Plasma Metabolomics Study in Lung Cancer Screening and Differential Diagnosis

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Abstract

[Objectives] By studying the plasma metabolomics of patients with different pulmonary nodules and healthy people, we can find the difference in plasma low-molecular metabolites among them.

[Methods] Patients with pulmonary nodules admitted to our department were divided into three groups: pulmonary metastatic carcinoma (PMC), benign pulmonary nodules (BPN), and primary lung cancer (PLC). Meanwhile healthy people were enrolled as healthy population group (HPG). PLC and HPG were equally divided into the Discovery Set and Validation set.

[Results] Five significant low-molecular metabolites were found by comparison of four groups as a whole. Four to six metabolites were selected by comparison of the three pulmonary nodule groups with healthy people respectively. The AUC of ROC of these metabolites were all 0.93. Each pairwise comparison within the three pulmonary nodule groups all found three metabolites, whose AUC of ROC were all 0.83. From the comparison of PLC and HPG in the discovery set, six metabolites were selected. Their AUC of ROC were all greater than 0.95 in the validation set, indicating that they had a strong ability to differentiate between primary lung cancer and healthy people.

[Conclusions] We can find the significant changes of some low-molecular metabolites among three pulmonary nodules and healthy people. These metabolites had the potential to be biomarkers for screening and differential diagnosis of lung cancer.

1. Introduction

The incidence and mortality of lung cancer have ranked first among all cancers in recent years[1]. Cancer screening can increase the likelihood of early detection for lung cancer in normal population. The main method is the chest low-dose CT scan[2]. After the detection of pulmonary nodules by chest CT, it is necessary to further determine the nature of pulmonary nodules according to the imaging characteristics and medical history of the patient. The common types of benign pulmonary nodules include tuberculoma, pulmonary hamartoma, sclerotic alveolar cell tumor and so on. While malignant pulmonary nodules generally include primary lung cancer, pulmonary metastatic lesions, etc. The imaging features of benign pulmonary nodules on chest CT mainly include geometric shape, calcification, long burr sign, and smooth cavity. The imaging features of primary lung cancer on chest CT mainly include ground glass component, short burr sign, nourishing vessels, pleural pulling or depression, and non-smooth cavities. Pulmonary metastatic carcinoma refers to the malignant tumor originating from other organs that have metastasized to the lung. The imaging features of pulmonary metastatic lesions on CT mainly include round shape, solid, single or multiple, and smooth surface, etc. Sometimes, the nature of pulmonary nodules cannot be accurately determined by chest CT scan[3]. Biomarkers for lung cancer, including CEA, NSE,CYFRA21-1, SCCA and so on, have the disadvantage of low sensitivity and specificity, resulting in their limited role in clinical application[4]. At present, the biomarkers for lung cancer which have the ability of screening and differential diagnosis are in urgent need.

Metabolomics is the study of the metabolites with molecular weight less than 1000 Da, which is an important complement to genomics, transcriptomics, and proteomics. Metabolomics had been widely used to study a variety of diseases, including major depression, type 2 diabetes, ulcerative colitis, and malignant tumors, et al. Metabolomics research about malignant tumors in various organs had been reported[5-8], such as colorectal cancer, liver cancer, pancreatic cancer and lung cancer[9]. Previous metabolomics research on lung cancer had focused on the following areas: the difference between lung cancer and healthy people[10, 11], or chronic obstructive pulmonary disease(COPD) [12], the association with pathological types and stages of lung cancer[13-15], the monitoring of treatment effects[16,

17], and the judgment of lung cancer prognosis[18]. These studies had some common shortcomings such as the same kind of research subjects, incomplete research scope, and lack of verification and repetitiveness.

The purpose of this study was to select some significant low-molecular metabolites in plasm as the potential biomarkers, by the comparison of metabolites in plasma samples from pulmonary metastatic carcinoma, benign pulmonary nodules, primary lung cancer, and healthy people. These selected metabolites may play a vital role in clinical screening of lung cancer and differential diagnosis of pulmonary nodules in the future.

2. Materials And Methods

2.1 Participants

This study was approved by the Ethics Committee of National Cancer Center, Chinese Academy of Medical Sciences and its number is 19/223-2007. The informed consent was obtained from all participants. The flow chart of this study is shown in Figure 1. Inclusion criteria were: lung nodules were accidentally found on chest CT, and the nature of the nodules was determined by paraffin pathology after surgical resection. Exclusion criteria were: the elderly and weak could not tolerate surgery, the patient required non-surgical treatment, the nature of the nodules was not confirmed by paraffin pathology, and the nature of the nodules was not in the range of pulmonary metastatic lesion, benign pulmonary nodule and primary lung cancer, such as lung lymphoma. A total of 250 patients with pulmonary nodules admitted to our department from February 1, 2019 to May 31, 2019 were collected and 208 people were selected according to the gender and age matching results. They were divided into pulmonary metastatic carcinoma (PMC), benign pulmonary nodules (BPN) and primary lung cancer (PLC) by the postoperative pathology. Meanwhile a total of 96 healthy people who underwent physical examination were enrolled as the healthy population group (HPG) in this study. PLC and HPG were equally divided into the discovery set and the validation set respectively by random sampling method. In the discovery set, there were 16 patients in PMC，32 patients in BPN, 80 patients in PLC, and 48 people in HPG; in the validation set, there were 80 patients in PLC and 48 people in HPG. The general characteristics of the subjects is shown in Table S1.

2.2 Sample Collection

In order to avoid the effects of food and time on low-molecular metabolites, blood samples of all subjects were taken under the fasting state in the morning. The blood samples were immediately placed in an EDTA anticoagulation tube, and then centrifuged at 4°C, 3000g for 5 minutes. Then the plasma was taken and stored in -80 ℃.

2.3 Sample Preparation

After thawing the plasma samples at 4°C, take 50ul of plasma and add 950ul of methanol/ acetonitrile (2/3, v/v) solution, and then vortex for 1 minute. After standing at 4°C for 24 hours, the sample was centrifuged at 19,000 g for 30 minutes. Take 50ul of the supernatant, add 250ul of dichloromethane and 500ul of ultrapure water and mix them. After vortexing for 30 seconds, centrifuge the sample at 1250g for 6 minutes. Transfer 75ul of the supernatant to a glass bottle and let it dry naturally at room temperature. The dried sample is re-dissolved by adding 150ul of 50% methanol solution.

2.4 Instrumental Test

For every 20 normal samples, a quality control(QC) sample was inserted to ensure the quality of the experiment. The QC sample were mixed from four different types of plasma samples. A total of 15 QC samples were tested in the whole

experiment. In addition, 4 blank samples were tested. The blank samples can be used to check the residues of the substance during the test.

2.5 Data Processing

The raw data of each sample in four groups were imported into the DataAnalysis 4.4 to obtain the corresponding peak spectrum. The typical peak spectra of samples from the four groups were shown in Figure S1. The metabolites which meet the criteria of 50-1000 Da in molecular weight and the absolute intensity value of greater than 1000 were selected. After deconvolution and combining isotopes, a total of 155 metabolites were obtained. Remove the metabolites which existed in less than 80% of all samples, then 80 different metabolites were left. For some metabolites did not exist in a minority of samples, their intensity values in such samples were set to half of the lowest absolute intensity value of the same metabolite in other samples. According to the HMDB database, isotopic abundance comparison, and biological characteristics of metabolites, 78 low-molecular metabolites were identified at last and the other two metabolites could not be interpreted.

2.6 Data Analysis

The data of each comparison group was normalized by MetaboAnalyst 4.0, and preliminary analysis was performed. Then the normalized data was imported into Simca 14.1 software for multivariate data statistical analysis, including Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA), etc. Before performing multivariate data statistical analysis, all data were log-transformed and Par-formatted to obtain more reliable and intuitive result. PCA can reflect the overall metabolic difference among groups and the variability degree of samples in the same group. OPLS-DA can filter signals that are irrelevant to the model classification, and obtain OPLS-DA model to clarify the relevant low-molecular metabolites. The quality of the model was tested by cross-validation, and the validity of the model was judged by the R2X and Q2 (representing the model's interpretable variables and the predictability respectively). The validity of the model is further tested by permutation experiments.

2.7 Biomarkers Selection

Calculate the VIP value (Variable Importance in the Projection) of metabolites that lead to the difference between two groups in the OPLS-DA model. It is generally considered that metabolites with VIP>1 have analytical significance. These low-molecular metabolites were statistically compared among the groups using SPSS 19.0 software. Kolmogorov-Smirnov test method was used to analyze the data, and it was found that the data of each group did not conform to normal distribution, so the non-parametric test method was adopted (comparisons between two groups were performed by the Mann-Whitney U test, comparisons among three or four groups were performed by the median test). Since there were more than one metabolites with VIP>1 in each comparison, and the statistical difference between the groups was tested more than once, the Bonferroni method was used to correct the threshold of p value. In each comparison between the groups, several most representative low-molecular metabolites were selected as biomarkers by the VIP value and p value, and their diagnostic sensitivity and specificity were judged by the ROC curve.

3. Results

3.1 *Comparison of General Characteristics*

The primary source of pulmonary metastatic carcinoma were rectal cancer (n=7), colon cancer (n=4), lower limb osteosarcoma (n=1), nasal cancer (n=1), breast cancer (n=2) and liver cancer (n=1). Postoperative pathological types in benign pulmonary nodule group included lymphadenopathy (n=11), tuberculous granulomatosis (n=4), sclerosing

alveolar cell tumor (n=2), organizing pneumonia (n=5), hamartoma (n=3), leiomyoma (n=2), solitary fibroma (n=1), fungal infection (n=1), atypical adenomatous hyperplasia (n=2) and epithelioid hemangioendothelioma (n=1).

The general characteristics, including smoking history, comorbid disease, history of malignant tumors, neoadjuvant therapy, were compared between the Discovery Set and Validation Set of primary lung cancer. There is no significant difference in all comparisons (p<0.05). In this study, the pathological type of primary lung cancer include six kinds: adenocarcinoma (n=131, 81.9%), squamous cell carcinoma (n=20, 12.5%), small cell lung cancer (n=4, 2.5%), carcinoid (n=3, 1.9%), carcinosarcoma (n=1, 0.6%), large cell lung cancer (n=1, 0.6%). The six types of pathology in the Discovery Set and Validation Set have no significant difference (p=0.669). The pathological stages of primary lung cancer include stage 0 (n=21, 13.1%), stage I (n=103, 64.4%), stage II (n=10, 6.3%), stage III (n=23, 14.4%), and stage IV (n=3, 1.8%). There was no significant difference in the pathological stages between the Discovery Set and the Validation Set (p=0.526). (Table S2).

3.2 Lung Cancer Screening In Healthy People

First compare the four groups of the discovery set as a whole, and obtain the overall difference among them. Then the healthy population group was compared with the other three groups, respectively. The differences in plasma metabolic profiles between them were analyzed, and the low-molecular metabolites that cause these differences were selected.

3.2.1 Overall comparison of the four groups

The data of four groups were normalized by MetaboAnalyst 4.0. The comparison of data before and after normalization is shown in Figure S2. The levels of some low-molecular metabolites in four groups were obviously different overall (Figure 3A and Figure S3). According to the VIP value and p value, five low-molecular metabolites were selected. Their intensity values in each group can be intuitively reflected in Figure 2. After non-parametric tests, the p values obtained were all less than 0.001, indicating that the level of the five metabolites in the four groups were significantly different.

3.2.2 Comparison of the healthy group with the three pulmonary nodule groups respectively

Three pulmonary nodule groups including PMC, BPN and PLC were compared with HPG respectively. The clear separation of two groups in each comparison can be seen(Figure 3B). The major low-molecular metabolites were selected in each comparison(Table 1).

In order to test the ability of these metabolites to discriminate between the healthy people and the pulmonary nodule lesions, these major low-molecular metabolites were drawn into ROC curves (Figure 4). The area under the curve (AUC) of every metabolite was greater than 0.9, indicating that these low-molecular metabolites all have a high discriminating ability. The optimal critical point of the ROC curve, and its corresponding sensitivity and specificity are shown in Table 1.

3.3 Differential Diagnosis of the Pulmonary Nodules

According to the pathology, the common pulmonary nodules were mainly divided into three types: primary lung cancer, benign pulmonary nodules, and pulmonary metastatic carcinoma. To help determine the nature of lung nodules before surgery, the three types of pulmonary nodules were compared by the means of plasma metabolomics.

3.3.1 Overall comparison of the three pulmonary nodule groups

The PMC, BPN and PLC were compared as a whole (Figure 3C). The validity of the model was further tested by permutation test. The number of tests was set to 200. The test result showed that the validity was good (Figure S4). The major low-molecular metabolites were selected from 27 metabolites (Figure S5): anabasine, octanoylcarnitine, 2-methoxyestrone, retinol, decanoylcarnitine, calcitroic acid, glycogen and austalide L.

3.3.2 Pairwise comparisons of the three pulmonary nodule groups

The PMC, BPN and PLC were compared in pairs. Firstly, the BPN was compared with PLC(Figure 3D), and the difference between the two groups was obvious. The main low-molecular metabolites selected from 26 metabolites were octanoylcarnitine, decanoylcarnitine and PGF2a ethanolamide. The difference of the three metabolites between the two groups can be seen directly in Figure 5A. ROC curves were drawn for these metabolites, and the AUC were 0.974, 0.965, and 0.881, respectively. The optimal critical points for the best sensitivity and specificity were 6.85, 3.50, and 6.89, accordingly (Table 1). Secondly, PMC and BPN were compared. Tyrosine, indoleacrylic acid and LysoPC(16:0) were selected. Their ROC curves and box plots were shown in Figure 5B. At last, the PMC was compared with PLC. The metabolites selected were octanoylcarnitine, retinol, and decanoylcarnitine. Figure 5C showed the obvious difference of these three metabolites between the two groups. Detailed information was shown in Table 1.

3.4 Validation of Metabolites in Primary Lung Cancer and Healthy People

A total of six major different low-molecular metabolites were found in the Discovery Set (Table 1). The ROC curves of these metabolites were drawn in the validation set and their AUC were all greater than 0.95, indicating that these six metabolites have a strong ability to differentiate primary lung cancer from healthy people (Figure 4D).

3.5 Further Analysis of Primary Lung Cancer

In this study, the pathological types of primary lung cancer included adenocarcinoma, squamous cell carcinoma, small cell lung cancer, carcinoid carcinoma, carcinosarcoma, and large cell lung cancer. All samples were grouped according to different pathological types. After multivariate statistical analysis of the data of each group, it was found that there was no significant difference in the spatial distribution of the fractional scatter plots of the six groups of samples. This indicates that there is no significant difference in the metabolic level of low-molecular metabolites among samples of different pathological types of lung cancer. The same operation and analysis were performed in the primary lung cancer of the Discovery Set and Validation Set respectively, and the same results were obtained.

All the samples of primary lung cancer were grouped according to different postoperative pathological stages (stage 0, I, II, III, IV). After multivariate statistical analysis of the data of each group, it was found that there was no significant difference in the spatial distribution of the fractional scatter plots of the five groups of samples. This indicates that there is no significant difference in the metabolic level of low molecular metabolites in the samples of different pathological stages. The same operation and analysis were performed in the primary lung cancer of the Discovery Set and Validation Set respectively, and the same results were obtained.

4. Discussion

According to the previous study[19], gender and age may cause changes in plasma metabolic profiles. In our study, there was no significant difference in gender and age between groups. In previous metabolomics studies on lung, it was common to compare primary lung cancer with healthy people[20]. A small number of literatures had reported benign pulmonary nodules or chronic obstructive pulmonary disease compared with primary lung cancer[21, 22]. However there was no reports on pulmonary metastatic carcinoma until now. This study was the first report on this and summarized the difference of plasma metabolites between three common pulmonary nodules and healthy people.

In the comparison of healthy people with the other three pulmonary nodule groups as a whole, five major low-molecular metabolites were selected (Figure 2). Decanoylcarnitine had the highest level in healthy people among the four groups. However, the other four metabolites in healthy people had the lowest level. Decanoylcarnitine is a type of acylcarnitine compound which is an organic compound containing fatty acids and belongs to endogenous lipids. The change in the metabolic level of decanoylcarnitine occurs during the development of many diseases, including ulcerative colitis, Crohn's disease, colorectal cancer, etc.[23, 24]. Klupczynska et al.[25] reported that patients with NSCLC had elevated carnitine levels and decreased amylcarnitine and propylcarnitine levels. Junjun Ni et al.[26] reported that the concentration of acylcarnitine in the plasma of lung cancer patients was significantly different from that of healthy people. Swee Ling Lim et al.[13] found that acylcarnitine was one of the main low-molecular metabolites that caused the difference in the metabolomics of lung tissues from different pathological types of lung cancer. Yanjie Li et al[27]. found differences in plasma concentrations of carnitine, fatty acids and fatty amides between lung cancer patients and healthy subjects, suggesting abnormal lipid metabolism in lung cancer patients. Lipid dysregulation is one of the most common metabolic changes in cancer[28].

Comparing the three pulmonary nodule lesions with healthy people, it was found that the major low-molecular metabolites in each comparison were mostly different. The major low-molecular metabolites between pulmonary metastatic carcinoma and healthy people were o-arachidonoyl ethanolamine, adrenoyl ethanolamide, tricin 7-diglucuronoside and p-coumaroyl vitisin A. The level of these four metabolites were all significantly higher in the patients with lung metastatic cancer than healthy people. O-arachidonoyl ethanolamine is the first endogenous cannabinoid isolated and acts on the same receptor as tetrahydrocannabinol. The area under the ROC curve of o-arachidonoyl ethanolamine and adrenoyl ethanolamide were 0.945 and 0.931, respectively, indicating that they can both distinguish pulmonary metastatic cancer from healthy people.

The main low-molecular metabolites found in comparison of benign pulmonary nodules with healthy people also include γ-glutamylphenylalanine and nitrosoglutathione. Glutamylphenylalanine is a dipeptide composed of glutamic acid and phenylalanine, which is a protein hydrolysate of a larger protein. Most dipeptides are only transient intermediates. After further proteolysis, they will enter a specific amino acid degradation pathway. Glutamine is involved in glutamate metabolism, which is one of the most altered metabolic pathways found in previous studies on lung cancer metabolism[29]. Glutamine is involved in the synthesis of purines, pyrimidines, glycine and other substances, and plays an important role in the growth of cancer cells, protein translation and macromolecule synthesis. Our study found that the content of glutamylphenylalanine in the plasma of patients with benign pulmonary nodules was significantly higher than that of healthy people, which may be related to the acceleration of amino acid metabolism under pathological conditions. The previous study of glutamylphenylalanine in patients with colorectal cancer and phenylketonuria were consistent with this conclusion[30]. Nitrosoglutathione is a circulating endogenous nitric oxide (NO) reservoir and may be a potential donor of nitric oxide with anti-platelet property. There was also a significant difference in the expression of nitrosoglutathione between primary lung cancer and healthy people. The study of Zhang et al.[31] showed that, as an endogenous NO carrier, nitrosoglutathione can induce apoptosis of lung cancer cells by nitrogenizing Prdx2. Glutathione is an important cellular antioxidant that regulates the body's redox state and immune response. Elevated glutathione levels are characteristic of some tumors[21] and are associated with an increase in reactive oxygen species in tumor cells.

The three pulmonary nodule groups were compared in pairs. Patients with lung metastases usually have a history of malignancy in other sites, but this is not a sufficient condition for the diagnosis of lung metastases, because there is a possibility that the patient may also have primary lung cancer . In our study, 15 of 160 patients with primary lung cancer were complicated with malignancies at other sites, which fully demonstrates this point. The common major metabolites between primary lung cancer with pulmonary metastatic carcinoma or benign pulmonary nodules were

octanoylcarnitine and decanoylcarnitine. The plasma levels of these two metabolites in patients with primary lung cancer were both significantly higher than pulmonary metastatic carcinoma and benign pulmonary nodule. Some studies had found the metabolic level of octanoylcarnitine in the blood of patients with obesity and inflammatory bowel disease had been significantly changed[23, 32]. PGF2a ethanolamide is a class of lipid compounds that occur naturally in animal and plant membranes. It can well distinguish benign pulmonary nodules from primary lung cancer. Its AUC of ROC is 0.881, and the sensitivity is 0.90 and specificity is 0.91, which shows a high ability of differential diagnosis. Retinol is one of the main plasma low-molecular metabolites for differentiating pulmonary metastatic cancer from primary lung cancer. Its plasma content in primary lung cancer is significantly higher than pulmonary metastatic cancer. Pamungkas et al.[33] reported the same conclusion with our study. Retinol is a yellow fat-soluble antioxidant vitamin, which belongs to the family of retinoids. Taken in the form of precursors by the human body, retinol and its derivatives play a crucial role in the reproductive process, immune response, bone growth, epithelial growth and differentiation, and the metabolic function of the retina.

Tyrosine, Indoleacrylic acid and LysoPC(16:0) were the main low-molecular metabolites that caused the difference between pulmonary metastatic carcinoma and benign pulmonary nodules. The areas under the ROC curve of these metabolites were 0.852, 0.869 and 0.836, respectively, showing a good ability of differential diagnosis. Tyrosine is an essential amino acid that can cross the blood-brain barrier and a precursor of the neurotransmitters such as dopamine, norepinephrine, and epinephrine. This study found that the level of tyrosine in the plasma of patients with pulmonary metastatic carcinoma was approximately 1.7 times that of pulmonary benign nodule. Its sensitivity and specificity were 0.938 and 0.719 respectively. Klupczynska et al[25] found that the concentration of tyrosine in the plasma of early non-small cell lung cancer was significantly different from that of normal people, but this could not distinguish squamous cell carcinoma and adenocarcinoma. Jian-Ming Hu et al.[34] conducted a metabolomics study of patients with advanced non-small cell lung cancer who underwent microwave ablation, and found that the plasma tyrosine content before treatment was significantly higher than that in the healthy control group, and after microwave ablation treatment, the level of tyrosine had dropped significantly.

Lysophosphatidylcholine (lysoPC or LPC) is present in a small amount in most tissues and is formed by the hydrolysis of phosphatidylcholine by the phospholipase A2. In this study, the plasma level of lysoPC (16:0) in patients with pulmonary metastatic carcinoma was significantly higher than that of benign pulmonary nodule, and its sensitivity and specificity were 0.938 and 0.687, showing its potential to the biomarker to distinguish the two. Zhiyi Yang et al.[35] reported a metabolomic research on malignant pleural effusion of advanced lung cancer, and found that 25 ether lipids, including phosphatidylcholine (PC), lysophosphatidylcholine (LPC) and phosphatidylthanolamine (PE), were significantly down-regulated in malignant pleural fluid, showing a good diagnostic potential. Ros-Mazurczyk et al.[36] found that the levels of lysoPC(18:2), lysoPC(18:1), and lysoPC(18:0) in patients with early-stage lung cancer were lower than those in healthy people.

A large number of nucleotides are needed as raw materials during the proliferation of malignant tumors. A class of synthetic antinucleotide drugs, including 5-fluorouracil and 6-mercaptopurine, have been effectively used as tumor chemotherapy drugs, indicating that nucleotide metabolism plays a crucial role in tumors. In our study, we found that the plasma concentration of CMP-N-glycoloylneuraminate in patients with primary lung cancer was significantly higher than that in healthy people, suggesting vigorous nucleotide metabolism. Yang Wang et al.[37] found that acetaldehyde dehydrogenase was overexpressed in lung adenocarcinoma cells, and the levels of nucleoside metabolites such as cytidine monophosphate (CMP), uridine monophosphate (UMP), adenosine monophosphate (AMP) and guanosine monophosphate (GMP) were increased, suggesting that enhanced nucleotide metabolism was beneficial to the survival and proliferation of tumor cells. Nucleotide metabolism may be the most relevant pathway for low molecular metabolism in lung cancer cells[38]. It is well known that the purine and pyrimidine nucleotide biosynthesis both require

a carbon unit, and purine and pyrimidine nucleotide biosynthesis are necessary for DNA and RNA synthesis. In order to ensure the proliferation of cancer cells, DNA and RNA biosynthesis required a large increase in nucleotides, so nucleotide supply is one of the key reasons why single-carbon metabolism is important for cancer cell survival.

In this study, different pathological types of primary lung cancer were compared, and it was found that there was no significant difference in the level of low-molecular metabolites in the plasma samples of each group. Swee Ling Lim et al.[13] reported that metabolic characteristics of four different pathological types (squamous cell carcinoma, adenocarcinoma, small cell lung cancer and large cell lung cancer) were significantly different. But unlike our study, the samples in their research were not plasma, but cancerous cell lines. This may be the main reason for the different results from our study, because differences in the levels of low-molecular metabolites in cancer cells are generally more pronounced than differences in plasma[39]. Tao Wen et al.[40] found significant differences in plasma levels of some low-molecular metabolites between early adenocarcinoma and healthy people, but the authors did not compare adenocarcinoma with other pathological types of lung cancer.

Different pathological stages of primary lung cancer were compared In this study, and we found that there was no significant difference in the levels of low-molecular metabolites in different lung cancer stages. Stanislaw Deja et al. [41] found significant differences in plasma low-molecular metabolic profiles between early primary lung cancer (stage I and II) and advanced lung cancer (stage III and IV). Plasma metabolites such as isoleucine, acetoacetic acid, lactic acid, creatinine, acetone, valine and isobutyric acid were decreased in advanced lung cancer. This suggests that with the progression of lung cancer, some metabolic processes in the body will change. Our study compared five groups of lung cancers in different stages without further merging them for further study, which may be one of the reasons why the results were different from this previous study.

5. Conclusion

By the metabolomics method, we comprehensively studied the changes in plasma levels of low-molecular metabolites from pulmonary metastatic carcinoma, benign pulmonary nodule, primary lung cancer, and healthy population. In particular, pulmonary metastatic carcinoma were included for the first time. It was found that the levels of some low-molecular metabolites were significantly different in the comparisons among the four groups. These major metabolites showed a good diagnostic ability with high sensitivity and specificity. They have the potential to become the biomarkers for the screening and differential diagnosis of lung cancer. This study laid a foundation for further research and clinical translation.

However, there are still some shortcomings in this study: First, the sample size of each group is small. Because metabolomics is the terminal stage of various biological changes, it is susceptible to various factors. The larger the sample size, the more reliable the results will be. Second, this research applies non-targeted metabolomics, so the low-molecular metabolites discovered can be further studied and identified by targeted metabolomics. Finally, although this study is based on clinical needs, the current research results are more at the stage of basic research, and it is necessary to find a suitable way for clinical transformation to have greater significance.

Declarations

Conflicts of Interest

Authors acknowledge no conflict of interest.

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Author Contribution

Yushun Gao and Zhili Li conceived and planned the experiments. Zixu Liu contributed to sample collection and wrote the manuscript. Lei Guo and Shuai Liu carried out the experiments. Zixu Liu and Lei Guo contributed to the interpretation of results. Minjun Du verified the analytical methods. Yicheng Liang supervised the findings of this work. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Ethics Declarations

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standard.

Availability of Data and Material

The metabolomics and metadata reported in this paper are available via MetaboLights (www.ebi.ac.uk/metabolights/MTBLS1581) study identifier MTBLS1581.

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Tables

Table 1 The Main Metabolites in the Comparisons of the Three Pulmonary Nodule Groups with the Healthy Group and the Three Pulmonary Nodule Groups In Pairs

	Metabolites	Fold Change	VIP	*P Value*	AUC of ROC	Critical Point	Sensitivity	Specificity
PMC vs HPG	O-Arachidonoyl ethanolamine	8.12	1.62	1.14E-7	0.945	1.30	0.875	1.000
	Adrenoyl ethanolamide	7.56	1.54	2.86E-7	0.931	43.77	0.813	1.000
	Tricin 7-diglucuronoside	5.45	1.46	4.82E-8	0.958	5.64	0.813	1.000
	p-Coumaroyl vitisin A	6.03	1.51	3.39E-8	0.964	2.13	0.875	0.979
BPN vs HPG	Glutamylphenylalanine	13.93	1.89	9.34E-13	0.973	2.09	0.875	0.958
	Nitroso-glutathione	43.14	2.18	4.39E-12	0.959	0.34	0.906	0.958
	Tricin 7-diglucuronoside	4.56	1.48	6.07E-13	0.977	2.24	0.969	0.896
	p-Coumaroyl vitisin A	4.98	1.55	7.53E-13	0.975	1.52	0.906	0.938
PLC vs HPG	Anabasine	32.42	2.25	1.23E-19	0.980	13.29	0.950	1.000
	1-Salicylate glucuronide	252.49	2.66	2.89E-17	0.947	12.01	0.850	1.000
	Nitroso-glutathione	89.30	2.36	2.34E-17	0.948	4.53	0.850	1.000
	Dihydrocaffeic acid 3-O-glucuronide	28.59	2.04	1.01E-16	0.939	2.37	0.850	1.000
	CMP-N-glycoloylneuraminate	15.08	1.93	7.16E-20	0.983	1.28	0.900	1.000
	Meloside L	8.91	1.72	5.45E-20	0.984	0.50	0.950	0.979
BPN vs PLC	Octanoylcarnitine	0.27	2.08	5.33E-15	0.974	6.85	0.875	1.000
	Decanoylcarnitine	0.19	2.52	1.71E-14	0.965	3.50	0.850	0.969
	PGF2a ethanolamide	0.48	1.33	3.39E-10	0.881	6.89	0.900	0.913
PMC vs BPN	Tyrosine	1.67	1.39	8.26E-5	0.852	1.71	0.938	0.719
	Indoleacrylic acid	1.76	1.40	3.57E-5	0.869	3.89	0.938	0.844
	LysoPC(16:0)	2.43	1.95	1.68E-4	0.836	0.53	0.938	0.687
PMC vs PLC	Octanoylcarnitine	0.25	1.65	2.71E-9	0.973	6.51	0.888	0.938
	Retinol	0.08	2.25	8.53E-10	0.988	0.99	1.000	0.938
	Decanoylcarnitine	0.21	1.81	9.39E-	0.956	3.01	0.875	1.000

Figures

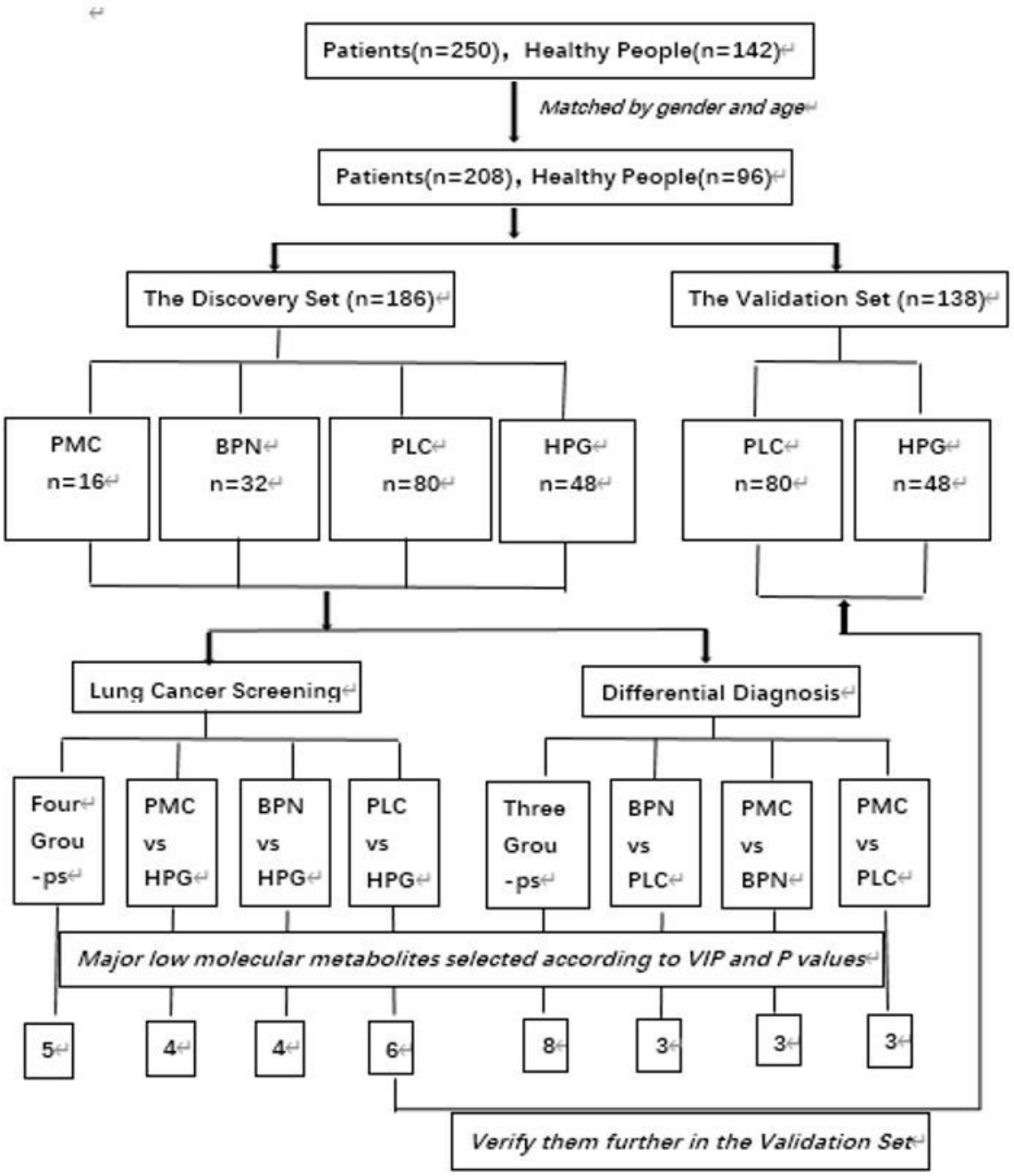

Figure 1

Flow chart of the study

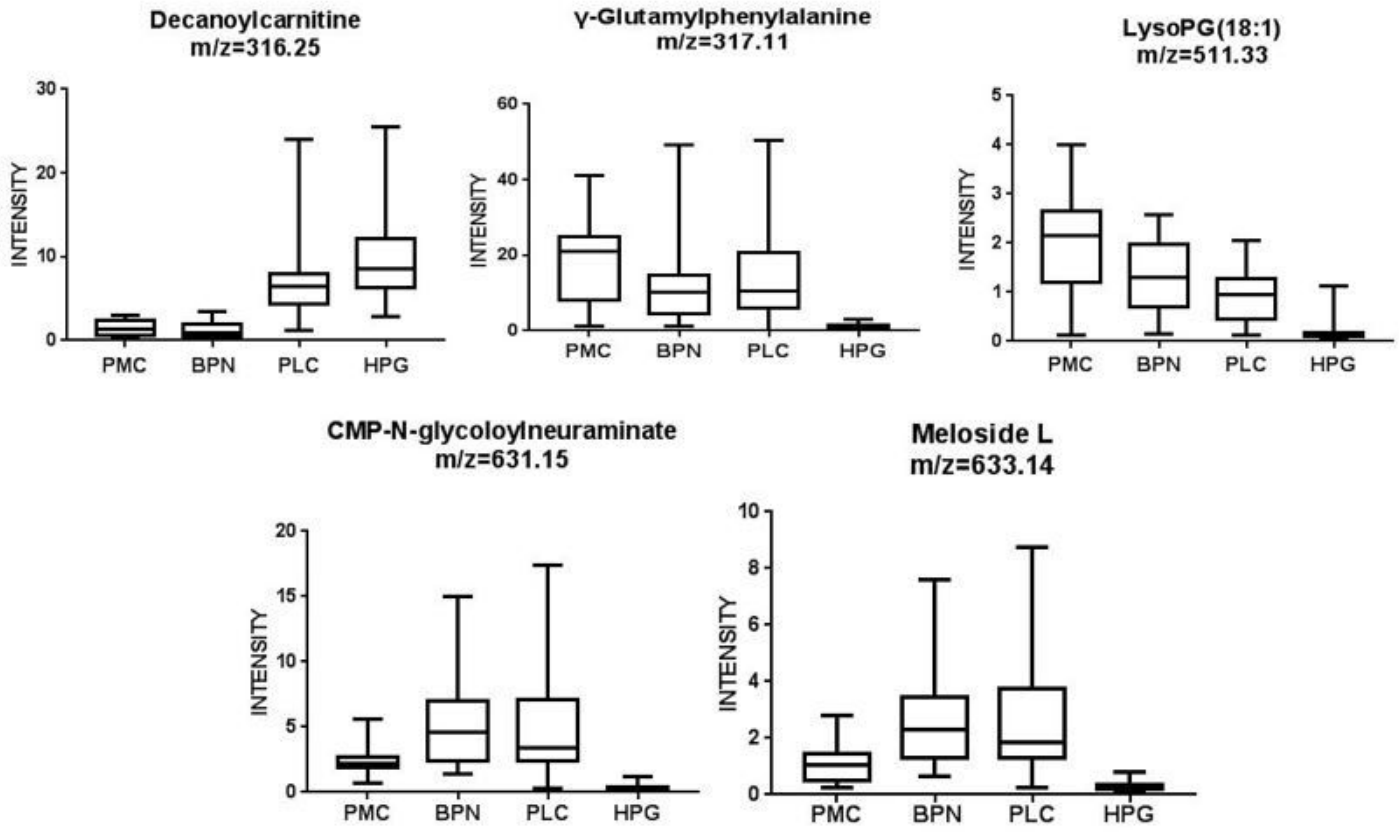

Figure 2

After the overall comparison of the four groups, five low-molecular metabolites were selected, namely decanoylcarnitine, γ-glutamylphenylalanine, lysophosphatidyl glycerol (18:1)、CMP-N-glycoloylneuraminate and meloside L. The intensity values of these five metabolites in each group can be intuitively reflected by box plots.

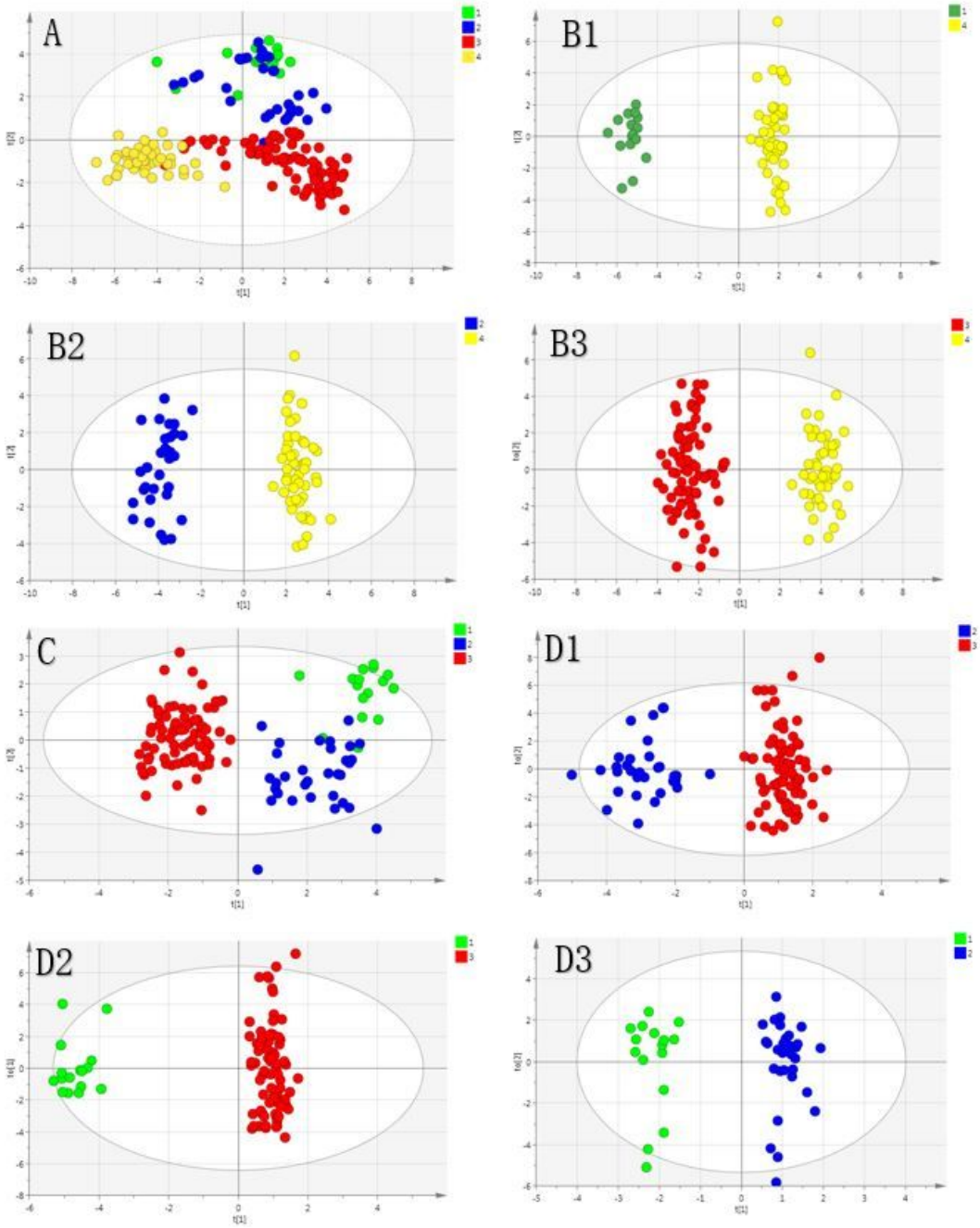

Figure 3

Score scatter plots by OPLS-DA analysis are shown. The number 1, 2, 3, and 4 in the figures represent PMC, BPN, PLC, and HPG, respectively. It can be seen that there is obvious difference in the distribution among the four groups. (A) Overall comparison of the four groups (B) Comparison of the healthy group with the three pulmonary nodule groups respectively (C) Overall comparison of the three pulmonary nodule groups (D) Pairwise comparisons of the three pulmonary nodule groups

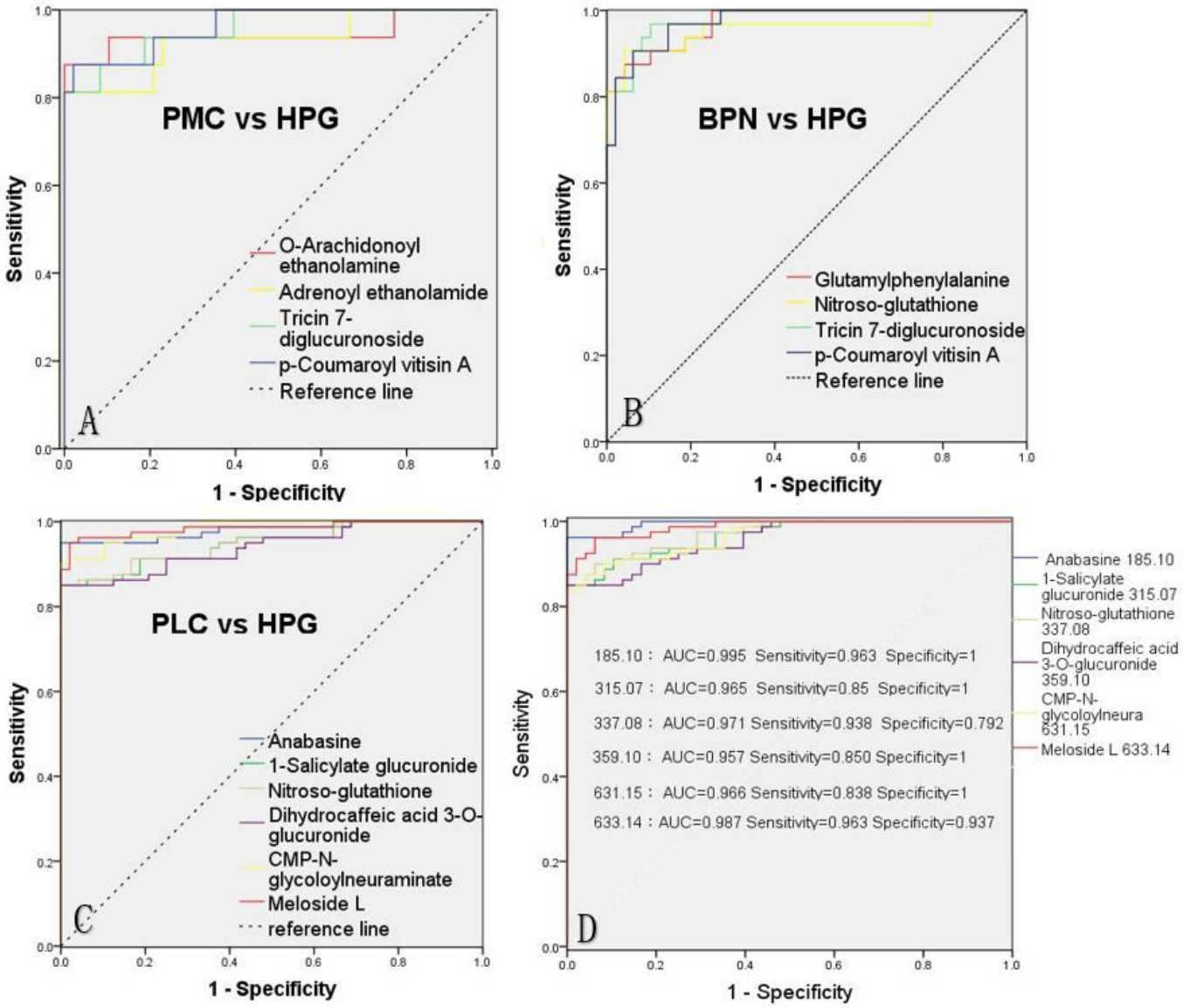

Figure 4

(A,B,C) ROC curves of the major low-molecular metabolites in the respective comparison between three pulmonary nodule groups and healthy people in the discovery set (D) ROC curves and relevant information of six low-molecular metabolites between PLC and HPG in the validation set

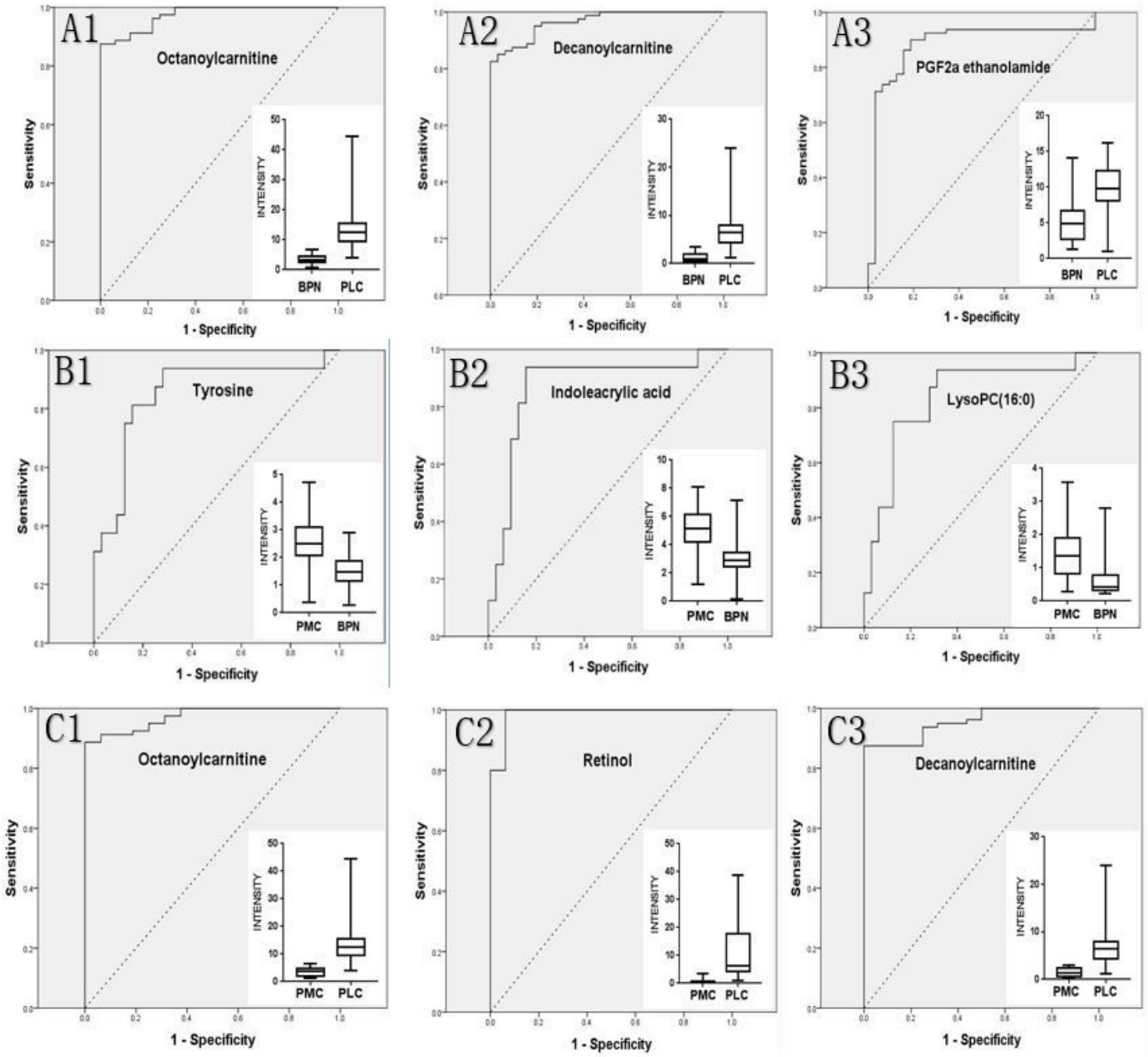

Figure 5

ROC curves and box plots of the major low-molecular metabolites in the comparison of BPN and PLC (A), PMC and BPN (B), PMC and PLC (C) for differential diagnosis of the pulmonary nodules.

Supplementary Files

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