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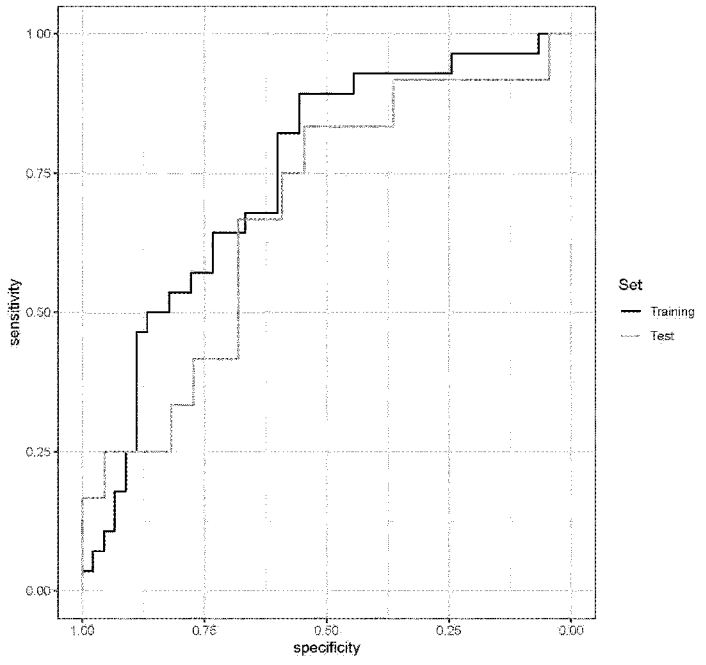

FIG 1

(57) Abstract: The present invention relates to the use of a marker set comprising at least three substances chosen from the group consisting of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, sucrose, scyllo-inositol, trigonelline, and acetone in an in-vitro method for differentiating between a prostate carcinoma and a benign prostate modification.

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Use of a marker set for differentiating between a prostate carcinoma and a benign prostate modification

Description

The present invention relates to the in-vitro use of a marker set for differentiating between a prostate carcinoma and a benign prostate modification according to the preamble of claim 1, to the further medical use of such a marker set according to the preamble of claim 6 as well as to an analysis method for differentiating between a prostate carcinoma and a benign prostate modification according to the preamble of claim 7.

Prostate cancer (PCa) affects 1 out of 6 to 8 men. 15 to 35 % of newly diagnosed prostate cancers are locally advanced and/or metastatic.

The prostate-specific antigen (PSA) is the most important clinical parameter associated with prostate cancer diagnosis. However, the reliability of the PSA test is still subject to controversy. In fact, PSA is not a tumor-specific but only a prostate specific protein. Therefore, elevated PSA levels are not necessarily indicative of prostate cancer but may also be caused by other conditions such as benign prostatic hyperplasia (BPH), urinary tract infections and prostatitis. This drawback leads to unnecessary biopsies that expose a patient to the risks associated with a biopsy.

Consequently, there exists a need for novel biomarkers to improve clinical decision-making and management of PCa.

It is an object of the present invention to provide novel methods and biomarkers for differentiating between a prostate carcinoma and benign prostate modifications.

This object is achieved with the in-vitro use of a marker set having the claim elements of claim 1. Such a marker set comprises at least three (e.g., 3, 4, 5, 6, or 7) substances chosen from the group consisting of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, sucrose, scyllo-inositol, trigonelline, and acetone. According to an aspect of the present invention, this marker set is used for differentiating between a prostate carcinoma and benign prostate modifications.

For this purpose, the concentration of the substances contained in the marker set is determined in a body fluid obtained from a patient. This concentration determination can be carried out by any appropriate measuring or analysis method, such as nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, and infrared spectroscopy such as Fourier-transform infrared (FT-IR) spectroscopy.

An alteration of concentration of at least three substances of the marker set with respect to the concentration in a control group or an alteration of a concentration ratio between at least three substances with respect to the concentration ratio of the same substances in a control group was correlated in a statistically significant way with the presence of a prostate carcinoma rather than a benign prostate modification.

For identifying the marker set and the substances that can make up the marker set, urine (as representative body fluid) of patients suffering from prostate cancer was used. These patients showed

- i) a PSA concentration in blood of more than 4 ng/ml and ii) a tumor detection by needle biopsy or an extension of the tumor beyond the prostate capsule and showing no signs of metastasis; or
- a newly diagnosed prostate cancer with high tumor burden, confirmed by i) an advanced local tumor, and ii) a PSA concentration in blood of more than 10 ng/ml, and iii) a Gleason score of at least 7 and/or bone metastases.

The Gleason scoring system is the most common grading system used to assess the aggressiveness of prostate cancer (i.e., for grading prostate cancer). It is based on the histological evaluation of the prostate tissue obtained after prostatectomy. The score ranges from 1 to 5 according to the pattern of cell growth of the tumor. The total score (Gleason sum) is the sum of two grades: A primary grade given to the predominant (most extensive) cell morphology and a secondary grade describing the cells of the next largest area of the tumor. The Gleason sum can range from 2 (non-aggressive cancer) to 10 (very aggressive cancer). The higher the score, the more aggressive is the cancer. The Gleason scoring system is described, e.g., in detail by Humphrey (Humphrey, P. A. (2004). Gleason grading and prognostic factors in carcinoma of the prostate. *Modern Pathology*, *17*(3), 292-306) and by Shah and Zhou (Shah, R. B., & Zhou, M. (2016). Recent advances in prostate cancer pathology: Gleason grading and beyond. *Pathology international*, *66*(5), 260-272).

The control group consisted of patients having a benign prostate modification. These patients showed i) a benign prostatic hyperplasia, evidenced by an enlarged prostate that is smooth, firm and slightly elastic but does not show palpable signs of tumor (such as irregular nodules or hard areas), and ii) a prostate volume of more than 40 ml measured by transrectal ultrasound, and iii) a PSA concentration in blood of less than 4 ng/ml.

When testing individual biomarkers of the substances contained in the marker set or two biomarkers of this marker set at the same time, no significant results could be obtained for distinguishing the two groups (prostate cancer versus benign prostate modification). Rather, the area under the curve (AUC) values of receiver operating characteristic (ROC) plots showed values mainly lying in a range of from 0.5 to 0.65. The AUC value of ROC plots is an aggregated metric that evaluates how well a logistic regression model classifies positive and negative outcomes at all possible cut-offs It can range from 0 to 1.0. An AUC value of 0 represents a prediction of the opposite of the trained correlation. An AUC value of 0.5 represents a random prediction. An AUC value of higher than 0.5 represents a classification of an event as fulfilling the trained correlation wherein higher values represent better classification.

The marker sets were tested against training datasets and test datasets and iteratively cross-validated. Cross-validation was performed by splitting the training dataset into, e.g., five parts and by using four parts for training (training subset) and the fifth part for testing (testing subset). The individual parts were iteratively removed from and returned to the training set so that each of the five parts belonged – in different training rounds – to the training subset and to the testing subset.

After all test and validation processes, 5-hydroxymethyl-2-furancarboxylic acid (Sumiki's acid), L-tyrosine, dimethylamine, sucrose, scyllo-inositol, trigonelline, and acetone turned out to be valid biomarkers for the underlying question (i.e., distinguishing a prostate carcinoma from a benign prostate modification), provided that the concentration of at least three of these biomarkers was determined at the same time (i.e., in one or more body fluid samples from the same patient obtained at the same time point). The concentration determination can be made with a method being able to determine the concentration of the substances by a single measurement or by a method requiring more than one measurement for such determination. NMR spectroscopy is particularly appropriate for such a concentration determination since it enables a highly accurate concentration determination in a body fluid by a single measurement in a very short measuring time.

In an embodiment, the concentration of the substances is standardized to the concentration of creatinine in the same sample or, alternatively, to the concentration of another substance that is naturally present in the sample.

In an embodiment, the benign prostate modification is chosen from the group consisting of prostatic intraepithelial neoplasia (PIN), atypical small acinar proliferation (ASAP), and benign prostatic hyperplasia (BPH).

In an embodiment, the marker set comprises or consists of at least three substances chosen from the group consisting of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, sucrose, scyllo-inositol, and acetone.

In an embodiment, the marker set comprises or consists of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, and scyllo-inositol. Such a marker set showed an AUC value of 0.678 in the test dataset and an AUC value of 0.744 in the training dataset (cf. Figure 1).

In an embodiment, the marker set comprises or consists of L-tyrosine, dimethylamine, and scyllo-inositol. Such a marker set showed an AUC value of 0.667 in the test dataset and an AUC value of 0.740 in the training dataset (cf. Figure 2). Thus, the use of 5-hydroxymethyl-2-furancarboxylic acid as additional biomarker does not significantly increase the AUC value. Rather, L-tyrosine, dimethylamine, and scyllo-inositol turned out to be very potent biomarkers without additional fourth marker.

In an embodiment, the marker set comprises L-tyrosine and dimethylamine.

In an embodiment, the marker set comprises L-tyrosine and scyllo-inositol.

In an embodiment, the marker set comprises scyllo-inositol and dimethylamine.

In an embodiment, the marker set comprises L-tyrosine.

In an embodiment, the marker set comprises dimethylamine.

In an embodiment, the marker set comprises scyllo-inositol.

Upon analyzing a plurality of NMR spectra for identifying appropriate biomarkers for the underlying question, the inventors were able to identify NMR signals in bin A232 that appeared to be highly appropriate for differentiating between a prostate carcinoma and a benign prostate

modification. This bin comprises a doublet signal around 5.60 ppm, wherein a first line of the doublet lies under exemplary measuring conditions at approximately 5.620 ppm and a second line of the doublet lies under the same measuring conditions at approximately 5.607 ppm. So far, the inventors were not yet successful in assigning a specific metabolite to these signals observed in bin A232. Therefore, the metabolite being responsible for the signals in bin A232 will be referred to in the following as substance Y.

In an independently claimed aspect, the present invention relates to uses of a marker set comprising the substances listed above and additionally comprising substance Y, as well as to related methods.

Therefore, in an independently claimed aspect, the present invention relates to the in-vitro use of a marker set comprising at least three (e.g., 3, 4, 5, 6, 7, or 8) substances chosen from the group consisting of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, sucrose, scyllo-inositol, trigonelline, acetone, and substance Y for differentiating between a prostate carcinoma and a benign prostate modification.

In an embodiment, the marker set comprises or consists of acetone, substance Y, sucrose, and scyllo-inositol. Such a marker set showed an AUC value of 0.655 in the test dataset and an AUC value of 0.776 in the training dataset (cf. Figure 3).

In an aspect, the present invention relates to the further medical use of a marker set comprising at least three (e.g., 3, 4, 5, 6, or 7) substances chosen from the group consisting of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, sucrose, scyllo-inositol, trigonelline, and acetone in in-vivo diagnostics for differentiating between a prostate carcinoma and a benign prostate modification.

In an aspect, the present invention relates to the further medical use of a marker set comprising at least three (e.g., 3, 4, 5, 6, 7, or 8) substances chosen from the group consisting of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, sucrose, scyllo-inositol, trigonelline, acetone, and substance Y in in-vivo diagnostics for differentiating between a prostate carcinoma and a benign prostate modification.

In an aspect, the present invention relates to a method for analyzing an isolated body fluid sample in vitro, comprising the steps explained in the following. This method is carried out on an isolated body fluid sample originating from an individual.

In a first step, the concentration of at least three (e.g., 3, 4, 5, 6, or 7) substances in the body fluid sample is determined by analyzing the body fluid sample with a suited measuring technique. The at least three substances are chosen from the group consisting of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, sucrose, scyllo-inositol, trigonelline, and acetone. A very well suited measuring technique for determining the concentration of the individual substances is nuclear magnetic resonance spectroscopy (NMR spectroscopy).

Afterwards, a score is calculated from the determined concentrations, wherein the score is indicative for the presence of a prostate carcinoma.

The score can be calculated by taking into consideration the concentrations measured or expected in a body fluid sample from a control group. To give a simple example, the score can be the median of the concentration ratios of the at least three substances between the body fluid test sample of the patient and corresponding control values of a body fluid control sample that have been measured the past. If the score is above a predetermined threshold value, a significant increase of the marker substances is present in the body fluid test sample that is indicative for a prostate carcinoma rather than for a benign prostate modification. It should be noted that other calculation methods as well as a weighting of individual marker concentrations with respect to other marker concentrations can also be performed in an embodiment.

A suited way to calculate the score is disclosed on pages 25 to 27 of WO 2012/045773 A9. Another suited way to calculate the score is the following:

$$score = \frac{1}{1+e^{-\omega}},$$

wherein

$$\omega = a + \sum_{x=1}^{n} b_x \cdot I_x$$

$$a = const.$$

$$b_x = substance\ specific\ coefficient$$

$$I = parameter\ being\ indicative\ for\ the\ concentration\ of\ substance\ x$$

Thereby, the individual factors a, b need to be adjusted according to the underlying model and can vary in dependence on the specific substances considered in the marker set. Parameter “I” can be, e.g., the signal intensity or signal integral of an according signal observed in the

evaluated measuring result. To give an example, "I" can be the signal intensity or signal integral of an NMR signal in an NMR spectrum if NMR spectroscopy is used as measuring technique.

In an embodiment, "I" is a ratio between two signal intensities or two signal integrals. In such a case, it is, e.g., possible to standardize the concentration of a first substance (or a plurality of substances) by the concentration of a second substance such as, e.g., creatinine.

The score is a (semi-)quantitative measure for distinguishing i) a prostate carcinoma requiring treatment from ii) an indolent prostate carcinoma not requiring treatment or benign prostate modifications not requiring treatment. Thus, the score serves for (semi-)quantitatively distinguishing i) a prostate carcinoma requiring treatment from ii) an indolent prostate carcinoma not requiring treatment or benign prostate modifications not requiring treatment.

Calculating the score comprises multiplying each of the concentrations of the substances by a substance-specific weighting factor to provide a plurality of weighted values and combining the weighted values into a risk equation. Afterwards, an output of the risk equation is compared to a predefined threshold. If the score is above the threshold, there is a likelihood that a prostate cancer requires treatment. In an embodiment, the likelihood is higher, the higher the score is (i.e., the likelihood increases with increasing distance of the score from the threshold).

In an embodiment, the calculated score is output and presented to the individual and/or to a third person such as a physician or medical staff. The output can be performed on a display (i.e., in an electronic way) or in printed form. Thereby, it is also possible to generate a report indicating the score, optionally in combination with a comparative scale of possible scores and their meaning with respect to the likelihood of the presence of a prostate carcinoma.

In an embodiment, the method is a computer-implemented method. In particular, all steps of spectral analysis and concentration determination as well as of score calculation are performed on a computer. Such steps are far too complex to be done in a manual way. The computer-implemented concentration determination is, in an embodiment, based on a spectral analysis, such as an analysis of NMR spectra. The spectral analysis and the further required steps until the score can be output can be done on the same computer that is used for controlling a spectrometer performing the spectral analysis or on a different computer.

In an embodiment, the body fluid sample is a urine sample or a blood sample. In an embodiment, the blood sample is a whole blood sample, a blood serum sample, a blood

plasma sample, or any other blood preparation derivable from whole blood or from other blood preparations.

In an embodiment, the body fluid sample (and therewith the patient from whom the body fluid sample originates) is grouped into one of at least two predefined groups based on the calculated score. Typically, one group encompasses patients suffering from a prostate carcinoma (prostate tumor), wherein the other group encompasses patients having a benign prostate alteration. The resulting grouping can also be indicated on an according report.

In an aspect, the present invention relates to another method for analyzing an isolated body fluid sample in vitro, comprising the steps explained in the following. This method is carried out on an isolated body fluid sample originating from an individual.

In a first step, the concentration of at least three (e.g., 3, 4, 5, 6, 7, or 8) substances in the body fluid sample is determined by analyzing the body fluid sample with a suited measuring technique. The at least three substances are chosen from the group consisting of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, sucrose, scyllo-inositol, trigonelline, acetone, and substance Y. A very well suited measuring technique for determining the concentration of the individual substances is nuclear magnetic resonance spectroscopy (NMR spectroscopy).

Afterwards, a score is calculated from the determined concentrations, wherein the score is indicative for the presence of a prostate carcinoma.

In an aspect, the present invention relates to a medical method for making a differential diagnosis between a prostate carcinoma and a benign prostate alteration. This method comprises the steps explained in the following.

In a first step, a body fluid sample is gathered from a patient. In a second step, the concentration of at least three (e.g., 3, 4, 5, 6, or 7) substances in the body fluid sample is determined by analyzing the body fluid sample with a suited measuring technique. The at least three substances are chosen from the group consisting of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, sucrose, scyllo-inositol, trigonelline, and acetone.

Afterwards, a score is calculated from the determined concentrations, wherein the score is indicative for the presence of a prostate carcinoma.

In a further method step, a differential diagnosis between a prostate carcinoma and a benign prostate alteration is made on the basis of the previously calculated score. The respective result is then output to the patient or to a third person like a physician or medical staff.

In an aspect, the present invention relates to another medical method for making a differential diagnosis between a prostate carcinoma and a benign prostate alteration. This method comprises the steps explained in the following.

In a first step, a body fluid sample is gathered from a patient. In a second step, the concentration of at least three (e.g., 3, 4, 5, 6, 7, or 8) substances in the body fluid sample is determined by analyzing the body fluid sample with a suited measuring technique. The at least three substances are chosen from the group consisting of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, sucrose, scyllo-inositol, trigonelline, acetone, and substance Y.

Afterwards, a score is calculated from the determined concentrations, wherein the score is indicative for the presence of a prostate carcinoma.

In a further method step, a differential diagnosis between a prostate carcinoma and a benign prostate alteration is made on the basis of the previously calculated score. The respective result is then output to the patient or to a third person like a physician or medical staff.

In a further aspect, the present invention relates to a decision support system for analyzing an isolated body fluid sample in vitro, the decision support system comprising:

a) a unit for providing a body fluid sample from an individual;

b) a unit for determining the concentration of at least three substances chosen from the group consisting of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, sucrose, scyllo-inositol, trigonelline, and acetone in the body fluid sample by analyzing the body fluid sample with a suited measuring technique; and

c) a unit for calculating a score from the determined concentrations, the score being indicative for a likelihood that the body fluid originates from a patient suffering from a prostate carcinoma or from a patient having a benign prostate modification.

In an embodiment, the unit for determining the concentration of the at three four substances is configured to determine the concentration of any substance combination of the embodiments explained above.

While some of the explained uses and methods are described as in vitro uses and methods and some of the explained uses and methods are described as in vivo uses and methods, it should be noted that each in vitro use or method can also be carried out as in vivo use or method, and vice versa.

All embodiments of the use of the marker set can be combined in any desired way and can be transferred either individually or in any arbitrary combination to the further medical use of the marker set as well as to the different methods and to the decision support system. Likewise, all embodiments of the further medical use of the marker set can be combined in any desired way and can be transferred either individually or in any arbitrary combination to the use of the marker set, to the different methods, and to the decision support system. Finally, all embodiments of the different methods can be combined in any desired way and can be transferred either individually or in any arbitrary combination to the use of the marker set, to the further medical use of the marker set, to any other of the described methods, and to the decision support system.

Further details of aspects of the present invention will be explained in the following making reference to exemplary embodiments and accompanying Figures. In the Figures:

Figure 1 shows an ROC plot of the ability of a first marker set for distinguishing a prostate carcinoma from a benign prostate alteration;

Figure 2 shows an ROC plot of the ability of a second marker set for distinguishing a prostate carcinoma from a benign prostate alteration; and

Figure 3 shows an ROC plot of the ability of a third marker set for distinguishing a prostate carcinoma from a benign prostate alteration.

All ROC plots shown in Figures 1 to 3 were obtained by analyzing urine samples of patients suffering from a prostate carcinoma requiring treatment. These patients showed

- i) a PSA concentration in blood of more than 4 ng/ml and ii) a tumor detection by needle biopsy or an extension of the tumor beyond the prostate capsule; or

- a newly diagnosed prostate cancer with high tumor burden, confirmed by i) an advanced local tumor, and ii) a PSA concentration in blood of more than 10 ng/ml, and iii) a Gleason score of at least 7 and/or bone metastases.

The control group consisted of patients having a benign prostate modification. These patients showed i) a benign prostatic hyperplasia, evidenced by an enlarged prostate that is smooth, firm and slightly elastic but does not show palpable signs of tumor (such as irregular nodules or hard areas), and ii) a prostate volume of more than 40 ml measured by transrectal ultrasound, and iii) a PSA concentration in blood of less than 4 ng/ml.

NMR measurements

All measurements were carried out on a Bruker Avance II+ 600MHz or a Bruker Avance III HD 600MHz NMR spectrometer, each using a PATXI 1H/D-13C/15N Z-GRD probe. All samples were kept at 5-7°C in the SampleJet and brought to the target temperature in the integrated preheating block before measurement. A standard pulse program with 30-degree excitation pulse and pre-saturation for water suppression was used (zgpr30).

Samples were measured in batches of up to 93 samples per run. In addition to the analytical samples, each run included one Axinon® urine calibrator sample and two Axinon® urine control samples (before and after the analytical urine samples, respectively) in order to assure ideal measurement conditions throughout the run.

Signal analysis

NMR spectra underwent automatic referencing, phase correction and baseline correction before further analysis.

Subsequently, the NMR spectra underwent an automatic standardization and calibration procedure to minimize between-device, between-day and between-run effects. The quality of each of these spectra was assessed by a custom spectrum qualification algorithm that analyzes general spectral properties, e.g., offset and tilt of the baseline in selected spectral regions, and properties of selected indicator signals, e.g., signal position, shape and width. Spectra that did not meet the predefined quality criteria were excluded from further analysis.

Successfully qualified spectra (typically covering a chemical shift from -5 to 14 ppm) were subjected to further modifications. In particular, broad background signals were separated with

a suitable algorithm, e.g., background intensities (such as generated from proteins) were subtracted from the spectra, resulting in spectral intensities devoid of such background signals.

The cohort (i.e., the plurality) of modified spectra was checked for regions in which the cohort does not show a significant number of signals. These regions – like the region of the water signal and the regions with signals arising from substances, e.g. buffer substances, added during sample preparation – were ignored in the steps explained in the following.

The remaining spectral regions were subject to an adaptive binning, which divides the spectrum in bins of differing size or extent (typically covering 0.01 to 0.05 ppm, but in extreme cases also covering 0.005 to 0.5 ppm).

Quantification of specific signal peaks (in particular signals resulting from dimethylamine) was done by fitting Pseudo-Voigt functions, which represent a linear combination of a Gaussian and a Lorentzian function, to each peak of interest. The resulting signal fits were checked for goodness of fit in order to reject results of insufficient fit quality.

Signals resulting from other substances were not fitted. Rather, quantification models making use of the previously assigned bins were applied. After substance identification, substance labels have been assigned to these bins. The quantification was then determined by the bin value, which calculates as [(sum of intensities in bin)/(number of data points in bin)]. The standardization by data points is used to compensate for a varying number of data points in the bins. The number of data points in a bin may vary by one data point due to shifts of the applied discretization grid. A true numerical integration would use a multiplication by the bin extent. However, this is a constant factor and can be omitted.

Test of identified marker substances

The identified marker substances were tested in different combinations to assess their suitability for differentiating between a prostate carcinoma and a benign prostate modification. In doing so, the result of the determination based on the marker substances (probability of prostate carcinoma or of a benign prostate modification) has been checked against clinical signs of a prostate tumor in the patient who donated the urine sample, as already explained above.

The results are summarized in receiver operating characteristic (ROC) plots. In these plots, the area under the curve (AUC) indicates the fitness of the prediction. If the AUC is 0.5, the

prediction is to be considered random and thus not well suited. The higher the AUC, the better is the prediction model.

All marker sets, the ROC plots of which are shown in Figures 1 to 3, were able to distinguish between the patient group and the control group in a statistically significant way. The results shown in Figures 1 to 3 are summarized in the following Table 1.

Table 1: Summary of results depicted in Figures 1 to 3.

Figure	Composition of marker set	AUC value (training dataset)	AUC value (test dataset)
1	5-hydroxymethyl-2-furancarboxylic acid L-tyrosine dimethylamine scyllo-inositol	0.744	0.678
2	L-tyrosine dimethylamine scyllo-inositol	0.740	0.667
3	acetone substance Y sucrose scyllo-inositol	0.776	0.655

Summarizing, the presented marker sets comprise highly appropriate biomarkers for distinguishing between a prostate carcinoma and a benign prostate modification.

Claims

1. Use of a marker set comprising at least three substances chosen from the group consisting of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, sucrose, scyllo-inositol, trigonelline, and acetone in an in-vitro method for differentiating between a prostate carcinoma and a benign prostate modification.

2. Use according to claim 1, **characterized** in that the benign prostate modification is chosen from the group consisting of prostatic intraepithelial neoplasia, atypical small acinar proliferation, and benign prostatic hyperplasia.

3. Use according to claim 1 or 2, **characterized** in that marker set comprises at least three substances chosen from the group consisting of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, sucrose, scyllo-inositol, and acetone.

4. Use according to any of the preceding claims, **characterized** in that marker set comprises 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, and scyllo-inositol.

5. Use according to any of the preceding claims, **characterized** in that marker set comprises L-tyrosine, dimethylamine, and scyllo-inositol.

6. Marker set comprising at least three substances chosen from the group consisting of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, sucrose, scyllo-inositol, trigonelline, and acetone for use in in-vivo differential diagnostics of prostate carcinoma versus a benign prostate modification.

7. Method for analyzing an isolated body fluid sample in vitro, comprising the following steps:

   a) determining the concentration of at least three substances chosen from the group consisting of 5-hydroxymethyl-2-furancarboxylic acid, L-tyrosine, dimethylamine, sucrose, scyllo-inositol, trigonelline, and acetone in an isolated body fluid sample from an individual by analyzing the body fluid sample with a suited measuring technique, and

   b) calculating a score from the determined concentrations, the score being indicative for a likelihood that the body fluid originates from a patient suffering from a prostate carcinoma or from a patient having a benign prostate modification.

8. Method according to claim 7, **characterized** in that the body fluid sample is a urine sample or a blood sample.

9. Method according to claim 7 or 8, **characterized** in that that the sample is grouped into one of at least two predefined groups on the basis of the calculated score.

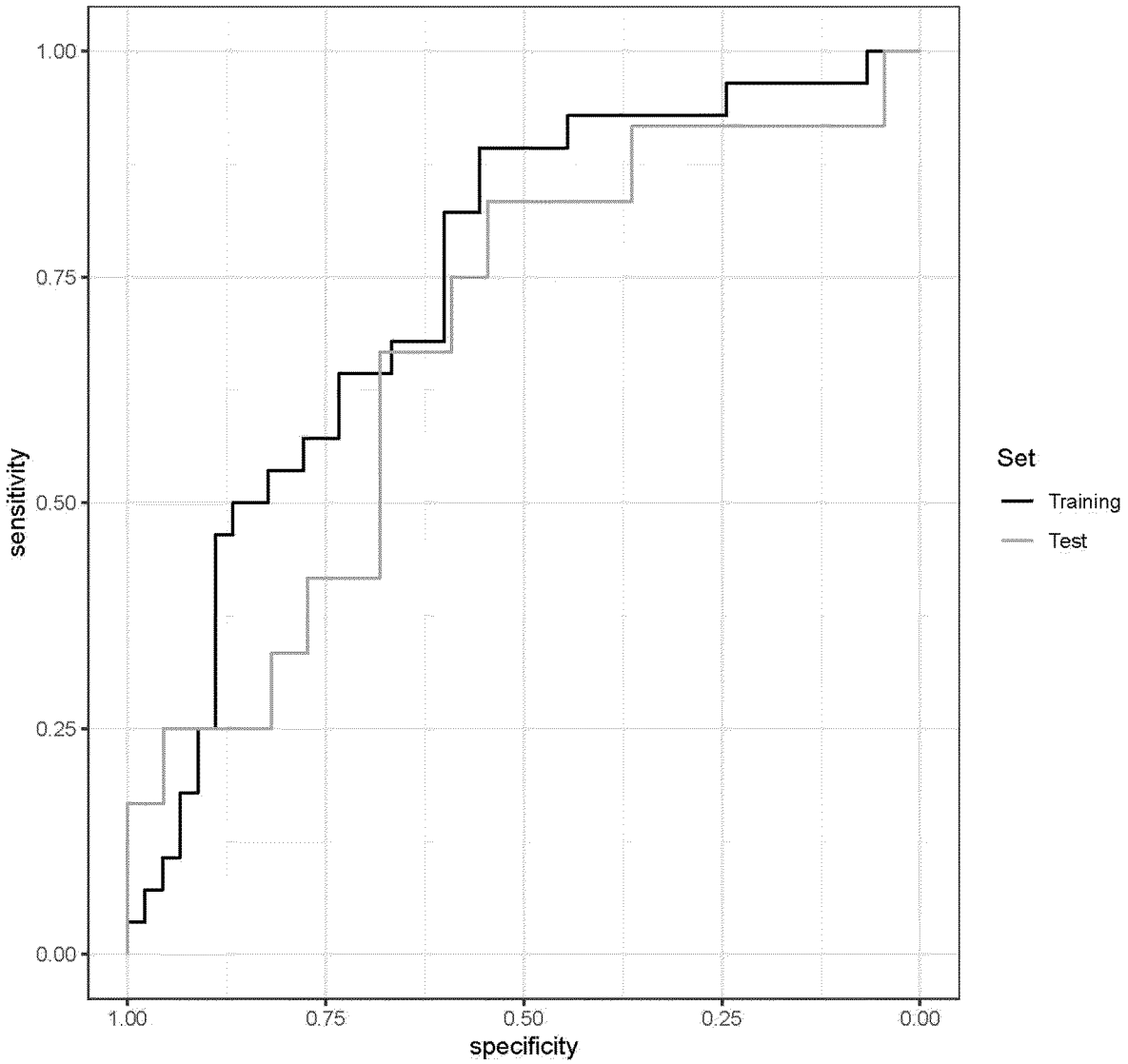
1.00
0.75
0.50
0.25
0.00
sensitivity
1.00
0.75
0.50
0.25
0.00
specificity
Set
Training
Test

FIG 1

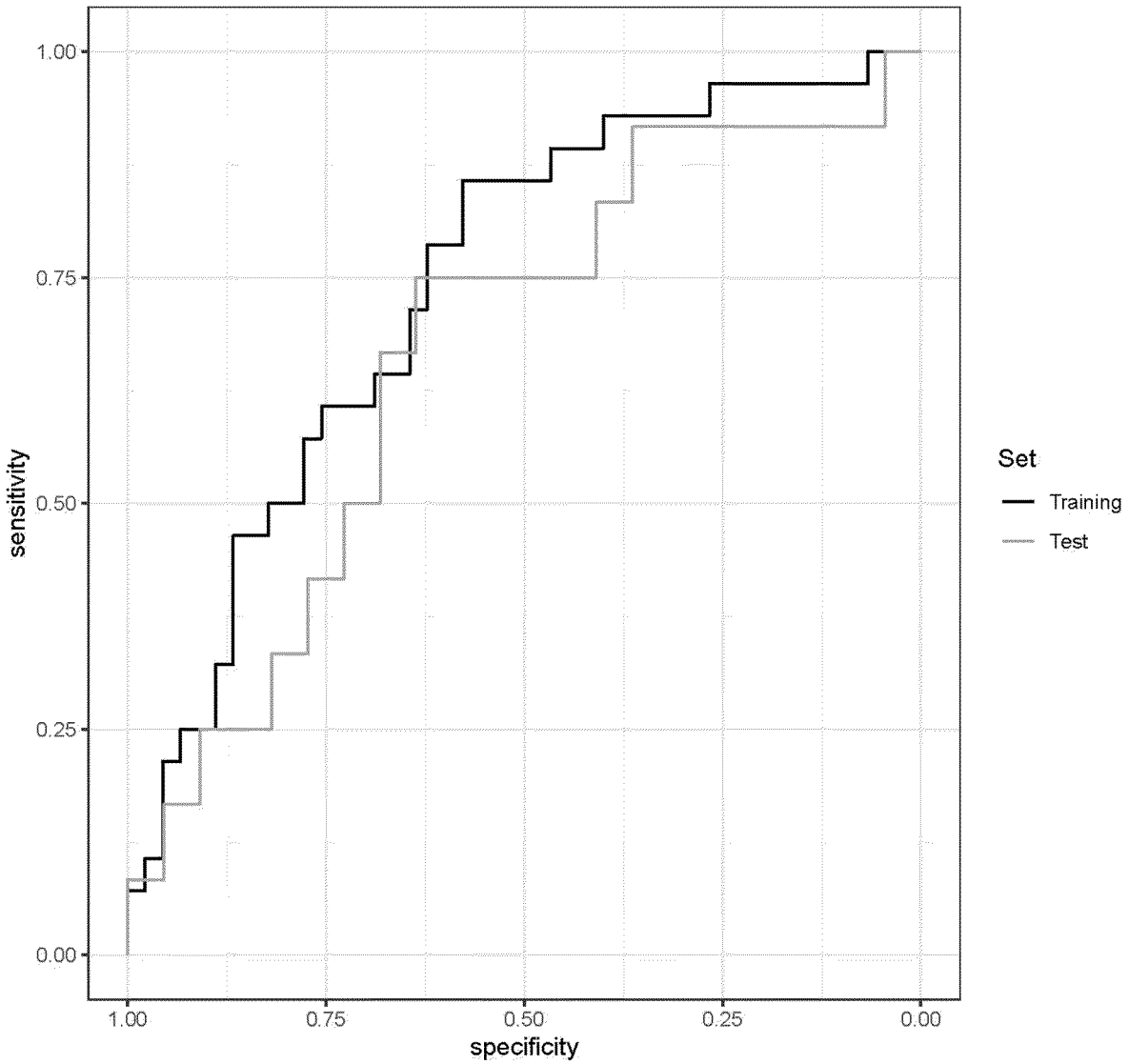
1.00
0.75
0.50
0.25
0.00
sensitivity
1.00
0.75
0.50
0.25
0.00
specificity
Set
Training
Test

FIG 2

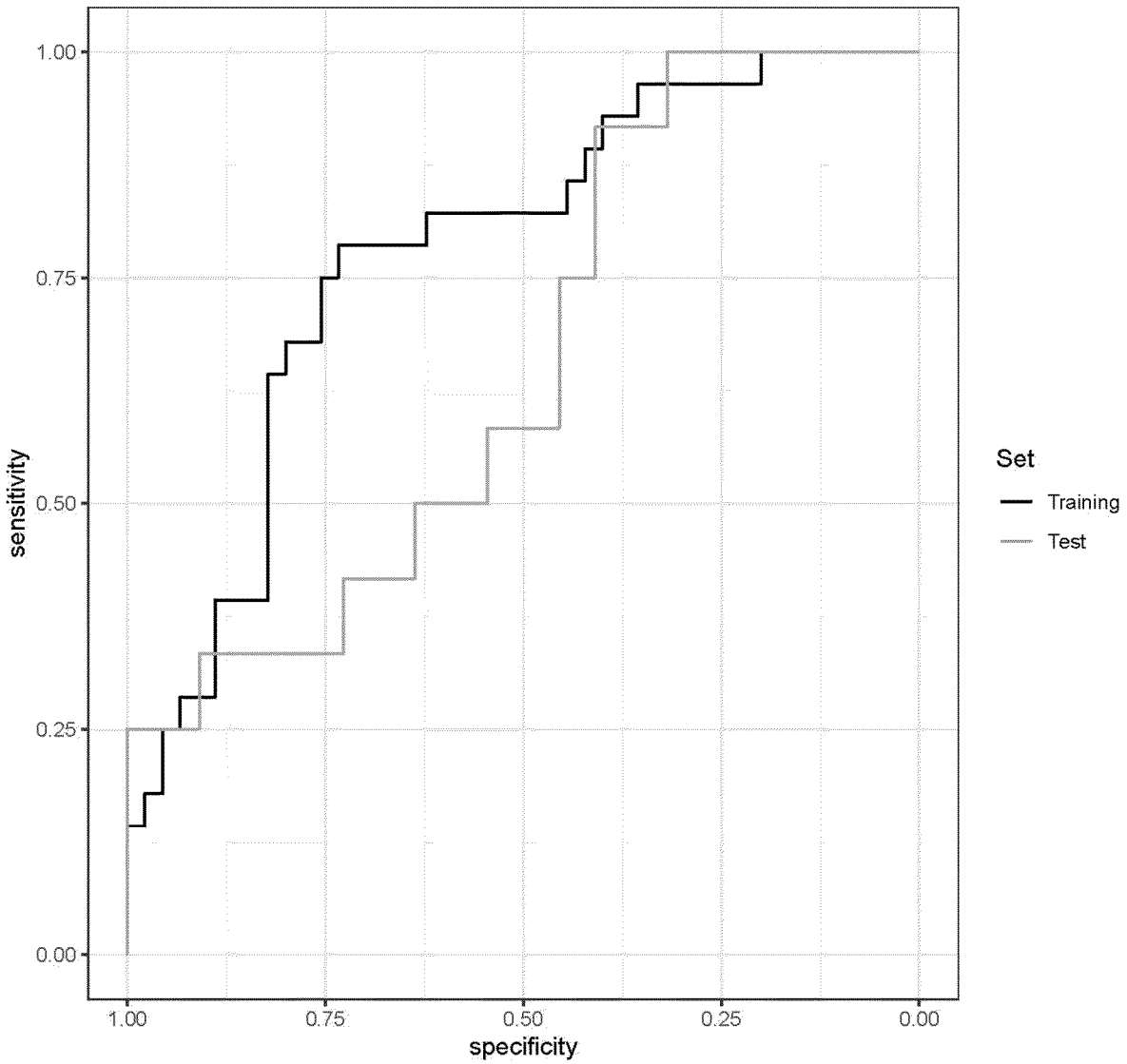
1.00
0.75
0.50
0.25
0.00
sensitivity
1.00
0.75
0.50
0.25
0.00
specificity
Set
Training
Test

FIG 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/076830

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/574
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	REDDY RRAVIKANTH ET AL: "Potential of nuclear magnetic resonance metabolomics in the study of prostate cancer", INDIAN JOURNAL OF UROLOGY, vol. 38, no. 2, 1 April 2022 (2022-04-01), page 99, XP093100958, IN ISSN: 0970-1591, DOI: 10.4103/iju.iju_416_21	1-3,6
Y	abstract; pg 100, right col, third par; pg 101, right col, third par; pg 102, left col, first par; figure 3; pg 103, right col, second par; table 1	1-9

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[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 January 2025	21/01/2025

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Motrescu-Hateley, E

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/076830

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LIMA ANA RITA ET AL: "Biomarker Discovery in Human Prostate Cancer: an Update in Metabolomics Studies", TRANSLATIONAL ONCOLOGY, vol. 9, no. 4, 1 August 2016 (2016-08-01), pages 357-370, XP093214946, United States ISSN: 1936-5233, DOI: 10.1016/j.tranon.2016.05.004 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5006818/pdf/main.pdf>	1-3,6
Y	abstract; table 1; table 4	1-9

Y	HE JINHUA ET AL: "Serum organic acid metabolites can be used as potential biomarkers to identify prostatitis, benign prostatic hyperplasia, and prostate cancer", FRONTIERS IN IMMUNOLOGY, vol. 13, 4 January 2023 (2023-01-04), XP093234273, Lausanne, CH ISSN: 1664-3224, DOI: 10.3389/fimmu.2022.998447 Retrieved from the Internet: URL:https://pmc.ncbi.nlm.nih.gov/articles/PMC9846500/pdf/fimmu-13-998447.pdf> abstract; pg 4, right col, second par; figure 4	1-4,6

Y	US 2016/258958 A1 (NARAIN NIVEN RAJIN [US] ET AL) 8 September 2016 (2016-09-08) abstract; par 0175	7-9

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/076830

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		WO 2016094425 A1	16-06-2016