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RESEARCH ARTICLE

Metabolomic Profiling of Leukemic Hematopoiesis: Effects of BNT162b2 mRNA COVID-19 Vaccine Administration

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Abstract: ***Background*:** Leukemia is marked by clonal hematopoietic stem cell expansion and metabolic reprogramming. The BNT162b2 mRNA COVID-19 vaccine has been proven effective, though questions remain about its broader physiological effects. This study investigates metabolomic alterations in leukemic bone marrow potentially associated with BNT162b2 vaccination.

***Objective*:** To compare the bone marrow metabolomic profiles of leukemia patients with and without BNT162b2 vaccination, and healthy unvaccinated controls, to explore potential metabolic differences.

***Methods*:** Bone marrow samples were obtained from three groups: vaccinated leukemia patients (n=7), unvaccinated leukemia patients without COVID-19 history (n=2), and unvaccinated healthy controls (n=7). Untargeted metabolomics was performed using LC-QTOF-MS. Data were analyzed using XCMS and MetaboAnalyst 5.0 to identify statistically significant metabolite differences and affected pathways. Fold change >1.5 and $p<0.05$ were considered significant.

***Results*:** Distinct metabolic profiles were observed between the leukemia and control groups. Increased glycolysis, pentose phosphate pathway activity, and altered tryptophan, lipid, and heme metabolism were noted in leukemia samples. Metabolic changes in vaccinated patients (ASL) were more similar to unvaccinated leukemia patients (LO) than to healthy controls, with minor vaccine-associated variations. Notable metabolites included 5-methoxyindoleacetate, phosphorylcholine, and tetrahydrofolic acid.

***Conclusion*:** This preliminary study identified altered metabolic pathways in leukemia bone marrow and suggests metabolomic differences associated with BNT162b2 vaccination. While the findings do not support a causal link between mRNA vaccination and leukemia development, they highlight the need for further studies to understand vaccine-induced metabolic modulation in hematological contexts.

Keywords: SARS-CoV-2, COVID-19, The Pfizer-BioNTech COVID-19 vaccine, Leukemia, Hematopoiesis, Metabolomics.

1. INTRODUCTION

Leukemia, a malignancy of hematopoietic cells, is characterized by the clonal proliferation of leukemic blast cells that exhibit altered cellular metabolism [1-3]. Recent advancements in systems biology, particularly the application of omics technologies, have significantly enhanced our understanding of the molecular underpinnings of leukemia [4, 5]. Among these, metabolomics, focused on the profiling of small molecules (<1500 Da), has emerged as a particularly valuable approach for studying leukemia [6-8]. Unlike genomics or transcriptomics, which primarily reflect static genetic information, metabolomics captures the dynamic, real-time changes in cellular metabolism, thus providing a more accurate depiction of cellular phenotype and the immediate biological response to internal and external stimuli [9, 10].

Metabolomic techniques, including nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), allow for the comprehensive analysis of complex metabolic networks, identifying alterations that can be directly linked to disease progression, therapeutic responses, and environmental influences [11, 12]. Leukemia pathogenesis is

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frequently associated with reprogrammed metabolic pathways, where leukemia stem cells (LSCs) preferentially utilize oxidative phosphorylation, in contrast to the reliance on anaerobic glycolysis observed in normal hematopoietic stem cells (HSCs) [8, 13, 14]. This metabolic reprogramming presents a compelling opportunity for targeted therapeutic interventions aimed at disrupting the metabolic advantages that sustain leukemic cell proliferation and survival [15].

SARS-CoV-2, a zoonotic coronavirus capable of cross-species transmission, emerged in December 2019 as a novel pathogen with significant global health implications. Sharing 96% nucleotide similarity with SARS-CoV, presumed to originate from bats, it was first identified through metagenomic sequencing of clinical samples from patients with unexplained atypical pneumonia [16-19]. Variants like Delta, Omicron, and Deltacron highlight SARS-CoV-2's adaptability through differences in transmissibility, immune evasion, and pathogenicity [20, 21]. SARS-CoV-2's pathogenic significance stems from its sophisticated molecular interaction with the angiotensin-converting enzyme 2 (ACE2) receptor, mediated by its spike glycoprotein complex. Upon binding to ACE2, SARS-CoV-2 initiates a cascade of immunological events, culminating in both an inflammatory response and a dysregulated cytokine release syndrome, commonly known as a cytokine storm, during COVID-19 infection [22, 23]. its receptor-binding domain (RBD-SD1) exhibits a particularly strong binding affinity for bone marrow cells [24-26]. Notably, the presence of comprehensive Renin-Angiotensin System (RAS) components within the bone marrow microenvironment, coupled with their regulatory role in hematopoiesis and cellular behavior, particularly in CD34+ cell proliferation and migration, suggests a complex interplay between SARS-CoV-2 infection and hematological homeostasis through RAS-mediated pathways [27-30]. The virus exhibits potential oncogenic mechanisms through perturbation of tumor suppressor proteins (pRb and p53) and induction of oxidative stress via ACE2 depletion [31-33].

The Pfizer-BioNTech COVID-19 vaccine has demonstrated efficacy in conferring immunological protection against SARS-CoV-2, including the Omicron variant, through the induction of robust humoral and cellular immune responses, thereby mitigating the risk of severe disease outcomes [20]. BNT162b2 utilizes lipid nanoparticles (LNPs) to deliver modified mRNA encoding a mutant spike protein (PS2) into host cells, where it is translated into viral protein and presented on the cell surface [34, 35]. This activates a T-cell-mediated immune response [36]. While mRNA vaccines are generally safe with low rates of severe adverse events, rare serious side effects have been reported, including myocardial infarction, Bell's palsy, thrombosis, Guillain-Barré syndrome, myocarditis, pulmonary embolism, and neurological complications [37-39]. Hematological issues such as thrombocytopenia, lymphadenopathy, and bleeding disorders, as well as reports of lymphoma and myeloid leukemia following vaccination, are notable concerns [40-43]. Although effective in generating immunity against SARS-CoV-2, the exact mechanisms behind these adverse events remain unclear, with lipid nanoparticles potentially causing broad tissue distribution and inflammation [35, 44].

In this study, we employed an LC-QTOF-MS-based untargeted metabolomics approach to analyze bone marrow aspiration samples from three groups: leukemia patients with a history of BNT162b2 vaccination, leukemia patients without such a history, and a healthy, unvaccinated control group. The objective was to explore metabolic variations between vaccinated and unvaccinated leukemia patients and compare these profiles with healthy controls. By investigating the metabolomic differences, we aim to gain insights into potential vaccine-associated metabolic changes in leukemia. While acknowledging the life-saving benefits of mRNA COVID-19 vaccines, our study focuses on understanding their effects to enhance vaccine safety and patient care.

2. MATERIALS AND METHODS

2.1. Collecting Samples

Bone marrow aspiration samples were obtained following the safe execution of clinically indicated procedures. The primary indication for these procedures was to perform flow cytometric analyses to identify cell surface antigens. The patients included in the study were divided into three groups: the first group consisted of leukemia patients with a history of mRNA COVID-19 vaccination (ASL), the second group included leukemia patients without a history of vaccination or COVID-19 infection (LO), and the third group served as the control group, comprising individuals without a history of mRNA COVID-19 vaccination, COVID-19 infection, or any malignancy (C). There were seven patients in the ASL group, two patients in the LO group, and seven patients in the C group. The demographic and general characteristics of the patients are presented in Table **1**.

2.2. Untargeted Metabolomics

Fresh bone marrow samples were centrifuged at 3,000 rpm for 20 minutes, after which the supernatant (liquid phase) was carefully collected. For metabolite extraction, 600 µL of methanol (MeOH) was added to 200 µL of the collected sample, leading to protein precipitation. Subsequently, 200 µL of the supernatant was transferred, and the solvent was evaporated using a vacuum centrifuge. After evaporation, the dried samples were reconstituted in an ACN: H_2O (1:1, v/v) mobile phase and transferred into glass vials for analysis.

In parallel, an extraction blank (containing only solvents) and a quality control (QC) sample pool (prepared by combining aliquots from all samples) were processed alongside the experimental samples. All chemicals used were of LC/MS grade or higher purity, ensuring analytical reliability. LC/MS analyses were

Table 1. Demographic and general characteristics of the patients.

Groups	Diagnosis	Age	Sex	Comorbidity	mRNA vaccine dose number	Time from the last mRNA vaccine to the onset of leukaemia	Covid-19
Control Group (C)	-	65	Male	-	-	-	-
	-	42	Female	-	-	-	-
	-	64	Male	Diabetes mellitus	-	-	-
	-	38	Male	-	-	-	-
	-	69	Female	Hypertension	-	-	-
	-	21	Male	-	-	-	-
	-	67	Female	-	-	-	-
Leukemia after a history of mRNA vaccination Group (ASL)	ALL	22	Female	-	2	52 days	-
	AML	74	Male	-	3	31 days	-
	AML	50	Male	-	2	63 days	+ 6 months ago
	CLL	59	Male	-	2	25 days	-
	AML	62	Male	-	3	47 days	-
	ALL	58	Female	-	3	21 days	+4 months ago
	AML	63	Male	Hypertension	2	15 days	-
Without a history of vaccination and covid Group (LO)	AML	52	Male	-	-	-	-
	AML	47	Female	-	-	-	-

Abbreviations: AML: Acute Myeloid Leukemia, ALL: Acute Lymphoblastic Leukemia, CLL: Chronic Lymphocytic Leukemia.

performed using LC-QTOF-MS (Agilent Technologies 6530, Santa Clara, US). Samples were injected in duplicate and random order, with a quality control sample every six injections. Analyses were performed using a BEH C18 column (2.1×50 mm 1.7 µm; Waters Acquity, Milford, MA). Mobile phases were water (phase A) and acetonitrile (phase B), consisting of 0.1% formic acid. The gradient program starts with 90% H_2O, decreases to 65% by the 3rd minute, decreases to 10% water by the 15th minute, and includes a 10-minute post-run. The flow rate was 0.4 mL/min. The injection volume was 10 µL. For the MS device, the scanning range was set to 100-1700 m/z, the column temperature was 350 °C, the drying gas temperature was 3500 °C, and the capillary voltage was 4000 V. To process the metabolomic data from LC-QTOF-MS analysis, we used the XCMS package in R, which involved importing raw data, detecting peaks, aligning retention times, grouping similar peaks, filling missing data, and normalizing peak intensities. This workflow allowed for the accurate identification and comparison of metabolite peaks across different sample groups.

2.3. Statistical Analysis

In this untargeted metabolomics study using LC-QTOF-MS, raw mass spectrometry data underwent preprocessing steps, including peak detection, alignment, and deconvolution, to create a comprehensive list of metabolite peaks. Data normalization was performed using total peak areas to standardize the metabolite peak areas to account for systematic variations and enable meaningful comparisons between samples. After normalization, the data were analyzed to identify metabolites showing significant changes between experimental groups [45-47].

Fold change (FC) analysis was conducted to quantify the magnitude of differences in metabolite levels between groups, where an FC of 1.5 or greater indicated a substantial alteration in metabolic regulation. To assess the statistical significance of these changes, t-tests were employed, with p-values used to indicate the likelihood that the observed differences were due to chance, with a significance threshold set at $p < 0.05$. Significant metabolites were further investigated to understand their biological roles and potential implications in the studied condition.

The extracted metabolite peaks that showed differences between groups were selected, grouped, normalized, and visualized using volcano plots. Volcano plots were used to visualize the data by integrating fold change and statistical significance criteria, which helped distinguish between actual biological differences and random fluctuations. Partial Least Squares Discriminant Analysis (PLS-DA) (including triple replicates of the analysis) was then performed to illustrate the overall variance and group separation based on the metabolomic profiles. Peaks that were statistically reliable and significantly altered, with t-test p-values < 0.05 and FC > 1.5, were putatively identified using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca). These significant metabolites were further processed for pathway analysis using IMPaLA (http://impala.molgen.mpg.de) to identify altered metabolic pathways.

3. RESULTS

In this metabolomics study, a comprehensive investigation was conducted using LC-QTOF-MS to analyze bone marrow samples from three groups (C, ASL, and LO). Metabolomic profiling was employed to elucidate the intricate biochemical changes occurring in these samples. Base peak chromatograms (Fig. **1**) were generated to capture the detailed metabolic profiles of the biological samples.

Notably, polar metabolites exhibited shorter retention times and eluted earlier in the chromatographic run, whereas apolar metabolites showed delayed elution, appearing later in the chromatogram. This clear differentiation in elution behavior between polar and apolar compounds highlights the effectiveness of the chromatographic method in separating metabolites based on their polarity characteristics. These chromatograms demonstrate that our chromatographic conditions were robust enough to effectively detect the extracted metabolites. Consistent identification of similar chromatograms across replicates is crucial in metabolomic analyses to ensure the reliability and reproducibility of the obtained data.

Data processing was conducted using XCMS, as described in the experimental section. XCMS is a widely adopted software tool specifically designed to analyze untargeted metabolomics data acquired through chromatography coupled to mass spectrometry. This powerful software facilitates peak detection, retention time alignment, and statistical analysis of metabolomic datasets, allowing for the extraction of meaningful biological insights from complex raw data. Under our experimental conditions, XCMS detected a total of 2,296 peaks. We focused on the peaks that exhibited differential abundance levels between groups C and LO, using criteria of statistical significance ($p < 0.05$) and an FC greater than 1.5 to evaluate the results. Fig. (**2**) presents volcano plots used to illustrate the differences in detected peaks between group C and LO in our untargeted metabolomics study.

In these plots, gray dots represent metabolite peaks where no significant differences were found between

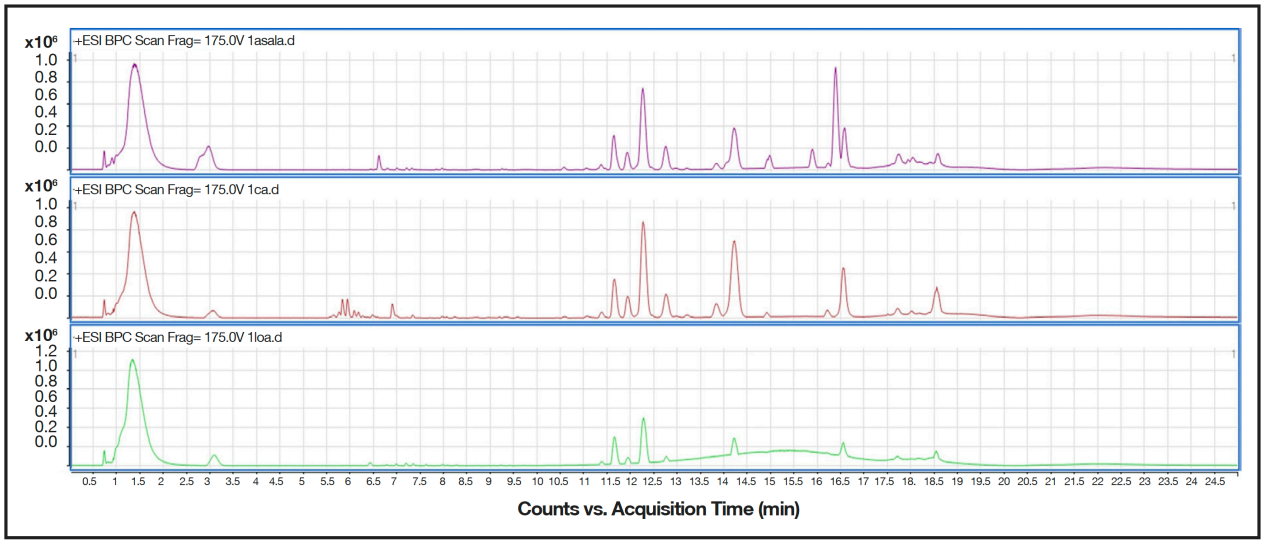

Fig. (1). Representative base peak chromatograms randomly selected under experimental conditions for control (C), leukemia patients with BNT162b2 vaccination (ASL), and leukemia patients without vaccination (LO) samples. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

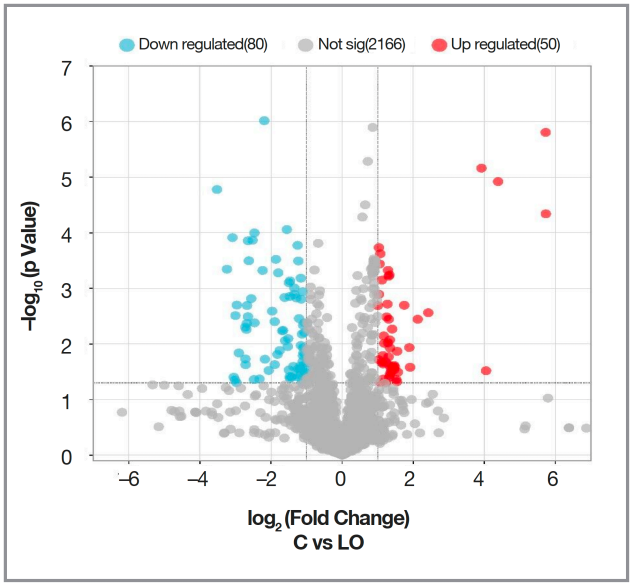

Fig. (2). Volcano plot showing differential metabolite abundance between control (C) and leukemia onset (LO) groups. The x-axis represents log2 fold change, and the y-axis represents -log10 p-value. Red and blue dots indicate significantly increased and decreased metabolites in the LO group, respectively, while gray dots represent non-significant changes. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

the groups, appearing in the central region of the plot. The predominance of gray dots in the volcano plot suggests that a substantial proportion of metabolite peaks maintain consistent abundance levels across the compared sample groups, despite the applied experimental conditions. This observation highlights the stability or lack of change in these metabolite levels in response to the factors studied. Notably, the higher count of gray dots compared to red and blue dots indicates that, while there are detectable differences, many metabolites remain similar between the groups. This outcome supports the robustness of our methodological approach in capturing both variations and consistencies in metabolite profiles across different experimental conditions. Table **2** presents the putatively identified (Level 3) list of metabolites using MetaboAnalyst 5.0. This putative identification is crucial for interpreting the biological relevance of the observed metabolic differences and provides a foundation for further targeted studies. The metabolites listed in Table **2** were selected based on their significant differences in abundance between the groups, as determined by the statistical and fold-change criteria applied during the analysis. Additionally, a clinical perspective was considered, supported by a comprehensive literature search, which provided further context for understanding how these metabolites might be linked to the biological processes under investigation. This combined approach of statistical rigor and clinical relevance enhances the robustness and applicability of our findings, paving the way for future targeted research.

PLS-DA (Fig. **3**) was used to analyze the metabolomic data, highlighting key metabolites that differentiate between groups. A key observation from the PLS-DA plot is the clear separation and distinct clustering of the C and LO groups, indicating markedly different metabolic profiles. Additionally, the proximity of the ASL group to the LO group suggests that the metabolic changes induced by the mRNA vaccine are relatively minor compared to the more significant alterations associated with the onset of leukemia.

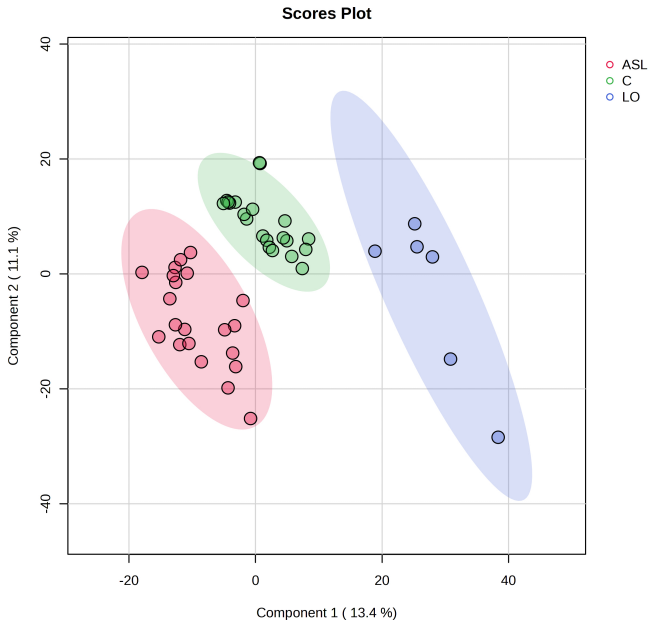

Fig. (3). PLS-DA score plot (including three replicates) showing the separation of metabolic profiles among three groups: control (C), leukemia patients with BNT162b2 vaccination (ASL), and leukemia patients without vaccination (LO). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

4. DISCUSSION

The primary aim of our study was to understand the potential metabolic differences between leukemia patients with and without a history of BNT162b2 vaccination and to compare these profiles with those of a healthy, unvaccinated control group. By examining the metabolomic profiles of these three groups, we sought to uncover any metabolic changes that might be associated with the mRNA COVID-19 vaccine in the context of leukemia. Using bone marrow as a unique biological sample allowed us to focus on the cellular environment most directly affected by leukemia, which could provide more precise insights into how the vaccine might influence leukemia's metabolic landscape [48, 49]. This approach, despite its challenges, aimed to offer a deeper understanding of the complex metabolic alterations in leukemia patients with varied vaccination histories.

Previous studies on leukemia have reported changes in energy metabolism, nucleotide synthesis,

Table 2. Putative identification of differentially abundant metabolites in leukemia patients with and without BNT162b2 vaccination and healthy controls.

No.	Metabolite	LO/C	LO/C*	ASL/C	ASL/C*	ASL/LO	ASL/LO*
		FC		FC		FC	
1	D-Glucose/ D-Fructose	1.93	+	1.09	+	1.77	-
2	5-Methoxyindoleacetate	1.55	+	1.03	+	1.50	-
3	D-Erythrose 4-phosphate	1.54	+	1.17	+	1.31	-
4	1-(sn-Glycero-3-phospho)-1D-myo-inositol	1.71	+	1.15	+	1.48	-
5	Uroporphyrinogen I/III	1.56	+	1.15	+	1.35	-
6	Dimethylarsinate	1.71	+	1.09	-	1.87	-
7	N-Acetyllactosamine	1.76	+	2.07	-	3.66	-
8	Chondroitin	2.09	+	2.21	-	4.64	-
9	Tetrahydrofolic acid	1.65	+	1.17	-	1.94	-
10	Delta 8.14 -Sterol	1.51	+	1.20	-	1.81	-
11	N-Formyl-L-glutamic acid/ N-Acetyl-L-aspartic acid	2.68	-	1.06	+	2.85	+
12	Phosphorylcholine	1.80	-	1.02	+	1.84	+

FC: Fold Change

*** +**: Indicates an increase in the level of the metabolite.

-: Indicates a decrease in the level of the metabolite.

and immune response, which are consistent with our findings. For instance, a study by Chen *et al*. (2015) highlighted the role of altered glycolysis in acute myeloid leukemia (AML) cells [50]. Similarly, our study observed increased levels of monosaccharides, suggesting altered glycolytic activity in leukemia patients. The regulation of glycolytic processes in AML blasts and leukemic stem cells (LSCs) involves multiple molecular mechanisms [51]. This metabolic reprogramming is orchestrated through enhanced expression and activity of key glycolytic enzymes, regulated by various factors including hypoxia-inducible factor 1 alpha (HIF1α) [52], the PI3K/mTOR signaling pathway [53] and epigenetic modifications mediated by ten-eleven translocation 3 (TET3) through 5-hydroxymethylcytosine (5hmC) [54].

Vieira *et al*. (2024) demonstrated that Ara-C-resistant AML cell lines exhibit distinct metabolic adaptations, with KG-1 Ara-R cells showing enhanced glycolytic phenotype and increased sensitivity to metabolic inhibitors, while MOLM13 Ara-R cells maintained baseline glucose metabolism, suggesting that targeting these metabolic alterations could provide new therapeutic strategies for chemoresistant AML patients [55].

Studies have demonstrated the importance of tryptophan metabolism in regulating immune responses in AML [56, 57]. In line with these findings, our observation of increased levels of 5-methoxy-indoleacetate, a tryptophan derivative, may indicate altered immune regulation in leukemia patients. This observation aligns with current understanding of tryptophan metabolism's crucial role in cancer progression. As Yan *et al*. (2024) describe, tryptophan metabolism occurs through three main pathways: the kynurenine pathway, the serotonin pathway, and the indole pathway. The dysregulation of these pathways can create an immunosuppressive tumor micro-environment. The kynurenine pathway is especially significant, as it produces immunosuppressive metabolites that interact with the aryl hydrocarbon receptor (AHR) to promote tumor growth and immune evasion [58]. Our findings of altered tryptophan metabolites in leukemia patients may reflect these broader mechanisms of immune modulation through tryptophan metabolism.

We observed increased levels of metabolites involved in the pentose phosphate pathway, such as D-erythrose 4-phosphate and sedoheptulose 1-phosphate, which aligns with previous findings in leukemia [59]. The pentose phosphate pathway is crucial for nucleotide synthesis and redox homeostasis, and its dysregulation may contribute to the survival and proliferation of leukemia cells [60, 61]. We observed increased levels of metabolites involved in the pentose phosphate pathway, such as D-erythrose 4-phosphate and sedoheptulose 1-phosphate, which aligns with previous findings in leukemia [59]. The pentose phosphate pathway is crucial for nucleotide synthesis and redox homeostasis, and its dysregulation may contribute to the survival and proliferation of leukemia cells [60].

1-(sn-Glycero-3-phospho)-1D-myo-inositol plays a crucial role in cellular membrane composition and serves as a precursor for phosphatidylinositol 3,4,5-trisphosphate (PIP3) synthesis [62]. PIP3 functions as a key signaling molecule that activates the PI3K/Akt/mTOR pathway, which has been implicated in leukemia pathogenesis and drug resistance [63]. Our study revealed altered levels of 1-(sn-Glycero-3-phospho)-1D-myo-inositol in both LO and ASL samples, with relatively higher concentrations observed in LO samples. Recent research by Gu *et al*. (2024) has further elucidated the significance of the PI3K pathway in AML, particularly highlighting the role of PI3Kγ in leukemia stem cells (LSCs) (64). Their findings demonstrated that PI3Kγ is highly enriched in LSCs and essential for their self-renewal, while remaining dispensable for normal hematopoietic stem cells. The mechanism involves PI3Kγ-AKT signaling promoting nuclear factor erythroid 2-related factor 2 (NRF2) nuclear accumulation, which subsequently induces 6-phosphogluconate dehydrogenase (PGD) and the pentose phosphate pathway, maintaining LSC stemness. The selective dependence of LSCs on PI3Kγ suggests that targeting this pathway could provide a promising therapeutic strategy for AML treatment without compromising normal hematopoiesis [64-66].

Uroporphyrinogen I or Uroporphyrinogen III, altered in LO samples and ASL, are metabolites involved in porphyrin biosynthesis [67]. A study found that mouse leukemic cells derived from hematopoietic progenitor cells with overexpression of MYCN cDNA (known as MYCN-HPCs) required heme/porphyrin biosynthesis for complete self-renewal and transformation into cancer cells [68]. Dysregulation of heme biosynthesis may impact cellular differentiation, proliferation, and drug resistance [69, 70] Uroporphyrinogen I or Uroporphyrinogen III, altered in LO samples and ASL, are metabolites involved in porphyrin biosynthesis [67]. A study found that mouse leukemic cells derived from hematopoietic progenitor cells with overexpression of MYCN cDNA (known as MYCN-HPCs) required heme/porphyrin biosynthesis for complete self-renewal and transformation into cancer cells [68]. Dysregulation of heme biosynthesis may impact cellular differentiation, proliferation, and drug resistance [69, 70].

Yamauchi *et al*. identified alterations in the pathways responsible for purine synthesis and salvage in AML, highlighting their potential role in meeting the increased nucleotide demand of rapidly dividing cells [71]. The observed decrease in tetrahydrofolic acid levels—a key metabolite involved in purine and pyrimidine synthesis—in the ASL samples may suggest a complex interaction between the mRNA vaccine-induced immune response and these metabolic pathways. These findings could have implications for understanding how the immune response to vaccines affects metabolism, particularly in individuals with leukemia, and open new avenues for research into the immunometabolism of vaccine response.

Several metabolites were significantly decreased in the LO group compared to the control group. The reduction in phosphorylcholine may indicate altered lipid metabolism, possibly due to leukemia. This finding is consistent with previous studies demonstrating lipid and amino acid metabolism changes in leukemia patients [72-74]. one study reported that LP-CAT1 levels were significantly reduced in bone marrow samples from AML patients compared to healthy controls.

The unique properties of mRNA vaccines may contribute to the metabolomic differences observed between leukemia cases in vaccinated and unvaccinated individuals. Various factors, including genetics, lifestyle, environment, and immune response, likely influence these differences. Since mRNA vaccines target the viral spike protein and stimulate an immune response, it is worth exploring whether the metabolite changes directly relate to the vaccine or reflect indirect effects on the immune response within the bone marrow microenvironment. It's important to emphasize that mRNA vaccines have been crucial in protecting against COVID-19, and this study aims to enhance our understanding of their effects in specific situations. Our research does not establish a direct link between mRNA vaccines and leukemia development; instead, it raises new questions about how the vaccine might influence the metabolic environment of the bone marrow, which could have implications for leukemia onset or progression.

Our pathway analysis, presenting the changes in glycolysis, the pentose phosphate pathway, tryptophan metabolism, lipid metabolism, and heme biosynthesis, underscores the complex metabolic reprogramming in leukemia. Our study observed increased glycolytic activity and upregulation of the pentose phosphate pathway, suggesting leukemia cells adapt their metabolism to support rapid proliferation and manage oxidative stress. Alterations in tryptophan and lipid metabolism further highlight the intricate relationship between metabolism and immune regulation in leukemia, potentially influenced by mRNA vaccination. The findings regarding the PI3K/Akt/mTOR pathway and heme biosynthesis pathways also provide a deeper understanding of the mechanisms that may

drive leukemia pathogenesis and drug resistance, suggesting possible targets for future therapeutic interventions.

Overall, these results underscore the importance of investigating the metabolic impact of mRNA vaccines within the context of leukemia and suggest that further targeted studies are necessary to elucidate the precise mechanisms underlying these metabolic changes.

LIMITATIONS OF THE STUDY

Several important limitations should be considered when interpreting our findings. First, the relatively small sample size (n=16), particularly the limited number of subjects in the LO group (n=2), constrained our ability to draw statistically robust conclusions. Second, the LC-QTOF-MS-based untargeted metabolomics approach provided only putative metabolite identifications (Level 3), necessitating validation through targeted studies. Third, the cross-sectional study design prevents the establishment of causal relationships between vaccination and metabolic changes. Additionally, temporal variations between vaccination and leukemia onset (15–63 days) may have influenced metabolic profiles. Fourth, potential confounding variables, including age, gender, comorbidities, and leukemia subtypes, could not be fully controlled. Fifth, the use of a single analytical platform may have limited the detection of certain metabolite classes, while the focus on liquid-phase metabolites potentially excluded relevant cellular component information.

These limitations highlight the preliminary nature of our findings and emphasize the need for larger-scale, longitudinal studies with targeted metabolomics approaches to validate our observations and better understand the biological significance of the metabolic differences observed in leukemia patients with and without prior mRNA COVID-19 vaccination

CONCLUSION

This study presents an untargeted LC-QTOF-MS metabolomics analysis, revealing metabolite profile differences among three groups: leukemia patients with prior BNT162b2 mRNA COVID-19 vaccination, unvaccinated leukemia patients, and healthy unvaccinated controls. The observed PLS-DA clustering patterns suggest potential metabolic variations between these groups. However, several important limitations should be acknowledged when interpreting our findings. The small sample size restricted the statistical robustness of our conclusions. Additionally, the LC-QTOF-MS-based untargeted metabolomics approach provided only putative metabolite identifications, which require further validation through targeted studies. The cross-sectional study design does not establish causal relationships between vaccination and metabolic changes. Despite these limitations, our findings provide preliminary data that may inform future research on how mRNA vaccines interact with bone marrow metabolism in the context of leukemia. Further studies with larger cohorts, validated metabolite identifications, and detailed pathway analyses are necessary to determine the biological relevance of these observations. It is important to emphasize that this study does not suggest any causal link between mRNA vaccination and leukemia development or progression. mRNA vaccines have played a crucial role in global public health by significantly reducing severe COVID-19 outcomes and saving millions of lives. Understanding their effects in various clinical settings is essential for optimizing patient care. Our findings lay the foundation for future investigations into these complex interactions, ultimately contributing to a more comprehensive understanding of vaccine safety and the metabolic dynamics of leukemia.

AUTHORS' CONTRIBUTIONS

The authors confirm their contribution to the paper as follows:

Study conception and design: MC, UYM, ICH

data collection: OK

analysis and interpretation of results: BBF

draft manuscript: BE

All authors reviewed the results and approved the final version of the manuscript.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was obtained from the Institutional Ethics Committee of Hacettepe University (protocol code SBA 24/030 on January 9, 2024).

HUMAN AND ANIMAL RIGHTS

All procedures performed in studies involving human participants were under the ethical standards of the institutional and/or research committee and with the 1975 Declaration of Helsinki, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was waived for this retrospective study due to the exclusive use of de-identified patient data, which posed no potential harm or impact on patient care.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. Raw mass spectrometry data files, processed metabolomic data, and relevant metadata are stored in a secure repository to ensure data integrity and accessibility. Researchers interested in accessing the data for further analysis or validation are encouraged to contact the corresponding author, providing a brief description of the intended use of the data. Data sharing is subject to compliance with ethical standards and data use agreements.

STANDARDS OF REPORTING

STROBE guidelines were followed.

FUNDING

None.

CONFLICT OF INTEREST

The author(s) declare no conflict of interest, financial or otherwise.

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