

Method for diagnosing endometriosis in a subject

Technical field of the invention

The present invention generally relates to the use of a panel of metabolic biomarkers for the diagnosis of endometriosis, and more specifically to an *ex vivo* method for diagnosing
5 endometriosis in a subject.

Background of the invention

Endometriosis (ICD-10 N80) is a complex, benign neoplastic, gynecological disease with ectopic growth of endometrium-like tissue that affects around 170 million women worldwide; around 40,000 new cases are observed annually only in Germany. It manifests itself with dysmenorrhea,
10 dyspareunia, increased risk of systemic or local inflammation, and chronic pelvic pain up to infertility (1, 2, 43). There are three main types of endometriosis: peritoneal endometriosis, ovarian endometriosis and deep infiltrating endometriosis depending on different location of ectopic endometrial tissue in the peritoneal cavity. The endometriosis can be as well manifested in a mixed form e.g. peritoneal and ovarian. Diagnosis is currently always invasive (with possible
15 complications) using laparoscopy and subsequent histological analyzes (3, 4). Treatment for pain relief, prevention of recurrence, and maintenance of fertility includes pain killers and hormonal approaches (5, 6). Due to the high individual variability and unspecific symptoms, which can also be related to other diseases, it takes an average of seven years before endometriosis is finally diagnosed (6, 7). Apart from the diagnostic difficulties mentioned above, there are currently no
20 reliable biomarkers that could predict the presence of endometriosis with high sensitivity and specificity (8, 9).

Present diagnostic procedures for endometriosis

The current gold standard in the present diagnostics is an invasive laparoscopy followed by histochemical analyses for pathology verification (10, 11). The laparoscopy may cause
25 complications (e.g. infections or internal bleeding), is expensive, laborious (needs weeks to months for the communication of final outcome), requires adequate and certified training of participating physicians and pathologist. Sole laparoscopic examination without histological verification of pathology was not recommended in the clinical diagnostic routine (12). Analyses of accuracy of laparoscopy-based diagnosis demonstrated a huge need for new biomarkers (13, 14).

Noninvasive methods like ultrasound and Magnetic Resonance Imaging (MRI) have been checked for applicability to diagnostics as noninvasive approaches despite their huge hardware requirements. Ultrasound and 3D-ultrasound approaches were tested and found to be applicable only to advanced stages of endometriosis. In the disease stages I-II and the III-IV the Area Under the Curve (AUC) was 0.68 and 0.84, respectively, but the methods were ranked as inadequate in routine diagnosis due to significant variability in the operator-dependent specificity and sensitivity (21, 22). The MRI analyses applied to detection of pelvic endometriosis suffered from the same issues in radiologists training. The MRI was found useful in diagnosing endometrial lesions with high specificity but poor sensitivity (23) and consequently not recommended as a replacement for laparoscopy (24).

Health care costs

Some aspects, referred to as indirect costs, cannot be directly calculated like that including loss of life quality due to pelvic pain, inflammation complications or infertility (15). The direct costs such as inpatient, outpatient, surgery, drug and other healthcare service vary among countries due to applied cost refund model. Indirect costs of endometriosis related to lost productivity at work ranged from \$3,314 per patient per year in Austria (16) to \$15,737 per patient per year in the USA (16) and \$17,484 per patient per year in Australia (17). Productivity loss was depicted as around 6,298€ per woman per year affected in Europe (18). The diagnostic golden standard (laparoscopy) is around \$3,313 (19). Ultrasound- and MRI-diagnostics is much more expensive than that by laparoscopy. Long delays in diagnosis of endometriosis may cause up to 34,600 USD all-cause costs (20).

Search for new diagnostic procedures

Plasma miRNA (hsa-miR-125b-5p, hsa-miR-28-5p and hsa-miR-29a-3p) was found to detect endometriosis in infertile women with AUC of 0.60 and not further recommended (25). Several peptides and proteins or antigens present in serum were intensively tested for diagnostics performance. Serum miR-17, IL-4, and IL-6 reveal remarkable AUC of 0.84 in early stages of endometriosis (26) but they are quite unspecific and may reflect inflammatory processes of other origin. A similar issue was found for BDNF (brain-derived neurotrophic factor) which is highly elevated in endometrial tissue (27). The issue is that the BDNF could be as well elevated in structural brain pathology, depression, or persistent nociception (28) or hypoxia (29). The ovarian carcinoma biomarker CA-125 was repurposed for the endometriosis diagnostics but was found to be increased

significantly only in stages III-IV with sensitivity of 46% at specificity of 89% and highly variable AUC in different cohorts (30). A combination of serum D-dimer, CA125 and data on neutrophil-to-lymphocyte ratio performed extremely well for the diagnostics of ovarian cancer (AUC 0.96) but not for the endometriosis (31). Genomic-approaches were so far unsuccessful in finding a single or
5 a combination of genetic feature like methylation markers explaining endometriosis (32-34).

In past research for diagnostic biomarkers of endometriosis, WO2013/178794 studied a single indication of ovarian endometriosis only. In the particular cohort studied it was discovered that metabolite ratios perform far better than single reference values of concentrations (44). It was found that eight lipid metabolites were endometriosis-associated biomarkers due to elevated levels
10 in patients compared with controls. A model containing hydroxysphingomyelin SMOH C16:1 and the ratio between phosphatidylcholine PCaa C36:2 to ether-phospholipid PCae C34:2, adjusted for the effect of age and the BMI, resulted in a sensitivity of 90.0%, a specificity of 84.3% and a ratio of the positive likelihood ratio to the negative likelihood ratio of 48.3. However, this discovery and the associated patent addressed only a single indication of ovarian endometriosis. The later is usually
15 co-discovered in the invasive treatment of ovary and oviduct disorders. Furthermore, the proposed diagnostic model was based on ratio of two metabolites only.

The unmet medical need

In several documented applications the golden standard procedures do not have very high diagnostic performances as described by the AUC, sensitivity or specificity, further by positive
20 predictive value or negative predictive value (35). Despite its wide use the AUC was judged as unreliable measure of screening performance because in practice the standard deviation of a screening or diagnostic test in affected and unaffected individuals can differ and instead detection rate (or sensitivity) and specificity should be used (36). For early cancer diagnostics the specificity, sensitivity or AUC the golden standard diagnostics markers might be really poorly performing but
25 are used because of lack of alternatives in these frequent human disorders.

So far reference values established for different molecular biomarkers like DNA-variants, miRNA, protein or metabolite concentrations were unsuccessful in the clinical practice and never entered clinical routine. WO 2013/178794 addresses a diagnosis of ovarian endometriosis only (sole one form of endometriosis) and was not very attractive to the diagnostic market. The pressing unsolved
30 issue is a procedure for unbiased detection of endometriosis types like peritoneal endometriosis and deep infiltrating endometriosis especially for patients where the endometriosis was not

presumed at the first visit or based on unspecific symptoms. Thus, there remains a significant need to provide innovative methods and means for a cheap, fast, reliable and accurate diagnostic of endometriosis in a subject, notably a human female. Early and unbiased diagnostics of endometriosis would facilitate early hormonal or palliative therapies improving female health.

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Summary of the invention

The present invention is based on the identification and use of a panel of metabolic biomarkers for the diagnosis of endometriosis. However, instead of comparing to reference values in healthy individuals, the present invention uses selected multiple metabolite ratios. Different combinations of metabolite combinations like two predictors (two pairs of two metabolites) and three predictors (three pairs of two metabolites, example is provided in Table 1) were tested for diagnostic performance, and this was surprisingly successful in biostatistical evaluations. This approach has the huge advantage of its insensitivity to human metabolome variability caused by confounders like ethnicity, age, nutrition, lifestyle or medication. The metabolite-based diagnosis method of the present invention provides for a cheap, fast, reliable and accurate way for diagnosing endometriosis in a subject (the diagnostic flow scheme is described in Figures 1 and 2).

The present inventor's findings further reveal the potential for the combination of individual metabolite ratios to provide biomarkers for semi-invasive diagnostics. Moreover, the combination of at least two pairs of metabolites, and more specifically the combination of metabolite ratios thereof, allow distinction of endometriosis from control cases and can be used in the diagnostics of this disease, and are independent of age, BMI and menstrual cycle.

The present invention thus provides in a first aspect the use of a combination of at least two pairs, preferably at least three pairs, of metabolic biomarkers for the diagnosis of endometriosis and/or any sub-type thereof in a subject. In the present invention abbreviations of metabolite names are used which are identifiable by their abbreviations or synonyms as defined in the Table 2 and are known to experts in the field. As there are no diagnostic relevant metabolite concentration reference values established in large human clinical studies for endometriosis, the metabolite ratios and their absolute values in diseased women are compared to that of control samples. The diagnosis is based on calculation of values according to models. For each medical indication only

one example is given. The example of use of metabolites in ratio composition may look like this (example taken from Figure 4):

metabolite1/metabolite2 + metabolite3/metabolite4 + metabolite 5/metabolite6

or using metabolite abbreviations:

- 5 LysoPC a C17:0/SM(OH) C16:1 + Arg/PC ae 36:0 + PC ae C38:0/PC ae C40:0

An overview of indications covered and the corresponding exemplary metabolite ratio compositions used for a diagnostic analysis is shown in table 1. There are further possible distinct metabolite ratio models for each specific type of endometriosis (specific medical indications) but only those with best AUC will be described in detail later. The number of metabolite combinations used is limited by the AUC threshold, i.e. not all possible metabolite combinations would pass biostatistics evaluation for selectivity and sensitivity calculated for AUC. All metabolite combinations with AUC close to 0.5 or less have no diagnostic value and are not listed.

Table 1. Examples of metabolite ratio compositions for best diagnostic performance.

Endometriosis Indication	Example of GLM model formula	AUC	Value for control	Value for case	Log2 Fold change observed
All types	LysoPC a C17:0_div_by_SM(OH) C16:1 + Arg_div_by_PC ae C36:0 + PC ae C38:0_div_by_PC ae C40:0	0.72	114.07	99.48	0.20
Peritoneal	Thr_div_by_SM(OH) C22:2 + PC aa C40:5_div_by_SFA_PC + LysoPC a C16:0_div_by_SM(OH) C16:1	0.83	28.99	36.30	-0.32
Peritoneal mixed	Orn_div_by_PC ae C38:0 + C4_div_by_PC aa C38:4 + Tyr_div_by_PC aa C42:2	0.68	293.09	265.04	0.15
Ovarian	PC aa C36:3_div_by_PC ae C40:5 + LysoPC a C14:0_div_by_PC aa C28:1 + Met_div_by_PC aa C36:3	0.71	37.18	35.89	0.05
Ovarian mixed	C10_div_by_PC aa C36:6 + SM C20:2_div_by_PUFA_PC + PC ae C42:3_div_by_SM(OH) C16:1	0.67	0.71	0.83	-0.23

GLM – generalized linear model, AUC – Area Under the Curve, metabolite abbreviations are explained in Table 2. Values for cases are calculated from concentrations of indicated metabolites according to the model formula. A Log2 fold change (numeric value, defined later as diagnostic score
5 DxS) is calculated according to the used model. The calculated value is used to discriminate between diseased and not affected patient. Negative or positive values in fold change describe the direction of differences of case versus control.

Calculation of ROC and AUC with GLM models and cross-validation of models

As the classic statistical approach proved not to be robustly efficient the metabolite selection was
10 performed by machine learning with randomForest (RF) on all metabolites and all possible metabolite ratios. All calculations are performed on the 10x cross validated data – this means data was randomly divided into 66% training data and 34% test data for each cross validation step. Therefore, every discovered model was validated in data not used for the creation of the model but in an independent data set. In order to narrow down the possible candidates for further modelling
15 with GLM and to obtain receiver-operator curves (ROC) with area under the curve (AUC) calculations with restrictive parameters assuring robust diagnostic performance (described in detail later) were undertaken. From the remaining candidates only those in the top 10% of the performance were selected. In the following all possible combinations for 3-predictor model for the GLM approach were calculated. This results in 67599 possible combinations for these GLMs when
20 leaving out metabolites/metabolite ratios which are derived total sums of measured metabolites. The later would be impractical to measure in a diagnostic assay and were excluded. The number of diagnostically relevant models is clearly limited by the AUC value which drops significantly if all combinations were included. Therefore only several models as listed later are relevant for diagnostics of each endometriosis indication. The GLMs were calculated on the response of samples
25 being in the control group or case group. Although the ROCs with their respective AUCs shown in the following pages show an AUC up to average 0.82 in the test data set, it is still worth to note that it is very well possible to distinguish the responses in the models with a rather fair accuracy by selecting the parameters of the GLMs by RF from all the possible metabolites and ratios. This is not a feasible approach for PLS-DA analysis due to the high likelihood of over-fitting the model (Figure
30 3). All results for cross-validation analyses of diagnostic models will be described for each medical indication in Figures 4-13.

Concept of diagnostic flow

Samples are collected from patients using standard procedures in outpatient and inpatient stations (Figure 1). Plasma is prepared and the metabolite analyses are undertaken with mass spectrometry apparatus. Data gained are undergoing processing with algorithm calculating values indicative of diagnostic status.

Concept of algorithm implementation

The algorithm constitutes of calculation of GLM-values for distinct endometriosis forms. In particular, the calculation can be performed for:

- Detection of any form of endometriosis
- Detection of specific form like ovarian or peritoneal
- Detection of mixed (multiple) forms like ovarian with coincidence of peritoneal and/or infiltrating

The algorithm can be implemented in parallel decision-making flow as depicted in Figure 2.

More specifically, the present invention provides the use of a combination of at least two pairs, preferably at least three pairs, of metabolic biomarkers selected from the group of pairs consisting of LysoPC a C17:0 and SM(OH) C16:1; Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0; LysoPC a C16:0 and SM C18:1; Thr and PC aa C34:3; C18 and LysoPC a C14:0; Ser and PC ae C44:3; Trp and PC ae C38:3; C8 and PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae C38:6; Arg and PC aa C36:6; Thr and SM(OH) C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM C18:0; PC aa C32:0 and PC aa C38:3; C6:1 and Pro; Arg and PC ae C34:0; C6:1 and LysoPC a C20:4; C5-M-DC and PC aa C42:5; LysoPC a C18:2 and PC ae C40:6; LysoPC a C18:2 and PC ae C40:4; PC ae C40:6 and CPT I ratio; LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg and PC ae C34:0; PC ae C34:1 and PC ae C42:0; Orn and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa C36:6; C5 and LysoPC a C17:0; C5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae C40:5; Pro and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and SM(OH) C16:1; Tyr and PC ae C38:0; PC aa C36:3 and PC ae C40:5; LysoPC a C14:0 and PC aa C28:1; Met and PC aa C36:3; PC aa C38:0 and PC ae C36:1; Thr and SM (OH) C22:1; PC aa C28:1 and PC ae C34:3; C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC ae C36:1; C10:1 and PC aa C36:1; PC ae C38:3 and SM C18:1; C12-DC and C14:2;

PC aa C38:3 and PC ae C44:5; C4 and C5:1; LysoPC a C20:4 and PC ae C32:1; LysoPC a C20:4 and PC aa C32:3; C10 and PC aa C36:6; PC ae C42:3 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and LysoPC a C20:4; LysoPC a C20:4 and PC ae C40:2; Ser and PC aa C38:3; C10 and LysoPC a C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC a C18:1 and PC aa C36:1; Gly and PC ae C34:1; Gln and PC

5 ae C30:2; LysoPC a C24:0 and PC ae C42:3; C10:1 and LysoPC a C24:0; Tyr and PC aa C42:4; C3-DC and C18; PC aa C42:1 and SM C22:3; Gly and SM C24:1; PC aa C32:0 and PC aa C40:1; PC aa C36:4 and PC aa C38:0; PC ae C44:3 and CPT I ratio; PC ae C34:0 and PC ae C40:3; C16:2-OH and SM C20:2; C6(C4:1-DC) and SM C16:1; Gly and PC aa C42:5; C0 and SM(OH) C22:2; PC ae C44:6 and SM C22:3; and C10:1 and C14:2-OH;

10 for the diagnosis of endometriosis and/or any sub-type thereof in a subject.

The present invention provides in a further aspect an *ex vivo* method of diagnosing endometriosis and/or any subtype thereof in a subject comprising quantifying in a sample obtained from said subject at least three pairs of metabolic biomarkers. More specifically, the present invention provides an *ex vivo* method of diagnosing endometriosis and/or any subtype thereof in a subject

15 comprising a) quantifying in a sample obtained from said of at least two pairs, preferably at least three pairs, of metabolic biomarkers, determining the ratio for each of the at least two pairs and b) obtaining a diagnostic score using a generalized linear model (GLM). More specifically, the present invention provides an *ex vivo* method of diagnosing endometriosis and/or any subtype thereof in a subject, the method comprising

20 10) quantifying in a sample obtained from said subject at least two pairs, preferably at least three pairs, of metabolic biomarkers selected from the group of pairs consisting of LysoPC a C17:0 and SM(OH) C16:1; Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0; LysoPC a C16:0 and SM C18:1; Thr and PC aa C34:3; C18 and LysoPC a C14:0; Ser and PC ae C44:3; Trp and PC ae C38:3; C8 and PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae

25 C38:6; Arg and PC aa C36:6; Thr and SM(OH) C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM C18:0; PC aa C32:0 and PC aa C38:3; C6:1 and Pro; Arg and PC ae C34:0; C6:1 and LysoPC a C20:4; C5-M-DC and PC aa C42:5; LysoPC a C18:2 and PC ae C40:6; LysoPC a C18:2 and PC ae C40:4; PC ae C40:6 and CPT I ratio; LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg and PC ae C34:0; PC ae C34:1 and PC ae C42:0; Orn

30 and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa C36:6; C5 and LysoPC a C17:0; C5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae C40:5; Pro

and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and SM(OH) C16:1; Tyr and PC ae C38:0; PC aa C36:3 and PC ae C40:5; LysoPC a C14:0 and PC aa C28:1; Met and PC aa C36:3; PC aa C38:0 and PC ae C36:1; Thr and SM (OH) C22:1; PC aa C28:1 and PC ae C34:3; C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC ae C36:1; C10:1 and PC aa C36:1; PC ae C38:3 and SM C18:1; C12-DC and C14:2; PC aa C38:3 and PC ae C44:5; C4 and C5:1; LysoPC a C20:4 and PC ae C32:1; LysoPC a C20:4 and PC aa C32:3; C10 and PC aa C36:6; PC ae C42:3 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and LysoPC a C20:4; LysoPC a C20:4 and PC ae C40:2; Ser and PC aa C38:3; C10 and LysoPC a C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC a C18:1 and PC aa C36:1; Gly and PC ae C34:1; Gln and PC ae C30:2; LysoPC a C24:0 and PC ae C42:3; C10:1 and LysoPC a C24:0; Tyr and PC aa C42:4; C3-DC and C18; PC aa C42:1 and SM C22:3; Gly and SM C24:1; PC aa C32:0 and PC aa C40:1; PC aa C36:4 and PC aa C38:0; PC ae C44:3 and CPT I ratio; PC ae C34:0 and PC ae C40:3; C16:2-OH and SM C20:2; C6(C4:1-DC) and SM C16:1; Gly and PC aa C42:5; C0 and SM(OH) C22:2; PC ae C44:6 and SM C22:3; and C10:1 and C14:2-OH;

and b) obtaining a diagnostic score using a generalized linear model (GLM).

The present invention may be further characterized by the following items:

1. Use of a combination of at least two pairs, preferably at least three pairs, of metabolic biomarkers for the diagnosis of endometriosis and/or any sub-type thereof in a subject.
2. Use according to item 1, wherein a combination of at least three pairs of metabolic biomarkers for the diagnosis of endometriosis and/or any sub-type thereof in a subject, wherein the diagnosis involves use of the quantification of at least three pairs of metabolic biomarkers in a sample obtained from said subject.
3. Use according to item 1 or 2, wherein the at least two pairs, preferably at least three pairs, of metabolic biomarkers are selected from the group of pairs consisting of LysoPC a C17:0 and SM(OH) C16:1; Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0; LysoPC a C16:0 and SM C18:1; Thr and PC aa C34:3; C18 and LysoPC a C14:0; Ser and PC ae C44:3; Trp and PC ae C38:3; C8 and PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae C38:6; Arg and PC aa C36:6; Thr and SM(OH) C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM C18:0; PC aa C32:0 and PC aa C38:3; C6:1 and Pro; Arg and PC ae C34:0; C6:1 and LysoPC a C20:4; C5-M-DC and PC aa C42:5; LysoPC a C18:2 and PC ae C40:6; LysoPC a C18:2 and PC ae C40:4; PC ae

C40:6 and CPT I ratio; LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg and PC ae C34:0; PC ae C34:1 and PC ae C42:0; Orn and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa C36:6; C5 and LysoPC a C17:0; C5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae C40:5; Pro and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and
 5 SM(OH) C16:1; Tyr and PC ae C38:0; PC aa C36:3 and PC ae C40:5; LysoPC a C14:0 and PC aa C28:1; Met and PC aa C36:3; PC aa C38:0 and PC ae C36:1; Thr and SM (OH) C22:1; PC aa C28:1 and PC ae C34:3; C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC ae C36:1; C10:1 and PC aa C36:1; PC ae C38:3 and SM C18:1; C12-DC and C14:2; PC aa C38:3 and PC ae C44:5; C4 and C5:1; LysoPC a C20:4 and PC ae C32:1; LysoPC a C20:4 and PC aa C32:3; C10
 10 and PC aa C36:6; PC ae C42:3 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and LysoPC a C20:4; LysoPC a C20:4 and PC ae C40:2; Ser and PC aa C38:3; C10 and LysoPC a C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC a C18:1 and PC aa C36:1; Gly and PC ae C34:1; Gln and PC ae C30:2; LysoPC a C24:0 and PC ae C42:3; C10:1 and LysoPC a C24:0; Tyr and PC aa C42:4; C3-DC and C18; PC aa C42:1 and SM C22:3; Gly and SM C24:1; PC aa C32:0 and PC aa C40:1; PC aa C36:4 and PC aa C38:0; PC ae C44:3 and CPT I ratio; PC ae C34:0 and PC ae C40:3; C16:2-
 15 OH and SM C20:2; C6(C4:1-DC) and SM C16:1; Gly and PC aa C42:5; C0 and SM(OH) C22:2; PC ae C44:6 and SM C22:3; and C10:1 and C14:2-OH;

for the diagnosis of endometriosis and/or any sub-type thereof.

4. The use according to any one of items 1 to 3, for diagnosing all endometriosis, wherein the
 20 at least two pairs, preferably at least three pairs, of metabolic biomarkers are selected from the group of pairs consisting of LysoPC a C17:0 and SM(OH) C16:1; Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0; LysoPC a C16:0 and SM C18:1; Thr and PC aa C34:3; C18 and LysoPC a C14:0; Ser and PC ae C44:3; Trp and PC ae C38:3; C8 and PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae C38:6; Arg and PC aa C36:6; Tyr and PC aa C42:4; C3-DC and C18; PC aa C42:1
 25 and SM C22:3; and C6(C4:1-DC) and SM C16:1.
5. The use according to any one of items 1 to 3, for diagnosing peritoneal endometriosis, wherein the at least two pairs, preferably at least three pairs, of metabolic biomarkers are selected from the group of pairs consisting of Thr and SM(OH) C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM C18:0; PC aa C32:0 and PC aa C38:3; C6:1 and Pro; Arg
 30 and PC ae C34:0; C6:1 and LysoPC a C20:4; C5-M-DC and PC aa C42:5; LysoPC a C18:2 and PC

ae C40:6; LysoPC a C18:2 and PC ae C40:4; PC ae C40:6 and CPT I ratio; LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg and PC ae C34:0; and PC ae C34:1 and PC ae C42:0.

6. The use according to any one of items 1 to 3, for diagnosing peritoneal mixed endometriosis, wherein the at least two pairs, preferably at least three pairs, of metabolic biomarkers are selected from the group of pairs consisting of Orn and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa C36:6; C5 and LysoPC a C17:0; C5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae C40:5; Pro and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and SM(OH) C16:1; Tyr and PC ae C38:0; SM C18:0 and C5; Gly and SM C24:1; PC aa C32:0 and PC aa C40:1; PC aa C36:4 and PC aa C38:0; Gly and PC aa C42:5; and C0 and SM(OH) C22:2.
7. The use according to any one of items 1 to 3, for diagnosing ovarian endometriosis, wherein the at least two pairs, preferably at least three pairs, of metabolic biomarkers are selected from the group of pairs consisting of PC aa C36:3 and PC ae C40:5; LysoPC a C14:0 and PC aa C28:1; Met and PC aa C36:3; PC aa C38:0 and PC ae C36:1; Thr and SM (OH) C22:1; PC aa C28:1 and PC ae C34:3; C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC ae C36:1; C10:1 and PC aa C36:1; PC ae C38:3 and SM C18:1; C12-DC and C14:2; PC aa C38:3 and PC ae C44:5; C4 and C5:1; LysoPC a C20:4 and PC ae C32:1; LysoPC a C20:4 and PC aa C32:3; C0 and C5-M-DC; C3 and PC ae 34:0.
8. The use according to any one of items 1 to 3, for diagnosing ovarian mixed endometriosis, wherein the at least two pairs, preferably at least three pairs, of metabolic biomarkers are selected from the group of pairs consisting of C10 and PC aa C36:6; PC ae C42:3 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and LysoPC a C20:4; LysoPC a C20:4 and PC ae C40:2; Ser and PC aa C38:3; C10 and LysoPC a C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC a C18:1 and PC aa C36:1; Gly and PC ae C34:1; Gln and PC ae C30:2; LysoPC a C24:0 and PC ae C42:3; C10:1 and LysoPC a C24:0; PC ae C44:3 and CPT I ratio; PC ae C34:0 and PC ae C40:3; C16:2-OH and SM C20:2; PC ae C44:6 and SM C22:3; and C10:1 and C14:2-OH.
9. The use according to any one of items 1 to 8, wherein the diagnosis involves use of a generalized linear model (GLM) based on the quantification of the at least two pairs, preferably at least three pairs, of metabolic biomarkers in a sample obtained from said subject.

10. The use according to item 9, wherein the generalized linear modelling (GLM) comprises i) determining the ratio of the concentrations for each of the at least two pairs, preferably at least three pairs, of metabolic biomarkers; and ii) calculating the sum of the obtained ratios (value for case).

5 11. The use according to item 10, wherein the generalized linear modelling (GLM) further comprises iii) obtaining a diagnostic score (DxS) calculated by forming the quotient between a predetermined reference value obtained from healthy subjects (value for control) and the sum of the obtained ratios (value for case)

$$DxS = \log_2 \left(\frac{\text{predetermined reference value (value for control)}}{\text{sum of the obtained ratios (value for case)}} \right)$$

10 12. Wherein said subject is diagnosed of having endometriosis or a sub-type thereof if the diagnostic score is different from zero ("0"), such as outside of the range 0 ± 0.03 .

13. An *ex vivo* method of diagnosing endometriosis and/or any subtype thereof in a subject comprising quantifying in a sample obtained from said subject at least three pairs of metabolic biomarkers.

15 14. The method according to item 12, which comprises quantifying in a sample obtained from said subject at least two pairs, preferably at least three pairs, of metabolic biomarkers.

14. The method according to item 12 or 13, wherein the at least two, preferably at least three pairs, of metabolic biomarkers are selected from the group of pairs consisting of LysoPC a C17:0 and SM(OH) C16:1; Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0; LysoPC a C16:0 and SM C18:1; Thr and PC aa C34:3; C18 and LysoPC a C14:0; Ser and PC ae C44:3; Trp and PC ae C38:3; C8 and PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae C38:6; Arg and PC aa C36:6; Thr and SM(OH) C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM C18:0; PC aa C32:0 and PC aa C38:3; C6:1 and Pro; Arg and PC ae C34:0; C6:1 and LysoPC a C20:4; C5-M-DC and PC aa C42:5; LysoPC a C18:2 and PC ae C40:6; LysoPC a C18:2 and PC ae C40:4; PC ae C40:6 and CPT I ratio; LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg and PC ae C34:0; PC ae C34:1 and PC ae C42:0; Orn and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa C36:6; C5 and LysoPC a C17:0; C5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae C40:5; Pro and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and SM(OH) C16:1; Tyr and PC ae C38:0; PC aa C36:3 and PC ae C40:5; LysoPC a

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C14:0 and PC aa C28:1; Met and PC aa C36:3; PC aa C38:0 and PC ae C36:1; Thr and SM (OH)
 C22:1; PC aa C28:1 and PC ae C34:3; C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC
 ae C36:1; C10:1 and PC aa C36:1; PC ae C38:3 and SM C18:1; C12-DC and C14:2; PC aa C38:3
 and PC ae C44:5; C4 and C5:1; LysoPC a C20:4 and PC ae C32:1; LysoPC a C20:4 and PC aa
 5 C32:3; C10 and PC aa C36:6; PC ae C42:3 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and
 LysoPC a C20:4; LysoPC a C20:4 and PC ae C40:2; Ser and PC aa C38:3; C10 and LysoPC a
 C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC a C18:1 and PC aa C36:1; Gly and PC ae C34:1;
 Gln and PC ae C30:2; LysoPC a C24:0 and PC ae C42:3; C10:1 and LysoPC a C24:0; Tyr and PC
 aa C42:4; C3-DC and C18; PC aa C42:1 and SM C22:3; Gly and SM C24:1; PC aa C32:0 and PC
 10 aa C40:1; PC aa C36:4 and PC aa C38:0; PC ae C44:3 and CPT I ratio; PC ae C34:0 and PC ae
 C40:3; C16:2-OH and SM C20:2; C6(C4:1-DC) and SM C16:1; Gly and PC aa C42:5; C0 and
 SM(OH) C22:2; PC ae C44:6 and SM C22:3; and C10:1 and C14:2-OH.

15. The method according to item 13 or 14, wherein the generalized linear modelling comprises
 i) determining the ratio of the concentrations for each of the at least two pairs and ii)
 15 calculating the sum of the obtained ratios (value for case).
16. The method according to item 15, wherein the generalized linear modelling (GLM) further
 comprises iii) obtaining a diagnostic score (DxS) calculated by forming the quotient between
 a predetermined reference value obtained from healthy subjects (value for control) and the
 sum of the obtained ratios (value for case)

$$20 \quad DxS = \log_2 \left(\frac{\text{predetermined reference value (value for control)}}{\text{sum of the obtained ratios (value for case)}} \right)$$

wherein said subject is diagnosed of having endometriosis or a sub-type thereof if the
 diagnostic score is different from zero ("0") in the range 0 ± 0.03 . The DxS is enabling
 mathematic values obtained from calculations of metabolite ratios according to models
 (GLMs in the diagnostics). DxS values in the range of 0 ± 0.03 are not facilitating diagnosis of
 25 specific indication of endometriosis type and other models have to be taken into the
 consideration as described in figures 1 and 2.

17. The method according to any one of items 12 to 16, comprising determining whether the
 subject is suffering from any type of endometriosis.
18. The method according to item 17, comprising

5 A1) quantifying in a sample obtained from said subject at least two pairs, preferably at least three pairs, of metabolic biomarkers selected from the group of pairs consisting of LysoPC a C17:0 and SM(OH) C16:1; Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0; LysoPC a C16:0 and SM C18:1; Thr and PC aa C34:3; C18 and LysoPC a C14:0; Ser and PC ae C44:3; Trp and PC ae C38:3; C8 and PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae C38:6; Arg and PC aa C36:6; and Tyr and PC aa C42:4; C3-DC and C18; PC aa C42:1 and SM C22:3; and C6(C4:1-DC) and SM C16:1; and

B1) obtaining a diagnostic score using a generalized linear model (GLM).

10 19. The method according to item 17 or 18, comprising any one of the following procedures (1) to (15):

- 15 (1) quantifying in a sample obtained from said subject the metabolites LysoPC a C17:0, SM(OH) C16:1, Arg, PC ae C36:0, PC ae C38:0 and PC ae C40:0; and performing GLM using the model formula $\text{LysoPC a C17:0_div_by_SM(OH) C16:1} + \text{Arg_div_by_PC ae C36:0} + \text{PC ae C38:0_div_by_PC ae C40:0}$;
- (2) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC ae C36:0, LysoPC a C16:0, SM C18:1, PC ae C38:0 and PC ae C40:0; and performing GLM using the model formula $\text{Arg_div_by_PC ae C36:0} + \text{LysoPC a C16:0_div_by_SM C18:1} + \text{PC ae C38:0_div_by_PC ae C40:0}$;
- 20 (3) quantifying in a sample obtained from said subject the metabolic biomarkers Thr, PC aa C34:3, LysoPC a C17:0, SM(OH) C16:1, Arg and PC ae C36:0; and performing GLM using the model formula $\text{Thr_div_by_PC aa C34:3} + \text{LysoPC a C17:0_div_by_SM(OH) C16:1} + \text{Arg_div_by_PC ae C36:0}$;
- (4) quantifying in a sample obtained from said subject the metabolic biomarkers Thr, PC aa C34:3, Arg, PC ae C36:0, LysoPC a C16:0 and SM C18:1; and performing GLM using the model formula $\text{Thr_div_by_PC aa C34:3} + \text{Arg_div_by_PC ae C36:0} + \text{LysoPC a C16:0_div_by_SM C18:1}$;
- 25 (5) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC aa C36:6, LysoPC a C17:0, SM(OH) C16:1, and PC ae C36:0; and

performing GLM using the model formula $\text{Arg_div_by_PC} \propto \text{C36:6} + \text{LysoPC} \propto \text{C17:0_div_by_SM(OH) C16:1} + \text{Arg_div_by_PC} \propto \text{C36:0}$;

- (6) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC $\propto \text{C17:0}$, SM(OH) C16:1, Arg, PC $\propto \text{C36:0}$, C18 and LysoPC $\propto \text{C14:0}$; and performing GLM using the model formula $\text{LysoPC} \propto \text{C17:0_div_by_SM(OH) C16:1} + \text{Arg_div_by_PC} \propto \text{C36:0} + \text{C18_div_by_LysoPC} \propto \text{C14:0}$;
- (7) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC $\propto \text{C16:0}$, SM C18:1, PC $\propto \text{C38:0}$, PC $\propto \text{C40:0}$, Ser and PC $\propto \text{C44:3}$; and performing GLM using the model formula $\text{LysoPC} \propto \text{C16:0_div_by_SM C18:1} + \text{PC} \propto \text{C38:0_div_by_PC} \propto \text{C40:0} + \text{Ser_div_by_PC} \propto \text{C44:3}$;
- (8) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC $\propto \text{C17:0}$, SM(OH) C16:1, Arg, PC $\propto \text{C36:0}$, Trp, PC $\propto \text{C38:3}$; and performing GLM using the model formula $\text{LysoPC} \propto \text{C17:0_div_by_SM(OH) C16:1} + \text{Arg_div_by_PC} \propto \text{C36:0} + \text{Trp_div_by_PC} \propto \text{C38:3}$;
- (9) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC $\propto \text{C36:0}$, LysoPC $\propto \text{C16:0}$, SM C18:1, C8 and PC $\propto \text{C30:0}$; and performing GLM using the model formula $\text{Arg_div_by_PC} \propto \text{C36:0} + \text{LysoPC} \propto \text{C16:0_div_by_SM C18:1} + \text{C8_div_by_PC} \propto \text{C30:0}$;
- (10) quantifying in a sample obtained from said subject the metabolic biomarkers Thr, PC $\propto \text{C36:5}$, Arg, PC $\propto \text{C36:0}$, LysoPC $\propto \text{C16:0}$ and SM C18:1; and performing GLM using the model formula $\text{Thr_div_by_PC} \propto \text{C36:5} + \text{Arg_div_by_PC} \propto \text{C36:0} + \text{LysoPC} \propto \text{C16:0_div_by_SM C18:1}$;
- (11) quantifying in a sample obtained from said subject the metabolite Arg, PC $\propto \text{C36:0}$, C18, LysoPC $\propto \text{C14:0}$, LysoPC $\propto \text{C16:0}$ and SM C18:1; and performing GLM using the model formula $\text{Arg_div_by_PC} \propto \text{C36:0} + \text{C18_div_by_LysoPC} \propto \text{C14:0} + \text{LysoPC} \propto \text{C16:0_div_by_SM C18:1}$;
- (12) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC $\propto \text{C36:0}$, C10, PC $\propto \text{C38:6}$, LysoPC $\propto \text{C16:0}$ and SM C18:1; and performing

- GLM using the model formula $\text{Arg_div_by_PC} \propto \text{C36:0} + \text{C10_div_by_PC} \propto \text{C38:6} + \text{LysoPC} \propto \text{C16:0_div_by_SM} \propto \text{C18:1}$;
- (13) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC aa C36:6, PC ae C36:0, LysoPC a C16:0 and SM C18:1; and performing GLM using the model formula $\text{Arg_div_by_PC} \propto \text{C36:6} + \text{Arg_div_by_PC} \propto \text{C36:0} + \text{LysoPC} \propto \text{C16:0_div_by_SM} \propto \text{C18:1}$;
- (14) quantifying in a sample obtained from said subject the metabolic biomarkers Tyr, PC aa C42:4, C3-DC, C18, PC aa C42:1 and SM C22:3; and performing GLM using the model formula $\text{Tyr_div_by_PC} \propto \text{C42:4} + \text{C3-DC_div_by_C18} + \text{PC} \propto \text{C42:1_div_by_SM} \propto \text{C22:3}$;
- (15) quantifying in a sample obtained from said subject the metabolic biomarkers C3-DC, C18, PC aa C42:1, SM C22:3, C6(C4:1-DC) and SM C16:1; and performing GLM using the model formula $\text{C3-DC_div_by_C18} + \text{PC} \propto \text{C42:1_div_by_SM} \propto \text{C22:3} + \text{C6} \propto \text{C4:1-DC_div_by_SM} \propto \text{C16:1}$.
20. The method according to any one of items 12 to 19, comprising determining whether the subject is suffering from peritoneal endometriosis.
21. The method according to item 20, comprising
- A2) quantifying in a sample obtained from said subject at least two pairs, preferably at least three pairs, of metabolic biomarkers selected from the group of pairs consisting of Thr and SM(OH) C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM C18:0; PC aa C32:0 and PC aa C38:3; C6:1 and Pro; Arg and PC ae C34:0; C6:1 and LysoPC a C20:4; C5-M-DC and PC aa C42:5; LysoPC a C18:2 and PC ae C40:6; LysoPC a C18:2 and PC ae C40:4; PC ae C40:6 and CPT I ratio; LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg and PC ae C34:0; and PC ae C34:1 and PC ae C42:0; and
- B2) obtaining a diagnostic score using a generalized linear model (GLM).
22. The method according to item 20 or 21, comprising any one of the following procedures (1) to (7):
- (1) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C16:0, SM(OH) C16:1, PC aa C32:0, SM C18:0, PC aa C32:0 and PC aa C38:3; and

- performing GLM using the model formula $\text{LysoPC a C16:0_div_by_SM(OH) C16:1} + \text{PC aa C32:0_div_by_SM C18:0} + \text{PC aa C32:0_div_by_PC aa C38:3}$;
- (2) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C16:0, SM(OH) C16:1, PC aa C32:0, SM C18:0, Arg and PC ae C34:0; and performing GLM using the model formula $\text{LysoPC a C16:0_div_by_SM(OH) C16:1} + \text{PC aa C32:0_div_by_SM C18:0} + \text{Arg_div_by_PC ae C34:0}$;
- (3) quantifying in a sample obtained from said subject the metabolic biomarkers C5-M-DC, PC aa C42:5, Arg, PC ae C34:0, LysoPC a C18:2 and PC ae C40:6; and performing GLM using the model formula $\text{C5-M-DC_div_by_PC aa C42:5} + \text{Arg_div_by_PC ae C34:0} + \text{LysoPC a C18:2_div_by_PC ae C40:6}$;
- (4) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C18:2, PC ae C40:4, PC ae C40:6, CPT I ratio, LysoPC a C17:0 and SM C18:0; and performing GLM using the model formula $\text{LysoPC a C18:2_div_by_PC ae C40:4} + \text{PC ae C40:6_div_by_CPT I ratio} + \text{LysoPC a C17:0_div_by_SM C18:0}$;
- (5) quantifying in a sample obtained from said subject the metabolic biomarkers C4, PC ae C30:2, Arg, PC ae C34:0, LysoPC a C18:2 and PC ae C40:6; and performing GLM using the model formula $\text{C4_div_by_PC ae C30:2} + \text{Arg_div_by_PC ae C34:0} + \text{LysoPC a C18:2_div_by_PC ae C40:6}$;
- (6) quantifying in a sample obtained from said subject the metabolic biomarkers PC ae C40:6, CPT I ratio, C4, PC ae C30:2, LysoPC a C18:2 and PC ae C40:6; and performing GLM using the model formula $\text{PC ae C40:6_div_by_CPT I ratio} + \text{C4_div_by_PC ae C30:2} + \text{LysoPC a C18:2_div_by_PC ae C40:6}$;
- (7) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C18:2, PC ae C40:4, Arg, PC ae C34:0, PC ae C34:1 and PC ae C42:0; and performing GLM using the model formula $\text{LysoPC a C18:2_div_by_PC ae C40:4} + \text{Arg_div_by_PC ae C34:0} + \text{PC ae C34:1_div_by_PC ae C42:0}$.
23. The method according to any one of items 12 to 22, comprising determining whether the subject is suffering from peritoneal mixed endometriosis.
24. The method according to item 23, comprising

- A3) quantifying in a sample obtained from said subject at least two pairs, preferably at least three pairs, of metabolic biomarkers selected from the group of pairs consisting of Orn and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa C36:6; C5 and LysoPC a C17:0; C5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae C40:5; Pro and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and SM(OH) C16:1; Tyr and PC ae C38:0; Gly and SM C24:1; PC aa C32:0 and PC aa C40:1; PC aa C36:4 and PC aa C38:0; Gly and PC aa C42:5; and C0 and SM(OH) C22:2; and
- B3) performing generalized linear modelling (GLM).
25. The method according to item 23 or 24, comprising any one of the following procedures (1) to (16):
- (1) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, C4, PC aa C38:4, Tyr and PC aa C42:2; and performing GLM using the model formula $\text{Orn_div_by_PC ae C38:0} + \text{C4_div_by_PC aa C38:4} + \text{Tyr_div_by_PC aa C42:2}$;
 - (2) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC aa C36:6, C5 and LysoPC a C17:0; and performing GLM using the model formula $\text{Arg_div_by_PC aa C36:6} + \text{C5_div_by_lysoPC a C17:0} + \text{C5_div_by_Arg}$;
 - (3) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, C5, LysoPC a C17:0 and Arg; and performing GLM using the model formula $\text{Orn_div_by_PC ae C38:0} + \text{C5_div_by_lysoPC a C17:0} + \text{C5_div_by_Arg}$;
 - (4) quantifying in a sample obtained from said subject the metabolic biomarkers C0, Gly, Orn, PC ae C38:0, Tyr and PC aa C42:2; and performing GLM using the model formula $\text{C0_div_by_Gly} + \text{Orn_div_by_PC ae C38:0} + \text{Tyr_div_by_PC aa C42:2}$;
 - (5) quantifying in a sample obtained from said subject the metabolic biomarkers SM C18:0, C5, LysoPC a C17:0 and Arg; and performing GLM using the model formula $\text{SM C18:0} + \text{C5_div_by_lysoPC a C17:0} + \text{C5_div_by_Arg}$;
 - (6) quantifying in a sample obtained from said subject the metabolic biomarkers C5, LysoPC a C17:0, Arg, Ser and SM(OH) C16:1; and performing GLM using the model formula $\text{C5_div_by_lysoPC a C17:0} + \text{C5_div_by_Arg} + \text{Ser_div_by_SM(OH) C16:1}$;

- (7) quantifying in a sample obtained from said subject the metabolic biomarkers SM C18:0, C0, Gly, Tyr and PC aa C42:2; and performing GLM using the model formula $SM\ C18:0 + C0_div_by_Gly + Tyr_div_by_PC\ aa\ C42:2$;
- 5 (8) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, C3, PC ae C40:5, Tyr and PC aa C42:2; and performing GLM using the model formula $Orn_div_by_PC\ ae\ C38:0 + C3_div_by_PC\ ae\ C40:5 + Tyr_div_by_PC\ aa\ C42:2$;
- 10 (9) quantifying in a sample obtained from said subject the metabolic biomarkers Pro, PC ae C34:0, Orn, PC ae C38:0, Tyr and PC aa C42:2; and performing GLM using the model formula $Pro_div_by_PC\ ae\ C34:0 + Orn_div_by_PC\ ae\ C38:0 + Tyr_div_by_PC\ aa\ C42:2$;
- (10) quantifying in a sample obtained from said subject the metabolic biomarkers C4, Ser, Orn, PC ae C38:0, Tyr and PC aa C42:2; and performing GLM using the model formula $C4_div_by_Ser + Orn_div_by_PC\ ae\ C38:0 + Tyr_div_by_PC\ aa\ C42:2$;
- 15 (11) quantifying in a sample obtained from said subject the metabolic biomarkers SM C18:0, Orn, PC ae C38:0, Tyr and PC aa C42:2; and performing GLM using the model formula $SM\ C18:0 + Orn_div_by_PC\ ae\ C38:0 + Tyr_div_by_PC\ aa\ C42:2$;
- (12) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, C4, PC ae C40:3, Tyr and PC aa C42:2; and performing GLM using the model formula $Orn_div_by_PC\ ae\ C38:0 + C4_div_by_PC\ ae\ C40:3 + Tyr_div_by_PC\ aa\ C42:2$;
- 20 (13) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, PC ae C42:3, SM(OH) C16:1, Tyr and PC aa C42:2; and performing GLM using the model formula $Orn_div_by_PC\ ae\ C38:0 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1 + Tyr_div_by_PC\ aa\ C42:2$;
- 25 (14) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, Tyr, PC ae C38:0 and PC aa C42:2; and performing GLM using the model formula $Orn_div_by_PC\ ae\ C38:0 + Tyr_div_by_PC\ ae\ C38:0 + Tyr_div_by_PC\ aa\ C42:2$;

- (15) quantifying in a sample obtained from said subject the metabolic biomarkers Gly, SM C24:1, PC aa C32:0, PC aa C40:1, PC aa C36:4 and PC aa C38:0; and performing GLM using the model formula $\text{Gly_div_by_SM C24:1} + \text{PC aa C32:0_div_by_PC aa C40:1} + \text{PC aa C36:4_div_by_PC aa C38:0}$;
- 5 (16) quantifying in a sample obtained from said subject the metabolic biomarkers Gly, PC aa C42:5, PC aa C36:4, PC aa C38:0SM, C0 and SM(OH) C22:2; and performing GLM using the model formula $\text{Gly_div_by_PC aa C42:5} + \text{PC aa C36:4_div_by_PC aa C38:0} + \text{C0_div_by_SM(OH) C22:2}$.
26. The method according to any one of items 12 to 25, comprising determining whether the
10 subject is suffering from ovarian endometriosis.
27. The method according to item 26, comprising
- A4) quantifying in a sample obtained from said subject at least two pairs, preferably at least three pairs, of metabolic biomarkers selected from the group of pairs consisting of PC aa C36:3 and PC ae C40:5; LysoPC a C14:0 and PC aa C28:1; Met and PC aa C36:3; PC aa C38:0
15 and PC ae C36:1; Thr and SM (OH) C22:1; PC aa C28:1 and PC ae C34:3; C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC ae C36:1; C10:1 and PC aa C36:1; PC ae C38:3 and SM C18:1; C12-DC and C14:2; PC aa C38:3 and PC ae C44:5; C4 and C5:1; LysoPC a C20:4 and PC ae C32:1; and LysoPC a C20:4 and PC aa C32:3; and
- B4) performing generalized linear modelling (GLM).
- 20 28. The method according to item 26 or 27, comprising any one of the following procedures (1) to (14):
- (1) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C36:3, PC ae C40:5, LysoPC a C14:0, PC aa C28:1 and Met; and performing GLM using the model formula $\text{PC aa C36:3_div_by_PC ae C40:5} + \text{LysoPC a C14:0_div_by_PC aa C28:1} + \text{Met_div_by_PC aa C36:3}$;
25
- (2) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C38:0, PC ae C36:1, Thr, SM (OH) C22:1, LysoPC a C14:0 and PC aa C28:1; and performing GLM using the model formula $\text{PC aa C38:0_div_by_PC ae C36:1} + \text{Thr_div_by_SM (OH) C22:1} + \text{LysoPC a C14:0_div_by_PC aa C28:1}$;

- (3) quantifying in a sample obtained from said subject the metabolic biomarkers Thr, SM (OH) C22:1, PC aa C28:1, PC ae C34:3, C18:2 and PC ae C34:3; and performing GLM using the model formula $\text{Thr_div_by_SM (OH) C22:1} + \text{PC aa C28:1_div_by_PC ae C34:3} + \text{C18:2_div_by_PC ae C34:3}$;
- 5 (4) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C36:3, PC ae C40:5, C3, PC ae C34:1, Met and PC aa C36:3; and performing GLM using the model formula $\text{PC aa C36:3_div_by_PC ae C40:5} + \text{C3_div_by_PC ae C34:1} + \text{Met_div_by_PC aa C36:3}$;
- 10 (5) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C36:3, PC ae C40:5, PC aa C28:1, PC ae C34:3, Gly and PC ae C36:1; and performing GLM using the model formula $\text{PC aa C36:3_div_by_PC ae C40:5} + \text{PC aa C28:1_div_by_PC ae C34:3} + \text{Gly_div_by_PC ae C36:1}$;
- 15 (6) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C38:0, PC ae C36:1, C18:2, PC ae C34:3, Met and PC aa C36:3; and performing GLM using the model formula $\text{PC aa C38:0_div_by_PC ae C36:1} + \text{C18:2_div_by_PC ae C34:3} + \text{Met_div_by_PC aa C36:3}$;
- 20 (7) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C38:0, PC ae C36:1, C10:1, PC aa C36:1, PC ae C38:3 and SM C18:1; and performing GLM using the model formula $\text{PC aa C38:0_div_by_PC ae C36:1} + \text{C10:1_div_by_PC aa C36:1} + \text{PC ae C38:3_div_by_SM C18:1}$;
- (8) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C38:0, PC ae C36:1, Gly, C3 and PC ae C34:1; and performing GLM using the model formula $\text{PC aa C38:0_div_by_PC ae C36:1} + \text{Gly_div_by_PC ae C36:1} + \text{C3_div_by_PC ae C34:1}$;
- 25 (9) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C38:0, PC ae C36:1, C12-DC, C14:2, PC ae C38:3 and SM C18:1; and performing GLM using the model formula $\text{PC aa C38:0_div_by_PC ae C36:1} + \text{C12-DC_div_by_C14:2} + \text{PC ae C38:3_div_by_SM C18:1}$;

- (10) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C36:3, PC ae C40:5, PC aa C38:3, PC ae C44:5 and Met; and performing GLM using the model formula $PC\ aa\ C36:3_div_by_PC\ ae\ C40:5 + PC\ aa\ C38:3_div_by_PC\ ae\ C44:5 + Met_div_by_PC\ aa\ C36:3$;
- 5 (11) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C38:0, PC ae C36:1, PC ae C38:3, SM C18:1, Met and PC aa C36:3; and performing GLM using the model formula $PC\ aa\ C38:0_div_by_PC\ ae\ C36:1 + PC\ ae\ C38:3_div_by_SM\ C18:1 + Met_div_by_PC\ aa\ C36:3$;
- 10 (12) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C28:1, PC ae C34:3, C18:2, PC ae C34:3, C4 and C5:1; and performing GLM using the model formula $PC\ aa\ C28:1_div_by_PC\ ae\ C34:3 + C18:2_div_by_PC\ ae\ C34:3 + C4_div_by_C5:1$;
- 15 (13) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C36:3, PC ae C40:5, LysoPC a C20:4, PC ae C32:1 and Met; and performing GLM using the model formula $PC\ aa\ C36:3_div_by_PC\ ae\ C40:5 + LysoPC\ a\ C20:4_div_by_PC\ ae\ C32:1 + Met_div_by_PC\ aa\ C36:3$;
- 20 (14) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C36:3, PC ae C40:5, LysoPC a C20:4, PC aa C32:3 and Met; and performing GLM using the model formula $PC\ aa\ C36:3_div_by_PC\ ae\ C40:5 + LysoPC\ a\ C20:4_div_by_PC\ aa\ C32:3 + Met_div_by_PC\ aa\ C36:3$.
29. The method according to any one of items 12 to 28, comprising determining whether the subject is suffering from ovarian mixed endometriosis.
30. The method according to item 29, comprising
- 25 A5) quantifying in a sample obtained from said subject at least two pairs, preferably at least three pairs, of metabolic biomarkers selected from the group of pairs consisting of C10 and PC aa C36:6; PC ae C42:3 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and LysoPC a C20:4; LysoPC a C20:4 and PC ae C40:2; Ser and PC aa C38:3; C10 and LysoPC a C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC a C18:1 and PC aa C36:1; Gly and PC ae C34:1; Gln and PC ae C30:2; LysoPC a C24:0 and PC ae C42:3; C10:1 and LysoPC a C24:0; PC ae C44:3 and CPT I ratio; PC

ae C34:0 and PC ae C40:3; C16:2-OH and SM C20:2; PC ae C44:6 and SM C22:3; and C10:1 and C14:2-OH; and

B5) performing generalized linear modelling (GLM).

31. The method according to item 23 or 24, comprising any one of the following procedures (1) to (13):
- 5
- (1) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC aa C36:6, Pro, PC ae C34:0, PC ae C42:3 and SM(OH) C16:1; and performing GLM using the model formula $C10_div_by_PC\ aa\ C36:6 + Pro_div_by_PC\ ae\ C34:0 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1$;
- 10
- (2) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC aa C36:6, PC ae C42:3, SM(OH) C16:1, C6:1 and LysoPC a C20:4; and performing GLM using the model formula $C10_div_by_PC\ aa\ C36:6 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1 + C6:1_div_by_LysoPC\ a\ C20:4$;
- (3) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC aa C36:6, PC ae C42:3, SM(OH) C16:1, LysoPC a C20:4 and PC ae C40:2; and performing GLM using the model formula $C10_div_by_PC\ aa\ C36:6 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1 + LysoPC\ a\ C20:4_div_by_PC\ ae\ C40:2$;
- 15
- (4) quantifying in a sample obtained from said subject the metabolic biomarkers Ser, PC aa C38:3, C10, PC aa C36:6, PC ae C42:3 and SM(OH) C16:1; and performing GLM using the model formula $Ser_div_by_PC\ aa\ C38:3 + C10_div_by_PC\ aa\ C36:6 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1$;
- 20
- (5) quantifying in a sample obtained from said subject the metabolic biomarkers C10, LysoPC a C18:1, PC aa C36:6, PC ae C42:3 and SM(OH) C16:1; and performing GLM using the model formula $C10_div_by_LysoPC\ a\ C18:1 + C10_div_by_PC\ aa\ C36:6 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1$;
- 25
- (6) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC aa C36:6, LysoPC a C24:0, PC ae C42:3, PC ae C42:3 and SM(OH) C16:1; and performing GLM using the model formula $C10_div_by_PC\ aa\ C36:6 + LysoPC\ a\ C24:0_div_by_PC\ ae\ C42:3 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1$;

- (7) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC aa C36:6, LysoPC a C18:1, PC aa C36:1, PC ae C42:3 and SM(OH) C16:1; and performing GLM using the model formula $C10_div_by_PC\ aa\ C36:6 + LysoPC\ a\ C18:1_div_by_PC\ aa\ C36:1 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1$;
- 5 (8) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC aa C36:6, + Gly, PC ae C34:1, PC ae C42:3 and SM(OH) C16:1; and performing GLM using the model formula $C10_div_by_PC\ aa\ C36:6 + Gly_div_by_PC\ ae\ C34:1 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1$;
- 10 (9) quantifying in a sample obtained from said subject the metabolic biomarkers Gln, PC ae C30:2, C10, PC aa C36:6, PC ae C42:3 and SM(OH) C16:1; and performing GLM using the model formula $Gln_div_by_PC\ ae\ C30:2 + C10_div_by_PC\ aa\ C36:6 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1$;
- 15 (10) quantifying in a sample obtained from said subject the metabolic biomarkers Pro, PC ae C34:0, LysoPC a C24:0, PC ae C42:3, PC ae C42:3 and SM(OH) C16:1; and performing GLM using the model formula $Pro_div_by_PC\ ae\ C34:0 + lysoPC\ a\ C24:0_div_by_PC\ ae\ C42:3 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1$;
- 20 (11) quantifying in a sample obtained from said subject the metabolic biomarkers C10:1, LysoPC a C24:0, LysoPC a C24:0, PC ae C42:3 and SM(OH) C16:1; and performing GLM using the model formula $C10:1_div_by_lysoPC\ a\ C24:0 + lysoPC\ a\ C24:0_div_by_PC\ ae\ C42:3 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1$;
- (12) quantifying in a sample obtained from said subject the metabolic biomarkers PC ae C44:3, CPT I ratio, PC ae C34:0, PC ae C40:3, C16:2-OH and SM C20:2; and performing GLM using the model formula $PC\ ae\ C44:3_div_by_CPT.I.ratio + PC\ ae\ C34:0_div_by_PC\ ae\ C40:3 + C16:2-OH_div_by_SM\ C20:2$;
- 25 (13) quantifying in a sample obtained from said subject the metabolic biomarkers PC ae C44:6, SM C22:3, PC ae C34:0, PC ae C40:3, C10:1 and C14:2-OH; and performing GLM using the model formula $PC\ ae\ C44:6_div_by_SM\ C22:3 + PC\ ae\ C34:0_div_by_PC\ ae\ C40:3 + C10:1_div_by_C14:2-OH$.
- 30 32. The method according to any one of items 12 to 31, wherein the sample is selected from blood, serum, plasma, saliva, urine, cerebrospinal fluid, condensates from respiratory air,

tears, mucosal tissue, mucus, vaginal tissue, endometrium, eutopic endometrium, skin, hair and hair follicle.

33. The method according to any one of items 12 to 31, wherein the sample is selected from blood, serum and plasma.
- 5 34. The method according to any one of items 12 to 31, wherein the sample is plasma.
35. The use according to any one of items 1 to 11 or the method according to any one of items 12 to 34, wherein the subject is a human subject.
36. The use or method according to item 35, wherein the human subject is a female.
37. The use or method according to item 35 or 36, wherein the human subject is of Caucasian
10 race.
38. The use or method according to any one of items 35 to 37, wherein the subject is suspected to suffer from endometriosis or to have a predisposition therefore.

Brief description of the figures

- 15 **Figure 1:** Process flow of diagnostic assay. Samples are collected from patients using standard procedures in outpatient and inpatient stations. Plasma is prepared and the metabolite analyses are undertaken with mass spectrometry apparatus. Data gained are undergoing processing with algorithm calculating values indicative of diagnostic status. DxS – calculated diagnostic score: $\log_2$ (ratio of GLM of control and GLM of patient).
- 20 **Figure 2:** Concept of algorithm implementation. The algorithm constitutes of calculation of GLM-values based on metabolite ratios measured in patient plasma. For distinct endometriosis forms different GLMs are indicative for the diagnosis. Should the DxS (ratio of GLM of control and GLM of patient) be zero these GLM can not be used for diagnosis and another GLM values are considered. All GLM models can be tested for the given sample in parallel. In particular, the calculation can be
25 performed for: 1. Detection of any form of endometriosis, 2. Detection of specific form like ovarian or peritoneal, 3. Detection of mixed (multiple) forms like ovarian with coincidence of peritoneal and/or infiltrating. DxS – calculated diagnostic score.

Figure 3: PLS-DA analysis for case vs control using absolute concentrations of metabolites. The calculated parameters indicate lack of separation according of this calculation: $R_y^2 = 0.355$, $R_x^2 = 0.445$, $Q_x^2 = -0.592$, $RMSE = 0.39$, $P_R^2 = -0.1515$, $P_Q^2 = -0.6585$.

Figure 4: Calculation of AUC for GLM model#1 for all cases of endometriosis for LysoPC a
5 C17:0_div_by_SM(OH) C16:1 + Arg_div_by_PC ae C36:0 + PC ae C38:0_div_by_PC ae C40:0

Figure 5: Calculation of AUC for GLM model#1 for all cases of endometriosis as composite plot with all test data sets (cross-validated) displayed for LysoPC a C17:0_div_by_SM(OH) C16:1 + Arg_div_by_PC ae C36:0 + PC ae C38:0_div_by_PC ae C40:0

Figure 6: Calculation of AUC for GLM model #1 for peritoneal endometriosis - LysoPC a
10 C16:0_div_by_SM(OH) C16:1 + PC aa C32:0_div_by_SM C18:0 + PC aa C32:0_div_by_PC aa C38:3

Figure 7: Calculation of AUC for GLM model #1 peritoneal endometriosis as composite plot with all test data sets (cross-validated) displayed for LysoPC a C16:0_div_by_SM(OH) C16:1 + PC aa C32:0_div_by_SM C18:0 + PC aa C32:0_div_by_PC aa C38:3

Figure 8: Calculation of AUC for GLM model#1 for peritoneal mixed endometriosis for
15 Orn_div_by_PC ae C38:0 + C4_div_by_PC aa C38:4 + Tyr_div_by_PC aa C42:2

Figure 9: Calculation of AUC for GLM model#1 for peritoneal mixed endometriosis as composite plot with all test data sets (cross-validated) displayed for Orn_div_by_PC ae C38:0 + C4_div_by_PC aa C38:4 + Tyr_div_by_PC aa C42:2

Figure 10: Calculation of AUC for GLM model#1 for ovarian endometriosis for PC aa
20 C36:3_div_by_PC ae C40:5 + lysoPC a C14:0_div_by_PC aa C28:1 + Met_div_by_PC aa C36:3

Figure 11: Calculation of AUC for GLM model#1 for ovarian endometriosis as composite plot with all test data sets (cross-validated) displayed for PC aa C36:3_div_by_PC ae C40:5 + lysoPC a C14:0_div_by_PC aa C28:1 + Met_div_by_PC aa C36:3

Figure 12: Calculation of AUC for GLM model#1 for ovarian mixed endometriosis for C10_div_by_PC
25 aa C36:6 + Pro_div_by_PC ae C34:0 + PC ae C42:3_div_by_SM(OH) C16:1

Figure 13: Calculation of AUC for GLM model#1 for ovarian mixed endometriosis as composite plot with all test data sets (cross-validated) displayed for: C10_div_by_PC aa C36:6 + Pro_div_by_PC ae C34:0 + PC ae C42:3_div_by_SM(OH) C16:1

The present invention is now described in more detail below.

5

Detailed description of the invention

As noted above, the present invention is based on the identification and use of a panel of metabolic biomarkers for the diagnosis of endometriosis. However, instead of comparing to reference values in healthy individuals, the present invention uses selected metabolite ratios. Different combinations of metabolite combinations like two predictors (two pairs of two metabolites) and three predictors (three pairs of two metabolites) were tested for diagnostic performance, and was successful in biostatistical evaluations. This approach has the huge advantage of its insensitivity to human metabolome variability caused by confounders like ethnicity, age, nutrition, lifestyle or medication. The metabolite-based diagnosis method of the present invention provides for a cheap, fast, reliable and accurate way for diagnosing endometriosis in a subject.

Specifically, the present inventors have identified the following pairs of metabolic biomarkers most suitable for the diagnosis of endometriosis and/or any sub-type thereof in a subject: LysoPC a C17:0 and SM(OH) C16:1; Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0; LysoPC a C16:0 and SM C18:1; Thr and PC aa C34:3; C18 and LysoPC a C14:0; Ser and PC ae C44:3; Trp and PC ae C38:3; C8 and PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae C38:6; Arg and PC aa C36:6; Thr and SM(OH) C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM C18:0; PC aa C32:0 and PC aa C38:3; C6:1 and Pro; Arg and PC ae C34:0; C6:1 and LysoPC a C20:4; C5-M-DC and PC aa C42:5; LysoPC a C18:2 and PC ae C40:6; LysoPC a C18:2 and PC ae C40:4; PC ae C40:6 and CPT I ratio; LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg and PC ae C34:0; PC ae C34:1 and PC ae C42:0; Orn and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa C36:6; C5 and LysoPC a C17:0; C5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae C40:5; Pro and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and SM(OH) C16:1; Tyr and PC ae C38:0; PC aa C36:3 and PC ae C40:5; LysoPC a C14:0 and PC aa C28:1; Met and PC aa C36:3; PC aa C38:0 and PC ae C36:1; Thr and SM (OH) C22:1; PC aa C28:1 and PC ae C34:3; C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC ae C36:1; C10:1 and PC aa C36:1; PC ae C38:3 and SM C18:1; C12-DC and C14:2; PC aa C38:3 and

PC ae C44:5; C4 and C5:1; LysoPC a C20:4 and PC ae C32:1; LysoPC a C20:4 and PC aa C32:3; C10 and PC aa C36:6; PC ae C42:3 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and LysoPC a C20:4; LysoPC a C20:4 and PC ae C40:2; Ser and PC aa C38:3; C10 and LysoPC a C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC a C18:1 and PC aa C36:1; Gly and PC ae C34:1; Gln and PC ae C30:2; LysoPC a C24:0 and PC ae C42:3; C10:1 and LysoPC a C24:0; Tyr and PC aa C42:4; C3-DC and C18; and PC aa C42:1 and SM C22:3; Gly and SM C24:1; PC aa C32:0 and PC aa C40:1; PC aa C36:4 and PC aa C38:0; PC ae C44:3 and CPT I ratio; PC ae C34:0 and PC ae C40:3; C16:2-OH and SM C20:2; C6(C4:1-DC) and SM C16:1; Gly and PC aa C42:5; C0 and SM(OH) C22:2; PC ae C44:6 and SM C22:3; and C10:1 and C14:2-OH.

10 Besides have generally identified pairs of metabolic biomarkers most suitable for the diagnosis of endometriosis, the present inventors have identified various subgroups of these pairs of metabolic biomarkers which allow for the diagnosis of any form of endometriosis (all endometriosis), the diagnosis of a specific form like ovarian or peritoneal, and/or the diagnosis of mixed (multiple) forms like ovarian with coincidence of peritoneal and/or infiltrating.

15 Specifically, the following pairs of metabolic biomarkers have been shown to provide a diagnostic score for diagnosing all endometriosis: LysoPC a C17:0 and SM(OH) C16:1; Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0; LysoPC a C16:0 and SM C18:1; Thr and PC aa C34:3; C18 and LysoPC a C14:0; Ser and PC ae C44:3; Trp and PC ae C38:3; C8 and PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae C38:6; Arg and PC aa C36:6; and Tyr and PC aa C42:4; C3-DC and C18; PC aa C42:1 and SM C22:3; and C6(C4:1-DC) and SM C16:1.

The following pairs of metabolic biomarkers have been shown to provide a diagnostic score for diagnosing peritoneal endometriosis: Thr and SM(OH) C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM C18:0; PC aa C32:0 and PC aa C38:3; C6:1 and Pro; Arg and PC ae C34:0; C6:1 and LysoPC a C20:4; C5-M-DC and PC aa C42:5; LysoPC a C18:2 and PC ae C40:6; LysoPC a C18:2 and PC ae C40:4; PC ae C40:6 and CPT I ratio; LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg and PC ae C34:0; and PC ae C34:1 and PC ae C42:0.

The following pairs of metabolic biomarkers have been shown to provide a diagnostic score for diagnosing peritoneal mixed endometriosis: Orn and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa C36:6; C5 and LysoPC a C17:0; C5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae C40:5; Pro and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and

SM(OH) C16:1; Tyr and PC ae C38:0; SM C18:0 and C5; Gly and SM C24:1; PC aa C32:0 and PC aa C40:1; PC aa C36:4 and PC aa C38:0; Gly and PC aa C42:5; and C0 and SM(OH) C22:2.

The following pairs of metabolic biomarkers have been shown to provide a diagnostic score for diagnosing ovarian endometriosis: PC aa C36:3 and PC ae C40:5; LysoPC a C14:0 and PC aa C28:1; 5 Met and PC aa C36:3; PC aa C38:0 and PC ae C36:1; Thr and SM (OH) C22:1; PC aa C28:1 and PC ae C34:3; C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC ae C36:1; C10:1 and PC aa C36:1; PC ae C38:3 and SM C18:1; C12-DC and C14:2; PC aa C38:3 and PC ae C44:5; C4 and C5:1; LysoPC a C20:4 and PC ae C32:1; LysoPC a C20:4 and PC aa C32:3; C0 and C5-M-DC; C3 and PC ae 34:0.

The following pairs of metabolic biomarkers have been shown to provide a diagnostic score for 10 diagnosing ovarian mixed endometriosis: C10 and PC aa C36:6; PC ae C42:3 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and LysoPC a C20:4; LysoPC a C20:4 and PC ae C40:2; Ser and PC aa C38:3; C10 and LysoPC a C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC a C18:1 and PC aa C36:1; Gly and PC ae C34:1; Gln and PC ae C30:2; LysoPC a C24:0 and PC ae C42:3; C10:1 and LysoPC a C24:0; PC ae C44:3 and CPT I ratio; PC ae C34:0 and PC ae C40:3; C16:2-OH and SM C20:2; PC ae C44:6 and 15 SM C22:3; and C10:1 and C14:2-OH.

Table 2. Metabolites used in accordance with the invention for diagnosing endometriosis.

Metabolite	Trivial name	HMDB ID	CAS number	Chemical Formula
PC aa C28:1	Phosphatidylcholine aa C28:1	HMDB07867	na	C36H70NO8P
PC ae C30:0	Phosphatidylcholine ae C30:0	HMDB13341	na	C38H78NO7P
PC aa C32:0	Phosphatidylcholine aa C32:0	HMDB00564	63-89-8	C40H80NO8P
PC aa C32:3	Phosphatidylcholine aa C32:3	HMDB07876	na	C40H74NO8P
PC aa C34:3	Phosphatidylcholine aa C34:3	HMDB08006	182820-31-1	C42H78NO8P
PC aa C36:1	Phosphatidylcholine aa C36:1	HMDB08037	na	C44H86NO8P
PC aa C36:3	Phosphatidylcholine aa C36:3	HMDB07980	na	C44H82NO8P
PC aa C36:4	Phosphatidylcholine aa C36:4	HMDB07982	na	C44H80NO8P
PC aa C36:6	Phosphatidylcholine aa C36:6	HMDB07892	na	C44H76NO8P
PC aa C38:0	Phosphatidylcholine aa C38:0	HMDB07893	na	C46H92NO8P
PC aa C38:3	Phosphatidylcholine aa C38:3	HMDB08046	na	C46H86NO8P
PC aa C38:4	Phosphatidylcholine aa C38:4	HMDB07988	na	C46H84NO8P
PC aa C40:1	Phosphatidylcholine aa C40:1	HMDB13433	na	C48H96NO7P
PC aa C40:4	Phosphatidylcholine aa C40:4	HMDB08054	na	C48H88NO8P
PC aa C40:5	Phosphatidylcholine aa C40:5	HMDB08055	na	C48H86NO8P
PC aa C42:1	Phosphatidylcholine aa C42:1	HMDB08059	na	C50H98NO8P
PC aa C42:2	Phosphatidylcholine aa C42:2	HMDB08570	na	C50H96NO8P
PC aa C42:4	Phosphatidylcholine aa C42:4	HMDB08572	na	C50H92NO8P
PC aa C42:5	Phosphatidylcholine aa C42:5	HMDB08287	na	C50H90NO8P
PC ae C30:2	Phosphatidylcholine ae C30:2	HMDB0013410	na	C38H74NO7P

Metabolite	Trivial name	HMDB ID	CAS number	Chemical Formula
PC ae C32:1	Phosphatidylcholine ae C32:1	HMDB0007898	na	C40H78NO7P
PC ae C34:0	Phosphatidylcholine ae C34:0	HMDB13405	na	C42H86NO7P
PC ae C34:1	Phosphatidylcholine ae C34:1	HMDB0013412	na	C42H84NO7P
PC ae C34:3	Phosphatidylcholine ae C34:3	HMDB0013413	na	C42H80NO7P
PC ae C36:0	Phosphatidylcholine ae C36:0	HMDB13406	na	C44H90NO7P
PC ae C36:1	Phosphatidylcholine ae C36:1	HMDB13427	na	C44H88NO7P
PC ae C36:5	Phosphatidylcholine ae C36:5	HMDB11222	na	C44H78NO7P
PC ae C38:0	Phosphatidylcholine ae C38:0	HMDB13408	na	C46H94NO7P
PC ae C38:3	Phosphatidylcholine ae C38:3	HMDB13439	na	C46H88NO7P
PC ae C38:6	Phosphatidylcholine ae C38:6	HMDB13409	na	C46H82NO7P
PC ae C40:0	Phosphatidylcholine ae C40:0	HMDB13421	na	C48H98NO7P
PC aa C40:1	Phosphatidylcholine aa C40:1	HMDB13433	na	C48H96NO7P
PC ae C40:2	Phosphatidylcholine ae C40:2	HMDB13437	na	C48H96NO7P
PC ae C40:3	Phosphatidylcholine ae C40:3	HMDB13445	na	C48H92NO7P
PC ae C40:4	Phosphatidylcholine ae C40:4	HMDB13442	na	C48H90NO7P
PC ae C40:5	Phosphatidylcholine ae C40:5	HMDB13444	na	C48H88NO7P
PC ae C40:6	Phosphatidylcholine ae C40:6	HMDB13422	na	C48H86NO7P
PC ae C42:0	Phosphatidylcholine ae C42:0	HMDB13443	na	C50H102NO7P
PC ae C42:3	Phosphatidylcholine ae C42:3	HMDB13459	na	C50H96NO7P
PC ae C44:3	Phosphatidylcholine ae C44:3	HMDB13449	na	C52H100NO7P
PC ae C44:5	Phosphatidylcholine ae C44:5	HMDB13456	na	C52H96NO7P
PC ae C44:6	Phosphatidylcholine ae C44:6	HMDB13457	na	C52H94NO7P
SM C16:1	Sphingomyelin C16:1	HMDB06317	na	C23H43NO4
SM(OH) C16:1	Hydroxysphingomyelin C16:1	HMDB13463	na	C39H77N2O7P
SM(OH) C22:2	Hydroxysphingomyelin C22:2	HMDB13467	na	C45H87N2O7P
SM C18:0	Sphingomyelin C18:0	HMDB01348	58909-84-5	C41H84N2O6P
SM C18:1	Sphingomyelin C18:1	HMDB12101	108392-10-5	C41H81N2O6P
SM C20:2	Sphingomyelin 20:2	HMDB13465	na	C43H83N2O6P
SM C22:3	Sphingomyelin C22:3	HMDB13468	na	C45H85N2O6P
SM C24:1	Sphingomyelin C24:1	HMDB12107	94359-13-4	C47H93N2O6P
lysoPC C14:0	Lysophosphatidylcholine a C14:0	HMDB10379	20559-16-4	C22H46NO7P
lysoPC C16:0	Lysophosphatidylcholine a C16:0	HMDB10382	17364-16-8	C24H50NO7P
LysoPC C17:0	Lysophosphatidylcholine a C17:0	HMDB12108	50930-23-9	C25H52NO7P
lysoPC C18:1	Lysophosphatidylcholine a C18:1	HMDB02815	19420-56-5	C26H52NO7P
lysoPC C18:2	Lysophosphatidylcholine a C18:2	HMDB10386	22252-07-9	C26H50NO7P
lysoPC C20:4	Lysophosphatidylcholine a C20:4	HMDB10395	60701-99-7	C28H50NO7P
C0	L-Carnitine (free carnitine)	HMDB00062	541-15-1	C7H15NO3

Metabolite	Trivial name	HMDB ID	CAS number	Chemical Formula
C3	Propionylcarnitine	HMDB00824	20064-19-1	C10H20NO4
C3-DC (C4-OH)	Hydroxybutyrylcarnitine	HMDB02095	910825-21-7	C10H17NO6
C4	Isobutyryl-L-carnitine	HMDB00736	25518-49-4	C11H21NO4
C5	Isovalerylcarnitine	HMDB00688	31023-24-2	C12H23NO4
C5:1	Tiglylcarnitine	HMDB02366	64681-36-3	C12H21NO4
C5-M-DC	Methylglutaryl-L-carnitine	HMDB00552	102673-95-0	C12H25NO5
C6:1	Hexenoylcarnitine	HMDB13161	na	C13H23NO4
C8	Octanoylcarnitine	HMDB0000791	25243-95-2	C15H30NO4
C10	Decanoylcarnitine	HMDB00651	1492-27-9	C17H33NO4
C10:1	Decenoylcarnitine	HMDB13205	na	C17H31NO4
C12-DC	Dodecanedioylcarnitine	HMDB13327	na	C19H35NO6
C14:1	Tetradecenoylcarnitine	HMDB02014	835598-21-5	C21H39NO4
C14:2	Tetradecadienylcarnitine	HMDB13331	na	C21H37NO4
C14:2-OH	Hydroxytetradecadienylcarnitine	HMDB240755	na	C21H37NO5
C16	Hexadecanoylcarnitine	HMDB00222	2364-67-2	C23H45NO4
C16:2-OH	Hydroxyhexadecadienylcarnitine	HMDB13335	na	C23H41NO5
C18	Stearoylcarnitine	HMDB00848	25597-09-5	C25H50NO4
C18:2	Octadecadienylcarnitine	HMDB06461	85114-47-2	C25H45NO4
Arg	L-Arginine	HMDB00517	74-79-3	C6H14N4O2
Gln	L-Glutamine	HMDB00641	56-85-9	C5H10N2O3
Gly	L-Glycine	HMDB00123	56-40-6	C2H5NO2
Met	Methionine	HMDB00696	63-68-3	C5H11NO2S
Orn	L-Ornithine	HMDB00214	3184-13-2	C5H12N2O2
Pro	L-Proline	HMDB00162	147-85-3	C5H9NO2
Ser	L-Serine	HMDB00187	56-45-1	C3H7NO3
Thr	L-Threonine	HMDB00167	72-19-5	C4H9NO3
Trp	L-Tryptophan	HMDB00929	73-22-3	C11H12N2O2
Tyr	L-Tyrosine	HMDB00158	60-18-4	C9H11NO3

Abbreviations used in the table are explained as follows: HMDB - Human Metabolome Database (<http://www.hmdb.ca>) which provides annotation of chemical and biological parameters of a metabolite; CAS - Chemical Abstracts Service (<https://www.cas.org>) which provides annotation of

chemical and physical parameters of a metabolite; na - not annotated, the "na" metabolite can be unequivocally measured but has not been described in the specific database.

The metabolites referred to herein are abbreviated using standard abbreviations well known in the art. Accordingly, "PC" abbreviates phosphatidylcholines, "LysoPC" abbreviates Lysophosphatidyl-
5 choline, "SM" abbreviates sphingomyelins and "CO" abbreviates free carnitine. The term "Cx:y" is used to describe the total number of carbons (x) and the number of double bonds (y) of all chains. Substitutions of side chains with hydroxy- (OH) residue are indicated. Glycerophospholipids are distinguished with respect to the presence of ester (a) and ether (e) bonds in the glycerol moiety, where two letters (aa=diacyl, ae=acyl-alkyl) denote that the two glycerol positions are each bound
10 to a fatty acid residue, while a single letter (a=acyl or e=alkyl) indicates the presence of a single fatty acid residue. For example "PC ae C34:1" denotes a glycerophosphatidylcholine with an acyl (a) and an ether (e) side chain, with 34 carbon atoms in both side chains and a single double bond in one of them. Amino acids are abbreviated in three letter code (e.g. Gln).

Further, the diagnostic approach according to the present invention involves use of a generalized
15 linear model (GLM) based on the quantification of the at least two pairs, preferably at least three pairs, of metabolic biomarkers in a sample obtained from said subject. GLM is a statistical approach which is well established and widely used. The GLM generalizes linear regression by allowing the linear model to be related to the response variable via a link function and by allowing the magnitude of the variance of each measurement to be a function of its predicted value. The generalized linear
20 models established by the present inventors allow the calculation of GLM-values characteristic for distinct endometriosis forms. The calculation can be performed for diagnosis of any form of endometriosis (all endometriosis), the diagnosis of a specific form like ovarian or peritoneal, and/or the diagnosis of mixed (multiple) forms like ovarian with coincidence of peritoneal and/or infiltrating.

25 With the GLM-based diagnostic approach of the present invention it is thus not only made possible to determine from a single sample of a subject whether said subject is generally suffering from any form of endometriosis (all endometriosis), but also whether said subject is suffering from a specific form, like ovarian or peritoneal, or a mixed (multiple) form. The determination of the various forms can thereby be implemented as illustrated in Figure 1 and 2.

30 The present invention thus provides in a first aspect the use of a combination of at least two pairs, preferably at least three pairs, of metabolic biomarkers for the diagnosis of endometriosis and/or

any sub-type thereof in a subject. More specifically, the present invention provides the use of a combination of at least two pairs, preferably at least three pairs, of metabolic biomarkers selected from the group of pairs consisting of LysoPC a C17:0 and SM(OH) C16:1; Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0; LysoPC a C16:0 and SM C18:1; Thr and PC aa C34:3; C18 and LysoPC a C14:0; 5 Ser and PC ae C44:3; Trp and PC ae C38:3; C8 and PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae C38:6; Arg and PC aa C36:6; Thr and SM(OH) C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM C18:0; PC aa C32:0 and PC aa C38:3; C6:1 and Pro; Arg and PC ae C34:0; C6:1 and LysoPC a C20:4; C5-M-DC and PC aa C42:5; LysoPC a C18:2 and PC ae C40:6; LysoPC a C18:2 and PC ae C40:4; PC ae C40:6 and CPT I ratio; LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg and PC ae C34:0; 10 PC ae C34:1 and PC ae C42:0; Orn and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa C36:6; C5 and LysoPC a C17:0; C5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae C40:5; Pro and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and SM(OH) C16:1; Tyr and PC ae C38:0; PC aa C36:3 and PC ae C40:5; LysoPC a C14:0 and PC aa C28:1; Met and PC aa C36:3; PC aa C38:0 and PC ae C36:1; Thr and SM (OH) C22:1; PC aa C28:1 and PC ae C34:3; C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC ae C36:1; C10:1 and PC aa C36:1; PC ae C38:3 and SM C18:1; C12-DC and C14:2; PC aa C38:3 and PC ae C44:5; C4 and C5:1; LysoPC a C20:4 and PC ae C32:1; LysoPC a C20:4 and PC aa C32:3; C10 and PC aa C36:6; PC ae C42:3 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and LysoPC a C20:4; LysoPC a C20:4 and PC ae C40:2; Ser and PC aa C38:3; C10 and LysoPC a C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC a C18:1 and PC aa C36:1; Gly and 20 PC ae C34:1; Gln and PC ae C30:2; LysoPC a C24:0 and PC ae C42:3; C10:1 and LysoPC a C24:0; Tyr and PC aa C42:4; C3-DC and C18; PC aa C42:1 and SM C22:3; Gly and SM C24:1; PC aa C32:0 and PC aa C40:1; PC aa C36:4 and PC aa C38:0; PC ae C44:3 and CPT I ratio; PC ae C34:0 and PC ae C40:3; C16:2-OH and SM C20:2; C6(C4:1-DC) and SM C16:1; Gly and PC aa C42:5; C0 and SM(OH) C22:2; PC ae C44:6 and SM C22:3; and C10:1 and C14:2-OH;

25 for the diagnosis of endometriosis and/or any sub-type thereof in a subject.

According to some embodiments, the present invention provides the use of a combination of at least two pairs, preferably at least three pairs, of metabolic biomarkers for diagnosing all endometriosis. More specifically, the present invention provides the use of a combination of at least two pairs, preferably at least three pairs, of metabolic biomarkers for diagnosing all 30 endometriosis, wherein the at least two pairs, preferably at least three pairs, of metabolic biomarkers are selected from the group of pairs consisting of LysoPC a C17:0 and SM(OH) C16:1;

Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0; LysoPC a C16:0 and SM C18:1; Thr and PC aa C34:3; C18 and LysoPC a C14:0; Ser and PC ae C44:3; Trp and PC ae C38:3; C8 and PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae C38:6; Arg and PC aa C36:6; and Tyr and PC aa C42:4; C3-DC and C18; PC aa C42:1 and SM C22:3; and C6(C4:1-DC) and SM C16:1.

5 According to some embodiments, the present invention provides the use of a combination of at least two pairs, preferably at least three pairs, of metabolic biomarkers for diagnosing peritoneal endometriosis. More specifically, the present invention provides the use of a combination of at least two pairs, preferably at least three pairs, of metabolic biomarkers for diagnosing peritoneal endometriosis, wherein the at least two pairs, preferably at least three pairs, of metabolic
10 biomarkers are selected from the group of pairs consisting of Thr and SM(OH) C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM C18:0; PC aa C32:0 and PC aa C38:3; C6:1 and Pro; Arg and PC ae C34:0; C6:1 and LysoPC a C20:4; C5-M-DC and PC aa C42:5; LysoPC a C18:2 and PC ae C40:6; LysoPC a C18:2 and PC ae C40:4; PC ae C40:6 and CPT I ratio; LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg and PC ae C34:0; and PC ae C34:1 and PC ae C42:0.

15 According to some embodiments, the present invention provides the use of a combination of at least two pairs, preferably at least three pairs, of metabolic biomarkers for diagnosing peritoneal mixed endometriosis. More specifically, the present invention provides the use of a combination of at least two pairs, preferably at least three pairs, of metabolic biomarkers for diagnosing peritoneal mixed endometriosis, wherein the at least two pairs, preferably at least three pairs, of metabolic
20 biomarkers are selected from the group of pairs consisting of Orn and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa C36:6; C5 and LysoPC a C17:0; C5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae C40:5; Pro and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and SM(OH) C16:1; Tyr and PC ae C38:0; SM C18:0 and C5; Gly and SM C24:1; PC aa C32:0 and PC aa C40:1; PC aa C36:4 and PC aa C38:0; Gly and PC aa C42:5; and C0 and SM(OH)
25 C22:2.

According to some embodiments, the present invention provides the use of a combination of at least two pairs, preferably at least three pairs, of metabolic biomarkers for diagnosing ovarian endometriosis. More specifically, the present invention provides the use of a combination of at least two pairs, preferably at least three pairs, of metabolic biomarkers for diagnosing ovarian
30 endometriosis, wherein the at least two pairs, preferably at least three pairs, of metabolic biomarkers are selected from the group of pairs consisting of PC aa C36:3 and PC ae C40:5; LysoPC

a C14:0 and PC aa C28:1; Met and PC aa C36:3; PC aa C38:0 and PC ae C36:1; Thr and SM (OH) C22:1; PC aa C28:1 and PC ae C34:3; C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC ae C36:1; C10:1 and PC aa C36:1; PC ae C38:3 and SM C18:1; C12-DC and C14:2; PC aa C38:3 and PC ae C44:5; C4 and C5:1; LysoPC a C20:4 and PC ae C32:1; LysoPC a C20:4 and PC aa C32:3; C0 and C5-M-DC; C3
 5 and PC ae 34:0.

According to some embodiments, the present invention provides the use of a combination of at least two pairs, preferably at least three pairs, of metabolic biomarkers for diagnosing ovarian mixed endometriosis. More specifically, the present invention provides the use of a combination of at least two pairs, preferably at least three pairs, of metabolic biomarkers for diagnosing ovarian
 10 mixed endometriosis, wherein the at least two pairs, preferably at least three pairs, of metabolic biomarkers are selected from the group of pairs consisting of C10 and PC aa C36:6; PC ae C42:3 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and LysoPC a C20:4; LysoPC a C20:4 and PC ae C40:2; Ser and PC aa C38:3; C10 and LysoPC a C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC a C18:1 and PC aa C36:1; Gly and PC ae C34:1; Gln and PC ae C30:2; LysoPC a C24:0 and PC ae C42:3; C10:1 and
 15 LysoPC a C24:0; PC ae C44:3 and CPT I ratio; PC ae C34:0 and PC ae C40:3; C16:2-OH and SM C20:2; PC ae C44:6 and SM C22:3; and C10:1 and C14:2-OH.

According to some embodiments, the diagnosis involves use of a generalized linear model (GLM) based on the quantification of the at least two pairs, preferably at least three pairs, of metabolic biomarkers in a sample obtained from said subject.

20 The present invention provides in a further aspect an ex vivo method of diagnosing endometriosis and/or any subtype thereof in a subject comprising a) quantifying in a sample obtained from said of at least two pairs, preferably at least three pairs, of metabolic biomarkers, determining the ratio for each of the at least two pairs and b) obtaining a diagnostic score using a generalized linear model (GLM). More specifically, the present invention provides an ex vivo method of diagnosing
 25 endometriosis and/or any subtype thereof in a subject, the method comprising

a) quantifying in a sample obtained from said subject at least two pairs, preferably at least three pairs, of metabolic biomarkers selected from the group of pairs consisting of LysoPC a C17:0 and SM(OH) C16:1; Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0; LysoPC a C16:0 and SM C18:1; Thr and PC aa C34:3; C18 and LysoPC a C14:0; Ser and PC ae C44:3; Trp and PC ae C38:3; C8
 30 and PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae C38:6; Arg and PC aa C36:6; Thr and SM(OH) C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM C18:0; PC aa C32:0 and PC aa C38:3;

C6:1 and Pro; Arg and PC ae C34:0; C6:1 and LysoPC a C20:4; C5-M-DC and PC aa C42:5; LysoPC a C18:2 and PC ae C40:6; LysoPC a C18:2 and PC ae C40:4; PC ae C40:6 and CPT I ratio; LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg and PC ae C34:0; PC ae C34:1 and PC ae C42:0; Orn and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa C36:6; C5 and LysoPC a C17:0; C5
5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae C40:5; Pro and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and SM(OH) C16:1; Tyr and PC ae C38:0; PC aa C36:3 and PC ae C40:5; LysoPC a C14:0 and PC aa C28:1; Met and PC aa C36:3; PC aa C38:0 and PC ae C36:1; Thr and SM (OH) C22:1; PC aa C28:1 and PC ae C34:3; C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC ae C36:1; C10:1 and PC aa C36:1; PC ae C38:3 and SM C18:1; C12-DC and C14:2; PC aa C38:3 and
10 PC ae C44:5; C4 and C5:1; LysoPC a C20:4 and PC ae C32:1; LysoPC a C20:4 and PC aa C32:3; C10 and PC aa C36:6; PC ae C42:3 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and LysoPC a C20:4; LysoPC a C20:4 and PC ae C40:2; Ser and PC aa C38:3; C10 and LysoPC a C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC a C18:1 and PC aa C36:1; Gly and PC ae C34:1; Gln and PC ae C30:2; LysoPC a C24:0 and PC ae C42:3; C10:1 and LysoPC a C24:0; Tyr and PC aa C42:4; C3-DC and C18; PC aa C42:1
15 and SM C22:3; Gly and SM C24:1; PC aa C32:0 and PC aa C40:1; PC aa C36:4 and PC aa C38:0; PC ae C44:3 and CPT I ratio; PC ae C34:0 and PC ae C40:3; C16:2-OH and SM C20:2; C6(C4:1-DC) and SM C16:1; Gly and PC aa C42:5; C0 and SM(OH) C22:2; PC ae C44:6 and SM C22:3; and C10:1 and C14:2-OH;

and b) obtaining a diagnostic score using a generalized linear model (GLM).

20 The method of the present invention may be performed to determined whether the subject is suffering from any type of endometriosis (all endometriosis), to determine whether the subject is suffering from a specific forms of endometriosis and/or to determine whether the subject is suffering from a mixed form of endometriosis. In other words, the method of the present invention may be performed to determine only one of any type of endometriosis (all endometriosis), a
25 specific forms of endometriosis and a mixed form of endometriosis, or may be performed to determine two or more (such as all) of any type of endometriosis (all endometriosis), a specific form of endometriosis and a mixed form of endometriosis.

Thus, according to some embodiments, the method according to the present invention comprises determining whether the subject is suffering from any type of endometriosis comprising

30 A1) quantifying in a sample obtained from said subject at least two pairs, preferably at least three pairs, of metabolic biomarkers selected from the group of pairs consisting of LysoPC a

C17:0 and SM(OH) C16:1; Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0; LysoPC a C16:0 and SM C18:1; Thr and PC aa C34:3; C18 and LysoPC a C14:0; Ser and PC ae C44:3; Trp and PC ae C38:3; C8 and PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae C38:6; Arg and PC aa C36:6; and Tyr and PC aa C42:4; C3-DC and C18; PC aa C42:1 and SM C22:3; and C6(C4:1-DC) and SM C16:1; and

B1) obtaining a diagnostic score using a generalized linear model (GLM).

According to some embodiments, the method according to the present invention (further) comprises determining whether the subject is suffering from peritoneal endometriosis comprising

A2) quantifying in a sample obtained from said subject at least two pairs, preferably at least three pairs, of metabolic biomarkers selected from the group of pairs consisting of Thr and SM(OH) C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM C18:0; PC aa C32:0 and PC aa C38:3; C6:1 and Pro; Arg and PC ae C34:0; C6:1 and LysoPC a C20:4; C5-M-DC and PC aa C42:5; LysoPC a C18:2 and PC ae C40:6; LysoPC a C18:2 and PC ae C40:4; PC ae C40:6 and CPT I ratio; LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg and PC ae C34:0; and PC ae C34:1 and PC ae C42:0; and

B2) obtaining a diagnostic score using a generalized linear model (GLM).

According to some embodiments, the method according to the present invention (further) comprises determining whether the subject is suffering from peritoneal mixed endometriosis comprising

A3) quantifying in a sample obtained from said subject at least two pairs, preferably at least three pairs, of metabolic biomarkers selected from the group of pairs consisting of Orn and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa C36:6; C5 and LysoPC a C17:0; C5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae C40:5; Pro and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and SM(OH) C16:1; Tyr and PC ae C38:0; Gly and SM C24:1; PC aa C32:0 and PC aa C40:1; PC aa C36:4 and PC aa C38:0; Gly and PC aa C42:5; and C0 and SM(OH) C22:2; and

B3) performing generalized linear modelling (GLM).

According to some embodiments, the method according to the present invention (further) comprises determining whether the subject is suffering from ovarian endometriosis comprising

5 A4) quantifying in a sample obtained from said subject at least two pairs, preferably at least three pairs, of metabolic biomarkers selected from the group of pairs consisting of PC aa C36:3 and PC ae C40:5; LysoPC a C14:0 and PC aa C28:1; Met and PC aa C36:3; PC aa C38:0 and PC ae C36:1; Thr and SM (OH) C22:1; PC aa C28:1 and PC ae C34:3; C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC ae C36:1; C10:1 and PC aa C36:1; PC ae C38:3 and SM C18:1; C12-DC and C14:2; PC aa C38:3 and PC ae C44:5; C4 and C5:1; LysoPC a C20:4 and PC ae C32:1; and LysoPC a C20:4 and PC aa C32:3; and

B4) performing generalized linear modelling (GLM).

10 According to some embodiments, the method according to the present invention (further) comprises determining whether the subject is suffering from ovarian mixed endometriosis comprising

15 A5) quantifying in a sample obtained from said subject at least two pairs, preferably at least three pairs, of metabolic biomarkers selected from the group of pairs consisting of C10 and PC aa C36:6; PC ae C42:3 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and LysoPC a C20:4; LysoPC a C20:4 and PC ae C40:2; Ser and PC aa C38:3; C10 and LysoPC a C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC a C18:1 and PC aa C36:1; Gly and PC ae C34:1; Gln and PC ae C30:2; LysoPC a C24:0 and PC ae C42:3; C10:1 and LysoPC a C24:0; PC ae C44:3 and CPT I ratio; PC ae C34:0 and PC ae C40:3; C16:2-OH and SM C20:2; PC ae C44:6 and SM C22:3; and C10:1 and C14:2-OH; and

20 B5) performing generalized linear modelling (GLM).

The generalized linear model(s) used according to the present invention may comprise determining the ratio of the concentrations for each of the at least two pairs, preferably at least three pairs, of metabolic biomarkers; and calculating the sum of the obtained ratios (value for case). The calculated sum of the obtained ratios (value for case) may then be compared to a
25 predetermined reference value established from healthy subjects (value for control) applying the same GLM on the respective metabolites quantified in samples of said healthy subjects.

Specifically, a diagnostic score (DxS) can then be calculated by forming the quotient between a predetermined reference value obtained from healthy subjects (value for control) and the sum of the obtained ratios (value for case)

$$DxS = \log_2 \left(\frac{\text{predetermined reference value (value for control)}}{\text{sum of the obtained ratios (value for case)}} \right)$$

The DxS is enabling mathematic values obtained from calculations of metabolite ratios according to models (GLMs in the diagnostics). DxS values in the range of 0 ± 0.03 are not facilitating diagnosis of specific indication of endometriosis type and other models have to be taken into the consideration as described in figures 1 and 2.

"Healthy subjects" in accordance with the present invention are subjects that do not have endometriosis. Accordingly, it will be appreciated that the term "healthy subject", in accordance with the present invention, does not require an overall healthy subject. Instead, a healthy subject in accordance with the present invention is a person not having endometriosis. Whether a subject has endometriosis can be ascertained by the presence of a plurality, such as e.g. at least three, more preferably at least four, such as at least five and most preferably all of the unspecific diagnostic parameters including: normal fertility, no pelvic pain or no pain in lower abdomen before menstruation, no pain with bowel movements, lack of inflammatory biomarkers, lack of extra menstrual bleeding. However, as final and dependable diagnosis of endometriosis depends on laparoscopic examination, which is an invasive operative procedure, it is preferred that the healthy subjects are subjects for which the absence of endometriosis has been confirmed by laparoscopic examination.

For example, samples may be taken from a sufficiently large group of healthy subjects, such as for example at least 10, more preferably at least 75 and most preferably at least 100 healthy subjects. The metabolite values obtained from this group, which are also referred to herein as reference values, are then correlated with the absence of endometriosis. It will be appreciated by the skilled person that determining these reference values in healthy subjects may be carried out prior to performing the present invention, such that the determined values may be used as a reference at later times whenever a sample is analysed in accordance with the present invention; or may be determined in parallel each time a sample is analysed in accordance with the present invention. Such reference values may also be determined only once and stored as a standard for all future tests.

Preferably, the reference values are derived from a population having the same racial background as the women to be diagnosed. For example, when employing the present invention in e.g. caucasian women, the reference values should be obtained from healthy caucasian subjects.

For example, using a group of caucasian (i.e. Slovenian and Austrian) females as shown in the
 5 appended examples, reference values were determined as shown in Tables 5, 8, 11, 14 and 17 below.

Accordingly, when employing the method of the present invention in a group of caucasian females, the above defined reference values for healthy subjects may for example be relied upon.

Generally, an indication of endometriosis or any of its sub-types is given when the diagnostic score
 10 is different from zero ("0"). In other words, if the diagnostic score has a positive or negative value, then the subject can be diagnosed as having endometriosis or the sub-type investigate. Conversely, if the diagnostic score is zero ("0"), then the subject is not suffering from endometriosis or the sub-type investigate.

According to some embodiments, the method according to the present invention comprises
 15 determining whether the subject is suffering from any type of endometriosis comprising any one of the following procedures (1) to (15):

- (1) quantifying in a sample obtained from said subject the metabolites LysoPC a C17:0, SM(OH) C16:1, Arg, PC ae C36:0, PC ae C38:0 and PC ae C40:0; and performing GLM using the model formula $\text{LysoPC a C17:0_div_by_SM(OH) C16:1} + \text{Arg_div_by_PC ae C36:0} + \text{PC ae C38:0_div_by_PC ae C40:0}$;
 20
- (2) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC ae C36:0, LysoPC a C16:0, SM C18:1, PC ae C38:0 and PC ae C40:0; and performing GLM using the model formula $\text{Arg_div_by_PC ae C36:0} + \text{LysoPC a C16:0_div_by_SM C18:1} + \text{PC ae C38:0_div_by_PC ae C40:0}$;
- 25 (3) quantifying in a sample obtained from said subject the metabolic biomarkers Thr, PC aa C34:3, LysoPC a C17:0, SM(OH) C16:1, Arg and PC ae C36:0; and performing GLM using the model formula $\text{Thr_div_by_PC aa C34:3} + \text{LysoPC a C17:0_div_by_SM(OH) C16:1} + \text{Arg_div_by_PC ae C36:0}$;

- (4) quantifying in a sample obtained from said subject the metabolic biomarkers Thr, PC aa C34:3, Arg, PC ae C36:0, LysoPC a C16:0 and SM C18:1; and performing GLM using the model formula $\text{Thr_div_by_PC aa C34:3} + \text{Arg_div_by_PC ae C36:0} + \text{LysoPC a C16:0_div_by_SM C18:1}$;
- 5 (5) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC aa C36:6, LysoPC a C17:0, SM(OH) C16:1, and PC ae C36:0; and performing GLM using the model formula $\text{Arg_div_by_PC aa C36:6} + \text{LysoPC a C17:0_div_by_SM(OH) C16:1} + \text{Arg_div_by_PC ae C36:0}$;
- 10 (6) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C17:0, SM(OH) C16:1, Arg, PC ae C36:0, C18 and LysoPC a C14:0; and performing GLM using the model formula $\text{LysoPC a C17:0_div_by_SM(OH) C16:1} + \text{Arg_div_by_PC ae C36:0} + \text{C18_div_by_LysoPC a C14:0}$;
- 15 (7) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C16:0, SM C18:1, PC ae C38:0, PC ae C40:0, Ser and PC ae C44:3; and performing GLM using the model formula $\text{LysoPC a C16:0_div_by_SM C18:1} + \text{PC ae C38:0_div_by_PC ae C40:0} + \text{Ser_div_by_PC ae C44:3}$;
- 20 (8) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C17:0, SM(OH) C16:1, Arg, PC ae C36:0, Trp, PC ae C38:3; and performing GLM using the model formula $\text{LysoPC a C17:0_div_by_SM(OH) C16:1} + \text{Arg_div_by_PC ae C36:0} + \text{Trp_div_by_PC ae C38:3}$;
- (9) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC ae C36:0, LysoPC a C16:0, SM C18:1, C8 and PC ae C30:0; and performing GLM using the model formula $\text{Arg_div_by_PC ae C36:0} + \text{LysoPC a C16:0_div_by_SM C18:1} + \text{C8_div_by_PC ae C30:0}$;
- 25 (10) quantifying in a sample obtained from said subject the metabolic biomarkers Thr, PC ae C36:5, Arg, PC ae C36:0, LysoPC a C16:0 and SM C18:1; and performing GLM using the model formula $\text{Thr_div_by_PC ae C36:5} + \text{Arg_div_by_PC ae C36:0} + \text{LysoPC a C16:0_div_by_SM C18:1}$;

- (11) quantifying in a sample obtained from said subject the metabolite Arg, PC ae C36:0, C18, LysoPC a C14:0, LysoPC a C16:0 and SM C18:1; and performing GLM using the model formula $\text{Arg_div_by_PC ae C36:0} + \text{C18_div_by_LysoPC a C14:0} + \text{LysoPC a C16:0_div_by_SM C18:1}$;
- 5 (12) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC ae C36:0, C10, PC ae C38:6, LysoPC a C16:0 and SM C18:1; and performing GLM using the model formula $\text{Arg_div_by_PC ae C36:0} + \text{C10_div_by_PC ae C38:6} + \text{LysoPC a C16:0_div_by_SM C18:1}$;
- 10 (13) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC aa C36:6, PC ae C36:0, LysoPC a C16:0 and SM C18:1; and performing GLM using the model formula $\text{Arg_div_by_PC aa C36:6} + \text{Arg_div_by_PC ae C36:0} + \text{LysoPC a C16:0_div_by_SM C18:1}$;
- 15 (14) quantifying in a sample obtained from said subject the metabolic biomarkers Tyr, PC aa C42:4, C3-DC, C18, PC aa C42:1 and SM C22:3; and performing GLM using the model formula $\text{Tyr_div_by_PC aa C42:4} + \text{C3-DC_div_by_C18} + \text{PC aa C42:1_div_by_SM C22:3}$;
- 20 (15) quantifying in a sample obtained from said subject the metabolic biomarkers C3-DC, C18, PC aa C42:1, SM C22:3, C6(C4:1-DC) and SM C16:1; and performing GLM using the model formula $\text{C3-DC_div_by_C18} + \text{PC aa C42:1_div_by_SM C22:3} + \text{C6 (C4:1-DC)_div_by_SM C16:1}$.

According to some embodiments, the method according to the present invention comprises determining whether the subject is suffering from peritoneal endometriosis comprising any one of the following procedures (1) to (7):

- 25 (1) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C16:0, SM(OH) C16:1, PC aa C32:0, SM C18:0, PC aa C32:0 and PC aa C38:3; and performing GLM using the model formula $\text{LysoPC a C16:0_div_by_SM(OH) C16:1} + \text{PC aa C32:0_div_by_SM C18:0} + \text{PC aa C32:0_div_by_PC aa C38:3}$;
- (2) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C16:0, SM(OH) C16:1, PC aa C32:0, SM C18:0, Arg and PC ae C34:0; and performing

GLM using the model formula $\text{LysoPC} \propto \text{C16:0_div_by_SM(OH)} \text{ C16:1} + \text{PC} \propto \text{C32:0_div_by_SM} \text{ C18:0} + \text{Arg_div_by_PC} \propto \text{C34:0}$;

- 5 (3) quantifying in a sample obtained from said subject the metabolic biomarkers C5-M-DC, PC $\propto$ C42:5, Arg, PC $\propto$ C34:0, LysoPC $\propto$ C18:2 and PC $\propto$ C40:6; and performing GLM using the model formula $\text{C5-M-DC_div_by_PC} \propto \text{C42:5} + \text{Arg_div_by_PC} \propto \text{C34:0} + \text{LysoPC} \propto \text{C18:2_div_by_PC} \propto \text{C40:6}$;
- 10 (4) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC $\propto$ C18:2, PC $\propto$ C40:4, PC $\propto$ C40:6, CPT I ratio, LysoPC $\propto$ C17:0 and SM C18:0; and performing GLM using the model formula $\text{LysoPC} \propto \text{C18:2_div_by_PC} \propto \text{C40:4} + \text{PC} \propto \text{C40:6_div_by_CPT I ratio} + \text{LysoPC} \propto \text{C17:0_div_by_SM} \text{ C18:0}$;
- (5) quantifying in a sample obtained from said subject the metabolic biomarkers C4, PC $\propto$ C30:2, Arg, PC $\propto$ C34:0, LysoPC $\propto$ C18:2 and PC $\propto$ C40:6; and performing GLM using the model formula $\text{C4_div_by_PC} \propto \text{C30:2} + \text{Arg_div_by_PC} \propto \text{C34:0} + \text{LysoPC} \propto \text{C18:2_div_by_PC} \propto \text{C40:6}$;
- 15 (6) quantifying in a sample obtained from said subject the metabolic biomarkers PC $\propto$ C40:6, CPT I ratio, C4, PC $\propto$ C30:2, LysoPC $\propto$ C18:2 and PC $\propto$ C40:6; and performing GLM using the model formula $\text{PC} \propto \text{C40:6_div_by_CPT I ratio} + \text{C4_div_by_PC} \propto \text{C30:2} + \text{LysoPC} \propto \text{C18:2_div_by_PC} \propto \text{C40:6}$;
- 20 (7) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC $\propto$ C18:2, PC $\propto$ C40:4, Arg, PC $\propto$ C34:0, PC $\propto$ C34:1 and PC $\propto$ C42:0; and performing GLM using the model formula $\text{LysoPC} \propto \text{C18:2_div_by_PC} \propto \text{C40:4} + \text{Arg_div_by_PC} \propto \text{C34:0} + \text{PC} \propto \text{C34:1_div_by_PC} \propto \text{C42:0}$.

According to some embodiments, the method according to the present invention comprises determining whether the subject is suffering from peritoneal mixed endometriosis comprising any
25 one of the following procedures (1) to (16):

- (1) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC $\propto$ C38:0, C4, PC $\propto$ C38:4, Tyr and PC $\propto$ C42:2; and performing GLM using the model formula $\text{Orn_div_by_PC} \propto \text{C38:0} + \text{C4_div_by_PC} \propto \text{C38:4} + \text{Tyr_div_by_PC} \propto \text{C42:2}$;

- (2) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC aa C36:6, C5 and LysoPC a C17:0; and performing GLM using the model formula $\text{Arg_div_by_PC aa C36:6} + \text{C5_div_by_lysoPC a C17:0} + \text{C5_div_by_Arg}$;
- 5 (3) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, C5, LysoPC a C17:0 and Arg; and performing GLM using the model formula $\text{Orn_div_by_PC ae C38:0} + \text{C5_div_by_lysoPC a C17:0} + \text{C5_div_by_Arg}$;
- (4) quantifying in a sample obtained from said subject the metabolic biomarkers C0, Gly, Orn, PC ae C38:0, Tyr and PC aa C42:2; and performing GLM using the model formula $\text{C0_div_by_Gly} + \text{Orn_div_by_PC ae C38:0} + \text{Tyr_div_by_PC aa C42:2}$;
- 10 (5) quantifying in a sample obtained from said subject the metabolic biomarkers SM C18:0, C5, LysoPC a C17:0 and Arg; and performing GLM using the model formula $\text{SM C18:0} + \text{C5_div_by_lysoPC a C17:0} + \text{C5_div_by_Arg}$;
- (6) quantifying in a sample obtained from said subject the metabolic biomarkers C5, LysoPC a C17:0, Arg, Ser and SM(OH) C16:1; and performing GLM using the model formula $\text{C5_div_by_lysoPC a C17:0} + \text{C5_div_by_Arg} + \text{Ser_div_by_SM(OH) C16:1}$;
- 15 (7) quantifying in a sample obtained from said subject the metabolic biomarkers SM C18:0, C0, Gly, Tyr and PC aa C42:2; and performing GLM using the model formula $\text{SM C18:0} + \text{C0_div_by_Gly} + \text{Tyr_div_by_PC aa C42:2}$;
- (8) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, C3, PC ae C40:5, Tyr and PC aa C42:2; and performing GLM using the model formula $\text{Orn_div_by_PC ae C38:0} + \text{C3_div_by_PC ae C40:5} + \text{Tyr_div_by_PC aa C42:2}$;
- 20 (9) quantifying in a sample obtained from said subject the metabolic biomarkers Pro, PC ae C34:0, Orn, PC ae C38:0, Tyr and PC aa C42:2; and performing GLM using the model formula $\text{Pro_div_by_PC ae C34:0} + \text{Orn_div_by_PC ae C38:0} + \text{Tyr_div_by_PC aa C42:2}$;
- 25 (10) quantifying in a sample obtained from said subject the metabolic biomarkers C4, Ser, Orn, PC ae C38:0, Tyr and PC aa C42:2; and performing GLM using the model formula $\text{C4_div_by_Ser} + \text{Orn_div_by_PC ae C38:0} + \text{Tyr_div_by_PC aa C42:2}$;

- (11) quantifying in a sample obtained from said subject the metabolic biomarkers SM C18:0, Orn, PC ae C38:0, Tyr and PC aa C42:2; and performing GLM using the model formula $SM\ C18:0 + Orn_div_by_PC\ ae\ C38:0 + Tyr_div_by_PC\ aa\ C42:2$;
- 5 (12) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, C4, PC ae C40:3, Tyr and PC aa C42:2; and performing GLM using the model formula $Orn_div_by_PC\ ae\ C38:0 + C4_div_by_PC\ ae\ C40:3 + Tyr_div_by_PC\ aa\ C42:2$;
- 10 (13) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, PC ae C42:3, SM(OH) C16:1, Tyr and PC aa C42:2; and performing GLM using the model formula $Orn_div_by_PC\ ae\ C38:0 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1 + Tyr_div_by_PC\ aa\ C42:2$;
- 15 (14) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, Tyr, PC ae C38:0 and PC aa C42:2; and performing GLM using the model formula $Orn_div_by_PC\ ae\ C38:0 + Tyr_div_by_PC\ ae\ C38:0 + Tyr_div_by_PC\ aa\ C42:2$;
- (15) quantifying in a sample obtained from said subject the metabolic biomarkers Gly, SM C24:1, PC aa C32:0, PC aa C40:1, PC aa C36:4 and PC aa C38:0; and performing GLM using the model formula $Gly_div_by_SM\ C24:1 + PC\ aa\ C32:0_div_by_PC\ aa\ C40:1 + PC\ aa\ C36:4_div_by_PC\ aa\ C38:0$;
- 20 (16) quantifying in a sample obtained from said subject the metabolic biomarkers Gly, PC aa C42:5, PC aa C36:4, PC aa C38:0SM, C0 and SM(OH) C22:2; and performing GLM using the model formula $Gly_div_by_PC\ aa\ C42:5 + PC\ aa\ C36:4_div_by_PC\ aa\ C38:0 + C0_div_by_SM(OH)\ C22:2$.

According to some embodiments, the method according to the present invention comprises
 25 determining whether the subject is suffering from ovarian endometriosis comprising any one of the following procedures (1) to (14):

- (1) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C36:3, PC ae C40:5, LysoPC a C14:0, PC aa C28:1 and Met; and performing GLM using the model formula $PC\ aa\ C36:3_div_by_PC\ ae\ C40:5 + LysoPC\ a\ C14:0_div_by_PC\ aa\ C28:1 + Met_div_by_PC\ aa\ C36:3$;
- 30

- (2) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C38:0, PC ae C36:1, Thr, SM (OH) C22:1, LysoPC a C14:0 and PC aa C28:1; and performing GLM using the model formula $PC\ aa\ C38:0_div_by_PC\ ae\ C36:1 + Thr_div_by_SM\ (OH)\ C22:1 + LysoPC\ a\ C14:0_div_by_PC\ aa\ C28:1$;
- 5 (3) quantifying in a sample obtained from said subject the metabolic biomarkers Thr, SM (OH) C22:1, PC aa C28:1, PC ae C34:3, C18:2 and PC ae C34:3; and performing GLM using the model formula $Thr_div_by_SM\ (OH)\ C22:1 + PC\ aa\ C28:1_div_by_PC\ ae\ C34:3 + C18:2_div_by_PC\ ae\ C34:3$;
- 10 (4) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C36:3, PC ae C40:5, C3, PC ae C34:1, Met and PC aa C36:3; and performing GLM using the model formula $PC\ aa\ C36:3_div_by_PC\ ae\ C40:5 + C3_div_by_PC\ ae\ C34:1 + Met_div_by_PC\ aa\ C36:3$;
- 15 (5) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C36:3, PC ae C40:5, PC aa C28:1, PC ae C34:3, Gly and PC ae C36:1; and performing GLM using the model formula $PC\ aa\ C36:3_div_by_PC\ ae\ C40:5 + PC\ aa\ C28:1_div_by_PC\ ae\ C34:3 + Gly_div_by_PC\ ae\ C36:1$;
- 20 (6) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C38:0, PC ae C36:1, C18:2, PC ae C34:3, Met and PC aa C36:3; and performing GLM using the model formula $PC\ aa\ C38:0_div_by_PC\ ae\ C36:1 + C18:2_div_by_PC\ ae\ C34:3 + Met_div_by_PC\ aa\ C36:3$;
- (7) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C38:0, PC ae C36:1, C10:1, PC aa C36:1, PC ae C38:3 and SM C18:1; and performing GLM using the model formula $PC\ aa\ C38:0_div_by_PC\ ae\ C36:1 + C10:1_div_by_PC\ aa\ C36:1 + PC\ ae\ C38:3_div_by_SM\ C18:1$;
- 25 (8) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C38:0, PC ae C36:1, Gly, C3 and PC ae C34:1; and performing GLM using the model formula $PC\ aa\ C38:0_div_by_PC\ ae\ C36:1 + Gly_div_by_PC\ ae\ C36:1 + C3_div_by_PC\ ae\ C34:1$;

- (9) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C38:0, PC ae C36:1, C12-DC, C14:2, PC ae C38:3 and SM C18:1; and performing GLM using the model formula $PC\ aa\ C38:0_div_by_PC\ ae\ C36:1 + C12-DC_div_by_C14:2 + PC\ ae\ C38:3_div_by_SM\ C18:1$;
- 5 (10) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C36:3, PC ae C40:5, PC aa C38:3, PC ae C44:5 and Met; and performing GLM using the model formula $PC\ aa\ C36:3_div_by_PC\ ae\ C40:5 + PC\ aa\ C38:3_div_by_PC\ ae\ C44:5 + Met_div_by_PC\ aa\ C36:3$;
- 10 (11) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C38:0, PC ae C36:1, PC ae C38:3, SM C18:1, Met and PC aa C36:3; and performing GLM using the model formula $PC\ aa\ C38:0_div_by_PC\ ae\ C36:1 + PC\ ae\ C38:3_div_by_SM\ C18:1 + Met_div_by_PC\ aa\ C36:3$;
- 15 (12) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C28:1, PC ae C34:3, C18:2, PC ae C34:3, C4 and C5:1; and performing GLM using the model formula $PC\ aa\ C28:1_div_by_PC\ ae\ C34:3 + C18:2_div_by_PC\ ae\ C34:3 + C4_div_by_C5:1$;
- 20 (13) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C36:3, PC ae C40:5, LysoPC a C20:4, PC ae C32:1 and Met; and performing GLM using the model formula $PC\ aa\ C36:3_div_by_PC\ ae\ C40:5 + LysoPC\ a\ C20:4_div_by_PC\ ae\ C32:1 + Met_div_by_PC\ aa\ C36:3$;
- (14) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C36:3, PC ae C40:5, LysoPC a C20:4, PC aa C32:3 and Met; and performing GLM using the model formula $PC\ aa\ C36:3_div_by_PC\ ae\ C40:5 + LysoPC\ a\ C20:4_div_by_PC\ aa\ C32:3 + Met_div_by_PC\ aa\ C36:3$.
- 25 According to some embodiments, the method according to the present invention comprises determining whether the subject is suffering from ovarian mixed endometriosis comprising any one of the following procedures (1) to (13):
- (1) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC aa C36:6, Pro, PC ae C34:0, PC ae C42:3 and SM(OH) C16:1; and

- performing GLM using the model formula $C10_div_by_PC \text{ aa } C36:6 + Pro_div_by_PC \text{ ae } C34:0 + PC \text{ ae } C42:3_div_by_SM(OH) \text{ C16:1}$;
- (2) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC aa C36:6, PC ae C42:3, SM(OH) C16:1, C6:1 and LysoPC a C20:4; and performing GLM using the model formula $C10_div_by_PC \text{ aa } C36:6 + PC \text{ ae } C42:3_div_by_SM(OH) \text{ C16:1} + C6:1_div_by_lysoPC \text{ a } C20:4$;
- (3) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC aa C36:6, PC ae C42:3, SM(OH) C16:1, LysoPC a C20:4 and PC ae C40:2; and performing GLM using the model formula $C10_div_by_PC \text{ aa } C36:6 + PC \text{ ae } C42:3_div_by_SM(OH) \text{ C16:1} + lysoPC \text{ a } C20:4_div_by_PC \text{ ae } C40:2$;
- (4) quantifying in a sample obtained from said subject the metabolic biomarkers Ser, PC aa C38:3, C10, PC aa C36:6, PC ae C42:3 and SM(OH) C16:1; and performing GLM using the model formula $Ser_div_by_PC \text{ aa } C38:3 + C10_div_by_PC \text{ aa } C36:6 + PC \text{ ae } C42:3_div_by_SM(OH) \text{ C16:1}$;
- (5) quantifying in a sample obtained from said subject the metabolic biomarkers C10, LysoPC a C18:1, PC aa C36:6, PC ae C42:3 and SM(OH) C16:1; and performing GLM using the model formula $C10_div_by_lysoPC \text{ a } C18:1 + C10_div_by_PC \text{ aa } C36:6 + PC \text{ ae } C42:3_div_by_SM(OH) \text{ C16:1}$;
- (6) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC aa C36:6, LysoPC a C24:0, PC ae C42:3, PC ae C42:3 and SM(OH) C16:1; and performing GLM using the model formula $C10_div_by_PC \text{ aa } C36:6 + LysoPC \text{ a } C24:0_div_by_PC \text{ ae } C42:3 + PC \text{ ae } C42:3_div_by_SM(OH) \text{ C16:1}$;
- (7) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC aa C36:6, LysoPC a C18:1, PC aa C36:1, PC ae C42:3 and SM(OH) C16:1; and performing GLM using the model formula $C10_div_by_PC \text{ aa } C36:6 + LysoPC \text{ a } C18:1_div_by_PC \text{ aa } C36:1 + PC \text{ ae } C42:3_div_by_SM(OH) \text{ C16:1}$;
- (8) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC aa C36:6, + Gly, PC ae C34:1, PC ae C42:3 and SM(OH) C16:1; and

performing GLM using the model formula $C10_div_by_PC \propto C36:6 + Gly_div_by_PC \propto C34:1 + PC \propto C42:3_div_by_SM(OH) C16:1$;

- 5 (9) quantifying in a sample obtained from said subject the metabolic biomarkers Gln, $PC \propto C30:2$, C10, $PC \propto C36:6$, $PC \propto C42:3$ and $SM(OH) C16:1$; and performing GLM using the model formula $Gln_div_by_PC \propto C30:2 + C10_div_by_PC \propto C36:6 + PC \propto C42:3_div_by_SM(OH) C16:1$;
- 10 (10) quantifying in a sample obtained from said subject the metabolic biomarkers Pro, $PC \propto C34:0$, LysoPC a C24:0, $PC \propto C42:3$, $PC \propto C42:3$ and $SM(OH) C16:1$; and performing GLM using the model formula $Pro_div_by_PC \propto C34:0 + lysoPC \propto C24:0_div_by_PC \propto C42:3 + PC \propto C42:3_div_by_SM(OH) C16:1$;
- (11) quantifying in a sample obtained from said subject the metabolic biomarkers C10:1, LysoPC a C24:0, LysoPC a C24:0, $PC \propto C42:3$ and $SM(OH) C16:1$; and performing GLM using the model formula $C10:1_div_by_lysoPC \propto C24:0 + lysoPC \propto C24:0_div_by_PC \propto C42:3 + PC \propto C42:3_div_by_SM(OH) C16:1$;
- 15 (12) quantifying in a sample obtained from said subject the metabolic biomarkers $PC \propto C44:3$, CPT I ratio, $PC \propto C34:0$, $PC \propto C40:3$, C16:2-OH and $SM C20:2$; and performing GLM using the model formula $PC \propto C44:3_div_by_CPT.I.ratio + PC \propto C34:0_div_by_PC \propto C40:3 + C16:2-OH_div_by_SM C20:2$;
- 20 (13) quantifying in a sample obtained from said subject the metabolic biomarkers $PC \propto C44:6$, $SM C22:3$, $PC \propto C34:0$, $PC \propto C40:3$, C10:1 and C14:2-OH; and performing GLM using the model formula $PC \propto C44:6_div_by_SM C22:3 + PC \propto C34:0_div_by_PC \propto C40:3 + C10:1_div_by_C14:2-OH$.

Means and methods for quantifying (i.e. determining the concentration) of metabolites in samples, such as e.g. in blood, are well known in the art. Preferably, quantifying the metabolic biomarkers includes measuring the absolute concentration of each of the biomarkers in the sample obtained from said subject.

25

Suitably, the metabolic biomarkers are to be quantified with mass spectrometry to ensure specificity of metabolite identification, quantification of metabolites and multiplexing. Thus, according to some embodiments, the concentrations of the metabolic biomarkers are determined by mass spectrometry.

30

Mass spectrometry and its use for determining the concentration of metabolites in a sample is well known in the art and has been described for example in (45 and 46). Mass spectrometry includes, for example, flow-injection analysis mass spectrometry (FIA-MS), tandem mass spectrometry, matrix assisted laser desorption ionization (MALDI) time-of-flight (TOF) mass spectrometry, MALDI-
5 TOF-TOF mass spectrometry, MALDI Quadrupole-time-of-flight (Q-TOF) mass spectrometry, electrospray ionization (ESI) -TOF mass spectrometry, ESI-Q-TOF, ESI-TOF-TOF, ESI-ion trap mass spectrometry, ESI Triple quadrupole mass spectrometry, ESI Fourier Transform mass spectrometry (FTMS), MALDI-FTMS, MALDI-Ion Trap-TOF, and ESI-Ion Trap TOF. At its most basic level, mass spectrometry involves ionizing a molecule and then measuring the mass of the resulting ions. Since
10 molecules ionize in a way that is well known, the molecular weight of the molecule can be accurately determined from the mass of the ions. In addition, by a comparison of data obtained from internal standards, a quantification of molecules of interest is possible, as detailed herein below.

According to some embodiments, the mass spectrometry is selected from flow-injection analysis
15 mass spectrometry (FIA-MS), liquid chromatography mass spectrometry (LC-MS or HPLC-MS) and tandem mass spectrometry (MS-MS).

The sample to be analysed may be any sample allowing the quantification of the metabolites. Non-limiting examples of suitable samples include blood, serum, plasma, saliva, urine, cerebrospinal fluid, condensates from respiratory air, tears, mucosal tissue, mucus, vaginal tissue, endometrium,
20 eutopic endometrium, skin, hair or hair follicle, of which blood, serum and plasma are preferred.

According to some embodiments, the sample is selected from blood, serum and plasma.

According to some embodiments, the sample is plasma.

According to some embodiments, the subject is suspected to suffer from endometriosis or to have a predisposition therefore.

25 According to some embodiments, the subject is a human subject, and preferably a human female.

According to some embodiments, the human subject, preferably human female, is of Caucasian race.

Certain definitions

The expression "AUC" as used herein means "area under the curve" and describes the quality of diagnostic model. The worst value is 0.5, the theoretically best 1.0 (41).

The expression "GLM" as used herein means generalized linear model (42).

The expression "CPT I ratio" as used herein is a ratio of (C18AC+C16AC)/C0, i.e. (octadecanoylcarnitine + hexadecanoylcarnitine) / free carnitine. It describes efficiency of import of metabolites to mitochondria (38).

"Variance" is the expectation of the squared deviation of a random variable from its mean.

$$\text{variance } \sigma^2 = \frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n}$$

where:

x_i = the i^{th} data point

$\bar{x}$ = the mean of all data points

n = the number of data points

" R_y^2 " describes explained x-variation. Should be above 0.75 and never negative.

10 " R_x^2 " describes explained y-variation. Should be above 0.75 and never negative.

" Q_x^2 " describes predicted variation. Should be above 0.4, never 1.0 and never negative.

"RMSE" means "root mean square error" of estimations and is an accuracy of the model. Should be below 0.25

15 " P_R^2 " - R^2 parameter after permutation testing of the sample grouping (2000 times). Has to be below 0.05 to produce valid PLS-DA.

" P_Q^2 " - Q^2 parameter after permutation testing of the sample grouping (2000 times). Has to be below 0.05 to produce a valid PLS-DA.

"Fold change" is expressed as log2 value to enable linear comparison (39, 40).

20 The expression "_div_by_" as used herein corresponds to the division of concentration of two metabolites.

Having generally described this invention, a further understanding can be obtained by reference to certain specific examples, which are provided herein for purposes of illustration only, and are not intended to be limiting unless otherwise specified.

Examples

Description of study and its replication

For the discovery and replication studies we ensured that controls and cases are matched for age and BMI as far as the clinical setting allows. Patients with other comorbidities were excluded. New
5 plasma samples were collected in Ljubljana (Slovenia) and Vienna (Austria) for the discovery phase and only in Ljubljana for the replication. Samples were measured with Biocrates p180 kit (37) in 287 plasma samples (discovery study) and in 245 plasma samples (replication study) obtained from controls and endometriosis patients at different stages. All measurements and primary data underwent quality assurance procedures and only a validated data set was used for biostatistical
10 analyses.

The data was calculated with R 4.0.2 (2020-06-22). Biostatistics analyses revealed no significant differences in age, BMI or menstrual cycle between control and case groups.

Quality control elements

NA imputation (missing data imputation) was performed for metabolites with less than 40% missing
15 values. Metabolites with more than 40% missing values were discarded.

Further, remaining metabolites were checked for coefficients of variation (CV%) of more than 25% and the affected metabolites discarded from the data set. The data was also log- transformed in order to check for lognormal data distribution by Shapiro-Wilks test, but log-normal data distribution was not detected.

20 *PLS-DA analysis case vs control*

Non-log transformed metabolite data was used to calculate the PLS-DA as given above in Figure 3. This PLS-DA does not include the metabolites which were found to be above the CV% threshold of 25% or were excluded due to being above the NA threshold of 40% (as described before). As is evident from the PLS-DA statistics, a separation by group is not possible if based on absolute
25 concentrations of metabolites.

Dedicated analysis for impact of confounders on plasma metabolome

Analyses from log transforming and autoscaling the metabolite concentrations of all samples for confounder effect like menstrual cycle, age, BMI, presence of other disease (cancer or diabetes) or medication revealed no detectable impact.

Statistics control vs case groups with false discovery rate

- 5 Log-transformation of the data does, according to Shapiro- Wilks test, lead to non-parametric data. Therefore, Mann- Whitney-U tests were performed on the data. When performing multiple testing correction by the FDR method, these significant results can be found. Without multiple testing correction, more metabolites appear to be significantly different.

Calculation of ROC and AUC with GLM models

- 10 As the classic statistical approach using absolute metabolite concentrations proved not sufficient for the data set presented, the metabolite selection was performed by machine learning with randomForest (RF) on all metabolites and all possible metabolite ratios.

All calculations are performed on the 10x cross validated data – this means data was randomly divided into 66% training data and 34% test data for each cross validation step.

- 15 In order to narrow down the possible candidates for further modelling with generalized linear models (GLM) and to obtain reporter-operator curves (ROC) with area under the curve (AUC) calculations, the following criteria were used for RF:

Number of calculated trees: 500

MeanDecreaseGini: > 0

- 20 From the remaining candidates only those in the top 10% of the performance were selected.
From the remaining candidates all possible combinations for 3-predictor model for the GLM were calculated. This results in 67599 possible combinations for these GLMs when leaving out metabolites/metabolite ratios which are derived from total sums of measured metabolites assigned within specific chemical classes. The later would be impractical to measure in a diagnostic assay
25 and were excluded.

The GLMs were calculated on the response of samples being in the control group or case group. Modelling with disease stage or disease type as response lead to over-fitting of the models.

Although the ROCs with their respective AUCs shown in the following pages only show an AUC up to average 0.82 in the test data set, it is still worth to note that it is very well possible to distinguish the responses in the models with a rather fair accuracy by selecting the parameters of the glms by RF from all the possible metabolites and ratios. This is not a feasible approach for PLS-DA analysis
 5 due to the high likelihood of over-fitting the model.

Description of GLM models and metabolites

The following chapters describe the GLMs identified for specific forms of endometriosis. The GLMs are annotated for performance (AUC and RMSE). The metabolites constituting the GLMs are
 10 extracted and annotated. Further the basis for diagnostic decisions is provided.

1. All types of endometriosis

Table 3: GLMs for all types of endometriosis

#	GLM	AUC average	RMSE
1	LysoPC a C17:0_div_by_SM(OH) C16:1 + Arg_div_by_PC ae C36:0 + PC ae C38:0_div_by_PC ae C40:0	0.7256	0.0427
2	Arg_div_by_PC ae C36:0 + lysoPC a C16:0_div_by_SM C18:1 + PC ae C38:0_div_by_PC ae C40:0	0.7240	0.0434
3	Thr_div_by_PC aa C34:3 + LysoPC a C17:0_div_by_SM(OH) C16:1 + Arg_div_by_PC ae C36:0	0.7159	0.0767
4	Thr_div_by_PC aa C34:3 + Arg_div_by_PC ae C36:0 + lysoPC a C16:0_div_by_SM C18:1	0.7120	0.0713
5	Arg_div_by_PC aa C36:6 + LysoPC a C17:0_div_by_SM(OH) C16:1 + Arg_div_by_PC ae C36:0	0.7087	0.0544
6	LysoPC a C17:0_div_by_SM(OH) C16:1 + Arg_div_by_PC ae C36:0 + C18_div_by_lysoPC a C14:0	0.7061	0.0540
7	lysoPC a C16:0_div_by_SM C18:1 + PC ae C38:0_div_by_PC ae C40:0 + Ser_div_by_PC ae C44:3	0.7040	0.0402

8	LysoPC a C17:0_div_by_SM(OH) C16:1 + Arg_div_by_PC ae C36:0 + Trp_div_by_PC ae C38:3	0.7037	0.0786
9	Arg_div_by_PC ae C36:0 + lysoPC a C16:0_div_by_SM C18:1 + C8_div_by_PC ae C30:0	0.7029	0.0559
10	Thr_div_by_PC ae C36:5 + Arg_div_by_PC ae C36:0 + lysoPC a C16:0_div_by_SM C18:1	0.7021	0.0503
11	Arg_div_by_PC ae C36:0 + C18_div_by_lysoPC a C14:0 + lysoPC a C16:0_div_by_SM C18:1	0.7013	0.0531
12	Arg_div_by_PC ae C36:0 + C10_div_by_PC ae C38:6 + lysoPC a C16:0_div_by_SM C18:1	0.7006	0.0465
13	Arg_div_by_PC aa C36:6 + Arg_div_by_PC ae C36:0 + lysoPC a C16:0_div_by_SM C18:1	0.7002	0.0523
14	Tyr_div_by_PC aa C42:4 + C3-DC_div_by_C18 + PC aa C42:1_div_by_SM C22:3	0.7397	0.0906
15	C3-DC_div_by_C18 + PC aa C42:1_div_by_SM C22:3 + C6 (C4:1-DC)_div_by_SM C16:1	0.7290	0.0969

GLM describes a model formula consisting of sum of three metabolite ratios. The models are listed according to average AUC (Area Under the curve) average and the RMSE (Root Mean Squared Error) less than 0,15. The AUC analyses for best model and its cross-validation are presented in figures 4 and 5.

5

Table 4: Performance of GLM models for all types of endometriosis

GLM model	AUC best	Sensitivity best	Specificity best	AUC average	Sensitivity average	Specificity average
1	0.76	0.87	0.74	0.72	0.85	0.72
2	0.76	0.84	0.72	0.72	0.87	0.73
3	0.83	0.84	0.78	0.71	0.88	0.71
4	0.82	0.84	0.76	0.71	0.87	0.71
5	0.76	0.87	0.74	0.70	0.86	0.71
6	0.75	0.87	0.74	0.70	0.88	0.72
7	0.75	0.36	0.67	0.70	0.73	0.67

8	0.75	0.84	0.75	0.70	0.87	0.71
9	0.73	0.82	0.76	0.70	0.82	0.68
10	0.76	0.87	0.74	0.70	0.87	0.71
11	0.75	0.84	0.70	0.70	0.85	0.70
12	0.73	0.89	0.74	0.70	0.86	0.70
13	0.72	0.89	0.73	0.70	0.87	0.71
14	0.84	0.77	0.68	0.74	0.68	0.65
15	0.83	0.84	0.64	0.72	0.66	0.65

Only for the first ten best GLM models the DxS values are calculated. In the development of GLMs we observed that further models, analysed for all types of endometriosis, are not contributing to the phenotype explanation significantly. In fact, we noticed that the performance drops continuously after several iterations, especially after the 10th model.

5

Table 5: Interpretation basis for diagnosis of all types endometriosis

GLM model	Reference value (Value for Control)	Value for Case	DxS - Fold change Log2
1	114.07	99.48	0.20
2	119.59	105.58	0.18
3	123.88	109.73	0.17
4	129.40	115.84	0.16
5	258.34	237.52	0.12
6	113.75	99.18	0.20
7	978.17	884.20	0.15
8	135.71	121.58	0.16
9	119.63	105.65	0.18
10	131.28	117.05	0.17

A numeric value is calculated according to the GLM model formula. The calculated value is used to discriminate between diseased and not affected patient. Negative or positive values of DxS describe the direction of differences of case versus control.

5

10 2. Peritoneal endometriosis

Table 6: GLMs for peritoneal endometriosis

#	GLM	AUC average	RMSE
1	lysoPC a C16:0_div_by_SM(OH) C16:1 + PC aa C32:0_div_by_SM C18:0 + PC aa C32:0_div_by_PC aa C38:3	0.8080	0.08836
2	lysoPC a C16:0_div_by_SM(OH) C16:1 + PC aa C32:0_div_by_SM C18:0 + Arg_div_by_PC ae C34:0	0.7949	0.09458
3	C5-M-DC_div_by_PC aa C42:5 + Arg_div_by_PC ae C34:0 + lysoPC a C18:2_div_by_PC ae C40:6	0.7901	0.10012
4	lysoPC a C18:2_div_by_PC ae C40:4 + PC ae C40:6_div_by_CPT I ratio + lysoPC a C17:0_div_by_SM C18:0	0.7875	0.08568
5	C4_div_by_PC ae C30:2 + Arg_div_by_PC ae C34:0 + lysoPC a C18:2_div_by_PC ae C40:6	0.7842	0.10440
6	PC ae C40:6_div_by_CPT I ratio + C4_div_by_PC ae C30:2 + lysoPC a C18:2_div_by_PC ae C40:6	0.7839	0.07258
7	lysoPC a C18:2_div_by_PC ae C40:4 + Arg_div_by_PC ae C34:0 + PC ae C34:1_div_by_PC ae C42:0	0.7806	0.08948

GLM describes a model formula consisting of sum of three metabolite ratios. The models are listed according to average AUC (Area Under the curve) average and the RMSE (Root Mean Squared Error) less than 0,15. The AUC analyses for best model and its cross-validation are presented in figures 6 and 7.

15

Table 7. Performance of GLM models for peritoneal endometriosis

GLM model	AUC best	Sensitivity best	Specificity best	AUC average	Sensitivity average	Specificity average
1	0.93	0.58	0.78	0.80	0.75	0.75
2	0.90	0.42	0.71	0.79	0.74	0.75
3	0.93	0.89	0.83	0.79	0.79	0.78
4	0.91	0.83	0.83	0.78	0.71	0.77
5	0.91	0.67	0.73	0.78	0.77	0.74
6	0.88	0.75	0.75	0.78	0.79	0.76
7	0.89	0.67	0.73	0.78	0.71	0.71

- 5 Only for the seven best GLM models the DxS values are calculated. In the development of GLMs we observed that further models, analysed for peritoneal endometriosis, are not contributing to the phenotype explanation significantly. In fact, we noticed that the performance drops continuously after several iterations, especially after the 7th model.

10 **Table 8:** Interpretation basis for diagnosis of peritoneal endometriosis

GLM model	Reference value (Value for Control)	Value for Case	DxS - Fold change Log2
1	20.32	27.15	-0.42
2	20.20	27.08	-0.42
3	95.57	101.91	-0.09
4	19.90	26.69	-0.42
5	95.64	102.05	-0.09
6	19.61	26.42	-0.43
7	85.00	87.59	-0.04

A numeric value is calculated according to the GLM model formula. The calculated value is used to discriminate between diseased and not affected patient. Negative or positive values of DxS describe the direction of differences of case versus control.

5 3. Peritoneal mixed endometriosis

Table 9: GLMs for peritoneal mixed endometriosis

#	GLM	AUC average	RMSE
1	Orn_div_by_PC ae C38:0 + C4_div_by_PC aa C38:4 + Tyr_div_by_PC aa C42:2	0.6805	0.0851
2	Arg_div_by_PC aa C36:6 + C5_div_by_lysoPC a C17:0 + C5_div_by_Arg	0.6793	0.0860
3	Orn_div_by_PC ae C38:0 + C5_div_by_lysoPC a C17:0 + C5_div_by_Arg	0.6721	0.0822
4	C0_div_by_Gly + Orn_div_by_PC ae C38:0 + Tyr_div_by_PC aa C42:2	0.6683	0.0648
5	SM C18:0 + C5_div_by_lysoPC a C17:0 + C5_div_by_Arg	0.6676	0.0956
6	C5_div_by_lysoPC a C17:0 + C5_div_by_Arg + Ser_div_by_SM(OH) C16:1	0.6669	0.0843
7	SM C18:0 + C0_div_by_Gly + Tyr_div_by_PC aa C42:2	0.6668	0.0961
8	Orn_div_by_PC ae C38:0 + C3_div_by_PC ae C40:5 + Tyr_div_by_PC aa C42:2	0.6662	0.0764
9	Pro_div_by_PC ae C34:0 + Orn_div_by_PC ae C38:0 + Tyr_div_by_PC aa C42:2	0.6649	0.0725
10	C4_div_by_Ser + Orn_div_by_PC ae C38:0 + Tyr_div_by_PC aa C42:2	0.6645	0.1073
11	SM C18:0 + Orn_div_by_PC ae C38:0 + Tyr_div_by_PC aa C42:2	0.6639	0.0760
12	Orn_div_by_PC ae C38:0 + C4_div_by_PC ae C40:3 + Tyr_div_by_PC aa C42:2	0.6637	0.1018
13	Orn_div_by_PC ae C38:0 + PC ae C42:3_div_by_SM(OH) C16:1 + Tyr_div_by_PC aa C42:2	0.6611	0.0700
14	Orn_div_by_PC ae C38:0 + Tyr_div_by_PC ae C38:0 + Tyr_div_by_PC aa C42:2	0.6581	0.0702
15	Gly_div_by_SM C24:1 + PC aa C32:0_div_by_PC aa C40:1 + PC aa C36:4_div_by_PC aa C38:0		

16 Gly_div_by_PC aa C42:5 + PC aa C36:4_div_by_PC aa C38:0 + C0_div_by_
SM(OH) C22:2

GLM describes a model formula consisting of sum of three metabolite ratios. The models are listed according to average AUC (Area Under the curve) average and the RMSE (Root Mean Squared Error) less than 0,15. The AUC analyses for best model and its cross-validation are presented in figures 8 and 9.

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Table 10: Performance of GLM models peritoneal mixed endometriosis

GLM model	AUC best	Sensitivity best	Specificity best	AUC average	Sensitivity average	Specificity average
1	0.81	0.81	0.76	0.68	0.69	0.64
2	0.77	0.74	0.70	0.67	0.73	0.73
3	0.75	0.74	0.74	0.67	0.71	0.65
4	0.74	0.68	0.63	0.66	0.67	0.64
5	0.79	0.77	0.80	0.66	0.69	0.63
6	0.78	0.71	0.76	0.66	0.68	0.63
7	0.80	0.77	0.75	0.66	0.70	0.64
8	0.80	0.87	0.73	0.66	0.68	0.62
9	0.78	0.81	0.68	0.66	0.70	0.63
10	0.79	0.77	0.75	0.66	0.70	0.65

10 Only for the first ten best GLM models the DxS values are calculated. In the development of GLMs we observed that further models, analysed for peritoneal mixed endometriosis, are not contributing to the phenotype explanation significantly. In fact, we noticed that the performance drops continuously after several iterations, especially after the 10th model.

Table 11: Interpretation basis for diagnosis of peritoneal mixed endometriosis

GLM model	Reference value	Value for Case	DxS - Fold change Log2
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	(Value for Control)		
1	293.09	265.04	0.15
2	139.35	134.95	0.05
3	32.90	34.57	-0.07
4	293.22	265.17	0.15
5	23.65	22.41	0.08
6	38.99	38.51	0.02
7	283.96	253.01	0.17
8	293.18	265.13	0.15
9	424.62	397.46	0.10
10	293.09	265.04	0.15

A numeric value is calculated according to the GLM model formula. The calculated value is used to discriminate between diseased and not affected patient. Negative or positive values of DxS describe the direction of differences of case versus control.

5

4. Ovarian endometriosis

Table 12: GLMs for ovarian endometriosis

#	GLM	AUC average	RMSE
1	PC aa C36:3_div_by_PC ae C40:5 + lysoPC a C14:0_div_by_PC aa C28:1 + Met_div_by_PC aa C36:3	0.7111	0.0776
2	PC aa C38:0_div_by_PC ae C36:1 + Thr_div_by_ SM (OH) C22:1+ lysoPC a C14:0_div_by_PC aa C28:1	0.6992	0.0782
3	Thr_div_by_ SM (OH) C22:1 + PC aa C28:1_div_by_PC ae C34:3 + C18:2_div_by_PC ae C34:3	0.6992	0.0982
4	PC aa C36:3_div_by_PC ae C40:5 + C3_div_by_PC ae C34:1 + Met_div_by_PC aa C36:3	0.6952	0.0893

5	PC aa C36:3_div_by_PC ae C40:5 + PC aa C28:1_div_by_PC ae C34:3 + Gly_div_by_PC ae C36:1	0.6952	0.0414
6	PC aa C38:0_div_by_PC ae C36:1 + C18:2_div_by_PC ae C34:3 + Met_div_by_PC aa C36:3	0.6948	0.1026
7	PC aa C38:0_div_by_PC ae C36:1 + C10:1_div_by_PC aa C36:1 + PC ae C38:3_div_by_SM C18:1	0.6928	0.0838
8	PC aa C38:0_div_by_PC ae C36:1 + Gly_div_by_PC ae C36:1 + C3_div_by_PC ae C34:1	0.6928	0.0712
9	PC aa C38:0_div_by_PC ae C36:1 + C12-DC_div_by_C14:2 + PC ae C38:3_div_by_SM C18:1	0.6924	0.1018
10	PC aa C36:3_div_by_PC ae C40:5 + PC aa C38:3_div_by_PC ae C44:5 + Met_div_by_PC aa C36:3	0.6920	0.1008
11	PC aa C38:0_div_by_PC ae C36:1 + PC ae C38:3_div_by_SM C18:1 + Met_div_by_PC aa C36:3	0.6916	0.0997
12	PC aa C28:1_div_by_PC ae C34:3 + C18:2_div_by_PC ae C34:3 + C4_div_by_C5:1	0.6912	0.1323
13	PC aa C36:3_div_by_PC ae C40:5 + lysoPC a C20:4_div_by_PC ae C32:1 + Met_div_by_PC aa C36:3	0.6912	0.0941
14	PC aa C36:3_div_by_PC ae C40:5 + lysoPC a C20:4_div_by_PC aa C32:3 + Met_div_by_PC aa C36:3	0.6900	0.1029

GLM describes a model formula consisting of sum of three metabolite ratios. The models are listed according to average AUC (Area Under the curve) average and the RMSE (Root Mean Squared Error) less than 0,15. The AUC analyses for best model and its cross-validation are presented in figures 10 and 11.

5

Table 13: Performance of GLM models for ovarian endometriosis

GLM model	AUC best	Sensitivity best	Specificity best	AUC average	Sensitivity average	Specificity average
1	0.80	0.93	0.81	0.71	0.92	0.77
2	0.82	0.96	0.82	0.69	0.77	0.73
3	0.77	0.96	0.82	0.69	0.92	0.80

4	0.80	0.89	0.81	0.69	0.97	0.78
5	0.72	0.93	0.76	0.69	0.97	0.77
6	0.86	0.93	0.84	0.69	0.73	0.80
7	0.80	0.89	0.78	0.69	0.97	0.79
8	0.82	0.22	0.50	0.69	0.90	0.75
9	0.80	0.93	0.79	0.69	0.82	0.73
10	0.81	0.93	0.79	0.69	0.98	0.77

Only for the first ten best GLM models the DxS values are calculated. In the development of GLMs we observed that further models, analysed for ovarian endometriosis, are not contributing to the phenotype explanation significantly. In fact, we noticed that the performance drops continuously after several iterations, especially after the 10th model.

Table 14: Interpretation basis for diagnosis of ovarian endometriosis

GLM model	Reference value (Value for Control)	Value for Case	DxS - Fold change Log2
1	37.18	35.89	0.05
2	9.66	8.98	0.10
3	8.42	7.61	0.15
4	36.07	34.72	0.06
5	88.29	82.40	0.10
6	0.77	0.80	-0.05
7	0.84	0.88	-0.08
8	52.63	48.16	0.13
9	4.35	3.78	0.20
10	56.62	55.51	0.03

A numeric value is calculated according to the GLM model formula. The calculated value is used to discriminate between diseased and not affected patient. Negative or positive values of DxS describe the direction of differences of case versus control.

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5. Ovarian mixed endometriosis

Table 15: GLMs for ovarian mixed endometriosis

#	GLM	AUC average	RMSE
1	C10_div_by_PC aa C36:6 + Pro_div_by_PC ae C34:0 + PC ae C42:3_div_by_SM(OH) C16:1	0.6656	0.0717
2	C10_div_by_PC aa C36:6 + PC ae C42:3_div_by_SM(OH) C16:1+ C6:1_div_by_lysoPC a C20:4	0.6612	0.0689
3	C10_div_by_PC aa C36:6 + PC ae C42:3_div_by_SM(OH) C16:1+ lysoPC a C20:4_div_by_PC ae C40:2	0.6593	0.0710
4	Ser_div_by_PC aa C38:3 + C10_div_by_PC aa C36:6 + PC ae C42:3_div_by_SM(OH) C16:1	0.6590	0.0789
5	C10_div_by_lysoPC a C18:1 + C10_div_by_PC aa C36:6 + PC ae C42:3_div_by_SM(OH) C16:1	0.6562	0.1236
6	C10_div_by_PC aa C36:6 + lysoPC a C24:0_div_by_PC ae C42:3 + PC ae C42:3_div_by_SM(OH) C16:1	0.6548	0.1142
7	C10_div_by_PC aa C36:6 + lysoPC a C18:1_div_by_PC aa C36:1 + PC ae C42:3_div_by_SM(OH) C16:1	0.6537	0.0830
8	C10_div_by_PC aa C36:6 + Gly_div_by_PC ae C34:1 + PC ae C42:3_div_by_SM(OH) C16:1	0.6517	0.0746
9	Gln_div_by_PC ae C30:2 + C10_div_by_PC aa C36:6 + PC ae C42:3_div_by_SM(OH) C16:1	0.6504	0.0846
10	Pro_div_by_PC ae C34:0 + lysoPC a C24:0_div_by_PC ae C42:3 + PC ae C42:3_div_by_SM(OH) C16:1	0.6474	0.0399

11	C10:1_div_by_lysoPC a C24:0 + lysoPC a C24:0_div_by_PC ae C42:3 + PC ae C42:3_div_by_SM(OH) C16:1	0.6386	0.0736
12	PC ae C44:3_div_by_CPT.I.ratio + PC ae C34:0_div_by_PC ae C40:3 + C16:2-OH_div_by_SM C20:2	0.7308	0.0868
13	PC ae C44:6_div_by_SM C22:3 + PC ae C34:0_div_by_PC ae C40:3 + C10:1_div_by_C14:2-OH	0.7045	0.1262

GLM describes a model formula consisting of sum of three metabolite ratios. The models are listed according to average AUC (Area Under the curve) average and the RMSE (Root Mean Squared Error) less than 0,15. The AUC analyses for best model and its cross-validation are presented in figures 12 and 13.

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Table 16: Performance of GLM models ovarian mixed endometriosis

GLM model	AUC best	Sensitivity best	Specificity best	AUC average	Sensitivity average	Specificity average
1	0.73	0.73	0.71	0.66	0.63	0.60
2	0.72	0.64	0.62	0.66	0.61	0.61
3	0.71	0.68	0.58	0.65	0.61	0.60
4	0.76	0.77	0.72	0.65	0.58	0.58
5	0.77	0.73	0.76	0.65	0.63	0.63
6	0.76	0.68	0.61	0.65	0.64	0.60
7	0.72	0.64	0.62	0.65	0.61	0.59
8	0.71	0.67	0.67	0.65	0.61	0.59

Only for the first eight best GLM models the DxS values are calculated. In the development of GLMs we observed that further models, for analysed mixed ovarian endometriosis, are not contributing to the phenotype explanation significantly. In fact, we noticed that the performance drops continuously after several iterations, especially after the 8th model.

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Table 17: Interpretation basis for diagnosis of ovarian mixed endometriosis

GLM model	Reference value	Value for Case	DxS - Fold change
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	(Value for Control)		Log2
1	135.99	129.81	0.07
2	0.71	0.83	-0.23
3	3.94	4.04	-0.04
4	4.13	4.17	-0.01
5	0.73	0.85	-0.22
6	1.47	1.61	-0.13
7	1.13	1.26	-0.15
8	37.37	37.21	0.01

A numeric value is calculated according to the GLM model formula. The calculated value is used to discriminate between diseased and not affected patient. Negative or positive values of DxS describe the direction of differences of case versus control.

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Claims

1. Use of a combination of at least three pairs of metabolic biomarkers for the diagnosis of endometriosis and/or any sub-type thereof in a subject, wherein the diagnosis involves use of the quantification of at least three pairs of metabolic biomarkers in a sample obtained from said subject.
2. Use according to claim 1, wherein the diagnosis involves use of a generalized linear model (GLM) based on the quantification of the at least three pairs of metabolic biomarkers in a sample obtained from said subject.
3. Use according to claim 1 or 2, wherein the at least three pairs of metabolic biomarkers are selected from the group of pairs consisting of LysoPC a C17:0 and SM(OH) C16:1; Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0; LysoPC a C16:0 and SM C18:1; Thr and PC aa C34:3; C18 and LysoPC a C14:0; Ser and PC ae C44:3; Trp and PC ae C38:3; C8 and PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae C38:6; Arg and PC aa C36:6; Thr and SM(OH) C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM C18:0; PC aa C32:0 and PC aa C38:3; C6:1 and Pro; Arg and PC ae C34:0; C6:1 and LysoPC a C20:4; C5-M-DC and PC aa C42:5; LysoPC a C18:2 and PC ae C40:6; LysoPC a C18:2 and PC ae C40:4; PC ae C40:6 and CPT I ratio; LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg and PC ae C34:0; PC ae C34:1 and PC ae C42:0; Orn and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa C36:6; C5 and LysoPC a C17:0; C5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae C40:5; Pro and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and SM(OH) C16:1; Tyr and PC ae C38:0; PC aa C36:3 and PC ae C40:5; LysoPC a C14:0 and PC aa C28:1; Met and PC aa C36:3; PC aa C38:0 and PC ae C36:1; Thr and SM (OH) C22:1; PC aa C28:1 and PC ae C34:3; C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC ae C36:1; C10:1 and PC aa C36:1; PC ae C38:3 and SM C18:1; C12-DC and C14:2; PC aa C38:3 and PC ae C44:5; C4 and C5:1; LysoPC a C20:4 and PC ae C32:1; LysoPC a C20:4 and PC aa C32:3; C10 and PC aa C36:6; PC ae C42:3 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and LysoPC a C20:4; LysoPC a C20:4 and PC ae C40:2; Ser and PC aa C38:3; C10 and LysoPC a C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC a C18:1 and PC aa C36:1; Gly and PC ae C34:1; Gln and PC ae C30:2; LysoPC a C24:0 and PC ae C42:3; C10:1 and LysoPC a C24:0; Tyr and PC aa C42:4; C3-DC and C18; PC aa C42:1 and SM C22:3; Gly and SM C24:1; PC aa C32:0 and PC aa C40:1; PC aa C36:4 and PC aa C38:0; PC ae C44:3 and CPT I ratio; PC ae C34:0 and PC ae C40:3; C16:2-OH and SM C20:2; C6(C4:1-DC)

and SM C16:1; Gly and PC aa C42:5; C0 and SM(OH) C22:2; PC ae C44:6 and SM C22:3; and C10:1 and C14:2-OH;

for the diagnosis of endometriosis and/or any sub-type thereof.

4. The use according to any one of claims 1 to 3, for diagnosing all endometriosis, wherein the
 5 at least three pairs of metabolic biomarkers are selected from the group of pairs consisting
 of LysoPC a C17:0 and SM(OH) C16:1; Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0;
 LysoPC a C16:0 and SM C18:1; Thr and PC aa C34:3; C18 and LysoPC a C14:0; Ser and PC ae
 C44:3; Trp and PC ae C38:3; C8 and PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae C38:6;
 Arg and PC aa C36:6; Tyr and PC aa C42:4; C3-DC and C18; PC aa C42:1 and SM C22:3; and
 10 C6(C4:1-DC) and SM C16:1.
5. The use according to any one of claims 1 to 3, for diagnosing peritoneal endometriosis,
 wherein the at least three pairs of metabolic biomarkers are selected from the group of pairs
 consisting of Thr and SM(OH) C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM
 C18:0; PC aa C32:0 and PC aa C38:3; C6:1 and Pro; Arg and PC ae C34:0; C6:1 and LysoPC a
 15 C20:4; C5-M-DC and PC aa C42:5; LysoPC a C18:2 and PC ae C40:6; LysoPC a C18:2 and PC ae
 C40:4; PC ae C40:6 and CPT I ratio; LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg
 and PC ae C34:0; and PC ae C34:1 and PC ae C42:0.
6. The use according to any one of claims 1 to 3, for diagnosing peritoneal mixed endometriosis,
 wherein the at least three pairs of metabolic biomarkers are selected from the group of pairs
 20 consisting of Orn and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa
 C36:6; C5 and LysoPC a C17:0; C5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae
 C40:5; Pro and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and SM(OH) C16:1;
 Tyr and PC ae C38:0; SM C18:0 and C5; Gly and SM C24:1; PC aa C32:0 and PC aa C40:1; PC
 aa C36:4 and PC aa C38:0; Gly and PC aa C42:5; and C0 and SM(OH) C22:2.
- 25 7. The use according to any one of claims 1 to 3, for diagnosing ovarian endometriosis, wherein
 the at least three pairs of metabolic biomarkers are selected from the group of pairs
 consisting of PC aa C36:3 and PC ae C40:5; LysoPC a C14:0 and PC aa C28:1; Met and PC aa
 C36:3; PC aa C38:0 and PC ae C36:1; Thr and SM (OH) C22:1; PC aa C28:1 and PC ae C34:3;
 C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC ae C36:1; C10:1 and PC aa C36:1; PC
 30 ae C38:3 and SM C18:1; C12-DC and C14:2; PC aa C38:3 and PC ae C44:5; C4 and C5:1; LysoPC

a C20:4 and PC ae C32:1; LysoPC a C20:4 and PC aa C32:3; C0 and C5-M-DC; C3 and PC ae 34:0.

8. The use according to any one of claims 1 to 3, for diagnosing ovarian mixed endometriosis, wherein the at least three pairs of metabolic biomarkers are selected from the group of pairs consisting of C10 and PC aa C36:6; PC ae C42:3 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and LysoPC a C20:4; LysoPC a C20:4 and PC ae C40:2; Ser and PC aa C38:3; C10 and LysoPC a C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC a C18:1 and PC aa C36:1; Gly and PC ae C34:1; Gln and PC ae C30:2; LysoPC a C24:0 and PC ae C42:3; C10:1 and LysoPC a C24:0; PC ae C44:3 and CPT I ratio; PC ae C34:0 and PC ae C40:3; C16:2-OH and SM C20:2; PC ae C44:6 and SM C22:3; and C10:1 and C14:2-OH.
9. The use according to any one of claims 1 to 8, wherein the generalized linear modelling (GLM) comprises i) determining the ratio of the concentrations for each of the at least three pairs of metabolic biomarkers; and ii) calculating the sum of the obtained ratios (value for case).
10. The use according to claim 9, wherein the generalized linear modelling (GLM) further comprises iii) obtaining a diagnostic score (DxS) calculated by forming the quotient between a predetermined reference value obtained from healthy subjects (value for control) and the sum of the obtained ratios (value for case)

$$DxS = \log_2 \left(\frac{\text{predetermined reference value (value for control)}}{\text{sum of the obtained ratios (value for case)}} \right)$$

wherein said subject is diagnosed of having endometriosis or a sub-type thereof if the diagnostic score is different from zero ("0"), such as outside of the range 0 ± 0.03 .

11. An *ex vivo* method of diagnosing endometriosis and/or any subtype thereof in a subject comprising quantifying in a sample obtained from said subject at least three pairs of metabolic biomarkers.
12. The method according to claim 11, which comprises a) quantifying in a sample obtained from said subject at least three pairs of metabolic biomarkers, and b) obtaining a diagnostic score using a generalized linear model (GLM).
13. The method according to any of claims 11 to 12, wherein the at least three pairs of metabolic biomarkers are selected from the group of pairs consisting of LysoPC a C17:0 and SM(OH)

- C16:1; Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0; LysoPC a C16:0 and SM C18:1; Thr
 and PC aa C34:3; C18 and LysoPC a C14:0; Ser and PC ae C44:3; Trp and PC ae C38:3; C8 and
 PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae C38:6; Arg and PC aa C36:6; Thr and SM(OH)
 C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM C18:0; PC aa C32:0 and PC aa
 5 C38:3; C6:1 and Pro; Arg and PC ae C34:0; C6:1 and LysoPC a C20:4; C5-M-DC and PC aa C42:5;
 LysoPC a C18:2 and PC ae C40:6; LysoPC a C18:2 and PC ae C40:4; PC ae C40:6 and CPT I ratio;
 LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg and PC ae C34:0; PC ae C34:1 and PC
 ae C42:0; Orn and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa C36:6;
 C5 and LysoPC a C17:0; C5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae C40:5;
 10 Pro and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and SM(OH) C16:1; Tyr and
 PC ae C38:0; PC aa C36:3 and PC ae C40:5; LysoPC a C14:0 and PC aa C28:1; Met and PC aa
 C36:3; PC aa C38:0 and PC ae C36:1; Thr and SM (OH) C22:1; PC aa C28:1 and PC ae C34:3;
 C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC ae C36:1; C10:1 and PC aa C36:1; PC
 ae C38:3 and SM C18:1; C12-DC and C14:2; PC aa C38:3 and PC ae C44:5; C4 and C5:1; LysoPC
 15 a C20:4 and PC ae C32:1; LysoPC a C20:4 and PC aa C32:3; C10 and PC aa C36:6; PC ae C42:3
 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and LysoPC a C20:4; LysoPC a C20:4 and PC ae
 C40:2; Ser and PC aa C38:3; C10 and LysoPC a C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC
 a C18:1 and PC aa C36:1; Gly and PC ae C34:1; Gln and PC ae C30:2; LysoPC a C24:0 and PC
 ae C42:3; C10:1 and LysoPC a C24:0; Tyr and PC aa C42:4; C3-DC and C18; PC aa C42:1 and
 20 SM C22:3; Gly and SM C24:1; PC aa C32:0 and PC aa C40:1; PC aa C36:4 and PC aa C38:0; PC
 ae C44:3 and CPT I ratio; PC ae C34:0 and PC ae C40:3; C16:2-OH and SM C20:2; C6(C4:1-DC)
 and SM C16:1; Gly and PC aa C42:5; C0 and SM(OH) C22:2; PC ae C44:6 and SM C22:3; and
 C10:1 and C14:2-OH.
14. The method according to claim 12 or 13, wherein the generalized linear modelling comprises
 25 i) determining the ratio of the concentrations for each of the at least three pairs and ii)
 calculating the sum of the obtained ratios (value for case).
15. The method according to claim 14, wherein the generalized linear modelling (GLM) further
 comprises iii) obtaining a diagnostic score (DxS) calculated by forming the quotient between
 a predetermined reference value obtained from healthy subjects (value for control) and the
 30 sum of the obtained ratios (value for case)

$$DxS = \log_2 \left(\frac{\text{predetermined reference value (value for control)}}{\text{sum of the obtained ratios (value for case)}} \right)$$

wherein said subject is diagnosed of having endometriosis or a sub-type thereof if the diagnostic score is different from zero ("0"), such as outside of the range 0 ± 0.03 .

16. The method according to any one of claims 11 to 15, comprising determining whether the subject is suffering from any type of endometriosis.
17. The method according to claim 16, comprising
 - A1) quantifying in a sample obtained from said subject at least three pairs of metabolic biomarkers selected from the group of pairs consisting of LysoPC a C17:0 and SM(OH) C16:1; Arg and PC ae C36:0; PC ae C38:0 and PC ae C40:0; LysoPC a C16:0 and SM C18:1; Thr and PC aa C34:3; C18 and LysoPC a C14:0; Ser and PC ae C44:3; Trp and PC ae C38:3; C8 and PC ae C30:0; Thr and PC ae C36:5; C10 and PC ae C38:6; Arg and PC aa C36:6; and Tyr and PC aa C42:4; C3-DC and C18; PC aa C42:1 and SM C22:3; and C6(C4:1-DC) and SM C16:1; and
 - B1) obtaining a diagnostic score using a generalized linear model (GLM).
18. The method according to claim 16 or 17, comprising any one of the following procedures (1) to (15):
 - (1) quantifying in a sample obtained from said subject the metabolites LysoPC a C17:0, SM(OH) C16:1, Arg, PC ae C36:0, PC ae C38:0 and PC ae C40:0; and performing GLM using the model formula $\text{LysoPC a C17:0_div_by_SM(OH) C16:1} + \text{Arg_div_by_PC ae C36:0} + \text{PC ae C38:0_div_by_PC ae C40:0}$;
 - (2) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC ae C36:0, LysoPC a C16:0, SM C18:1, PC ae C38:0 and PC ae C40:0; and performing GLM using the model formula $\text{Arg_div_by_PC ae C36:0} + \text{LysoPC a C16:0_div_by_SM C18:1} + \text{PC ae C38:0_div_by_PC ae C40:0}$;
 - (3) quantifying in a sample obtained from said subject the metabolic biomarkers Thr, PC aa C34:3, LysoPC a C17:0, SM(OH) C16:1, Arg and PC ae C36:0; and performing GLM using the model formula $\text{Thr_div_by_PC aa C34:3} + \text{LysoPC a C17:0_div_by_SM(OH) C16:1} + \text{Arg_div_by_PC ae C36:0}$;

- (4) quantifying in a sample obtained from said subject the metabolic biomarkers Thr, PC aa C34:3, Arg, PC ae C36:0, LysoPC a C16:0 and SM C18:1; and performing GLM using the model formula $\text{Thr_div_by_PC aa C34:3} + \text{Arg_div_by_PC ae C36:0} + \text{LysoPC a C16:0_div_by_SM C18:1}$;
- 5 (5) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC aa C36:6, LysoPC a C17:0, SM(OH) C16:1, and PC ae C36:0; and performing GLM using the model formula $\text{Arg_div_by_PC aa C36:6} + \text{LysoPC a C17:0_div_by_SM(OH) C16:1} + \text{Arg_div_by_PC ae C36:0}$;
- 10 (6) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C17:0, SM(OH) C16:1, Arg, PC ae C36:0, C18 and LysoPC a C14:0; and performing GLM using the model formula $\text{LysoPC a C17:0_div_by_SM(OH) C16:1} + \text{Arg_div_by_PC ae C36:0} + \text{C18_div_by_LysoPC a C14:0}$;
- 15 (7) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C16:0, SM C18:1, PC ae C38:0, PC ae C40:0, Ser and PC ae C44:3; and performing GLM using the model formula $\text{LysoPC a C16:0_div_by_SM C18:1} + \text{PC ae C38:0_div_by_PC ae C40:0} + \text{Ser_div_by_PC ae C44:3}$;
- 20 (8) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C17:0, SM(OH) C16:1, Arg, PC ae C36:0, Trp, PC ae C38:3; and performing GLM using the model formula $\text{LysoPC a C17:0_div_by_SM(OH) C16:1} + \text{Arg_div_by_PC ae C36:0} + \text{Trp_div_by_PC ae C38:3}$;
- (9) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC ae C36:0, LysoPC a C16:0, SM C18:1, C8 and PC ae C30:0; and performing GLM using the model formula $\text{Arg_div_by_PC ae C36:0} + \text{LysoPC a C16:0_div_by_SM C18:1} + \text{C8_div_by_PC ae C30:0}$;
- 25 (10) quantifying in a sample obtained from said subject the metabolic biomarkers Thr, PC ae C36:5, Arg, PC ae C36:0, LysoPC a C16:0 and SM C18:1; and performing GLM using the model formula $\text{Thr_div_by_PC ae C36:5} + \text{Arg_div_by_PC ae C36:0} + \text{LysoPC a C16:0_div_by_SM C18:1}$;

- (11) quantifying in a sample obtained from said subject the metabolite Arg, PC ae C36:0, C18, LysoPC a C14:0, LysoPC a C16:0 and SM C18:1; and performing GLM using the model formula $\text{Arg_div_by_PC ae C36:0} + \text{C18_div_by_LysoPC a C14:0} + \text{LysoPC a C16:0_div_by_SM C18:1}$;
- 5 (12) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC ae C36:0, C10, PC ae C38:6, LysoPC a C16:0 and SM C18:1; and performing GLM using the model formula $\text{Arg_div_by_PC ae C36:0} + \text{C10_div_by_PC ae C38:6} + \text{LysoPC a C16:0_div_by_SM C18:1}$;
- 10 (13) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC aa C36:6, PC ae C36:0, LysoPC a C16:0 and SM C18:1; and performing GLM using the model formula $\text{Arg_div_by_PC aa C36:6} + \text{Arg_div_by_PC ae C36:0} + \text{LysoPC a C16:0_div_by_SM C18:1}$;
- 15 (14) quantifying in a sample obtained from said subject the metabolic biomarkers Tyr, PC aa C42:4, C3-DC, C18, PC aa C42:1 and SM C22:3; and performing GLM using the model formula $\text{Tyr_div_by_PC aa C42:4} + \text{C3-DC_div_by_C18} + \text{PC aa C42:1_div_by_SM C22:3}$;
- 20 (15) quantifying in a sample obtained from said subject the metabolic biomarkers C3-DC, C18, PC aa C42:1, SM C22:3, C6(C4:1-DC) and SM C16:1; and performing GLM using the model formula $\text{C3-DC_div_by_C18} + \text{PC aa C42:1_div_by_SM C22:3} + \text{C6 (C4:1-DC)_div_by_SM C16:1}$.
19. The method according to any one of claims 11 to 18, comprising determining whether the subject is suffering from peritoneal endometriosis.
20. The method according to claim 19, comprising
- 25 A2) quantifying in a sample obtained from said subject at least three pairs of metabolic biomarkers selected from the group of pairs consisting of Thr and SM(OH) C22:2; LysoPC a C16:0 and SM(OH) C16:1; PC aa C32:0 and SM C18:0; PC aa C32:0 and PC aa C38:3; C6:1 and Pro; Arg and PC ae C34:0; C6:1 and LysoPC a C20:4; C5-M-DC and PC aa C42:5; LysoPC a C18:2 and PC ae C40:6; LysoPC a C18:2 and PC ae C40:4; PC ae C40:6 and CPT I ratio; LysoPC a C17:0 and SM C18:0; C4 and PC ae C30:2; Arg and PC ae C34:0; and PC ae C34:1 and PC ae C42:0;
- 30 and

B2) obtaining a diagnostic score using a generalized linear model (GLM).

21. The method according to claim 19 or 20, comprising any one of the following procedures (1) to (7):

- 5 (1) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C16:0, SM(OH) C16:1, PC aa C32:0, SM C18:0, PC aa C32:0 and PC aa C38:3; and performing GLM using the model formula $\text{LysoPC a C16:0_div_by_SM(OH) C16:1} + \text{PC aa C32:0_div_by_SM C18:0} + \text{PC aa C32:0_div_by_PC aa C38:3}$;
- 10 (2) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C16:0, SM(OH) C16:1, PC aa C32:0, SM C18:0, Arg and PC ae C34:0; and performing GLM using the model formula $\text{LysoPC a C16:0_div_by_SM(OH) C16:1} + \text{PC aa C32:0_div_by_SM C18:0} + \text{Arg_div_by_PC ae C34:0}$;
- 15 (3) quantifying in a sample obtained from said subject the metabolic biomarkers C5-M-DC, PC aa C42:5, Arg, PC ae C34:0, LysoPC a C18:2 and PC ae C40:6; and performing GLM using the model formula $\text{C5-M-DC_div_by_PC aa C42:5} + \text{Arg_div_by_PC ae C34:0} + \text{LysoPC a C18:2_div_by_PC ae C40:6}$;
- (4) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C18:2, PC ae C40:4, PC ae C40:6, CPT I ratio, LysoPC a C17:0 and SM C18:0; and performing GLM using the model formula $\text{LysoPC a C18:2_div_by_PC ae C40:4} + \text{PC ae C40:6_div_by_CPT I ratio} + \text{LysoPC a C17:0_div_by_SM C18:0}$;
- 20 (5) quantifying in a sample obtained from said subject the metabolic biomarkers C4, PC ae C30:2, Arg, PC ae C34:0, LysoPC a C18:2 and PC ae C40:6; and performing GLM using the model formula $\text{C4_div_by_PC ae C30:2} + \text{Arg_div_by_PC ae C34:0} + \text{LysoPC a C18:2_div_by_PC ae C40:6}$;
- 25 (6) quantifying in a sample obtained from said subject the metabolic biomarkers PC ae C40:6, CPT I ratio, C4, PC ae C30:2, LysoPC a C18:2 and PC ae C40:6; and performing GLM using the model formula $\text{PC ae C40:6_div_by_CPT I ratio} + \text{C4_div_by_PC ae C30:2} + \text{LysoPC a C18:2_div_by_PC ae C40:6}$;
- (7) quantifying in a sample obtained from said subject the metabolic biomarkers LysoPC a C18:2, PC ae C40:4, Arg, PC ae C34:0, PC ae C34:1 and PC ae

C42:0; and performing GLM using the model formula $\text{LysoPC a C18:2_div_by_PC ae C40:4} + \text{Arg_div_by_PC ae C34:0} + \text{PC ae C34:1_div_by_PC ae C42:0}$.

22. The method according to any one of claims 11 to 21, comprising determining whether the subject is suffering from peritoneal mixed endometriosis.
- 5 23. The method according to claim 22, comprising
- A3) quantifying in a sample obtained from said subject at least three pairs of metabolic biomarkers selected from the group of pairs consisting of Orn and PC ae C38:0; C4 and PC aa C38:4; Tyr and PC aa C42:2; Arg and PC aa C36:6; C5 and LysoPC a C17:0; C5 and Arg; C0 and Gly; Ser and SM(OH) C16:1; C3 and PC ae C40:5; Pro and PC ae C34:0; C4 and Ser; C4 and PC ae C40:3; PC ae C42:3 and SM(OH) C16:1; Tyr and PC ae C38:0; Gly and SM C24:1; PC aa C32:0 and PC aa C40:1; PC aa C36:4 and PC aa C38:0; Gly and PC aa C42:5; and C0 and SM(OH) C22:2; and
- 10 B3) performing generalized linear modelling (GLM).
24. The method according to claim 22 or 23, comprising any one of the following procedures (1) to (16):
- 15 (1) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, C4, PC aa C38:4, Tyr and PC aa C42:2; and performing GLM using the model formula $\text{Orn_div_by_PC ae C38:0} + \text{C4_div_by_PC aa C38:4} + \text{Tyr_div_by_PC aa C42:2}$;
- 20 (2) quantifying in a sample obtained from said subject the metabolic biomarkers Arg, PC aa C36:6, C5 and LysoPC a C17:0; and performing GLM using the model formula $\text{Arg_div_by_PC aa C36:6} + \text{C5_div_by_lysoPC a C17:0} + \text{C5_div_by_Arg}$;
- (3) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, C5, LysoPC a C17:0 and Arg; and performing GLM using the model
- 25 formula $\text{Orn_div_by_PC ae C38:0} + \text{C5_div_by_lysoPC a C17:0} + \text{C5_div_by_Arg}$;
- (4) quantifying in a sample obtained from said subject the metabolic biomarkers C0, Gly, Orn, PC ae C38:0, Tyr and PC aa C42:2; and performing GLM using the model formula $\text{C0_div_by_Gly} + \text{Orn_div_by_PC ae C38:0} + \text{Tyr_div_by_PC aa C42:2}$;

- (5) quantifying in a sample obtained from said subject the metabolic biomarkers SM C18:0, C5, LysoPC a C17:0 and Arg; and performing GLM using the model formula $SM\ C18:0 + C5_div_by_lysoPC\ a\ C17:0 + C5_div_by_Arg$;
- 5 (6) quantifying in a sample obtained from said subject the metabolic biomarkers C5, LysoPC a C17:0, Arg, Ser and SM(OH) C16:1; and performing GLM using the model formula $C5_div_by_lysoPC\ a\ C17:0 + C5_div_by_Arg + Ser_div_by_SM(OH)\ C16:1$;
- (7) quantifying in a sample obtained from said subject the metabolic biomarkers SM C18:0, C0, Gly, Tyr and PC aa C42:2; and performing GLM using the model formula $SM\ C18:0 + C0_div_by_Gly + Tyr_div_by_PC\ aa\ C42:2$;
- 10 (8) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, C3, PC ae C40:5, Tyr and PC aa C42:2; and performing GLM using the model formula $Orn_div_by_PC\ ae\ C38:0 + C3_div_by_PC\ ae\ C40:5 + Tyr_div_by_PC\ aa\ C42:2$;
- 15 (9) quantifying in a sample obtained from said subject the metabolic biomarkers Pro, PC ae C34:0, Orn, PC ae C38:0, Tyr and PC aa C42:2; and performing GLM using the model formula $Pro_div_by_PC\ ae\ C34:0 + Orn_div_by_PC\ ae\ C38:0 + Tyr_div_by_PC\ aa\ C42:2$;
- (10) quantifying in a sample obtained from said subject the metabolic biomarkers C4, Ser, Orn, PC ae C38:0, Tyr and PC aa C42:2; and performing GLM using the model formula $C4_div_by_Ser + Orn_div_by_PC\ ae\ C38:0 + Tyr_div_by_PC\ aa\ C42:2$;
- 20 (11) quantifying in a sample obtained from said subject the metabolic biomarkers SM C18:0, Orn, PC ae C38:0, Tyr and PC aa C42:2; and performing GLM using the model formula $SM\ C18:0 + Orn_div_by_PC\ ae\ C38:0 + Tyr_div_by_PC\ aa\ C42:2$;
- (12) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, C4, PC ae C40:3, Tyr and PC aa C42:2; and performing GLM using the model formula $Orn_div_by_PC\ ae\ C38:0 + C4_div_by_PC\ ae\ C40:3 + Tyr_div_by_PC\ aa\ C42:2$;
- 25 (13) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, PC ae C42:3, SM(OH) C16:1, Tyr and PC aa C42:2; and

- performing GLM using the model formula $\text{Orn_div_by_PC ae C38:0} + \text{PC ae C42:3_div_by_SM(OH) C16:1} + \text{Tyr_div_by_PC aa C42:2}$;
- (14) quantifying in a sample obtained from said subject the metabolic biomarkers Orn, PC ae C38:0, Tyr, PC ae C38:0 and PC aa C42:2; and performing GLM using the model formula $\text{Orn_div_by_PC ae C38:0} + \text{Tyr_div_by_PC ae C38:0} + \text{Tyr_div_by_PC aa C42:2}$;
- (15) quantifying in a sample obtained from said subject the metabolic biomarkers Gly, SM C24:1, PC aa C32:0, PC aa C40:1, PC aa C36:4 and PC aa C38:0; and performing GLM using the model formula $\text{Gly_div_by_SM C24:1} + \text{PC aa C32:0_div_by_PC aa C40:1} + \text{PC aa C36:4_div_by_PC aa C38:0}$;
- (16) quantifying in a sample obtained from said subject the metabolic biomarkers Gly, PC aa C42:5, PC aa C36:4, PC aa C38:0SM, C0 and SM(OH) C22:2; and performing GLM using the model formula $\text{Gly_div_by_PC aa C42:5} + \text{PC aa C36:4_div_by_PC aa C38:0} + \text{C0_div_by_SM(OH) C22:2}$.
25. The method according to any one of claims 11 to 24, comprising determining whether the subject is suffering from ovarian endometriosis.
26. The method according to claim 25, comprising
- A4) quantifying in a sample obtained from said subject at least three pairs of metabolic biomarkers selected from the group of pairs consisting of PC aa C36:3 and PC ae C40:5; LysoPC a C14:0 and PC aa C28:1; Met and PC aa C36:3; PC aa C38:0 and PC ae C36:1; Thr and SM (OH) C22:1; PC aa C28:1 and PC ae C34:3; C18:2 and PC ae C34:3; C3 and PC ae C34:1; Gly and PC ae C36:1; C10:1 and PC aa C36:1; PC ae C38:3 and SM C18:1; C12-DC and C14:2; PC aa C38:3 and PC ae C44:5; C4 and C5:1; LysoPC a C20:4 and PC ae C32:1; and LysoPC a C20:4 and PC aa C32:3; and
- B4) performing generalized linear modelling (GLM).
27. The method according to claim 24 or 25, comprising any one of the following procedures (1) to (14):
- (1) quantifying in a sample obtained from said subject the metabolic biomarkers PC aa C36:3, PC ae C40:5, LysoPC a C14:0, PC aa C28:1 and Met; and

- performing GLM using the model formula $PC_{aa\ C36:3_div_by_PC\ ae\ C40:5} + LysoPC_{a\ C14:0_div_by_PC\ aa\ C28:1} + Met_{div_by_PC\ aa\ C36:3}$;
- (2) quantifying in a sample obtained from said subject the metabolic biomarkers $PC_{aa\ C38:0}$, $PC_{ae\ C36:1}$, Thr, SM (OH) C22:1, $LysoPC_{a\ C14:0}$ and $PC_{aa\ C28:1}$; and performing GLM using the model formula $PC_{aa\ C38:0_div_by_PC\ ae\ C36:1} + Thr_{div_by_SM\ (OH)\ C22:1} + lysoPC_{a\ C14:0_div_by_PC\ aa\ C28:1}$;
- (3) quantifying in a sample obtained from said subject the metabolic biomarkers Thr, SM (OH) C22:1, $PC_{aa\ C28:1}$, $PC_{ae\ C34:3}$, C18:2 and $PC_{ae\ C34:3}$; and performing GLM using the model formula $Thr_{div_by_SM\ (OH)\ C22:1} + PC_{aa\ C28:1_div_by_PC\ ae\ C34:3} + C18:2_{div_by_PC\ ae\ C34:3}$;
- (4) quantifying in a sample obtained from said subject the metabolic biomarkers $PC_{aa\ C36:3}$, $PC_{ae\ C40:5}$, C3, $PC_{ae\ C34:1}$, Met and $PC_{aa\ C36:3}$; and performing GLM using the model formula $PC_{aa\ C36:3_div_by_PC\ ae\ C40:5} + C3_{div_by_PC\ ae\ C34:1} + Met_{div_by_PC\ aa\ C36:3}$;
- (5) quantifying in a sample obtained from said subject the metabolic biomarkers $PC_{aa\ C36:3}$, $PC_{ae\ C40:5}$, $PC_{aa\ C28:1}$, $PC_{ae\ C34:3}$, Gly and $PC_{ae\ C36:1}$; and performing GLM using the model formula $PC_{aa\ C36:3_div_by_PC\ ae\ C40:5} + PC_{aa\ C28:1_div_by_PC\ ae\ C34:3} + Gly_{div_by_PC\ ae\ C36:1}$;
- (6) quantifying in a sample obtained from said subject the metabolic biomarkers $PC_{aa\ C38:0}$, $PC_{ae\ C36:1}$, C18:2, $PC_{ae\ C34:3}$, Met and $PC_{aa\ C36:3}$; and performing GLM using the model formula $PC_{aa\ C38:0_div_by_PC\ ae\ C36:1} + C18:2_{div_by_PC\ ae\ C34:3} + Met_{div_by_PC\ aa\ C36:3}$;
- (7) quantifying in a sample obtained from said subject the metabolic biomarkers $PC_{aa\ C38:0}$, $PC_{ae\ C36:1}$, C10:1, $PC_{aa\ C36:1}$, $PC_{ae\ C38:3}$ and SM C18:1; and performing GLM using the model formula $PC_{aa\ C38:0_div_by_PC\ ae\ C36:1} + C10:1_{div_by_PC\ aa\ C36:1} + PC_{ae\ C38:3_div_by_SM\ C18:1}$;
- (8) quantifying in a sample obtained from said subject the metabolic biomarkers $PC_{aa\ C38:0}$, $PC_{ae\ C36:1}$, Gly, C3 and $PC_{ae\ C34:1}$; and performing GLM using the model

formula $PC_{aa\ C38:0_div_by_PC_{ae\ C36:1}} + Gly_{div_by_PC_{ae\ C36:1}} + C3_{div_by_PC_{ae\ C34:1}}$;

- 5 (9) quantifying in a sample obtained from said subject the metabolic biomarkers $PC_{aa\ C38:0}$, $PC_{ae\ C36:1}$, C12-DC, C14:2, $PC_{ae\ C38:3}$ and SM C18:1; and performing GLM using the model formula $PC_{aa\ C38:0_div_by_PC_{ae\ C36:1}} + C12-DC_{div_by_C14:2} + PC_{ae\ C38:3_div_by_SM\ C18:1}$;
- 10 (10) quantifying in a sample obtained from said subject the metabolic biomarkers $PC_{aa\ C36:3}$, $PC_{ae\ C40:5}$, $PC_{aa\ C38:3}$, $PC_{ae\ C44:5}$ and Met; and performing GLM using the model formula $PC_{aa\ C36:3_div_by_PC_{ae\ C40:5}} + PC_{aa\ C38:3_div_by_PC_{ae\ C44:5}} + Met_{div_by_PC_{aa\ C36:3}}$;
- (11) quantifying in a sample obtained from said subject the metabolic biomarkers $PC_{aa\ C38:0}$, $PC_{ae\ C36:1}$, $PC_{ae\ C38:3}$, SM C18:1, Met and $PC_{aa\ C36:3}$; and performing GLM using the model formula $PC_{aa\ C38:0_div_by_PC_{ae\ C36:1}} + PC_{ae\ C38:3_div_by_SM\ C18:1} + Met_{div_by_PC_{aa\ C36:3}}$;
- 15 (12) quantifying in a sample obtained from said subject the metabolic biomarkers $PC_{aa\ C28:1}$, $PC_{ae\ C34:3}$, C18:2, $PC_{ae\ C34:3}$, C4 and C5:1; and performing GLM using the model formula $PC_{aa\ C28:1_div_by_PC_{ae\ C34:3}} + C18:2_{div_by_PC_{ae\ C34:3}} + C4_{div_by_C5:1}$;
- 20 (13) quantifying in a sample obtained from said subject the metabolic biomarkers $PC_{aa\ C36:3}$, $PC_{ae\ C40:5}$, LysoPC a C20:4, $PC_{ae\ C32:1}$ and Met; and performing GLM using the model formula $PC_{aa\ C36:3_div_by_PC_{ae\ C40:5}} + lysoPC_{a\ C20:4_div_by_PC_{ae\ C32:1}} + Met_{div_by_PC_{aa\ C36:3}}$;
- 25 (14) quantifying in a sample obtained from said subject the metabolic biomarkers $PC_{aa\ C36:3}$, $PC_{ae\ C40:5}$, LysoPC a C20:4, $PC_{aa\ C32:3}$ and Met; and performing GLM using the model formula $PC_{aa\ C36:3_div_by_PC_{ae\ C40:5}} + lysoPC_{a\ C20:4_div_by_PC_{aa\ C32:3}} + Met_{div_by_PC_{aa\ C36:3}}$.

28. The method according to any one of claims 11 to 27, comprising determining whether the subject is suffering from ovarian mixed endometriosis.

29. The method according to claim 28, comprising

- A5) quantifying in a sample obtained from said subject at least three pairs of metabolic biomarkers selected from the group of pairs consisting of C10 and PC aa C36:6; PC ae C42:3 and SM(OH) C16:1; Pro and PC ae C34:0; C6:1 and LysoPC a C20:4; LysoPC a C20:4 and PC ae C40:2; Ser and PC aa C38:3; C10 and LysoPC a C18:1; LysoPC a C24:0 and PC ae C42:3; LysoPC a C18:1 and PC aa C36:1; Gly and PC ae C34:1; Gln and PC ae C30:2; LysoPC a C24:0 and PC ae C42:3; C10:1 and LysoPC a C24:0; PC ae C44:3 and CPT I ratio; PC ae C34:0 and PC ae C40:3; C16:2-OH and SM C20:2; PC ae C44:6 and SM C22:3; and C10:1 and C14:2-OH; and
- B5) performing generalized linear modelling (GLM).
30. The method according to claim 28 or 29, comprising any one of the following procedures (1) to (13):
- (1) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC aa C36:6, Pro, PC ae C34:0, PC ae C42:3 and SM(OH) C16:1; and performing GLM using the model formula $C10_div_by_PC\ aa\ C36:6 + Pro_div_by_PC\ ae\ C34:0 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1$;
- (2) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC aa C36:6, PC ae C42:3, SM(OH) C16:1, C6:1 and LysoPC a C20:4; and performing GLM using the model formula $C10_div_by_PC\ aa\ C36:6 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1 + C6:1_div_by_LysoPC\ a\ C20:4$;
- (3) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC aa C36:6, PC ae C42:3, SM(OH) C16:1, LysoPC a C20:4 and PC ae C40:2; and performing GLM using the model formula $C10_div_by_PC\ aa\ C36:6 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1 + lysoPC\ a\ C20:4_div_by_PC\ ae\ C40:2$;
- (4) quantifying in a sample obtained from said subject the metabolic biomarkers Ser, PC aa C38:3, C10, PC aa C36:6, PC ae C42:3 and SM(OH) C16:1; and performing GLM using the model formula $Ser_div_by_PC\ aa\ C38:3 + C10_div_by_PC\ aa\ C36:6 + PC\ ae\ C42:3_div_by_SM(OH)\ C16:1$;
- (5) quantifying in a sample obtained from said subject the metabolic biomarkers C10, LysoPC a C18:1, PC aa C36:6, PC ae C42:3 and SM(OH) C16:1; and

- performing GLM using the model formula $C10_div_by_lysoPC \propto C18:1 + C10_div_by_PC \propto C36:6 + PC \propto C42:3_div_by_SM(OH) \propto C16:1$;
- (6) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC $\propto$ C36:6, LysoPC $\propto$ C24:0, PC $\propto$ C42:3, PC $\propto$ C42:3 and SM(OH) C16:1; and performing GLM using the model formula $C10_div_by_PC \propto C36:6 + LysoPC \propto C24:0_div_by_PC \propto C42:3 + PC \propto C42:3_div_by_SM(OH) \propto C16:1$;
- (7) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC $\propto$ C36:6, LysoPC $\propto$ C18:1, PC $\propto$ C36:1, PC $\propto$ C42:3 and SM(OH) C16:1; and performing GLM using the model formula $C10_div_by_PC \propto C36:6 + LysoPC \propto C18:1_div_by_PC \propto C36:1 + PC \propto C42:3_div_by_SM(OH) \propto C16:1$;
- (8) quantifying in a sample obtained from said subject the metabolic biomarkers C10, PC $\propto$ C36:6, + Gly, PC $\propto$ C34:1, PC $\propto$ C42:3 and SM(OH) C16:1; and performing GLM using the model formula $C10_div_by_PC \propto C36:6 + Gly_div_by_PC \propto C34:1 + PC \propto C42:3_div_by_SM(OH) \propto C16:1$;
- (9) quantifying in a sample obtained from said subject the metabolic biomarkers Gln, PC $\propto$ C30:2, C10, PC $\propto$ C36:6, PC $\propto$ C42:3 and SM(OH) C16:1; and performing GLM using the model formula $Gln_div_by_PC \propto C30:2 + C10_div_by_PC \propto C36:6 + PC \propto C42:3_div_by_SM(OH) \propto C16:1$;
- (10) quantifying in a sample obtained from said subject the metabolic biomarkers Pro, PC $\propto$ C34:0, LysoPC $\propto$ C24:0, PC $\propto$ C42:3, PC $\propto$ C42:3 and SM(OH) C16:1; and performing GLM using the model formula $Pro_div_by_PC \propto C34:0 + lysoPC \propto C24:0_div_by_PC \propto C42:3 + PC \propto C42:3_div_by_SM(OH) \propto C16:1$;
- (11) quantifying in a sample obtained from said subject the metabolic biomarkers C10:1, LysoPC $\propto$ C24:0, LysoPC $\propto$ C24:0, PC $\propto$ C42:3 and SM(OH) C16:1; and performing GLM using the model formula $C10:1_div_by_lysoPC \propto C24:0 + lysoPC \propto C24:0_div_by_PC \propto C42:3 + PC \propto C42:3_div_by_SM(OH) \propto C16:1$;
- (12) quantifying in a sample obtained from said subject the metabolic biomarkers PC $\propto$ C44:3, CPT I ratio, PC $\propto$ C34:0, PC $\propto$ C40:3, C16:2-OH and SM C20:2; and

performing GLM using the model formula $PC_{ae\ C44:3_div_by_CPT.I.ratio} + PC_{ae\ C34:0_div_by_PC_{ae\ C40:3} + C16:2-OH_div_by_SM\ C20:2}$;

(13) quantifying in a sample obtained from said subject the metabolic biomarkers $PC_{ae\ C44:6}$, $SM\ C22:3$, $PC_{ae\ C34:0}$, $PC_{ae\ C40:3}$, $C10:1$ and $C14:2-OH$; and
 5 performing GLM using the model formula $PC_{ae\ C44:6_div_by_SM\ C22:3} + PC_{ae\ C34:0_div_by_PC_{ae\ C40:3} + C10:1_div_by_C14:2-OH}$.

31. The method according to any one of claims 11 to 30, wherein the sample is selected from blood, serum, plasma, saliva, urine, cerebrospinal fluid, condensates from respiratory air, tears, mucosal tissue, mucus, vaginal tissue, endometrium, eutopic endometrium, skin, hair
 10 and hair follicle.

32. The method according to any one of claims 11 to 30, wherein the sample is selected from blood, serum and plasma.

33. The method according to any one of claims 11 to 30, wherein the sample is plasma.

34. The use according to any one of claims 1 to 10 or the method according to any one of claims
 15 11 to 33, wherein the subject is a human subject.

35. The use or method according to claim 34, wherein the human subject is a female.