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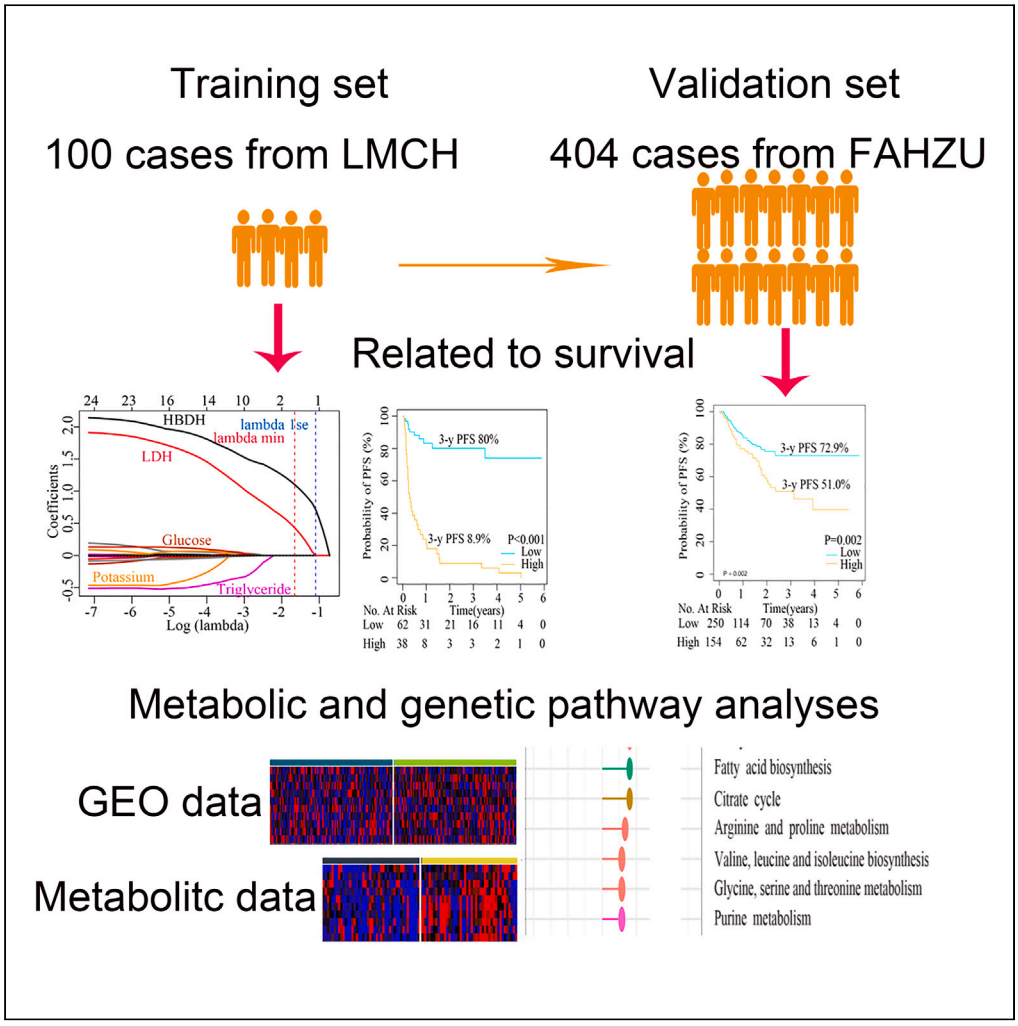

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Highlights

HBDH levels were correlated with adverse survival for DLBCL patients

Overexpression of LDHB, but not LDHA, was linked to poorer outcomes for DLBCL patients

HBDH expression was related to metabolic pathways of lymphoma progression

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The prognostic value of hydroxybutyrate dehydrogenase in diffuse large B cell lymphoma

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SUMMARY

In our investigation, we examined biochemical parameters and identified hydroxybutyrate dehydrogenase (HBDH) as a significant predictor for diffuse large B cell lymphoma (DLBCL) patients. A group of 100 patients was used to explore potential biomarkers related to progression-free survival (PFS). Subsequently, an independent cohort of 404 patients from a separate hospital was recruited to validate our findings. Our results revealed a strong association between elevated HBDH levels and poor PFS. Furthermore, although overexpression of LDHB, but not LDHA, was notably linked to poorer outcomes, HBDH expression emerged as a more robust predictor of clinical prognosis compared to LDH expression. Our investigations, which included metabolic and genetic pathway enrichment analyses, indicated that patients exhibiting heightened HBDH expression were characterized by distinct pathways related to energy metabolism and lymphoma progression. In conclusion, elevated HBDH levels were correlated with adverse survival and might serve as an independent parameter for evaluating patient outcomes.

INTRODUCTION

The 5-year survival rate of patients with diffuse large B cell lymphoma (DLBCL) is approximately 70%, largely due to 30% of patients experiencing relapsed and refractory disease after frontline therapies.[1] DLBCL is recognized as a clinically heterogeneous disease.[2] To improve survival rates, it is imperative to identify novel parameters for the early identification of high-risk patients. Clinically, the international prognostic index (IPI) has served as a robust tool for stratifying DLBCL patients into low-, intermediate-, and high-risk groups.[3] This prognostic model incorporates factors such as age, disease stage, extranodal involvement, poor performance status, and elevated lactate dehydrogenase (LDH) to compute IPI scores.[4] Among these parameters, LDH plays a crucial role as an enzyme in glycolysis, facilitating the conversion of pyruvate to lactic acid and in turn promoting glycolysis.[5] Notably, malignant lymphoma cells exhibit increased glycolysis, leading to increased lactate production and uncontrolled proliferation.[6,7] Thus, LDH is not only an important component of the IPI but also a biological marker that contributes to the aggressiveness of lymphomatous disease and poor prognosis.[8]

Generally, LDH enzymes consist of LDH-M and LDH-H subunits, forming five tetrameric isozymes: LDH1 (4H), LDH2 (3H, 1 M), LDH3 (2H, 2 M), LDH4 (1H, 3 M), and LDH5 (4 M) subunits. LDH-H subunits exhibit a high affinity for α-ketoacid,[9] whereas hydroxybutyrate dehydrogenase (HBDH) predominantly comprises LDH-H subunits. Therefore, HBDH is assessed using α-ketoacid as a substrate, providing insights into the activity of LDH1 and LDH2, known as biochemical markers of tumor progression in lymphoma patients.[10] Therefore, doctors have gradually focused on the application of LDH isozymes in clinical practice.[11–13] Some studies have suggested that LDH2 and LDH3 isoenzymes have prognostic significance in lymphoma patients[11,12]; others have indicated that LDH1 and LDH2 are somewhat associated with lymphoma disease progression.[10]

Notably, HBDH primarily reflects the activities of LDH1 and LDH2 isozymes in the myocardium, red blood cells, and kidneys.[9] Recent studies have shown elevated serum HBDH levels in patients with neoplastic conditions like malignant melanoma,[14] lymphoma,[15] germinomas,[16] and acute leukemia.[17–19] However, the predictive value of circulating HBDH levels in DLBCL patients remains unexplored. Therefore, this study aims to systematically analyze the clinical significance of serum HBDH in DLBCL patients.

RESULTS

HBDH was identified as a predictor in biochemical tests

In this study, we explored the prognostic significance of biochemical parameters in 100 newly diagnosed DLBCL patients. The median HBDH value was 155.5 U/L, ranging from 98.0 U/L to 840.0 U/L. The nonnormal distribution of HBDH values is shown in Figures S1A and S1B. The normal reference range was determined to be 72 U/L to 182 U/L based on measurements of HBDH levels in 260 healthy controls in Beijing

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Table 1. Clinical characteristics of DLBCL patients

Variables	Low group	High group	*p* value
Number	62	38	
HBDH (U/L, median[IQR])	131.00 [113.75, 150.00]	308.50 [219.00, 406.75]	<0.001
Sex, male, n (%)	35 (56.5)	22 (57.9)	1.000
Age, years	62.00 [49.00, 72.00]	68.50 [61.00, 79.75]	0.009
LDH (U/L, median [IQR])	188.50 [160.25, 224.75]	336.00 [277.50, 471.75]	<0.001
ECOG-PS>2, n (%)	21 (36.2)	23 (60.5)	0.023
Ann Arbor stage III–IV, n (%)	41 (66.1)	36 (94.7)	0.001
Extranodal disease, n (%)	43 (69.4)	29(76.3)	0.499
IPI, n (%)			<0.001
Low	20 (32.3)	3 (7.9)	
Low-intermediate	17 (27.4)	5 (13.2)	
High-intermediate	16 (25.8)	7 (18.4)	
High	9 (14.5)	23 (60.5)	
Double-expressor lymphoma, n (%)	16 (25.8)	12 (31.6)	0.647
Non-GCB subtype, n (%)	40 (64.5)	30 (78.9)	0.177
Treatment response, n (%)			<0.001
CR	56 (90.3)	14 (36.8)	
PD	4 (6.5)	6 (15.8)	
PR	0 (0.0)	7 (18.4)	
SD	2 (3.2)	11 (28.9)	

HBDL, hydroxybutyrate dehydrogenase; IQR, interquartile range; ECOG-PS, eastern cooperative oncology group performance status; LDH, lactate dehydrogenase; IPI, International Prognostic Index; CR, complete remission; PD, progressive disease; PR, partial response; SD, stable disease. Extranodal involvement: the bone marrow; CNS, liver/GI tract, spleen, lung, and other sites.

according to the manufacturer's instructions. This normal range was also confirmed in 110 healthy individuals in Zhejiang Province (Figure S1C). Elevated HBDH was defined as a value exceeding 182 U/L, in accordance with the standards established by our laboratory. Subsequently, our patients were classified into a low group (<182 U/L) and a high group (≥182 U/L), with 62 patients (62%) falling into the former and 38 patients (38%) into the latter group. The clinical characteristics of these patients are summarized in Table 1. Patients in the high group exhibited characteristics of being older ($p = 0.009$), having elevated levels of lactate dehydrogenase (LDH, $p < 0.001$), a higher incidence of poor Eastern Cooperative Oncology Group (ECOG) performance status (60.5% vs. 36.2%, $p = 0.023$), advanced disease (stage III–IV, 94.7% vs. 66.1%, $p = 0.001$), higher IPI scores ($p < 0.001$), and lower rates of achieving complete response ($p < 0.001$). Regarding the biochemical features (Table S1), patients in the high group had lower levels of albumin ($p = 0.012$), prealbumin ($p < 0.001$), and chloride ($p = 0.003$) compared to those in the low group. In contrast, the high group displayed elevated levels of aspartate aminotransferase (AST, $p = 0.001$), gamma glutamyl transpeptidase (GGT, $p = 0.011$), alkaline phosphatase (ALP, $p = 0.032$), direct bilirubin ($p = 0.028$), and fatty acid ($p = 0.003$). Additionally, there was no statistically significant correlation between HBDH levels and other variables, including sex, double-expressor lymphoma (DEL), Hans classification (GCB vs. Non-GCB), total protein, triglyceride, creatinine, urea nitrogen, and so on (Tables 1 and S1). As expected, we found that liver function biomarkers, such as ALT, AST, ALP, and GGT, as well as renal function biomarkers, such as uric acid, urea nitrogen, creatinine, and uric acid, were closely correlated with each other ($p < 0.05$, Figure S2).

A total of 24 biomarkers related to liver and renal function were identified in this study (Table S1; Figure S2). Through the LASSO regression model, we determined that two variables were significantly correlated with PFS: HBDH and LDH (Figures 1A and 1B). Notably, HBDH demonstrated higher accuracy in predicting the 5-year PFS rate than LDH (Figures 1C and 1D). Therefore, we next delved deeper into the clinical significance of HBDH in this investigation.

High HBDH levels are associated with poor survival in DLBCL patients

In this study, the 3-year PFS and overall survival (OS) rates of 100 patients were 49% and 76%, respectively. A significant association was observed between the IPI and event rates for PFS ($p < 0.001$) or OS ($p < 0.001$) (Figure S3). As depicted in Figure 2, patients in the low HBDH subgroup exhibited longer PFS and OS durations compared to those in the high HBDH subgroup (80% vs. 8.9%, $p < 0.001$ for the 3-year PFS rate; 87% vs. 51%, $p < 0.001$ for the 3-year OS rate). Furthermore, the IPI score, HBDH score, and DEL showed significant differences based on the univariate analysis (Table S2). Importantly, the independent prognostic value of HBDH levels still remained in the multivariate models even after adjusting for sex, IPI score, Hans classification, and DEL (Table S3).

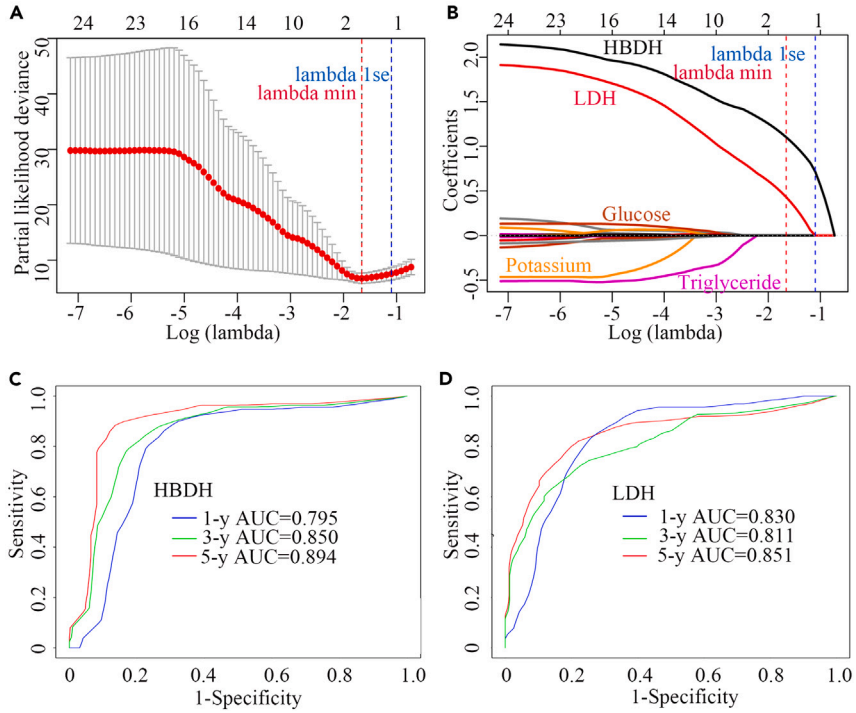

Figure 1. HBDH is identified as the most significant parameter in routine biochemical tests

The "LASSO" regression model with cross-validation algorithms was utilized to identify intriguing biomarkers (A). LDH and HDBH emerged as the most significantly correlated biomarkers with PFS (B). Receiver operating characteristic curve analyses using the "survivalROC" package indicated that HBDH (C) exhibited higher accuracy in predicting the 1-year, 3-year, and 5-year PFS rates than LDH (D).

HBDH as a predictor was validated in another cohort of DLBCL patients

We enrolled 404 patients to validate the abovementioned results; their 3-year PFS and OS rates were 65% and 86%, respectively. Patients in this validated cohort had somewhat different clinical characteristics (Table S4). Specifically, patients were younger ($p < 0.001$) and had a lower ECOG performance score >2 ($p < 0.001$), Ann Arbor stage III–IV disease ($p < 0.001$), extranodular disease ($p < 0.001$), low IPI scores ($p < 0.001$), and higher rate of complete remission ($p < 0.010$) in this validated cohort. As shown in Figure S3, the three-year survival rates of patients in the low-risk group (89.1% vs. 100%) were longer for PFS and OS than those in the intermediate-risk group (46.0% vs. 78.0%, $p < 0.001$) or high-risk group (30.5% vs. 16.5%, $p < 0.001$). Notably, levels of HBDH were similar between these two cohorts. We categorized patients into 250 (61.9%) in the low-risk group and 154 (38.1%) in the high-risk group based on the same cutoff value of HBDH; their 3-year PFS and OS rates were 72.9% and 85.5%, respectively, for the low group and 51.0% and 82.5%, respectively, for the high group (Figure 2). The clinical characteristics of patients in the high and low groups are summarized in Table S5. Remarkably, patients in the high group were older ($p = 0.031$), had higher frequencies of non-GCB subtypes ($p = 0.013$), and had lower rates of complete remission ($p = 0.027$). There was no statistically significant correlation between HBDH levels and other variables, including sex, ECOG performance status, cell-of-origin (COO) classification, or DEL subtypes. We also found that the IPI and DEL were associated with poor outcomes in univariate analyses for PFS and OS (Tables S6 and S7). Additionally, in multivariate analyses, high HBDH levels were significantly associated with poor PFS after adjusting for sex, IPI, COO classification, and DEL subtypes (Table S6).

LDHB, but not LDHA, expression was associated with shorter survival

The LDH-H and LDH-M subunits are encoded by the *LDHB* and *LDHA* genes, respectively. Given that HBDH predominantly represents the activity of LDH1 and LDH2, we questioned whether there was an association between *LDHA* and *LDHB* gene expression and patient outcomes. Therefore, we assessed the impact of *LDHA* and *LDHB* expression on the survival of DLBCL patients using data from nine previously published gene expression datasets.[20–27] Patients with DLBCL were stratified into low- and high-expression groups based on the mean value of each gene. Notably, above-mean *LDHB* expression in the high group was linked to poor survival in a meta-analysis of 2,835 patients [hazard ratio (HR) and 95% confidence interval (CI): 1.23 (1.01, 1.50); $p = 0.038$ for the random effects model; Figure S4]. Conversely, *LDHA* expression did not show a significant correlation with patient outcome [HR (95% CI): 0.97 (0.75; 1.25); $p = 0.797$ for the random effects model, Figure S5].

To avoid potential statistical biases, we treated the expression values of these genes as continuous variables. Furthermore, as depicted in Figure 3, meta-analyses of all nine datasets revealed an overall HR of 1.25 (95% CI, 1.01–1. 54) with a corresponding p value of 0.043 for *LDHB* expression and an overall HR of 0.88 (95% CI, 0.70–1.10) with a p value of 0.237 for *LDHA* expression. Additionally, we conducted a reanalysis

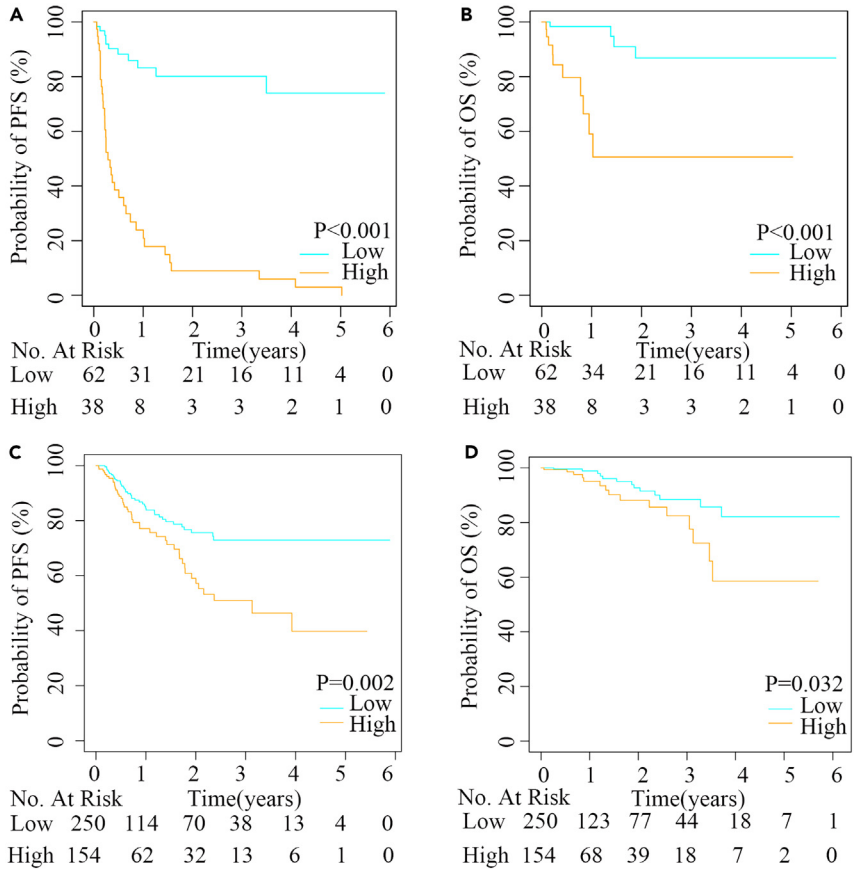

Figure 2. High HBDH levels were associated with poor survival outcomes in DLBCL patients

Kaplan-Meier curves revealed that patients in the LMCH cohort in the high HBDH subgroup had shorter PFS (A) and OS (B) compared to those in the low HBDH subgroup. Similarly, in the FAHZU cohort, Kaplan-Meier curves revealed that patients in the high HBDH subgroup had shorter PFS (C) and OS (D) durations than those in the low HBDH subgroup.

involving 15 additional patients for whom LDHB data were accessible through data-independent acquisition (DIA) mass spectrometry (https://link.springer.com/article/10.1007/s43657-022-00075-w). We observed a significant correlation between LDHB protein and HBDH concentrations (R = 0.55, p = 0.034; Figure S6). Additionally, we found that both high HDBH and high LDHB levels were more prevalent in patients with a non-responsive status. This result was derived from a small cohort of DLBCL patients and needs to be validated in future studies. Furthermore, we found that the adjusted IPI using HBDH, rather than LDH, showed better predictive value for survival in DLBCL patients. As illustrated in Figure S7, the net benefits for the IPI score and adjusted IPI score were observed between threshold probabilities of 10%–75% and 10%–95%, respectively, in the LMCH cohort. Similarly, in the FAHZU cohort, the net benefits for the IPI and adjusted IPI were seen between threshold probabilities of 10%–60% and 10%–75%, respectively.

To identify the differentially expressed genes (DEGs) between patients with low and high *LDHB* expression, we conducted a meta-analysis of nine datasets.[20–27] The analysis revealed 1,770 upregulated and 857 downregulated coexpressed genes, as shown in Table S8. Subsequently, we performed KEGG pathway analysis on these DEGs and found that they were commonly involved in 63 upregulated pathways (Table S9). Some of these genes were associated with energy metabolism, including pyruvate metabolism; oxidative phosphorylation; metabolic pathways; the citrate cycle; and glycine, serine, and threonine metabolism. Additionally, some of pathways such as the Epstein-Barr virus infection pathway, HIF-1 signaling pathway, and B cell receptor signaling pathway were found to promote lymphoma progression (Table S9). Furthermore, we identified eight KEGG pathways, such as osteoclast differentiation and the PI3K-Akt signaling pathway, that were associated with the downregulated genes in this study (Table S10).

Metabolic changes related to high HBDH levels

We measured serum metabolic profiles in groups with high (n = 5) and low (n = 5) HDBH levels. The detailed clinical information is summarized in Table S11. In this retrospective study, frozen serum samples were obtained from 30 patients at the time of their disease diagnosis. Among these 30 patients, five individuals with elevated HBDH levels were enrolled for the analysis of small molecular metabolites. Next, we applied propensity score analysis to match DLBCL patients with high and low HBDH, and matching was based on factors such as age, sex, extranodal

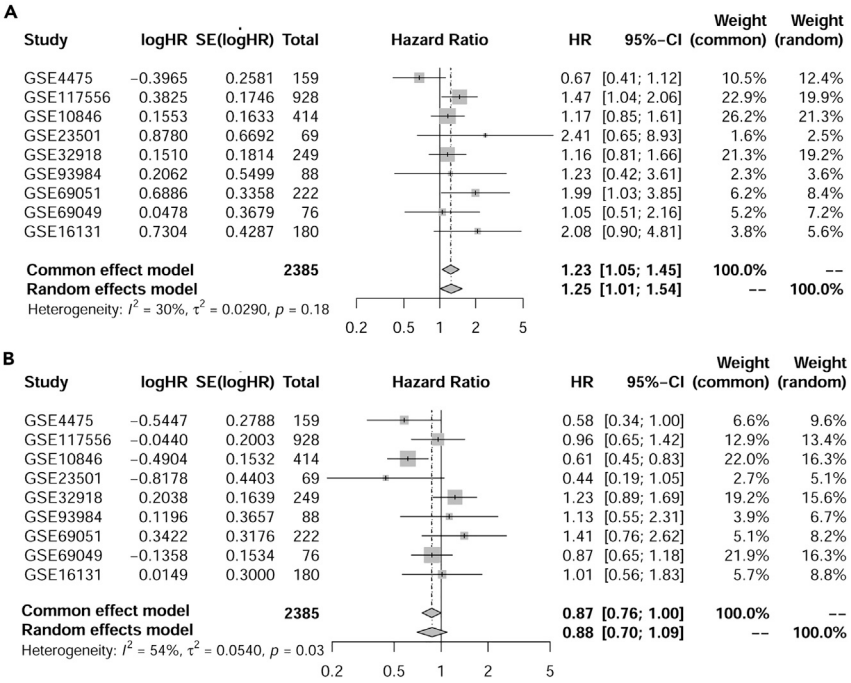

Figure 3. Prognostic values of LDHB and LDHA expressions

Meta-analyses of LDHB (A) and LDHA (B) expression in nine published gene expression datasets. High LDHB expression, as a continuous variable, was associated with poor survival in a meta-analysis of 2,835 patients. In contrast, LDHA expression did not show significant association with patient outcomes.

disease, Ann Arbor staging, ECOG performance status, Hans classification, and LDH levels, which might affect HBDH levels (Table S11). Thus, we selected five samples with high HBDH and five matching samples with low HBDH to assess the differences in serum metabolites. We found 24 metabolic signatures and metabolic pairs with statistically significant changes. Notably, the high HBDH group exhibited significant upregulation of pyruvic acid, pyruvic acid to lactic acid ratio, myo-inositol, glyceraldehyde, oxalic acid, and other metabolites, as depicted in Figure 4. Moreover, we performed KEGG metabolic-pathway-based differential abundance (DA) analysis to investigate the dysregulation of metabolic pathways (Figure 5). Consistent with the genes regulated by *LDHB*, elevated HBDH levels were associated with the upregulation of several pathways including glycolysis/gluconeogenesis, the citrate cycle, cysteine and methionine metabolism, pyruvate metabolism, and others.

DISCUSSION

In clinical practice, we routinely assess various biochemical parameters, including markers of liver, renal and heart function at the time of disease diagnosis. Among these parameters, LDH has been commonly integrated into the IPI score for evaluating the prognosis of patients with DLBCL. However, besides LDH, it remains unclear whether other biochemical parameters can serve as predictive markers. To answer this question, we conducted a systematic analysis of 24 biochemical markers to search for significant biomarkers. Our analysis revealed that both LDH and HBDH exhibited significant potential in predicting patient outcomes (Figures 1A and 1B). Notably, HBDH demonstrated superior accuracy in predicting survival compared to LDH (Figures 1C and 1D). Therefore, we focused on investigating the prognostic significance of HBDH in DLBCL patients. Our findings indicated that patients with elevated HBDH levels experienced shorter PFS compared to those with lower HBDH levels, and this result was further verified in an independent cohort of DLBCL patients (Figure 2).

Lactate dehydrogenase (LDH), a crucial enzyme in anaerobic glycolysis, consists of five isoenzymes: LDH1, LDH2, LDH3, LDH4, and LDH5. Additionally, LDH isoenzymes have LDH-M and LDH-H subunits, which are encoded by the *LDHA* and *LDHB* genes, respectively. Here, we found that high *LDHB*, but not *LDHA*, expression was positively associated with poor survival in 2,385 DLBCL patients in a meta-analysis (Figure 3). These results may help us to explain why HBDH levels were a more accurate predictor of patient outcomes than LDH levels. Previous studies have shown that double knockout of *LDHA* and *LDHB* completely inhibited LDH activity and lactate secretion,[28] indicating that *LDHA* and *LDHB* synergistically contribute to the biological function of LDH. Additionally, since *LDHA* and *LDHB* have distinct prognostic values for DLBCL patients and are coregulated, the prognostic value of LDH, which is mainly driven by *LDHA* expression, may be somewhat limited by the inability of *LDHA* expression to predict survival. Conversely, our findings indicated *LDHB* expression might help with HBDH to predict patients' outcomes. *LDHB* is necessary for cellular autophagy and cancer cell proliferation in oxidative and glycolytic cancer cells.[29] However, the biological role of *LDHB* in DLBCL is still unclear. Hence, we also analyzed KEGG pathways related to altered *LDHB* expression in nine published gene expression datasets. The pathways upregulated by *LDHB* were predominantly involved in energy metabolism processes,

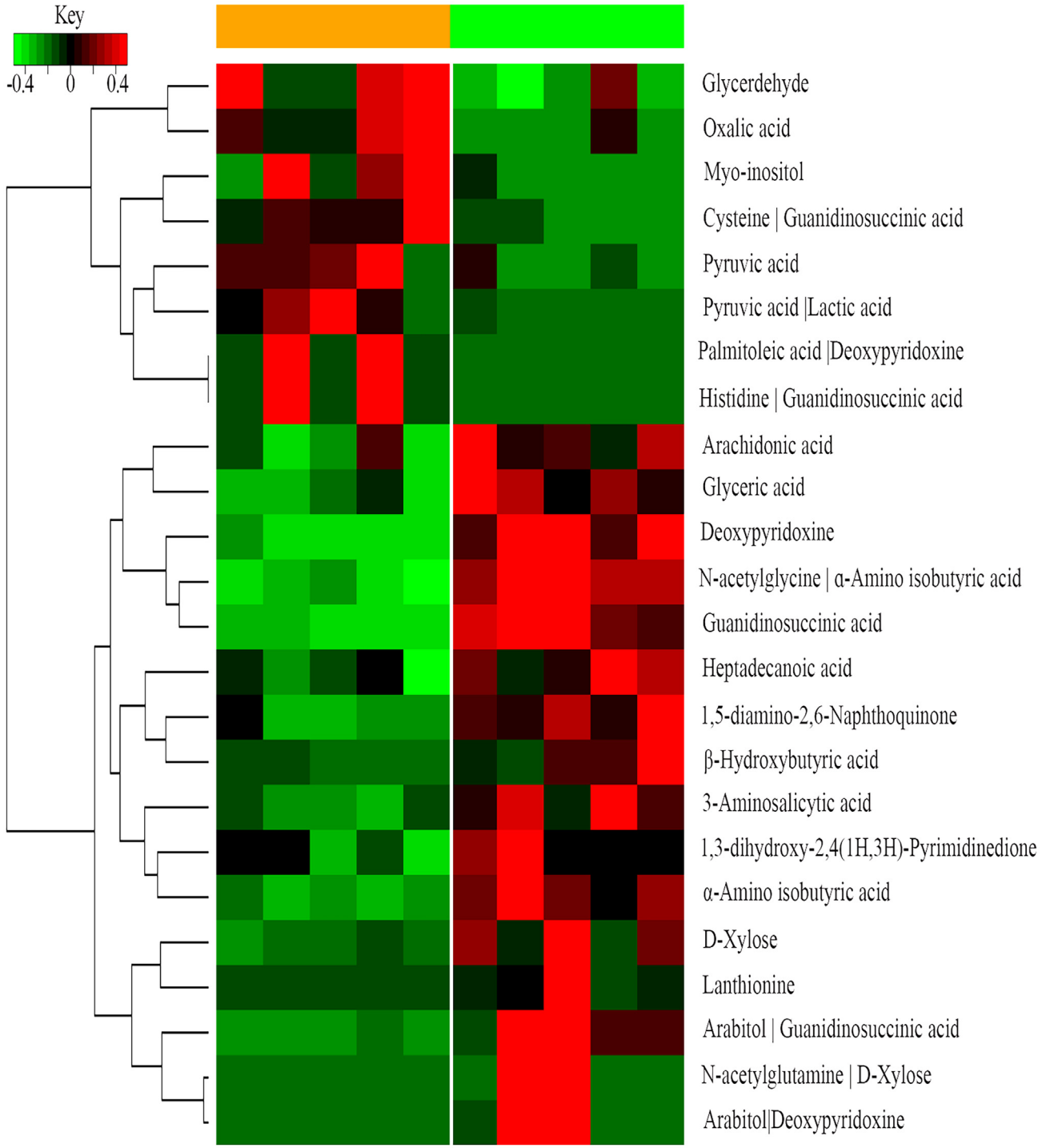

Figure 4. The heatmap of metabolites was different between the high-HBDH group and the low-HBDH group

The orange bar represents patients with high HBDH levels, whereas the green bar represents patients with low HBDH levels.

such as pyruvate metabolism, oxidative phosphorylation, metabolic pathways, the citrate cycle, and glycine, serine and threonine metabolism (Table S8). Additionally, we also identified that elevated *LDHB* expression is associated with the progression of lymphoma, including Epstein-Barr virus infection, the HIF-1 signaling pathway, and the B cell receptor signaling pathway (Table S8). To corroborate these results, metabolic profiling was also conducted on patients with high and low levels of HBDH. Our investigation revealed an upregulation of genes involved in glycolysis, the tricarboxylic acid (TCA) cycle, fatty acid biosynthesis, and other metabolic processes among patients with elevated HBDH.

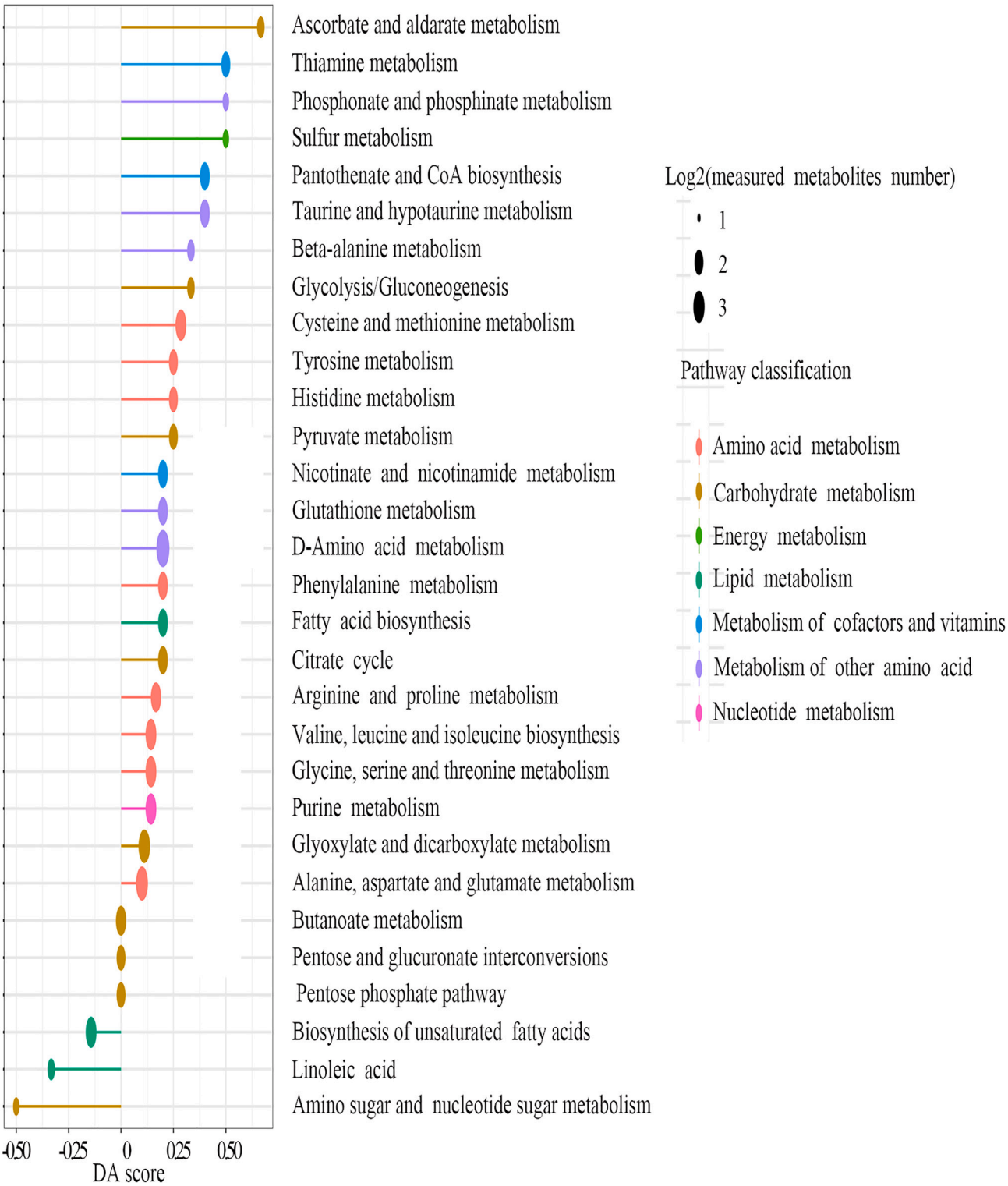

Figure 5. KEGG metabolic-pathway-based differential abundance (DA) analysis was used to investigate the dysregulation of metabolic pathways

These results collectively imply that *LDHB,* responsible for encoding the LDH-H subunit and primarily contributing to HBDH production, might actively participate in promoting energy metabolism and disease progression in individuals with DLBCL. In summary, our investigation uncovered a significant association between HBDH levels and poor PFS in DLBCL patients. As such, HBDH levels hold promise as a clinical parameter for evaluating patient outcomes.

Limitations of the study

Despite the significance of our study, several limitations warrant consideration. Firstly, we did not account for genetic mutation subtypes among our patient cohort, thereby failing to exclude molecular biomarkers like the MCD and N1 subtypes, which have adverse prognostic implications that could potentially confound HBDH levels in DLBCL patients. Secondly, our conclusions were drawn from a retrospective analysis. A prospective multicenter cohort is imperative to corroborate and validate these findings. Thirdly, the evaluation of the levels of the five LDH isozymes is still important for predicting responses to immunotherapy. Therefore, a thorough review of our findings is warranted.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to the lead contact, Jinghan Wang (1513084@zju.edu.cn).

Materials availability

This study did not generate new materials.

Data and code availability

The clinical datasets used in this study are available in the main text and supplementary data. The metabolic data can be obtained from www.ebi.ac.uk/metabolights/MTBLS10446.

This paper does not report original code.

Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

J.H.W. and J.Y.Z. designed this study and wrote the manuscript. B.C.C., C.J.Z., M.P.Z., and Z.M.F. followed the patient. L.J.L. and J.J. were responsible for the management of patients and approved patient information, and all authors critically read and approved the draft manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Methanol(Optima® LCMS grade)	Fisher Scientific	A456-4
Acetonitrile(Optima® LCMS grade)	Fisher Scientific	A955-4
2-Propanol(Optima® LCMS grade)	Fisher Scientific	A461-4
Formic Acid(98%)	Anpel laboratory technologies	UN1779
Critical commercial assays		
HBDH Kit	Biosino Bio-technology and sicience INC	9P0258
Deposited data		
Metabolomic data	https://www.ebi.ac.uk/metabolights/MTBLS10446	MTBLS10446

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Patients

We enrolled 100 patients from Lishui Municipal Central Hospital (LMCH) and 404 patients from the First Affiliated Hospital, Zhejiang University School of Medicine (FAHZU) from 2015 to 2020 to evaluate the prognostic value of biochemical parameters. Liver, heart, and renal function were measured in the included patients at the time of disease diagnosis. We retrospectively collected clinical and laboratory information from the Han Chinese patients' electronic medical records, including age, sex, disease stage, IPI scores, and liver, kidney, and heart biochemical marker data. The detailed variables are shown in Table S1. Patients with severe hepatic, heart, or renal insufficiency, HIV infection, primary central nervous system lymphoma, or transformed indolent lymphoma were excluded. Patients with a previous malignancy or pregnancy and those who did not agree to participate in the follow-up were also excluded. Disease staging was investigated by computed tomography (CT) scans and/or positron emission tomography-CT and bone marrow biopsy. The cell-of-origin (COO) classification was determined by the Hans algorithm.[30] Patients with more than 40% MYC-positive cells and 50% BCL2-positive cells were identified as having double expressor lymphoma (DEL). Patients included in this study were treated with rituximab-based immunochemotherapy. The treatment regimens for all patients were as follows: rituximab 375 mg/m^2 on Day 0, cyclophosphamide 750 mg/m^2, doxorubicin 50 mg/m^2, vincristine 1.4 mg/m^2 on Day 1, and prednisone 60 mg/m^2 orally on Days 1-5. Treatment response was defined according to the Revised Response Criteria for Malignant Lymphoma.[31] Written informed consents were obtained from all patients and the study was approved by the Institutional Review Boards of the Lishui Municipal Central Hospital(2023-439).

HBDH measurements

Biochemistry tests at the time of disease diagnosis were performed using the ARCHITECT c16000 Full Automated Biochemical Analyzer. HBDH concentrations were measured by the enzymatic rate method from serum samples collected on the following morning after admission.

mRNA datasets

We screened mRNA expression datasets of DLBCL patients with survival information. Nine datasets met the criteria and were selected for subsequent analysis of the prognostic value of *LDHA* and *LDHB*. The datasets included were GSE4475,[20] GSE117556,[21] GSE10846,[22] GSE23501,[23] GSE32918,[24] GSE93984,[25] GSE69051,[26] GSE69049,[26] and GSE16131.[27]

Metabolomic profiles

Metabolomic profiling of serum samples was conducted on GC-TOFMS platforms (Metabo-Profile, Shanghai, China) -. 400 μl serum samples collected at the time of disease diagnosis were stored frozen at −80°C until use. Serum was added into a 1.5 ml of tube followed by the addition of 400 μl of acetone for protein precipitation. The mixture was stirred by vortex for 30 s and centrifuged at 10000 rpm for 10 min. A 400-μl supernatant was transferred to a 500 μl of glass tube and dried under vacuum. The dried analytes were dissolved in 80 μl of methoxylamine hydrochloride for 90 min at 30°C and then silylated with 80 μl N,O-bis-trimethylsilyl-trifluoroacetamideand Trimethylchlorosilane (in a ratio of 99:1) (Supelco) for 2 h at 70°C. Each 70μl aliquot of hexane was added to the derivatization bottles. After the sample was stirred for 1 min and kept at room temperature for an hour, 1-μl aliquot of the solution was injected into a PerkinElmer gas chromatography coupled with a TurboMass-Autosystem XL mass spectrometer in the splitless mode. A DB-5MS capillary column coated with 5% Diphenyl cross-linked

95% dimethylpolysiloxane was used for separation. Both the injection temperature and the interface temperature were set to 260°C, and the ion source temperature was adjusted to 200°C. Initial GC oven temperature was set at 80°C for 2 min after injection, and was raised up to 285°C with 5°C/min and maintained at 285°C for 7 min. Helium at a flow rate of 1 mL/min was used as the carrier gas. The measurements were made with electron impact ionization (70 eV) in the full scan mode (m/z 30−550). A total of 109 metabolites were identified by comparison with the internal library built with the standard reference compounds. Wilcoxon test analysis was used to test for metabolites significantly different among between each two groups.

METHOD DETAILS

This study evaluated the prognostic impact of biochemical parameters in patients with diffuse large B-cell lymphoma (DLBCL). A total of 504 patients were enrolled, including 100 from Lishui Municipal Central Hospital and 404 from the First Affiliated Hospital of Zhejiang University School of Medicine, covering the period from 2015 to 2020. Liver, heart, and kidney functions were measured at the time of diagnosis, and clinical and laboratory data from electronic medical records were collected. Patients with severe liver, heart, or kidney dysfunction, HIV infection, and other specific conditions were excluded.The patients received rituximab-based immunochemotherapy, and treatment response was assessed according to the Revised Response Criteria for Malignant Lymphoma. The study focused on analyzing the prognostic impact of serum HBDH (α-hydroxybutyrate dehydrogenase) on progression-free survival (PFS). Various statistical methods, including Cox regression analysis and Kaplan-Meier survival analysis, were used to validate differences between the high- and low-HBDH groups. In addition, metabolomic and genetic pathway analyses were conducted to further explore the role of HBDH.

QUANTIFICATION AND STATISTICAL ANALYSIS

Patient characteristics were summarized using descriptive statistics, including frequency counts, medians and interquartile ranges. Categorical variables were compared using Fisher's exact test, while continuous variables were analyzed using a nonparametric t test. The primary objective of this study was to evaluate the prognostic impact of one selected biochemical variable, HBDH, on the progression-free survival (PFS) of DLBCL patients. PFS was defined as the time from the date of diagnosis until removal from the study due to incomplete remission, relapse, or death. Overall survival (OS) was defined as the time from the date of diagnosis until death due to any cause or the last follow-up. The prognostic impact of the high and low groups was investigated by the log-rank test in the Kaplan−Meier survival model. The proportional hazards assumption was verified for each variable before fitting the Cox models. Univariate and multivariate analyses with a Cox proportional hazard model were performed to identify significant predictors. Differentially expressed metabolites between the high-HBDH group and low-HBDH group were compared using the Wilcoxon test. KEGG pathway analysis was conducted on the platform (https://toppgene.cchmc.org/prioritization.jsp). The R package "MNet" was used to analyze the metabolic pathways (https://tuantuangui.github.io/index.html). Receiver operating characteristic (ROC) curves were generated and analyzed using the "survivalROC" package (https://cran.r-project.org/web/packages/survivalROC/index.html). All statistical analyses were conducted using the R statistical package.