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Article

Keywords: Acute lymphoblastic leukemia, Pediatric, Metabolomics, Lipid metabolic remodeling, Minimal residual disease

Posted Date: October 5th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10891030/v1>

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Additional Declarations: No competing interests reported.

Dynamic Lipid Metabolic Remodeling During Induction Chemotherapy Is Associated With Early Treatment Response and Minimal Residual Disease in Children With Acute Lymphoblastic Leukemia

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[Abstract] Objective: Metabolic reprogramming may contribute to leukemic cell survival and treatment response in acute lymphoblastic leukemia (ALL). This study aimed to characterize dynamic metabolic remodeling during induction chemotherapy and evaluate its association with early treatment response. **Methods:** Sixteen children with newly diagnosed ALL were enrolled. Paired serum samples were collected before and at the end of induction chemotherapy and analyzed using untargeted liquid chromatography – mass spectrometry-based metabolomics. Differential metabolites were identified using multivariate and univariate analyses, followed by pathway enrichment analysis. Changes in selected metabolites were correlated with hematological recovery velocities and minimal residual disease (MRD) status on days 15 and 33. **Results:** Induction chemotherapy induced pronounced metabolic remodeling, with lipid-related metabolites showing the most prominent alterations. Differential metabolites were mainly enriched in phospholipid-, glycerophospholipid-, ether lipid-, and sphingolipid-related pathways. Six representative lipid metabolites, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), lysophosphatidylcholine (LysoPC), and sphingomyelin (SM) species, were further analyzed. Spearman analysis showed that several metabolite changes were moderately

associated with hematological recovery. The change in PC(16:0/18:1) was negatively correlated with platelet recovery velocity ($r = -0.671$) and positively correlated with day-33 MRD status ($r = 0.575$). PC(P-16:0/18:1) was negatively correlated with day-33 MRD status ($r = -0.547$). Changes in PE(18:0/18:2) were negatively correlated with WBC and neutrophil recovery velocities ($r = -0.590$ and -0.574 , respectively). **Conclusions:** Induction chemotherapy is associated with substantial lipid metabolic remodeling in children with ALL. Dynamic alterations in specific lipid metabolites are associated with hematological recovery and MRD status, providing preliminary evidence for further investigation of lipid metabolism in early treatment response and MRD persistence.

[Keywords] Acute lymphoblastic leukemia; Pediatric; Metabolomics; Lipid metabolic remodeling; Minimal residual disease

1.Introduction

Acute lymphoblastic leukemia (ALL) represents the predominant form of leukemia in children, with an annual incidence of approximately 3 – 4 cases per 100,000 children and accounting for nearly 75% – 80% of pediatric leukemias^[1]. Over recent decades, advances in risk-adapted multiagent chemotherapy, supportive care, and treatment stratification incorporating minimal residual disease (MRD) have markedly improved the prognosis of childhood ALL, with 5-year event-free survival (EFS) exceeding 85% in some high-income countries^[2, 3]. Despite these improvements, relapse remains a major obstacle to long-term cure. International multicenter studies have reported overall relapse rates of approximately 12% – 20%^[4, 5], and outcomes after relapse remain unsatisfactory, with 5-year overall survival generally below 50%. Similarly, Chinese multicenter studies based on standardized protocols, including CCCG-ALL-2015 and CCLG-2008, have reported cumulative relapse rates of approximately 15% during 5 – 8 years of follow-up^[6]. These observations highlight the need to better characterize treatment-associated biological changes and to identify markers reflecting early therapeutic response, particularly those associated with persistent MRD, which remains one of the most important indicators of treatment sensitivity and relapse risk in pediatric ALL.

In recent years, metabolic reprogramming has increasingly been recognized as an important biological feature of tumor development and treatment response^[7, 8]. Under conditions of rapid proliferation and chemotherapy-induced stress, leukemic cells continuously adapt multiple metabolic networks, including glucose, nucleotide, and lipid metabolism, to maintain cellular proliferation and survival^[9-11]. Previous studies have shown that induction chemotherapy can induce extensive alterations in plasma metabolites and complex lipids in children with ALL, with lipid molecules such as phosphatidylethanolamines and triacylglycerols showing prominent treatment-associated changes. These findings suggest that metabolomics can provide a comprehensive view of biological changes occurring during ALL treatment^[12-14]. However, current metabolomic studies in pediatric ALL have mainly focused on disease diagnosis, metabolic differences between treatment stages, or metabolic features at individual time points, whereas the relationship between dynamic metabolic changes during induction chemotherapy and early treatment response, hematological recovery, and MRD remains insufficiently characterized. Therefore, investigating treatment-associated metabolic remodeling from a dynamic perspective may provide valuable insights into its clinical relevance in pediatric ALL.

Based on this rationale, we performed a longitudinal paired untargeted serum metabolomic analysis to compare serum metabolic profiles before and after induction chemotherapy in children with ALL and systematically characterize treatment-associated metabolic remodeling and the major metabolic pathways involved. Representative lipid metabolites were subsequently selected, and their dynamic changes during induction therapy were evaluated in relation to hematological recovery and MRD status on days 15 and 33. This study aimed to explore the potential association between induction chemotherapy-associated lipid metabolic remodeling and early treatment response in pediatric ALL and to provide a basis for further investigation of the biological mechanisms through which lipid metabolism may contribute to treatment response and MRD persistence.

2. Materials and Methods

2.1 Blood collection and sample preparation

The study population was recruited from the First Affiliated Hospital of Xinjiang Medical University. Children with newly diagnosed ALL who received induction chemotherapy at our institution between January 2025 and June 2025 were enrolled. A total of 16 children with ALL were included. Paired serum samples were collected at two time points: one day before the initiation of induction chemotherapy (pre-induction) and day 33 of induction therapy (post-induction), yielding a total of 32 serum samples. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University. Written informed consent was obtained from the parents or legal guardians of all participants before enrollment and initiation of treatment. All blood samples were collected under non-fasting conditions. After complete clotting, blood samples were centrifuged at $1,500 \times g$ for 15 min at -5°C . The serum was carefully separated, aliquoted, and immediately stored at -80°C until metabolomic analysis.

2.2 Serum Sample Preparation and Metabolomic Analysis

Serum samples were thawed on ice prior to metabolite extraction. An aliquot of $100 \mu \text{L}$ serum was thoroughly mixed with $400 \mu \text{L}$ of pre-cooled methanol/acetonitrile (1:1, v/v) by vortexing. The mixture was subsequently sonicated for 1 h in an ice bath and incubated at -20°C for 1 h. The samples were then centrifuged at $14,000 \times g$ for 20 min at 4°C . The resulting supernatants were carefully collected and dried under vacuum. The dried extracts were reconstituted in 50% acetonitrile and filtered through a disposable $0.22\text{-}\mu \text{m}$ cellulose acetate membrane before LC-MS analysis.

2.3 UHPLC-MS/MS Analysis

Metabolomic profiling was performed using an ultra-high-performance liquid chromatography – mass spectrometry (UHPLC-MS/MS) platform consisting of a Nexera X2 LC-30AD UHPLC system (Shimadzu, Japan) coupled with a Q Exactive Plus mass spectrometer (Thermo Scientific, San Jose, CA, USA). Chromatographic separation was performed using an ACQUITY UPLC HSS T3 column ($2.1 \times 100 \text{ mm}$, $1.8 \mu \text{m}$; Waters, Milford, MA, USA), with water containing 0.1% formic acid and acetonitrile as the mobile phases. Data were acquired in both positive and

negative electrospray ionization (ESI) modes over an m/z range of 70 – 1050. Full-scan MS data were acquired at a resolution of 70,000, followed by MS/MS acquisition for metabolite identification.

2.4 Data Preprocessing and Metabolite Identification

Raw LC-MS data were processed using MS-DIAL for peak detection, retention time correction, peak alignment, and peak area extraction. Metabolite features were identified based on accurate mass measurements and MS/MS spectral information by matching against publicly available databases, including the Human Metabolome Database (HMDB) and MassBank, as well as an in-house metabolite library. Metabolite features detected in more than 50% of the samples were retained for subsequent analyses. Quality control (QC) samples were prepared by pooling aliquots of the study samples and were processed and analyzed using the same procedures as the experimental samples to assess the stability and reproducibility of the analytical system.

2.5 Differential Metabolite and Pathway Analysis

Multivariate statistical analyses were performed to characterize the overall metabolic differences between paired pre-induction and post-induction samples. Principal component analysis (PCA) was initially performed to evaluate the overall distribution of samples and identify potential outliers. Orthogonal partial least-squares discriminant analysis (OPLS-DA) was subsequently performed to characterize treatment-associated metabolic separation, and model robustness was assessed using 200 permutation tests to evaluate potential overfitting. Differential metabolites were identified based on a variable importance in projection (VIP) score >1.0 and a two-sided Student's t -test $P < 0.05$. Fold changes (FCs) were calculated to characterize the magnitude and direction of metabolic alterations between pre-induction and post-induction samples. Differential metabolites were further annotated and mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database for pathway enrichment analysis^[15-17]. Enriched pathways were evaluated using Fisher's exact test, with a false discovery rate (FDR)-adjusted $P < 0.05$ considered statistically significant.

2.6 Clinical Correlation Analysis

To further investigate the clinical relevance of chemotherapy-associated metabolic alterations, six representative lipid metabolites were selected based on their differential expression patterns, lipid metabolic pathway enrichment, and biological relevance. For each selected metabolite, the change during induction therapy (Δ metabolite) was calculated as the difference between post-induction and pre-induction levels. The associations between changes in selected lipid metabolites and hematological recovery kinetics were subsequently assessed. Hematological recovery velocities were calculated based on changes in peripheral blood cell counts during the early treatment period, including the intervals from baseline to day 15 and from baseline to day 33. Spearman's rank correlation analysis was used to evaluate the relationships between Δ metabolite values and hematological recovery parameters, including white blood cell, neutrophil, lymphocyte, red blood cell, hemoglobin, and platelet recovery velocities. The associations between metabolite changes and minimal residual disease (MRD) status on days 15 and 33 were also evaluated using Spearman's rank correlation analysis. A two-sided $P < 0.05$ was considered statistically significant.

3. Results

3.1 Study design and global metabolic profiling of pediatric ALL patients

Sixteen pediatric patients with newly diagnosed acute lymphoblastic leukemia (ALL) were included in the metabolomic analysis. Paired serum samples were collected from each patient before and after induction chemotherapy. Untargeted liquid chromatography – mass spectrometry (LC-MS) was subsequently performed to characterize the metabolic profiles, followed by peak detection, alignment, normalization, metabolite annotation, differential metabolite analysis, pathway enrichment, and correlation with clinical hematological outcomes (Figure 1A). To assess the overall metabolic differences between the pre- and post-induction samples, multivariate analyses were performed. The OPLS-DA score plot demonstrated a clear separation between the two metabolic states, with the pre-induction and post-induction samples forming distinct clusters (Figure 1B). Similarly, the PLS-DA

model showed an apparent separation between the two groups, although partial overlap was observed between the confidence regions (Figure 1C). Permutation testing supported the robustness of the model, with an observed cumulative R^2 of 0.9789 and Q^2 of -0.57 (Figure 1C - D). These findings indicate that induction chemotherapy was associated with substantial alterations in the global serum metabolic profile.

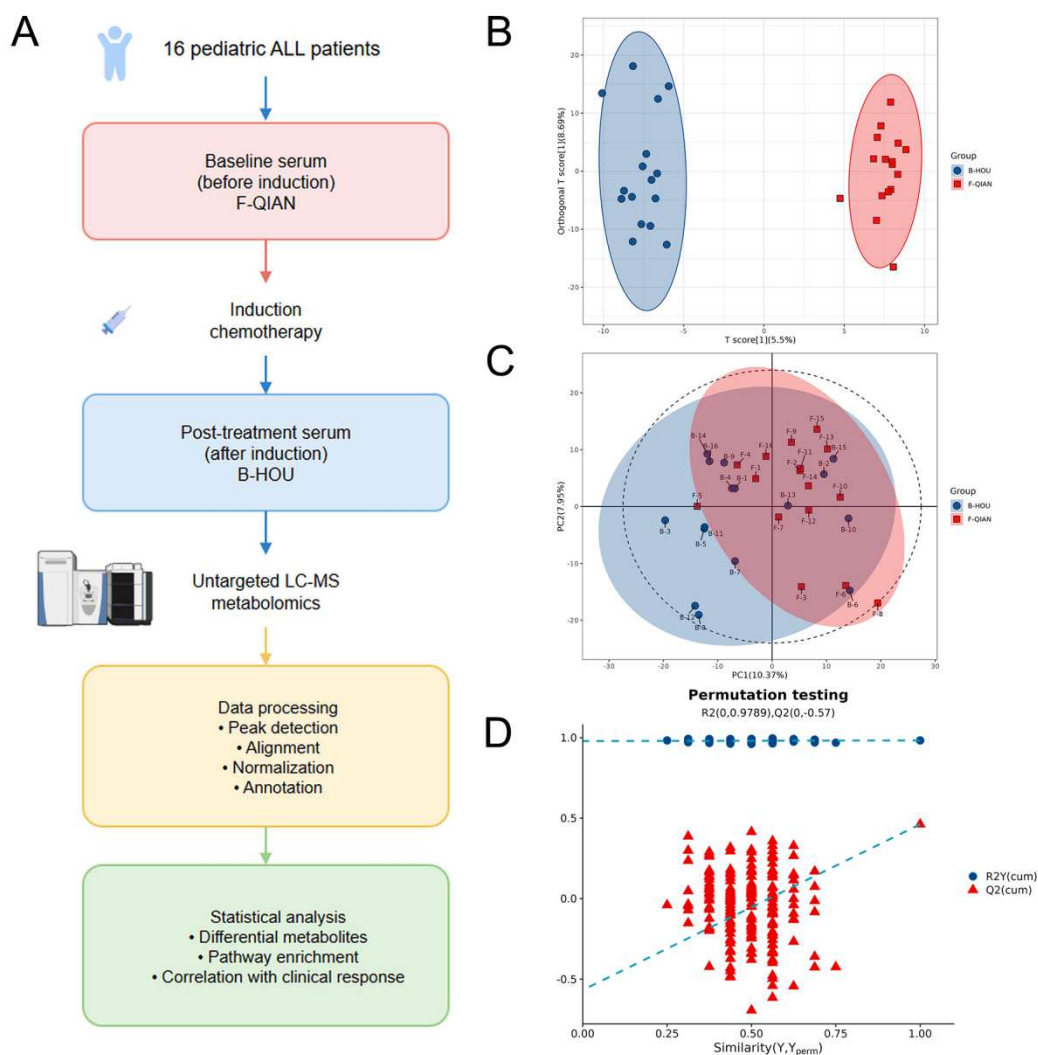


Figure 1. Study design and multivariate analysis of serum metabolomic profiles before and after induction chemotherapy. (A) Study workflow of the untargeted LC - MS metabolomics analysis. (B) OPLS-DA score plot of pre- and post-induction samples. (C) PCA score plot of the two groups. (D) Permutation test of the OPLS-DA model.

Abbreviations: ALL, acute lymphoblastic leukemia; LC – MS, liquid chromatography – mass spectrometry; PCA, principal component analysis; OPLS-DA, orthogonal partial least squares discriminant analysis.

3.2 Global metabolic profiling revealed distinct metabolic patterns following induction chemotherapy

Hierarchical clustering analysis demonstrated distinct metabolic patterns between serum samples obtained before and after induction chemotherapy (Figure 2A). Samples from the two treatment time points exhibited broadly different abundance patterns across the detected metabolites, with several clusters showing consistent increases or decreases after treatment. The differential analysis further identified a substantial number of metabolites that differed between the pre- and post-induction samples. The volcano plot showed that numerous metabolites were significantly altered, with both increased and decreased metabolites observed after induction chemotherapy (Figure 2B – C). Notably, several metabolites exhibited relatively large fold changes and high statistical significance, indicating that induction chemotherapy was associated with marked alterations in serum metabolic composition.

Among the differential metabolites, lipids and lipid-like molecules constituted the largest molecular class, accounting for 53 metabolites (48.18%), followed by organic acids and derivatives (21, 19.09%) and organoheterocyclic compounds (13, 11.82%) (Figure 2D). Benzene-related compounds accounted for 7 metabolites (6.36%), while other molecular classes represented smaller proportions. Overall, these findings suggested that lipid metabolism represented a major metabolic component affected by induction chemotherapy in pediatric ALL patients.

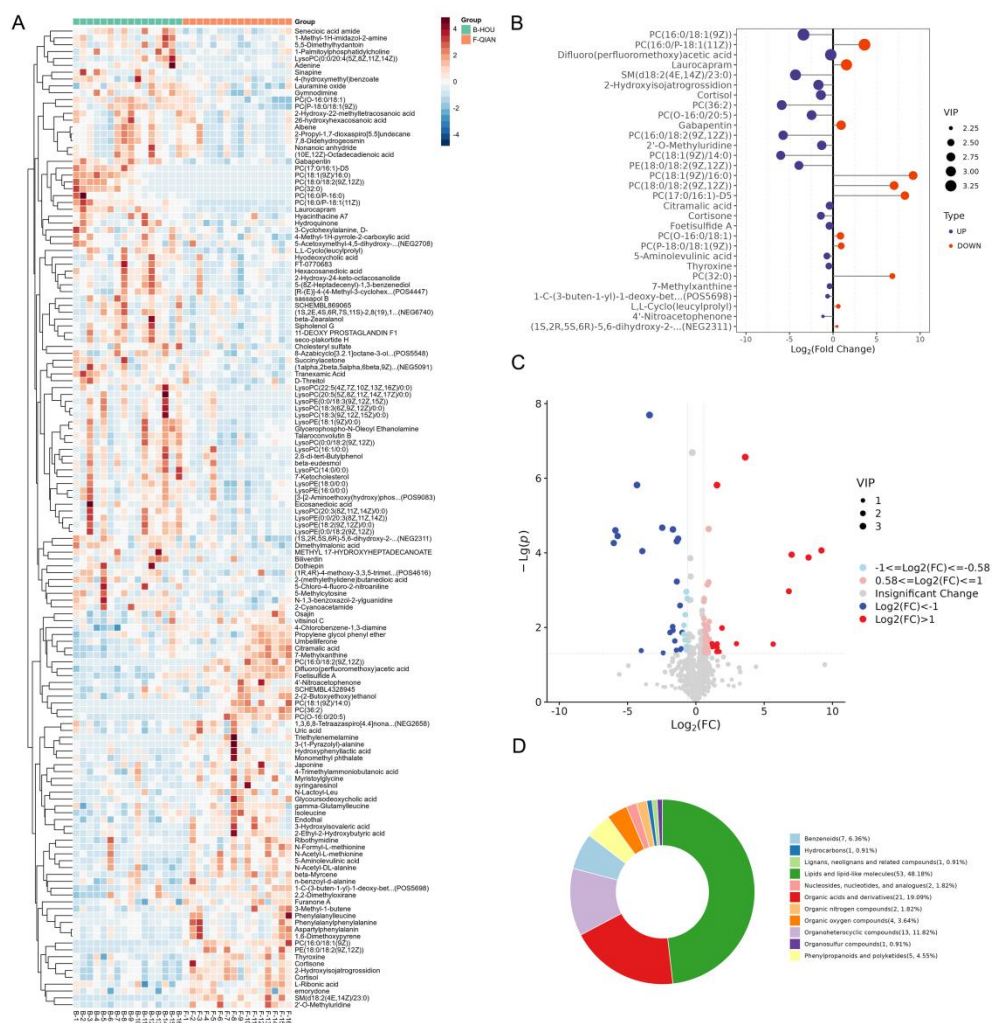


Figure 2. Differential metabolite profiles before and after induction chemotherapy.

(A) Hierarchical clustering heatmap of differential metabolites. (B) Fold changes and VIP values of representative differential metabolites. (C) Volcano plot of differential metabolites. (D) Chemical classification of the differential metabolites.

Abbreviations: VIP, variable importance in projection; FC, fold change.

3.3 Identification of representative differential metabolites

To further characterize the metabolites most strongly associated with the metabolic differences between the two treatment time points, metabolites were prioritized according to their variable importance in projection (VIP) values and statistical significance. Several metabolites exhibited high VIP values together with strong statistical significance (Figure 3A). Among these, PC(16:0/18:1(9Z)), PC(16:0/P-18:1(11Z)), SM(d18:2(4E,14Z)/23:0), PC(16:0/18:2(9Z,12Z)), PE(18:0/18:2(9Z,12Z)), and LysoPC(0:0/18:2(9Z,12Z)) were identified as

representative lipid-related metabolites and selected for subsequent clinical correlation analyses.

Direct comparison of the relative abundances further demonstrated pronounced changes in several of these lipid metabolites between the two treatment time points. PC(16:0/18:1(9Z)) showed a marked increase after induction chemotherapy ($P = 2.02 \times 10^{-8}$; Figure 3B), whereas PC(16:0/P-18:1(11Z)) was substantially decreased ($P = 2.72 \times 10^{-7}$; Figure 3C). PE(18:0/18:2(9Z,12Z)) was significantly increased following treatment ($P = 8.98 \times 10^{-5}$; Figure 3D). LysoPC(0:0/18:2(9Z,12Z)) showed a significant reduction after induction chemotherapy ($P = 9.93 \times 10^{-3}$; Figure 3F), whereas SM(d18:2(4E,14Z)/23:0) exhibited a marked increase in the post-induction samples ($P = 1.52 \times 10^{-6}$; Figure 3G). PC(16:0/18:2(9Z,12Z)) was also among the highly prioritized metabolites based on its VIP value and statistical significance and was therefore included in the subsequent clinical correlation analyses. Taken together, these findings indicated that induction chemotherapy was accompanied by pronounced alterations in phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, and sphingomyelin metabolism.

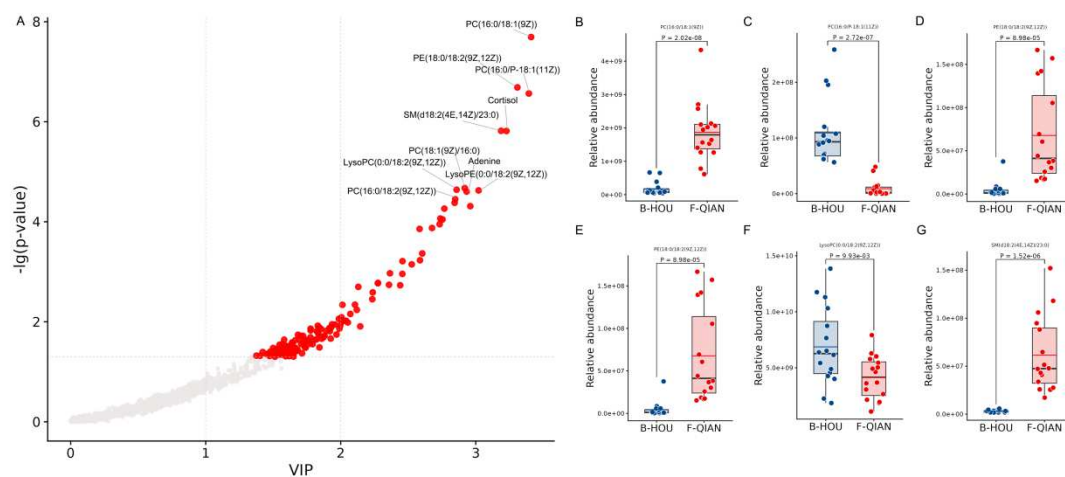


Figure 3. Identification and relative abundance of representative lipid metabolites altered after induction chemotherapy.

(A) Distribution of metabolites according to VIP values and statistical significance. (B – G) Relative abundances of six representative lipid metabolites before and after induction chemotherapy. P values are shown in the corresponding panels.

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; LysoPC, lysophosphatidylcholine.

3.4 Pathway enrichment analysis revealed alterations in lipid-related metabolic pathways

To further investigate the biological pathways associated with the altered metabolites, pathway enrichment analysis was performed using the identified differential metabolites (Figure 4A). The enriched pathways involved multiple biological processes, including lipid metabolism, amino acid metabolism, nucleotide metabolism, membrane transport, signaling and interaction, and metabolic pathways related to cofactors and vitamins. Among these pathways, lipid metabolism represented one of the major metabolic categories affected by induction chemotherapy. The pathway analysis further demonstrated enrichment of metabolites involved in several metabolic networks, suggesting that the metabolic alterations observed after induction chemotherapy were not restricted to individual metabolites but involved coordinated changes across interconnected biochemical pathways. A network-based analysis was subsequently constructed to visualize the relationships among the altered metabolites and their associated metabolic pathways (Figure 4B). The network revealed extensive interactions among lipid-related metabolites and other metabolic components, further supporting the involvement of interconnected metabolic pathways following induction chemotherapy.

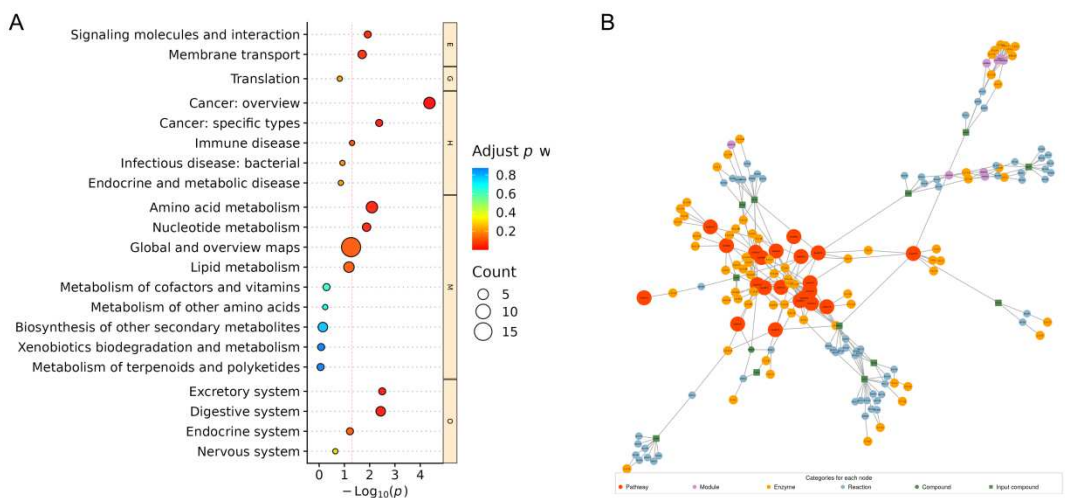


Figure 4. Pathway enrichment and network analysis of differential metabolites.

(A) KEGG pathway enrichment analysis of differential metabolites. Bubble size represents the number of metabolites, and color indicates the adjusted P value. (B) Metabolic network of the differential metabolites and their associated pathways, enzymes, reactions, and compounds.

Abbreviation: KEGG, Kyoto Encyclopedia of Genes and Genomes.

3.5 Clinical correlation of lipid metabolites with hematological recovery and minimal residual disease

To investigate whether treatment-associated metabolic alterations were associated with dynamic clinical changes and treatment response, the six representative lipid metabolites were further examined for correlations with the change velocities of hematological and biochemical parameters, including WBC, neutrophils (NEU), platelets (PLT), lactate (Lac), and albumin (ALB), as well as minimal residual disease (MRD) measurements (Figure 5). Spearman correlation analysis revealed several significant associations between lipid metabolites and hematological recovery. PC(16:0/18:1(9Z)) was negatively correlated with NEU change velocity during days 0 - 15 ($\rho = -0.51$, $P < 0.05$) and showed an even stronger negative correlation with PLT change velocity during the same period ($\rho = -0.67$, $P < 0.01$). SM(d18:2(4E,14Z)/23:0) was negatively correlated with WBC change velocity during days 0 - 33 ($\rho = -0.55$, $P < 0.05$). PE(18:0/18:2(9Z,12Z)) showed consistent negative correlations with hematological recovery, including WBC change velocities during days 0 - 15 ($\rho = -0.56$, $P < 0.05$) and 0 - 33 ($\rho = -0.59$, $P < 0.05$), as well as NEU change velocity during days 0 - 15 ($\rho = -0.57$, $P < 0.05$). Similarly, LysoPC(0:0/18:2(9Z,12Z)) was negatively correlated with WBC change velocities during both days 0 - 15 ($\rho = -0.53$, $P < 0.05$) and days 0 - 33 ($\rho = -0.55$, $P < 0.05$). In contrast, PC(16:0/P-18:1(11Z)) and PC(16:0/18:2(9Z,12Z)) showed no significant correlations with the evaluated hematological recovery indices.

Notably, specific lipid metabolites were also associated with MRD at day 33. PC(16:0/18:1(9Z)) was positively correlated with MRD33 ($\rho = 0.57$, $P < 0.05$), whereas PC(16:0/P-18:1(11Z)) was negatively correlated with MRD33 ($\rho = -0.55$, $P < 0.05$). No significant correlations were observed between the six lipid metabolites

and MRD15. Collectively, these findings indicate that treatment-associated alterations in selected lipid metabolites were associated with distinct patterns of hematological recovery and, in the case of PC(16:0/18:1(9Z)) and PC(16:0/P-18:1(11Z)), with treatment response as reflected by MRD33 in pediatric ALL.

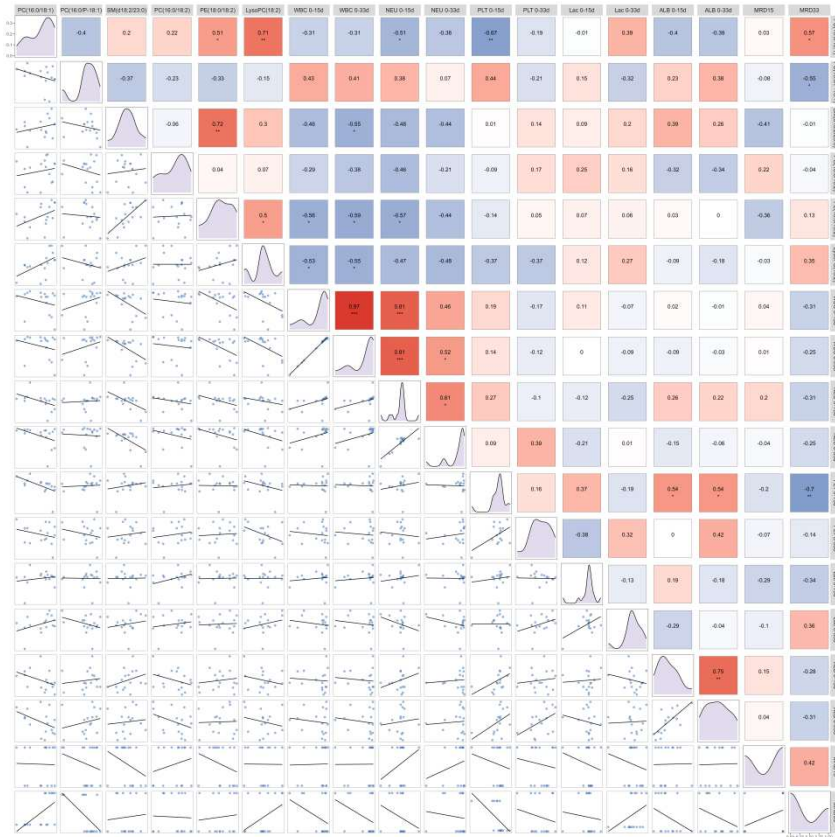


Figure 5. Correlations between representative lipid metabolites and clinical parameters during induction chemotherapy. Spearman correlation analysis was performed between six representative lipid metabolites, clinical change velocities, and minimal residual disease (MRD). The upper triangle shows Spearman's correlation coefficients (ρ), the lower triangle shows scatter plots, and the diagonal shows variable distributions. Red and blue indicate positive and negative correlations, respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. For readability, simplified lipid labels were used in the figure: PC(16:0/18:1), PC(16:0/P-18:1), SM(d18:2/23:0), PC(16:0/18:2), PE(18:0/18:2), and LysoPC(18:2), corresponding to PC(16:0/18:1(9Z)), PC(16:0/P-18:1(11Z)), SM(d18:2(4E,14Z)/23:0), PC(16:0/18:2(9Z,12Z)), PE(18:0/18:2(9Z,12Z)), and LysoPC(0:0/18:2(9Z,12Z)), respectively.

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; LysoPC, lysophosphatidylcholine; WBC, white blood cell count; NEU, neutrophil count; PLT, platelet count; Lac, lactate; ALB, albumin; MRD, minimal residual disease.

4. Discussion

Metabolic reprogramming is increasingly recognized as an important feature of ALL^[18, 19]. Current metabolomic studies in pediatric ALL have mainly focused on distinguishing patients from healthy controls or comparing metabolic differences across different treatment stages^[20, 21]. With the continuous optimization of induction treatment regimens and improvements in MRD remission rates in pediatric ALL, further exploration of the relationship between metabolic alterations and MRD is of potential clinical importance^[22]. In the present study, paired metabolomic analysis revealed substantial metabolic remodeling during induction chemotherapy, with particularly prominent alterations in glycerophospholipid and sphingolipid metabolism. Multiple PC, PE, LysoPC, and SM species showed significant treatment-associated changes and were further correlated with dynamic hematological parameters and MRD, suggesting that lipid remodeling during induction therapy may not only reflect chemotherapy-related metabolic alterations but may also be associated with hematopoietic recovery and early treatment response.

Previous studies have demonstrated that induction chemotherapy induces broad metabolic alterations in pediatric ALL. Saito et al. analyzed more than 1,200 metabolites and complex lipids before and after induction therapy and found that more than 20% were significantly altered, with lipid metabolism, particularly phosphatidylethanolamine-related metabolism, among the most prominently affected pathways^[23]. Similarly, Papadopoulou et al. compared plasma metabolic profiles at diagnosis and day 33 and identified significant alterations in fatty acid metabolism, amino acid metabolism, glycerophospholipid metabolism, and glutaminolysis/TCA cycle activity^[24]. More recently, Yan et al. reported pronounced abnormalities in phospholipid and sphingolipid metabolism in children with ALL, involving multiple PC, PE, ceramide, and SM species^[25]. Collectively, these findings indicate that lipid

metabolism represents an important component of metabolic remodeling in pediatric ALL. Our study further showed that different molecular species within the same lipid class did not necessarily change in the same direction, suggesting that treatment-associated lipid remodeling may be molecular-species specific. Krull et al. reported a significant increase in SM after induction therapy in a prospective study of children with ALL. Lin et al. further suggested that SM may promote SLC2A1-dependent glucose uptake and glycolysis and contribute to apoptosis resistance in ALL cells through CASP3 lactylation^[26]. Consistent with these observations, a specific SM species identified in our study increased after induction therapy, suggesting that the biological significance of sphingolipid metabolism may depend more on individual molecular species than on overall changes in the entire lipid class.

Another important finding of the present study was the association between specific lipid species and the kinetics of hematological changes during induction therapy. Previous metabolomic studies in pediatric ALL have largely focused on associations between metabolite abundance and static clinical parameters. Yan et al. reported that altered lipids and metabolites were associated with peripheral blood indices, with several ceramides and nucleoside-related metabolites showing positive correlations with WBC counts but negative correlations with hemoglobin and platelet counts^[25]. In contrast, our study further evaluated the change velocities of WBC, neutrophils, and platelets during the D0 – D15 and D0 – D33 intervals. PC(16:0/18:1(9Z)) was negatively correlated with early NEU and PLT change velocities; PE(18:0/18:2(9Z,12Z)) was negatively correlated with WBC and NEU change velocities; LysoPC(0:0/18:2(9Z,12Z)) was associated with WBC change velocities; and SM(d18:2(4E,14Z)/23:0) was associated with WBC changes during D0 – D33. These findings suggest that treatment-related lipid alterations may be linked to dynamic hematopoietic recovery during induction therapy. Previous studies by Kaiser, Raza, Ettel, and colleagues have shown that glycerophospholipid and sphingolipid metabolism are involved in myeloid differentiation, while fatty acid oxidation also contributes to the maturation of neutrophils^[27-29]. In addition,

megakaryocyte differentiation and platelet production depend on phospholipid remodeling and fatty acid uptake^[30, 31]. Therefore, the lipid – hematological associations observed in our study may reflect coordinated changes in metabolic adaptation and hematopoietic recovery during treatment.

The relationship between lipid metabolites and MRD further highlights their potential clinical relevance. MRD at the end of induction is an important indicator of early treatment response in pediatric ALL. Schraw et al. performed metabolomic profiling of diagnostic bone marrow plasma and found abnormalities in central carbon and amino acid metabolism in patients who remained MRD-positive at the end of induction, suggesting that metabolic characteristics were independently associated with MRD^[32]. Wang et al. also identified distinct post-induction metabolic profiles between MRD-positive and MRD-negative pediatric B-ALL patients, with arginine metabolism and ether lipid metabolism among the significantly enriched pathways ^[33]. These findings suggest a close relationship between metabolic state, chemotherapy sensitivity, and residual leukemia burden. In this context, our study found that PC(16:0/18:1(9Z)) was positively correlated with MRD33, whereas PC(16:0/P-18:1(11Z)) was negatively correlated with MRD33. The opposite associations of these two PC species with MRD33 further suggest that treatment response may be related to specific lipid molecular signatures rather than to overall phosphatidylcholine abundance.

In summary, our study not only demonstrated marked remodeling of phospholipid and sphingolipid metabolism after induction chemotherapy but also showed that selected lipid species were associated with the kinetics of hematological changes and end-induction MRD. These findings suggest that longitudinal metabolomic profiling may provide complementary information beyond that obtained from a single static metabolic measurement. Future studies integrating baseline and post-induction metabolite levels, within-patient metabolic changes, blood cell recovery trajectories, and MRD measurements may help determine the potential value of dynamic lipid signatures for evaluating treatment response in pediatric ALL. Several limitations should be acknowledged. First, the present study was based on

correlation analyses and therefore cannot establish causal relationships between specific lipid species and hematopoietic recovery or leukemia clearance. Second, circulating lipid profiles during induction therapy may be influenced by multiple treatment-related factors, including glucocorticoids, L-asparaginase, nutritional status, hepatic metabolism, inflammation, and infection. Further validation in independent cohorts using targeted lipidomic approaches is therefore warranted, together with mechanistic studies to clarify the relationship between these PC, PE, LysoPC, and SM species and treatment response in ALL.

Ethics Statements:

This prospective study was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (approval number: 240522-93). Written informed consent was obtained from the parents or legal guardians of all participants before enrollment and sample collection. The study complied with the ethical standards of the Declaration of Helsinki.

Funding:

This work was supported by the Science and Technology Department of Xinjiang Uygur Autonomous Region. The funded projects include Impact Analysis of Pre-chemotherapy Serum Metabolomics and Nutritional Index on the Prognosis of Children with Acute Lymphoblastic Leukemia (2024D01C169) and Research on Basic Science of Major Childhood Diseases, Special Disease Cohort Studies, and Early Warning Model Construction (2024B03038). This work was supported by the Youth Research Seed Fund of the First Affiliated Hospital of Xinjiang Medical University (Grant No. 2022YFY-QKQN-03).

Abbreviations:

ALB, albumin;
ALL, acute lymphoblastic leukemia;
EFS, event-free survival;
ESI, electrospray ionization;
FC, fold change;
FDR, false discovery rate;

443 HMDB, Human Metabolome Database;
444 KEGG, Kyoto Encyclopedia of Genes and Genomes;
445 Lac, lactate;
446 LC-MS, liquid chromatography–mass spectrometry;
447 LysoPC, lysophosphatidylcholine;
448 MRD, minimal residual disease;
449 NEU, neutrophil count;
450 OPLS-DA, orthogonal partial least-squares discriminant analysis;
451 PC, phosphatidylcholine;
452 PCA, principal component analysis;
453 PE, phosphatidylethanolamine;
454 PLS-DA, partial least-squares discriminant analysis;
455 PLT, platelet count;
456 QC, quality control;
457 SM, sphingomyelin;
458 TCA, tricarboxylic acid;
459 VIP, variable importance in projection;
460 WBC, white blood cell count.

461 **Conflicts of interest**

462 The authors declare that they have no conflicts of interest.

463 **Consent for publication**

464 Not applicable

465 **Availability of data and materials**

466 The datasets generated and/or analyzed during the current study are not publicly
467 available due to ethical restrictions but are available from the corresponding author on
468 reasonable request.

469 **Author contributions**

470 Changda Zhao: Conceptualization, Methodology, Formal analysis, Investigation,
471 Data curation, Visualization, Writing – original draft. Mei Yan: Investigation, Data
472 curation, Formal analysis. Yingbin Yue: Investigation, Data curation. Li Zhao:

Investigation, Data curation. Yu Liu: Conceptualization, Supervision, Project administration, Writing – review & editing. All authors read and approved the final manuscript.

Acknowledgements

None

Clinical trial number

Not applicable

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