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Explore potential plasma biomarkers of acute respiratory distress syndrome (ARDS) using GC–MS metabolomics analysis

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ABSTRACT

Objectives: The aim of this study was to analyse the metabolomics of patients with acute respiratory distress syndrome (ARDS) for the identification of metabolic markers with potential diagnostic and prognostic value.
Methods: The enrolled subjects included adult patients with ARDS that met the Berlin definition and healthy controls matched based on age, gender, and body mass index (BMI). Plasma samples were collected from 37 patients with ARDS and 28 healthy controls. The plasma metabolites were detected with gas chromatography–mass spectrometry (GC–MS), and the relevant metabolic pathways were predicted using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.
Results: A total of 222 metabolites were identified in our study, of which 128 were significantly altered in patients with ARDS compared with healthy controls. Phenylalanine, aspartic acid, and carbamic acid levels were significantly different between all groups of patients with ARDS classified from mild to severe. Furthermore, four metabolites, ornithine, caprylic acid, azetidine, and iminodiacetic acid, could serve as biomarkers to potentially predict the severity of ARDS. We discovered 92 pathways that were significantly different between ARDS and control groups, including 57 pathways linked to metabolism.
Conclusions: Plasma metabolomics may improve our understanding of ARDS biology. Specific products related to hypoxia may serve as early biomarkers for ARDS prediction, while the metabolites with significant correlations with partial pressure of arterial oxygen (PaO_2)/percentage of inspired oxygen (FiO_2) may play a role in determining ARDS severity. This study suggests that metabolomic analysis in patients at risk of ARDS or those with early ARDS may provide new insight into disease pathogenesis or prognosis.

1. Introduction

Acute respiratory distress syndrome (ARDS) is among the most common conditions encountered in intensive care unit (ICU). The absence of predictive biomarkers has restricted the identification and development of effective pharmacotherapies aimed at reducing the associated morbidity and mortality. We speculated the existence of a relationship between ARDS and microenvironmental changes and hypothesised that such a relationship may provide an insight in ARDS pathology. The alterations in the metabolic processes of tissues and cells such as endothelial, T, and B cells in ARDS may be analysed with the substances from the gastrointestinal tract [1–3] and some inflammatory regulatory factors [4,5]. The novel inflammation-immunity-metabolism axis may be useful to propose new therapeutic implications for ARDS and provide a different and deeper understanding of ARDS. Genome-wide association study (GWAS) has been used for the identification of variants of 25 candidate genes that have been associated with ARDS [6–9], but only one follow-up study has demonstrated the association with a single nucleotide polymorphism (SNP) near PPFIA1 in trauma-associated ARDS using hypothesis-free GWAS [10]. Proteomics is an important field of omics and non-targeted studies of proteomics in ARDS have been recently attempted. Only eight original proteomic studies on ARDS have been conducted. The plasma acute-phase proteins, inflammatory/immune-associated proteins, and

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coagulation proteins related to uncontrolled inflammation have played important roles. A common endpoint with T and B cells for multiple heterogeneous pathophysiological cascades as an essential feature of ARDS was the core reason for the limitation of GWAS and proteomics [11,12]. Metabolomics is a new, rapidly expanding field of systems biology. The application of untargeted metabolomics for biomarker discovery is well suited with the complexity of ARDS because metabolomics may detect as many as several hundreds of metabolites, depending on the analytical platform, from a single sample with minimal bias and no preconception of the sample composition [13–15]. Currently, only two studies on exhaled breath and oedema fluid in the metabolism of ARDS have determined different metabolites and three volatile biological markers in the samples [16,17]. However, the acquisition of samples was relatively difficult and has restricted further clinical applications. In the present study, we used untargeted gas chromatography mass spectrometry (GC–MS) metabolomics to determine the endogenous metabolites in the plasma samples collected from patients with ARDS and healthy controls. Moreover, we used peripheral blood samples, the most common specimens in the clinic, to identify the biomarkers that may be useful for the prediction of early ARDS onset, disease severity, and prognosis.

2. Materials and methods

2.1. Study design

The patients or their family members were informed of the study details and provided written consent. All serum samples were collected as soon as the patients were hospitalised before the start of treatment. Patients with ARDS were enrolled in the ICU of the First Affiliated Hospital of Chongqing Medical University from October 2015 to February 2016. The healthy controls were volunteers from the Healthy Center of the First Affiliated Hospital of Chongqing Medical University. The control and ARDS groups were matched by gender, age, and body mass index (BMI). Healthy control volunteers were not required to be hospitalised and showed normal results in physical check-ups and laboratory examinations. Blood samples from patients with ARDS were collected at three different time intervals as follows: immediately upon admission to the ICU, the third day (if possible), and the seventh day (if possible) after admission. All plasma samples were prepared from the blood samples collected using the standard venous blood sampling protocol. The collected blood was centrifuged at 3000 ×*g* for 10 min at 4 °C, and the plasma was transferred to clean tubes and stored at −80 °C until use.

2.2. GC–MS

2.2.1. Sample preparation

Plasma samples were thawed on ice for several hours. For deproteinisation during sample preparation for GC–MS analysis, 100 μL samples were diluted 1:1 with 100 μL of a 0.2% formic acid solution. Four internal standards, 10 mM DL-alanine-2,3,3,3–4 (Sigma, USA), DL-phenylalanine (CIL, USA), DL-tyrosine (CIL, USA), and octanoic acid (Sigma, USA), were added to each thawed sample. The samples were vortexed, then centrifuged at 14,000 ×*g* for 15 min at 4 °C, and placed on ice. Approximately 60 μL of each supernatant was taken from two tubes and transferred into a new tube and immediately placed on ice. The sample was mixed for quality control (QC) sample preparation and stored in a − 20 °C refrigerator. Prior to GC–MS analysis, the prepared samples were thawed on ice and centrifuged at low speed to remove the foam and precipitates.

2.2.2. GC–MS procedure

The prepared samples were thawed on ice and centrifuged at low speed. The foam and sediments were removed; the samples were concentrated with SpeedVac vacuum concentrators (Thermo Scientific Savant, MA, USA) and subsequently derivatised by methyl chloride methyl ester (MCF, Sigma, USA). GC–MS conditions were as follows: ZB-1701 gas-phase capillary column (30 m × 320 μm × 0.25 μm, Phenomenex, Torrance, CA,USA), inlet for Agilent splitter/splitless inlet, inlet temperature of 250 °C, flame ionisation detector (FID) temperature of 250 °C, nitrogen flow rate of 40 mL/min, hydrogen flow rate of 40 mL/min, air flow rate of 450 mL/min, split ratio of 20:1, and sample injection volume of 1 μL. The program temperature was as follows: initial temperature of 80 °C, 25 °C/min to 200 °C, followed by 3 °C/min to 215 °C; the metabolites were finally analysed at 2 °C/min to 230 °C.

2.2.3. Statistical analysis

Automated Mass Spectral Deconvolution and Identification System (AMDIS) software (version 2.71, National Institute of Standards and Technology, Gaithersburg, MD, USA) was used to deconvolve the GC–MS spectra, whereas MS library of the MCF-identified compounds was derived from Smart et al. [18]. The metabolites were labelled with MCF-treated derivatives of the mass spectrum and the corresponding mass retention time. The relative intensities of the identified metabolites were calculated by the XCMS-based R-script, and the selected base ion of the reference ion was within the appropriate retention time. The abundance of metabolites was normalised by the internal standard number of each sample, and the batch error was removed by the median number. The identified metabolites were from the MS library, and those not recognised in the MS library could be found in NIST. The least squares discriminant analysis (PLSDA) and receiver operating curve (ROC) were generated by Excel inserted into Multi-base (Digital Dynamics, Japan). One-way analysis of variance (ANOVA) & Tukey's honestly significant difference (HSD) test (log) were used to describe heat-map for severity of ARDS. Once the metabolites were identified with MCF in Kyoto Encyclopedia of Genes and Genomes (KEGG), all the metabolic pathways linked to the metabolites were considered. The Student's *t*-test and the false discovery rate were used to determine significant differences ($p < .05$, $q < 0.01$) in the metabolite expression and pathways between the ARDS and control samples. The resulting data plots were generated by gplots and ggplot2 R program. Statistical and correlation analysis was calculated by GraphPad Prism 6.0 software.

3. Results

3.1. Subjects

To accomplish the goals of the present study, plasma samples were obtained from 37 patients with ARDS and 28 healthy controls from the ICU and Medical Examination Center of the First Affiliated Hospital of Chongqing Medical University (Table 1). Of all ARDS samples, 37 were collected upon admission in the ICU, 18 were collected on day 3, while eight samples were obtained on day 7. Patients with ARDS were assigned to three categories based on the threshold values for partial pressure of arterial oxygen (PaO_2)/percentage of inspired oxygen (FIO_2) at ARDS onset and at 24 h as follows: mild ($200 \leq PaO_2/FIO_2 < 300$ with positive end-expiratory pressure [PEEP] ≥ 5 cm H_2O), moderate ($100 \leq PaO_2/FIO_2 < 200$ with PEEP ≥5 cm H_2O), and severe ($PaO_2/FIO_2 < 100$ with PEEP ≥10 cm H_2O). The mild group comprised 20 patients, while the moderate and severe groups had 13 and four patients, respectively (PEEP was filtered through a ventilator after collection from patients). The control group had fewer patients than the ARDS group owing to the limitations of recruiting subjects for this relatively invasive procedure; however, the groups did not differ in terms of age ($p = .8825$ by Mann-Whitney test), gender ($p > .9999$ by Mann-Whitney test), and BMI ($p = .4495$ by Mann-Whitney test).

Table 1
Demographic characteristics of ARDS patients and healthy control subjects.

Variables	ARDS	Controls
Sample size	37	28
Gender		
Female	13(35%)	11(39%)
Male	24(65%)	17(61%)
Age(mean)	61.68 ± 18.7y	57.79 ± 19.1y
BMI(mean)	23.6 ± 4.6	22.9 ± 2.6
ARDS etiology (%)		N/A
sepsis	15(41%)	
aspiration	4(11%)	
pneumonia	6(16%)	
trauma	3(8%)	
others	9(24%)	
APACHEIII score(mean)	65.31 ± 27.1	N/A
PaO_2/FIO_2 ratio	202.38 ± 55.8	N/A

[a)]Data as a percentage of patients or mean.
[b)]ARDS: acute respiratory distress syndrome; APACHE: Acute Physiology and Chronic Health Evaluation; BMI: body mass index; $PaCO_2$: arterial carbon dioxide tension; PaO2: arterial oxygen tension; FiO_2: inspiratory oxygen fraction.

3.2. Feature selection and training of algorithm

3.2.1. Metabolomics of plasma samples

3.2.1.1. Metabolites of ARDS were different from those of healthy controls. The metabolomic profile of the plasma was extremely different between the patients with ARDS and control subjects (Fig. 1A). A total of 222 compounds were detected in the sera, and 94 metabolites were excluded owing to their instability or presence of contaminants. The remaining 128 metabolites showed significantly altered levels between patients with ARDS and healthy controls ($p < .05$), including 27 metabolites from carbohydrate metabolism, 30 from lipid metabolism, 23 from amino acid metabolism, one vitamin, one inorganic compound, one organic compound, and four "others." We positively selected 77 metabolites with $p < .001$ as the point of distinction (Fig. 1B and C).

3.2.1.2. Relation of plasma metabolites in different PaO_2/FiO_2 of patients with ARDS. Five metabolites showed significant correlations with PaO_2/FiO_2 ($p < .05$) among 128 metabolites, which displayed significantly altered levels in patients with ARDS, compared with healthy controls. These metabolites were 2-hydroxybutyric acid (Fig. 2A), methionine, 2,4-imidazolidinedione, 1-methyl-70.9, decanoic acid, and phenethyl acetate; all the q-values were > 98%.

3.2.1.3. Comparison between metabolites from different categories of ARDS. The comparison of metabolites from patients with different ARDS categories and healthy controls revealed elevation in three of the five metabolites (phenylalanine, carbamic acid, and aspartic acid) ($p < .05$) in ARDS (Fig. 2B).

3.2.1.3.1. Results of the KEGG database. Analysis of the KEGG database revealed two pathways involving phenylalanine, namely the phenylalanine metabolism and tyrosine and tryptophan biosynthesis (Fig. 2B). However, no other pathways were linked to carbamic acid or aspartic acid in KEGG pathway database.

3.2.1.3.2. ROC curves. ROC curves of three different amino acids were generated. For phenylalanine, the area under the ROC curve was 0.9112 (95% confidence interval was 0.8319 to 0.9905, $p < .0001$, specificity [SP] 64%, sensitivity [SE] 86.9%). For aspartic acid, the area under the ROC curve was 0.9112 (95% confidence interval was 0.8390 to 0.9834, $p < .0001$, SP 66.1%, SE 84.8%), while the area under the ROC curve for carbamic acid was 0.9102 ($p < .0001$, 95% confidence interval was 0.8355 to 0.9850, SP 64.1%, SE 87.1%) (Fig. 2B).

3.2.1.4. Comparison of metabolites between patients with ARDS with different severity levels. We compared mild, moderate, and severe groups and obtained three significant groups of metabolites (Fig. 3). Correlation analyses showed that ornithine, caprylic acid, azetidine, n-dimethylcarbamoyl-(93.9), and iminodiacetic acid had positive correlations with PaO_2/FiO_2 ($p < .05$) but exhibited no correlation with gender, age, and BMI ($p > .05$).

3.2.1.4.1. Results of KEGG database. We retrieved one pathway linked to ornithine, namely the D-arginine and D-ornithine metabolism (Fig. 2C). No pathway was linked to caprylic acid, azetidine, N-dimethylcarbamoyl-(93.9), and iminodiacetic acid.

3.2.1.4.2. ROC curves. ROC curves were generated for the four amino acids. For ornithine, the area under the ROC curve was 0.5569 (95% confidence interval was 0.4128 to 0.7011, $p > .05$), while the area under the ROC curve was 0.5077 for caprylic acid (95% confidence interval was 0.4053 to 0.6505, $p > .05$). For azetidine and N-dimethylcarbamoyl-(93.9), the area under the ROC curve was 0.5569 (95% confidence interval was 0.4128 to 0.7011, $p > .05$), while the area under the ROC curve for iminodiacetic acid was 0.5116 (95% confidence interval was 0.3684 to 0.6547, $p > .05$).

3.2.1.5. Metabolites at different points from the same patient with ARDS. Eight patients (sepsis [2/8], aspiration [1/8], trauma [2/8], and SAP [3/8]) were hospitalised in ICU for longer than 7 days. We performed a self-control comparison of plasma metabolites from the samples collected on days 1, 3, and 7 for each patient and found that amylene hydrate (66.1%) was significantly different over the three time points ($p = .046905894$). The remaining top four metabolites were margaric acid ($p = .05515987$), 11,14,17-eicosatrienoic acid ($p = .079884954$), arachidonic acid ($p = .079884954$), and fumaric acid ($p = .097048926$).

3.2.2. Metabolic pathway analysis

The KEGG alignment revealed 154 pathways that were linked to the above-detected metabolites. In comparison with the healthy controls, the ARDS group showed statistical differences in 92 of 154 pathways. From the 92 pathways, 57 were related to metabolism, including carbohydrate metabolism [9], amino acid metabolism [6], other amino acids [3], lipid metabolism [4], energy metabolism [4], and nucleotide metabolism [1]. The remaining 30 pathways were associated with other types of metabolisms such as metabolism of cofactors and vitamins, metabolism of terpenoids and polyketides, and biosynthesis of other secondary metabolites (Fig. 4A).

In comparison with the control group, the ARDS group showed an increase in most of the pathways related to carbohydrate metabolism (8/9, 88.9%), including pentose and glucuronate interconversions, pentose phosphate pathway, pyruvate metabolism, glycolysis/gluconeogenesis, ascorbate and aldarate metabolism, butanoate metabolism, and propanoate metabolism. Furthermore, the pathways linked to amino acids and their intermediates (such as phenylalanine metabolism, tyrosine metabolism, histidine metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, and D-glutamine and D-glutamate metabolism) and nucleotide metabolism (pyrimidine metabolism) were upregulated in ARDS samples. We also found upregulation in four other pathways of energy metabolism (including sulphur metabolism, photosynthesis, nitrogen metabolism, and circadian entrainment). The metabolism of cofactors and vitamins (such as vitamin B6, nicotinate, and nicotinamide) also increased (Fig. 4B).

In contrast, compared with the control group, the ARDS group showed downregulation of four pathways linked to lipid metabolism (including arachidonic acid metabolism, steroid hormone biosynthesis, biosynthesis of unsaturated fatty acids, and sphingolipid metabolism). Furthermore, naphthalene degradation and biosynthesis of siderophore group, and non-ribosomal peptide pathways were downregulated (Fig. 4B).

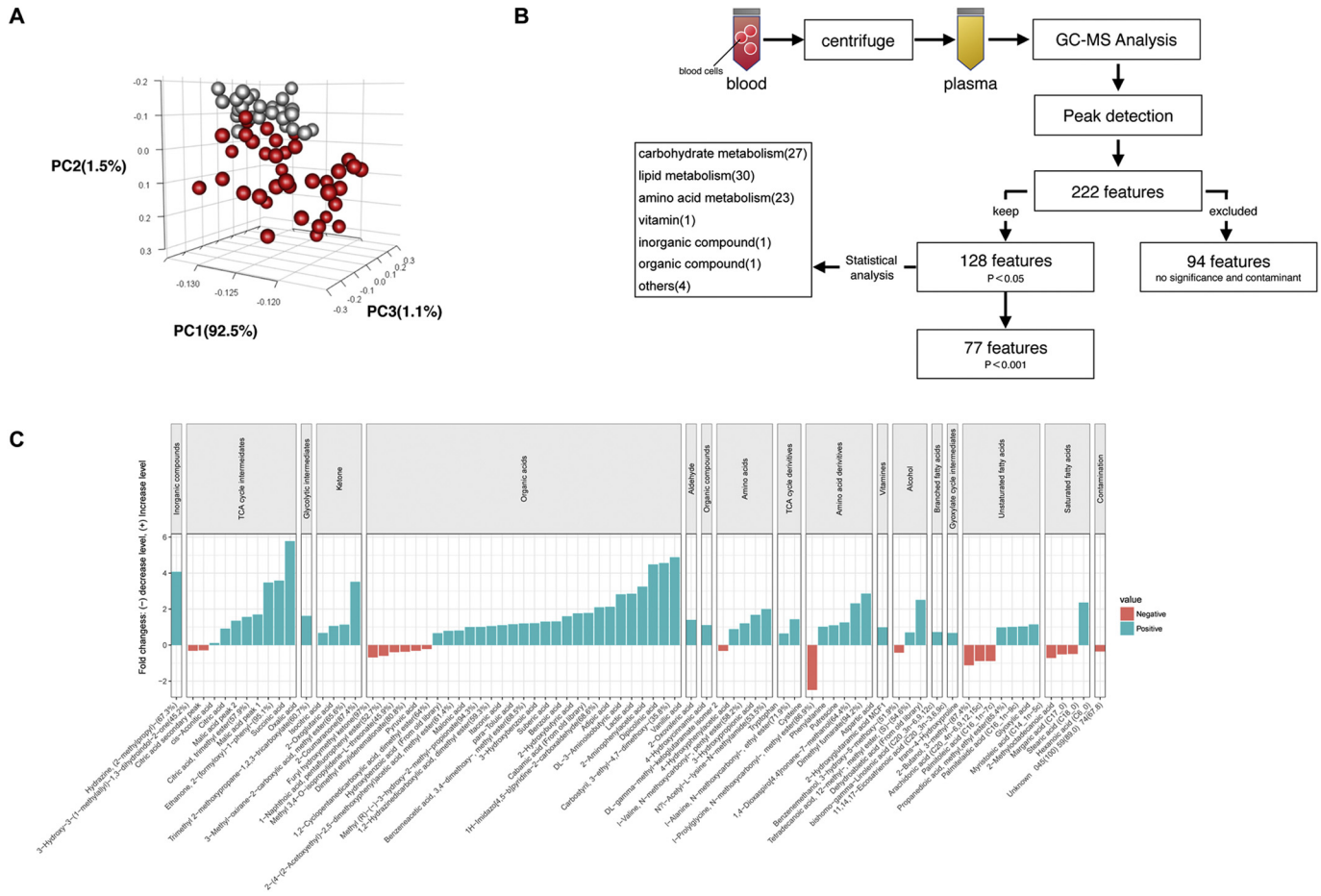


Fig. 1. Metabolites of ARDS were different from those of healthy controls. A, The overall data of metabolite comparison between ARDS and healthy controls via partial least squares-discriminate analysis (PLS-DA). Red represents ARDS, and grey represents healthy controls. The picture indicates significant differences between two groups. B, Metabolites of ARDS were different from those of healthy controls. C, Heatmap of significant metabolites between ARDS and healthy controls. The left side of the figure indicates the name of the metabolite. The right side of the figure indicates the classification of metabolite. The average of the metabolites was calculated by LOG equation. The two groups of metabolites were compared (ARDS/healthy control); red (negative) represents the concentrations of metabolites that decreased in the ARDS group and blue (positive) indicates that the concentrations of metabolites that increased in the ARDS group; (−) indicates downregulation, (+) indicates upregulation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

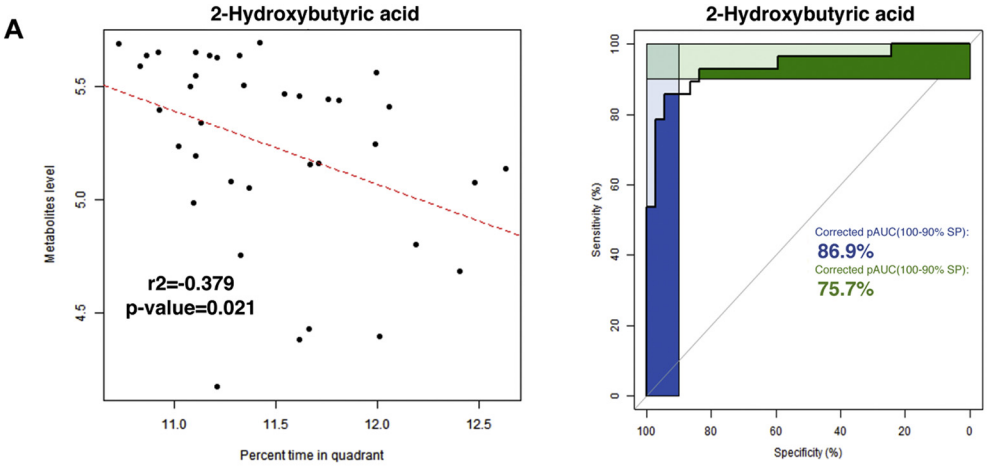
3.2.3. Prognostic attribution analysis of ARDS

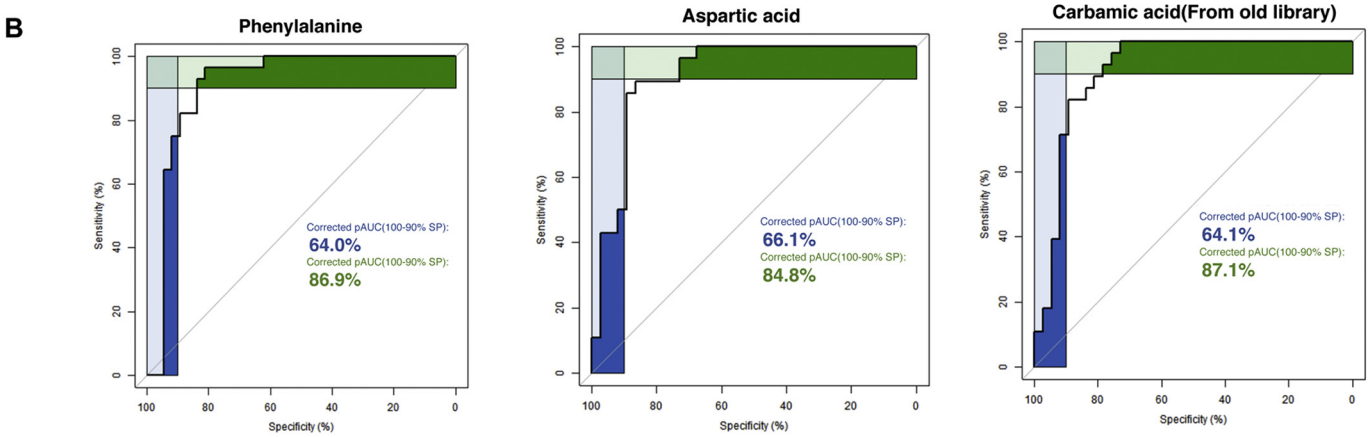
Of the 37 patients with ARDS enrolled in this study, 15 died; the 28-day mortality rate was 40.5% (15/37). We compared plasma metabolites between the non-survivors and surviving patients and identified no difference in metabolites between the two groups (Fig. 4C).

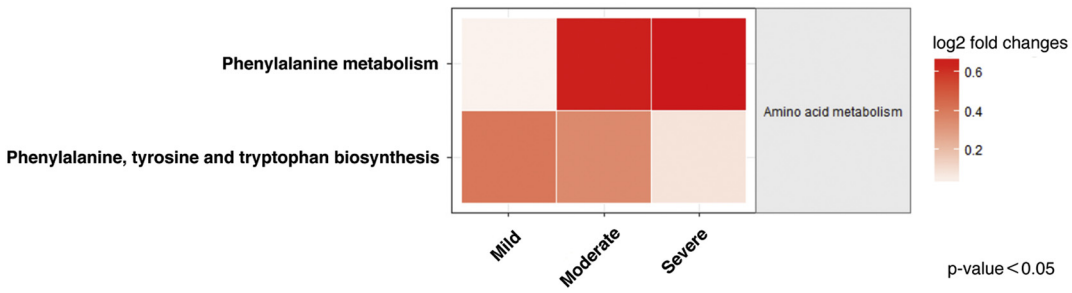
4. Discussion

Early effective diagnosis is the premise for the treatment of ARDS. The use of metabolomics as a possible diagnostic tool for ARDS has been investigated in several studies, including exhaled breath and oedema fluid analyses. However, the discovery of a specific biological marker with early diagnostic significance is desirable. The present study shows that the blood plasma comprises several metabolites that are amenable to detection by GC–MS. We observed significant differences in the metabolomics and metabolic pathways between the control and ARDS groups (Fig. 1). This differential expression was closely related with hypoxia of ARDS. Our findings suggest the potential utility of metabolomics to identify biomarkers that predict early ARDS onset, progression, severity, and prognosis.

Herein, phenylalanine, aspartic acid, and carbamic acid levels were significantly different between all groups of patients with ARDS classified from mild to severe as per the Berlin definition (Fig. 2). Hypoxia is one of the important pathological features of ARDS. The increase in lactate level is closely related to hypoxia. Recent studies have confirmed the association between high levels of lactate and phenylalanine in sepsis [19]. The significant increase in free phenylalanine level was the consequence of autophagy activation in response to severe acute normobaric hypoxia [20]. Phenylalanine filtered herein was a strong indicator and an early biomarker of ARDS (Fig. 2B). The urea cycle (ornithine cycle) is a pathway that produces urea ($(NH_2)_2CO$) from ammonia (NH_3). Under hypoxic conditions, the urea cycle is downregulated [21]. A condensation reaction occurs between the amino group of aspartate and the carbonyl group of citrulline to form argininosuccinate. During hypoxia, this step is affected, and aspartic acid takes part in the urea cycle with the consumption of ATP. During amino acid degradation, the urea cycle requires two amino groups, one of which is provided by aspartic acid. Hence, the increase in aspartic acid level is reflective of metabolic disturbances (via urea circulation) in patients with hypoxic ARDS. In addition, carbamic acid is an intermediate in the production of urea that is involved in the reaction between carbon dioxide and ammonia. Thus, the downregulation of the urea cycle may result in the decrease in the production of urea. Therefore, the level of carbamic acid may increase in patients with ARDS (Fig. 2B and C). As a consequence, aspartic acid and carbamic acid filtered herein may serve as the other two dynamic biomarkers of ARDS, although the underlying mechanisms are unclear. This observation shows that these three metabolites that respond to the







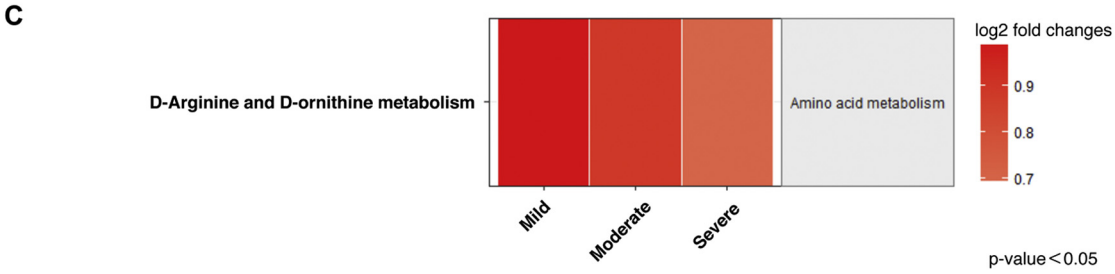


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Fig. 2. Comparison of metabolites in different categories and severity of ARDS. A, The plasma metabolite level of 2-hydroxybutyric acid in different PaO_2/FiO_2 of patients with ARDS. B, Phenylalanine, aspartic acid, and carbamic acid levels were elevated in patients with ARDS as compared with the healthy controls ($p < .05$). Two pathways associated with phenylalanine, namely, phenylalanine metabolism and tyrosine and tryptophan biosynthesis ($p < .05$). C, One pathway linked to ornithine was detected, namely, the D-arginine and D-ornithine metabolism ($p < .05$).

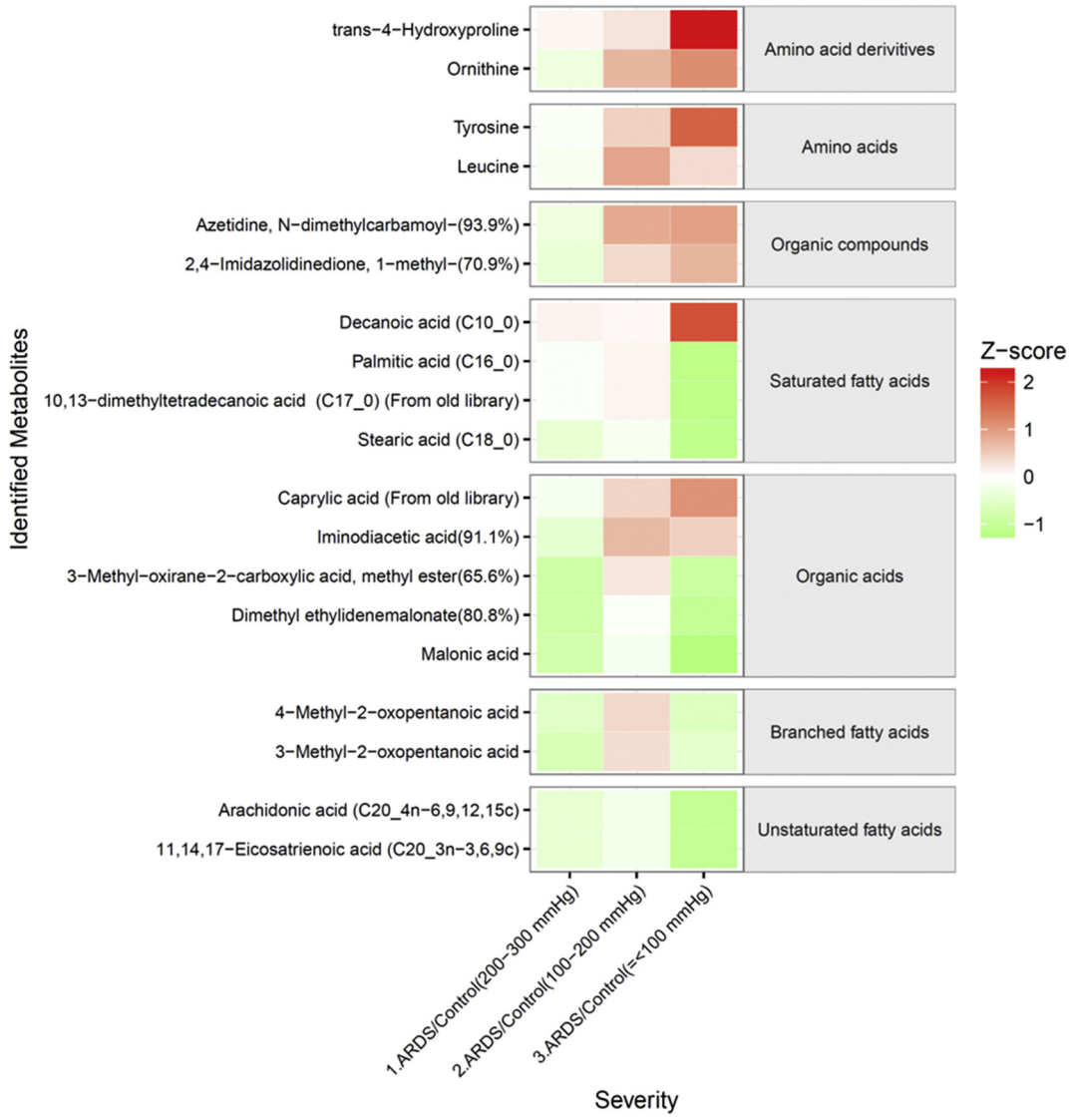

Fig. 3. Metabolites at different time points in the same patient with ARDS.

changes in the internal environment may act as early biological markers of ARDS.

Further analysis showed that four other metabolites, namely, ornithine, caprylic acid, azetidine, and iminodiacetic acid, may be useful as biomarkers to predict the severity of ARDS (Fig. 4). In the urea cycle, the carbamoyl phosphate group is donated to ornithine, resulting in the release of a phosphate group in response to the action of ornithine transcarbamoylase. Ornithine is a central part of the urea cycle and disposes the excess of nitrogen in the mitochondria. During hypoxia, mitochondria may rupture, eventually leading to decreased activity of urea cycle. Ornithine is a central indicator that may respond to the changes in urea cycle levels under such conditions. Therefore, ornithine may act as an important biomarker to predict the severity of ARDS. The correlation between ornithine and PaO_2/FiO_2 observed herein could further support this hypothesis. In general, the severity of hypoxia is positively related to insufficiency of lipid oxidation. As a saturated fatty acid, caprylic acid is an intermediate metabolite that showed significant correlation with PaO_2/FiO_2. Thus, the caprylic acid level must be examined to predict the severity of ARDS. Considering the complexity of metabolic networks, the mechanistic relationship remains unclear, although azetidine and iminodiacetic acid levels were significantly correlated with PaO_2/FiO_2.

Our study revealed several important findings. First, the plasma samples were profiled and the features that showed strong links to ARDS were selected, as per the latest recommendations for metabolomics [22,23]. We recognised and described the heterogeneity of ARDS plasma. To the best of our knowledge, these are the most elaborate human ARDS plasma metabolomic data to date that demonstrate the complex, multifaceted processes involved in ARDS. Second, we focused on the effects of ARDS severity. The Berlin definition is more effective to refine ARDS severity and facilitates recognition of advanced ARDS in the clinic. We show that some metabolites tend to change in response to the severity of ARDS. Three plasma metabolites (phenylalanine, aspartic acid, and carbamic acid) that were closely related to

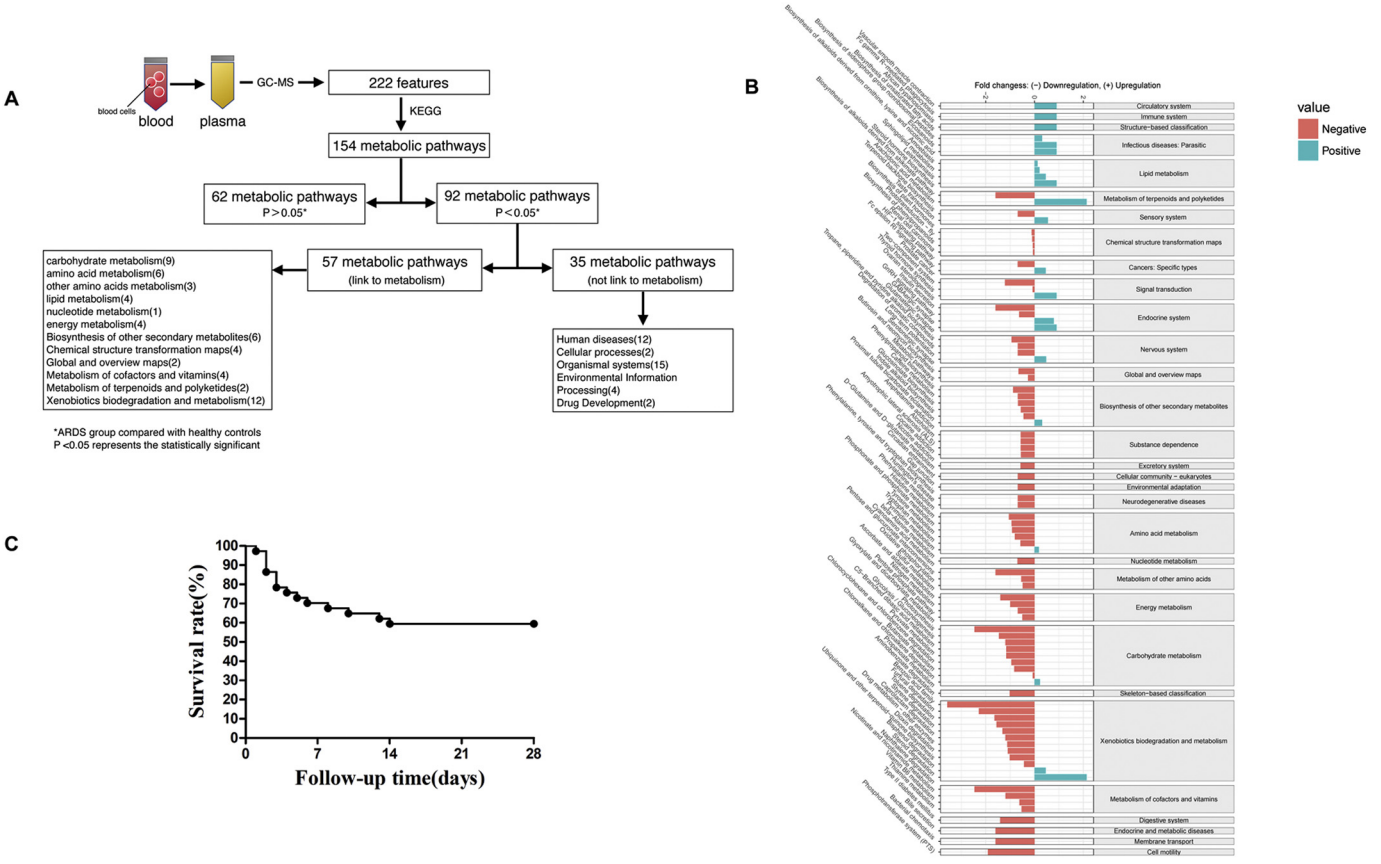

Fig. 4. Metabolic pathway analysis. A, Schematic of the data processing workflow of pathways. Plasma samples were extracted by centrifugation and used for GC–MS analysis to evaluate features through peak detection. KEGG database was used to evaluate differentially expressed pathways. Of these, 92 pathways had a value of *p < .05, while 57 pathways were linked to metabolism. *ARDS group compared with healthy controls. B, Heatmap of significant pathways between patients with ARDS and healthy controls, *p < .05. (−) indicates downregulation, (+) denotes regulation. C, Survivorship curve of the 37 patients with ARDS by 28-day follow-up.

hypoxia, significantly increased under different severities of ARDS. Third, the acquisition of plasma is a common invasive procedure in ICU and is much easier than other methods.

There are some limitations associated with our study, mainly the small sample size. However, as this was a controlled paired study, the interference caused by small samples was reduced as much as possible. Another challenge was the sample, which was unpredictable. The difference in the pathogenesis of ARDS is currently a major constraint to the in-depth studies of this syndrome. The effects of mechanical ventilation (MV) and the interference of various variables on further treatment acted as interfering factors in the evaluation of metabolite levels in the plasma samples of patients with ARDS. To minimise this variable, we used the plasma samples that were collected early in the course of ARDS. Although the obtained area under the curve value may be limited, the most important aspect of our study is that the plasma metabolomic analysis using GC–MS may be used for the diagnosis and treatment of ARDS. On the other hand, given the differences in the essential metabolic process between children and adults, these metabolites may show differences under diseased conditions. Therefore, the results of our research were limited for adults. Future research should take paediatric respiratory distress syndrome into account that may help to discover the metabolic characteristics of paediatric respiratory distress syndrome and highlight the differences from ARDS in adults.

This study suggests potential promising avenues for future research in large-scale studies. Our ultimate goal is to construct a metabolomic strategy to completely characterise the ARDS metabolome to drive biomarker discovery and identify drug targets or monitor target opportunities to improve the outcome of patients with ARDS. We recognised the possibility of carrying out metabolomic tests with small samples. In some pulmonary diseases (such as chronic obstructive pulmonary disease and asthma), metabolomic approaches have been successful in the identification of potential markers of disease severity or therapeutic candidates such as purines, sphingolipids, and amino acids, but only limited information is obtained [24]. A systems biology- or pathway-based approach is needed to reveal the potential mechanisms [25]. As most of the metabolites were processed substances and the resulting metabolic pathways were derived from databases such as KEGG, metabolomic analysis of ARDS provided clues to explore the potential pathophysiological and molecular mechanisms. The choice of sample type, including gas, alveolar fluid, and plasma, is crucial and the results presented may be inconsistent [16,17,26]. The use of metabolites to determine the respiratory functions of ARDS ought to be combined with more specific data on respiratory monitoring. The accumulation of research data may be potentially advantageous to search for biomarkers from the metabolites to judge disease severity. We could rely on in vitro monitoring technologies, including an array of sensors, infrared spectral recognition technology, and rapid mass-spectrometric techniques [16,27], and use artificial intelligence to achieve the dynamic monitoring and evaluation of ARDS.

5. Conclusions

Plasma metabolomics has the potential to improve our understanding of ARDS biology. Specific products related to hypoxia may serve as early biomarkers for ARDS prediction, while particular products that show significant correlations with PaO_2/FiO_2 may play a role

as biomarkers to evaluate the severity of ARDS. These biomarkers could facilitate early detection of ARDS and help evaluate its severity.

Author contributions

Study conception and design, S.-H.L., F.X. Application for clinical ethics, M.Z. Clinical ethics supervision, M.Z., Q.L. Clinical sample collection, X.Y. Clinical data acquisition, J.Z. Metabolomics experiment study, C.-J.W., M.L. Metabolomics analysis, T.-L.H., H.W. Computer analysis and programming, H.W. Data management, X.Y.,S.-H.L.,F.X. Data analysis and interpretation,S.-H.L.,F.X. Manuscript writing, S.-H.L.,F.X.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in the studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards, and approved by the Ethics Committees of the First Affiliated Hospital of Chongqing Medical University.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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