ARTICLE

Serum NMR metabolomics in distinct subtypes of hematologic malignancies

Ayse Zehra Gul[a]*, Sahabettin Selek[a], Somer Bekiroglu[b], Metin Demirel[a,c], Fatma Betul Cakir[d], and Bulent Uyanik[e]

[a]*Department of Medical Biochemistry, Bezmialem Vakif University Faculty of Medicine, Istanbul, Turkey;* [b]*National Biological and Chemical Test Center, TÜBİTAK Marmara Research Center, Gebze, Kocaeli, Turkey;* [c]*Health Sciences Institute, Bezmialem Vakif University, Istanbul, Turkey;* [d]*Department of Pediatrics, Bezmialem Vakif University Faculty of Medicine, Istanbul, Turkey;* [e]*Department of Medical Genetics Department, Bezmialem Vakif University Faculty of Medicine, Istanbul, Turkey*

Hematologic malignancies encompass a diverse array of subtypes, contributing to substantial heterogeneity that poses challenges in predicting clinical outcomes. Leveraging the capabilities of nuclear magnetic resonance holds substantial promise in the detection of serum biomarkers and individual metabolic alterations in patients. This study involved the analysis of the sera from patients with acute myeloid leukemia, chronic lymphocytic leukemia, and non-Hodgkin lymphoma to investigate the affected metabolites and their associated pathways. The quantitative one-dimensional (1D) 1H nuclear magnetic resonance method was employed to identify alterations. Metabolite annotations were validated using two-dimensional (2D) analyses. Discriminating chemometric models and receiver operating characteristic curves were created using the MetaboAnalyst platform. The findings revealed significant alterations in the serum levels of amino acid catabolism products, citrate cycle intermediates, and phospholipids. The acute myeloid leukemia group showed differences in glucogenic amino acids related to the glycolysis pathway, whereas the chronic lymphocytic leukemia and non-Hodgkin lymphoma groups displayed variances in fumarate and acetate levels linked to the citrate cycle pathway. In the leukemia groups, higher levels of products from the protein degradation pathway were observed. The biomarker panels for each malignancy group exhibited outstanding discrimination from controls. Healthy individuals differed distinctly from patients, indicating commonly observed metabolic adaptation patterns among frequent hematologic malignancies. The small cohort study using nuclear magnetic resonance metabolomics in various hematologic malignancy subtypes revealed significant changes in serum amino acid and protein degradation end-product levels, suggesting prolonged leukocyte lifespan and increased energy demand.

HIGHLIGHTS

- Metabolomics tools have allowed for global serum profiling of different hematologic malignancy subtypes.
- Serum levels of amino acid catabolism products, citrate cycle intermediates, and phospholipids were altered in patients with AML, CLL, and NHL.
- Patients with hematologic malignancy showed increased lifespan and energy demand of leukocytes, indicating universal metabolic adaptation patterns of tumor formation.

Hematologic malignancies (HMs) make up the fourth most common type of cancer, accounting for 9.4% of all cancer-related deaths [1]. Stemming from abnormal blood cell growth, these conditions vary in age of onset and clinical presentations for affected individuals. The most essential aspects of cancer formation include genetic predisposition, molecular anomalies, and epigenetic regulation of specific genes. However, the complex organizational structure of white blood cells and their rapid turnover make it difficult to track the origin of carcinogenic cells and identify etiological causes.

Patients with HM with similar mutational backgrounds may have different clinical presentations and cannot be grouped into a common subclass [2]. In such cases with the high complexity across disease subgroups, using the personalized medicine approaches has an immense potential to reveal individual driving forces leading to outcome. Metabolomics applications in identifying precise therapeutic targets can address subgroup heterogeneity by exploring bioactive molecule interactions in both healthy and diseased states. Rationally designed studies limiting the confounding factors may yield valuable information about individuals at the intersect of genomic and epigenetic exposure [3].

Address correspondence to Ayse Zehra Gul, Department of Medical Biochemistry, Bezmialem Vakif University Faculty of Medicine, 34093 Istanbul, Turkey; E-mail: aysezehragul@gmail.com

0301-472X/

https://doi.org/10.1016/j.exphem.2025.104710

The biomarker research has kept its crucial place at the center of metabolomics studies to advance early diagnosis and to define phenotypic signatures. Discovering biomarkers and unknown molecules in diseases, nuclear magnetic resonance (NMR) and mass spectrometry (MS) provide high-throughput data for heuristic metabolomics. MS enables ultrasensitive detection of metabolites in complex biological samples, facilitating the identification of trace molecules in clinical specimens. In parallel, NMR provides reproducible results with ease of use and exceptional instrumental stability [4].

The NMR metabolomics study aimed to compare serum biochemistry in patients with non-Hodgkin lymphoma (NHL), chronic lymphocytic leukemia (CLL), and acute myeloid leukemia (AML) for a personalized characterization of the phenotypic landscape. Using robust NMR methodology, discriminant metabolites, along with their associated metabolic pathways and biomarker models, were identified for each HM group. Integrating NMR methodology significantly elevates laboratory practices in the growing field of metabolomics, resulting in an efficient workflow that enhances research outcomes.

METHODS

Patient Recruitment and Sample Collection

Experiments were conducted at the TÜBİTAK Marmara Research Center with the approval of the Bezmialem Vakif University (BVU) Ethics Committee (No: 2021/391). All participants were given informed consent to the clinical study following the Declaration of Helsinki protocol.

Each group had 30 individuals considering pilot trials and studies involving same malignancy subgroups [5]. Fasting serum samples were collected from individuals following a regular diet. To prevent contamination, serum specimens were analyzed for hemolysis, icterus, and lipemia (HIL) indices by spectrophotometric assessment, and any samples exhibiting these incidents were excluded. Inclusion criteria involved initial diagnosis of patients with HM on their active disease phase with no previous chemotherapy treatment. The exclusion criteria included the absence of drug or smoking use, secondary tumors, malnutrition, or chronic inflammatory disease.

Sample Preparation

One milliliter of each serum sample was mixed with 1:2 (vol/vol) ultrapure methanol and centrifuged for 1.5 hours at 10,000 *g* force to remove protein precipitate [6]. The collected supernatant was left for evaporation under vacuum concentrator until total dryness. A total of 600 μL NMR buffer (60 mM sodium phosphate, pH = 7.4) and 100 μL sodium trimethylsilylpropanesulfonate (1.75 mM, in D_2O) were added to the pellet. The resulting mixture was transferred to 5-mm NMR tubes.

NMR EXPERIMENTS

One-dimensional (1D) and two-dimensional (2D) experiments were recorded with 700 MHz Bruker AVIII instrument equipped with a QCI cryoprobe. 1D NOESY pulse sequence with a 2-sec presaturation was used for quantitative ^{1}H NMR (qNMR) spectra acquisition (128 scans, 64 data collections, 4 sec acquisition time, 3 seconds relaxation delay, 0.2 sec mixing time, and 101 receiver gain).

The peak assignment was carried out using the 2D experiment data (heteronuclear single quantum coherence [^{1}H-^{13}C HSQC] and total correlated spectroscopy [^{1}H-^{1}H TOCSY]) and the Human Metabolome Database. 2D methods were performed on serum pools obtained by extracting each sample from experimental groups [7]. The spectral widths were set as to 11.7 and 185 ppm for the 1H (F2) and 13C (F1) dimensions in HSQC and to 11.7 and 12.0 ppm in TOCSY, with 256 increments recorded for each spectrum. Sixty-four scans with 1.5 sec relaxation delay in HSQC and 48 scans with 2 sec in TOCSY experiments were applied. The mixing time was set to 80 msec in TOCSY.

The resulting spectra (Topspin ASCII) were transferred, and assignments were confirmed by reference to the online database COLMARm using default peak picking/ fitting/ chemical shift difference cutoff parameters. 2D-authenticated metabolites were quantitatively analyzed with the Chenomx NMR Suite based on the integrality of internal standard (DSS). Sixty metabolites from various chemical classes were extracted for statistical analysis (Supplementary Table E2).

Spectra Preprocessing

Fourier transformation, baseline correction, and peak alignment steps were performed in TopSpin (v. 4.1.3) and Chenomx NMR Suite (v.9.02). The line broadening was set to 0.3 Hz with the zero-filling factor to 2 (132 K data points). Chemical shift frequencies of the analytes were calibrated using DSS set to 0.0 ppm. Quality control criteria were met by visually inspecting the spectra of serum pool samples and referencing the lactate signal at $\delta = 1.33$ ppm. Data were autoscaled, and normal distribution on metabolite intensities was achieved after the log-transformation.

Statistical Analysis

Univariate statistics of clinical parameters and NMR metabolite concentration values were performed using SPSS (version 27.0). Multivariate statistical analysis was conducted using the MetaboAnalyst R package on the NMR data. The Student *t* test was applied to define the significance values. Principal component analysis (PCA) was performed to determine distribution trends of samples between groups and to exclude extreme values. Analytes with variable significance score (VIP) above 1 were selected and enhanced models covering these were created with the partial least squares regression discriminant analysis (PLS-DA). VIP-PLS-DA model performance validation was demonstrated by cross-validated analysis of variance (CV-ANOVA) fitting degree values (Supplementary Table E6). Biomarker models were built in the receiver operating characteristic (ROC) curves of the VIP-PLS-DA model. Pathway analysis was conducted using the KEGG database (MetaboAnalyst) in each HM compared with controls.

RESULTS

Because of sample loss during preparation, the analysis involved 29 patients with AML, 30 patients with CLL, and 27 patients with NHL with 30 control individuals. The sociodemographic data are presented in Supplementary Table E1a.

The results of the correlation analysis between metabolites and clinical data are presented in Supplementary Table E5. Patients with AML with a complex karyotype showed strong positive correlation with the European Leukemia Net risk score. In both the CLL and

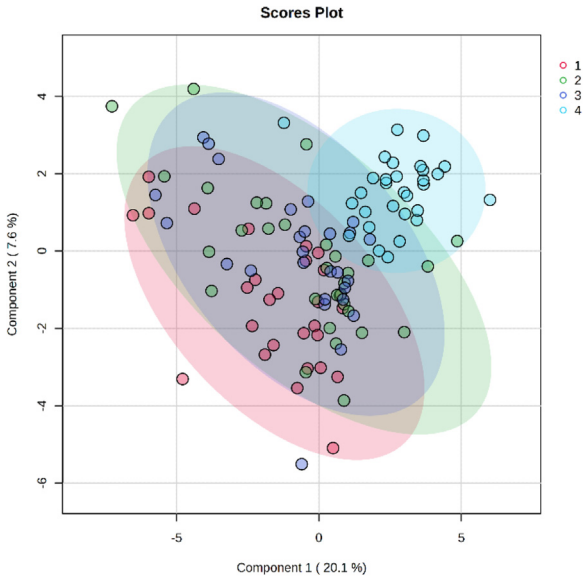

Figure 1 The all-groups PLS-DA plot demonstrates a clear separation between the HM groups and the controls. (1) AML; (2) CLL; (3) NHL; (4) controls.

NHL groups, the International Prognostic Index (IPI) risk score demonstrated a strong positive correlation with clinical stage. In alignment with existing literature, patients with NHL exhibiting elevated lactate dehydrogenase (LDH) enzyme levels showed a significant positive correlation with the IPI risk score.

The PLS-DA analysis in all groups indicates a clear distinction between the HM groups and the control group (Figure 1, Supplementary Table E6). The significant metabolites are shown in Supplementary Table E3 according to the post hoc analysis (Tukey's honestly significant difference [HSD]).

Comparing the HM groups, the Student *t* test results revealed no significant differences in serum molecules between patients with AML-NHL and CLL-NHL. 3-Hydroxyisovalerate stands out as the only differential molecule between the AML and CLL groups (Figure 2).

Compared with the control group, the serum NMR analysis revealed increased levels of methyl guanidine, glycine, and urea in the AML and CLL groups. Betaine levels were elevated in patients with CLL and NHL. There were additional increases in creatinine for CLL and in creatine, glutamine, and succinate for patients with NHL. Pyroglutamate was commonly decreased in all HM groups, whereas fumarate and acetate were lower in CLL and NHL groups. Acetoacetate and phosphoethanolamine (PEA) were decreased in NHL, whereas alanine, lactate, and 3-hydroxyisovaleric acid were decreased in AML. The level of 3-hydroxy-3-methylglutarate was reduced in sera of both patients with AML and patients with NHL (Table 1).

As expected, serum NMR analysis resulted in diagnostic biomarker models that showed outstanding discrimination. The models included the top 10 metabolites for AML, 3 for CLL, and 20 for NHL (Figures 3, 4, and 5). To ensure the actual performance, these models must be tested against an independent group of new samples.

DISCUSSION

Despite their complex subgroups and diverse clinical characteristics, HMs exhibit similar metabolic changes related to hematopoiesis [8]. The adaptation processes commonly observed may reflect the general dynamics of oncogenic blasts and white blood cells. In this context, the current study findings pertain to the universal changes observed in many tumors. These changes involve essential metabolic functions that rely on specific analytes for cell division and survival, increased energy demand in cancer cells, defense mechanisms against reactive oxygen species (ROS), and the production of angiogenesis-inducing substances [9]. The serum NMR study showed common altered metabolic pathways in individuals with HMs compared with controls, including glycolysis/gluconeogenesis; alanine, aspartate, and glutamate metabolism; glycine, serine, and threonine metabolism; pyruvate metabolism; arginine metabolism; glutathione (GSH) metabolism; and purine metabolism [10]. It is intriguing to note that for the CLL and NHL groups, the citrate cycle and tyrosine

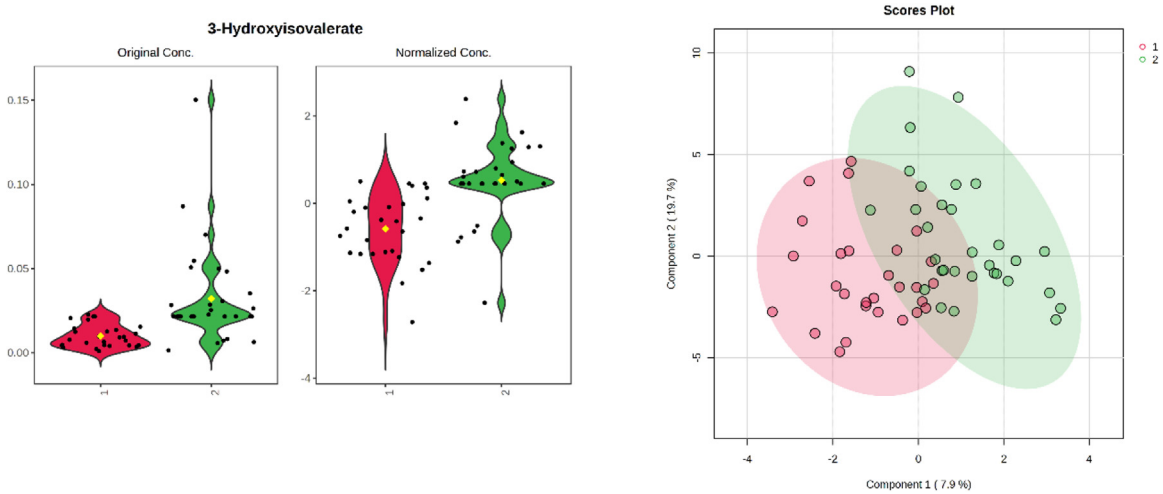

Figure 2 3-Hydroxyisovalerate as a distinct serum metabolite in differentiating between patients with AML and CLL (upper left); the PLS-DA model of the comparison (upper right). (1) AML; (2) CLL.

Table 1 Significant serum NMR metabolites in each HM/C comparisons ($p < 0.05$).

	t statistics	*p* value	−log10(*p*)	FDR
AML				
Pyroglutamate	−5.6974	0.000000546	6.2626	0.0000268
3-Hydroxyisovalerate	−5.4709	0.00000124	5.9066	0.0000304
Alanine	−4.6229	0.0000247	4.6081	0.000403
Methyl guanidine	4.4097	0.0000509	4.293	0.000624
3-Hydroxy-3-methylglutarate	−4.0098	0.000191	3.7181	0.001876
Isobutyrate	3.4213	0.001208	2.9181	0.009862
Glycine	2.9799	0.004345	2.362	0.029836
Lactate	−2.9388	0.004871	2.3124	0.029836
Urea	2.8205	0.006733	2.1718	0.036655
CLL				
Fumarate	−8.9568	0.00000000000212	11.674	0.000000000108
Pyroglutamate	−4.4355	0.0000434	4.362	0.000945
Betaine	4.2895	0.0000715	4.146	0.000945
Methyl guanidine	4.2788	0.0000741	4.1302	0.000945
Urea	4.1639	0.000109	3.9624	0.001112
Glycine	3.2232	0.002116	2.6746	0.017983
Creatinine	2.8628	0.005898	2.2293	0.042913
Acetate	−2.8144	0.006732	2.1719	0.042913
NHL				
Acetate	−6.8869	0.00000000686	8.1638	0.000000398
Fumarate	−5.1324	0.00000416	5.381	0.000121
Acetoacetate	−4.7169	0.0000178	4.7488	0.000345
Pyroglutamate	−3.9941	0.000201	3.6959	0.002921
Phosphoethanolamine	−3.7974	0.000378	3.4224	0.003655
Glutamine	3.3583	0.001458	2.8362	0.012082
Succinate	2.9898	0.004227	2.374	0.02724
Creatine	2.9003	0.005416	2.2663	0.031411
3-Hydroxy-3-methylglutarate	−2.766	0.007795	2.1082	0.037771
Betaine	2.765	0.007815	2.1071	0.037771

metabolism stand out as two of the top five affected serum biochemistry pathways, unlike the AML group. Previous studies have supported this finding by mentioning aberrations in the citrate cycle for various lymphoblastic malignancies [5,11,12]. Tyrosine metabolism was also highly implicated in the two groups. This may indicate the consumption of amino acids for energy production in the citrate cycle and the specific metabolic needs of lymphoblastic cells [10]. In AML serum chemistry, the most affected pathways were GSH and selenocompound metabolism, emphasizing the unique metabolic dependencies of myeloblasts. In general, various amino acids that play a role in essential survival processes and intermediates in energy production pathways were affected in all HMs [10]. The antioxidant defense system (GSH and selenocompound metabolism) was the primary differential pathway for AML, whereas the citrate cycle was for CLL and NHL groups.

Shared and Differential Metabolic Changes Across HM Subtypes

The comparisons between HM groups did not show clear distinctions in the PLS-DA models regarding serum metabolites. Among the few differential metabolites between the HM groups, the levels of 3-hydroxyisovalerate and 3-hydroxymethylglutarate were found to be higher in CLL compared with AML. 3-Hydroxyisovalerate is a byproduct of the leucine degradation pathway and its production depends on a biotin-dependent carboxylase [13]. Inactivated enzyme leads to accumulated 3-hydroxyisovalerate that eventually acts as a

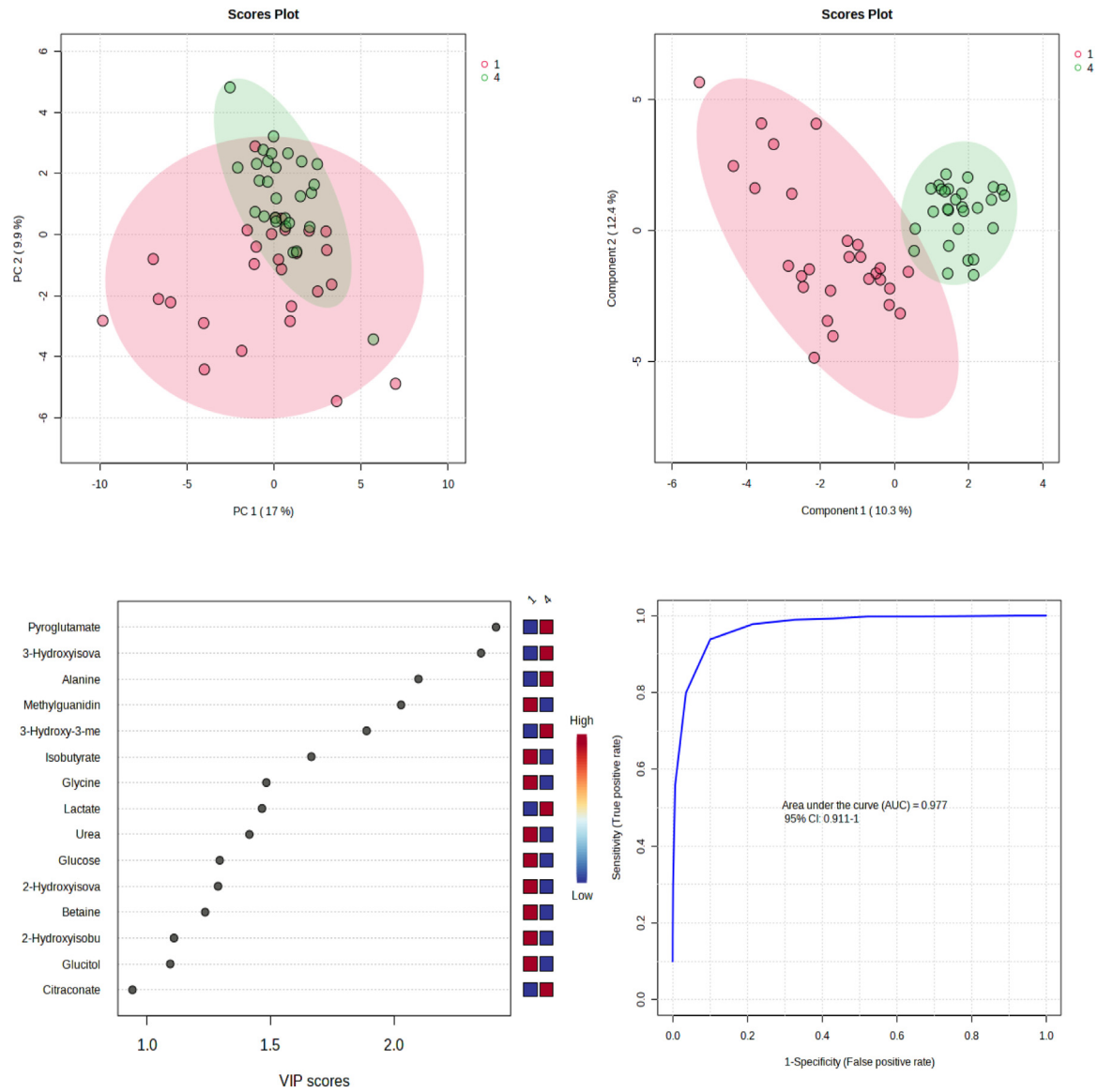

Figure 3 PCA (upper left) and PLS-DA (upper right) score plots between AML and control groups. (1) AML; (4) controls. The variance explanatory components are shown as principal component 1 (PC1) and principal component 2 (PC2). Metabolites with a VIP score >1 in the PLS-DA model (below left). Biomarker model created with 10 metabolites with the highest area under the curve (AUC) value.

metabotoxin in the mitochondria. Biotin metabolism dysfunction and lymphogenesis processes specific to CLL [14] may explain how stimulating survival in the presence of biotin deficiency led to higher serum acid levels in CLL. Furthermore, the increased breakdown of branched-chain amino acids and leucine utilization in AML [15] could result in reduced levels of serum 3-hydroxyisovalerate during the catabolic process to form acetoacetate and acetyl coenzyme A, which are then incorporated into the citrate cycle [16]. In line with the premise, sera from patients with CLL showed a strong inverse correlation between valine levels and the IPI risk score. This may suggest that highly proliferating lymphoblasts increase branched-chain amino acid utilization, such as valine, as the same relationship was shown in a study investigating the serum of patients with acute lymphocytic leukemia (ALL) [17].

CLL was the sole HM group that did not exhibit a decrease in serum 3-hydroxymethylglutarate levels compared with the control group. This metabolite is involved in the leucine degradation pathway as a byproduct of the deficient or ineffective intramitochondrial 3-hydroxy-3-methylglutaryl-CoA lyase enzyme, which catalyzes the final step of leucine degradation [18]. Unlike 3-hydroxyisovalerate, 3-hydroxymethylglutarate is an off-product of the pathway and has been linked to mitochondrial toxicity and early diabetic diseases [19].

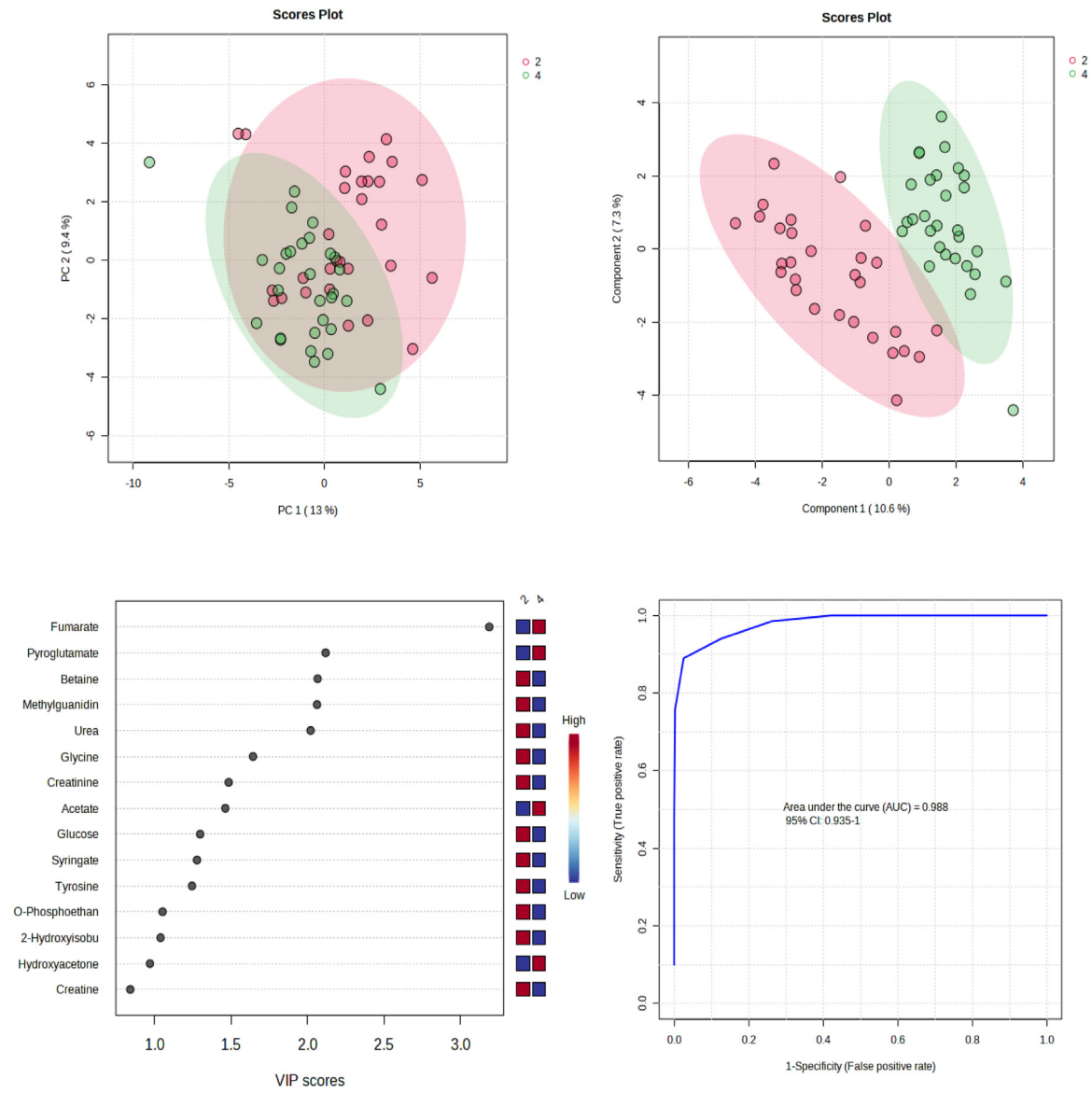

Figure 4 PCA (upper left) and PLS-DA (upper right) score plots between CLL and control groups. (2) CLL; (4) controls. The variance explanatory components are shown as principal component 1 (PC1) and principal component 2 (PC2). Metabolites with a VIP score >1 in the PLS-DA model (below left). Biomarker model created with 3 metabolites with the highest area under the curve (AUC) value.

Together with 3-hydroxyisovalerate, the amino acid metabolism intermediates that were not found altered in CLL indicate a significant metabolic deviation from the AML and control groups. Functional studies are necessary to investigate the potential causes of this difference given the lack of information about these two intermediates in the metabolomics literature related to HM pathogenesis.

The rapid turnover of serum proteins observed in the AML and CLL leukemia subgroups highlights a significant discovery, indicated by increased protein degradation metabolites such as urea and methyl guanidine. Sera from patients with NHL not showing this pattern could result from NHL being more of a solid and localized form of lymphoid tumors. The natural course of the disease progression does not exhibit the typical leukemia phenotype of aberrant leukocytes in circulation [20]. Given the extended lifespan of leukocytes in AML and CLL, patient sera are likely to show the accumulation of protein turnover end-products, as observed in the study.

Although the HM groups were not distinguishable in the all-groups PLS model, healthy individuals were clearly separable from HM subtypes. The division of healthy individuals' serum profile could be evaluated as an expected result because HMs exhibit the common metabolic characteristics of oncogenesis [8].

Glucose Metabolism

The current study showed higher serum glucose levels and correlations between glucose and glucogenic amino acids in the AML group. The elevated serum glucose may have been caused by the fact that elderly individuals newly diagnosed with AML are known to be

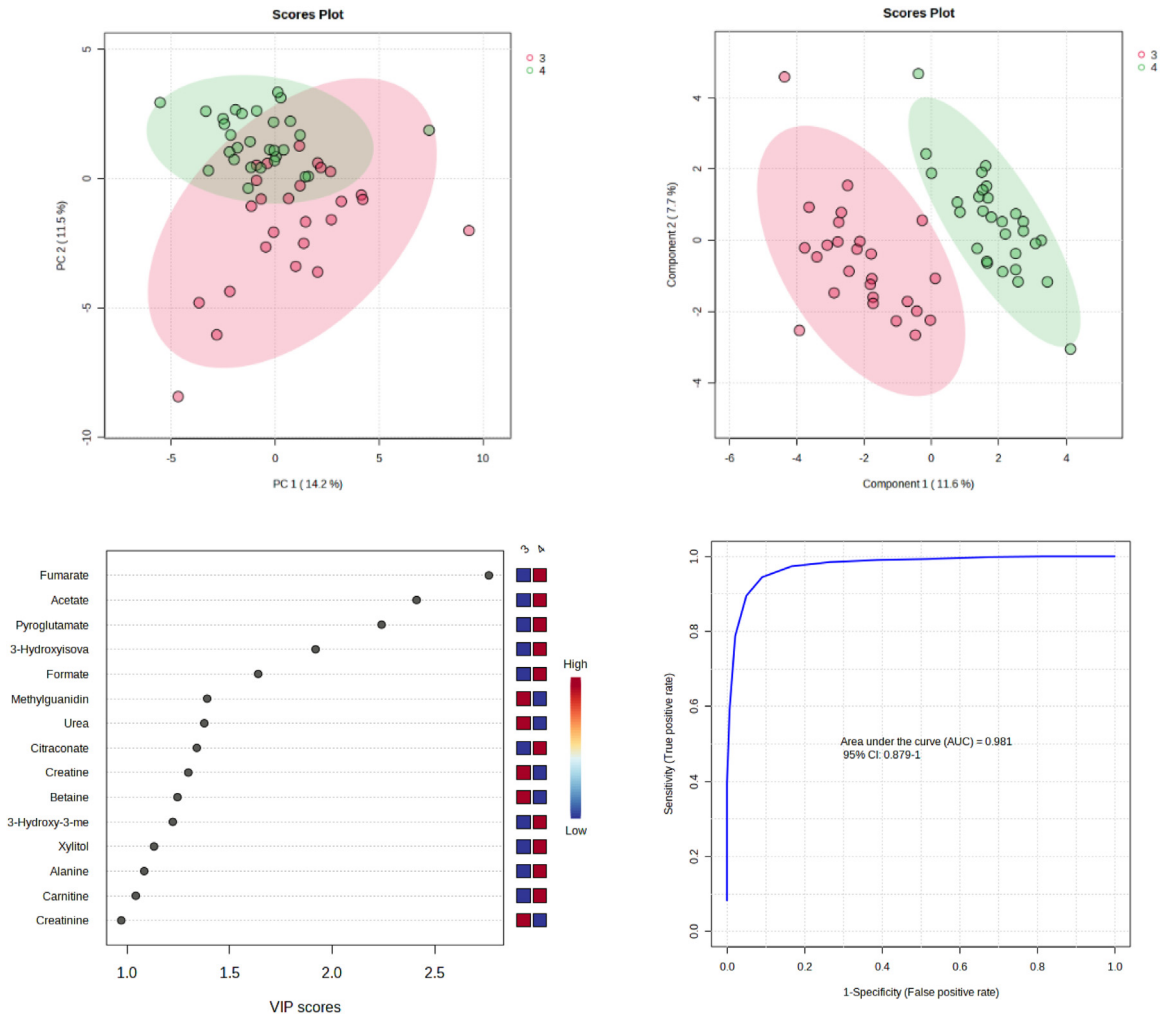

Figure 5 PCA (upper left) and PLS-DA (upper right) score plots between NHL and control groups. (3) NHL; (4) controls. The variance explanatory components are shown as principal component 1 (PC1) and principal component 2 (PC2). Metabolites with a VIP score >1 in the PLS-DA model (below left). Biomarker model created with 5 metabolites with the highest area under the curve (AUC) value.

predisposed to glucose metabolism disorders [21]. The positive correlation of glucose with valine [22], and the negative correlation with pyroglutamate, may indicate changes in gluconeogenic amino acids in the sera of patients with AML, as previously reported [23,24].

Compared with the controls, sera of patients with AML showed lower levels of lactate and higher levels of 2-hydroxyisovalerate. Multiple serum NMR studies have confirmed lower lactate levels in AML sera [10,22,24]. Despite the increase in serum 2-hydroxyisovalerate, a marker for lactic acidosis follow-up [5], the low levels of lactate in AML sera indicate high consumption of lactate by glycolysis-dependent AML blasts [23]. Furthermore, the concurrent reduction in serum lactate along with glucogenic amino acids like alanine and pyroglutamate suggests that these molecules are used to meet the heightened carbon skeleton requirement for gluconeogenesis in patients with AML [10]. In addition, sera from patients with CLL showed a marked inverse correlation between clinical stage and lactate levels, suggesting substantial lactate consumption by high-turnover lymphoblasts.

In all types of HMs (Supplementary Table E4), the glycolysis/gluconeogenesis pathway underwent significant changes, aligning with the widely observed Warburg effect in universal tumorigenesis processes. Overall, comparative analysis reveals that patients with AML exhibit a higher prevalence of glucose metabolism abnormalities than CLL and NHL groups. This underscores the importance of targeting glucose metabolism for a deeper understanding of pathogenesis and the development of effective therapeutic interventions across various HM subtypes.

Amino Acid Metabolism

Beyond the classical Warburg effect, activation of the citrate cycle is frequently observed as a cellular adaptation process in cancer cells [25]. Amino acid catabolism products play a crucial role in fueling the cycle, serving as essential carbon sources. Their contributions to energy production, macromolecule biosynthesis, and redox balance are indispensable for promoting increased proliferation and survival. In this context, the study finding of a decreased serum alanine level in

patients with AML represents a substantial change in amino acid metabolism, further supported by NMR metabolomics studies [26]. The decreased alanine levels may refer to its nutrient role in the energy production via the alanine–pyruvate conversion pathway [27]. Increased consumption of alanine in macromolecule synthesis, especially for gluconeogenesis, is another prominent feature in cancer cells [28]. Reduced serum alanine levels could be a direct consequence of the enhanced gluconeogenetic processes commonly associated with AML [29].

The levels of serum pyroglutamate were lower in all HM groups compared with the control group. During prolonged sample preparation and NMR spectra acquisition, pyroglutamate was found to be the primary form in which glutamine cyclizes [30]. With ultrafiltration or protein precipitation methods, glutamine levels may decrease by up to 75%, and the cyclization was found to vary among different portions of samples from the same sample. The research advocates for the measurement of the combined concentrations of glutamine and pyroglutamate in samples when conducting serum biomarker research. The groups showed no significant difference in the total levels of serum glutamine and pyroglutamate according to the current results. In the AML group, pyroglutamate showed a strong negative correlation with the karyotype, indicating lower amino acid levels in patients with a complex karyotype (Supplementary Table E5). Considering cyclization phenomenon, it is imperative that this finding be validated through additional research studies.

Asparagine is a proteogenic amino acid that plays a key role in regulating the activity of mTORC1, which is involved in tumor growth and survival in leukemia [31]. It is closely related to alanine, aspartate, and glutamate metabolism, which is among the most affected pathways in all HM groups. However, serum NMR analysis showed no detection of asparagine in any study groups. One reason for the absence could be the high demand for protein synthesis during periods of glutamine deprivation within the tumor environment [32]. Asparagine is also known to be susceptible to deamidation under physiological conditions owing to its carboxamide side-chain, making selective tagging applications preferable in NMR methodology [33]. Asparagine is a topic of specific interest in the hematology literature because of its depletion-based therapeutic options for patients with ALL [34]. The exceptional success of asparaginase depends on the low expression of asparagine synthetase (ASNS), which renders ALL cells reliant on extracellular asparagine. In contrast, the expression of ASNS was found to be significantly higher in AML cells compared with ALL [35]. In the context of B-cell lymphomas, it has been observed that physiological concentrations of asparagine hinder de novo biosynthesis of asparagine, regardless of ASNS expression [36]. This mechanism contributes to the resistance of this group of HMs to asparaginase treatment. The role of asparagine depletion therapies in non-ALL groups is still unclear, making it difficult to draw firm conclusions.

In the AML and CLL groups, elevated serum glycine levels were detected. Glycine is a primary component of methyl groups used for the biosynthesis of GSH, proteins, purines, and DNA/histone methylation [37]. With these crucial roles, glycine constitutes an essential nutrient for tumor growth, particularly in leukemia stem cells [38]. Several studies have demonstrated an increase in intracellular levels and an upregulation of the serine/glycine pathway in these cells [38,39]. In support of this, the metabolism of glycine, serine, and threonine was found to be among the top affected pathways in all HMs subtypes compared with controls (Supplementary Table E4).

The current study could not contribute to the literature regarding serum levels of aspartate and its association with distinct HM subtypes, as it was not detectable in the serum NMR analysis. Aspartate is primarily synthesized from glutamate through the action of aspartate aminotransferase (AST). It serves a critical role in the biosynthesis of purines, acting as a nitrogen donor, and is also involved in the synthesis of asparagine and arginine. The gene expression analysis of AST showed reduced levels in tumor samples from patients with AML accessed through The Cancer Genome Atlas, as well as affecting enzymes that use aspartate in biosynthesis [40]. The study indicated strong clinical evidence for aspartate-derived amino acid vulnerabilities, particularly in myeloid leukemias. In patients newly diagnosed with diffuse large B-cell lymphoma, decreased plasma aspartate levels showed high diagnostic performance alongside glucose levels [11]. Under hypoxic conditions, the increased availability of aspartate acts as an internal metabolic limitation that helps replenish oxaloacetate and NADH during glycolysis, which could have important implications for the pathogenesis of HMs [41].

The only phospholipid molecule that was differentially altered from controls was PEA, which was elevated in sera from patients with CLL. Various cancer cell lines, including breast and pancreatic cancers, exhibited elevated PEA levels because of increased ethanolamine kinase activity, making this reaction a potential therapeutic target [42]. The increased levels were also shown to be correlated with a poor prognosis in leukemia cell lines [43]. Based on these findings, the elevated serum PEA levels observed in patients with CLL may be attributed to an accelerated biosynthesis. A former phosphorus-NMR study supports this with increased PEA levels in CLL lymphocytes [44].

A notable finding was found in the serum samples of patients with NHL, showing a decrease in carnitine levels. Carnitine functions as a carrier of acetyl and acyl groups between the cytosol and the mitochondria during the β-oxidation process [45]. Because enhanced β-oxidation is a hallmark of cancer cell metabolism [29], the low serum carnitine level in patients with NHL may indicate increased energy needs in lymphoma, compensated by an increase in β-oxidation. Although several studies have demonstrated decreased serum carnitine levels in acute leukemias [4,29], the current result referring to patients with NHL represents a novel finding.

In the CLL and NHL groups, there was a significant decrease in serum levels of fumarate and acetate. The two intermediates of the citrate cycle had the highest VIP score and were key components of the most significant pathways for lymphoma groups (Figures 4 and 5, Supplementary Table E4). A validation study demonstrated decreased serum fumarate levels in patients with mutated CLL, indicating intense consumption of Krebs cycle intermediates in proliferating leukemic cells [12]. The evidence suggests a metabolic shift to the citrate cycle, crucial for obtaining energy for proliferation and developing chemoresistance, beyond the Warburg effect [25].

Further analysis of the extensive utilization of citrate cycle elements in the extended lifespan of lymphoblasts revealed a reduction in acetate levels within the NHL group. Serum acetate emerged as a differential compound between the NHL and AML groups. According to a previous study, acetate from bone marrow stromal cells supports the citrate cycle and lipid biosynthesis in AML cell lines [46]. Although acetate was not found to be differentially altered in the sera of patients with AML, it is crucial to investigate its biological relevance in leukemia pathogenesis. In patients with NHL, previous NMR metabolomics study confirmed decreased serum acetate levels in the same HM groups [5].

In comparison to the AML group, sera from patients with CLL and NHL exhibited the highest impact on tyrosine metabolism, along with notable alterations in pyruvate-alanine metabolism and the citrate cycle. These findings may further emphasize the increased protein turnover in lymphoblastic tumor growth [47].

Lipid Metabolism

The noticeable finding regarding the lipid metabolism was recorded in patients with NHL with lower serum PEA levels. Former phosphorus magnetic resonance spectroscopy studies reported elevated PEA levels in vivo for NHL cells [48] with reduced precursor levels in patient sera, indicating the sustained phospholipid metabolism. The increased cell content of PEA and phosphocholine was also found to be a negative prognostic variable for future treatment response and these phospholipid precursors were linked to the triggered apoptosis processes in NHL tumor tissues [49,50]. PEA is a lipid chaperone that initiates caspase-independent cellular autophagy [51] and is shown to be elevated prior to apoptosis triggering treatment with menaquinone in the cell lines of acute lymphoblastic leukemia [52]. Synthetic PEA was demonstrated to induce apoptosis, reduce tumor growth [53], and to have antiangiogenic and antimetastatic activities [54] in several cancer cell lines [42,55], including leukemia and malignant lymphomas [56,57]. Although complementary measurements of related molecules are required (especially in cellular compartment) to reveal the causality of the decline in serum PEA levels, the current finding may indicate a potential vulnerability for synthetic therapeutical intervention for patients with NHL [57].

CONCLUSION

The preliminary study globally evaluating the serum metabolome in various HMs has identified several potential biomarkers, within the amino acid molecules. The highlighted metabolic pathways in HMs included glycolysis/gluconeogenesis in the AML group and the citrate cycle pathway in the CLL and NHL groups. The distinct biochemical changes within specific groups and the shared alterations offer valuable indications about the intrinsic characteristics of various HM types. Yet, emerging findings need to be validated with larger cohorts and integrated with other omics data to understand the genomic and proteomic landscape for assessing individual disease drivers within the context of personalized medicine.

Conflict of Interest Disclosure

The authors do not have any conflicts of interest to declare in relation to this work.

Acknowledgments

We are grateful to Ahmet Balci, Ece Kolay Cayir, and Erdem Akalin for putting their significant effort during the experimental procedures.

Author Contributions

AZG: methodology, formal analysis, and writing—review and editing. SS: conceptualization, methodology, formal analysis, and writing—review and editing. SB: methodology. MD: formal analysis. FBC: data curation. BU: data curation.

Data Availability

Data are available upon request.

Funding

This study was supported by BVU Scientific Research Projects Unit under the Grant No. 20211203 and Scientific and Technological Research Council of Turkey (TÜBİTAK) under the Grant No. 122S7442, 01/09. The authors thank to TÜBİTAK for their supports.

Ethics Approval Statement

The study was performed at BVU Pharmaceutical Application and Research Center and TÜBİTAK Marmara Research Center between 27 February 2023 and 5 June 2023 with the permission of BVU Ethics Committee (No: 2021/391). All participants were given informed consent to the clinical study following the Declaration of Helsinki protocol. The manuscript has been read and approved by all authors.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.exphem.2025.104710.

REFERENCES

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin 2023;73:17–48.
2. Ball M, List AF, Padron E. When clinical heterogeneity exceeds genetic heterogeneity: thinking outside the genomic box in chronic myelomonocytic leukemia. Blood 2016;128:2381–7.
3. Alonso A, Marsal S, Julià A. Analytical methods in untargeted metabolomics: state of the art in 2015. Front Bioeng Biotechnol 2015;3:23.
4. Arévalo C, Rojas L, Santamaria M, et al. Untargeted metabolomic and lipidomic analyses reveal lipid dysregulation in the plasma of acute leukemia patients. Front Mol Biosci 2023;10:1235160.
5. Wojtowicz W, Chachaj A, Olczak A, et al. Serum NMR metabolomics to differentiate haematologic malignancies. Oncotarget 2018;9:24414–27.
6. Gowda GAN, Raftery D. Quantitating metabolites in protein precipitated serum using NMR spectroscopy. Anal Chem 2014;86:5433–40.
7. Bingol K, Li DW, Zhang B, Brüschweiler R. Comprehensive metabolite identification strategy using multiple two-dimensional NMR spectra of a complex mixture implemented in the COLMARm web server. Anal Chem 2016;88:12411–8.
8. Zuo F, Yu J, He X. Single-cell metabolomics in hematopoiesis and hematological malignancies. Front Oncol 2022;12:931393.
9. Komura D, Kawabe A, Fukuta K, et al. Universal encoding of pan-cancer histology by deep texture representations. Cell Rep 2022;38:110424.
10. Musharraf SG, Siddiqui AJ, Shamsi T, Choudhary MI, Rahman AU. Serum metabonomics of acute leukemia using nuclear magnetic resonance spectroscopy. Sci Rep 2016;6:30693.
11. Fei F, Zheng M, Xu Z, et al. Plasma metabolites forecast occurrence and prognosis for patients with diffuse large B-cell lymphoma. Front Oncol 2022;12:894891.
12. MacIntyre DA, Jiménez B, Lewintre EJ, et al. Serum metabolome analysis by 1H-NMR reveals differences between chronic lymphocytic leukaemia molecular subgroups. Leukemia 2010;24:788–97.
13. 3-Hydroxyisovaleric acid (HMDB0000754). Human Metabolome Database site. XXXX. Available at: https://hmdb.ca/metabolites/HMDB0000754. Accessed October 29, 2023.

14. Griffin JB, Zempleni J. Biotin deficiency stimulates survival pathways in human lymphoma cells exposed to antineoplastic drugs. J Nutr Biochem 2005;16:96–103.
15. Kikushige Y, Miyamoto T, Kochi Y, et al. Human acute leukemia uses branched-chain amino acid catabolism to maintain stemness through regulating PRC2 function. Blood Adv 2023;7:3592–603.
16. Neinast M, Murashige D, Arany Z. Branched chain amino acids. Annu Rev Physiol 2019;81:139–64.
17. Chen S, He G, Zhang M, Tang N, Zeng Y. Causal relationship between branched-chain amino acids and leukemia risk: insights from a two-sample Mendelian randomization study. Hematology 2024;29:2433904.
18. Wishart DS, Guo A, Oler E, et al. HMDB 5.0: the Human Metabolome Database for 2022. Nucleic Acids Res 2022;50:D622–31.
19. Singh H, Miyamoto S, Darshi M, et al. Gut microbial changes in diabetic db/db mice and recovery of microbial diversity upon pirfenidone treatment. Microorganisms 2020;8:1347.
20. Guillerman RP, Voss SD, Parker BR. Leukemia and lymphoma. Radiol Clin North Am 2011;49:767–97.
21. Wang Y, Zhang L, Chen WL, et al. Rapid diagnosis and prognosis of de novo acute myeloid leukemia by serum metabonomic analysis. J Proteome Res 2013;12:4393–401.
22. Duan Z, Chen Y, Ye M, et al. Differentiation and prognostic stratification of acute myeloid leukemia by serum-based spectroscopy coupling with metabolic fingerprints. FASEB J 2022;36:e22416.
23. Chen WL, Wang JH, Zhao AH, et al. A distinct glucose metabolism signature of acute myeloid leukemia with prognostic value. Blood 2014;124:1645–54.
24. Wojcicki AV, Kasowski MM, Sakamoto KM, Lacayo N. Metabolomics in acute myeloid leukemia. Mol Genet Metab 2020;130:230–8.
25. De Mets F, Van Melderen L, Gottesman S. Regulation of acetate metabolism and coordination with the TCA cycle via a processed small RNA. Proc Natl Acad Sci U S A 2019;116:1043–52.
26. Valin L, Kanefsky J, Martinez E, et al. Acute myeloid leukemia cells rely on the glycolytic enzyme PGK1 to support energy production and amino acid metabolism. Blood 2022;140(Suppl 1):8694–5.
27. Vettore L, Westbrook RL, Tennant DA. New aspects of amino acid metabolism in cancer. Br J Cancer 2020;122:150–6.
28. Taherizadeh M, Khoshnia M, Shams S, Hesari Z, Joshaghani H. Clinical significance of plasma levels of gluconeogenic amino acids in esophageal cancer patients. Asian Pac J Cancer Prev 2020;21:2463–8.
29. Morad HM, Abou-Elzahab MM, Aref S, El-Sokkary AMA. Diagnostic value of 1H NMR-based metabolomics in acute lymphoblastic leukemia, acute myeloid leukemia, and breast cancer. ACS Omega 2022;7:8128–40.
30. Gowda GAN, Gowda YN, Raftery D. Massive glutamine cyclization to pyroglutamic acid in human serum discovered using NMR spectroscopy. Anal Chem 2015;87:3800–5.
31. Meng D, Yang Q, Wang H, et al. Glutamine and asparagine activate mTORC1 independently of Rag GTPases. J Biol Chem 2020;295:2890–9.
32. Jiang J, Pavlova NN, Zhang J. Asparagine, a critical limiting metabolite during glutamine starvation. Mol Cell Oncol 2018;5:e1441633.
33. Cao C, Chen JL, Yang Y, Huang F, Otting G, Su XC. Selective ^{15}N-labeling of the side-chain amide groups of asparagine and glutamine for applications in paramagnetic NMR spectroscopy. J Biomol NMR 2014;59:251–61.
34. Chen C, Zhang J. Enhancing leukemia treatment: the role of combined therapies based on amino acid starvation. Cancers 2024;16:1171.
35. Kaspers GJL. Acute myeloid leukaemia niche regulates response to L-asparaginase. Br J Haematol 2019;186:397–9.
36. Grima-Reyes M, Vandenberghe A, Nemazanyy I, et al. Tumoral microenvironment prevents de novo asparagine biosynthesis in B cell lymphoma, regardless of ASNS expression. Sci Adv 2022;8:eabn6491.
37. Amelio I, Cutruzzolá F, Antonov A, Agostini M, Melino G. Serine and glycine metabolism in cancer. Trends Biochem Sci 2014;39:191–8.
38. Verstraete P, De Keersmaecker K, Kampen KR. Drivers of de novo serine/glycine synthesis in acute leukemia. FEBS Lett 2023;597:2145–6.
39. Kampen KR, Fancello L, Girardi T, et al. Translatome analysis reveals altered serine and glycine metabolism in T-cell acute lymphoblastic leukemia cells. Nat Commun 2019;10:2542.
40. Bhingarkar A, Vangapandu HV, Rathod S, Hoshitsuki K, Fernandez CA. Amino acid metabolic vulnerabilities in acute and chronic myeloid leukemias. Front Oncol 2021;11:694526.
41. Garcia-Bermudez J, Baudrier L, La K, et al. Aspartate is a limiting metabolite for cancer cell proliferation under hypoxia and in tumours. Nat Cell Bio 2018;20:775–81.
42. Shah T, Krishnamachary B, Wildes F, Wijnen JP, Glunde K, Bhujwalla ZM. Molecular causes of elevated phosphoethanolamine in breast and pancreatic cancer cells. NMR Biomed 2018;31:e3936.
43. Lo Presti C, Fauvelle F, Jacob MC, Mondet J, Mossuz P. The metabolic reprogramming in acute myeloid leukemia patients depends on their genotype and is a prognostic marker. Blood Adv 2021;5:156–66.
44. Franks SE, Smith MR, Arias-Mendoza F, et al. Phosphomonoester concentrations differ between chronic lymphocytic leukemia cells and normal human lymphocytes. Leuk Res 2002;26:919–26.
45. Melone MAB, Valentino A, Margarucci S, Galderisi U, Giordano A, Peluso G. The carnitine system and cancer metabolic plasticity. Cell Death Dis 2018;9:228.
46. Vilaplana-Lopera N, Cuminetti V, Almaghrabi R, et al. Crosstalk between AML and stromal cells triggers acetate secretion through the metabolic rewiring of stromal cells. eLife 2022;11:e75908.
47. Wang D, Tan G, Wang H, Chen P, Hao J, Wang Y. Identification of novel serum biomarker for the detection of acute myeloid leukemia based on liquid chromatography-mass spectrometry. J Pharm Biomed Anal 2019;166:357–63.
48. Negendank WG, Padavic-Shaller KA, Li CW, et al. Metabolic characterization of human non-Hodgkin's lymphomas in vivo with the use of proton-decoupled phosphorus magnetic resonance spectroscopy. Cancer Res 1995;55:3286–94.
49. Arias-Mendoza F, Smith MR, Brown TR. Predicting treatment response in non-Hodgkin's lymphoma from the pretreatment tumor content of phosphoethanolamine plus phosphocholine1. Acad Radiol 2004;11:368–76.
50. Arias-Mendoza F, Brown T. Predicting treatment response for non-Hodgkin's lymphoma: A multi-institutional MR spectroscopy study. Clin Cancer Res 2014;13:A20.
51. Patel D, Witt SN. Ethanolamine and phosphatidylethanolamine: partners in health and disease. Oxid Med Cell Longev 2017;2017:4829180.
52. Dhakshinamoorthy S, Dinh N-T, Skolnick J, Styczynski MP. Metabolomics identifies the intersection of phosphoethanolamine with menaquinone-triggered apoptosis in an in vitro model of leukemia. Mol Biosyst 2015;11:2406–16.
53. Mambelli LI, Teixeira SF, Jorge SD, et al. Phosphoethanolamine induces caspase-independent cell death by reducing the expression of C-RAF and inhibits tumor growth in human melanoma model. Biomed Pharmacother 2018;103:18–28.
54. Ferreira AK, Freitas VM, Levy D, et al. Anti-angiogenic and anti-metastatic activity of synthetic phosphoethanolamine. PLoS One 2013;8:e57937.
55. Luna ACdL, Saraiva GKV, Chierice GO, Hesse H, Maria DA. Antiproliferative and proapoptotic effects of DODAC/synthetic phosphoethanolamine on hepatocellular carcinoma cells. BMC Pharmacol Toxicol 2018;19:44.
56. de Oliveira Conceição T, da Silva MGL, Maria DA. Phosphomonoester phosphoethanolamine induces apoptosis in human chronic myeloid leukemia cells. J Pharm Pharmacol 2019;7:434–50.
57. Ferreira AK, Santana-Lemos BAA, Rego EM, Filho OMR, Chierice GO, Maria DA. Synthetic phosphoethanolamine has in vitro and in vivo anti-leukemia effects. Br J Cancer 2013;109:2819–28.