Identification of potential endometriosis biomarkers in peritoneal fluid and blood plasma via shotgun lipidomics

Natalia Starodubtseva, Vitaliy Chagovets, Anna Borisova, Dinara Salimova, Natalia Aleksandrova, Konstantin Chingin, Huanwen Chen, Vladimir Frankevich

PII: S2376-9998(18)30046-1
DOI: https://doi.org/10.1016/j.clinms.2019.05.007
Reference: CLINMS 75

To appear in: Clinical Mass Spectrometry

Received Date: 19 October 2018
Revised Date: 30 May 2019
Accepted Date: 30 May 2019

Please cite this article as: N. Starodubtseva, V. Chagovets, A. Borisova, D. Salimova, N. Aleksandrova, K. Chingin, H. Chen, V. Frankevich, Identification of potential endometriosis biomarkers in peritoneal fluid and blood plasma via shotgun lipidomics, Clinical Mass Spectrometry (2019), doi: https://doi.org/10.1016/j.clinms.2019.05.007

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Identification of potential endometriosis biomarkers in peritoneal fluid and blood plasma via shotgun lipidomics

Natalia Starodubtseva\textsuperscript{1,2}; Vitaliy Chagovets\textsuperscript{1}; Anna Borisova\textsuperscript{1}; Dinara Salimova\textsuperscript{1}; Natalia Aleksandrova\textsuperscript{1}; Konstantin Chingin\textsuperscript{3}; Huanwen Chen\textsuperscript{3}; *Vladimir Frankevich\textsuperscript{1}

\textsuperscript{1}V. I. Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology, Ministry of Healthcare of the Russian Federation, Moscow
\textsuperscript{2}Moscow Institute of Physics and Technology, Moscow, Russia
\textsuperscript{3}Jiangxi Key Laboratory for Mass Spectrometry and Instrumentation, East China University of Technology, Nanchang, China

*corresponding authors: vfrankevich@gmail.com

Graphical abstract

Abstract

Endometriosis is a recurrent and benign gynecological disorder, defined by the ectopic presence of endometrium. About 10\% of reproductive-aged women suffer from endometriosis. There are no non-invasive or minimally invasive tests available in clinical practice to accurately diagnose endometriosis today.
Here, we present our efforts to determine the diagnostic accuracy of biomarkers in peritoneal fluid and blood plasma using flow injection analysis with electrospray ionization tandem mass spectrometry (ESI-MS/MS) in 70 women with endometriosis and 20 women from a control group. The presence of endometriosis was confirmed by surgical findings and post-operative pathological examination. A qualitative and quantitative evaluation of the lipids in peritoneal fluids and blood plasma was carried out using electrospray ionization mass spectrometry (ESI-MS). The analysis revealed more than 140 molecular species of lipids, most of which pertained to five classes: phosphatidylcholines, phosphatidylethanolamines, sphingomyelins, di- and triglycerides. The data were analyzed using a statistical multifactorial method (i.e., PLS-DA). It was found that 9 potential biomarkers of endometriosis (LPC 16:0, PE O-20:0, PE O 34:1, PC 36:2, PC 36:4, PC 36:5, PC 38:4, PC 38:6 and SM 34:1) are common in blood plasma and peritoneal fluid, supporting connection with the pathological process. The sensitivity of the method developed for plasma was 93% with a specificity of 95%; for peritoneal fluid, the sensitivity was 90% and the specificity 95%. Accordingly, plasma is the most suitable biological fluid for clinical diagnostics of endometriosis. Further validation of these lipids as serologic biomarkers may enhance non-invasive diagnostic tools for patients with suspected endometriosis and reduce the frequency of diagnostic laparoscopy.

**Abbreviations :**

BMI- Body Mass Index  
DG- Diglyceride  
EDTA - Ethylenediaminetetraacetic Acid  
ESI – Electrospray Ionization  
FDR – False Discovery Rate  
LOOCV -Leave-One-Out Cross-Validation  
LPC - Lysophosphatidylcholine  
MS – Mass Spectrometry
MS/MS – Tandem Mass Spectrometry  
m/z – Mass to Charge Ratio  
PC- Phosphatidylcholine  
PCA –Principal Component Analysis  
PLS-DA - Partial Least Squares-Discriminant Analysis  
PE- Phosphoethanolamine  
QC- Quality Control  
RPM- Revolutions per Minute  
SM- Sphingomyelin  
SMPD - Sphingomyelin Phosphodiesterase  
TG- Triglycerides  
TIC – Total Ion Current

1. Introduction

Endometriosis is a recurrent and benign gynecological disorder, defined by the ectopic presence of endometrium [1]. The inflammatory process, caused by the spread of endometrial cells outside the endometrium, as well as changes in other physiological processes, including increased cell adhesion, proliferation, invasion and angiogenesis, extracellular matrix degradation, apoptosis disorder, increased oxidative stress, and increased steroid hormone biosynthesis are the basis of pathophysiology of the disease [1–3]. As a result of these processes, endometrial cells can survive and multiply in ectopic sites [3].

Endometriosis is taking the leading position among proliferative diseases of the pelvic organs [4]. About 10% of reproductive-aged women suffer from endometriosis, which is equivalent to 176 million women worldwide. Moreover, between 30% to 50% of women with infertility and / or chronic pelvic pain suffer from endometriosis [5].

Laparoscopy, with subsequent histological confirmation, is the gold standard for diagnosis of endometriosis [6]. Currently, there are no non-invasive or minimally invasive tests available in clinical practice to accurately diagnose
endometriosis. For example, routine gynecological examination has low predictive value, and visualization technologies are inadequate for detection of pelvic adhesions or superficial peritoneal implants [4,6]. This fact results in a mean latency of 7-11 years from onset of symptoms to definitive diagnosis [2]. The World Society for Endometriosis has endorsed development of a reliable non-invasive diagnostic tool for endometriosis as one of the top priorities of scientific research in gynecology [7].

In spite of the fact that more than 100 potential biomarkers of endometriosis have been proposed, none have shown sufficient diagnostic predictive value [3,7][5, 7]. The level of five different classes of lipids were shown to be significantly different in the ectopic, compared to the eutopic, endometrium: phosphatidylcholine (PC 32:1, PC O-36:3, PC 38:7, PC 38:6, PC 40:8, PC 40:7, PC 40:6, PC O-42:1), phosphoethanolamine (PE O-20:0), sphingomyelin (SM 34:1), diglyceride (DG 44:9) and triglycerides (TG 41:2, TG 49:4, TG 52:3) [8–10]. Phosphatidylcholines and sphingomyelin may be potential markers of the endometrial expansion, since these lipids are associated with apoptosis suppression, oxidative stress and cell proliferation [11]. These suspicions were validated by Dutta et al. [12], who also described an increased concentration of phosphatidylcholines in serum samples in patients with endometriosis. Moreover, the involvement of sphingolipids in the pathophysiology of endometriosis was also postulated by Lee et al. [13], who found changes in the metabolism of sphingolipids in ectopic endometrium, serum blood and peritoneal fluid in patients with endometriosis.

In this study, we performed lipid content analysis of peritoneal fluid and plasma from patients with endometriosis. Peritoneal fluid in women with endometriosis has its own characteristics due to direct contact with the foci of endometriosis and is a promising biological environment for the detection of potential biomarkers. The attractiveness of using blood plasma for the diagnosis of various human diseases is undisputed, due to the fact that blood reflects the phenotype of the organism and its condition in the present. The metabolites,
previously identified in the ectopic endometrium, were assessed in the blood and peritoneal fluid of women with endometriosis. Their role in the pathophysiology of endometriosis was also studied, together with the possibility of creating a diagnostic model. The results indicate that the developed method is promising for the non-invasive diagnosis of endometriosis based on metabolic analysis of biological fluids.

2. Materials and methods

2.1 Study design

Fertile women with different stages with endometriosis (according to American Society for Reproductive Medicine classification system, 1997) were enrolled in the study. All women (n=20) underwent laparoscopic surgical examination of the abdominal cavity and complete excision of endometriotic tissue in the Department of a Gynecologic Surgery at V. I. Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology, Ministry of Healthcare of the Russian Federation. The diagnosis was confirmed by surgical findings and post-operative pathological examination. Laparoscopic examination of the abdominal cavity excluded the presence of any other pelvic pathology that could potentially confound the data. In order to extract lipids we collected samples of blood plasma and peritoneal fluid. The inclusion criteria for all participants of the study were: reproductive age (19-45 years) and regular menstrual cycle (the length of cycle varied from 25 to 35 days). The exclusion criteria were: abnormal uterine bleeding, hormonal treatment within 6 months prior to surgery, pelvic inflammatory diseases, endometrial pathologies (e.g., endometrial hyperplasia, endometrial cancer, intrauterine adhesions), suspicion of adenomiosis presence according to ultrasound investigation and severe somatic pathology, malignant neoplasms. The final number of patients in the endometriosis test group who met the inclusion and exclusion criteria and were included in the study was 70. Demographic and clinical characteristics of patients are presented in Table 1. The
age of patients with endometriosis ranged between 22 to 41 years (mean age 31±6 years) and the BMI varied between 18.2 to 28.6 kg/m² (mean 22.4±1.1 kg/m²).

Table 1. Demographic and clinical data of the 90 patients included in the study. The values are presented as Mean±SD, and number (percent of group).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Endometriosis (n=70)</th>
<th>Control (n=20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31±6</td>
<td>33±5</td>
<td>3.96</td>
</tr>
<tr>
<td>Caucasian</td>
<td>70 (100 %)</td>
<td>19 (95 %)</td>
<td>13.23</td>
</tr>
<tr>
<td>Others</td>
<td>0 (0 %)</td>
<td>1 (5 %)</td>
<td>4.2</td>
</tr>
<tr>
<td>Body mass index, (kg/m²)</td>
<td>22.4±1.1</td>
<td>24.1±1.2</td>
<td>10.4</td>
</tr>
<tr>
<td>I-II stage of endometriosis</td>
<td>35 (50 %)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III-IV stage of endometriosis</td>
<td>35 (50 %)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Infertility I</td>
<td>49 (70 %)</td>
<td>4 (20 %)</td>
<td>0.04</td>
</tr>
<tr>
<td>Infertility II</td>
<td>21 (30 %)</td>
<td>2 (10 %)</td>
<td>0.1</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>14 (20 %)</td>
<td>3 (15 %)</td>
<td>0.5</td>
</tr>
<tr>
<td>Chronic pelvic pain syndrome</td>
<td>63 (90 %)</td>
<td>1 (5 %)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The control group was selected from 50 fertile women without endometriosis who underwent surgery for myomectomy. The absence of endometriosis was confirmed by meticulous examination of the pelvic and extrapelvic peritoneum, ovaries, intestine and diaphragm in order to detect typical or atypical endometriotic lesions. Women affected by pelvic inflammatory disease, sexually transmitted infections, severe somatic, oncological or endocrine pathologies (including obesity), as well as women with endometrial hyperplasia were excluded from the study. None of the women had received any form of hormone therapy for at least 6 months prior to the study. The final number of women in the control group was 20. The average age of women in the control group was 33±5 years and the BMI varied between 18.8 and 29.8 kg/m² (mean 24.1±1.2 kg/m²).

All procedures and study methods were approved by the Commission of Biomedical Ethics at V. I. Kulakov National Medical Research Center for
Obstetrics, Gynecology and Perinatology, Ministry of Healthcare of the Russian Federation. All patients provided written informed consent.

2.2 Sample collection

Venous blood was collected from the antecubital vein just before anesthesia on the operating table after 12 hours of fasting. The puncture was carried out with a disposable syringe with a capacity of 3 ml. The blood was placed in a vacuum sterile tube with EDTA - sodium (0.5 ml of a 1.5% solution per 10 ml of blood) and centrifuged for 10 minutes at 2500 RPM. The resultant supernatant was collected and poured into a sterile cryovial (Corning).

Peritoneal fluid was obtained at the time of laparoscopy by aspiration using a laparoscopic cannula before any surgical intervention in order to minimize blood contamination and was placed into a separate sterile cryovial (Corning). Vials were immediately immersed in liquid nitrogen to prevent oxidation of lipids, transported to the laboratory, and stored at -80°C.

2.3 Lipid extraction

Lipid extracts were prepared according to the modified Folch method [14]: 200 μL of sample (blood plasma or peritoneal fluid) were added to 4 mL chloroform-methanol (2:1, v/v), incubated for 10 minutes, and filtered using filter paper. 800 μL of an aqueous solution of NaCl (1 mol/L) was added to the filtrate to induce phase separation. The mixture was centrifuged at 3000 RPM for 5 minutes at ambient temperature. The organic lower layer containing the lipids was dried in a nitrogen stream, re-dissolved in acetonitrile-2-propanol (1:1, v/v) for subsequent mass spectrometric analysis. All extraction procedures were performed in glass tubes.

Equal amounts of all samples were pooled as a QC sample for MS system conditioning and quality control.

2.4 Electrospray ionization mass spectrometric analysis
Lipid composition of samples was determined by flow injection analysis with ESI-MS using Maxis Impact qTOF mass spectrometer (Bruker Daltonics, Bremen, Germany). Constant flow of methanol/H2O 9/1 was supplied at the rate of 10 µL/min by Dionex UltiMate 3000 binary pump and 20 µL of sample was injected by a Dionex UltiMate 3000 autosampler (ThermoScientific, Bremen, Germany). Mass spectra were obtained in the positive ion mode over the mass range $m/z$ 400-1000 with resolution of 50,000 and the following ion source settings: capillary voltage 4.1 kV, spray gas pressure 0.7 bar, drying gas flow rate 6 l/min, the temperature of the drying gas was 200°C.

Tandem MS (MS/MS) was performed using data dependent analysis with the following characteristics: five the most abundant peaks were chosen after a full mass scan and subjected to MS/MS analysis with collision induced dissociation applying 35 eV collision energy, 3 Da isolation window and 1 minute of mass exclusion time.

QC samples were injected among the samples and used to evaluate the experimental quality.

2.5 Identification of lipids and statistical analysis

100 mass spectra were averaged over the total ion current (TIC) maximum and transformed into the abundance-$m/z$ table for further analysis. Each peak abundance was normalized to the TIC.

Multivariate data analysis of the MS data was performed with principal components analysis (PCA) to detect outliers, and partial least squares-discriminant analysis (PLS-DA) with Pareto scaling using “ropls” library (release 3.8) to find out if the MS data was sufficient for the classification of samples under the study [15]. The PLS-DA builds a statistical model on a training sample set with known diagnosis. Then each new sample can be tested using an already existing model. PLS-DA approach allows not only classification of the samples, but also the ability to identify ions (MS peaks, $m/z$) in the mass spectra responsible for differences between the groups (with the greatest VIP values according to the PLS-
DA model). As a result, potential biomarkers can be determined. The quality of
the PLS-DA model was estimated by \( R^2 \) and \( Q^2 \) values. \( Q^2 \) was calculated by 7-
fold leave-one-out cross-validation (LOOCV).

The statistical significance of the ions (m/z) proposed as potential
biomarkers by the PLS-DA model was confirmed by a Mann-Whitney test
(p<0.05). Bonferroni correction and false discovery rate (FDR) correction for
multiple comparisons was also determined. The lipids, corresponding to these ions,
were annotated with in-lab created R code (the RStudio version was 1.1.463 and
the R language version was 3.5.2), which compares measured accurate m/z values
with theoretical computer-generated values, using the Lipid Maps database with a
10 ppm mass accuracy [16]. The code searched for records within 10 ppm of the
experimental m/z. Proton, sodium and potassium ion adducts were considered.
More precise identification was made based on the MS/MS data for the peak under
consideration, if it had undergone MS/MS analysis. Lipidiomic nomenclature
throughout the paper is in accordance with LIPID MAPS [17] terminology and
shorthand notation summarized by Liebisch et al [18].

3. Results and discussion

Blood plasma and peritoneal fluid samples from 70 women with
endometriosis (main group) and 20 women of control group were investigated
using ESI-MS to obtain information about molecular composition. In the mass
spectra of positive ions from the 90 blood plasma and 90 peritoneal fluid samples,
the most intense peaks corresponded to phosphatidylcholines. Among 148 detected
metabolites, 53 significant lipid peaks were identified and divided into five
different classes: phosphatidylcholines, phosphatidylethanolamines,
sphingomyelins, di- and triglycerides. The identification [19] was provided
according to accurate mass within 10 ppm mass accuracy and MS/MS information
about the fragmentation pattern (Fig. S1). Mass peak on m/z 184 is characteristic
for lipids with the choline residue in the headgroup. These are
phosphatidylcholines, lysophosphatidylcholines and sphingomyelins. Neutral loss of 141 Da is characteristic for phosphatidylethanolamines and lysophosphatidylethanolamines [20].

PCA of QC samples was performed to assess experiment quality. PCA showed that the pooled QC samples were clustered (Fig. S2), indicating that the MS analysis process met the required qualifications. The obtained MS data was analyzed using PLS-DA (Fig. 1). The model performance was assessed by its ability to fit (R²) and predict (Q²) variance of the data. The models effectively differentiated between the groups based on data from plasma (R²X = 0.50, R²Y = 0.99, Q² = 0.95) and peritoneal fluid (R²X = 0.52, R²Y = 0.99, Q² = 0.89). The metrics of the models are summarized in Table S1. The points corresponding to the samples from patients with endometriosis and the control group were visually divided into two clusters. Moreover, these results did not depend on the stage of the endometriosis. According to cross-validation analysis, the sensitivity of the model for plasma data was 93% with a specificity of 95%. The sensitivity of the model for peritoneal fluid data was 90% with a specificity of 95%.
Figure 1. PLS-DA score plot of blood plasma (A) and peritoneal fluid (B) samples in patients with endometriosis (red, 70 patients) and control group (blue, 20 patients).

Figure 2 shows the level of lipids, corresponding to PLS-DA model variables (m/z) that give the largest contribution to the projection on hidden structures (VIP variables) with significantly (p < 0.05 by pairwise Mann-Whitney test) different level in biofluids (plasma, peritoneal fluid) for studied groups (endometriosis vs control). Bonferroni and false discovery rate (FDR) correction for multiple comparisons was also provided for these lipids (Tables S2, S3). It turned out that there are 6 lipids with local FDR less than 0.05 in plasma and 4 lipids in peritoneal fluid out of 13 lipids with a Mann-Whitney p-value less than 0.05 for plasma and 14 for peritoneal fluid. The VIP scores and corresponding p-
values, q-values and local FDR of the lipids are summarized in Tables S2, S3. These compounds are proposed as possible biomarkers in blood for minimally invasive endometriosis diagnosis.

The most significant in the peritoneal fluid were 14 lipids: lysophosphatidylcholine (LPC 16:0), phosphoethanolamines (PE O-20:0, PE O 34:1), diglycerides (DG 32:2, DG 38:2), phosphatidycholines (PC 34:1, PC 34:2, PC 36:2, PC 36:3, PC 36:4, PC 36:5, PC 38:4, PC 38:6) and sphingomyelin (SM 34:1). For the blood plasma, the most important were 13 lipid peaks: lysophosphatidylcholines (LPC 16:0, LPC 20:5), phosphoethanolamines (PE O-20:0, PE O-34:1), diglyceride (DG 40:5), phosphatidylcholines (PC 36:2, PC 36:4, PC 36:5, PC 38:5, PC 38:6, PC 38:7, PC 40:9) and sphingomyelin (SM 34:1). Thus, 9 potential biomarkers of endometriosis (LPC 16:0, PE O-20:0, PE O 34:1, PC 36:2, PC 36:4, PC 36:5, PC 38:4, PC 38:6 and SM 34:1) are common between blood plasma and peritoneal fluid indicating their connection with the pathological process.

Interestingly, the level of phosphoethanolamine PE O-20:0 was increased not only in blood and peritoneal fluid, but also in the ectopic endometrium [8], which indicates a high diagnostic value for this lipid. Despite an almost 2-fold increase of SM 34:1 levels in ectopic versus eutopic endometrium [8–13], the reduction in the level of SM was found in blood plasma and peritoneal fluid of patients with endometriosis when compared with control samples. The same was observed for LPC 16:0, PE O-34:1 and PC 36:4. The peritoneal fluid in patients with endometriosis is characterized by increased oxidative stress [2,21,22], which could explain why our experimental data shows reductions in the level of SM. Oxidative stress promotes the conversion of sphingomyelins to ceramide by translocation of acidic sphingomyelinase from lysosomes on the outer layer of the cell membrane [23]. Enhanced formation of ceramides is also facilitated by an increase in the level of sphingomyelin phosphodiesterase 3 (SMPD3), observed in endometrial tissue in patients with endometriosis [13,24]. Thus, ceramides can be converted to sphingosine-1-phosphate (S-1P), a bioactive lipid involved in
angiogenesis, cell migration and apoptosis, immunomodulation and cell differentiation regulation [25]. Dysregulation in the sphingosine pathway has been noted in ectopic endometrium, leading to a decrease in the catabolism of sphingosine-1-phosphate (S-1P) [25].

Notably, the level of phospholipids (phosphatidylcholines PC 36: 5, PC 36: 2, PC 38: 5, PC 38: 6, PC 40: 9 and lysophosphatidylcholine LPC 20:5) was higher in the plasma of patients with endometriosis than that found in their peritoneal fluid. The decrease in total lipid levels in the peritoneal fluid may be due to lipid peroxidation specific to patients with endometriosis [21]. Significant increases in the intensity of free radical oxidation with a simultaneous decrease in the activity of antioxidant protection leads to the accumulation of toxic products of lipid peroxidation, which compromise cell membrane integrity, ion permeability, and energy mechanisms, resulting in dysregulation of cell functions [22]. These mechanisms are known to provoke the spread and progression of endometriosis [22].

Decreases in PC levels in the peritoneal fluid may indicate their role as a source for increasing prostaglandin production [21]. This hypothesis is supported by an increase in the expression of phospholipase A2 (PLA2G2F, PLA2G1B, PLA2G5, PLA2G2D, PLA2G2A) observed in the ectopic endometrium [2,10,11].

Despite the fact that the level of lysophosphatidylcholine (LPC) in the peritoneal fluid of patients with endometriosis turned out to be lower than in the control group, a lower ratio between the total phosphatidylcholine and lysophosphatidylcholine content was also observed, which also supports our hypothesis. In addition, phosphatidylcholine can serve as a source for the synthesis of sphingomyelin by transmitting a signal from phosphocholine to ceramides. This reaction is catalyzed by sphingomyelin synthase 1 (SGMS1), which has also been detected in high concentrations in endometriosis patients [11–13] and leads to a decrease in the overall PC level in the peritoneal fluid. On the other hand, reduced PC levels may be due to low expression of the enzyme alkylglycerone phosphate synthase (AGPS), which catalyzes the first step in the synthesis of PC [24].
Figure 2. Comparison of lipid levels with the greatest impact on separation between the endometriosis and control groups in blood plasma (A) and in peritoneal fluid (B).

Conclusion

The development of metabolomic technologies, including lipidomics, based on mass spectrometric analysis, has led to the emergence of new methods that are capable of rapid and highly accurate specification of the molecular composition of any biological sample. Metabolites present the molecular phenotype of an organism, as they are substrates, intermediates and the final products of biochemical reactions. All changes in the body are reflected in the metabolome, as if it were a "molecular mirror". Lipids and fatty acids are involved in virtually all
processes occurring in the human body. These metabolites are a structural element of cell membranes, hormones and secondary messengers, involved in transportation of hydrophobic and amphiphilic substances.

We have discovered that the levels of some sphingo- and phospholipids (LPC 16:0, PE O-20:0, PE O 34:1, PC 36:2, PC 36:4, PC 36:5, PC 38:4, PC 38:6 and SM 34:1) are coordinately elevated or attenuated in the biological fluids of patients with endometriosis. These lipids are involved directly in implantation, inner heightened proliferation and decreased apoptotic properties of endometriosis tissues.

In spite of the fact that the endometriosis foci are primarily within the peritoneal fluid, plasma would be the most suitable biological fluid to analyze using mass spectrometry in order to diagnose endometriosis with high accuracy. Our data revealed lipid alterations in the plasma of patients with endometriosis with a higher sensitivity than from peritoneal fluid.

Further validation of studied lipids as serologic biomarkers may enhance non-invasive diagnostic tools for patients with suspected endometriosis and may reduce demand for the invasive procedure of diagnostic laparoscopy.

**Declaration of interest**

All authors have nothing to disclose.

**Acknowledgement**

This work was supported by RSF (No.18-75-10097) (study design, ESI-MS/MS experiments, lipid identification) the 111 Project (No. D17006), the Project of Jiangxi Provincial Department of Education (No. GJJ160544), National Natural Science Foundation of China (Nos. 21565004, 21765001), and Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT) (No. IRT_17R20) (statistical analysis).

**References**


**Highlights**

- 9 potential biomarkers of endometriosis that are common in blood plasma and peritoneal fluid were identified using high-resolution electrospray ionization tandem mass spectrometry analysis.
- High-resolution electrospray ionization tandem mass spectrometry in combination with statistical analysis is proposed as a non-invasive diagnostic tool for endometriosis.
- The sensitivity and specificity using these biomarkers for diagnosis of endometriosis from plasma was 93% and 95%, respectively; and from peritoneal fluid, 90% and 95%, respectively.