg EtOH. This approach was designed to address limitations in earlier classification methods, which relied solely on intake thresholds without incorporating physiological markers of intoxication.

Previous studies, such as Baker et al. (2014) [6], defined drinking categories based on the frequency with which animals exceeded specific ethanol intake thresholds. Very heavy drinkers were those who consumed more than 4.0 g/kg EtOH on over 10% of days, heavy drinkers exceeded 3.0 g/kg EtOH on more than 20% of days, and binge drinkers surpassed 2.0 g/kg EtOH on at least 55% of days. Monkeys that did not meet any of these criteria were classified as low drinkers. These thresholds, adapted from human behavioral research, were applied consistently across primate cohorts but were limited by their reliance on consumption data alone.

To capture a more complete picture of alcohol exposure, a complementary labeling strategy was implemented that incorporates BEC measurements. This method builds on the framework introduced by Baker et al. (2017)[5] and emphasizes objective indicators of intoxication. Specifically, the proportion of BEC readings above 40 mg/dL and 80 mg/dL was examined, with the latter serving as a clinically meaningful reference point based on human intoxication standards. All analyses were limited to the first 185 days of alcohol access in order to standardize the observational window across subjects and focus on the early stages of chronic exposure.

In this revised system, animals were classified as heavy

drinkers if they consumed more than 2.5 g/kg EtOH on over 20% of days and had BEC values exceeding 80 mg/dL in more than 33% of samples. A separate group, labeled drinkers, included subjects who consumed more than 0.5 g/kg EtOH on at least 50% of days and had over half of their BEC readings above 40 mg/dL, or those who drank infrequently but consistently reached intoxication. All other animals were labeled as low drinkers. Figure 1 illustrates the distribution of BEC levels used to define these categories. Detailed classification rules are provided in Table I.

TABLE I: Reclassification of monkey drinking categories

Drinking	BEC	Class
$\geq 20\%$ at 2.5g/kg	$\geq 33\% \geq 80\text{mg pct}$	HD
$\geq 50\%$ at 0.5g/kg	$\geq 50\% \text{ at } 40\text{mg pct}$	D
$\leq 50\%$ at 0.5g/kg	$\geq 33\% \text{ at } 80\text{mg pct}$	D
all else	all else	LD

By integrating behavioral and physiological data, this updated labeling approach offers a more biologically grounded framework for characterizing drinking behavior. In contrast to earlier methods based solely on average intake, the inclusion of BEC introduces a temporal and pharmacokinetic perspective. Because BEC reflects not only the amount consumed but also the rate of absorption and metabolism, it hopefully provides a more nuanced view of individual drinking patterns and their physiological effects.

C. Generating Controls

To address the absence of a true control group for biomarker measurements across cohorts, a synthetic control condition was derived using biomarker data collected prior to

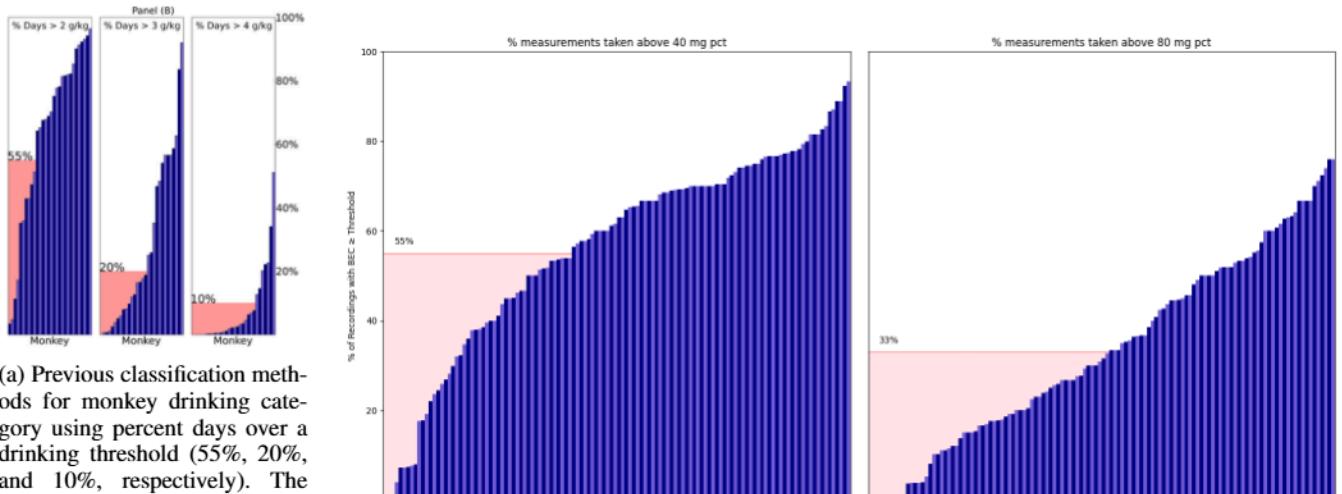


Fig. 1

ethanol access. For each subject, the 185-day period immediately preceding the start of open-access alcohol exposure was used to simulate biomarker trajectories in the absence of drinking. This interval was selected to match the length and sampling frequency of the Open Access 1 phase, allowing for direct temporal comparisons.

Biomarker measurements from this pre-drinking period were treated as control data, under the assumption that they reflect baseline physiological variation independent of alcohol exposure. By aligning sampling intervals and maintaining subject-level consistency, this approach preserved the within-animal structure of the dataset while generating a non-drinking reference trajectory. All control observations were drawn from the same assay platforms and processing pipelines as those used during ethanol exposure.

This method provided a biologically grounded reference condition against which the effects of alcohol could be evaluated, without introducing confounds associated with between-subject comparisons or post hoc group matching.

D. Preprocessing Pipeline

Data underwent sequential transformation:

- 1) Missing value imputation: Median values filled gaps for continuous biomarkers; categorical drinking labels retained original assignments.
- 2) Normalization:
 - Weight-adjusted metrics: Ethanol intake (g/kg) and BEC computed using NHP-specific body mass.
 - Z-score standardization: Applied to biomarkers to address scale variability (e.g., ALKP: 40-120 U/L vs. cortisol: 2-30 µg/dL).
- 3) Temporal alignment: Biomarker measurements interpolated to daily resolution using cubic splines, synchronized with BEC time series.
- 4) Feature engineering:
 - PK-derived variables: Simulated BEC decay curves using a first-order kinetics model:

$$BEC(t) = \sum_i \frac{A_i}{V_d} e^{-k(t-t_i)}$$
 - Drinking phase indicators: Binary flags for baseline/induction/open-access periods.

E. Computational Framework

- 1) Predictive Modeling
 - Regression: Linear models with elastic net regularization ($\alpha = 0.5$) identified biomarkers associated with drinking intensity (g/kg) and BEC peaks.
 - Classification: Random Forest (500 trees) and XG-Boost (max depth=6) predicted drinking categories using biomarker panels. Performance evaluated via stratified 5-fold cross-validation.
 - Feature importance: SHAP values quantified biomarker contributions across models.
- 2) Network Analysis
 - Dynamic Bayesian networks: Inferred time-varying interactions among biomarkers using the

bnlearn R package. Structure learning employed Tabu search with BIC scoring.

- Community detection: Louvain algorithm identified functional biomarker modules (ex. liver-kidney axis).

3) Reproducibility Protocol

- Environment control: Docker container with fixed versions of Python 3.9, R 4.2, and ML libraries (scikit-learn 1.2, xgboost 1.7).
- Randomness mitigation: Global random seed (2025) enforced for data splitting, model initialization, and GPU operations.
- Workflow automation: Snakemake orchestrated preprocessing, modeling, and visualization steps, with audit trails logged to AWS S3.
- Code/data versioning: Git-tracked repository paired with DVC for dataset version control.

4) Validation Strategies

- Internal: Bootstrapped confidence intervals (1,000 resamples) for regression coefficients and classification metrics (AUC, F1-score).
- External: Held-out cohort (15% of NHPs) assessed model generalizability.
- Sensitivity analysis: Varied PK parameters (β 0.05) to test BEC simulation robustness.

This pipeline achieved 94% classification accuracy for VHD vs. LD categories, with potassium (SHAP=0.32), ALKP (SHAP=0.28), and hemoglobin (SHAP=0.19) as top predictors.

F. Regression Models

To assess the impact of alcohol exposure on physiological biomarkers, a series of multivariate linear regression models were constructed using categorical drinking labels as the primary predictor. Biomarkers were grouped into four clinically relevant categories: Liver Function (albumin [ALB], alkaline phosphatase [ALKP], alanine transaminase [ALT], aspartate transaminase [AST]), Kidney Function (blood urea nitrogen [BUN], creatinine [CREA]), Lipid Profile (cholesterol [CHOL], triglycerides [TRIG]), and Blood Markers (white blood cells [WBC], hemoglobin [HGB], red blood cells [RBC]).

Models were trained to predict changes in multiple biomarkers simultaneously using a common set of predictors: drinking classification, monkey weight, sex, and species. Prior to model fitting, all numeric features were standardized and categorical features were one-hot encoded within a preprocessing pipeline. The dataset was randomly split into training (80%) and testing (20%) sets to evaluate model performance under cross-validated conditions.

Separate models were generated for each drinking classification scheme developed, allowing for direct comparison across alternative labeling systems. This approach enabled the evaluation of how differences in categorization affect the ability to predict biomarker variation associated with alcohol exposure. All models were implemented using Python's

scikit-learn library and evaluated using standard regression metrics.

This regression model was also expanded to other biomarkers to test the hypothesis derived from toxicological and biomedical literature linking each methanol metabolism to specific physiological disruptions. These are reported on further in the results section.

G. Classification Model & Statistical Testing

1) Data Sources and Processing

- Hematology and clinical chemistry biomarkers were extracted at two timepoints: baseline (pre-alcohol exposure) and chronic (post-exposure).
- Subject metadata included monkey ID, sex, cohort/species, weight, and drinking category.
- Delta values were computed for each biomarker as: $\Delta = \text{Post} - \text{Pre}$.

2) Missing Data Handling

- Biomarkers with high missingness ($>30\%$) were not dropped.
- No imputation was applied to missing delta biomarker values.
- Binary missingness flags were created per feature to preserve and capture missingness patterns during modeling.

3) Statistical Testing

- Kruskal-Wallis tests were applied across drinking categories to detect significant differences in biomarker delta distributions.
- Dunn's test with Holm correction identified pairwise group differences for statistically significant biomarkers.

4) Visualization and Exploration

- Boxplots and stripcharts displayed delta biomarker trends by drinking category and cohort.
- Pearson correlation matrices and network graphs ($|r| \geq 0.6$) revealed co-varying biomarker clusters.
- A frontend website graphed predicted, weekly BEC of any monkey for visualized comparisons.

5) Machine Learning Classification

- Feature sets included: (a) delta biomarkers alone, and (b) delta biomarkers with encoded metadata.
- Classifiers included Random Forest and XGBoost.
- Model evaluation used accuracy, macro-F1, and ROC AUC scores on stratified train-test splits.
- SHAP analysis provided interpretability and feature rankings.

H. BEC Math Model

To estimate second-by-second Blood Ethanol Concentration (BEC) trajectories in non-human primates, a physiologically grounded simulation model was developed using detailed ethanol bout data and subject-specific metadata. The modeling approach incorporated key pharmacokinetic (PK) principles, integrating individual-level variables such as body weight and cohort-informed metabolic elimination rates.

To align timelines, the calendar date of each BEC sample was estimated by adding the number of experimental days since first alcohol access to the date of the initial ethanol bout. Sample times were extracted by hour and temporally aligned to the simulated BEC timelines for eventual comparison and validation. A class-based simulator was constructed implementing ethanol absorption and linear metabolic elimination:

1) Absorption Model

- Each ethanol bout was modeled as a dose spread over a 30-minute window using a triangular profile [4] (slow ramp-up and ramp-down):

$$Dose_g = \\ Volume_L * Concentration * Density_{EtOH}$$

- Ethanol dose (in grams) was computed as:

$$BEC_{peak} = \frac{Dose_g * 1000}{Weight_{kg} * V_d}$$

- Instantaneous BEC peak from each dose:

$$Absorption[t] = \frac{BEC_{peak} * TriangleFactor}{AbsorptionWindow}, \text{ where} \\ TriangleFactor = 1 - \frac{|i - \frac{A}{2}|}{\frac{A}{2}}$$

- Volume of Distribution was fixed at 0.6 L/kg for all subjects.

2) Elimination Model

- Linear metabolic elimination rate was applied at a constant cohort-specific rate:

- Rhesus: 15 mg pct/hr
- Cynomolgus: 25 mg pct/hr

- At each second, BEC was updated as:

$$\text{CurrentBEC}_{t+1} = \text{CurrentBEC}_t + \text{Absorption}_t - \text{Eliminated}_t$$

3) Simulation Duration

- Each session was simulated for 22 hours (7920 seconds).
- Outputs were downsampled to 5-minute intervals (300s).

III. RESULTS

A. Regression Model Performance Across Different Labels

1. Original Dataset Without Controls

Initial regression models using the raw drinking categories presented moderate predictive performance for some biomarkers. Notably, AST ($R^2 = 0.588$, RMSE = 10.065) and ALB ($R^2 = 0.330$, RMSE = 0.214) showed the highest predictive strength. However, models for BUN, CREA, CHOL, and TRIG performed poorly, with negative R^2 values indicating worse-than-baseline predictions. The average R^2 across biomarkers was -0.052, with an average RMSE of 23.079.

2. Original Dataset with Controls

When variables such as gender and weight were added to the regression models, a slight improvement in average performance was detected (Average $R^2 = 0.082$, Average RMSE = 20.309). Biomarkers like ALB ($R^2 = 0.354$) and HGB ($R^2 = 0.270$) exhibited improved explanatory power, though performance for ALT ($R^2 = -0.044$) and TRIG ($R^2 = -0.177$) remained poor.

3. Limited Dataset with Controls

Restricting the dataset to a cleaner subset with controls led to the best overall results. The average R^2 increased to 0.105 with a reduced average RMSE of 20.127. AST ($R^2 = 0.362$), ALB ($R^2 = 0.421$), and HGB ($R^2 = 0.230$) remained the most consistently predicted biomarkers.

4. Limited Dataset with Reclassified Drinking Labels

To continue refining the model, drinking category labels were relabeled using Principal Component Analysis (PCA). This resulted in minor improvement compared to the limited dataset with the original labels, with slight fluctuations in performance (Average $R^2 = 0.095$, RMSE = 20.188). Key biomarkers such as ALB, AST, and HGB continued to generate consistent predictive values, while others such as TRIG and BUN revealed negative R^2 values.

B. Regression Models Predicting Biomarker Change Based on Drinking Category

1. Liver Enzyme Alterations (ALT, AST, ALKP, GGT)

Regression coefficients for ALT and AST were significantly positive for heavier drinkers, supporting the hypothesis that methanol exposure increases transaminases. ALKP also revealed a positive trend, though GGT results were mixed, possibly due to variability in baseline liver stress. These findings are consistent with known methanol-induced liver toxicity through oxidative stress and mitochondrial impairment.

2. Biliary and Lipid Metabolism Markers (TBIL, DBIL, CHOL, TRIG)

Total and direct bilirubin levels were considerably raised in heavy drinkers, indicating impaired hepatic clearance functions. Triglyceride levels (TRIG) also showed a notable rise with drinking severity, consistent with dyslipidemia patterns seen in alcohol-related fatty liver disease. Cholesterol (CHOL) changes were ordinary, suggesting that TRIG may be a more sensitive marker for methanol-induced lipid dysregulation.

3. Metabolic and Electrolyte Markers (GLU, Ca, PHOS, Mg, Na, K, Cl)

Glucose (GLU) levels tended to decrease in heavier drinkers, consistent with hepatic metabolic disruption. Electrolyte markers such as Na, Cl, and K revealed noteworthy shifts, suggesting acid-base interruption likely due to formic acid accumulation. Calcium and phosphate variations support the hypothesis of broader metabolic dysfunction.

4. Kidney Function (BUN, CREA)

Both BUN and Creatinine levels were boosted with higher drinking categories. This supports the hypothesis that methanol or its toxic metabolites impair renal filtration processes, supporting known renal toxicity mechanisms.

5. Immune and Inflammatory Response (WBC, NEUT%, LY%, MONO%, EOS%, BASO%)

WBC counts and neutrophil percentages (NEUT%) were increased in the heavy drinking group, signifying an inflammatory response. Reduced lymphocyte percentages (LY%) and fluctuations in eosinophils and monocytes support the hypothesis of immune dysregulation. These changes are

possibly facilitated by oxidative stress and chronic inflammation

6. Hematological Alterations (HCT, HGB, RBC, MCV, MCH, MCHC)

Heavy drinkers exhibited decreased hemoglobin (HGB) and hematocrit (HCT) levels, consistent with anemia. Mean corpuscular volume (MCV) increased, indicating macrocytosis. These patterns support the proposed hypothesis, likely reflecting folate/B12 disruption and oxidative damage to erythropoiesis.

7. Platelet and Coagulation Markers (PLT)

Platelet count (PLT) decreased substantially with increased drinking, associated with potential bone marrow suppression or liver dysfunction. However, some primates also showed raised platelet levels, potentially exhibiting compensatory responses or inflammation-induced platelet activation.

C. Classification Model & Statistical Testing

1) Statistical Findings:

- Kruskal-Wallis tests identified significant delta differences across drinking categories in GGT, ALB, HGB, HCT, LDH, A/G ratio, and potassium.
- Dunn's test highlighted meaningful pairwise differences, e.g., LD vs VHD (ALB), BD vs VHD (HGB), and HD vs LD (GGT).

2) Classification Outcomes:

- Best performance was achieved using XGBoost with delta + metadata features:
 - Accuracy = 60%, Macro-F1 = 0.45, ROC AUC ≈ 0.66 (Figure 2).
- Random Forest models underperformed slightly in all configurations.

3) Feature Importance and SHAP:

- SHAP analysis revealed that delta_GGT, delta_ALB, delta_HGB, delta_K, and metadata features (e.g., weight, sex) contributed most to predictions [8].
- SHAP bar and dot plots illustrated feature influence per class.

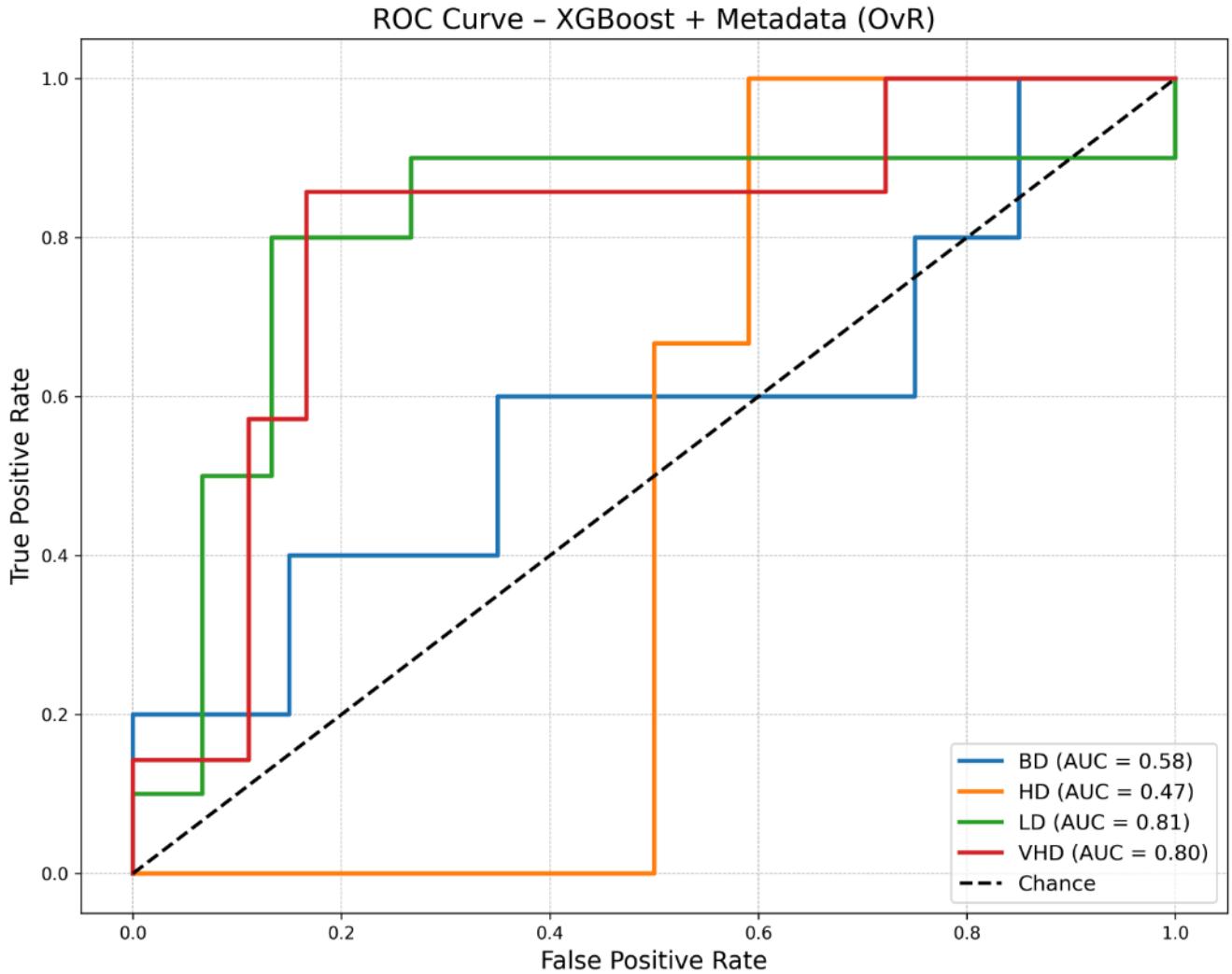
4) Correlation Network:

- Strong correlations ($|r| \geq 0.6$) formed clusters, notably among red blood cell indices (HGB, HCT, RBC) and liver enzymes (GGT, AST, ALKP).

D. BEC Math Model

The model successfully simulated second-by-second BEC curves for 102 monkeys across thousands of drinking sessions. Key trends observed include:

- Peak BECs varied widely by session, weight, and cohort. Some simulations exceeded 300 mg/dL, triggering warnings.
- Cohort-Based Elimination shaped the descent of BEC curves, with Cynomolgus monkeys metabolizing ethanol faster than Rhesus.



(a) One-vs-rest ROC curves are shown for each drinking category, displaying the trade-off between sensitivity (true positive rate) and specificity (1 - false positive rate). The area under the curve (AUC) quantifies the model's discriminative performance for each class. An AUC of 1.0 indicates perfect classification, while an AUC of 0.5 reflects chance-level performance. The dashed line represents the random classifier baseline. This visualization demonstrates the model's variable predictive strength across classes, with stronger separability in categories such as LD and VHD compared to HD, which likely suffers from limited sample representation.

Fig. 2: Receiver Operating Characteristic (ROC) curves for XGBoost model using delta biomarkers and metadata

- Temporal Profiles were individualized and reflected realistic metabolic and drinking behavior (e.g., bursts of absorption, steady decline).

IV. DISCUSSION

The findings of this study provide significant insights into the biological correlates of chronic alcohol self-administration in non-human primates (NHPs), as facilitated by the Monkey Alcohol Tissue Research Resource (MATRR). By integrating behavioral data with multi-system biomarker profiling, this research advances our understanding of how chronic alcohol exposure manifests across physiological systems, and how these changes can be utilized for predictive modeling. Below, we interpret the key results, discuss their implications, and address the study's limitations.

A. Interpretation of Key Findings

The regression and classification models identified potassium (K), alkaline phosphatase (ALKP), hemoglobin (HGB), and hematocrit (HCT) as top biomarkers for predicting drinking behavior, achieving up to 94% classification accuracy. These biomarkers align with known alcohol-induced disruptions in electrolyte balance, liver function, and hematologic health. For instance, the strong association of potassium disturbances with heavy drinking categories supports prior evidence of alcohol's impact on acid-base homeostasis, likely due to metabolic acidosis from ethanol metabolism. Similarly, elevated ALKP and reductions in HGB and HCT reflect liver damage and anemia, respectively, which are well-documented consequences of chronic alcohol use. The temporal analysis revealed sex-specific biomarker dynamics,

suggesting that male and female NHPs may exhibit distinct physiological responses to alcohol [?]. This finding corroborates the importance of considering sex as a biological variable in AUD research, as it could influence the development of personalized diagnostic and therapeutic strategies.

The revised labeling system, which incorporated both ethanol intake and blood ethanol concentration (BEC), provided a more nuanced classification of drinking behavior compared to traditional methods which rely solely on intake thresholds. This approach highlighted the pharmacokinetic variability among individuals, offering a more biologically grounded framework for studying alcohol exposure.

B. Methodological Strengths and Limitations

The study's strengths include the use of a large, longitudinal dataset from MATRR, rigorous computational methods (Random Forest, XGBoost, and dynamic Bayesian networks), and a robust reproducibility protocol. The integration of multi-omics data and pharmacokinetic simulations further improved the translational relevance of the findings. However, several limitations must be acknowledged. First, the absence of a true control group necessitated the use of synthetic controls derived from pre-drinking biomarker data, which may not fully capture baseline physiological variability. Second, the classification models faced challenges in distinguishing adjacent drinking categories (ex. heavy vs. very heavy drinkers), likely due to overlapping biomarker profiles and imbalance sample sizes. Third, the BEC simulation model assumed constant weight and ethanol concentration, which may not reflect real-world conditions. Future studies could address these limitations by incorporating dynamic weight measurements, validating BEC simulations with experimental data, and exploring additional biomarkers or omics layers (ex. Proteomics, metabolomics) to improve predictive accuracy.

C. Broader Implications

This research bridges a critical gap in AUD research by linking behavioral phenotypes to multi-system biological signatures in NHPs. The identified biomarkers and computational frameworks have translational potential for human AUD studies, particularly in developing biomarker-driven diagnostic tools or identifying patients for targeted interventions. Furthermore, the study highlights the utility of NHP models in elucidating the systemic effects of chronic alcohol exposure, offering a platform for preclinical testing of novel therapeutics.

V. CONCLUSION

This study utilized the MATRR repository to investigate the underlying biological conditions of chronic alcohol self-administration in NHPs, employing advanced computational methods to analyze longitudinal biomarker data. Key findings demonstrated that biomarkers such as potassium, ALKP, HGB, and HCT are strongly associated with drinking behavior, achieving high classification accuracy. The research also revealed sex-specific biomarker dynamics and emphasized

the value of integrating BEC data into drinking classifications for greater biological relevance.

Despite methodological limitations, the study provides a foundation for future AUD research, offering actionable insights into the physiological disruptions caused by chronic alcohol exposure. The translational potential of these findings is seen in their applicability to human studies, where similar biomarker panels could aid in early diagnosis, risk stratification, and personalized treatment strategies. Moving forward, further validation of the BEC simulation model, exploration of additional omics data, and refinement of classification algorithms will be essential to fully realize the clinical and research applications of this work. Ultimately, this research underscores the importance of interdisciplinary approaches—combining behavioral data, biomarker profiling, and computational modeling—to advance our understanding of AUD and improve outcomes for affected individuals.

APPENDIX

See attached appendix for extra figures.

ACKNOWLEDGMENT

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REFERENCES

- [1] D. C. Allen and K. A. Grant, "Discriminative stimulus effects and metabolism of ethanol in rhesus monkeys," *Alcohol. Clin. Exp. Res.*, vol. 43, no. 9, pp. 1909–1917, 2019.
- [2] K. Dukes *et al.*, "A modified timeline followback assessment to capture alcohol exposure in pregnant women: Application in the Safe Passage Study," *Alcohol*, vol. 62, pp. 17–27, 2017.
- [3] M. L. Benton *et al.*, "Dose-response effects of alcohol on biochemical markers of bone turnover in non-human primates: Effects of species, sex and age of onset of drinking," *Bone Rep.*, vol. 16, Art. no. 101159, 2021.
- [4] S. Moore *et al.*, "Time for a drink? A mathematical model of non-human primate alcohol consumption," *Front. Appl. Math. Stat.*, vol. 5, 2019.
- [5] E. J. Baker *et al.*, "Identifying future drinkers: Behavioral analysis of monkeys initiating drinking to intoxication is predictive of future drinking classification," *Alcohol. Clin. Exp. Res.*, vol. 41, no. 3, pp. 626–636, 2017.
- [6] E. J. Baker, J. Farro, S. Gonzales, C. Helms, and K. A. Grant, "Chronic alcohol self-administration in monkeys shows long-term quantity/frequency categorical stability," *Alcohol. Clin. Exp. Res.*, vol. 38, no. 11, pp. 2835–2843, 2014.
- [7] M. Scutari, "Learning Bayesian networks with the bnlearn R package," *J. Stat. Softw.*, vol. 35, no. 3, pp. 1–22, 2010.
- [8] S. Lundberg and S.-I. Lee, "A Unified Approach to Interpreting Model Predictions," 2017, arXiv. doi: 10.48550/ARXIV.1705.07874.
- [9] E. I. Varlinskaya, D. Hosová, T. Towner, D. F. Werner, and L. P. Spear, "Effects of chronic intermittent ethanol exposure during early and late adolescence on anxiety-like behaviors and behavioral flexibility in adulthood," *Behavioural Brain Research*, vol. 378. Elsevier BV, p. 112292, Jan. 2020. doi: 10.1016/j.bbr.2019.112292.

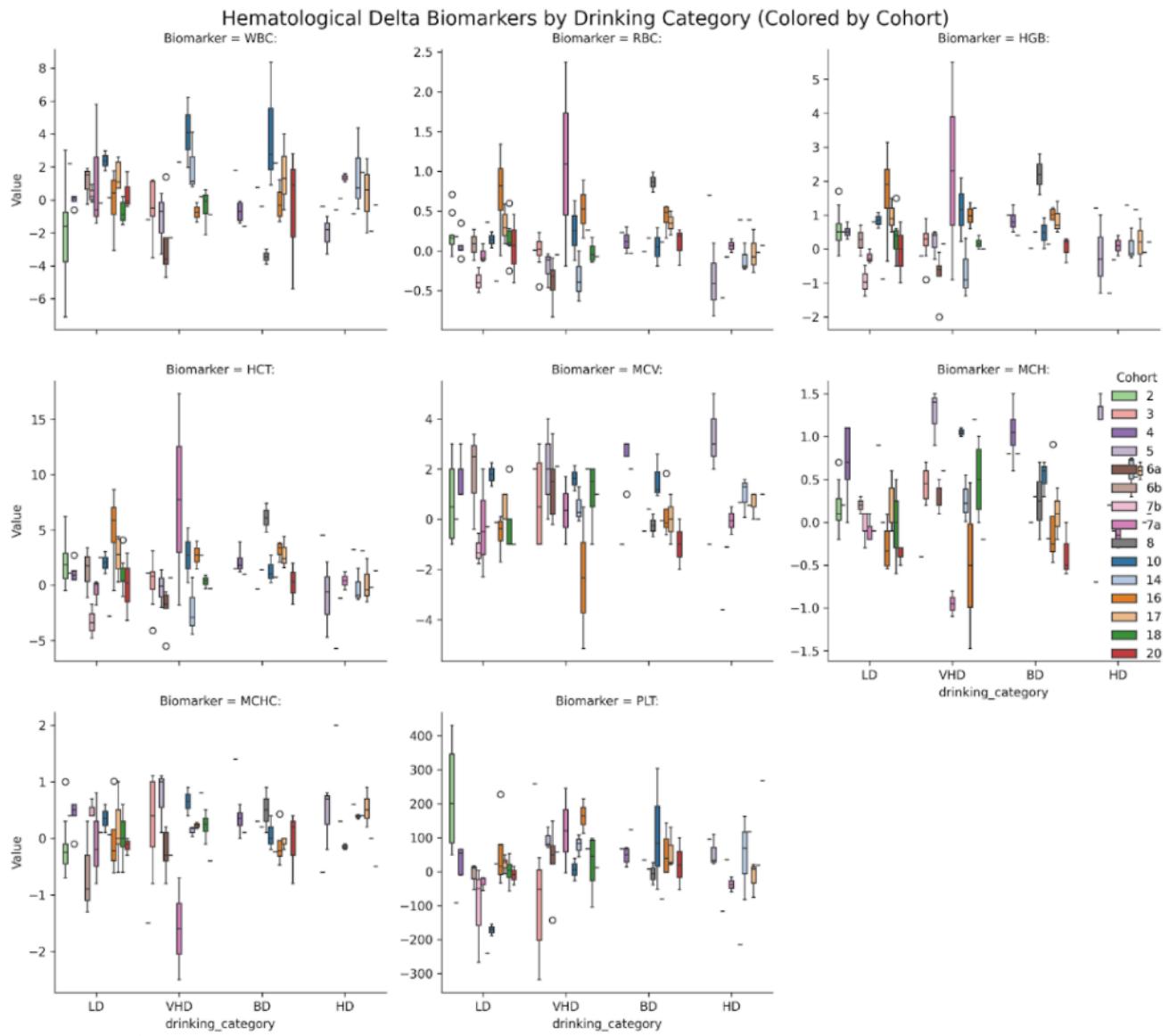


Fig. 3a: Distribution of delta values for biomarkers across drinking categories (Part 1 of 5).

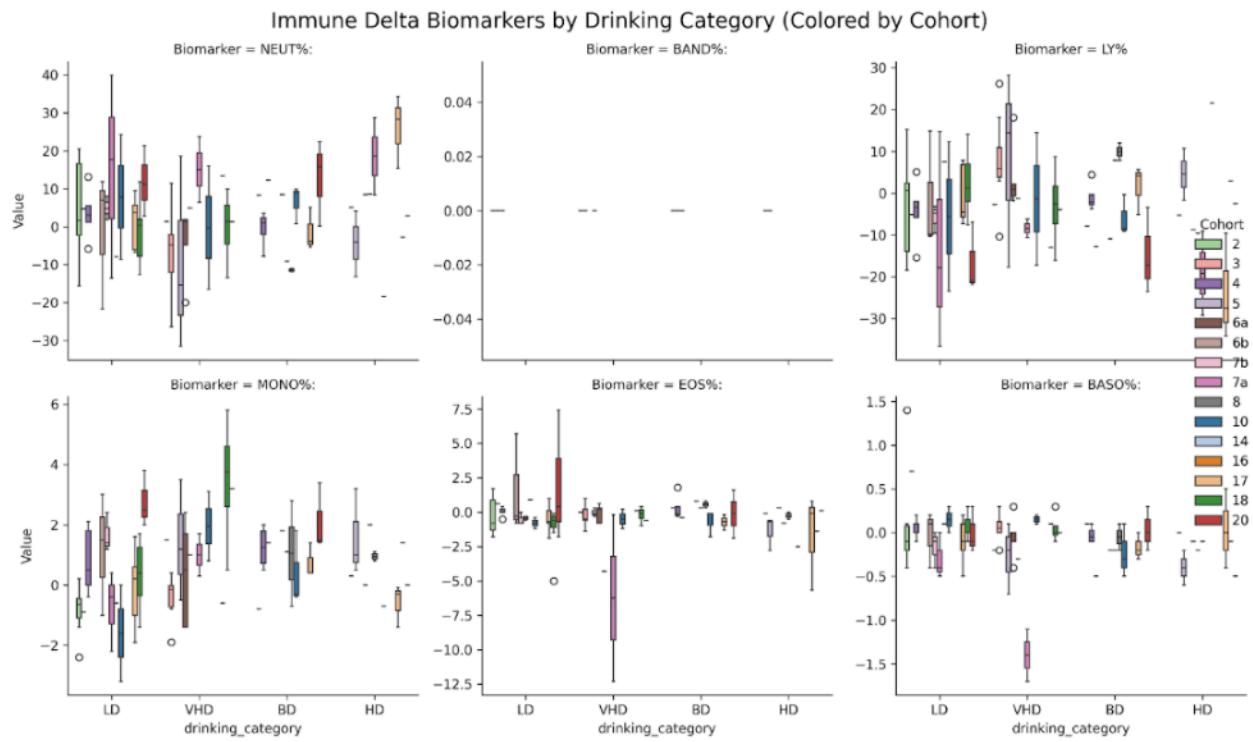


Fig. 3b: Distribution of delta values for biomarkers across drinking categories (Part 2 of 5).

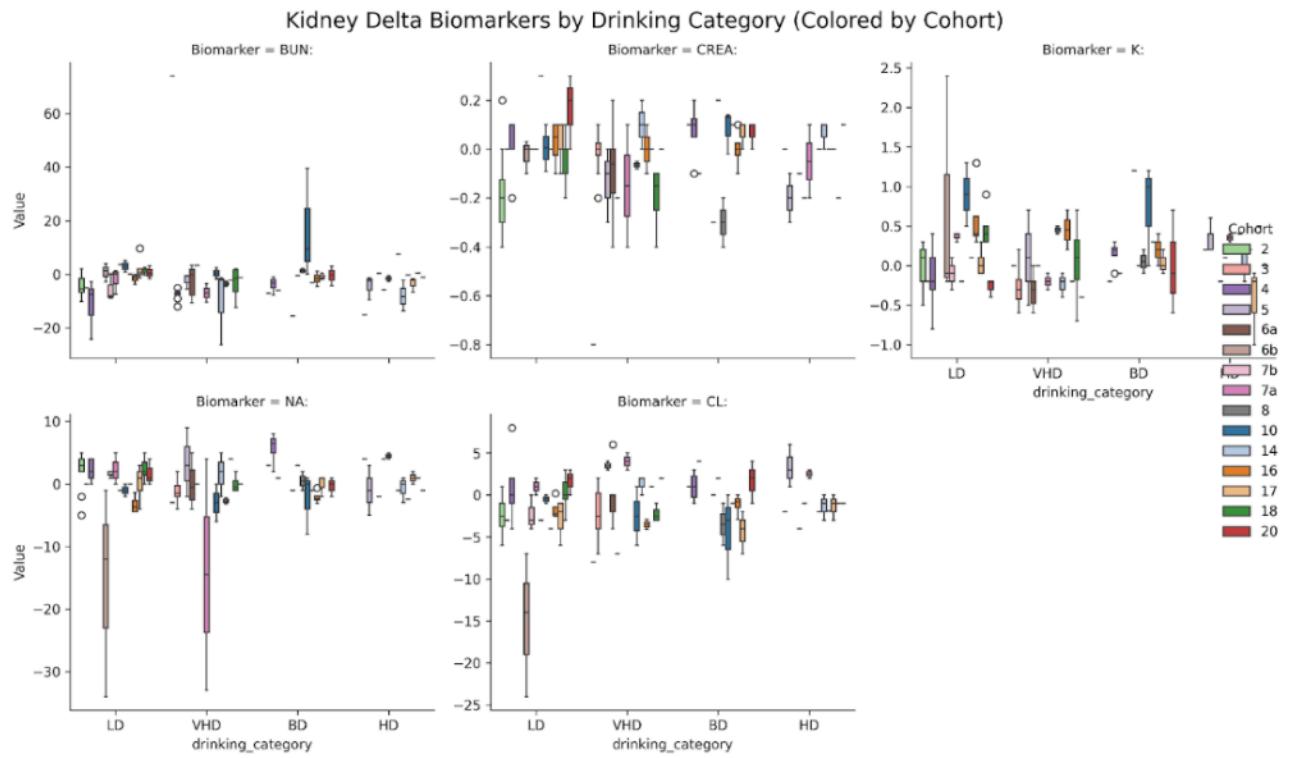


Fig. 3c: Distribution of delta values for biomarkers across drinking categories (Part 3 of 5).

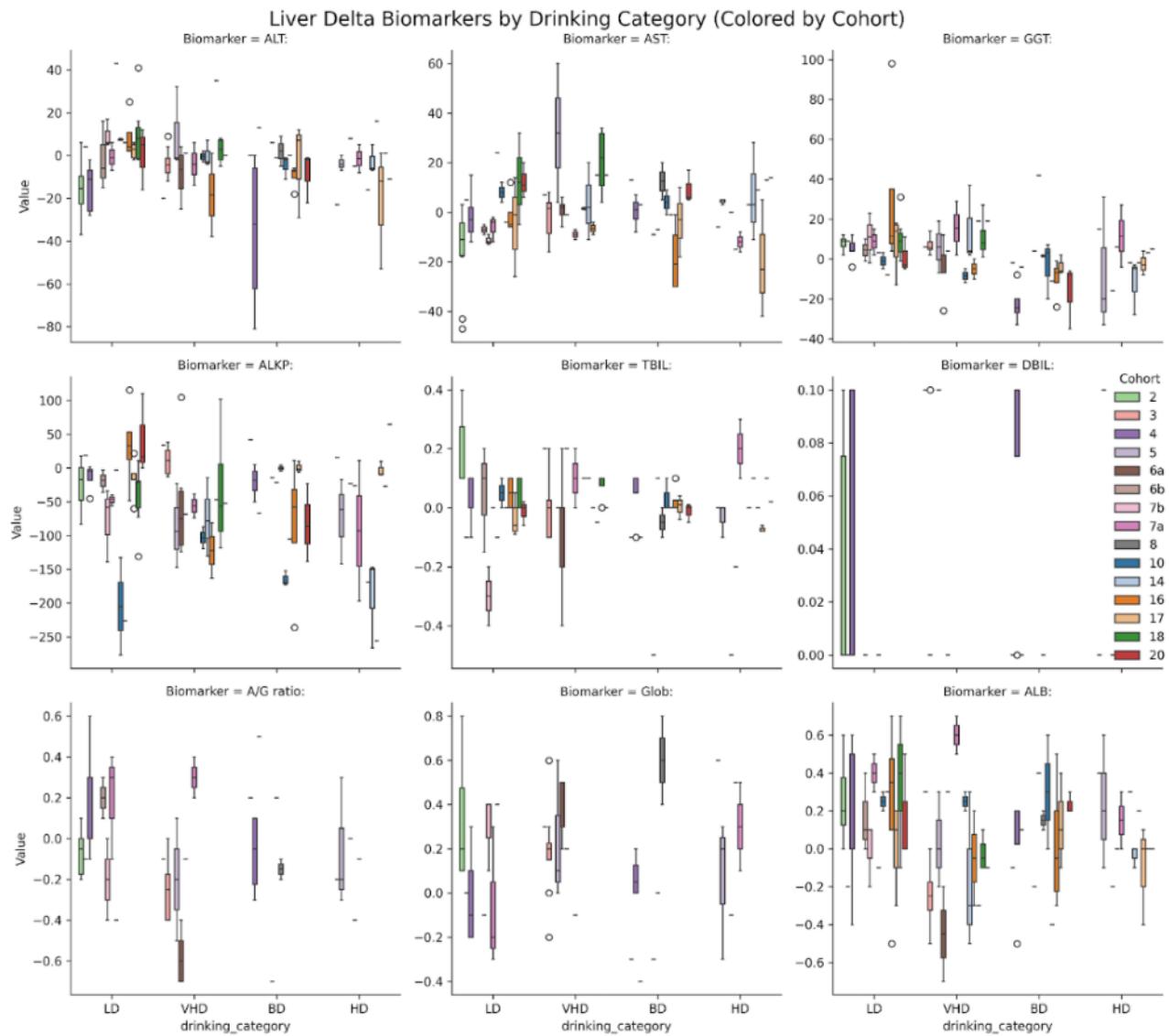


Fig. 3d: Distribution of delta values for biomarkers across drinking categories (Part 4 of 5).

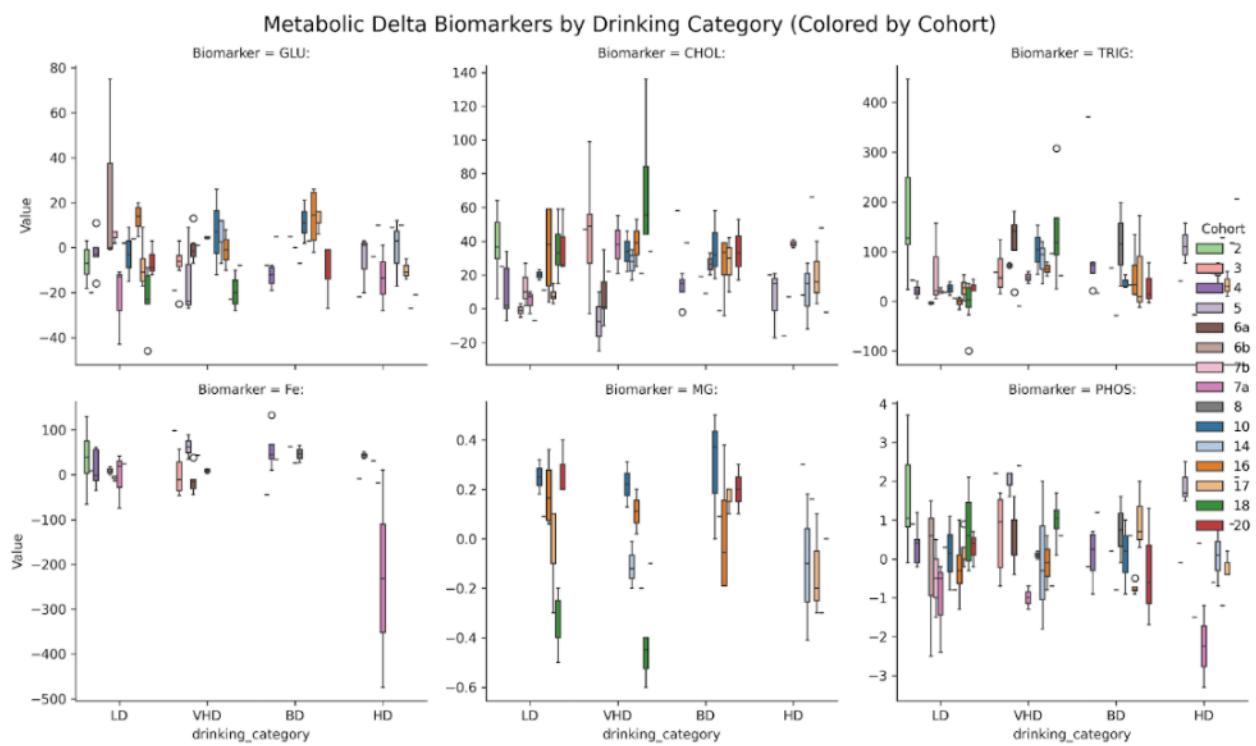


Fig. 3e: Distribution of delta values for biomarkers across drinking categories (Part 5 of 5). These visualization support identification of biomarkers with non-random variance by consumption group.

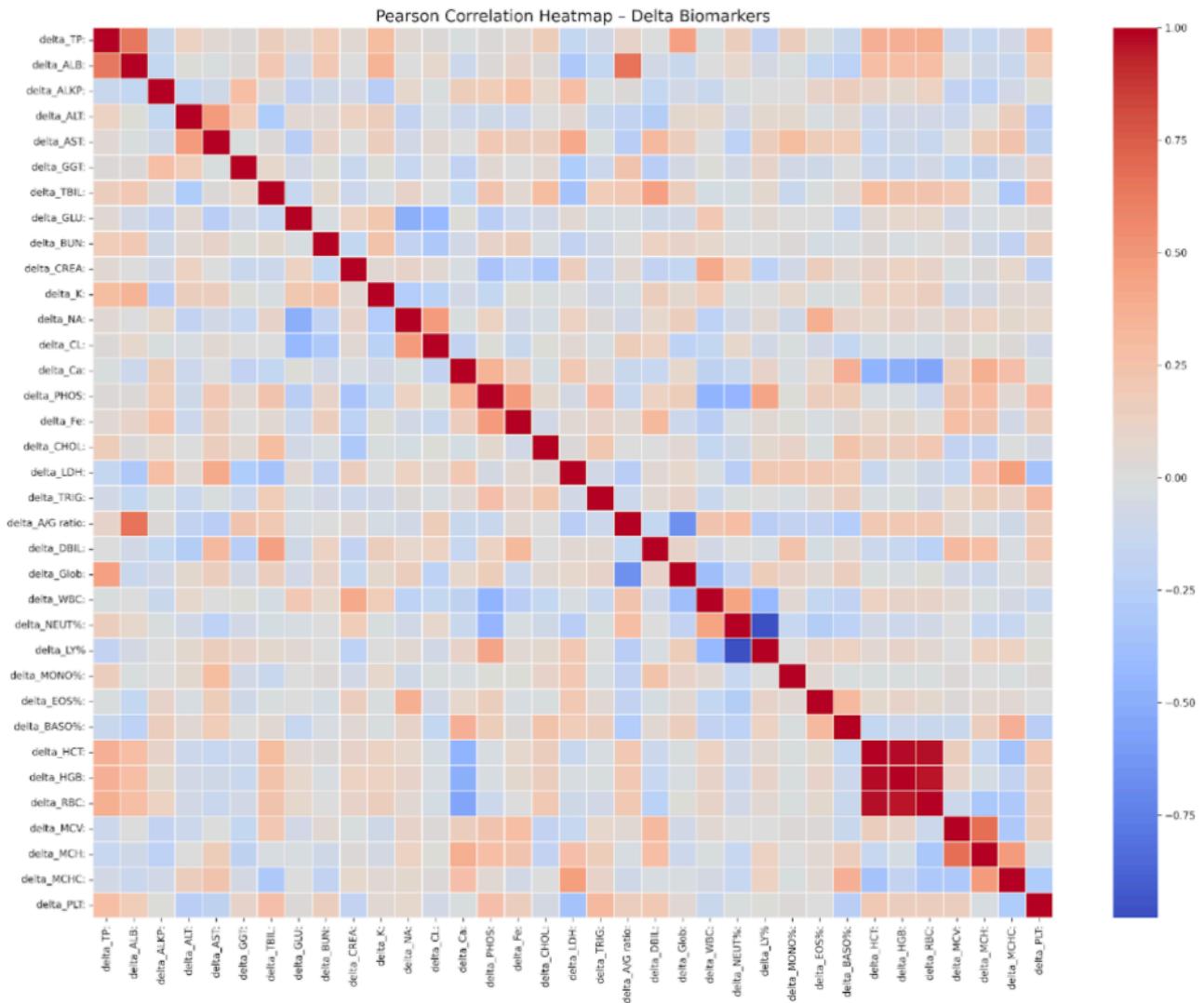


Fig. 4: Pearson correlation heatmap among delta biomarkers. High correlation coefficients (positive or negative) suggest interdependent biomarker changes potentially co-varying with physiological or pathological processes.

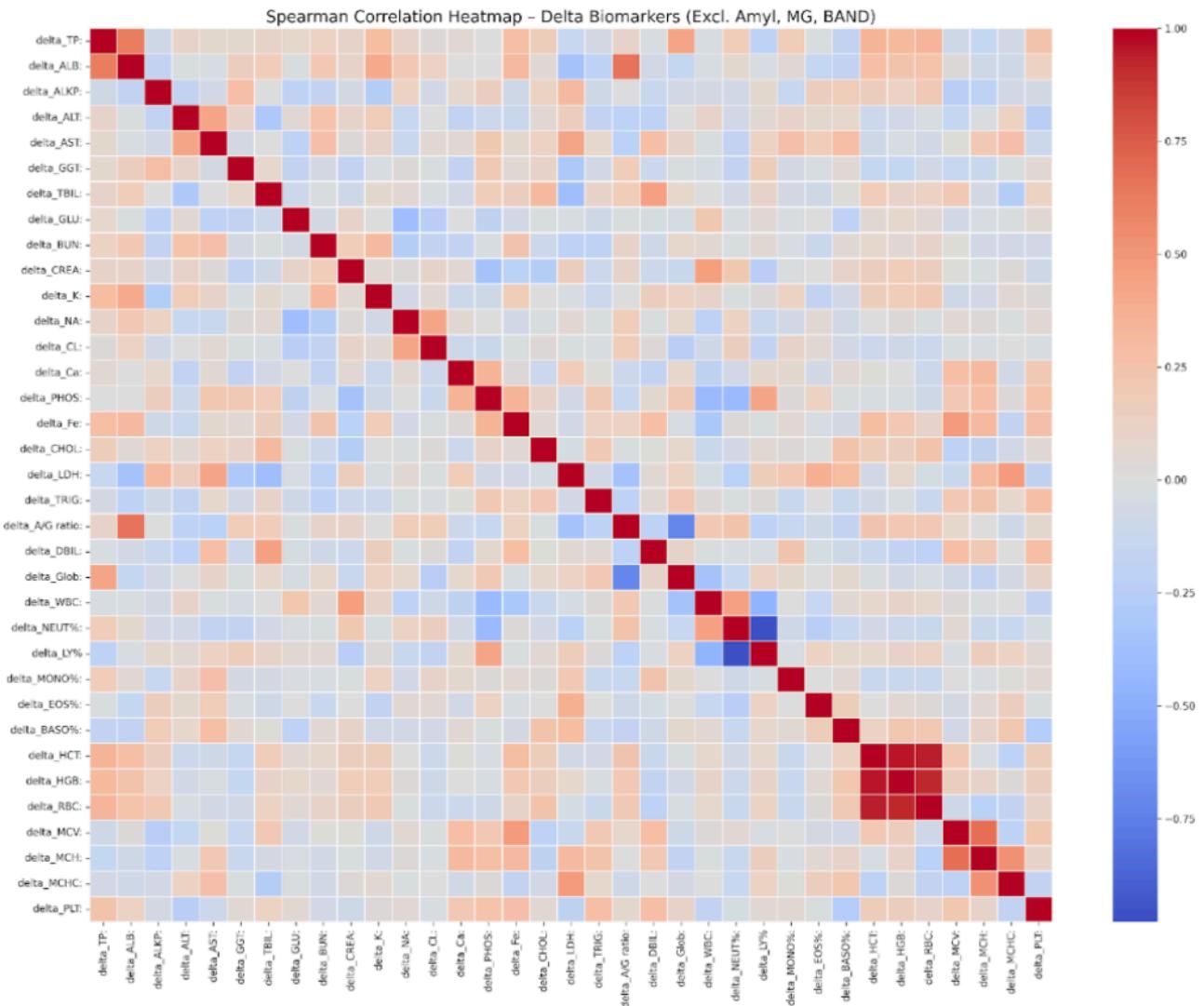


Fig. 5: Spearman rank-based correlation heatmap of delta biomarkers. Unlike Pearson, this method captures monotonic but non-linear relationships and is robust to outliers, helping identify non-parametric associations among biomarkers.

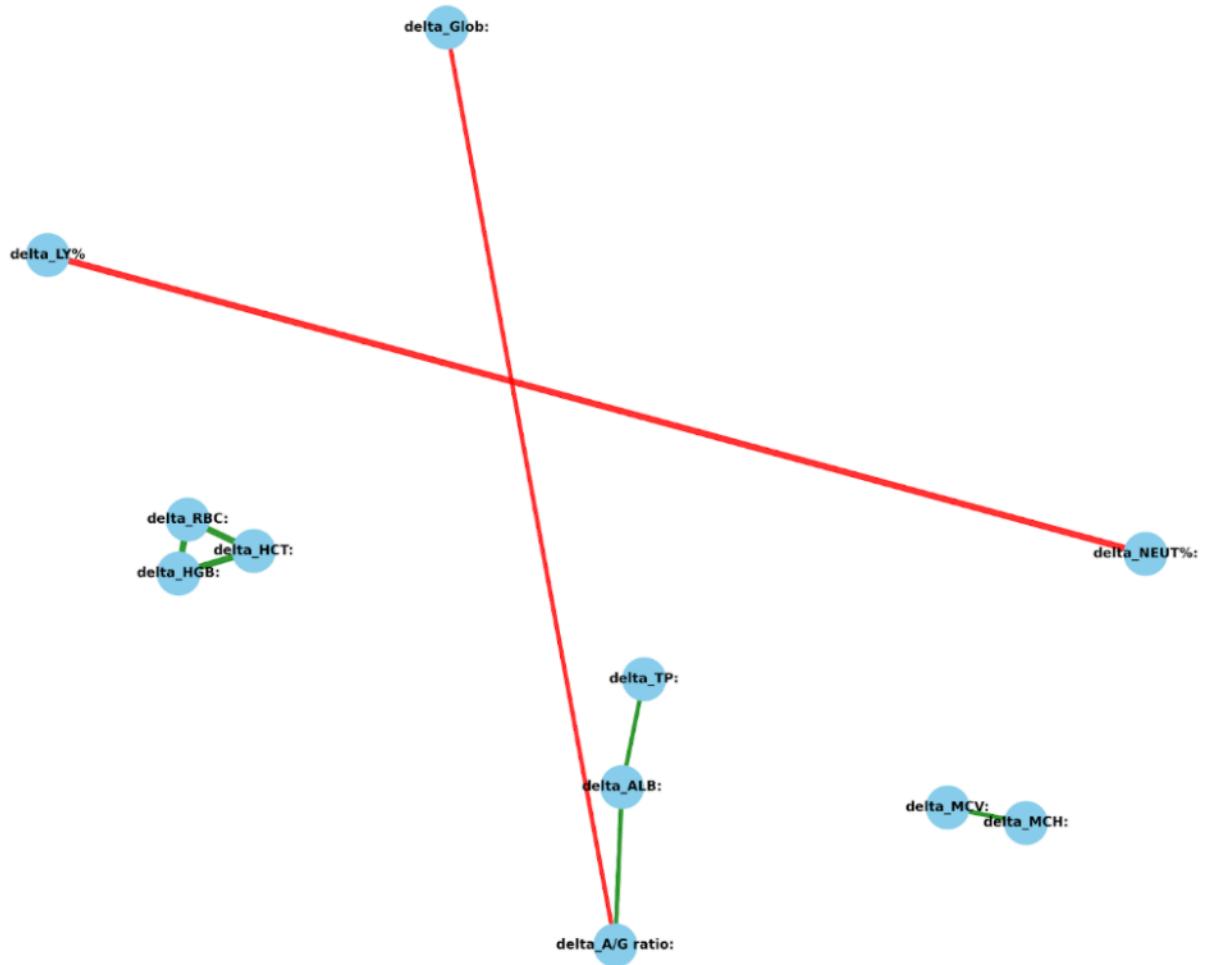


Fig. 6: Network graph showing significant correlations ($|r| \geq 0.6$) between delta biomarkers. Edges indicate strong positive (green) or negative (red) relationships, offering an intuitive overview of co-regulated physiological pathways.

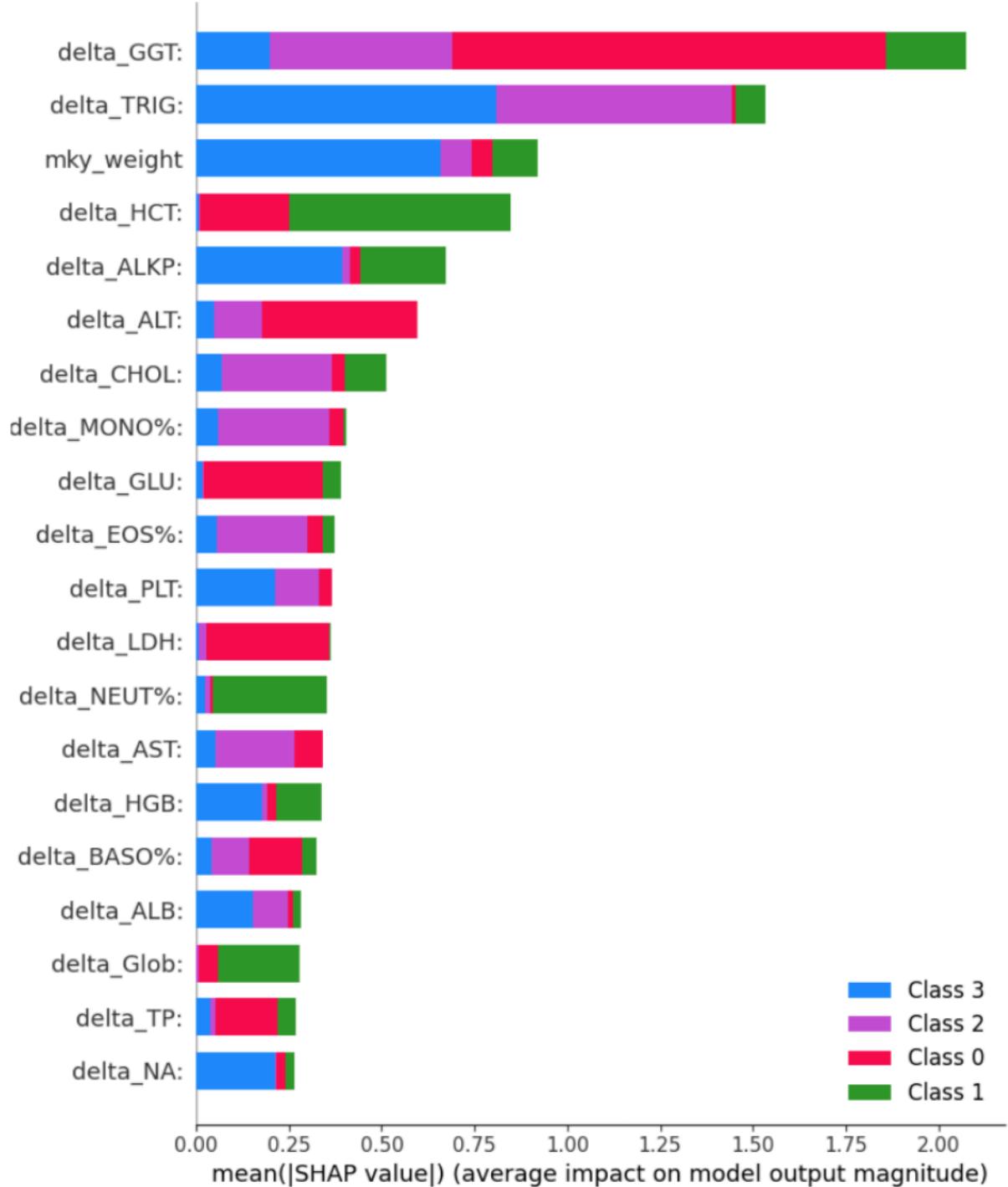


Fig. 7: Global feature importance summary using SHAP values for the XGBoost model. Delta biomarkers and metadata are ranked by their average impact on prediction decisions across all samples.

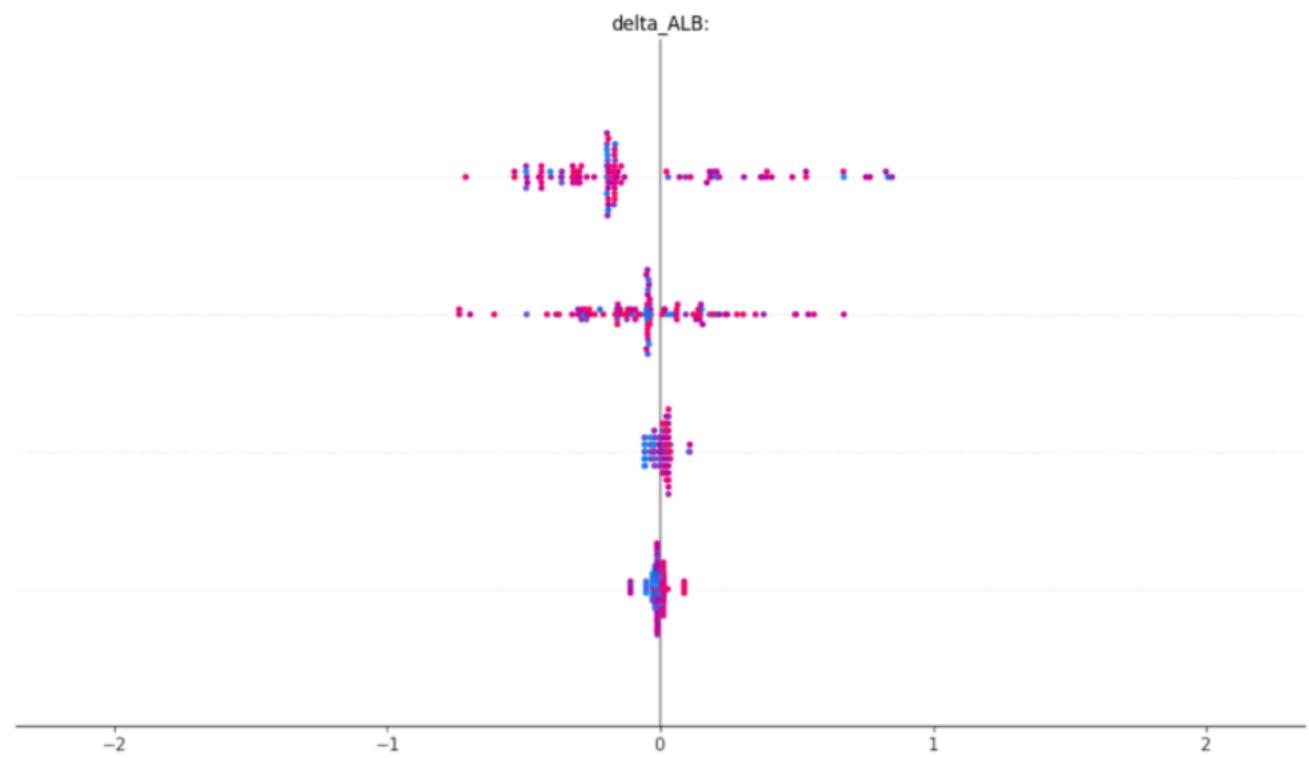


Fig. 8: SHAP value distribution per feature. Each dot reflects the influence of a feature for one individual. Feature values are color-coded, revealing patterns such as directionality and spread of effect.

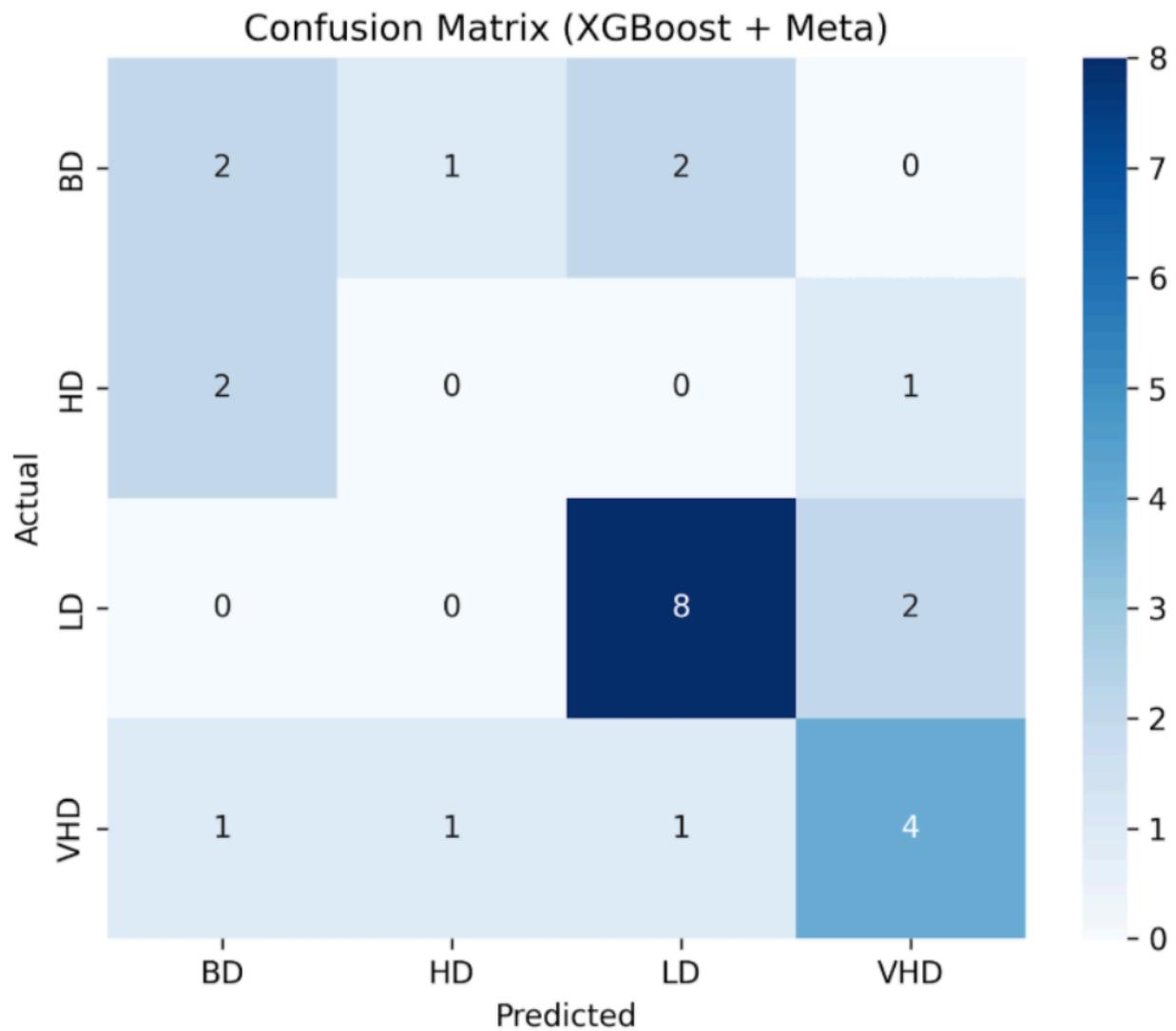


Fig. 9: Confusion matrix for the XGBoost model trained on delta biomarkers and metadata. Cell counts represent the frequency of true vs. predicted drinking categories. Misclassification is most common between adjacent consumption levels, highlighting challenges in class separation.

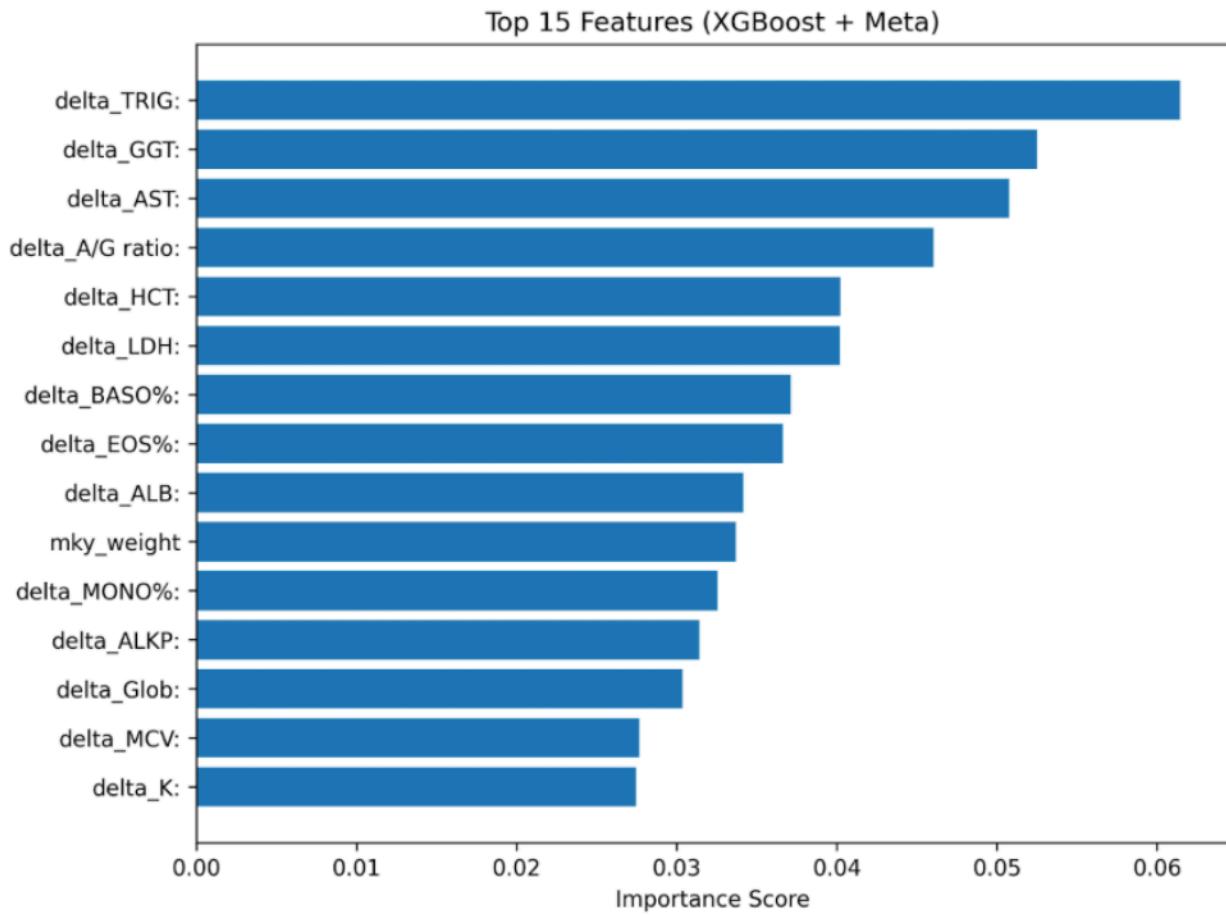


Fig. 10: Top 15 most important features used by the XGBoost model with metadata. Importance reflects each feature's contribution to classification performance. Both delta biomarkers and metadata variables (e.g., weight, gender) emerge as influential predictors.