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Emerging Roles of GC-MS-Detected Aromatic Metabolites in Clinical Diagnostics

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INTRODUCTION & AIM

Microbial metabolites, particularly those derived from gut microbiota, are increasingly recognized as crucial mediators between host and microbial communities. These metabolites, such as phenolic compounds, play significant roles in inflammation and infection development, influencing clinical outcomes in various patient populations, such as those with sepsis [1], post-surgical complications [2], and post-COVID-19 syndrome [3]. Understanding their profiles and functions is essential for advancing personalized medicine and improving diagnostic and prognostic strategies in clinical practice.

Modern studies utilize gas chromatography–mass spectrometry (GC-MS) as a primary tool to quantitatively assess microbial metabolites within blood serum.This technique is prized for its sensitivity, specificity, and reproducibility, allowing for comprehensive detection of both phenolic compounds and dicarboxylic acids. Enhanced analytical power is achieved through careful sample processing, including extraction and derivatization, while advanced multivariate statistical approaches relate metabolite data to patient outcomes.

This research seeks to elucidate the role of gut microbiota–derived metabolites in inflammation and infection development, and to assess their potential as diagnostic and prognostic biomarkers for personalized medical approaches.

Materials and Methods

Study Design and Participants: the research was conducted the Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology. Observational studies encompassed blood serum samples sepsis patients [1], with cardiac surgery patients [2], and post-COVID-19 syndrome individuals [3]. For patients with post-COVID-19 syndrome (n = 39), the median age was 56 (49–71) years, of which 8 (28%) were men [3]. Infectious complications were studied in 62 patients of Post-Operative Complications after Cardiac Surgery were included in the study, [2].The median (interquartile range 25, 75%) age was 62 (57, 68) years. The study of biotransformation of metabolites included 10 patients (9 men and 1 woman) [1]. The mean age of the patients was 43 (34–60) years.

GC-MS Analytical Methods: Metabolite quantification utilized gas chromatography-mass spectrometry with electron ionization following trimethylsilyl derivatization to enhance volatility of polar compounds. Gas chromatography-mass spectrometry (GC-MS) analyses using a Trace GC 1310 gas chromatograph and ISQ LT mass spectrometer from the Thermo Electron Corporation (Santa Clara, CA, USA) were conducted to measure the concentration of various aromatic and dicarboxylic acids (benzoic acid—BA, phenylacetic acid—PhAA, phenylpropionic acid—PhPA, phenyllactic acid—PhLA, 4-hydroxybenzoic acid—p-HBA, 4-hydroxyphenylacetic acid—p-HPhAA, 4-hydroxyphenylpropionic acid—p-HPhPA, homovanillic acid—HVA, 4-hydroxyphenyllactic acid—p-HPhLA, succinic acid—SA, and fumaric acid—FA).

Statistical Analysis: Metabolite concentrations were compared between groups using Mann-Whitney U tests and multivariate analysis including principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) for post-surgical complications [2]. The Shapiro–Wilk test was used to assess the normality of the data distribution, and it was revealed that all quantitative indicators of parametric comparison criteria were inapplicable due to the small number of outcomes, and the comparative intergroup analysis was carried out using nonparametric statistics. Independent group differences were explored using the Mann–Whitney U test. The Wilcoxon signed-rank test was used to compare two related groups for post-COVID-19 syndrome patients [3]. The T-Wilcoxon test was used for comparison between groups of paired samples. The Mann-Whitney test was used for between-group comparisons of independent samples for patients with sepsis [1].

RESULTS & DISCUSSION

A study published in [1] investigated septic patients and compared the biotransformation of aromatic microbial metabolites between the gut microbiota of septic patients (pathobiota) and healthy individuals (normobiota). We found that the normal microbiota could biotransform sepsis-associated aromatic metabolites such as phenyl lactic acid (PhLA) and 4-hydroxyphenyl lactic acid (4-HPhLA), whereas the microbiota from septic patients lost this function. The proportion of sepsis-associated aromatic metabolites in patients with sepsis was significantly higher than normal and significance of 40%, while in donors it did not exceed 5% figure1.

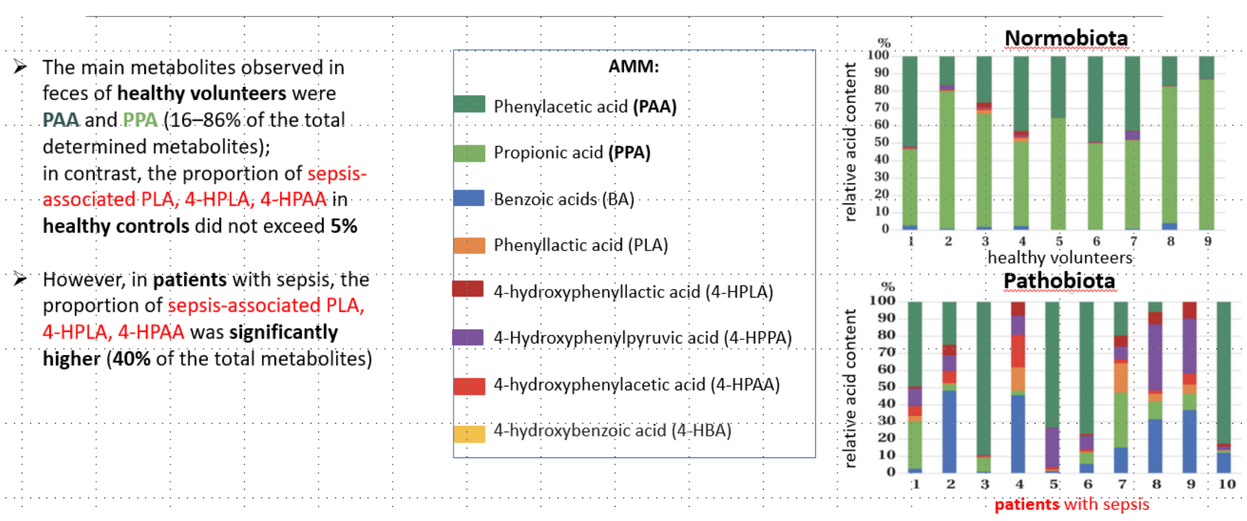

Figure 1 Composition of Aromatic Microbial Metabolites in Samples

This loss of biotransformation capacity leads to the accumulation of potentially harmful metabolites, correlating with organ dysfunction and increased mortality risk. Therefore, gut microbiota dysfunction is crucial in sepsis pathogenesis. In patients with sepsis, the levels of phenyllactic (p=0,002) and 4-hydroxyphenyllactic (p=0,001) in the intestine were statistically significantly higher compared to healthy volunteers (pathobiota vs normobiota) figure 2.

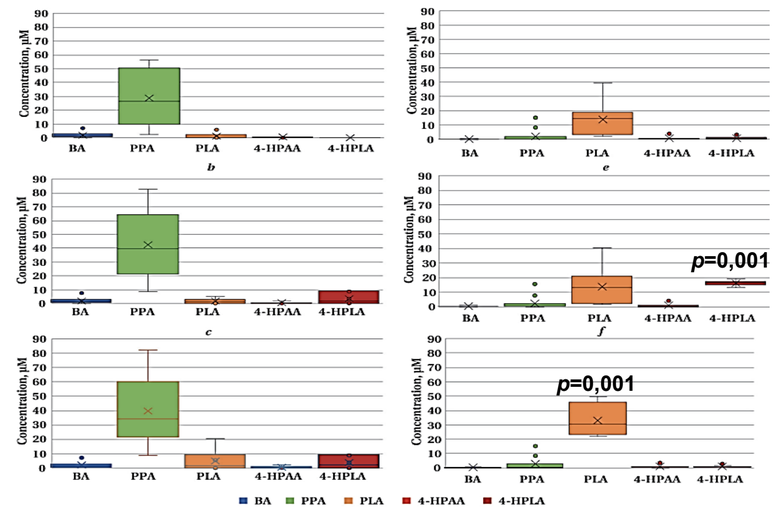

Figure 2. The Levels of Microbial Metabolites of Aromatic Amino Acids

Patients after cardiac surgery [2] exhibit altered microbial and mitochondrial metabolite levels, for example, higher concentration of sum of sepsis- associated metabolites, and succinic and fumaric serum concentrations than healthy individuals. Multivariate prognostic model, predicting the presence or absence of all types of post-operative complications that was built using all data (clinical data, concentrations of metabolites and biomarkers) demonstrated ROC-AUC of 0.85 with 81 % sensitivity and 79% specificity table 1 [2].

Table 1. Characteristics of multi- and univariate predictive models for the post-operative cardiac complications.

Models	ROC-AUC, 95% CI	Sensitivity, 95% CI	Specificity, 95% CI
Multivariate models			
All data	0.85 (0.78, 0.92)	0.81 (0.77, 0.85)	0.79 (0.75, 0.83)
Clinical data	0.80 (0.71, 0.89)	0.66 (0.63, 0.69)	0.78 (0.72, 0.84)
olites and biomarkers	0.71 (0.56, 0.86)	0.72 (0.69, 0.75)	0.47 (0.43, 0.51)
Univariate models			
SOFA	0.79 (0.73, 0.85)	0.67 (0.62, 0.72)	0.84 (0.75, 0.93)
ΔΣ3AMM	0.70 (0.62, 0.78)	0.59 (0.55, 0.73)	0.79 (0.75, 0.83)
Lactate	0.62 (0.42, 0.82)	0.74 (0.66, 0.82)	0.61 (0.56, 0.66)
Δp-HPhLA	0.58 (0.47, 0.69)	0.85 (0.80, 0.90)	0.48 (0.41, 0.55)
EuroScore2	0.54 (0.38, 0.70)	0.49 (0.3, 0.55)	0.63 (0.59, 0.67)

Post-COVID-19, patients [3] In post-COVID-19 syndrome patients, microbial metabolite patterns reflected ongoing inflammation and gut dysbiosis. For example, serum levels of 4-hydroxybenzoic, succinic, and fumaric acids figure 3, which were increased in patients on the day of admission for the non-specific rehabilitation program, did not reach the level of healthy people providing insights for targeted interventions.

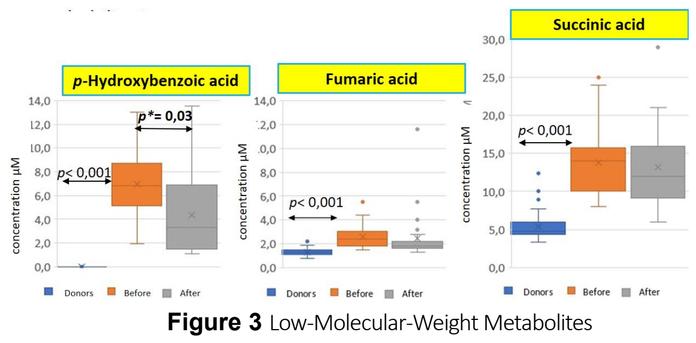

Figure 3 Low-Molecular-Weight Metabolites

CONCLUSION

The integrated data from these studies emphasize the critical role of microbial metabolites across diverse post-infectious and critical illness conditions. Persistent metabolite alterations in post-COVID-19 Syndrome illustrate the long-term impact of viral infection on gut microbial metabolism. In cardiac infectious complications, microbial metabolites actively modulate vascular inflammation, influencing recovery and complications after surgery. In sepsis, loss of microbial metabolic capacity exacerbates systemic toxicity. These findings collectively advocate for microbiota-focused diagnostic and therapeutic strategies, including metabolite profiling and microbiome modulation, to severity disease burden and improve patient prognosis.

FUTURE WORK / REFERENCES

[1] Chernevskaya, E. *et al.* Sepsis-Associated Metabolites and Their Biotransformation by Intestinal Microbiota. *General Reanimatology,* 2023, 19, 4-12. https://doi.org/10.15360/1813-9779-2023-6-4-12.
[2] Meinarovich, P. *et al.* An Integrated Approach Based on Clinical Data Combined with Metabolites and Biomarkers for the Assessment of Post-Operative Complications after Cardiac Surgery. *Journal of Clinical Medicine,* 2024, 13, 5054. https://doi.org/10.3390/jcm13175054.
[3] Sorokina, E. *et al.* Promising Markers of Inflammatory and Microbiota Disorders in Patients with Post-COVID-19 Syndrome. *Journal of Personalized Medicine,* 2023, 13, 971. https://doi.org/10.3390/jpm13060971.