

1. A method of diagnosing cancer based on lipidomic analysis of a body fluid taken from the body of a patient, wherein the body fluid is selected from serum, plasma, blood, urine, and wherein the cancer is pancreatic cancer or kidney cancer, characterized in that said method comprises the steps of:
  - spiking of the sample with a set of internal standards comprising at least one internal standard for each lipid class present in the sample,
  - subsequently processing the sample by liquid-liquid lipidomic extraction or by solid phase lipidomic extraction,
  - measurement of the processed sample by a mass spectrometry method,
  - determining concentrations for at least 51 lipids, more preferably for all lipids present at a level above detection threshold of the mass spectrometry method, using the internal standards for the corresponding lipid classes for the quantitation, or using relative concentrations, or using ratios of concentrations and/or signal intensities,
  - statistical evaluation of the determined concentrations of the lipids, said statistical evaluation determining the level of probability of the patient suffering from kidney or pancreatic cancer, based on the determined lipid concentrations compared to a specific cancerous pattern, and a non-cancerous pattern, wherein the specific cancerous pattern and the non-cancerous pattern are determined by statistical analysis of the lipid concentrations for a group of known cancer samples and non-cancer samples, and wherein the statistical evaluation is done separately for males and females.

2. The method according to claim 1, wherein the at least 51 lipids include the following lipids:

| Sphingolipids        |          |         |              |                  |                  |
|----------------------|----------|---------|--------------|------------------|------------------|
| Cer 34:1             | SM 34:2  | SM 40:1 | SM 42:2      | Sul 40:1         | Sul 42:1         |
| Cer 42:1             | SM 36:1  | SM 40:2 | SM 42:3      | Sul 40:1(OH)     | Sul 42:1(2OH)    |
| Cer 40:1             | SM 36:2  | SM 41:1 | SM 43:1      | Sul 40:2(OH)     | Sul 42:1(OH)     |
| Cer 42:2             | SM 38:1  | SM 41:2 | Sul 34:0(OH) | Sul 41:1         |                  |
| SM 34:1              | SM 39:1  | SM 42:1 | Sul 34:2(OH) | Sul 41:1(OH)     |                  |
| Glycerophospholipids |          |         |              |                  |                  |
| LPC 16:0             | LPC 18:2 | PC 34:1 | PC 36:3      | PC O-34:2/P-34:1 | PC O-36:5/P-36:4 |
| LPC 18:0             | PC 32:0  | PC 34:2 | PC 36:4      | PC O-34:3/P-34:2 | PE 34:1          |
| LPC 18:1             | PC 32:2  | PC 36:2 | PC 38:4      | PC O-36:3/P-36:2 |                  |
| Glycerolipids        |          |         |              |                  |                  |
| TG 49:1              | TG 50:4  | TG 50:5 | TG 51:4      | TG 53:4          | TG 55:5          |

3. A method of diagnosing cancer, wherein pancreatic cancer is diagnosed based on lipidomic analysis of a body fluid taken from the body of a patient, wherein the body fluid is selected from serum, plasma, blood, urine, characterized in that it comprises the steps of:

- spiking of the sample with a set of internal standards comprising at least one internal standard for each lipid class present in the sample,
- subsequently processing the sample by liquid-liquid lipidomic extraction,
- measurement of the processed sample by a mass spectrometry method,
- determining concentrations for at least 31 lipids, more preferably for all lipids present at a level above detection threshold of the mass spectrometry method, using the internal standards for the corresponding lipid classes for the quantitation, or using relative concentrations of lipids, or using ratios of concentrations and/or signal intensities,
- statistical evaluation of the determined concentrations of the lipids, said statistical evaluation determining the level of probability of the patient suffering from pancreatic cancer based on the determined lipid concentrations compared to a pancreatic cancerous pattern and a non-cancerous pattern, wherein the pancreatic cancerous pattern and the non-cancerous pattern are determined by statistical analysis of the lipid concentrations for a group of known pancreatic cancer samples and non-cancer samples, and wherein the statistical evaluation is done separately for males and females.

4. The method according to claim 3, wherein the at least 31 lipids include:

| Lipid                                      |
|--------------------------------------------|
| HexCer d18:1/16:0; HexCer d18:1/15:1 (1OH) |
| SM 34:1                                    |
| HexCer d18:1/24:1; HexCer d18:1/23:2 (1OH) |
| PE 34:1; PE P-35:0                         |
| PC 32:0; PC O-33:0                         |
| PE 36:4; PE P-37:3                         |
| PC 34:1; PC P-35:0                         |
| PE 34:2; PE P-35:1                         |
| PC P-34:0; PC 33:1                         |
| DG 36:2                                    |
| Cer d18:1/24:1; Cer d18:1/23:2 (1OH)       |
| CE 16:0                                    |
| SM 41:1                                    |
| SM 41:2                                    |
| PC 34:4; PC P-35:3                         |
| HexCer d18:1/24:0; HexCer d18:1/23:1 (1OH) |
| PE P-36:3; PE 35:4                         |
| PE P-38:4; PE 37:5                         |
| PE P-38:5; PE 37:6                         |
| PE P-36:2; PE 35:3                         |
| SM 32:1                                    |
| HexCer d18:1/22:0; HexCer d18:1/21:1 (1OH) |

|                               |
|-------------------------------|
| PE P-38:3; PE 37:4            |
| PC P-38:2; PC 37:3            |
| PE P-40:5; PE 39:6            |
| PE 36:0; PE P-38:6            |
| PE 38:0; PE P-40:6, PE O-39:0 |
| PE P-36:3; PE 35:4            |
| PC P-34:1; PC 33:2            |
| PC P-36:2; PC 35:3            |
| DG 34:3                       |

5. The method according to any one of the preceding claims, wherein the patient is a mammal, preferably a human.

5

6. The method according to any one of the preceding claims, wherein the internal standards are exogenous lipid compounds, having the polar head structure typical for the relevant lipid class and containing fatty acyls with shorter chains than naturally occurring lipids, preferably chains 12:0 or 14:0, or fatty acyls with odd number of carbon atoms, preferably chains 17:0, 17:1 or 19:1, or the internal standards are isotopically labelled analogues of the lipids of the relevant lipid class, preferably D7-Chol, D7-CE 16:0.

10

7. The method according to any one of the preceding claims, wherein a set of internal standards contains:

|                    |                        |
|--------------------|------------------------|
| D7-CE 16:0         | MG 19:1                |
| Cer d18:1/12:0     | PA 14:0/14:0           |
| DG 12:1/12:1       | PC 14:0/14:0           |
| Hex2Cer d18:1/12:0 | PE 14:0/14:0           |
| HexCer d18:1/12:0  | PG 14:0/14:0           |
| D7-Chol            | PS 14:0/14:0           |
| LPC 17:0           | SM d18:1/12:0          |
| LPE 14:0           | SulfoHexCer d18:1/12:0 |
| LPG 14:0           | TG 19:1/19:1/19:1      |
| LPA 14:0           | LPS 17:1               |

8. The method according to any one of the preceding claims, wherein the mass spectrometry method is selected from shotgun mass spectrometry, ultrahigh-performance liquid chromatography – mass spectrometry, ultrahigh-performance supercritical fluid chromatography – mass spectrometry, and matrix-assisted laser desorption/ionization mass spectrometry.

15

9. The method according to any one of the preceding claims, wherein a pooled sample prepared by mixing identical volumes of several samples is used and processed in the same way as the measured samples,

20

wherein the pooled sample includes samples from pancreatic cancer patients and healthy volunteers, and wherein the pooled sample is used for observing intra-day accuracy and intra-day precision, and/or for inter-day accuracy and inter-day precision, and/or for determining the lower limit of quantitation and the upper limit of quantitation, and/or for quality control during measurements of sample set.

5

10. The method according to any one of the preceding claims, wherein the order of samples is randomized in sample measurement sequences.

11. The method according to any one of the preceding claims, wherein the step of determining concentrations for all lipids present at a level above lower limit of quantitation of the mass spectrometry method, using the internal standards for the corresponding lipid classes for the quantitation, comprises one or more of the following procedures and corrections:

- isotopic correction,  
 - zero filling procedure in which the signals of lipid species which are not detected for particular sample are replaced by 50 to 100 %, preferably by 60 to 100% or by 70 to 100 %, more preferably by 80 to 90 %, or by 80 %, of the minimum concentration observed for said lipid species in all samples.

12. The method according to any one of the preceding claims, wherein the statistical evaluation involves  
 - data pre-processing such as centering, scaling, and/or transformation;  
 - PCA analysis for identification of influential factors such as outliers or measurement failures,  
 - discrimination analysis by OPLS-DA for group separation of pancreatic cancer patients and healthy volunteers, preferably performed for males and females separately,  
 - assignment of the statistical parameters as well as the evaluation of the prediction power.

25