and 2293 females). The authors stated that among females, there was no relationship between biomarkers and cancer incidence.

Summary

In an aspect, there is provided a method for predicting the risk of getting cancer in a subject that does not suffer from cancer in a subject comprising: determining the level of Pro-Tachykinin, its splice variants or fragments thereof of at least 5 amino acids in a bodily fluid obtained from said subject; and correlating said level of Pro-Tachykinin, its splice variants or fragments thereof with risk for getting cancer, wherein a reduced level is predictive for an enhanced risk of getting cancer, the term "reduced level" meaning a level below a certain threshold level.

In another aspect, there is provided an assay for determining Pro-Tachykinin, its splice variants or fragments thereof of at least 5 amino acids in a sample comprising two binders that bind to two different regions within the region of Pro-Tachykinin that is Pro-Tachykinin 1-37 (SEQ ID NO. 2), wherein said assay is additionally for determining Pro-Neurotensin or fragments thereof of at least 5 amino acids in a sample further comprising two binders that bind to two different regions within the region of Pro Neurotensin that is Pro Neurotensin 1-117 (SEQ ID No. 18).

Detailed Description

A subject of the present invention was to investigate the prognostic and diagnostic power of Pro-Tachykinin for the prediction of cancer incidence and the prediction of the risk of reoccurrence of cancer. To address this issue, stable fragments of Pro-Tachykinin (Ernst *et al.*, 2008) in fasting plasma were measured in said Swedish prospective cohort study (Malmö Diet and Cancer Study) and related baseline level of this biomarker to breast-cancer incidence during 15 years of follow-up.

Surprisingly, it has been shown that Pro-Tachykinin is a powerful and highly significant biomarker for predicting the risk of getting cancer in a subject that does not suffer from cancer or alternatively diagnosing cancer in a subject.

Thus, subject matter of the present invention is a method for predicting the risk of getting cancer in a subject that does not suffer from cancer or alternatively diagnosing cancer in a subject comprising:

CLAIMS

1. A method for predicting the risk of getting cancer in a subject that does not suffer from cancer in a subject comprising:

- determining the level of Pro-Tachykinin, its splice variants or fragments thereof of at least 5 amino acids in a bodily fluid obtained from said subject; and

- correlating said level of Pro-Tachykinin, its splice variants or fragments thereof with risk for getting cancer, wherein a reduced level is predictive for an enhanced risk of getting cancer, the term "reduced level" meaning a level below a certain threshold level.

2. A method according to claim 1 wherein the following steps are further comprised:

- determining the level of Pro-Neurotensin or fragments thereof of at least 5 amino acids in a bodily fluid obtained from said subject; and

- correlating additionally Pro-Neurotensin or fragments thereof of at least 5 amino acids with a risk for getting cancer, wherein an increased level of Pro-Neurotensin or fragments thereof is predictive for an enhanced risk of getting cancer, the term "increased level" meaning a level above a certain threshold level.

3. A method according to claim 1 or 2 wherein the following steps are further comprised:

- determining the level of Pro-Enkephalin or fragments thereof of at least 5 amino acids in a bodily fluid obtained from said subject; and

- correlating additionally Pro-Enkephalin or fragments thereof of at least 5 amino acids with a risk for getting cancer, and wherein an reduced level of Pro-Enkephalin or fragments thereof is predictive for an enhanced risk of getting cancer.

4. A method according to any of claims 1 to 3 wherein the following steps are further comprised:

- determining the level of Insulin in a bodily fluid obtained from said subject; and

- correlating additionally Insulin with a risk for getting cancer, wherein an reduced level of Insulin is predictive for an enhanced risk of getting cancer.

5. A method according to any of claims 2 to 4 wherein additionally correlating means a combined analysis of the determined biomarker levels by taking into account the relative risk factors for cancer development obtained by the individual biomarkers.

6. A method according to any of claims 1-5 wherein a reduced level of Pro-Tachykinin, its splice variants or fragments thereof is a level below a threshold wherein said threshold is about or below 100 pmol/l, preferably about or below 80 pmol/L, preferably about or below 60 pmol/L, preferably about or below 50 pmol/L, preferably about or below 45,6 pmol/L, preferably about 40 pmol/L

7. A method according to claims 2 to 6 wherein an increased level of Pro-Neurotensin or fragments thereof is a level above a threshold wherein said threshold is about or above 78 pmol/l, preferred about or above 100 pmol/l, more preferred about 150 pmol/l.

8. A method according to any of claims 3 to 7 wherein a reduced level of Pro-Enkephalin or fragments thereof is a level below a threshold wherein said threshold is about or below 100 pmol/l, preferably about or below 75 pmol/L, preferably about or below 50 pmol/L, preferably about 40,4 pmol/L.

9. A method according to any of claims 4 to 8 wherein a reduced level of Insulin is a level below a threshold wherein said threshold is about 70 pmol/l.

10. A method according to any of claims 1 to 9 wherein said subject is female.

11. A method according to claim 10, wherein said cancer is breast cancer.

12. A method according to any of claims 1 to 10 wherein said cancer is lung cancer.

13. A method according to any of claims 1 to 12, wherein said subject has never had a history of diagnosis of cancer at the time the sample of bodily fluid is taken from said subject.

14. A method according to claims 1 to 12, wherein said subject has had a history of diagnosis of cancer and has been cured at the time the sample of bodily fluid is taken from said subject and the risk of reoccurrence of getting cancer is determined or alternatively the reoccurrence of breast cancer is determined.

15. A method according to claims 1 to 14, wherein at the time the sample of bodily fluid is taken from said subject, said subject has been diagnosed as having a cardiovascular disease or diabetes.

16. A method according to claims 1 to 15, wherein additionally at least one clinical parameter is determined selected from the group comprising: age, presence of diabetes mellitus, current smoking.

17. A method according to any of claims 1 to 16, wherein the level of Pro Tachykinin, its splice variants or fragments thereof and Pro-Neurotensin or fragments thereof and/or Pro-Enkephalin or fragments thereof and/or Insulin is measured with an immunoassay.

18. A method according to any of claims 1 to 17 wherein said a method is performed more than once in order to monitor the risk of getting cancer in a subject or in order to monitor the course of treatment.

19. A method according to claim 18 wherein said monitoring is performed in order to evaluate the response of said subject to preventive and/or therapeutic measures taken.

20. A method according to any of claims 1 to 19 in order to stratify said subjects into risk groups.

21. A method according to any of claims 1-20 wherein the bodily fluid is blood or plasma or serum.

22. An assay for determining Pro Tachykinin, its splice variants or fragments thereof of at least 5 amino acids in a sample comprising two binders that bind to two different regions within the region of Pro-Tachykinin that is Pro-Tachykinin 1-37 (SEQ ID NO. 2), wherein said assay is additionally for determining Pro-Neurotensin or fragments thereof of at least 5 amino acids in a sample further comprising two binders that bind to two

different regions within the region of Pro Neurotensin that is Pro Neurotensin 1-117 (SEQ ID No. 18).

23. An assay for determining Pro-Tachykinin, its splice variants or fragments thereof of at least 5 amino acids according to claim 22 wherein said assay is additionally for determining Pro-Enkephalin and Pro-Enkephalin fragments in a sample comprising two binders that bind to two different regions within the region of Pro-Enkephalin that is amino acid 133-140 (LKELLETG, SEQ ID NO. 22) and amino acid 152-159 (SDNEEEVS, SEQ ID NO. 23) wherein each of said regions comprises at least 4 or 5 amino acids.

24. An assay for determining Pro-Tachykinin, its splice variants or fragments thereof of at least 5 amino acids according to any of claims 22 to 23 wherein said assay is additionally for determining Insulin in a sample comprising two binders that bind to two different regions of insulin.

CLAIMS

1. A method for predicting the risk of getting cancer in a subject that does not suffer from cancer or alternatively diagnosing cancer in a subject comprising:

- determining the level of Pro-Tachykinin, its splice variants or fragments thereof of at least 5 amino acids in a bodily fluid obtained from said subject; and

- correlating said level of Pro-Tachykinin, its splice variants or fragments thereof with risk for getting cancer, wherein a reduced level is predictive for an enhanced risk of getting cancer or alternatively diagnosing cancer wherein a reduced level is correlated with the diagnosis of cancer.

2. A method according to claim 1 wherein the following steps are further comprised:

- determining the level of Pro-Neurotensin or fragments thereof of at least 5 amino acids in a bodily fluid obtained from said subject; and

- correlating additionally Pro-Neurotensin or fragments thereof of at least 5 amino acids with a risk for getting cancer, wherein an increased level of Pro-Neurotensin or fragments thereof is predictive for an enhanced risk of getting cancer or alternatively diagnosing cancer wherein an increased level of Pro-Neurotensin or fragments thereof is correlated with the diagnosis of cancer.

3. A method according to claim 1 or 2 wherein the following steps are further comprised:

- determining the level of Pro-Enkephalin or fragments thereof of at least 5 amino acids in a bodily fluid obtained from said subject; and

- correlating additionally Pro-Enkephalin or fragments thereof of at least 5 amino acids with a risk for getting cancer, and wherein an reduced level of Pro-Enkephalin or fragments thereof is predictive for an enhanced risk of getting cancer or alternatively

diagnosing cancer wherein an reduced level of Pro-Enkephalin or fragments thereof is correlated with the diagnosis of cancer.

4. A method according to any of claims 1 to 3 wherein the following steps are further comprised:

- determining the level of Insulin in a bodily fluid obtained from said subject; and
- correlating additionally Insulin with a risk for getting cancer, wherein an reduced level of Insulin is predictive for an enhanced risk of getting cancer or alternatively diagnosing cancer wherein an reduced level of Insulin is correlated with the diagnosis of cancer.

5. A method according to any of claims 2 to 4 wherein additionally correlating means a combined analysis of the determined biomarker levels by taking into account the relative risk factors for cancer development obtained by the individual biomarkers.

6. A method according to any of claims 1-5 wherein a reduced level of Pro-Tachykinin, its splice variants or fragments thereof is a level below a threshold wherein said threshold is about or below 100 pmol/l, preferably about or below 80 pmol/L, preferably about or below 60 pmol/L, preferably about or below 50 pmol/L, preferably about or below 45,6 pmol/L, preferably about 40 pmol/L

7. A method according to claims 2 to 6 wherein an increased level of Pro-Neurotensin or fragments thereof is a level above a threshold wherein said threshold is about or above 78 pmol/l, preferred about or above 100 pmol/l, more preferred about 150 pmol/l.

8. A method according to any of claims 3 to 7 wherein a reduced level of Pro-Enkephalin or fragments thereof is a level below a threshold wherein said threshold is about or below 100 pmol/l, preferably about or below 75 pmol/L, preferably about or below 50 pmol/L, preferably about 40,4 pmol/L.

9. A method according to any of claims 4 to 8 wherein a reduced level of Insulin is a level below a threshold wherein said threshold is about 70 pmol/l.

10. A method according to any of claims 1 to 9 wherein said subject is female.

11. A method according to claim 10, wherein said cancer is breast cancer.

12. A method according to any of claims 1 to 10 wherein said cancer is lung cancer.

13. A method according to any of claims 1 to 12, wherein said subject has never had a history of diagnosis of cancer at the time the sample of bodily fluid is taken from said subject.

14. A method according to claims 1 to 12, wherein said subject has had a history of diagnosis of cancer and has been cured at the time the sample of bodily fluid is taken from said subject and the risk of reoccurrence of getting cancer is determined or alternatively the reoccurrence of breast cancer is determined.

15. A method according to claims 1 to 14, wherein at the time the sample of bodily fluid is taken from said subject, said subject has been diagnosed as having a cardiovascular disease or diabetes.

16. A method according to claims 1 to 15, wherein additionally at least one clinical parameter is determined selected from the group comprising: age, presence of diabetes mellitus, current smoking.

17. A method according to any of claims 1 to 16, wherein the level of Pro Tachykinin, its splice variants or fragments thereof and Pro-Neurotensin or fragments thereof and/or Pro-Enkephalin or fragments thereof and/or Insulin is measured with an immunoassay.

18. A method according to any of claims 1 to 17 wherein said a method is performed more than once in order to monitor the risk of getting cancer in a subject or in order to monitor the course of treatment.

19. A method according to claim 18 wherein said monitoring is performed in order to evaluate the response of said subject to preventive and/or therapeutic measures taken.

20. A method according to any of claims 1 to 19 in order to stratify said subjects into risk groups.

21. A method according to any of claims 1-20 wherein the bodily fluid is blood or plasma or serum.

22. An assay for determining Pro-Tachykinin, its splice variants or fragments thereof of at least 5 amino acids in a sample comprising two binders that bind to two different regions within the region of Pro-Tachykinin that is Pro-Tachykinin 1-37 (SEQ ID NO. 2).

23. An assay for determining Pro Tachykinin, its splice variants or fragments thereof of at least 5 amino acids according to claim 22 wherein said assay is additionally for determining Pro-Neurotensin or fragments thereof of at least 5 amino acids in a sample further comprising two binders that bind to two different regions within the region of Pro Neurotensin that is Pro Neurotensin 1-117 (SEQ ID No. 18).

24. An assay for determining Pro-Tachykinin, its splice variants or fragments thereof of at least 5 amino acids according to claim 22 or 23 wherein said assay is additionally for determining Pro-Enkephalin and Pro-Enkephalin fragments in a sample comprising two binders that bind to two different regions within the region of Pro-Enkephalin that is amino acid 133-140 (LKELLETG, SEQ ID NO. 22) and amino acid 152-159 (SDNEEEVS, SEQ ID NO. 23) wherein each of said regions comprises at least 4 or 5 amino acids.

25. An assay for determining Pro-Tachykinin, its splice variants or fragments thereof of at least 5 amino acids according to any of claims 22 to 24 wherein said assay is additionally for determining Insulin in a sample comprising two binders that bind to two different regions of insulin.

AMENDED CLAIMS
received by the International Bureau on 15 August 2014 (15.08.2014)

1. A method for predicting the risk of getting cancer in a subject that does not suffer from cancer in a subject comprising:

   - determining the level of Pro-Tachykinin, its splice variants or fragments thereof of at least 5 amino acids in a bodily fluid obtained from said subject; and

   - correlating said level of Pro-Tachykinin, its splice variants or fragments thereof with risk for getting cancer, wherein a reduced level is predictive for an enhanced risk of getting cancer.

2. A method according to claim 1 wherein the following steps are further comprised:

   - determining the level of Pro-Neurotensin or fragments thereof of at least 5 amino acids in a bodily fluid obtained from said subject; and

   - correlating additionally Pro-Neurotensin or fragments thereof of at least 5 amino acids with a risk for getting cancer, wherein an increased level of Pro-Neurotensin or fragments thereof is predictive for an enhanced risk of getting cancer.

3. A method according to claim 1 or 2 wherein the following steps are further comprised:

   - determining the level of Pro-Enkephalin or fragments thereof of at least 5 amino acids in a bodily fluid obtained from said subject; and

   - correlating additionally Pro-Enkephalin or fragments thereof of at least 5 amino acids with a risk for getting cancer, and wherein an reduced level of Pro-Enkephalin or fragments thereof is predictive for an enhanced risk of getting cancer.

4. A method according to any of claims 1 to 3 wherein the following steps are further comprised:

   - determining the level of Insulin in a bodily fluid obtained from said subject; and

- correlating additionally Insulin with a risk for getting cancer, wherein an reduced level of Insulin is predictive for an enhanced risk of getting cancer.

5. A method according to any of claims 2 to 4 wherein additionally correlating means a combined analysis of the determined biomarker levels by taking into account the relative risk factors for cancer development obtained by the individual biomarkers.

6. A method according to any of claims 1-5 wherein a reduced level of Pro-Tachykinin, its splice variants or fragments thereof is a level below a threshold wherein said threshold is about or below 100 pmol/l, preferably about or below 80 pmol/L, preferably about or below 60 pmol/L, preferably about or below 50 pmol/L, preferably about or below 45,6 pmol/L, preferably about 40 pmol/L

7. A method according to claims 2 to 6 wherein an increased level of Pro-Neurotensin or fragments thereof is a level above a threshold wherein said threshold is about or above 78 pmol/l, preferred about or above 100 pmol/l, more preferred about 150 pmol/l.

8. A method according to any of claims 3 to 7 wherein a reduced level of Pro-Enkephalin or fragments thereof is a level below a threshold wherein said threshold is about or below 100 pmol/l, preferably about or below 75 pmol/L, preferably about or below 50 pmol/L, preferably about 40,4 pmol/L.

9. A method according to any of claims 4 to 8 wherein a reduced level of Insulin is a level below a threshold wherein said threshold is about 70 pmol/l.

10. A method according to any of claims 1 to 9 wherein said subject is female.

11. A method according to claim 10, wherein said cancer is breast cancer.

12. A method according to any of claims 1 to 10 wherein said cancer is lung cancer.

13. A method according to any of claims 1 to 12, wherein said subject has never had a history of diagnosis of cancer at the time the sample of bodily fluid is taken from said subject.

14. A method according to claims 1 to 12, wherein said subject has had a history of diagnosis of cancer and has been cured at the time the sample of bodily fluid is taken from said subject and the risk of reoccurrence of getting cancer is determined or alternatively the reoccurrence of breast cancer is determined.

15. A method according to claims 1 to 14, wherein at the time the sample of bodily fluid is taken from said subject, said subject has been diagnosed as having a cardiovascular disease or diabetes.

16. A method according to claims 1 to 15, wherein additionally at least one clinical parameter is determined selected from the group comprising: age, presence of diabetes mellitus, current smoking.

17. A method according to any of claims 1 to 16, wherein the level of Pro Tachykinin, its splice variants or fragments thereof and Pro-Neurotensin or fragments thereof and/or Pro-Enkephalin or fragments thereof and/or Insulin is measured with an immunoassay.

18. A method according to any of claims 1 to 17 wherein said a method is performed more than once in order to monitor the risk of getting cancer in a subject or in order to monitor the course of treatment.

19. A method according to claim 18 wherein said monitoring is performed in order to evaluate the response of said subject to preventive and/or therapeutic measures taken.

20. A method according to any of claims 1 to 19 in order to stratify said subjects into risk groups.

21. A method according to any of claims 1-20 wherein the bodily fluid is blood or plasma or serum.

22. An assay for determining Pro-Tachykinin, its splice variants or fragments thereof of at least 5 amino acids in a sample comprising two binders that bind to two different regions within the region of Pro-Tachykinin that is Pro-Tachykinin 1-37 (SEQ ID NO. 2).

23. An assay for determining Pro Tachykinin, its splice variants or fragments thereof of at least 5 amino acids according to claim 22 wherein said assay is additionally for

determining Pro-Neurotensin or fragments thereof of at least 5 amino acids in a sample further comprising two binders that bind to two different regions within the region of Pro Neurotensin that is Pro Neurotensin 1-117 (SEQ ID No. 18).

24. An assay for determining Pro-Tachykinin, its splice variants or fragments thereof of at least 5 amino acids according to claim 22 or 23 wherein said assay is additionally for determining Pro-Enkephalin and Pro-Enkephalin fragments in a sample comprising two binders that bind to two different regions within the region of Pro-Enkephalin that is amino acid 133-140 (LKELLETG, SEQ ID NO. 22) and amino acid 152-159 (SDNEEEVS, SEQ ID NO. 23) wherein each of said regions comprises at least 4 or 5 amino acids.

25. An assay for determining Pro-Tachykinin, its splice variants or fragments thereof of at least 5 amino acids according to any of claims 22 to 24 wherein said assay is additionally for determining Insulin in a sample comprising two binders that bind to two different regions of insulin.