Alterations in Lipid Profile Upon Uterine Fibroids and Its Recurrence

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Research Article

Keywords: lipidomics, uterine fibroids, diagnostics, mass spectrometry

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Alterations in lipid profile upon uterine fibroids and its recurrence

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Abstract

Background. Uterine fibroids (UF) is the most common (about 70% cases) type of gynecological disease, with the recurrence rate varying from 11% to 40%. Because UF has no distinct symptomatology and is often asymptomatic, the specific and sensitive diagnosis of UF as well as the assessment for the probability of UF recurrence pose considerable challenge.

Aim. The aim of this study was to characterize alterations in the lipid profile of tissues associated with the first-time diagnosed UF and recurrent uterine fibroids (RUF) and to explore the potential of mass spectrometry (MS) lipidomics analysis of blood plasma samples for the sensitive and specific determination of UF and RUF with low invasiveness of analysis.

Materials and methods. MS analysis of lipid levels in the myometrium tissues, fibroids tissues and blood plasma samples was carried out on 66 patients, including

35 patients with first-time diagnosed UF and 31 patients with RUF. The control group consisted of 15 patients who underwent surgical treatment for the intrauterine septum. Fibroids and myometrium tissue samples were analyzed using direct MS approach. Blood plasma samples were analyzed using high performance liquid chromatography hyphened with mass spectrometry (HPLC/MS). MS data were processed by discriminant analysis with projection into latent structures (OPLS-DA).

Results. Significant differences were found between the first-time UF, RUF and control group in the levels of lipids involved in the metabolism of glycerophospholipids, sphingolipids, lipids with an ether bond, triglycerides and fatty acids. Significant differences between the control group and the groups with UF and RUF were found in the blood plasma levels of cholesterol esters, triacylglycerols, (lyso) phosphatidylcholines and sphingomyelins. Significant differences between the UF and RUF groups were found in the blood plasma levels of cholesterol esters, phosphotidylcholines, sphingomyelins and triacylglycerols. Diagnostic models based on the selected differential lipids using logistic regression showed sensitivity and specificity of 88% and 86% for the diagnosis of first-time UF and 95% and 79% for RUF, accordingly.

Conclusion. This study confirms the involvement of lipids in the pathogenesis of uterine fibroids. A diagnostically significant panel of differential lipid species has been identified for the diagnosis of UF and RUF by low-invasive blood plasma analysis. The developed diagnostic models demonstrated high potential for clinical use and further research in this direction.

Keywords: lipidomics; uterine fibroids; diagnostics; mass spectrometry

1. Introduction

Uterine fibroids (UF), also referred to as myomas, is the most common (about 70% cases) type of gynecological disease [1–6]. 25% of UF patients of reproductive age undergo surgery [1–3]. UF has no distinct symptomatology and is often

asymptomatic, which makes it difficult to diagnose [1,2]. The common complaints of patients with UF include painful and heavy menstruation, abnormal uterine bleeding, pain in the lower abdomen, infertility, miscarriage, impaired function of adjacent organs, dyspareunia, etc.[7–9].

The pathogenesis of UF remains unknown. The roles of genetic mutations [10,11], hormonal disorders (estrogen-progesterone imbalance), neoangiogenesis [12], and growth factors [13] have been reported. Risk factors of UF include early menarche, late reproductive age, obesity, tamoxifen therapy, high parity, menopause, smoking, combined oral contraceptives, inflammatory processes [1,12,14–16].

For the UF patients who are planning pregnancy, myomectomy remains the main treatment. However, the recurrence of UF is possible after organ-preserving treatment. The recurrence rate of UF varies from 11% to 40%. A second surgery is necessary in 1.3-27% of cases [17]. UF belongs to diseases with a genetic predisposition [18]. A family history of UF was shown to increase the risk of UF recurrence [18,19]. Risk factors for UF recurrence include the presence of more than 3 fibroids, age from 30 to 40 years, rapid tumor growth before surgery, as well as certain histological types of UF [20]. Surgery can trigger the development of new myomatous nodes, because damage to the myometrium activates the expression of mitogenic and angiogenic growth factors. High level of Ki-67 (Ki-67), progesterone (PgR) and vascular endothelial growth factor (VEGF) in the myometrium and fibroids are pathogenetic factors of UF recurrence [20–23].

To increase the effectiveness of organ-preserving treatment of UF patients in the reproductive period, it is important to assess the risk of recurrence [24]. The search of new UF biomarkers is mainly done by metabolomics and proteomics approaches, because these approaches allow determination of the molecular composition for any biological sample with high accuracy [25]. Shotgun lipidomics based on electrospray ionization mass spectrometry (ESI-MS) allows deep molecular profiling of a sample without significant losses of chemical information [26,27]. The high diagnostic potential of lipidomics has been shown in many areas

of medicine, particularly in oncology: lung, thyroid gland, breast, stomach, pancreas, colorectal, liver, kidney, prostate, ovarian, and endometrium cancer [28,29,38–47,30,48–51,31–37]. MS studies of lipid profiles in tissues and blood plasma have revealed new promising biomarkers of endometriosis (benign gynecological disorder) [26,27,32,33,37,44,47]. Heinonen et al. found that homocarnosine level was reduced in all fibroid subtypes studied; sphingolipids, phosphatidylserines, vitamin A and C levels were reduced in MED12 mutated fibroids [52]. A significant decrease in the level of lipids in the tumor tissue may lead to a small size of subtype MED 12 UF [52,53]. A comparative MS study of lipid profiles of blood plasma, tissues of fibroids and myometrium may reveal new molecular markers for the diagnosis and prediction of the course of UF.

This study presents the lipidomic profiling of myometrium tissues, fibroids tissues, and blood plasma samples from patients with UF and recurrent uterine fibroids (RUF).

2. Materials and methods

2.1 Study design

The study of fibroids and myometrium tissues included 35 women with uterine fibroids (UF) diagnosed for the first time and 31 patients diagnosed with recurrent uterine fibroids (RUF). A control group for a comparative analysis of blood plasma included 15 patients operated for infertility and for the intrauterine septum. All patients (n=81) were examined in the department of Operative Gynecology of National Medical Reseach Center for Obstetrics, Gynecology and Perinatology named after Academician V.I. Kulakov of the Ministry of Healthcare of Russian Federation. All patients signed an informed consent to participate in the study, approved by the Ethics Committee of National Medical Reseach Center for Obstetrics, Gynecology and Perinatology named after Academician V.I. Kulakov. We confirm that all methods were performed in accordance with the relevant guidelines and regulations.

Inclusion criteria for the UF and RUF groups were: reproductive age (18-45 years), uterine fibroids, organ-preserving surgery, lack of hormone therapy for 6 months or more before surgery. Exclusion criteria were: systemic autoimmune diseases, severe somatic pathology, cancer, inflammatory processes, concomitant gynecological pathology. All women underwent laparoscopic myomectomy. The indications for surgical treatment were heavy menstruation leading to anemia, severe pain syndrome, lack of effect from previous conservative therapy, and infertility.

2.2 Sample collection

Myometrial and fibroids samples were collected during surgery. Tissue samples were placed in a sterile cryovials (Corning), transported in liquid nitrogen to a Biobank, and stored in a freezer at the temperature of -80 °C until analysis. Blood sampling was performed on an empty stomach on the eve of surgery. Blood was collected in a sterile vacuum tube with EDTA-sodium and centrifuged for 10 min at 2500 rpm to obtain plasma. Plasma was stored in sterile cryovials (Corning) in a freezer at -80 °C until analysis.

2.3 Sample preparation for lipidome analysis

Plasma and tissue lipid extracts were prepared according to the modified Folch method [26,27,54]. Briefly, after homogenization of 50 mg of tissue in liquid nitrogen, 5 μL of internal standard and 4 mL of a chloroform-methanol (2:1, v/v) were added, incubated for 10 min, and filtered. Then, 800 μL of 1 M NaCl solution in water was added and centrifuged. An organic layer containing lipids was collected, vacuum dried, and redissolved in 500 μL 2-propanol-acetonitrile (1:1,v/v) for MS analysis.

For plasma samples, 480 μL and 5 μL of internal standard 1 of chloroform-methanol (2:1,v/v) was added to 40 μL of a plasma. The mixture was sonicated for 10 min. Then, 150 μL of H_2O was added. The mixture was centrifuged for 5 min at 15000 rpm at ambient temperature. An organic layer was collected, vacuum dried and then redissolved in 200 μL 2-propanol-acetonitrile (1:1, v/v) for MS analysis.

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

2.4 Mass spectrometry analysis of lipid extracts

The molecular composition of tissue lipid extracts was determined using electrospray ionization mass spectrometry (ESI-MS) on a Maxis Impact qTOF mass spectrometer (Bruker Daltonics, Bremen, Germany). Mass spectra were obtained in both positive and negative ion detection modes in the *m/z* range of 100-1800 with the following settings: 4.1 kV capillary voltage in positive ion mode (3.0 kV in negative ion mode), spray gas pressure 0.7 bar, drying gas flow rate 6 L/min, the temperature of the drying gas 200°C [26,27].

The molecular composition of plasma lipid fraction was determined by HPLC-MS using a Dionex UltiMate 3000 liquid chromatograph (Thermo Scientific, Germany) connected to a Maxis Impact qTOF mass analyzer with an ESI ion source (Bruker Daltonics, Germany). Lipids were separated by reverse phase chromatography on a Zorbax C18 column (150x2.1 mm, 5 μm, Agilent, USA) with a linear gradient of 30% to 90% eluent B (acetonitrile/2-propanol/water, 90:8:2, v/v/v, with 0.1% formic acid and 10 mM ammonium formate) in 20 min. Acetonitrile/water (60:40, v/v) with of 0.1% formic acid and 10 mM ammonium formate was used as eluent A. The elution flow rate was 40 μL/min. The volume of the injected sample was 3 μL. 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 is 200ºC. Quality control samples were injected randomly between the samples and used to evaluate the quality of our experiments.

Tandem MS analysis (MS/MS) was done using data dependent analysis mode. Five the most abundant peaks were chosen after full MS scan and subjected to MS/MS analysis (CID) with 35 eV collision energy, 3 Da isolation window and mass exclusion time of 1 min.

2.5 Statistical analysis

Lipids from myometrium and fibroids tissues were identified with in-lab created R code (the RStudio version was 1.1.463 and the R language version was 3.5.2) by exact mass within 10 ppm mass accuracy using the theoretical computer-generated database of mass lipids for a given ion, class, total length of fatty acid residues and characteristic tandem mass spectra (MS/MS). Blood plasma lipids were identified using the Lipid Match R-script [55] for the exact mass within 10 ppm mass accuracy [56] and for the tandem mass spectra (MS/MS).

Statistical significance of lipid level changes was studied by a non-parametric two-way Mann-Whitney U-test ($p<0.05$). To determine the metabolic pathways enriched in uterine fibroids, lipid, with significant differences in tissue and plasma, were analyzed by the online resource Metaboanalyst 4.0 (https://www.metaboanalyst.ca/) using hypergeometric test methods and KEGG library for Homo Sapience.

The classification models were built using the discriminant analysis method with orthogonal projection on latent structures (OPLS-DA) for lipids with a significant difference in levels. 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). Potential lipid markers included lipids with the greatest importance of the independent variable for projection (VIP) values according to the OPLS-DA model (VIP>1). The selected lipids were used for creation diagnostic models based on logistic regression with the formula $y = \frac{1}{1+e^{-(\beta_o+\beta*I^t)}}$, where y is variable response with values 0 in cases of control group and 1 in cases of myoma, β_o is free coefficient, β is vector of coefficients, and I is the vector of marker's intensity. Sensitivity and specificity of the models were evaluated by leave-one-out cross-validation.

3. Results and discussion

3.1 Clinical data

The study included 81 women divided into three groups. The first group included 35 women with uterine fibroids (UF) diagnosed for the first time. The

second group included 31 patients diagnosed with recurrent uterine fibroids (RUF). The third control group included 15 patients operated for infertility and for the intrauterine septum.

The patients included in the study were of reproductive age (more than 80% were 36-45 years old). The average age of patients with uterine fibroids was 37.9 ± 5.5 years, and patients with recurrence of myoma - 39.9 ± 5.2 years (Table 1). The clinical diagnosis of patients was done on the basis of an objective examination, ultrasound data and finally verified according to the data of histological examination (Figure 1S). All patients underwent organ-preserving treatment with endoscopic access.

Table 1. Clinical and demographical data for UF and RUF patients.

	First-time diagnosed uterine fibroids (n=35)	Recurrent uterine fibroids (n=31)	p-value
Age, years	37,6±5,5	39,8±5,9	p>0,5
Body mass index	24±5,0	25±4,0	p<0,039
Menarche, years	12,9±1,0	12,8±1,3	p>0,5
Menstrual cycle length, days	27,8±2,4	27,6±3,1	
Duration of menstruation, days	5,4±1,2	5,3±1,4	
Number of pregnancies	1,3±1,8	1,3±1,5	p>0,5
Infertility complaints, %	21,3	31,9	p=0,015
Uterine fibroids in close relatives, %	49,2	56,45	p<0,05
Duration of surgery, min	94,6±39,4	122,7±61,4	p=0,001
Blood loss, mL	234,7	299,2	p=0,001

We discovered that the first myomectomy was performed in the age from 36 to 41 years and the myomectomy caused by RUF was performed in the age from 42 to 45 years.Significant (p <0.039) excess of BMI was observed in patients with RUF. Pain syndrome and problems with the onset and bearing of pregnancy were most pronounced in the RUF group. Patients from the UF group complained of infertility with an average duration of 5.6 ± 4.4 years. In the RUF group, infertility occurred in 31.9% of cases with an average duration of 7 ± 4.5 years.

Diabetes and uterine fibroids are significantly more frequent in the closest relatives ($p<0.05$) for UF and RUF groups compared to control group. The data obtained confirm the presence of a family predisposition of UF. The frequency of detection of submucous and interstitial-submucous fibroids during ultrasound examination is higher for RUF group compared to UF group. Similar data were obtained by assessing the intraoperative localization of nodes. This observation can be explained by the presence of hormonally active tissue near the endometrium and the result of the previous operation (reduction of myometrial tissue and growth of fibroids towards the uterine cavity).

For RUF, a long duration of surgical treatment was observed. This indicates the complexity of the repeated organ-preserving surgery, considerable intraoperative blood loss and more frequently used reinfusion of erythrocytes. The number of removed fibroids was greater in the RUF group, but the size of the removed nodes was higher in the UF group.

According to our data, the percentage of recurrence in the group of UF diagnosed for the first time was 7.9% after 12 months and 15.8% after 2 years. The percentage of recurrence in the group of RUF was 15.8%, after 12 months and 31.2% after 24 months.

During the follow-up period after myomectomy over 12-18 months, pregnancy occurred in 9.7% of cases in the group of first recurrence (second-time diagnosed UF) and in 34.2% of cases in the group of first-time diagnosed UF.

3.2 Uterine myometrium and fibroids lipidomics (ESI-MS/MS)

The total of 296 lipid species was identified in the tissue of the myometrium and fibroids (Table 2). Out of the 296 identified lipid species, 66 lipid species showed statistically significant abundance variation between the diagnosed UF and its recurrence in the tissues of the fibroids and 39 lipid species showed statistically significant abundance variation between the diagnosed UF and its relapse in the tissues of the myometrium (Table 2). Thus, the greatest alterations in the lipid composition during fibroids recurrence were observed in the tumor tissue. The level of 20 lipid species changed significantly both in the myometrium and in the

myomatous nodes during the disease recurrence (Figure 1). For 19 out of these 20 species the increase in expression was found.

Table 2. Lipid species with differential abundance in the tissues with first-time diagnosed UF and RUF.

Lipid class	Number of lipid species		
	Total	Differential lipid species in myometrium	Differential lipid species in the fibroids
All	296	39	66
Ceramides (Cer)	13	2	6
Diacylglycerides (DG)	9	0	0
Fatty acid (FA)	20	10	14
(lyso) phosphotidylcholines (LPC)	(12) 70	(4) 8	14
(lyso) phosphotidylethanolamines (LPE)	(5) 42	7	(1) 9
Phosphotidylserines (PS)	19	4	2
Phosphotidyl Acids (PA)	6	1	2
Sphingomyelins (SM)	18	1	5
Triacylglycerides (TG)	99	3	14
Plasmalogens (PCO, PEO)	57	5	9

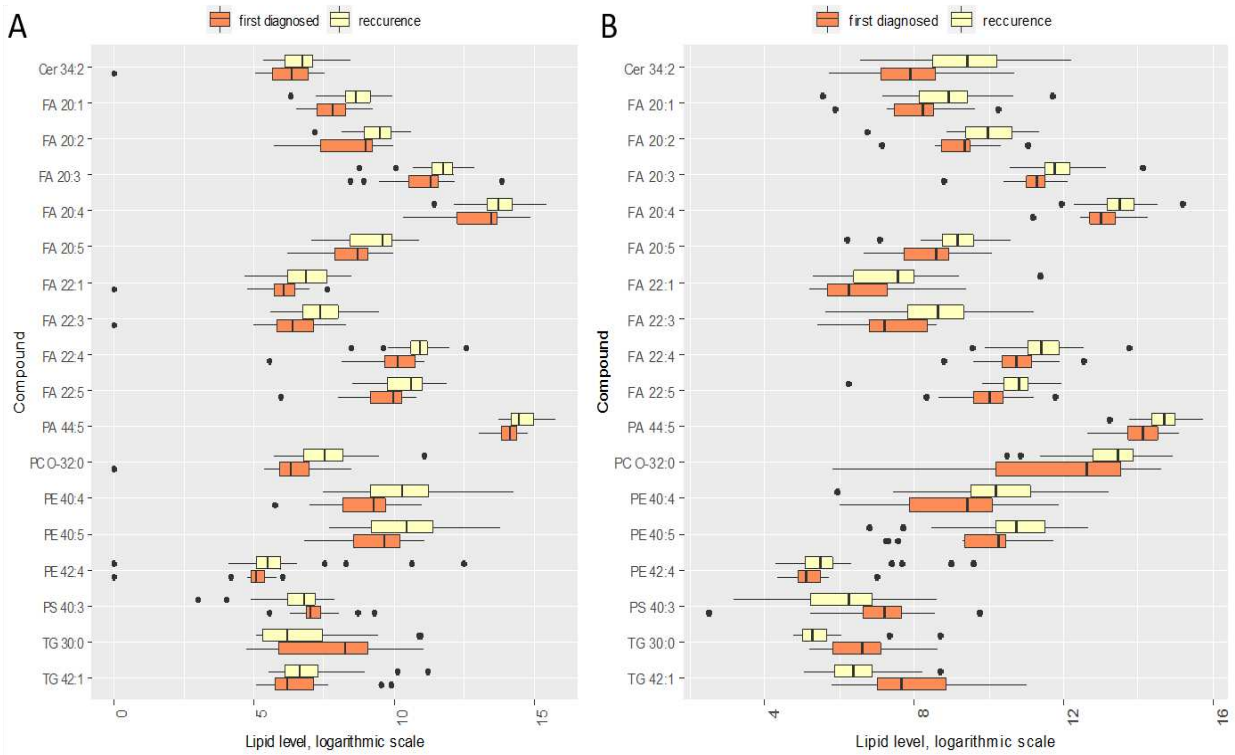

Figure 1. Relative intensity of marker lipids ($p<0.05$) in the mass spectrum of A) myometrium and B) uterine fibroids. Orange color corresponds to the first-time diagnosed UF. Yellow color corresponds to RUF. The diagram shows Q1–1.5*IQR, Q1, Me, Q3, Q3+1.5*IQR. Black dots correspond to outliers.

Enrichment of the linoleic acid, glycerophospholipids, ether lipids, sphingolipids metabolism was shown for benign tumor tissue during recurrence of UF (Figure 2S). Differential lipid species that are statistically significant for both myometrium and fibroids were found to be mainly involved in the metabolism of glycerophospholipids and sphingolipids (Figure 2). This indicates the similarity of metabolic processes for myometrium and fibroids during recurrence of fibroids. In contrast, linoleic acid metabolism undergoes changes only in UF cells. Differences in the metabolism of linoleic acid in UF cells compared with myometrial cells, as well as a changes in the fatty acid profile of the cells were previously noted by Islam and Castellucci [57].

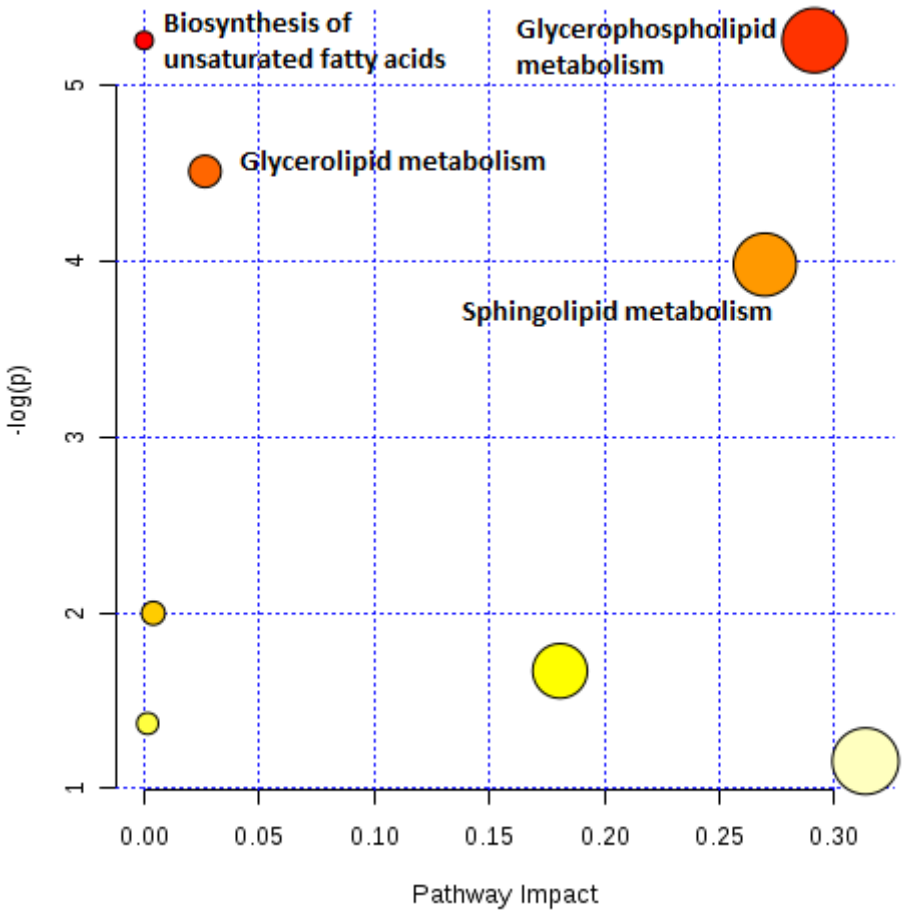

Figure 2. Diagram of metabolic pathways for lipid species with statistically significant abundance variation common for benign tumor and myometrium during recurrence of fibroids.

3.3 Blood plasma lipidomics in recurrent uterine fibroids (HPLC-MS/MS)

The total of 267 lipid species was identified in blood plasma samples. The lipid levels were tested by pairwise Mann-Whitney U-test: “control group vs. first-time diagnosed fibroids”, “control group vs. recurrent fibroids” and “first-time diagnosed fibroids vs. recurrent fibroids”. Statistically significant differences were found for 43 lipid species in the first case (control vs. first-time UF), 64 in the second case (control vs. RUF) and 87 for the third case (first-time UF vs. RUF). OPLS-DA models were constructed to classify patients (Figure 3).

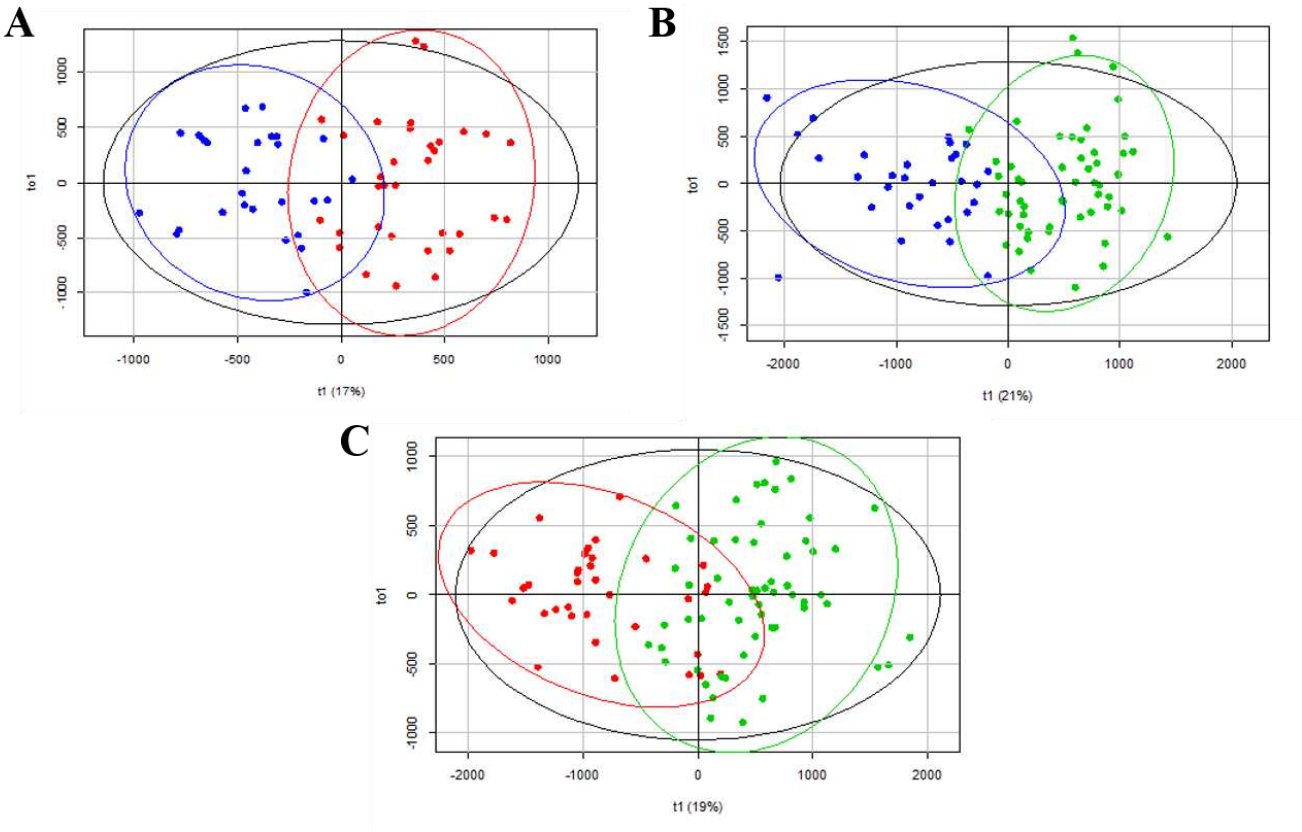

Figure 3. OPLS-DA score plots of plasma lipidomic data (blue dots correspond to control group, red dots correspond to the UF group, and green dots correspond to RUF): A) Control group vs. first-time diagnosed UF B) Control group vs. RUF C) First-time diagnosed UF vs. RUF.

For the OPLS-DA models distinguishing between control group and UF group (Figure 3A) and between control group and RUF group (Figure 3B), 70% and 67% of data were included (R2Y). The expected classification accuracy for new samples (Q2Y) was 63% and 60%, accordingly. The values of R2Y> 50% and Q2Y> 40% suggest that there are significant changes in the lipid profile of blood plasma upon UF. For the OPLS-DA model distinguishing between UF and RUF groups, parameters R2Y and Q2Y were equal to 61% and 47%, respectively (Table 3). Thus, our data indicate that the recurrence of fibroids is accompanied by significant changes in lipid metabolism in the whole body.

Table 3. The parameters of OPLS-DA models.

	Lipids with VIP>1	R^2X	R^2Y	Q^2Y
control group vs first-time diagnosed UF	LPC 18:2, PC 16:0_20:3, PC 18:0_18:1, PC 18:0_20:3, SM d18:1/22:0, SM d18:1/22:1, SM d18:1/24:0, TG 18:0_18:1_18:1	0.49	0.70	0.63
control group vs RUF	PC 16:0_22:6, PC 16:0_18:2, PC 16:0_20:3, PC 18:0_20:3, PC 18:0_18:1, SM d12:0/14:1, SM d18:1/24:1, SM d18:2/24:1	0.36	0.67	0.60
first-time diagnosed UF vs RUF	CE 18:2, CE 20:4, PC 16:0_22:6, PC 18:0_18:2, SM d12:0/14:1, SM d18:1/22:0, SM d18:1/22:1, SM d18:1/24:0, SM d18:1/24:1, SM d18:2/16:0, SM d18:2/24:1, TG 14:1_18:1_18:2, TG 16:0_16:1_18:2, TG 16:0_18:1_18:2, TG 16:1_18:0_18:1, TG 16:1_18:0_18:3, TG 18:1_18:2_18:3	0.32	0.61	0.47

The largest contribution (VIP>1) to the differentiation between the control group and the UF group was provided by phosphotidylcholines and sphingomyelins. Three lipid species, including PC 16:0_20:3, PC 18:0_20:3 and PC 18:0_18:1, were significantly decreased in the blood plasma of UF patients compared to control group (Figures 3S and 4S).

Diagnostic models based on the selected lipid species using logistic regression (Table 4 and Table 5) show sensitivity and specificity of 88% and 86% for the diagnosis of first-time UF and 95% and 79% for the diagnosis of RUF. These results indicate the potential suitability of the lipid profiling of blood plasma for the low-invasive diagnosis of uterine fibroids.

Table 4. Coefficients for logistic regression of diagnostic model “control group/first-time diagnosed UF”

	β	CI β	Z stat	p

Free coefficient	3.49E1	1.84E1 - 6.07E1		
LPC 18:2	-6.46E-6	-2.22E-5 - 7.94E-6	-0.87	0.38
PC 16:0_20:3	-5.72E-6	-2.02E-5 - 6.64E-6	-0.88	0.38
PC 18:0_18:1	-1.65E-6	-2.11Ee-5 - 1.57E-5	-0.18	0.85
PC 18:0_20:3	-1.83E-5	-7.98E-5 - - 1.43E-5	-1.95	0.05
SM d18:1/22:0	-4.23E-5	-7.98E-5 - - 1.43E-5	-2.63	0.01
SM d18:1/22:1	-2.78E-5	-6.16E-5 - - 6.22E-6	-1.97	0.05
SM d18:1/24:0	-9.91E-6	-3.79E-5 - 1.36E-5	-0.79	0.43
TG 16:0_16:1_18:1	-3.22E-6	-1.57E-5 - 7.93E-6	-0.57	0.57

Table 5. Coefficients for the logistic regression of diagnostic model “control group/RUF group”

	β	CI β	Z stat	p
Free coefficient	-1.24E1	-2.33E1 - -4.82E0		
PC 16:0_22:6	2.09E-6	4.36E-7 - 4.10E-6	2.28	0.02
PC 16:0_18:2	2.74E-6	1.08E-6 - 6.0E-6	2.32	0.02
PC 16:0_20:3	-1.21E-6	-9.00E-6 - 4.16E-6	-0.39	0.70
PC 18:0_20:3	8.17E-6	-4.36E-6 2.30E-5	1.21	0.23
SM d12:0/14:1	5.32E-6	2.17E-6 - 1.03E-5	2.64	0.01
SM d18:1/24:1	-5.74E-6	-1.75E-5 - 5.08E-6	-1.02	0.31

SM d18:2/24:1	2.87E-5	2.14E-6 - 6.03E-5	1.99	0.05
PC 18:0_18:1	-1.61E-5	-3.07E-5 - -3.74E-6	-2.39	0.02

Lipid species identified as potentially significant in blood plasma for the differentiation between first-time UF and RUF include cholesterol esters, phosphotidylcholines, sphingomyelins and triglycerides (Table 3, Figure 4). Triglyceride levels were significantly increased in the RUF group. The diagnostic significance of triglycerides alterations in blood plasma upon UF remains a controversial issue. The relationship between the deviations in energy metabolism and the UF development has been demonstrated in earlier studies [58,59].

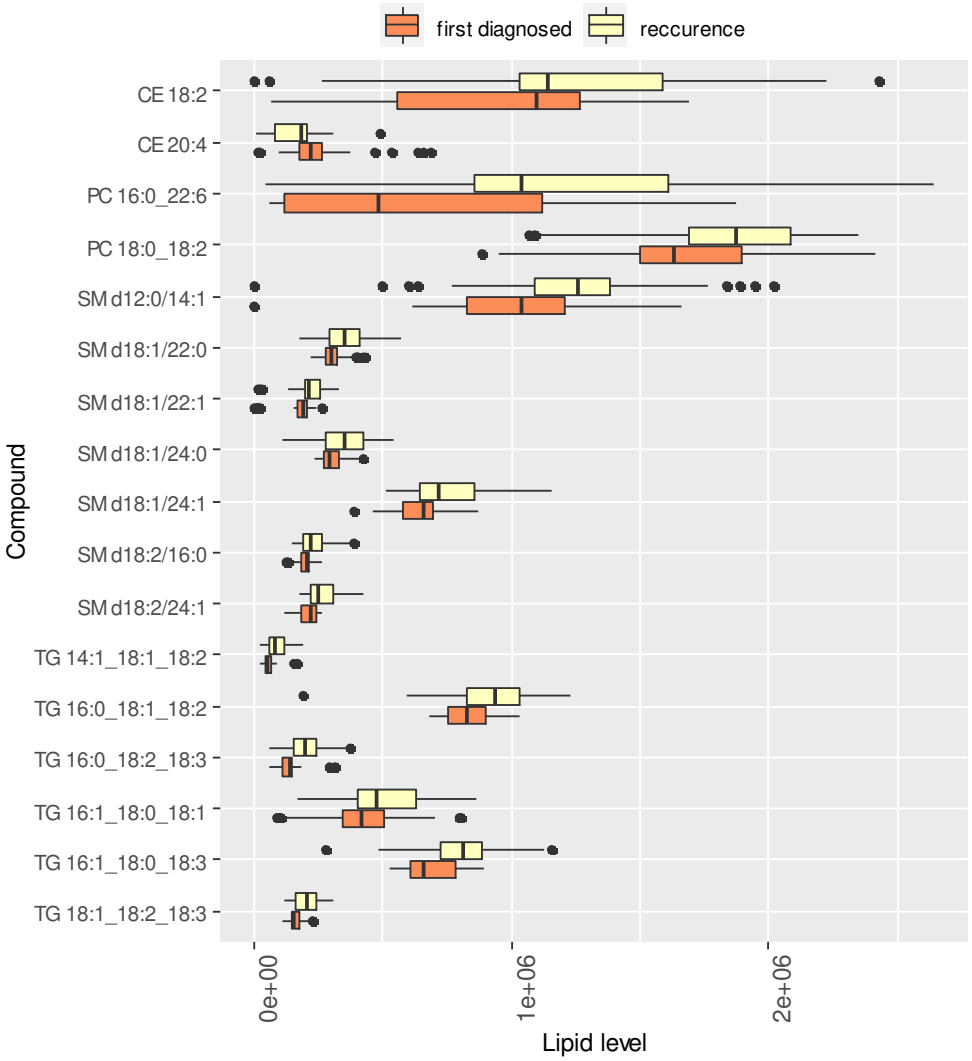

Figure 4. Relative intensity of marker plasma lipids in the OPLS-DA model classifying UF and RUF groups. Orange color corresponds to first-time diagnosed

UF, and yellow color corresponds to the RUF. The diagram shows Q1–1.5*IQR, Q1, Me, Q3, Q3+1.5*IQR. Black dots correspond to outliers.

Conclusions

The results of our comparative study of lipid profiles in blood plasma, UF tissues and myometrium tissues suggest new potential molecular markers for the prediction of UF recurrence. The greatest changes in the lipid composition associated with the UF recurrence were observed in the UF tumor tissue. The level of 20 lipid species showed significant changes both in the myometrium and in the myomatous nodes during the UF recurrence. For 19 out of the 20 differential lipid species the increase in expression was found. In fibroids and myometrium samples, alterations in the level of lipids related to the metabolism of glycerophospholipids and sphingolipids were prominent. In fibroids tissues, linoleic acid metabolism was also notably altered. A number of phospholipids, sphingomyelins, cholesterol esters and triglycerides displayed significantly different levels in blood plasma of women with UF, RUF and the control group. Diagnostic models based on the selected lipids using logistic regression show sensitivity and specificity of 88% and 86% for the diagnosis of first-time UF and 95% and 79% for RUF. These results indicate the potential of the lipid profiling of blood plasma for the low-invasive diagnosis of fibroids. Determination of significant molecular alterations in the tissues of fibroids will make it possible to give recommendations regarding further treatment, rehabilitation and reproductive function correction. Further study of molecular processes in the myometrium and in the fibroids as well as the determination of the ratio of proliferation and apoptosis processes will enhance our mechanistic understanding of UF and its recurrence.

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Conflict of interests. The authors declare that there are no conflicts of interest.

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Figures

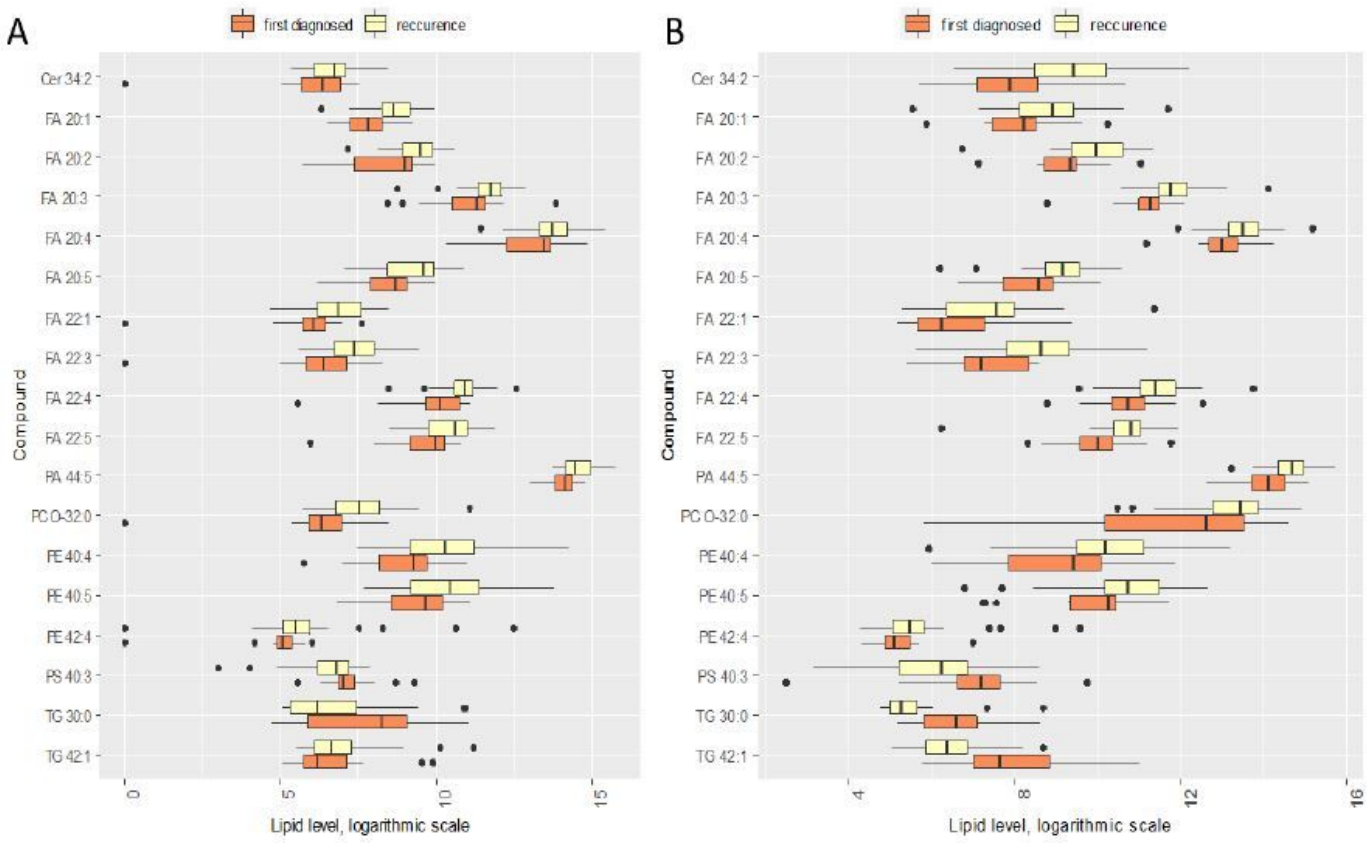

Figure 1

Relative intensity of marker lipids ($p<0.05$) in the mass spectrum of A) myometrium and B) uterine fibroids. Orange color corresponds to the first-time diagnosed UF. Yellow color corresponds to RUF. The diagram shows Q1−1.5*IQR, Q1, Me, Q3, Q3+1.5*IQR. Black dots correspond to outliers.

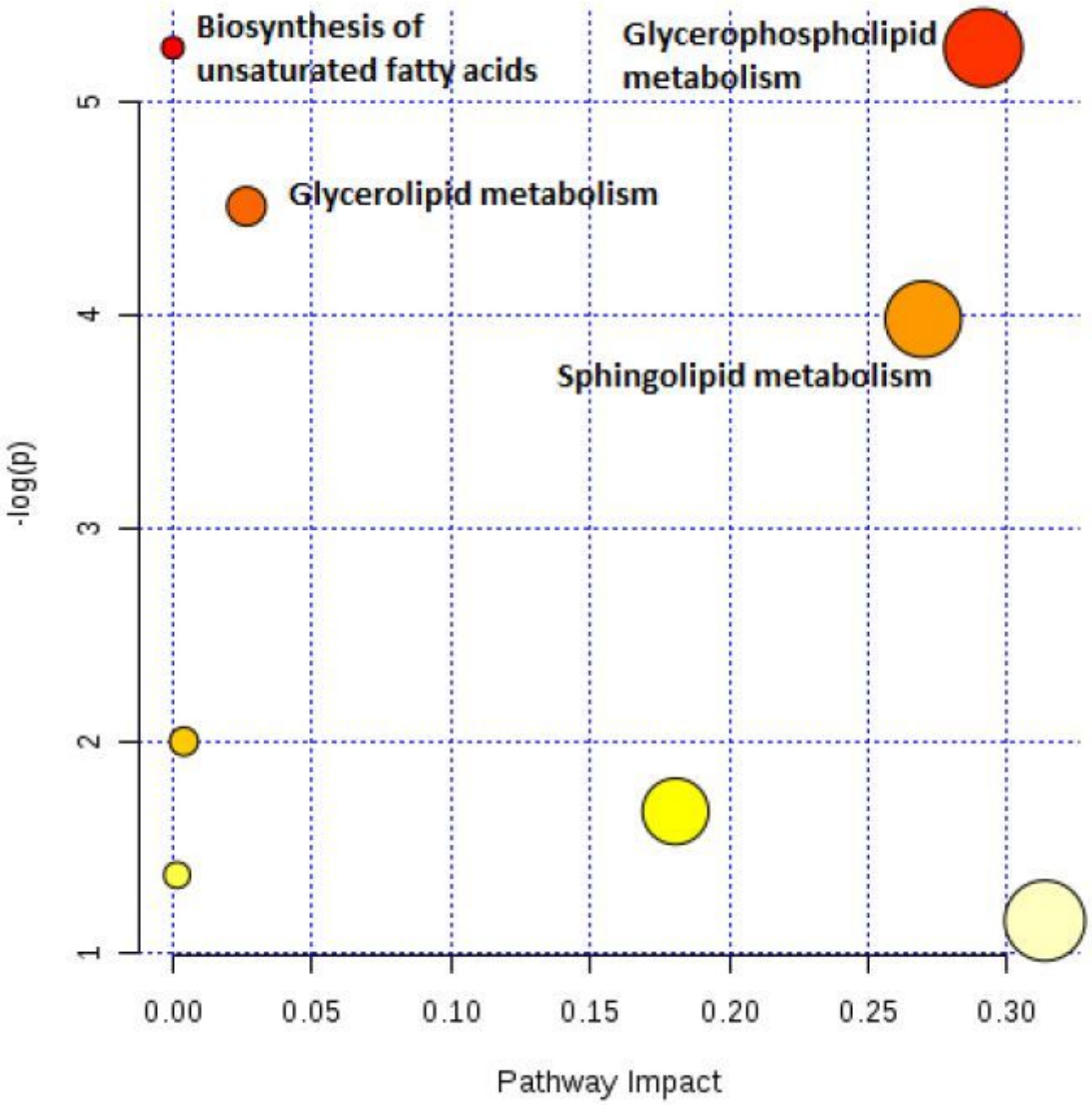

Figure 2

Diagram of metabolic pathways for lipid species with statistically significant abundance variation common for benign tumor and myometrium during recurrence of fibroids.

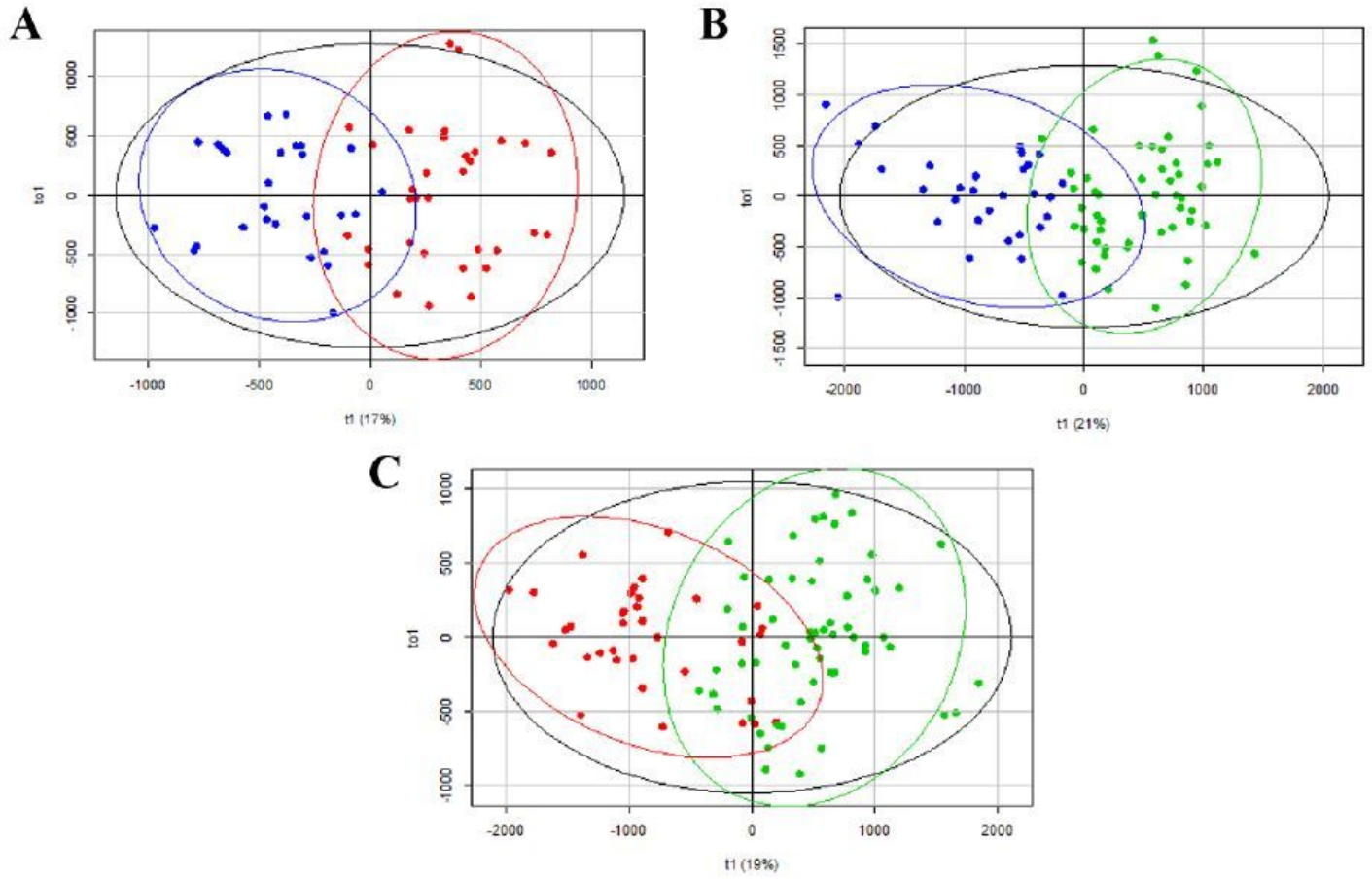

Figure 3

OPLS-DA score plots of plasma lipidomic data (blue dots correspond to control group, red dots correspond to the UF group, and green dots correspond to RUF): A) Control group vs. first-time diagnosed UF B) Control group vs. RUF C) First-time diagnosed UF vs. RUF.

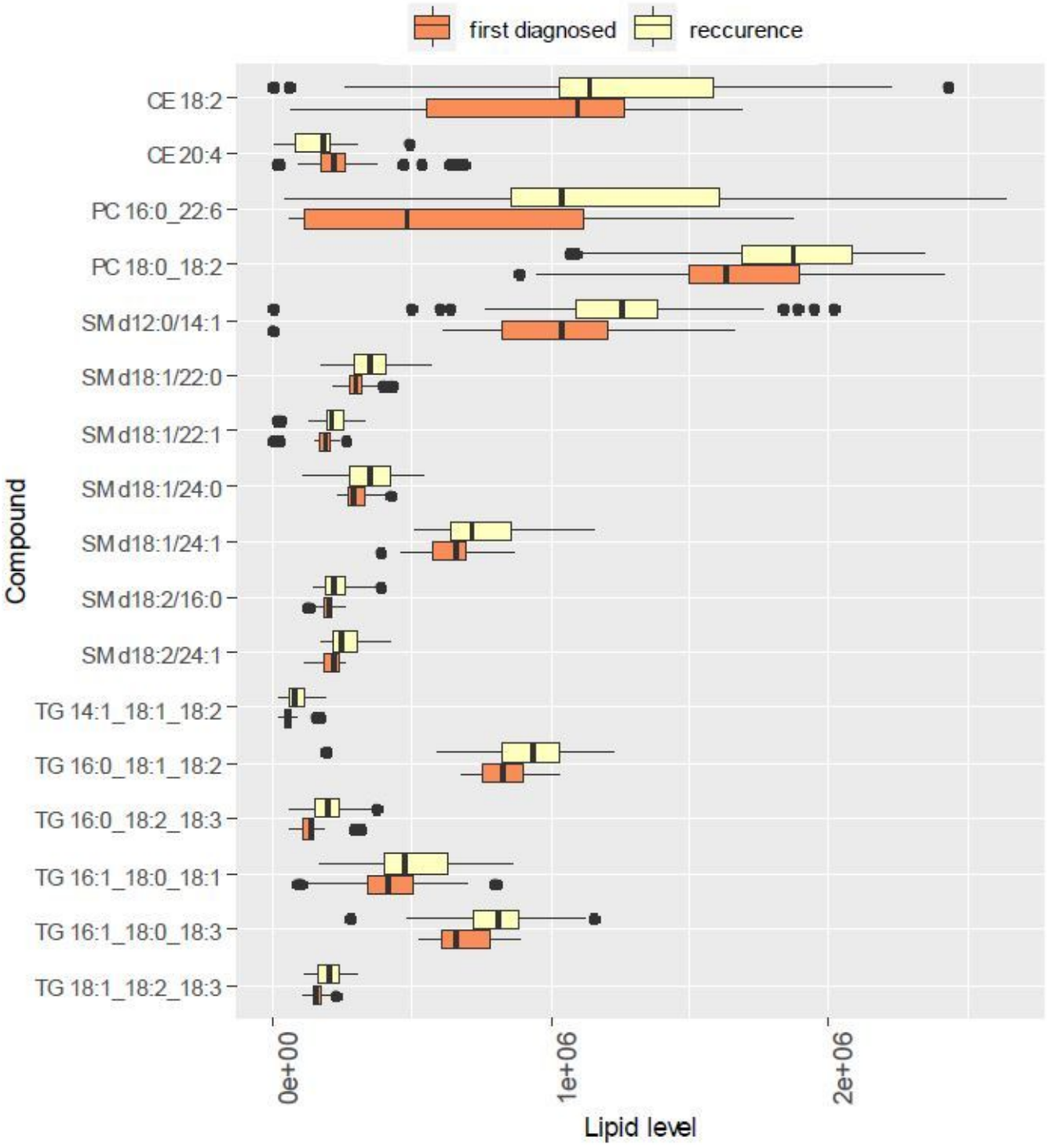

Figure 4

Relative intensity of marker plasma lipids in the OPLS-DA model classifying UF and RUF groups. Orange color corresponds to first-time diagnosed UF, and yellow color corresponds to the RUF. The diagram shows Q1 – 1.5*IQR, Q1, Me, Q3, Q3+1.5*IQR. Black dots correspond to outliers.

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