In 2012, the United States Preventative Services Taskforce (USPTF) issued a recommendation against routine prostate cancer screening using the PSA test. This led to a decrease in the number of men proceeding to biopsy following elevated PSA test results and an increase in the proportion of men presenting with aggressive prostate cancer (Fleshner & Carlsson, Nature Reviews Urology, volume 15, pages 532–534, 2018).

A general need exists for more convenient, reliable and/or accurate diagnostic tests capable of discerning between aggressive and non-aggressive forms of prostate cancer and for detecting aggressive prostate cancer.

Summary ~~of the Invention~~

The present disclosure has~~inventors have~~ identified combinations of biomarker/s and clinical variable/s effective for detecting aggressive prostate cancer. Accordingly, the biomarker/clinical variable combinations disclosed herein can be used to detect the presence or absence of aggressive prostate cancer in a subject. In one aspect, the present disclosure provides a method for diagnosing aggressive prostate cancer (CaP) in a test subject, comprising: (a) obtaining an analyte level for one or more analytes in a biological sample obtained from the test subject, and a measurement of one or more clinical variables having been obtained from the test subject; and (b) applying a suitable algorithm and/or transformation to a combination of the clinical variable measurements and analyte level/s of the test subject to thereby generate a test subject score value for comparison to a threshold value; and (c) determining whether the test subject has aggressive CaP by comparison of the subject test score value and the threshold value, wherein: the one or more analyte/s comprise or consist of WAP four-disulfide core domain protein 2 (WFDC2 (HE4)), and optionally total prostate specific antigen (PSA), the one or more clinical variables comprise at least one of: %Free PSA, DRE, Prostate Volume (PV) and the threshold value was determined by: measuring said one or more analyte/s in a series of biological samples obtained from a population of subjects having aggressive CaP and from a population of control subjects not having aggressive CaP, to thereby obtain an analyte level for each said analyte in each said biological sample of the series; combining each said analyte level of the series with measurements of said one or more clinical variables obtained from each said subject of the populations, in a manner that allows discrimination between aggressive CaP and an absence of aggressive CaP, to thereby generate the threshold value. In another aspect, the present disclosure provides a method for discerning whether a test subject has non-aggressive or aggressive prostate cancer (CaP), comprising: (a) obtaining an analyte level for one or more analytes in a biological sample obtained from the test subject, and a measurement of one or more clinical variables having been obtained from the test subject; and (b) applying a suitable algorithm and/or transformation to a combination of the clinical variable measurements and analyte level/s of the test subject to thereby generate a test subject score value for comparison to a threshold value; and (c) determining whether the test subject has aggressive CaP by comparison of the subject test score value and the threshold value,

wherein: the one or more analyte/s comprise or consist of WFDC2 (HE4), and optionally total PSA, the one or more clinical variables comprise at least one of: %Free PSA, DRE, Prostate Volume (PV) and the threshold value was determined by: measuring said one or more analyte/s in a series of biological samples obtained from a population of subjects having aggressive CaP and from a population of control subjects having non-aggressive CaP, to thereby obtain an analyte level for each said analyte in each said biological sample of the series; combining each said analyte level of the series with measurements of said one or more clinical variables obtained from each said subject of the populations, in a manner that allows discrimination between aggressive CaP and non-aggressive CaP, to thereby generate the threshold value.

The present ~~invention~~disclosure also describes ~~relates at least to~~ the following series of numbered ~~embodiment~~examples below:

~~Embodiment~~Example 1. A method for diagnosing aggressive prostate cancer (CaP) in a test subject, comprising:

(a) obtaining an analyte level for one or more analytes in the test subject's biological sample, and obtaining a measurement of one or more clinical variables from the test subject; and

(b) applying a suitable algorithm and/or transformation to a combination of the clinical variable measurements and analyte level/s of the test subject to thereby generate a test subject score value for comparison to a threshold value; and

(c) determining whether the test subject has aggressive CaP by comparison of the subject test score value and the threshold value,

wherein:

the one or more analyte/s comprise or consist of WAP four-disulfide core domain protein 2 (WFDC2 (HE4)), and optionally total prostate surface antigen (PSA),

the one or more clinical variables comprise at least one of: %Free PSA, DRE, Prostate Volume (PV), and

the threshold value was determined by:

measuring said one or more analyte/s in a series of biological samples obtained from a population of subjects having aggressive CaP and from a population of control subjects not having aggressive CaP, to thereby obtain an analyte level for each said analyte in each said biological sample of the series;

combining each said analyte level of the series with measurements of said one or more clinical variables obtained from each said subject of the populations, in a manner that allows discrimination between aggressive CaP and an absence of aggressive CaP, to thereby generate the threshold value.

~~Embodiment~~Example 2. The method of ~~embodiment~~example 1, wherein the population of control subjects comprises subjects that do not have prostate cancer and subjects that have non-aggressive prostate cancer

~~Embodiment~~Example 3. A method for discerning whether a test subject has non-aggressive or aggressive prostate cancer (CaP), comprising:

(a) obtaining an analyte level for one or more analytes in the test subject's biological sample, and obtaining a measurement of one or more clinical variables from the test subject; and

(b) applying a suitable algorithm and/or transformation to a combination of the clinical variable measurements and analyte level/s of the test subject to thereby generate a test subject score value for comparison to a threshold value; and

(c) determining whether the test subject has aggressive CaP by comparison of the subject test score value and the threshold value,

wherein:

the one or more analyte/s comprise or consist of WFDC2 (HE4), and optionally total PSA,

the one or more clinical variables comprise at least one of: %Free PSA, DRE, Prostate Volume (PV), and

the threshold value was determined by:

measuring said one or more analyte/s in a series of biological samples obtained from a population of subjects having aggressive CaP and from a population of control subjects having non-aggressive CaP, to thereby obtain an analyte level for each said analyte in each said biological sample of the series;

combining each said analyte level of the series with measurements of said one or more clinical variables obtained from each said subject of the populations, in a manner that allows discrimination between aggressive CaP and non-aggressive CaP, to thereby generate the threshold value.

~~Embodiment~~Example 4. The method of ~~embodiment~~example 1 or ~~embodiment~~example 3, wherein the population of control subjects has non-aggressive CaP as defined by a Gleason score of 3+3.

~~Embodiment~~Example 5. The method of any one of ~~embodiment~~examples 1 to 4, wherein the threshold value is determined prior to performing the method.

~~Embodiment~~Example 6. The method of any one of ~~embodiment~~examples 1 to 5, wherein the one or more clinical variables and the one or more analyte/s comprise or consist of any one of the following:

- WFDC2 (HE4) and %Free PSA

- WFDC2 (HE4) and DRE
- WFDC2 (HE4) and PV
- WFDC2 (HE4), %Free PSA, and DRE
- WFDC2 (HE4), %Free PSA, and PV
- WFDC2 (HE4), total PSA and %Free PSA
- WFDC2 (HE4), total PSA and PV
- WFDC2 (HE4), total PSA and DRE
- WFDC2 (HE4), total PSA, %Free PSA, and PV, or
- WFDC2 (HE4), total PSA, %Free PSA, and DRE.

~~Embodiment~~Example 7. The method of any one of ~~embodiment~~examples 1 to 6, comprising selecting a subset of the combined analyte/s and/or clinical variable measurements to generate the threshold value.

~~Embodiment~~Example 8. The method of any one of ~~embodiment~~examples 1 to 7, wherein said combining of each said analyte level of the series with said measurements of the one or more clinical variables comprises combining a logistic regression score of the clinical variable measurements and analyte level/s in a manner that maximizes said discrimination, in accordance with the formula:

(i)

$$\text{Logit (P)} = \text{Log(P/1-P)}$$

$$= \boldsymbol{intercept} + \sum_{i=1}^{N}(\boldsymbol{coefficient_i} \times \boldsymbol{transformed}\,(\boldsymbol{variable_i})$$

$$\boldsymbol{P} = \frac{\boldsymbol{exp(Logit(P))}}{\boldsymbol{1+exp(Logit(P))}}$$

wherein:

P is probability of that the test subject has aggressive prostate cancer,

the coefficient$_i$ is the natural log of the odds ratio of the variable,

the transformed variable$_i$ is the natural log of the variable$_i$ value; or

(ii)

$$\text{Logit (P)} = \text{Log(P/1-P)}$$

$$= \boldsymbol{intercept} + \sum_{i=1}^{N}(\boldsymbol{coefficient_i} \times \boldsymbol{transformed}\,(\boldsymbol{variable_i}) + coefficient_{DRE} \times DRE$$

$$\boldsymbol{P} = \frac{\boldsymbol{exp(Logit(P))}}{\boldsymbol{1+exp(Logit(P))}}$$

wherein:

P is probability that the test subject has aggressive prostate cancer,

the coefficient$_i$ is the natural log of the odds ratio of the variable,

the transformed variable$_i$ is the natural log of the variable$_i$ value,

a DRE value of 1 indicates abnormal, while DRE value of 0 indicates normal.

~~Embodiment~~Example 9. The method of any one of ~~embodiment~~examples 1 to 8, wherein said applying a suitable algorithm and/or transformation to the combination of the clinical variable measurements and analyte level/s comprises use of an exponential function, a logarithmic function, a power function and/or a root function.

~~Embodiment~~Example 10. The method according to any one of ~~embodiment~~examples 1 to 9, wherein the suitable algorithm and/or transformation applied to the combination of the clinical variable measurements and analyte level/s of the test subject is in accordance with the formula:

(i)

$$\text{Logit (P)} = \text{Log(P/1-P)}$$

$$= \boldsymbol{intercept} + \sum_{i=1}^{N}(\boldsymbol{coefficient_i} \times \boldsymbol{transformed}\,(\boldsymbol{variable_i})$$

$$\boldsymbol{P} = \frac{\boldsymbol{exp(Logit(P))}}{\boldsymbol{1+exp(Logit(P))}}$$

wherein:

P is probability of that the test subject has aggressive prostate cancer,

the coefficient$_i$ is the natural log of the odds ratio of the variable,

the transformed variable$_i$ is the natural log of the variable$_i$ value; or

(ii)

$$\text{Logit (P)} = \text{Log(P/1-P)}$$

$$= \boldsymbol{intercept} + \sum_{i=1}^{N}(\boldsymbol{coefficient_i} \times \boldsymbol{transformed}\,(\boldsymbol{variable_i}) + coefficient_{DRE} \times DRE$$

$$\boldsymbol{P} = \frac{\boldsymbol{exp(Logit(P))}}{\boldsymbol{1+exp(Logit(P))}}$$

wherein:

P is probability of that the test subject has aggressive prostate cancer,

the coefficient$_i$ is the natural log of the odds ratio of the variable,

the transformed variable$_i$ is the natural log of the variable$_i$ value,

a DRE value of 1 indicates abnormal, while DRE value of 0 indicates normal;

and wherein said suitable algorithm and/or transformation is used to generate the subject test score that is compared to the threshold value to thereby determine whether or not the test subject has aggressive prostate cancer.

~~Embodiment~~Example 11. The method according to any one of ~~embodiment~~examples 1 to 10, wherein said combining of each said analyte level of the series with measurements of said one or more clinical variables obtained from each said subject of the populations maximizes said discrimination.

~~Embodiment~~Example 12. The method of any one of ~~embodiment~~examples 1 to 11, wherein said combining of each said analyte level of the series with the measurements of one or more clinical variables obtained from each said subject of the populations is conducted in a manner that:

(i) reduces the misclassification rate between the subjects having aggressive CaP and said control subjects; and/or

(ii) increases sensitivity in discriminating between the subjects having aggressive CaP and said control subjects; and/or

(iii) increases specificity in discriminating between the subjects having aggressive CaP and said control subjects.

~~Embodiment~~Example 13. The method of ~~embodiment~~example 12, wherein said combining in a manner that reduces the misclassification rate between the subjects having aggressive CaP and said control subjects comprises selecting a suitable true positive and/or true negative rate.

~~Embodiment~~Example 14. The method of ~~embodiment~~example 12, wherein said combining in a manner that reduces the misclassification rate between the subjects having aggressive CaP and said control subjects minimizes the misclassification rate.

~~Embodiment~~Example 15. The method of ~~embodiment~~example 12, wherein said combining in a manner that reduces the misclassification rate between the subjects having aggressive CaP and said control subjects comprises minimizing the misclassification rate between the subjects having aggressive CaP and said control subjects by identifying a point where the true positive rate intersects the true negative rate.

~~Embodiment~~Example 16. The method of ~~embodiment~~example 12, wherein said selecting the threshold value from the combined clinical variable measurement/s and combined analyte level/s in a manner that increases sensitivity in discriminating between the subjects having aggressive CaP and said control subjects increases or maximizes said sensitivity.

~~Embodiment~~Example 17. The method of ~~embodiment~~example 12, wherein said selecting the threshold value from the combined clinical variable measurement/s and combined analyte level/s in a manner that increases specificity in discriminating between the subjects having aggressive CaP and said control subjects increases or maximizes said specificity.

~~Embodiment~~Example 18. The method according to any one of ~~embodiment~~examples 1 to 17, wherein the one or more clinical variables and the one or more analytes comprise or consist of:

- total PSA, %free PSA, DRE, WFDC2 (HE4)
- total PSA, %free PSA, PV, WFDC2 (HE4), or
- total PSA, %free PSA, DRE, PV, WFDC2 (HE4).

~~Embodiment~~Example 19. The method according to any one of ~~embodiment~~examples 1 to 18, wherein the test subject has previously received a positive indication of prostate cancer.

~~Embodiment~~Example 20. The method according to any one of ~~embodiment~~examples 1 to 19, wherein the test subject has previously received a positive indication of prostate cancer by digital rectal exam (DRE) and/or by PSA testing.

~~Embodiment~~Example 21. The method according to any one of ~~embodiment~~examples 1 to 19, wherein the test subject has a PSA level of 2-10 ng/mL blood, or 4-10 ng/mL blood.

~~Embodiment~~Example 22. The method according to any one of ~~embodiment~~examples 1 to 21, wherein the series of biological samples obtained from each said population and/or the test subject's biological sample are selected from; whole blood, serum, plasma, saliva, tear/s, urine, and tissue.

~~Embodiment~~Example 23. The method according to any one of ~~embodiment~~examples 1 to 22, wherein said test subject, said population of subjects having aggressive CaP, and said population of control subjects are human.

~~Embodiment~~Example 24. The method of any one of ~~embodiment~~examples 1 to 23, further comprising measuring one or more analyte/s in the test subject's biological sample to thereby obtain the analyte level for each said one or more analytes.

~~Embodiment~~Example 25. The method according to ~~embodiment~~example 24, wherein said measuring of one or more analyte/s in the test subject's biological sample comprises:

(i) measuring one or more fluorescent signals indicative of each said analyte level;

(ii) obtaining a measurement of weight/volume of said analyte/s in the biological sample;

(iii) measuring an absorbance signal indicative of each said analyte level; or

(iv) using a technique selected from the group consisting of: electrochemiluminescence, mass spectrometry, a protein array technique, high performance liquid chromatography (HPLC), gel electrophoresis, radiolabeling, and any combination thereof.

~~Embodiment~~Example 26. The method according to ~~embodiment~~example 24 or ~~embodiment~~example 25, wherein the test subject's biological sample is contacted, or the series of biological samples was contacted, with first and second antibody populations for detection of each said analyte, wherein each said antibody population has binding specificity for one of said analytes, and the first and second antibody populations have different analyte binding specificities.

~~Embodiment~~Example 27. The method according to ~~embodiment~~example 26, wherein the first and/or second antibody populations are labelled.

~~Embodiment~~Example 28. The method according to ~~embodiment~~example 27, wherein the first and/or second antibody populations comprise a label selected from the group consisting of a radiolabel, a

fluorescent label, a biotin-avidin amplification system, a chemiluminescence system, microspheres, and colloidal gold.

~~Embodiment~~Example 29. The method according to any one of ~~embodiment~~examples 26 to 28, wherein binding of each said antibody population to the analyte is detected by a technique selected from the group consisting of: immunofluorescence, radiolabeling, immunoblotting, Western blotting, enzyme-linked immunosorbent assay (ELISA), flow cytometry, immunoprecipitation, immunohistochemistry, biofilm test, affinity ring test, antibody array optical density test, and chemiluminescence.

~~Embodiment~~Example 30. The method of any one of ~~embodiment~~examples 24 to 29, wherein said measuring of each said analyte in the biological sample from the test subject or the series of biological samples obtained from each said population comprises measuring the analytes directly.

~~Embodiment~~Example 31. The method of any one of ~~embodiment~~examples 24 to 29, wherein said measuring of each said analyte in the biological sample from the test subject or the series of biological samples obtained from each said population comprises detecting a nucleic acid encoding the analytes.

~~Embodiment~~Example 32. The method of any one of ~~embodiment~~examples 1 to 31, further comprising measuring the two one or more clinical variables in the test subject.

~~Embodiment~~Example 33. The method of any one of ~~embodiment~~examples 1 to 32, further comprising determining said threshold value.

~~Embodiment~~Example 34. The method of ~~embodiment~~example 33, wherein determining said threshold value comprises measuring said one or more analyte/s in a series of biological samples obtained from a population of subjects having aggressive CaP and from a population of control subjects not having aggressive CaP, to thereby obtain an analyte level for each said analyte in each said biological sample of the series.

Brief Description of the Figures

Preferred embodiments of the present invention will now be described, by way of example only, with reference to the accompanying figures wherein:

Figure One depicts a ROC curve analysis based on PSA levels (model fitting: logistic regression) generated to differentiate [aggressive prostate cancer (AgCaP) versus non-aggressive prostate cancer (NonAgCaP)].

Figure Two depicts depicts a ROC curve analysis based on DRE status (model fitting: logistic regression) generated to differentiate [aggressive prostate cancer (AgCaP) versus non-aggressive prostate cancer (NonAgCaP)].

CLAIMS

1. A method for diagnosing aggressive prostate cancer (CaP) in a test subject, comprising:

(a) obtaining an analyte level for one or more analytes in a biological sample obtained from the test subject~~'s biological sample~~, and ~~obtaining~~ a measurement of one or more clinical variables having been obtained from the test subject; and

(b) applying a suitable algorithm and/or transformation to a combination of the clinical variable measurements and analyte level/s of the test subject to thereby generate a test subject score value for comparison to a threshold value; and

(c) determining whether the test subject has aggressive CaP by comparison of the subject test score value and the threshold value,

wherein:

the one or more analyte/s comprise or consist of WAP four-disulfide core domain protein 2 (WFDC2 (HE4)), and optionally total prostate specific antigen (PSA),

the one or more clinical variables comprise at least one of: %Free PSA, DRE, Prostate Volume (PV) and

the threshold value was determined by:

measuring said one or more analyte/s in a series of biological samples obtained from a population of subjects having aggressive CaP and from a population of control subjects not having aggressive CaP, to thereby obtain an analyte level for each said analyte in each said biological sample of the series;

combining each said analyte level of the series with measurements of said one or more clinical variables obtained from each said subject of the populations, in a manner that allows discrimination between aggressive CaP and an absence of aggressive CaP, to thereby generate the threshold value.

2. The method of claim 1, wherein the population of control subjects comprises subjects that do not have prostate cancer and subjects that have non-aggressive prostate cancer

3. A method for discerning whether a test subject has non-aggressive or aggressive prostate cancer (CaP), comprising:

(a) obtaining an analyte level for one or more analytes in a biological sample obtained from the test subject~~'s biological sample~~, and ~~obtaining~~ a measurement of one or more clinical variables having been obtained from the test subject; and

(b) applying a suitable algorithm and/or transformation to a combination of the clinical variable measurements and analyte level/s of the test subject to thereby generate a test subject score value for comparison to a threshold value; and

(c) determining whether the test subject has aggressive CaP by comparison of the subject test score value and the threshold value,

wherein:

the one or more analyte/s comprise or consist of WFDC2 (HE4), and optionally total PSA,

the one or more clinical variables comprise at least one of: %Free PSA, DRE, Prostate Volume (PV) and

the threshold value was determined by:

measuring said one or more analyte/s in a series of biological samples obtained from a population of subjects having aggressive CaP and from a population of control subjects having non-aggressive CaP, to thereby obtain an analyte level for each said analyte in each said biological sample of the series;

combining each said analyte level of the series with measurements of said one or more clinical variables obtained from each said subject of the populations, in a manner that allows discrimination between aggressive CaP and non-aggressive CaP, to thereby generate the threshold value.

4. The method of claim 1 or claim 3, wherein the population of control subjects has non-aggressive CaP as defined by a Gleason score of 3+3.

5. The method of any one of claims 1 to 4, wherein the threshold value is determined prior to performing the method.

6. The method of any one of claims 1 to 5, wherein the one or more clinical variables and the one or more analyte/s comprise or consist of any one of the following:

- WFDC2 (HE4) and %Free PSA
- WFDC2 (HE4) and DRE
- WFDC2 (HE4) and PV
- WFDC2 (HE4), %Free PSA, and DRE
- WFDC2 (HE4), %Free PSA, and PV
- WFDC2 (HE4), total PSA and %Free PSA

- WFDC2 (HE4), total PSA and DRE
- WFDC2 (HE4), total PSA and PV
- WFDC2 (HE4), total PSA, %Free PSA, and DRE, or
- WFDC2 (HE4), total PSA, %Free PSA, and PV.

7. The method of any one of claims 1 to 6, comprising selecting a subset of the combined analyte/s and/or clinical variable measurements to generate the threshold value.

8. The method of any one of claims 1 to 7, wherein said combining of each said analyte level of the series with said measurements of the one or more clinical variables comprises combining a logistic regression score of the clinical variable measurements and analyte level/s in a manner that maximizes said discrimination, in accordance with the formula: (i)

$$\text{Logit (P)} = \text{Log(P/1-P)}$$

$$= \boldsymbol{intercept} + \sum_{i=1}^{N}(\boldsymbol{coefficient_i} \times \boldsymbol{transformed}\,(\boldsymbol{variable_i})$$

$$\boldsymbol{P} = \frac{\boldsymbol{exp(Logit(P))}}{\boldsymbol{1+exp(Logit(P))}}$$

wherein:

P is probability of that the test subject has aggressive prostate cancer,

the coefficient$_i$ is the natural log of the odds ratio of the variable,

the transformed variable$_i$ is the natural log of the variable$_i$ value; or

(ii)

$$\text{Logit (P)} = \text{Log(P/1-P)}$$

$$= \boldsymbol{intercept} + \sum_{i=1}^{N}(\boldsymbol{coefficient_i} \times \boldsymbol{transformed}\,(\boldsymbol{variable_i}) + coefficient_{DRE} \times DRE$$

$$\boldsymbol{P} = \frac{\boldsymbol{exp(Logit(P))}}{\boldsymbol{1+exp(Logit(P))}}$$

wherein:

P is probability that the test subject has aggressive prostate cancer,

the coefficient$_i$ is the natural log of the odds ratio of the variable,

the transformed variable$_i$ is the natural log of the variable$_i$ value,

a DRE value of 1 indicates abnormal, while DRE value of 0 indicates normal.

9. The method of any one of claims 1 to 8, wherein said applying a suitable algorithm and/or transformation to the combination of the clinical variable measurements and

analyte level/s comprises use of an exponential function, a logarithmic function, a power function and/or a root function.

10. The method according to any one of claims 1 to 9, wherein the suitable algorithm and/or transformation applied to the combination of the clinical variable measurements and analyte level/s of the test subject is in accordance with the formula:

(i)

$$\text{Logit (P)} = \text{Log(P/1-P)}$$

$$= \boldsymbol{intercept} + \sum_{i=1}^{N}(\boldsymbol{coefficient_i} \times \boldsymbol{transformed}\,(\boldsymbol{variable_i})$$

$$\boldsymbol{P} = \frac{\boldsymbol{exp(Logit(P))}}{\boldsymbol{1+exp(Logit(P))}}$$

wherein:

P is probability of that the test subject has aggressive prostate cancer,

the coefficient$_i$ is the natural log of the odds ratio of the variable,

the transformed variable$_i$ is the natural log of the variable$_i$ value; or

(ii)

$$\text{Logit (P)} = \text{Log(P/1-P)}$$

$$= \boldsymbol{intercept} + \sum_{i=1}^{N}(\boldsymbol{coefficient_i} \times \boldsymbol{transformed}\,(\boldsymbol{variable_i}) + coefficient_{DRE} \times DRE$$

$$\boldsymbol{P} = \frac{\boldsymbol{exp(Logit(P))}}{\boldsymbol{1+exp(Logit(P))}}$$

wherein:

P is probability of that the test subject has aggressive prostate cancer,

the coefficient$_i$ is the natural log of the odds ratio of the variable,

the transformed variable$_i$ is the natural log of the variable$_i$ value,

a DRE value of 1 indicates abnormal, while DRE value of 0 indicates normal;

and wherein said suitable algorithm and/or transformation is used to generate the subject test score that is compared to the threshold value to thereby determine whether or not the test subject has aggressive prostate cancer.

11. The method according to any one of claims 1 to 10, wherein said combining of each said analyte level of the series with measurements of said one or more clinical variables obtained from each said subject of the populations maximizes said discrimination.

12. The method of any one of claims 1 to 11, wherein said combining of each said analyte level of the series with the measurements of one or more clinical variables obtained from each said subject of the populations is conducted in a manner that:

(i) reduces the misclassification rate between the subjects having aggressive CaP and said control subjects; and/or

(ii) increases sensitivity in discriminating between the subjects having aggressive CaP and said control subjects; and/or

(iii) increases specificity in discriminating between the subjects having aggressive CaP and said control subjects.

13. The method of claim 12, wherein said combining in a manner that reduces the misclassification rate between the subjects having aggressive CaP and said control subjects:

comprises selecting a suitable true positive and/or true negative rate; or

~~14. The method of claim 12, wherein said combining in a manner that reduces the misclassification rate between the subjects having aggressive CaP and said control subjects~~ minimizes the misclassification rate; or

~~15. The method of claim 12, wherein said combining in a manner that reduces the misclassification rate between the subjects having aggressive CaP and said control subjects~~ comprises minimizing the misclassification rate between the subjects having aggressive CaP and said control subjects by identifying a point where the true positive rate intersects the true negative rate.

14~~16~~. The method of claim 12, wherein:

said selecting the threshold value from the combined clinical variable measurement/s and combined analyte level/s in a manner that increases sensitivity in discriminating between the subjects having aggressive CaP and said control subjects increases or maximizes said sensitivity; or

~~17. The method of claim 12, wherein~~ said selecting the threshold value from the combined clinical variable measurement/s and combined analyte level/s in a manner that increases specificity in discriminating between the subjects having aggressive CaP and said control subjects increases or maximizes said specificity.

15~~18~~. The method according to any one of claims 1 to 14~~17~~, wherein the one or more clinical variables and the one or more analytes comprise or consist of:

- total PSA, %free PSA, DRE, WFDC2 (HE4)
- total PSA, %free PSA, PV, WFDC2 (HE4), or
- total PSA, %free PSA, DRE, PV, WFDC2 (HE4).

16~~19~~. The method according to any one of claims 1 to 15~~18~~, wherein the test subject: has previously received a positive indication of prostate cancer, such as ~~.~~

~~20. The method according to any one of claims 1 to 19, wherein the test subject has previously received a positive indication of prostate cancer~~ by digital rectal exam (DRE) and/or by PSA testing; or~~.~~

~~21. The method according to any one of claims 1 to 19, wherein the test subject~~ has a PSA level of 2-10 ng/mL blood, or 4-10 ng/mL blood.

17~~22~~. The method according to any one of claims 1 to 16~~21~~, wherein the series of biological samples obtained from each said population and/or the test subject's biological sample are selected from; whole blood, serum, plasma, saliva, tear/s, urine, and tissue.

18~~23~~. The method according to any one of claims 1 to 17~~22~~, wherein said test subject, said population of subjects having aggressive CaP, and said population of control subjects are human.

19~~24~~. The method of any one of claims 1 to 18~~23~~, further comprising measuring one or more analyte/s in the biological sample obtained from the test subject~~'s biological sample~~ to thereby obtain the analyte level for each said one or more analytes, optionally ~~.~~

~~25. The method according to claim 24,~~ wherein said measuring of one or more analyte/s in the test subject's biological sample comprises:

(i) measuring one or more fluorescent signals indicative of each said analyte level;

(ii) obtaining a measurement of weight/volume of said analyte/s in the biological sample;

(iii) measuring an absorbance signal indicative of each said analyte level; or

(iv) using a technique selected from the group consisting of: electrochemiluminescence, mass spectrometry, a protein array technique, high performance liquid chromatography (HPLC), gel electrophoresis, radiolabeling, and any combination thereof.

2026. The method according to claim ~~24 or claim 25~~19, wherein the test subject's biological sample is contacted, or the series of biological samples was contacted, with first and second antibody populations for detection of each said analyte, wherein each said antibody population has binding specificity for one of said analytes, and the first and second antibody populations have different analyte binding specificities, and ~~.~~

~~27. The method according to claim 26,~~ wherein the first and/or second antibody populations are optionally labelled such as with ~~.~~

~~28. The method according to claim 27, wherein the first and/or second antibody populations comprise a label selected from the group consisting of~~ a radiolabel, a fluorescent label, a biotin-avidin amplification system, a chemiluminescence system, microspheres, ~~and~~ or colloidal gold.

2129. The method according to ~~any one of claims 26 to 28~~claim 20, wherein binding of each said antibody population to the analyte is detected by a technique selected from the group consisting of: immunofluorescence, radiolabeling, immunoblotting, Western blotting, enzyme-linked immunosorbent assay (ELISA), flow cytometry, immunoprecipitation, immunohistochemistry, biofilm test, affinity ring test, antibody array optical density test, and chemiluminescence.

2230. The method of any one of claims 1924 to 2129, wherein said measuring of each said analyte in the biological sample from the test subject or the series of biological samples obtained from each said population comprises:

measuring the analytes directly; or~~.~~

~~31. The method of any one of claims 24 to 29, wherein said measuring of each said analyte in the biological sample from the test subject or the series of biological samples obtained from each said population comprises~~ detecting a nucleic acid encoding the analytes.

2332. The method of any one of claims 1 to 2231, further comprising ~~measuring~~ the two ~~one~~ or more clinical variables having been measured in the test subject.

3324. The method of any one of claims 1 to 2332, further comprising determining said threshold value, optionally ~~.~~

~~34. The method of claim 33,~~ wherein determining said threshold value comprises measuring said one or more analyte/s in a series of biological samples obtained from a population of subjects having aggressive CaP and from a population of control subjects not having aggressive CaP, to thereby obtain an analyte level for each said analyte in each said biological sample of the series.