Translational Oncology 19 (2022) 101393

Contents lists available at ScienceDirect

Translational Oncology

journal homepage: www.elsevier.com/locate/tranon

Original Research

Prediction of response and adverse drug reaction of pemetrexed plus platinum-based chemotherapy in lung adenocarcinoma by serum metabolomic profiling

Wei-Jing Gong [a,b,1], Peng Cao [a,b,1], Qi-Lin Zhang [a,b], Xiao-Yu Han [c,d], Shuo-Wen Wang [e], Yi-Fei Huang [a,b], San-Lan Wu [a,b], Qiang Li [a,b], Rui Zhang [a,b], Shuang-Bing Xu [f], Ya-Ni Liu [a,b], Shao-Jun Shi [a,b], Yu Zhang [a,b,*]

[a] Department of Pharmacy, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China
[b] Hubei Province Clinical Research Center for Precision Medicine for Critical Illness, Wuhan 430022, China
[c] Department of Radiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China
[d] Hubei Province Key Laboratory of Molecular Imaging, Wuhan 430022, China
[e] Department of Clinical Pharmacy, Shanghai General Hospital, School of Medicine, Shanghai Jiao Tong University, No. 100 Haining Road, Shanghai 200080, China
[f] Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

ARTICLE INFO

Keywords:
Lung adenocarcinoma
Pemetrexed plus platinum
Metabolomics
Prediction model

ABSTRACT

Background: Pemetrexed plus platinum doublet chemotherapy regimen remains to be the standard first-line treatment for lung adenocarcinoma patients. However, few biomarkers can be used to identify potential beneficiaries with maximal efficacy and minimal toxicity. This study aimed to explore potential biomarker models predictive of efficacy and toxicity after pemetrexed plus platinum chemotherapy based on metabolomics profiling.

Methods: A total of 144 patients who received at least two cycles of pemetrexed plus platinum chemotherapy were enroled in the study. Serum samples were collected before initial treatment to perform metabolomics profiling analysis. Logistic regression analysis was performed to establish prediction models.

Results: 157 metabolites were found to be differentially expressed between the response group and the nonresponse group. A panel of Phosphatidylserine 20:4/20:1, Sphingomyelin d18:1/18:0, and Phosphatidic Acid 18:1/20:0 could predict pemetrexed and platinum chemotherapy response with an Area Under the Receiver Operating Characteristic curve (AUROC) of 0.7968. 76 metabolites were associated with hematological toxicity of pemetrexed plus platinum chemotherapy. A panel incorporating triglyceride 14:0/22:3/22:5, 3-(3-Hydroxyphenyl) Propionate Acid, and Carnitine C18:0 was the best predictive ability of hematological toxicity with an AUROC of 0.7954. 54 differential expressed metabolites were found to be associated with hepatotoxicity of pemetrexed plus platinum chemotherapy. A model incorporating stearidonic acid, Thromboxane B3, L-Homocitrulline, and phosphoinositide 20:3/18:0 showed the best predictive ability of hepatotoxicity with an AUROC of 0.8186.

Conclusions: This study established effective and convenient models that can predict the efficacy and toxicity of pemetrexed plus platinum chemotherapy in lung adenocarcinoma patients before treatment delivery.

Abbreviations: AUROC, Area Under the Receiver Operating Characteristic curve; RECIST, Response Evaluation Criteria In Solid Tumor; NCI-CTC, National Cancer Institute Common Toxicity Criteria; UPLC-ESI-MS/MS, ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometry; PCA, Principal Component Analysis; AIC, Akaike Information Criterion; ROC, Receiver Operating Characteristic curve; KEGG, Kyoto Encyclopedia of Genes and Genomes; PR, Partial Response; SD, Stable Disease; PD, Progressive Disease; ADR, Adverse Drug Reaction; QC, Quality Control; TIC, Total Ion Current; PS, Phosphatidylserine; SM, Sphingomyelin; PA, Phosphatidic Acid; CI, Confidence Interval; FC, Fold Change; TC, Triglyceride; PI, Phosphoinositide.

* Corresponding author at: Department of Pharmacy, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China.
E-mail address: zhangwkp@163.com (Y. Zhang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.tranon.2022.101393
Received 8 November 2021; Received in revised form 9 February 2022; Accepted 4 March 2022

Introduction

Lung cancer is one of the most commonly diagnosed and fatal malignancies, which is a great public health burden worldwide [1]. Lung adenocarcinoma is the most common histological type, accounting for almost half of primary lung cancer [2]. Although great progress in diagnosis has been achieved, many patients are diagnosed at an advanced stage (IIIB or IV). Unfortunately, there is no curative treatment for those advanced stage patients. Pemetrexed plus platinum doublet chemotherapy regimen remains to be the standard first-line treatment for those lung adenocarcinoma patients who are not eligible for target therapy. However, the response rates of the chemotherapy regimen are about 30%−40%, and many of them suffer unpredictable severe side effects [3]. Thus, potential biomarkers predicting the response or side effects of patients to pemetrexed plus platinum doublet therapy are urgently needed.

Metabolomics is an-omics technology that allows for a global assessment of small-molecule endogenous metabolites in biofluids. Metabolites are stable and quantifiable, which have the potential to be biomarkers to predict the efficacy and toxicity of chemotherapy. Compared to genomics, transcriptomics, and proteomics, metabolomics can directly reflect the current status of an organism, which provides a strong link between genotype and phenotype [4]. So far, metabolomics-based approaches have been a hotspot for the screening of biomarkers guiding precision medicine. Wei et al. developed a prediction model by combining nuclear magnetic resonance and mass spectrometry-derived metabolites, which could correctly identify 80% of breast cancer patients without complete response to neoadjuvant chemotherapy [5]. Tian et al. developed a discriminant model based on metabolomics profiling that can accurately predict the efficacy and survival outcomes of pemetrexed plus platinum doublet chemotherapy [6]. Ghini et al. identified the metabolomic fingerprint of serum as a predictive “collective” biomarker for immune checkpoint inhibitor response with $>$ 80% accuracy [7].

In this study, we use a metabolomics approach to predict the response and toxicity to pemetrexed plus platinum-based chemotherapy in lung adenocarcinoma.

Materials and methods

Study participants

From December 1, 2018 to December 16, 2019, a total of 144 patients were initially enroled from the cancer center of Wuhan Union Hospital (Wuhan, Hubei, China), Shanghai General Hospital (Shanghai, China), or The First Affiliated Hospital of Sun Yat-sen University (Guangzhou, Guangdong, China). All patients were histologically or cytologically diagnosed with primary lung adenocarcinoma and subjected to at least two cycles of pemetrexed plus platin (cisplatin/carboplatin/nedaplatin) based chemotherapy as primary treatment. The heparin anticoagulated plasma samples were collected before treatment, centrifugated at 3500 rpm for 10 min at 4 °C, followed by pipetting the supernatant plasma and immediately transferred to a −80 °C freezer until metabolic analysis. A total of 130 plasma samples were tested in the present study, the rest 14 samples were not analyzed due to hemolysis or degradation. The tumor response to chemotherapy after two cycles of treatment was evaluated using the Response Evaluation Criteria In Solid Tumor (RECIST) guidelines. The severity of toxicity at each cycle of chemotherapy was assessed according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 3.0. Finally, 108 patients with RECIST evaluation results were employed to establish a chemotherapy efficacy model while 113 patients with NCI-CTC evaluation results were used to establish a hematological toxicity model or a hepatotoxicity model. We primarily focused on hematological toxicity and hepatotoxicity. This study protocol had been approved by the Ethics Committee of the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, and written informed consent was obtained from all patients.

Materials for sample extraction and metabolomic analysis

HPLC-grade methanol, acetonitrile, ethanol, acetic acid, ammonium methyl acetate, chloroform, and methyl tert-butyl ether (MTBE) were purchased from Merck (Germany). Standard chemicals were bought from Sigma-Aldrich (America).

Targeted metabolomic detection

Methods for the extraction of hydrophilic compounds

The plasma samples were thawed on ice and mixed with 3 vol of ice-cold methanol, the mixture was then whirled for 3 min and centrifuged with 12,000 rpm at 4 °C for 10 min. Then the supernatant was collected and centrifuged at 12,000 rpm at 4 °C for 5 min. Finally, the supernatant was collected again for subsequent analysis.

Separation conditions of hydrophilic compounds

The sample extracts were analyzed using an ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS) system (UPLC, Shim-pack UFLC SHIMADZU CBM30A system; MS, QTRAP® System). The analytical conditions were as follows: UPLC: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm*100 mm); column temperature, 40 °C; flow rate, 0.4 mL/min; injection volume, 5 μL; solvent system, water (0.1% formic acid): acetonitrile (0.1% formic acid); gradient program, 95:5 v/v at 0 min, 10:90 v/v at 11.0 min, 10:90 v/v at 12.0 min, 95:5 v/v at 12.1 min, 95:5 v/v at 14.0 min.

Targeted lipidomic detection

Methods for the extraction of hydrophobic compounds

The plasma samples were melted on ice, vortexed for 10 s, and then centrifuged with 3000 rpm at 4 °C for 5 min. 50 μL of each sample was taken and homogenized with 1 mL mixture (including methanol, methyl tert-butyl ether, and internal standard). The mixture was whirled for 2 min, followed by the addition of 500 μL water, and whirled again for 1 min. After centrifugation with 12,000 rpm at 4 °C for 10 min, 500 μL supernatant of each sample was taken and concentrated. Next, dissolve the extract with 100 μL mobile phase B, then stored in −80 °C. Finally, take the dissolving solution into the sample bottle for subsequent analysis.

Separation conditions of hydrophobic compounds

As mentioned above, the sample extracts were analyzed using an LC-ESI-MS/MS system. The analytical conditions were as follows, UPLC: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm*100 mm); column temperature, 40 °C; flow rate, 0.4 mL/min; injection volume, 5 μL; solvent system, water (0.04% acetic acid): acetonitrile (0.04% acetic acid); gradient program, 95:5 v/v at 0 min, 5:95 v/v at 11.0 min, 5:95 v/v at 12.0 min, 95:5 v/v at 12.1 min, 95:5 v/v at 14.0 min.

Mass spectrometry conditions

A triple quadrupole-linear ion trap mass spectrometer (QTRAP) LC-MS/MS System equipped with an ESI Turbo Ion-Spray interface was used to analyze the samples in positive and negative ion mode and controled by Analyst 1.6.3 software (Sciex). The ESI source operation parameters were as follows: source temperature 500 °C; ion spray voltage (IS) 5500 V (positive), −4500 V (negative); ion source gas I (GSI), gas II (GSII), and curtain gas (CUR) were set at 55, 60, and 25.0 psi, respectively; the collision gas (CAD) was high. Instrument tuning and mass calibration were performed with 10 and 100 μmol/L polypropylene glycol solutions in QQQ and LIT modes, respectively. A

specific set of multiple reaction monitoring (MRM) transitions was monitored for each period according to the metabolites eluted within this period.

Statistical analysis

The clinical characteristics of participants were analyzed by two-tailed unpaired Student's *t*-test or Chi-square test in SPSS 23.0 software, and statistical significance was considered at $P < 0.05$. The data of endogenous metabolites in terms of homogeneity and reproducibility was visualized by Principal Component Analysis (PCA). The logistic regression analysis was performed to evaluate the diagnostic value of the combined biomarkers model. To establish a more reliable model, candidate metabolites were tested according to the Akaike Information Criterion (AIC). A lower AIC value indicates a better model effect. Model performance was assessed by the Receiver Operating Characteristic curve (ROC) was plotted using R software (Belgium, Version 12.4.2.0). The analysis of metabolic pathways was conducted by the Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.kegg.jp/) [8].

Results

Baseline of the study population

The clinical characteristics of the participants were displayed according to the chemotherapy efficacy model, hematological toxicity model, and hepatotoxicity model, respectively. 24 patients were evaluated as chemotherapy responders (partial response (PR)) and the other 84 patients were non-responders (stable disease (SD) or progressive disease (PD)). As shown in Table 1, there were no statistical differences between these two populations on their age, body mass index (BMI), gender, smoking history, and disease stage. Among the 113 patients with records of adverse drug reaction (ADR) after chemotherapeutic treatment, 76 of them had blood ADR, but only 23 of the 113 patients had liver ADR. There was no significant difference between patients with or without ADR on their clinical features. Except that, patients with liver ADR were significantly younger than those without liver ADR ($p = 0.009$).

Determination of plasma endogenous metabolites using targeted metabolomics and lipidomic methods

This study was conducted based on an integrated platform targeting the metabolome and lipidome. The database of this platform contains more than 3000 characteristic compounds, of which 1141 compounds were detected in the present study (Supplementary File 1).

A total of 130 plasma samples were tested in the present study. Besides, quality control samples (QC) were prepared by mixing the sample extracts. In this study, 18 QC samples were inserted into the queue to monitor the repeatability of the analysis method every 100 min, which was judged by overlaying the total ion current diagrams (TIC diagrams) of different QC samples. The results showed that the curves of the TIC diagrams were highly overlapped, and the retention time and peak intensity were consistent, indicating that the signal was stable throughout the analysis process (Fig. 1A–D).

In addition, PCA was used to estimate the overall metabolic difference and the degree of variability. The results showed that the QC samples were not separated from each other, demonstrating the stability of the analysis method (Fig. 1E).

Predictive effect of plasma endogenous metabolites on pemetrexed chemotherapy response

A total of 108 patients with RECIST chemotherapeutic evaluation results were analyzed here to find the metabolites with predictive effects, among which 24 of them were assessed as PR and the remaining 84 patients were SD or PD. Firstly, 157 kinds of differentially expressed metabolites with *P* value<0.05 were screened out, which could be enriched in the pathways of phospholipid biosynthesis, glycerolipid metabolism, etc. (Supplementary Fig. 1).

Secondly, a logistic regression model was established for 15 metabolites with the smallest *P* values, all of which were classified as lipids. The predictive effects of them were evaluated by a ROC diagnostic analysis (Table 2), the results showed that their Area Under the Receiver Operating Characteristic curve (AUROC) was between 0.6918 and 0.7386. To establish a more reliable model to predict the chemotherapeutic response, different combinations of these 15 candidate metabolites were tested using logistic regression analysis. Finally, a panel including three metabolites was selected due to its lowest AIC value. The regression equation for response prediction was as following: Logit (P) = 7.086 - 0.000011 * phosphatidylserine (PS) (20:4/20:1) - (9.9014E-08) * sphingomyelin (SM) (d18:1/18:0) - 0.000042 * phosphatidic acid (PA) (18:1/20:0). The response prediction model showed an AUROC of 0.7968 (95% confidence interval (CI): 0.6775–0.9161) with an optimal cut-off at 1.150 (Table 2, Supplementary Fig. 2).

Table 1
The clinical characteristics of the enroled participants in this study.

Variables	Chemotherapy efficacy model			Hematological toxicity model			Hepatotoxicity model		
	Responders	Non-responders	*P* value	Normal blood Group	Blood ADR Group	*P* value	Normal Liver Group	Liver ADR Group	*P* values
Number	24	84		37	76		90	23	
Age	54.4 ± 11.2	57.8 ± 10.8	0.176	57.5 ± 8.3	55.6 ± 9.8	0.316	57.3 ± 9.2	51.7 ± 8.3	0.009
BMI	22.6 ± 3.1	22.6 ± 3.1	0.983	22.9 ± 2.6	22.3 ± 3.4	0.495	22.3 ± 3.1	23.7 ± 3.0	0.207
Gender			0.205			0.867			0.053
Male	12	54		23	46		59	10	
Female	12	30		14	30		31	13	
Smoking History			0.376			0.915			0.142
Smokers	4	21		10	21		27	4	
Non-smokers	18	50		20	43		46	17	
Ex-smokers	2	13		7	12		17	2	
Stage			0.919			1.000			0.798
I	1	3		2	3		5	0	
II	0	1		0	1		1	0	
III	3	15		7	16		18	5	
IV	20	65		28	56		66	18	

Footnote: *P* values indicate differences between different groups. The clinical characteristics of participants were analyzed by two-tailed unpaired Student's *t*-test or Chi-square test in SPSS 23.0 software. $P < 0.05$ was considered statistically significant.
body weight index (BMI), adverse reactions (ADR).

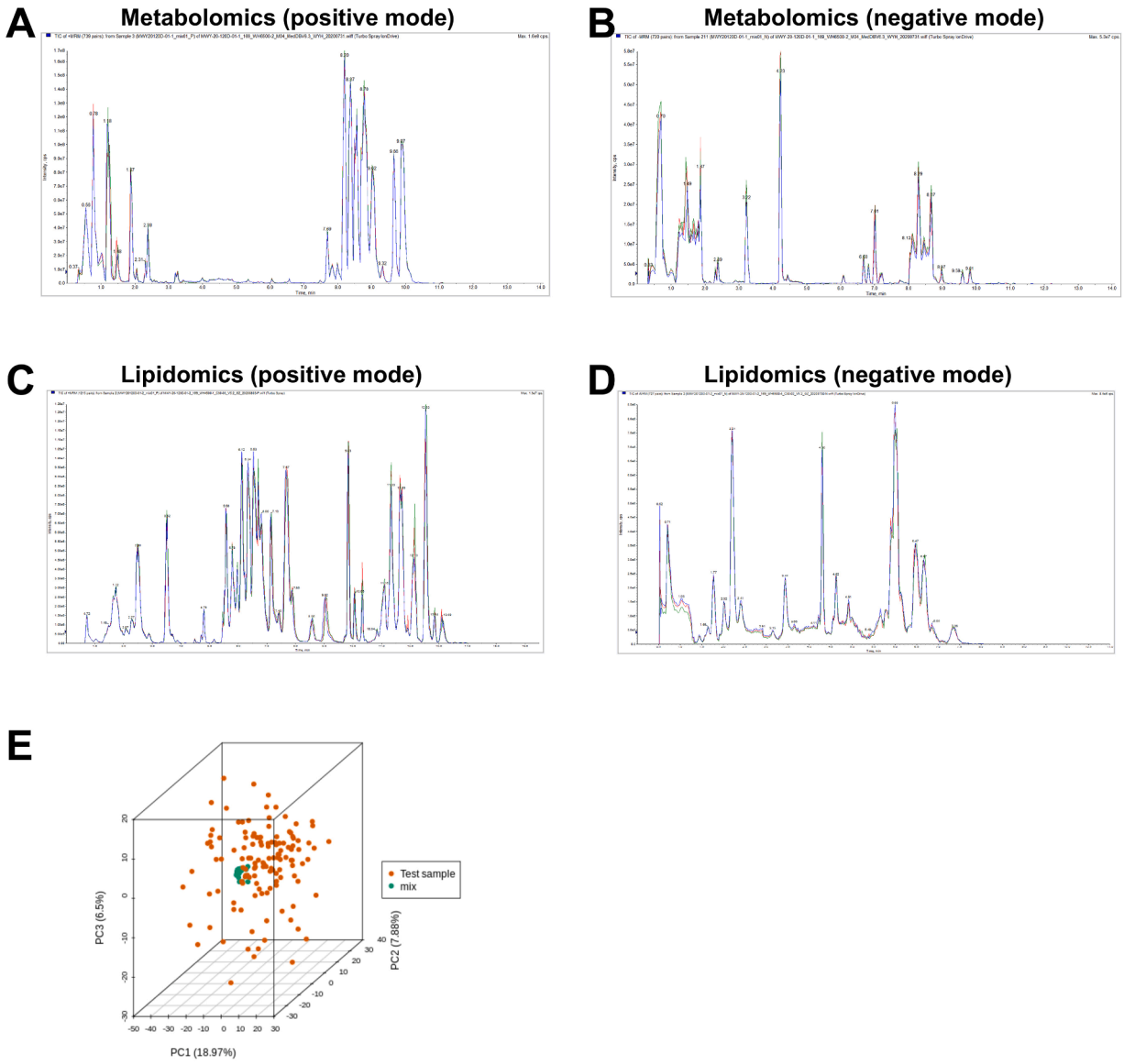

Fig. 1. The presentative overlapping TIC diagrams of QC samples monitored by (A) targeted metabolomics in positive mode detection; (B) targeted metabolomics in negative mode detection; (C) targeted lipidomics in positive mode detection and (D) targeted lipidomics in negative mode detection. (E) 3D PCA score chart of tested and mixed samples (X-axis represents the first principal component, Y-axis represents the second principal component).

Predictive effect of plasma endogenous metabolites on blood ADR of pemetrexed chemotherapy

Based on the records of the electronic medical record system, 114 of the enroled patients were divided into groups with/without liver/blood ADR. One sample was separated from the other ones and excluded from the following analysis. Therefore, 113 patients were included herein to discover differential expressed metabolites with an effective ADR predictive role.

There were 76 patients with blood adverse reactions (Blood ADR Group) and 37 patients without blood adverse reactions (Blood Normal Group). All 1141 detected endogenous metabolites were compared between the two groups using the Student's *t*-test, and 76 differential expressed metabolites were screened out ($P < 0.05$), which could be enriched in pathways of alanine metabolism, glutathione metabolism, etc. (Supplementary Fig. 3).

In addition, 15 metabolites with the smallest *P* values, most of which were classified as lipids with AUROC ranging from 0.6600 to 0.7092, were selected for subsequent analysis. A logistic regression model was established for different kinds of combinations of these 15 metabolites. According to the AIC selection standard, a panel incorporating three metabolites was selected. The regression equation for predicting blood ADR was as following: Logit (P) = 3.522 - 0.00003 * triglyceride (TG) (14:0/22:3/22:5) + 0.000012 * 3-(3-Hydroxyphenyl) Propionate Acid - 0.000002 * Carnitine C18:0. The blood ADR prediction model showed an AUROC of 0.7954 (95% CI: 0.6971–0.8936) with an optimal cut-off at 0.7499 (Table 3, Supplementary Fig. 4).

Predictive effect of plasma endogenous metabolites on hepatotoxicity of pemetrexed chemotherapy

There were 23 patients with hepatotoxicity (Liver ADR Group) and 90 patients without hepatic adverse reactions (Liver Normal Group). Likewise, all 1141 detected endogenous metabolites were compared between the two groups using the Student's *t*-test, and 54 differential expressed metabolites were screened out ($P < 0.05$), which could be enriched in pathways of galactose metabolism, lactose degradation, ketone body metabolism, etc. (Supplementary Fig. 5).

In addition, 15 candidate metabolites were also selected according to the *P* values. As displayed in Table 4, the AUROC of them ranged from

Table 2
Diagnostic ability of plasma endogenous compounds on chemotherapeutic efficacy of pemetrexed.

Compounds	Class	FC	*P* value	AUROC
PS(20:4/20:0)	Lipids	0.7609	0.0005	0.7386
SM(d18:0/14:0)	Lipids	0.7060	0.0002	0.7366
PE(20:4/22:2)	Lipids	0.6622	0.0017	0.7172
PC(O-18:3/18:2)	Lipids	0.8129	0.0027	0.7159
Cer(d18:1/16:0)	Lipids	0.8322	0.0007	0.7146
SM(d18:1/16:0)	Lipids	0.8909	0.0020	0.7126
PE(P-18:2/20:2)	Lipids	0.7193	0.0008	0.7112
PS(20:4/20:1)	Lipids	0.7031	0.0005	0.7019
SM(d18:1/18:0)	Lipids	0.8609	0.0105	0.7019
LPE(0:0/18:1)	Lipids	0.7832	0.0065	0.7012
PA(18:1/20:0)	Lipids	0.6713	0.0002	0.6999
PC(O-16:0/14:1)	Lipids	0.8833	0.0150	0.6992
PC(O-18:2/18:1)	Lipids	0.7403	0.0017	0.6918
SM(d18:1/16:1)	Lipids	0.8683	0.0041	0.6918
Lysope 18:1	Lipids	0.7920	0.0126	0.6918
Panel A				0.7968

Footnote: FC, fold change of group (PD+SD)/group PR, Area Under the Receiver Operating Characteristic curve (AUROC), phosphatidylserine (PS), sphingomyelin (SM), phosphatidylethanolamine (PE), phosphatidylcholine (PC), ceramide (Cer), sphingomyelin (SM), lysophatidylethanolamine (LPE), phosphatidic acid (PA).
Panel *A* = 7.086–0.000011*a-(9.9014E-08)*b-0.000042*c, a: PS (20:4/20:1), b: SM (d18:1/18:0), c: PA (18:1/20:0).

Table 3
Diagnostic ability of plasma endogenous compounds on the occurrence of blood ADR after pemetrexed treatment.

Compounds	Class	FC	*P*-value	AUROC
TG(14:0/22:3/22:5)	Lipids	0.6365	0.0002	0.7092
TG(14:0/20:4/22:2)	Lipids	0.7644	0.0041	0.6925
TG(14:0/20:4/22:1)	Lipids	0.7558	0.0067	0.6837
TG(14:0/20:4/22:3)	Lipids	0.7419	0.0031	0.6815
3-(3-Hydroxyphenyl) Propionate Acid	Organic acid	2.2853	0.0385	0.6815
TG(14:0/20:4/22:0)	Lipids	0.7707	0.0131	0.6767
PE(20:4/22:2)	Lipids	0.7165	0.0089	0.6687
Glycine	Amino acid	1.2295	0.0072	0.6682
PE(20:1/20:4)	Lipids	0.7109	0.0061	0.6659
LysoPC 22:5 (2n isomer3)	Lipids	0.8097	0.0279	0.6659
LysoPC 22:5 (2n isomer2)	Lipids	0.8097	0.0279	0.6659
L-Alanine	Amino acid	1.2169	0.0090	0.6648
β-Alanine	Amino acid	1.2169	0.0090	0.6648
Carnitine C18:0	Lipids	0.8392	0.0124	0.6628
LysoPC 18:2 (2n isomer1)	Lipids	1.0358	0.0042	0.6600
Panel B				0.7954

Footnote: FC, fold change of group (Blood ADR)/group (Blood Normal), Area Under the Receiver Operating Characteristic curve (AUROC), triglyceride (TG), phosphatidylethanolamine (PE), lysophosphatidylcholine (LysoPC).
Panel *B* = 3.522–0.00003*d-1170+0.000012*e-0.000002*f, d: TG (14:0/22:3/22:5), e: 3-(3-Hydroxyphenyl) Propionate Acid, f: Carnitine C18:0.

0.6800 to 0.7550. Subsequent logistic regression analysis using different combinations of candidate compounds based on AIC screen standard found that a model incorporating four metabolites showed the best predictive ability. The regression equation for predicting hepatotoxicity was as following: Logit (P) = 15.801 - 0.000102 * Stearidonic Acid - 0.000016 * TxB3 −0.000051 * L-Homocitrulline + 0.000013 * phosphoinositide (PI) (20:3/18:0). The hepatotoxicity prediction model showed an AUROC of 0.8186 (95% CI: 0.7294–0.9078) with an optimal cut-off at −6.865 (Table 4, Supplementary Fig. 6).

Table 4
Diagnostic ability of plasma endogenous compounds on the occurrence of hepatotoxicity after pemetrexed treatment.

Compounds	Class	FC	*P*-value	AUROC
Stearidonic Acid	Lipids	0.9080	0.0006	0.7550
Carnitine C13:0	Lipids	0.5085	0.0026	0.7436
3-Hydroxy-decenoyl- carnitine	CAR	0.6737	0.0092	0.7261
TxB3	Eicosanoid	0.5945	0.0049	0.7136
L-Homocitrulline	Amino acid	0.6945	0.0123	0.7011
N-Methyl-L-Glutamate	Amino acid	0.7475	0.0097	0.6968
Carnitine C11:0	Lipids	0.6550	0.0114	0.6918
DG(14:1/16:0/0:0)	DG	0.8113	0.0065	0.6900
N-acetylornithine	Organic acid	0.8585	0.0252	0.6900
3-hydroxy-cis-5-octenoyl-carnitine	CAR	0.7086	0.0196	0.6832
PI(20:3/18:0)	PI	1.5326	0.0088	0.6829
D-Glucoronic Acid	Carboxylic acids	0.7920	0.0168	0.6825
D-Malic acid	Organic acid	0.7702	0.0217	0.6804
Carnitine C15:0	Lipids	0.9296	0.0083	0.6800
N-Acetylthreonine	Amino acid	0.8575	0.0312	0.6800
Panel C				0.8186

Footnote: FC, fold change of group (Liver ADR)/group (Liver Normal), Area Under the Receiver Operating Characteristic curve (AUROC), thromboxane B3 (TxB3), diglyceride (DG), phosphoinositide (PI).
Panel C = 15.801–0.000102*g-0.000016*h-0.000051*i + 0.000013*j, g: Stearidonic Acid, h: TxB3, i: L-Homocitrulline, j: PI (20:3/18:0).

Discussion

Metabolomics provides a strong link between genotype and phenotype, and is sensitive to many factors. It reflects alterations of biological states. Metabolomics enables identify small-molecule metabolites in biofluids that might be feasible and early markers of drug efficacy and toxicity [9]. In the study, we developed effective discriminant models that could predict the efficacy and blood/liver toxicity of pemetrexed plus platinum chemotherapy regimens in lung adenocarcinoma patients using metabolomic analysis. These predictive models might be used to identify potential pemetrexed plus platinum chemotherapy beneficiaries with minimal toxicity.

Although target therapy and immunotherapy have been used for the treatment of lung adenocarcinoma, pemetrexed plus platinum chemotherapy is still recommended as the first-line treatment. However, the response rate is only about 35%. A great number of studies tried to identify biomarkers that could be used to predict chemotherapy response [10]. But few biomarkers have been verified and used in the clinic. Our study found 157 kinds of differentially expressed metabolites between the response group (PR) and the non-response group (PD+SD), which were mainly enriched in pathways of phospholipid biosynthesis and glycerolipid metabolism. 15 lipid metabolites were found to be potential predictive biomarkers with their AUROC ranging from 0.6918 to 0.7386. Multiple logistic regression analysis found an effective model based on a panel of PS (20:4/20:1), SM (d18:1/18:0), and PA (18:1/20:0) that could predict pemetrexed and platinum chemotherapy response with an AUROC of 0.7968. PS, a component of the bilayer cell membrane, is normally sequestered to the inner leaflet. PS exposure on the external leaflet not only triggers rapid removal by phagocytic engulfment in apoptotic cells by also participates in immune regulation in nonapoptotic cells. PS is aberrantly increased in the tumor microenvironment, which contributes to innate immunosuppressive properties and facilitates tumor growth and metastasis that might antagonize the efficacy of chemotherapy [11,12]. SM (d18:1/18:0) is a sphingomyelin with systematic name N-(octadecanoyl)-sphing-4-enine-1-phosphocholine [13]. Ceramide is composed of a sphingosine long-chain base. Ceramide which acts as the central hub of sphingolipid metabolism, mediates cancer cell death based on the subcellular localization of ceramide and the availability of downstream targets of ceramide [14]. Ceramide can be generated in response to many stressors, including environmental stress, cytokines, and chemotherapy treatment. Many studies showed ceramide might be key to overcome resistance to current drug therapies [15]. PA is a central metabolite in the synthesis of

membrane phospholipids and is required for the stability and activity of mTOR complexes. PA plays key roles in numerous essential cellular functions, such as vesicular trafficking, exocytosis, autophagy, regulation of cellular metabolism, and tumorigenesis [16]. Suppression of the de novo synthesis of PA can lead to G1 cell cycle arrest [17]. It was reported that PA could increase the apoptotic potential of doxorubicin [18]. For those patients with a response prediction model value less than 1.150, they might have great potential beneficiaries with a great chance of efficacy. While those patients with a response prediction model value higher than 1.150 might avoid pemetrexed plus platinum chemotherapy because of high risk of non-response based on our response prediction model.

Unpredictable severe side effects greatly impeded the use of platinum-based chemotherapy. What's more, the incidence and severity of toxicities differ greatly between individuals. Biomarkers predicting the toxicities of platinum-based chemotherapy are urgently needed. In the study, we found 76 differential expressed metabolites to be associated with hematological toxicity of pemetrexed plus platinum chemotherapy. Those metabolites were mainly enriched in pathways of alanine metabolism and glutathione metabolism. A panel incorporating TG (14:0/22:3/22:5), 3-(3-Hydroxyphenyl) Propionate Acid, and Carnitine C18:0 were found to be the best predictive ability with an AUROC of 0.7954. Numbers of studies found serum triglyceride was involved in the pathogenesis of lung, rectal, thyroid, prostate, and gynecological cancers [19]. Serum triglyceride concentration was inversely related to the hemoglobin concentration in chronic myeloid leukemia patients with chemotherapy treatment [20]. 3-(3-Hydroxyphenyl) Propionate Acid was reported to be capable of altering the mesenchymal stem cell differentiation program and bone cell senescence [21]. 3-(3-Hydroxyphenyl) Propionate Acid had a strong protective ability against Cadmium-induced erythrocyte cytotoxicity [22]. L-carnitine can be effective in protecting against platinum-induced myelosuppression in bone marrow cells and reduce hematological toxicity in gastrointestinal cancer patients who received LFP chemotherapy [23,24]. For those patients with a blood ADR prediction model value less than 0.7499, they might have great potential beneficiaries with a low risk of hematological toxicity. Those patients with a blood ADR prediction model value higher than 0.7499 should be cautious or change the treatment regimen.

Hepatotoxicity such as aspartate aminotransferase elevation is a common adverse side effect of pemetrexed plus platinum chemotherapy. It is reported that hepatotoxicity is a major dosing-limiting factor when high dose platinum chemotherapy has been continued [25]. In the study, 54 differential expressed metabolites were found to be associated with hepatotoxicity of pemetrexed plus platinum chemotherapy. Those metabolites were mainly enriched in pathways of galactose metabolism, lactose degradation, and ketone body metabolism. A model incorporating stearidonic acid, TxB3, L-Homocitrulline, and PI (20:3/18:0) showed the best predictive ability with an AUROC of 0.8186. Stearidonic acid could enhance the cytotoxic effects of chemotherapy agents such as docetaxel in human prostate cancer cells and canine lymphoid tumor cells [26,27]. Thromboxane B3 is the stable hydrolysis product of Thromboxane A3 synthesized from eicosapentaenoic acid by COX and thromboxane synthase. L-Homocitrulline is metabolized to homoarginine through homoargininosuccinate via the urea cycle pathway and its metabolic abnormality could lead to Lysinuric Protein Intolerance. Phosphatidylinositols are glycerophospholipids that contain a glycerol backbone, two non-polar fatty acid tails, and a polar inositol head group. They represent approximately 10% of total cellular phospholipids. Phosphatidylinositols can be phosphorylated on their inositol rings to produce phosphoinositides, which have been implicated in calcium regulation, vesicle trafficking, mitogenesis, cell survival, and rearrangement of actin. For those patients with a hepatotoxicity prediction model value less than −6.865, they might have great potential beneficiaries with a low risk of hepatotoxicity. Those patients with a hepatotoxicity prediction model value less than −6.865 should take precautions or change the treatment regimen.

It must be admitted that the present study had some limitations. First, because of the relatively small population, the prediction models need to be further verified in different races and large samples. Second, owing to the complexity of the mechanism of pemetrexed plus platinum, multi-omics studies such as genomic, proteomic, and metabolomics are needed. Third, those metabolic markers were selected based on the qualitative comparison. The quantitative analysis lacks. Fourth, this study lacked a group of healthy human plasma samples for comparisons with lung adenocarcinoma patients. Last but not least, the precise mechanisms of selected biomarkers are still unknown.

Conclusion

We established predicted models of drug response and toxicity in lung adenocarcinoma patients with pemetrexed plus platinum chemotherapy. Those models with high accuracy might be a feasible approach to identify potential beneficiaries with maximal efficacy and minimal toxicity. For those patients with a response prediction model value less than 1.150, a blood ADR prediction model value less than 0.7499, and a hepatotoxicity prediction model value less than −6.865, they might have a great chance of efficacy and low risk of toxicity. However, the prediction models still need to be further verified in different races and large samples.

CRediT authorship contribution statement

Wei-Jing Gong: Data curation, Formal analysis, Writing – original draft. **Peng Cao:** Conceptualization, Formal analysis, Data curation, Writing – original draft. **Qi-Lin Zhang:** Data curation, Formal analysis, Data curation. **Xiao-Yu Han:** Data curation, Formal analysis. **Shuo-Wen Wang:** Formal analysis. **Yi-Fei Huang:** Data curation, Formal analysis. **San-Lan Wu:** Data curation, Formal analysis, Formal analysis. **Qiang Li:** Data curation, Formal analysis. **Rui Zhang:** Data curation. **Shuang-Bing Xu:** Data curation. **Ya-Ni Liu:** Conceptualization, Formal analysis, Data curation. **Shao-Jun Shi:** Formal analysis. **Yu Zhang:** Conceptualization, Formal analysis, Writing – original draft.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Findings

This study was supported by the National Key R&D Program of China (2017YFC0909900), National Natural Science Foundation of China (No. 82003868), and Hubei Provincial Natural Science Foundation of China (No. 2020CFB388). We sincerely thank Wuhan Metware Biotechnology Co., Ltd. for its technical support.

Ethical statement

This study was approved by the Ethics Committee of the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. All patients enroled in this study signed an informed consent form.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2022.101393.

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