약학석사학위논문

# HPLC-QTOF-MS와 GC-MS를 이용한 수혈 대사체학 분석

## HPLC-QTOF-MS and GC-MS based metabolomics on blood transfusion study

2015년 8월

서울대학교 대학원

약학과 약품분석학전공

Wang, Haiping

# HPLC-QTOF-MS와 GC-MS를 이용한 수혈 대사체학 분석

# HPLC-QTOF-MS and GC-MS based metabolomics on blood transfusion study

지도교수 권성원

이 논문을 약학석사학위논문으로 제출함

2015년 5월

서울대학교 대학원
약학과 약품분석학전공
Wang, Haiping

Wang, Haiping의 약학석사학위논문을 인준함

2015년 6월

위 원 장 박 정 일 (인)
부 위 원 장 송 준 명 (인)
위 원 권 성 원 (인)

# Abstract

수혈은 대부분의 임상적 상황에서 유익하다. 수혈은 일시적으로 환자의 신체 상태를 호전시켜주지만, 면역 및 비면역 시스템 전반적으로 영향을 미친다. 최근 perioperative 동종 이형 수혈이 면역 기능 저하와 연관되어 있으며, 이러한 것이 '치유적'인 암 절제술 이후의 재발률 증가를 일으킨다는 자료가 증가하였다. 그럼에도 불구하고, 지금까지 수혈의 잠재적인 위험성에 대한 근거를 나타내는 전체적인 대사체 변화에 대해 잘 알려지지 않았다.분자량이 작은 대사체들의 전체적인 분석에 중점을 주는 방법으로서, 대사체학은 생물계에서 biomarker들을 찾는 매우 효과적인 접근법이다. 본 연구에서는 처음으로 rat model을 이용하여 동종 이형 수혈을 통한 대사체 변화에 대해 대사체학을 기반으로 하여 조사하였다. 또한 수술 중 동종 이형 수혈을 받은 암환자에 대한 잠재적인 부정적 영향력을 이러한 biomarker들과 연관하여 조사하였다. 수혈 후 "대사체" 를 profile하기 위해, liquid chromatography-quadrupole time-of-flight high definition mass spectrometry와 gas chromatography mass spectrometry를 이용하였다. Performing principal component analysis (PCA)와 partial least squares-discriminant analysis (PLS-DA)를 이용하여 동종 이형 수혈 집단에서 총 12개의 대사체들이 자가 수혈 집단에 비하여 유의한 차이가 있음을 확인하였다. 본 연구 결과 두 집단이 명확하게 차이가 나며, 대사체 변화는 암의 재발과 연관성이 있을 가능성이 있음을 알 수 있었다. 또한 수혈에 대해 더 명확히 이해할 수 있었으며, 수혈이 암 수술 후 재발에 중요한 영향을 미칠 수 있다는 것을 알 수 있었다.

주요어: 동종 이형 수혈; HPLC-QTOF-MS; GC-MS; 대사체학

학번: 2013-23936

# Contents

# List of Figures

# List of Tables

# Ⅰ. Introduction

Blood transfusion is a supportive and compensatory therapeutic treatment which transfers the blood products from one person to another person intravenously. Blood transfusion therapy was usually conducted between the individuals with the same blood type, which is so called "allergenic blood transfusion". The blood was usually examined through infectious diseases test before transfusion, however there is no absolute safety in blood transfusion therapy because infections and complications may happen in any phase of treatment. Even though blood transfusion is a life-saving therapy in some circumstances, there still exist significant risks, including allergic reactions, incompatibility, transmission of infectious agents, and coagulopathy.

In evaluating clinical studies relating blood transfusion, especially allergenic blood transfusion to outcomes, it is worth being aware that the circumstances under which patients are given blood products perioperatively are likely to influence the cancer occurrence or recurrence. Nevertheless, the potential impacts of these important confounding factors on cancer recurrence are complicated and difficult to understand from previous research. Besides, most of the relative investigation results to date are from clinical research, and little is well known in mechanism study of the impacts of the blood transfusion on cancer occurrence.

Metabolomics provides a qualitative understanding of the metabolic components of integrated living organisms and its dynamic responses to the changes of both endogenous factors and

exogenous factors. Therefore, by profiling the metabolic variation, metabolomic technique may reveal the changing circumstances after blood transfusion.

The purpose of this study is to investigate the metabolic changing between autologous blood transfusion and allergenic blood transfusion based on rat model. Besides, to profile comprehensive metabolites, both gas chromatography combined with mass spectrometry (GC-MS) and high performance liquid chromatography-quadrupole-time-of-flight combined with mass spectrometry (LC-QTOF-MS) were used to achieve the sample determination. On the basis of multivariate statistical analysis, the impacts of allergenic blood transfusion were discussed. Moreover, the correlation of the identified biomarkers was also studied to investigate the possible reaction mechanism from a new perspective.

# Ⅱ. Experiment

## 1. Experiment Animals

Six male Lewis rats and six Sprague Dawley (SD) rats were blood donors and other twelve male Lewis rats were blood recipients. All rats weighed from 160 g to 180 g and were housed in cages for two-week observation before study. The experimental animals in this study were approved and reviewed with the Approval ID: INHA 140321-283

## 2. Chemicals and Materials

### 2-1. Chemicals

Chemicals used in GC-MS analysis

- Chloroform (Avantor Performance Materials, USA)
- Methanol (Avantor Performance Materials, USA)
- Water (Avantor Performance Materials, USA)
- Methoxyamine Hydrochloride (Sigma Aldrich, St. Louis, USA)
- N,O-Bis (trimethylsilyl) trifluoroacetamide with trimethylchlorosilane (1% TMCS)( Sigma Aldrich, St. Louis, USA)
- Pyridine (Sigma Aldrich, St. Louis, USA)

Chemicals used in LC-QTOF-MS analysis

- Methanol (Avantor Performance Materials, USA)
- Water (Avantor Performance Materials, USA)
- Formic acid (Sigma Aldrich, St. Louis, Mo, USA)

Standards used for identification

- Glucose (Sigma Aldrich, St. Louis, Mo, USA)
- Lactic acid (Sigma Aldrich, St. Louis, Mo, USA)
- Phosphoric acid (Sigma Aldrich, St. Louis, Mo, USA)
- L-alanine (Sigma Aldrich, St. Louis, Mo, USA)
- L-glutamic acid (Sigma Aldrich, St. Louis, Mo, USA)
- Lysophosphatidylcholine (LysoPC (17:0)) (Sigma Aldrich, St. Louis, Mo, USA)

## 2-2. Experimental Supplies

- Adjust pipette (0.5~10 μL, 20~200 μL, 100~1000 μL, Eppendorf AG, Hamburg, Germany)
- Centrifuge (Eppendorf AG, Hamburg, Germany)
- Centrifuge tube (2mL, Eppendorf AG, Hamburg, Germany)
- Conical tube (15mL, SPL Life Sciences Co. Ltd)
- Liquid nitrogen
- Nitrogen purge (EYELA MG-2200)
- Pipette tips (0.5~10 μL, 20~200 μL, 100~1000 μL, Eppendorf AG, Hamburg, Germany)
- Vacuum pump (ULVAC DTC-21)
- Vacuum manifold
- Vortex Mixer (Vortex Genie 2)

## 2-3. Analytical Instruments

- GC-MS QP2010 (SHIMADZU, Germany)

  Column: DB-5MS (30 m × 0.25 mm, 0.25 $\mu$m, Agilent, USA)

- HPLC (Agilent)-QTOF-MS (6510 ESI-QTOF-MS, MAXIS, Bruker)

  Column: ODS (100 × 2.1 mm, 1.7$\mu$m)

# 3. Experimental Method

## 3-1. Animal Experiment

In this study, eight male Lewis rats and eight Sprague Dawley (SD) rats weighing from 160 g to 180 g were used as blood donors, while other sixteen Lewis rats weighing from 160 g to 180 g were used as blood recipients. In test group, blood was obtained from the blood donor SD rats, and then 1 mL of the blood was transferred into the blood recipients Lewis rats via tail vein. Seven days after blood transfusion, the Lewis rats (blood recipients) were sacrificed and 1 mL blood sample was collected by vena cava puncture. The sampled blood was kept in heparin tube for continuous determination with analytical instruments. Alternatively, in the control group, the whole process of animal experiment was the same with the test group except for the blood donors, which were change to Lewis rats (Figure 1).

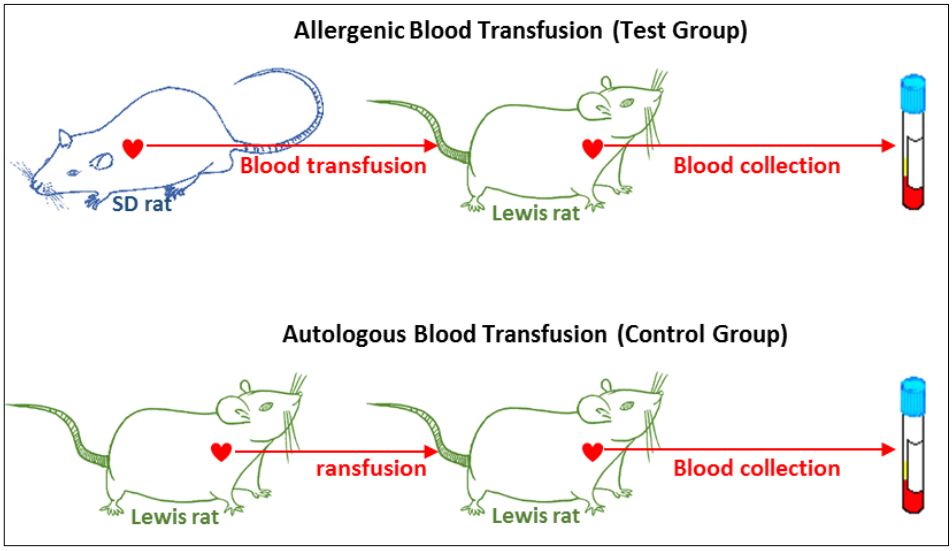


Figure 1. The process of blood transfusion based on rat model

## 3-2. Plasma Treatment

***GC-MS.*** Figure 2 shows the plasma treatment method for GC-MS. 250 μL of solvent (v:v:v, 2.5:1:1, methanol, water and chloroform) was added to each 100 μL aliquots of plasma, followed by vortex for 1 min. After keeping at 60 ℃ for 30 min, the sample was centrifuged at 16,000 rpm under 4 ℃ for 5 min. 250 μL of supernatant was transferred to a centrifuge tube and evaporated with nitrogen at room temperature. The residue was oximated with 40 μL methoxyamine hydrochloride (20 mg/mL in pyridine) and then kept at 60 ℃ for 60 min, followed by adding 20 μL of BSTFA-TMCS. The mixture was then kept at 60 ℃ for another 45 min and filtered at a centrifugation of 16,000 rpm under 20 ℃ for 10 min. The supernatant was collected for injection.

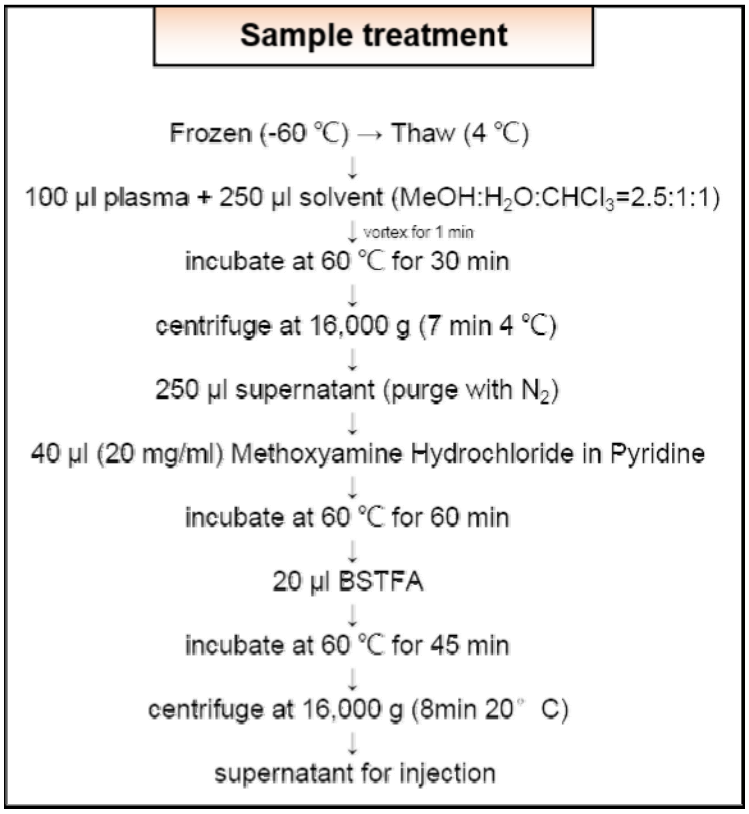


Figure 2. The plasma handling method for GC-MS detection.

***LC-QTOF-MS.*** Figure 3 shows the plasma treatment method for LC-QTOF-MS. Protein was precipitated with 25% acetonitrile, followed by vortexing for 2 min, and centrifuged with 16,000 rpm at 4℃ for 20 min. The supernatant was dried by purging with nitrogen. For analysis, samples were reconstituted in 100 μL acetonitrile/water (4:1) solvent. Mixture of test and control group pooled sample in 1:1 (v:v) was used as quality control (QC) samples.

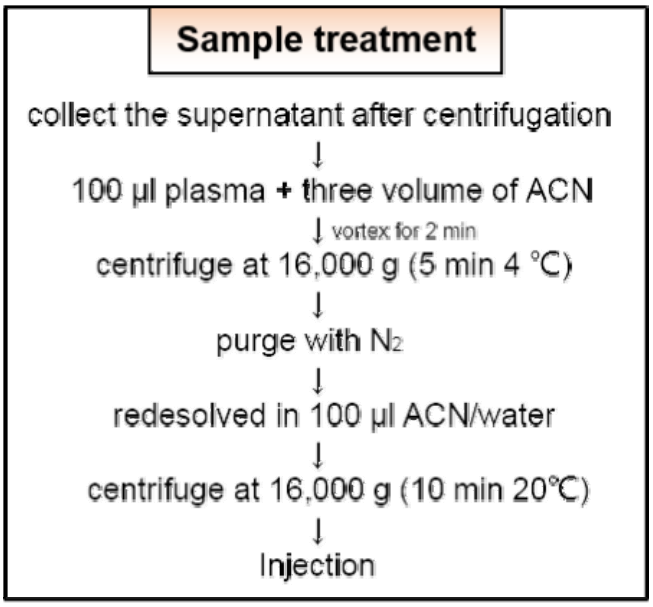


Figure 3. The plasma handling method for LC-QTOF-MS detection.

## 3-3. Instrumental Analysis

***GC-MS.*** GC-MS analysis was performed by using the GCMS-QP2010 system (Table 1). Chromatographic separation was performed with a DB-5MS column (30 m×0.25 mm, 0.25 μm). The GC oven temperature was firstly held at 100 ℃ for 5 min, increased to 180 ℃ at the rate of 5 ℃ /min, then increased to 300 ℃ at the rate of 5 ℃ /min and held at the final temperature for 5 min. A 1 μL sample was injected in split mode and helium (99.9999% He) was used as a carrier gas at a flow of 1 mL/min. The injection temperature and ion source temperatures were set at 300 ℃ and 200 ℃, respectively. With a 3 min solvent delay, mass spectra were obtained at 5,000 scans per second at a mass range of m/z 30-880. The ionization energy was 70 eV in electron impact mode. The standards were injected for the identification.

Table 1. GC-MS analysis parameters.

| | |
|---|---|
| **Instrument** | GCMS-QP2010 (Shimadzu, Germany) |
| **Column** | DB-5MS (0.25 mm × 30 m, 0.25 μm) |
| **GC parameter** | Column oven temperature: 100 ℃<br>Injection temperature: 300 ℃<br>Injection mode: Split<br>Pressure: 72.9 Kpa<br>Column flow: 1.00 mL/min<br>Gradient: start with 100 ℃ (2 min)<br>increase the temp. to 180 ℃ with the rate of 5 ℃/min<br>increase the temp. to 300 ℃ with the rate of 5 ℃/min and hold 5 min |
| **MS parameter** | Injection volume: 1 μL<br>Ion source temperature: 200 ℃<br>Interface temperature: 300 ℃<br>Ionization mode: 70 eV<br>Mass range: 30 – 600 m/z |

***LC-QTOF-MS.*** HPLC-Q-TOF-MS analysis was performed on a High Performance Liquid Chromatography system equipped with a ODS column (100×2.1 mm, 1.7 μm) and coupled to an electron spray ionization quadrupole TOF-mass spectrometer (6510 ESI-Q-TOF-MS). The column temperature was 50℃ with a flow rate of 0.35 mL/min. In first 26 min, a linear gradient changing from 98% A (0.1% formic acid in water) to 100% B (acetonitrile), then the final condition was held for 20 min. The mass spectrometer was operated in ESI positive ionization mode, with ultrahigh-purity nitrogen as nebulizer gas and drying gas (8.0 L/min). Scan mode was applied and the scan mass range is from 50 to 1000 m/z. For the verification of data reliability, the quality control (QC) sample was injected at regular intervals during sequence running. Then the data-dependent MS/MS was performed by a collision energy ramp from 10 eV to 50 eV. The standard LysoPC (17:0) was chosen to confirm the identification result. The whole process was performed using chromatography software (Bruker Daltonics, Germany).

Table 2. HPLC-Q-TOF-MS analysis parameters.

| | |
|---|---|
| **Instrument** | ACQUITY HPLC (Waters, USA) |
| **Mass spectrometry** | micrO-TOF QII (Bruker, Germany) |
| **Column** | ACQUITY HPLC BEH C18<br>(2.1 mm × 100 mm, 1.7 μm, 50 ℃) |
| **Flow rate** | 0.35 mL/min |
| **LC parameter** | Solvent : (A) Water + 0.1% formic acid<br>(B) Acetonitrile<br>Gradient : started with 98% A to 100% B (in 26 min)<br>100% B (25~30 min) |
| **MS parameter** | Injection volume: 5 μL<br>Dry gas: 8.0 L/min<br>Transfer time: 100 μs<br>Mass range: 30 – 1500 m/z<br>Nebulizer: 1.2 bar<br>Dry temp.: 200 ℃<br>Pre puls storage: 5 μs |

## 3-4. Data Processing

After raw data acquisition, the alignment was accomplished by using MZmine 2.10. The alignment process and parameters for GC-MS and LC-QTOF-MS are shown in Table 3. The multivariate statistical analysis was then carried out via SPSS and Simca-P+ with the aligned data. Partial least-squares discriminant analysis (PLS-DA) was used as the classification method for discrimination. The variable importance in the projection score (VIP score>1) shows the most important variables over the mode. T-test was conducted individually, setting the p-value less than 0.05 as the level of statistical significance. Finally, two lists of biomarker candidates for GC-MS and LC-QTOF-MS were obtained, respectively. The workflow of data processing is shown in the Figure 4.

Table 3. The alignment process and parameters for GC-MS and LC-QTOF-MS.

| | GC-MS | LC-QTOF-MS |
|---|---|---|
| **Conversion** | Noise level: $7.0\times10^4$ | Noise level: $5.0\times10^4$ |
| **Chromatogram construction** | Minimum height: $7.0 \times 10^4$<br>m/z tolerance: 0.02 | Minimum height: $5.0 \times 10^4$<br>m/z tolerance: 0.01 |
| **Peak recognition** | Minimum peak height: $7.0 \times 10^4$<br>Derivative threshold level: 80% | Minimum peak height: $5.0 \times 10^4$<br>Derivative threshold level: 80% |
| **Peak alignment** | m/z tolerance at 0.02<br>Retention time tolerance: 0.2 min<br>RANSAC iterations: 30000 | m/z tolerance at 0.01<br>Retention time tolerance: 0.1 min<br>RANSAC iterations: 30000 |
| **Peak gap filling** | m/z tolerance: 0.02 | m/z tolerance: 0.01 |

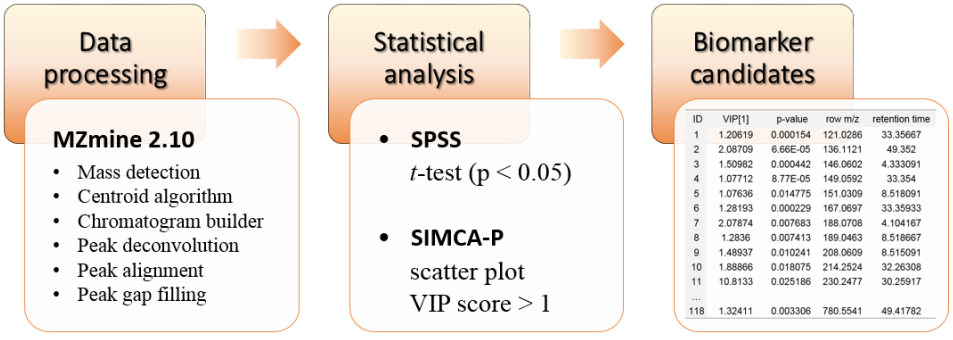


Figure 4. The workflow of data processing.

### 3-5. Biomarker Identification

With LC-QTOF-MS data, based on the accurate mass information of biomarker candidates, the Human Metabolome Database (HMDB), METLIN, Lipid Maps, and, PubChem Compound were searched, with a mass accuracy tolerance of 5 ppm. MS/MS spectra contributed to discriminate between database hits, which emphasized neutral losses and product ions. While for GC-MS data, comparison was conducted with GC-MS internal spectral library firstly.

The standards were finally determined to confirm the identification results by a comparison of their mass spectra and chromatographic retention time with that of plasma samples.

# Ⅲ. Results

## 1. Animal Observation

Seven days after blood transfusion, the rats were all in good conditions and no abnormal situation was found. No hemolysis phenomenon occurred in the process of sample collection.

## 2. Total Ion Chromatogram

To compare the chromatogram of each group, the overlaid total ion chromatograms of LC-QTOF-MS and GC-MS are shown in Figure 5 and Figure 6. The test group and control group represent allergenic blood transfusion group and autologous blood transfusion group, respectively.

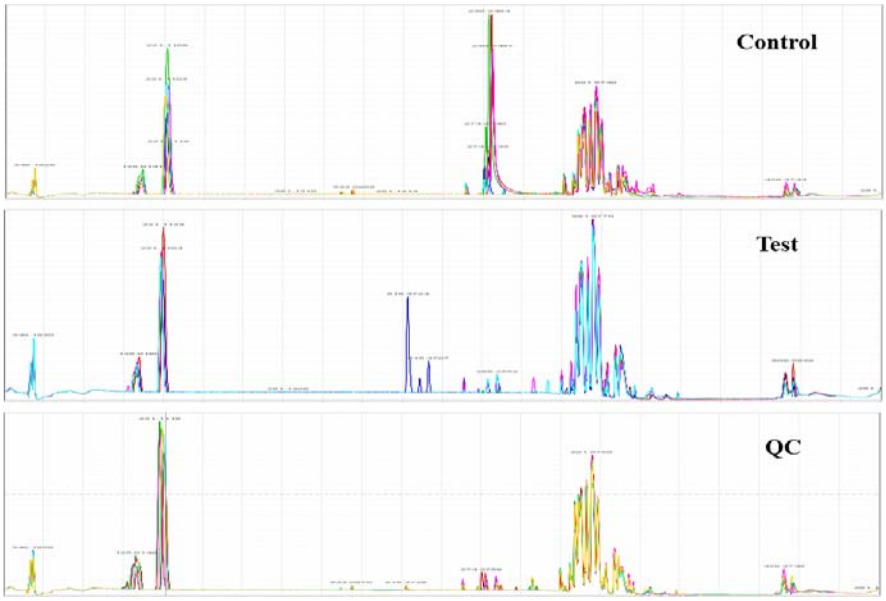


Figure 5. Overlaid total ion chromatograms of LC-QTOF-MS.

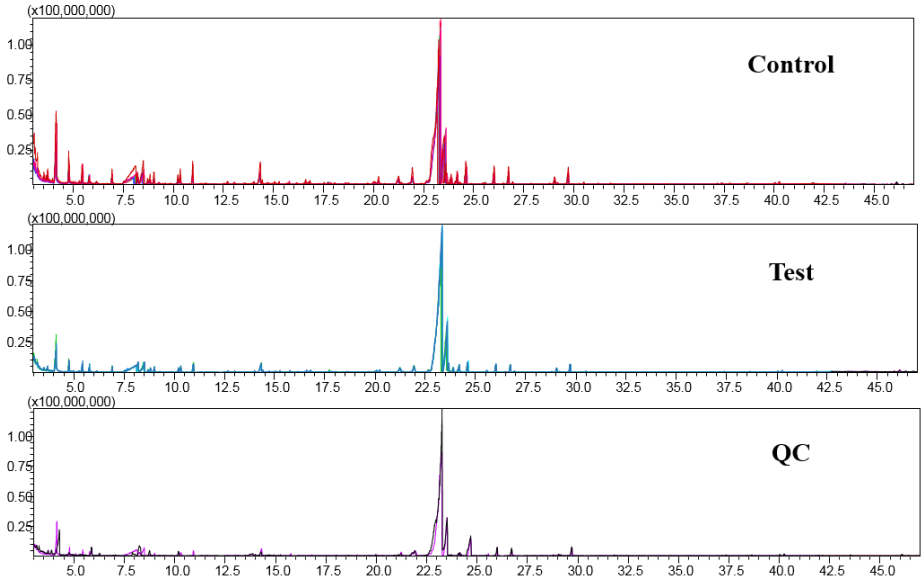


Figure 6. Overlaid total ion chromatograms of GC-MS.

# 3. Data Processing results

## 3-1. PLS-DA Score Plots and Scatter Diagrams

Supervised PLS-DA classification model using one PLS component and one orthogonal component was performed based on nontargeted metabolite profile data derived from allogeneic (test) and autologous (control) blood transfusion with GC-MS (Figure 7) and LC-QTOF-MS (Figure 8) data. The PLS-DA score plots show the significant discrimination between the two groups.

The scatter diagram (Figure 9 and Figure 10) show the significant variables between the two groups. According to VIP score, the dots with red circle represent the important biomarker candidates which may play critical roles in discrimination of the two groups.

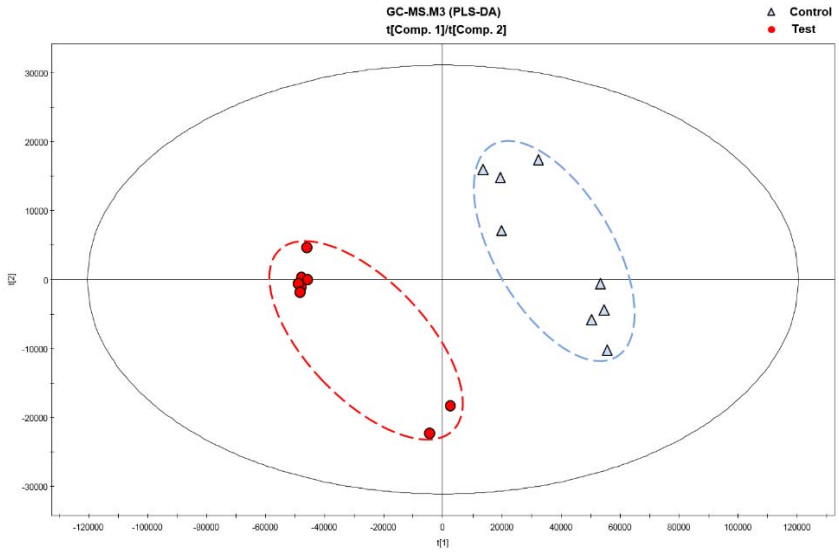


Figure 7. The PLS-DA score plot of GC-MS.

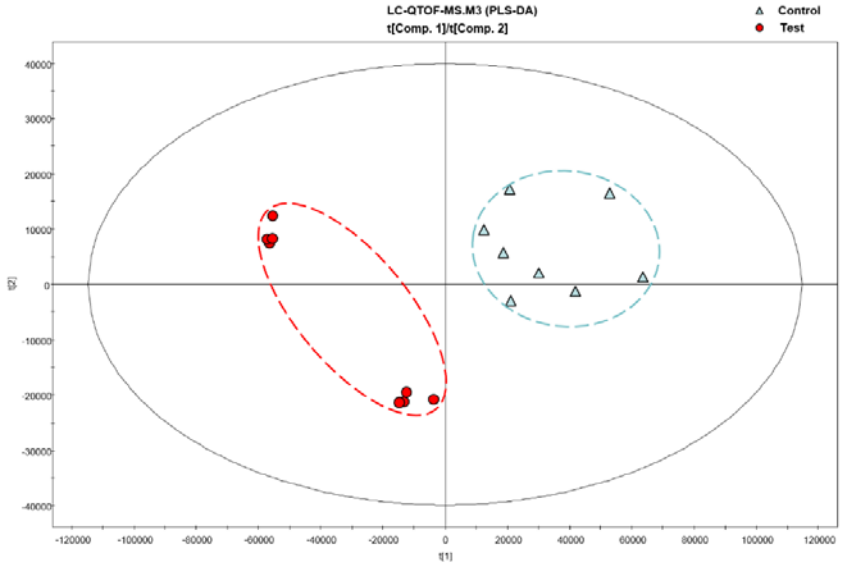


Figure 8. The PLS-DA score plot of LC-QTOF-MS.

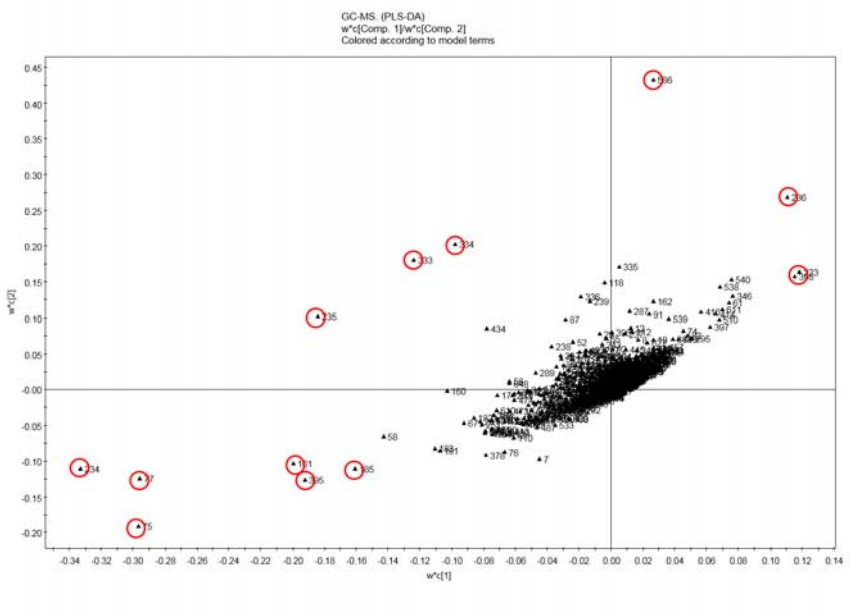


Figure 9. The scatter diagram of GC-MS.

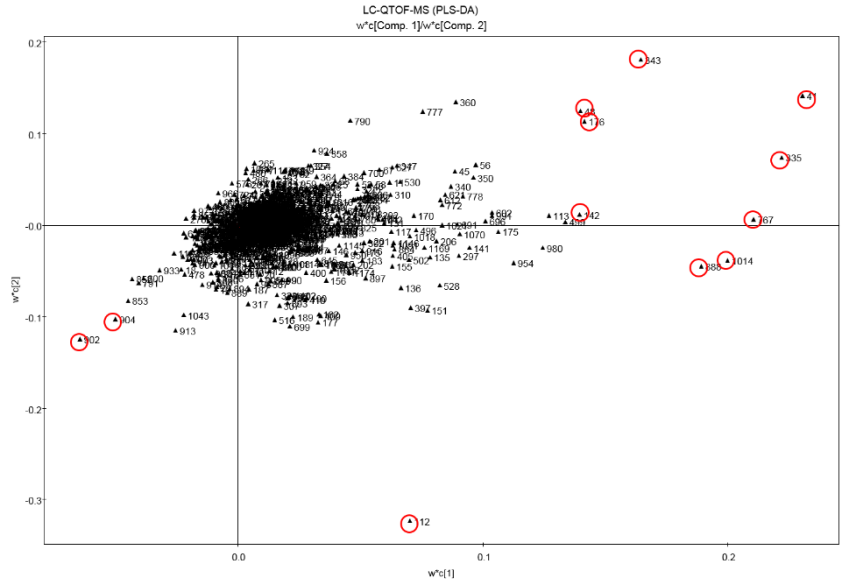


Figure 10. The scatter diagram of LC-QTOF-MS.

## 3-2. Biomarker Mining

The mass data and spectra of biomarker candidates were then compared with the library and databases online. Also standards were determined for verification of the identification results. Figure 11 shows the GC-MS identification process of *L*-Alanine with comparison of standard and sample on their chromatogram and mass spectra. The characteristic fragments of LysoPC (16:1) detected from LC-QTOF-MS is displayed in Figure 12.

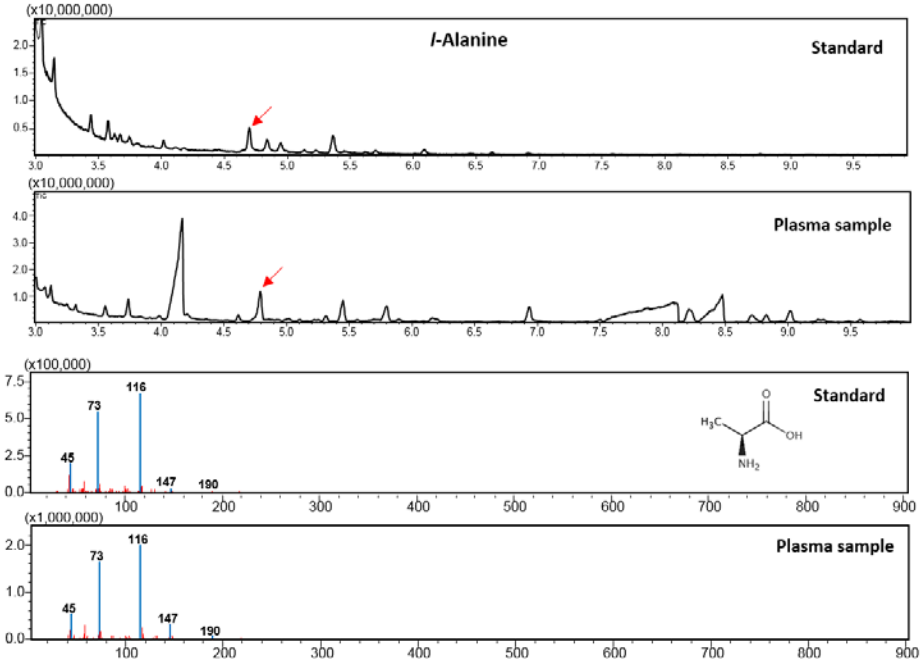


Figure 11. Analysis of L-Alanine by GC-MS with its standard.

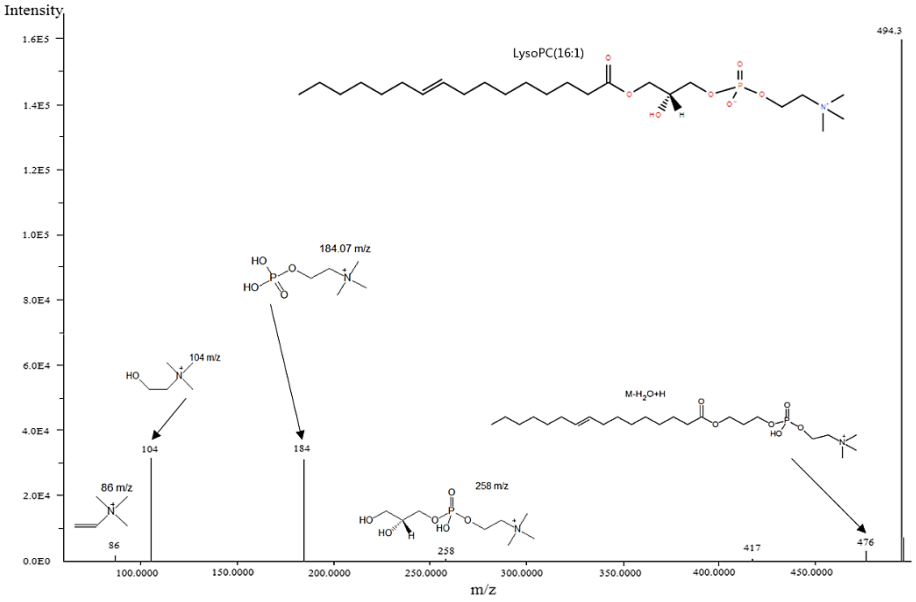


Figure 12. HPLC-Q-TOF-MS/MS spectrum of LysoPC (16:1) in plasma sample with characteristic ion m/z 184.

Finally, fourteen biomarkers for discrimination of allergenic transfusion group and autologous transfusion group were identified with standards and libraries (Table 4). Additional important metabolites were also listed in Table 5 with p-value less than 0.05. These metabolites were identified by comparing with the GC-MS internal library and database online.

Table 4. Identification results of plasma metabolites from rats between test and control group.

| | Biomarkers | VIP value | Retention time | p-value | Change direction |
|---|---|---|---|---|---|
| GC-MS | Glucose[a] | 12.93 | 23.19 | 0.0003 | Up |
| | Lactic acid[a] | 3.71 | 4.14 | 0.035 | Down |
| | L-Alanine[a] | 3.52 | 4.77 | 0.043 | Down |
| | Urea[b] | 2.12 | 8.01 | 0.018 | Up |
| | Phosphoric acid[a] | 1.22 | 8.47 | 0.005 | Up |
| LC-QTOF-MS | LysoPC(16:0)[c] | 13.07 | 36.97 | 0.006 | Down |
| | LysoPC(18:2)[c] | 12.98 | 36.27 | 0.002 | Down |
| | LysoPC(18:1)[c] | 11.77 | 37.35 | 0.001 | Down |
| | LysoPC(20:4)[c] | 10.89 | 36.22 | 0.001 | Down |
| | N-acetylneuraminyl-Galactosylceramide[b] | 9.65 | 37.06 | 0.004 | Down |
| | LysoPC(18:0)[c] | 7.72 | 38.71 | 0.007 | Down |
| | LysoPC(14:0)[c] | 4.58 | 35.05 | 0.001 | Down |
| | Eicosanoids Prostaglandins[b] | 3.12 | 28.91 | 0.004 | Down |
| | C17 Sphinganine[a] | 2.86 | 31.28 | 0.001 | Up |

[a]The biomarkers identified with standards; [b]the biomarkers identified with database and system internal library; [c]the biomarkers identified with similar compounds and characteristic ions.

Table 5. Other important metabolites detected from plasma.

| | Important metabolites | VIP value | Retention time | p-value | Change direction |
|---|---|---|---|---|---|
| GC-MS | Myo-inositol | 0.11 | 26.74 | 0.025 | Up |
| | L-Leucine | 0.06 | 8.18 | 0.039 | Down |
| | Acetamide | 1.75 | 3.18 | 0.001 | Down |
| | Glycine | 1.97 | 8.95 | 0.039 | Up |
| | serine | 1.13 | 10.15 | 0.022 | Up |
| LC-QTOF-MS | Unknown (517.3 m/z) | 2.47 | 35.66 | 0.001 | Down |
| | PI (Glycerophosphoinositols) | 2.26 | 28.92 | 0.003 | Down |
| | Unknown (167.1 m/z) | 1.67 | 33.36 | 0.00003 | Up |
| | Unknown (560.3 m/z) | 1.28 | 31.82 | 0.001 | Down |
| | PI-Cer(d18:0/16:0) | 1.11 | 38.55 | 0.002 | Up |
| | L-Acetylcarnitine | 1.07 | 1.71 | 0.004 | Down |

# Ⅳ. Discussion

## Comparison of allergenic and autologous groups

The heat map (Figure 13) was achieved based on the top 50 biomarker candidates of importance. Difference is revealed between the allergenic (test) and autologous (control) groups, with verified keys marked on the left.

The changing conditions of each metabolites are shown in Figure 14. The dysregulation of glucose, l-lactic acid and phosphoric acid are reported have association with several diseases. The increase of these metabolites shown in scattergram may exert side impacts after allergenic blood transfusion. Moreover, LysoPC plays an important role in immune system, which participates in humoral and cellular immune responses. Therefore the decrease of total LysoPC indicates the reduction of the ability of immune system.

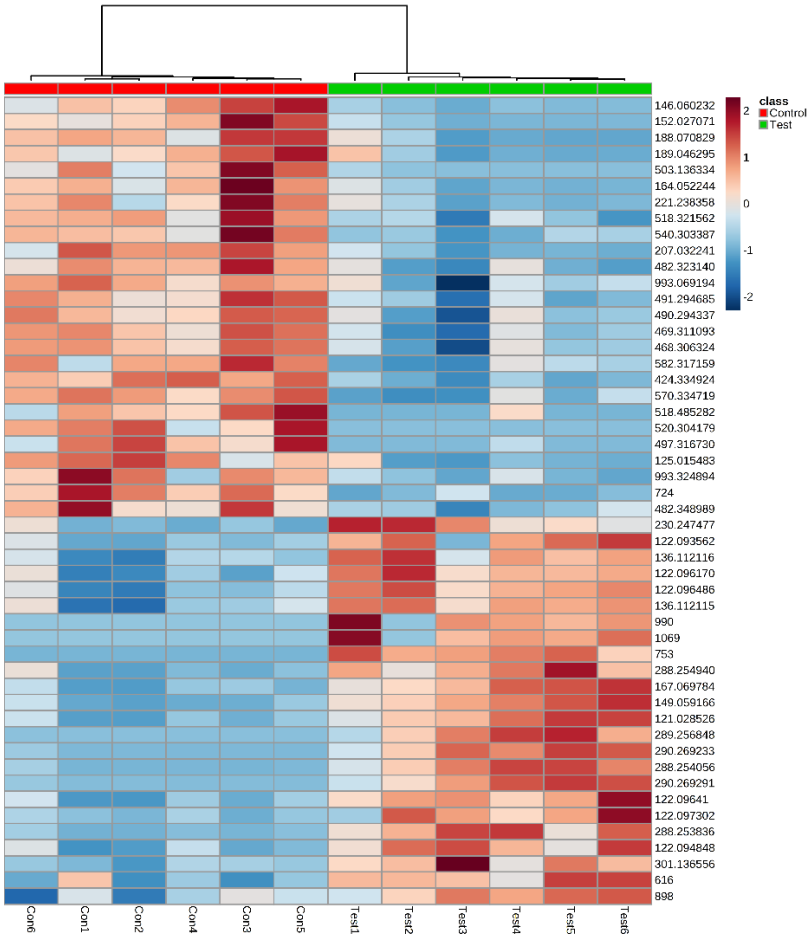


Figure 13. Heat map visualization of 50 significantly changed features between two groups.

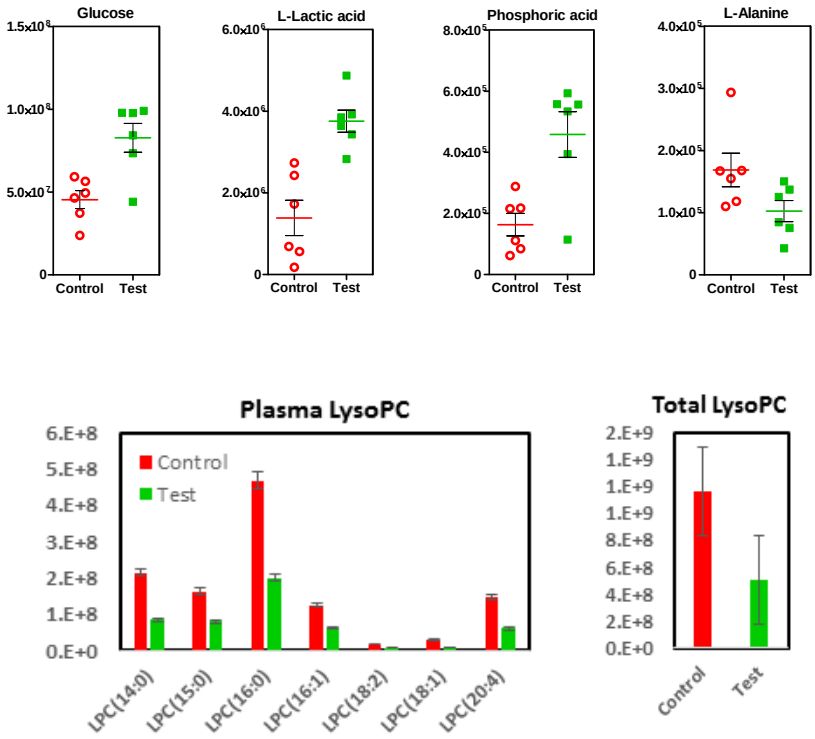


Figure 14. The scattergram and bar chart of significant metabolites.

## Metabolomic Data Analysis with MetaboAnalyst 3.0

Metabolite Set Enrichment Analysis is a way to identify biologically meaningful patterns that are signicantly enriched in quantitative metabolomic data. Over Representation Analysis (ORA) is performed when a list of compound names is provided. The list of compound list can be obtained through conventional feature selection methods (pathway-associated metabolite sets), to investigate if some biologically meaningful patterns can be identified. ORA was implemented using the hypergeometric test to evaluate whether a particular metabolite set is represented more than expected by chance within the given compound list. *P*-values are provided after adjusting for multiple testing. Figure 15 below summarizes the result. In Figure 16, the metabolites associated disease pathways resulted from over representation analysis are displayed for further study.

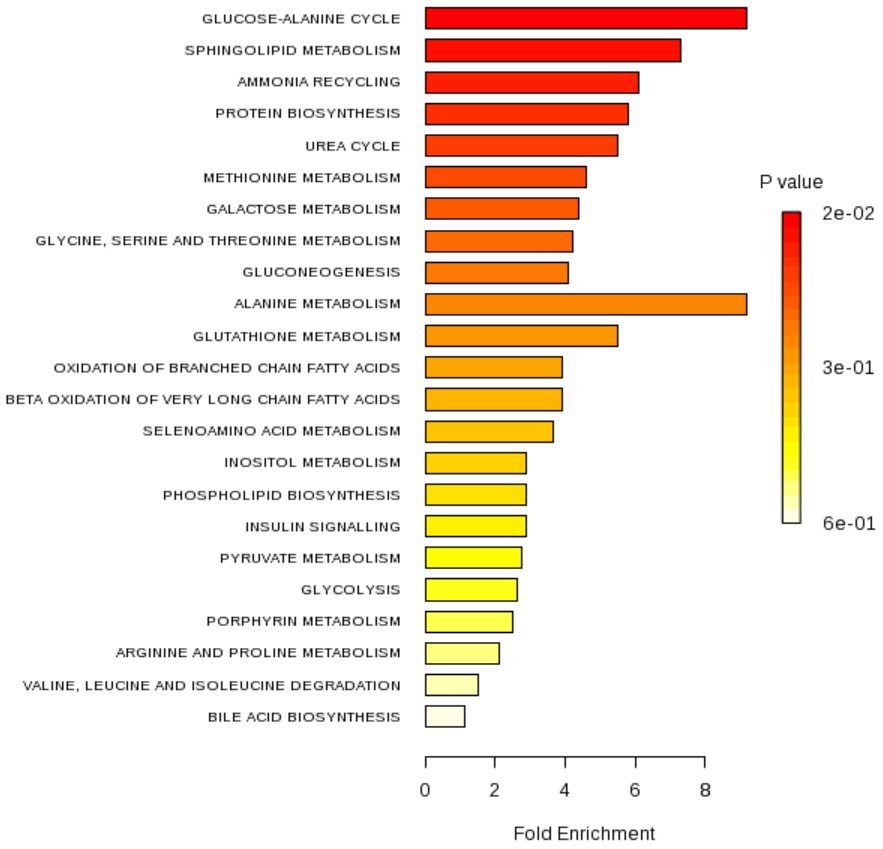


Figure 15. Pathway-associated metabolic summary plot for over representation analysis (ORA).

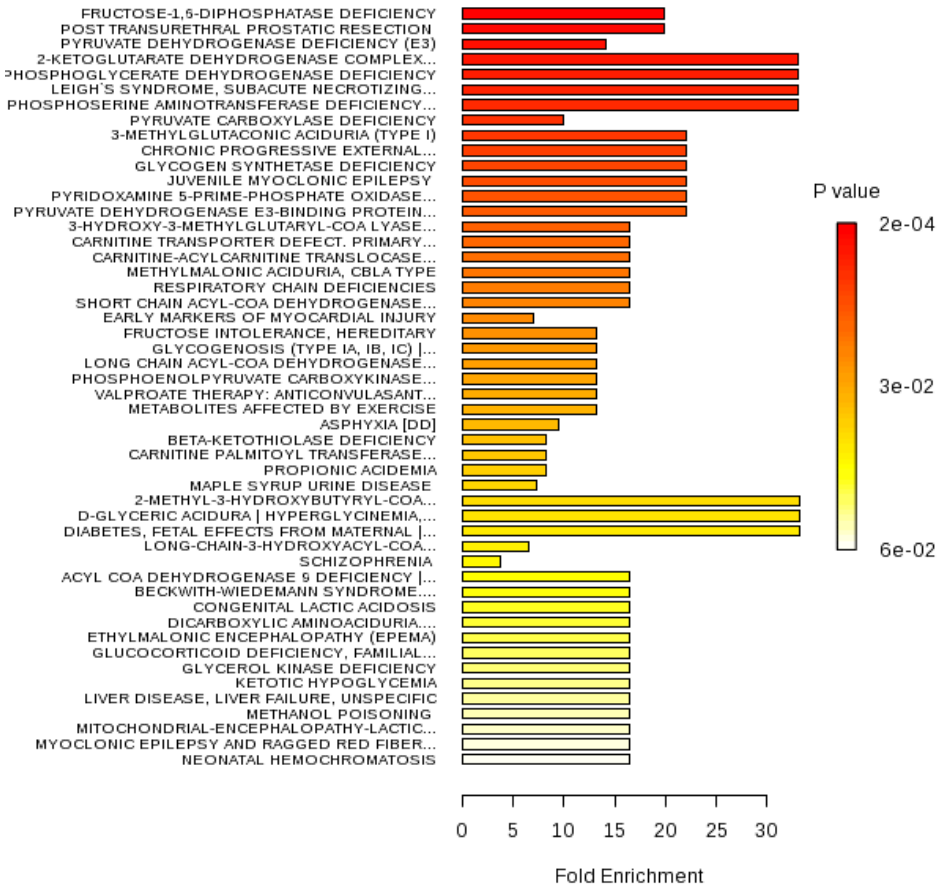


Figure 16. Disease associated metabolic pathway result from over representation analysis.

# V. Conclusion

1. Plasma metabolomic study on the transfused rat model showed that the metabolic profiles could differentiate the autologous transfused and allogeneic transfused rats, and several metabolic biomarkers were identified.

2. The dysregulation of the biomarkers such as LysoPC, glucose, and some amino acids show correlations with pre-existing biological pathways, of which are associated with some diseases.

3. Explanations offered here provide foundations for the adverse impacts of allogeneic blood transfusion and more studies are needed to demonstrate the clinical significance of side impacts of allogeneic blood transfusion. If confirmed, the benefits must be weighed against risks. Even though in some cases blood transfusion is a "have-to" therapy, blood using still should be paid sufficient attention to avoid unnecessary risk.

# VI. References


1) Al-Refaie WB, Parsons HM, Markin A, Abrams J, Habermann EB. Blood transfusion and cancer surgery outcomes: a continued reason for concern. *Surgery*. 2012; 152(3):344-354.

2) Vamvakas EC. Allogeneic blood transfusion and cancer recurrence: 20 years later. *Transfusion*. 2014; 54(9):2149-2153.

3) River C. *Research Animal Models*. August 2012.

4) Dunn WB, Broadhurst D, Begley P, et al. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. *Nat Protoc*. 2011; 6(7):1060-1083.

5) Nishiumi S, Shinohara M, Ikeda A, et al. Serum metabolomics as a novel diagnostic approach for pancreatic cancer. *Metabolomics*. 2010; 6(4):518-528.

6) Calderon-Santiago M, Priego-Capote F, Luque de Castro MD. Enhanced detection and identification in metabolomics by use of LC-MS/MS untargeted analysis in combination with gas-phase fractionation. *Anal Chem*. 2014; 86(15):7558-7565.

7) Yea K, Kim J, Yoon JH, et al. Lysophosphatidylcholine activates adipocyte glucose uptake and lowers blood glucose

levels in murine models of diabetes. *J Biol Chem.* 2009; 284(49):33833-33840.

8) Karl DM. Aquatic ecology. Phosphorus, the staff of life. *Nature.* 2000; 406(6791):31, 33.

9) Heasley LE, Thaler S, Nicks M, Price B, Skorecki K, Nemenoff RA. Induction of cytosolic phospholipase A2 by oncogenic Ras in human non-small cell lung cancer. *J Biol Chem.* 1997; 272(23):14501-14504.

10) Jo KI, Shin JW, Choi TY, Park YJ, Youm W, Kim MJ. Eight-year experience of bloodless surgery at a tertiary care hospital in Korea. *Transfusion.* 2013; 53(5):948-954.

11) Saida Mebarek, Abdelkarim Abousalham, David Magne. Phospholipases of Mineralization Competent Cells and Matrix Vesicles: Roles in Physiological and Pathological Mineralizations. *Int. J. Mol. Sci.* 2013, 14(3), 5036-5129.

# Abstract

## HPLC-QTOF-MS and GC-MS based metabolomics on blood transfusion study

Wang, Haiping
Department of Pharmacy, Pharmaceutical Analysis
The Graduate School
Seoul National University

Blood transfusions are beneficial in many clinical circumstances, however they temporarily improve the physical state of the patients but exert wide spread effects on immune and non-immune systems. Recently, data has accumulated to suggest that allogeneic blood transfusions are connected with regulations in the immune functions, which have increased rate of recurrence of cancer. Nevertheless, to date, little is known about the global metabolomic alterations that indicate the possible reasons for hidden risks about blood transfusion. As a discipline focused on comprehensive analysis of small molecule metabolites, metabolomics provides a powerful approach to seek out biomarkers in biosystem. In this thesis, we attempt to investigate the metabolite changings generated by allogeneic blood transfusion based on rat model through using metabolomics technology, and explored potential

negative impacts associated with these biomarkers on cancer patients who received allogeneic blood transfusion during surgery. To further profile the “metabolome” after blood transfusions, we used both liquid chromatography-quadrupole time-of-flight high definition mass spectrometry and gas chromatography mass spectrometry. The level of total twelve metabolites were found significantly changed in allogeneic groups compared to autologous groups by performing principal component analysis (PCA) coupled with partial least squares-discriminant analysis (PLS-DA). The results showed that two groups were clearly discriminated from each other and metabolic variations may have correlations with cancer occurrence, leading to a better understanding of blood transfusion and its significance for recurrence after cancer surgery.

Key words: Blood transfusions; LC-QTOF-MS; GC-MS; Metabolomics

학번: 2013-23936