

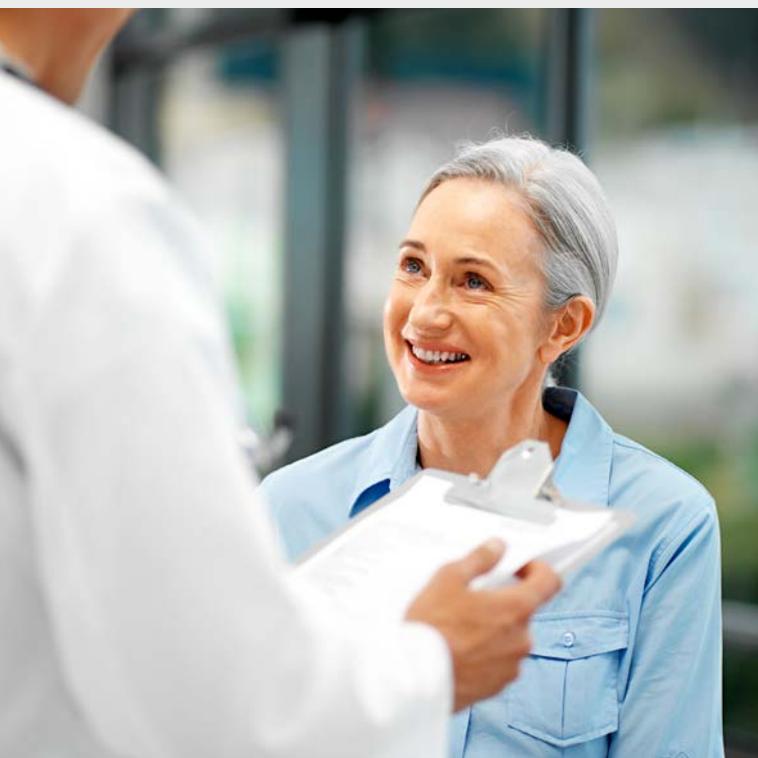


sphingotest[®] pro-NT/pro-ENK

Simple detection
in the risk of breast cancer

Detect risk factors for breast cancer fast

In the United States 1 in every 8 women develops breast cancer during her lifetime; with approx. 230,000 cases of invasive breast cancer expected to be diagnosed each year. Over 88% of breast cancer cases occur at ages 45+*. We know that the earlier the development of this cancer is detected, the higher the chances of treating it successfully. It is also crucial to know the risk factors in the development of the disease including age, time of the first menstruation or menopause, density of breast tissue and genetic changes (mutation of the breast cancer gene) that cannot be influenced. Other factors, include choice of HRT treatment, Body Mass Index (BMI) and smoking, for example. Further lifestyle decisions can also encompass levels of physical activity, eating habits and alcohol consumption. With the aid of empirical models, factors such as these can be compiled and individual risk calculated using the data and information at hand. With proneurotensin and proenkephalin, new constituents have now been found which are independent of, and linked to, the risk of breast cancer more than any other known factors.



Should a woman have a higher risk of developing breast cancer, closer monitoring, a change in lifestyle and additional testing may be warranted.

*National Cancer Institute

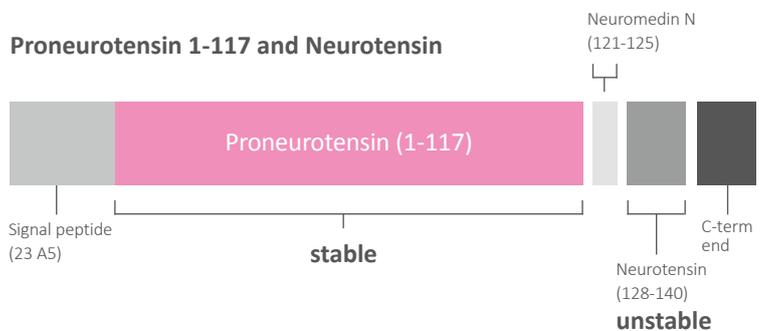


Endogenous hormone neurotensin causes uncontrolled cell growth

Neurotensin is a hormone peptide composed of 13 amino acids, which is essentially formed in the N cells of the small intestine. The release of this hormone into the body's circulation by the small intestine is primarily influenced by animal fats and sugar. It also acts as a satiety hormone. Independent of its function as "insulin of the fat", neurotensin stimulates the growth of breast tumor cells and is also seen as having a directly anti-apoptotic effect on breast cancer cell growth. The NT1 receptor and neurotensin is overexpressed in malignant breast cancer cells. As neurotensin is unstable *in vivo* and *in vitro*, its direct determination is not suitable for laboratory testing.

Proneurotensin 1-117 (pro-NT), on the other hand, is a stable fragment of the neurotensin precursor molecule and is formed in the same ratio as neurotensin. This means it presents a useable surrogate marker for neurotensin even in the laboratory routine.

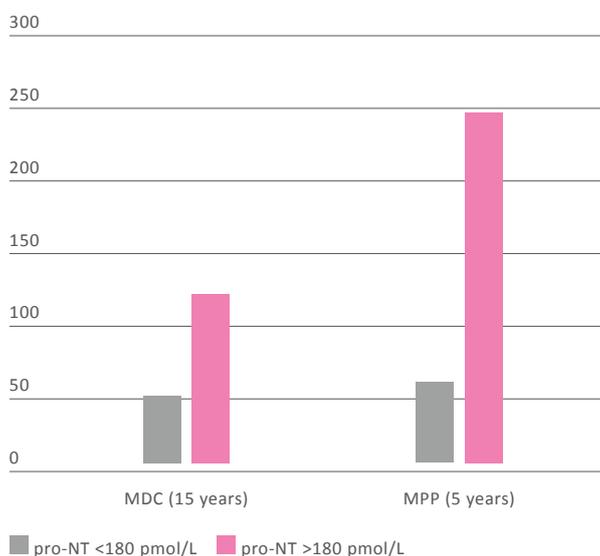
Proneurotensin 1-117 and Neurotensin



Proneurotensin: an indicator in detecting the risk of breast cancer

Results from the Malmö Diet and Cancer Study (MDC)¹ in 2012 clearly prove the correlation between the concentration of pro-NT in the blood and the risk of developing breast cancer. In this prospective cohort study blood plasma was taken from approx. 2,000 women from the normal healthy female population. This was then stored in a sample bank and in 2012 the respective pro-NT concentration was determined using the sphingotest[®] pro-NT. These values were compared with the information collected over approx. 16 years on the health/illness status of the women involved in the study. A total of 123 of the women developed breast cancer during this time of observation. The results showed that an increased pro-NT concentration demonstrated that there was an almost threefold higher risk in developing breast cancer in the following 10-15 years. These results were also confirmed in the further study conducted by the Malmö Prevention Project Study (MPP)².

Breast Cancer Events per 1,000 women



1 Melander, O, et al., Plasma Proneurotensin and Incidence of Diabetes, Cardiovascular Disease, Breast Cancer, and Mortality. *JAMA (Journal of the American Medical Association)*. 10th October 2012, vol. 308, 14, p. 1469-75.

2 Melander, O, et al., Validation of plasma proneurotensin as a novel biomarker for the prediction of incident breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2014 Jun 12. pii: cebp.1200.2013. [Epub ahead of print]



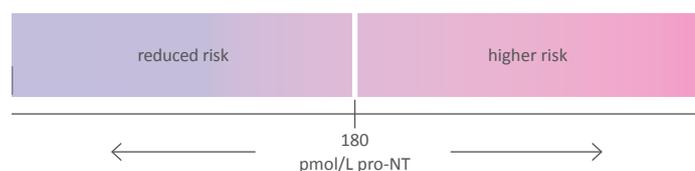
pro-NT: less is more

The results from the Malmö Prevention Project Study further demonstrated that there is an especially strong signal of breast cancer risk prediction at a 5-year follow up. The data from the study showed, compared to a low concentration of pro-NT (<180 pmol/L), a 390% greater risk in the development of breast cancer when a high concentration of pro-NT (>180 pmol/L) was detected. With the aid of detecting a pro-NT concentration in these women, the 10-year risk in the development of breast cancer could be reclassified as follows:

- 26% of the women with breast cancer developed later were elevated to a higher category and
- 21% of women who did not develop breast cancer later were classified in a lower category

The risk calculation was adjusted for known risk factors in a multivariate analysis and demonstrated to be an independent predictor of breast cancer³. There was no direct correlation between pro-NT and BMI as one of the important risk parameters according to the study data. The determination of pro-NT therefore delivers additional information to the risk factors already known and surpasses these in their significance.

The pro-NT concentration detected allows a categorization which varies from the “normal” breast cancer risk in women.



³ e.g. age, use of blood-pressure lowering drugs, hormone replacement therapy, oral contraceptives, education level, age of menarche, age at first pregnancy, number of children, menopause status, systolic blood pressure, BMI, diabetes mellitus, smoking habits, existing cardiovascular disease, hereditary predisposition, concentration of HDL, LDL and insulin.

Enkephalin adds predictive power

The measurement of the endogenous hormone peptide enkephalin significantly adds to the predictive power present in the breast cancer risk indicator pro-NT.

sphingotec has developed the pro-ENK assay (sphingotest[®] pro-ENK), a marker to determine the level of enkephalin in the body. Enkephalin plays a major role in a variety of physiological processes, such as perception of pain, regulation of stress, cardiovascular functions, bone formation and immune response.





A tiny drop of knowledge

Knowledge is power

Using the knowledge that enkephalin and its receptors are expressed in cancerous cells, sphingotec builds on the fact that the substance stimulates cell apoptosis (Programmed Cell Death or PCD). We know that apoptosis is necessary and confers advantages during an organism's lifecycle. It is also something that can be marked in the detection of cancer as an insufficient amount of apoptosis results in uncontrolled cell proliferation, such as cancer.

Detecting stratification systems

With enkephalin levels illustrating how efficiently apoptosis takes place, we can see if sufficient levels lead to PCD, vital for the prevention of cancer cell growth. During examinations, we can see that lower levels of proenkephalin can lead to the unhindered growth of cancer cells as apoptosis does not take place as it should.

pro-ENK is a stable mid-regional fragment of proenkephalin, a surrogate marker for the unstable enkephalin.

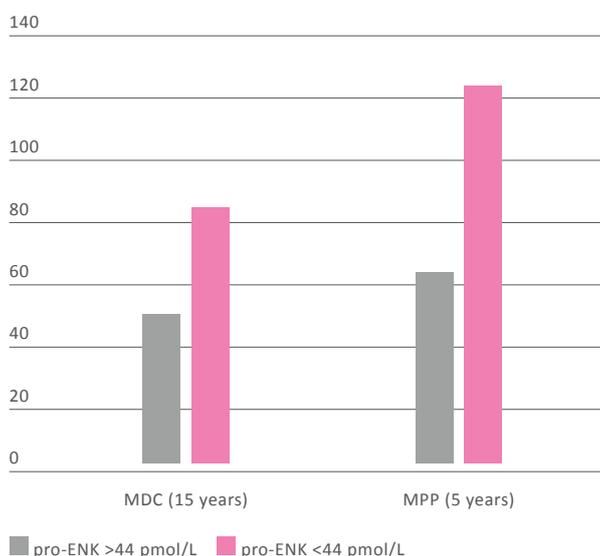
The sphingotec pro-ENK assay (sphingotest® pro-ENK) measures the level of proenkephalin by an immunoassay using monoclonal antibodies specific to pro-ENK. Only a small sample volume of less than 100 µL per test is required.

sphingotest® pro-ENK

Key findings of the population studies

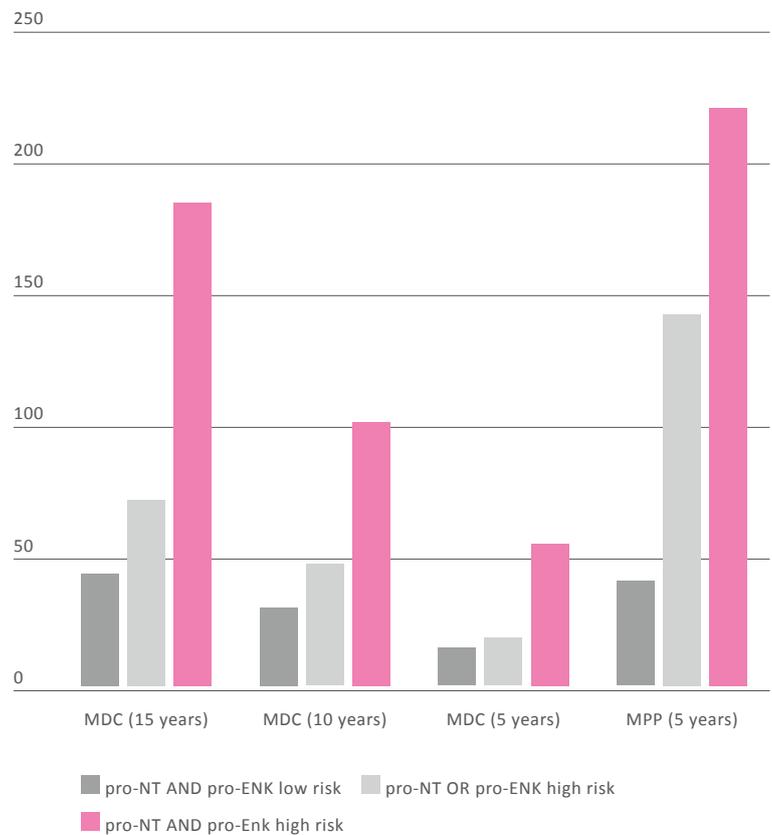
Two general population studies, the Malmö Diet And Cancer Study (MDC) and the Malmö Preventive Project (MPP), resulted in significant findings on the correlation of proneurotensin and proenkephalin on the development of breast cancer. In these two independent general population studies, the simple blood tests sphingotest® pro-NT and sphingotest® pro-ENK determined the concentration of two hormones in the blood: neurotensin and enkephalin. As enkephalin helps the body find diseased cells and fight them, it is imperative that enkephalin levels are not too low. However these levels can be depressed if a patient makes certain lifestyle choices including taking certain medication for pain relief or consuming too much alcohol. These factors can inhibit the release of enkephalin into the circulation, which can lead to an increased risk of breast cancer.

Breast Cancer Events per 1,000 women



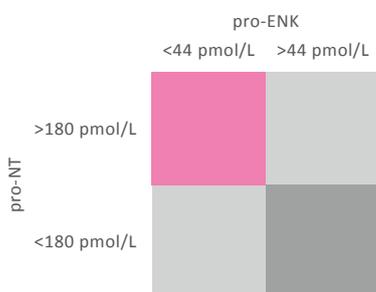
Combining pro-NT + pro-ENK for breast cancer risk prediction

Breast Cancer Events per 1,000 women
Combination of pro-NT and pro-ENK



The studies have shown that elevated pro-NT and lowered pro-ENK are strong and independent risk factors for the development of incident breast cancer. pro-ENK significantly adds prognostic value to pro-NT ($p < 0.0001$) and vice versa ($p < 0.0001$).

Using the measurement system of determining the levels of pro-NT and/or pro-ENK allows physicians to individually adapt measures of early diagnosis and/or prevention of breast cancer:



- Frequency of diagnostic measures (mammography, ultrasound etc)
- Lifestyle (nutrition, exercise, drug consumption)
- Chemoprevention
- HRT guidance

What information is provided by the pro-NT/pro-ENK value?

The evidence of hormone peptides gives women and their clinicians important information on whether they have a higher risk of the disease, regardless of other factors, including genetic factors. By measuring the concentration of pro-NT and pro-ENK, an objective measurable condition is identified in the body. This may not present a health problem at the time of reading but can, over a period of time, favor or signal the development of breast cancer.



Elevated pro-NT and reduced pro-ENK can favor an increased risk of breast cancer in the near term. This information can aid in clinical decision-making on lifestyle and other changes.

Determining pro-NT and pro-ENK identifies risk factors that we can influence



Regular monitoring and screening are ways to guide patients who are concerned about their breast cancer risk. Not only can fears be addressed scientifically but doctors are provided with insight and the necessary information to advise patients about certain lifestyle choices. Taking substantial amounts of painkillers, for example, can lower the amount of enkephalin in the body, effectively reducing the organism's ability to carry out programmed cell death. Biomarkers can be used to independently and reliably predict the risk of breast cancer in women in the menopause.

Baseline fasting plasma samples measured using sphingotest® pro-NT and sphingotest® pro-ENK in independent general populations studies have proven that elevated pro-NT and lowered pro-ENK can indicate the risk of breast cancer.

There is a correlation between nutrition, levels of medication consumption including pain killers, as well as other lifestyle factors and the development of cancer, including breast cancer. Numerous studies aim to further clarify the link between diet and cancer as well as other chronic diseases in order to make more specific recommendations on healthy lifestyles. By detecting the levels of pro-NT and pro-ENK, there is the possibility of identifying the risk resulting from these factors. This gives physicians time to prescribe or adjust HRT therapies, provide advice on lifestyle choices and, if necessary, provide chemoprevention.

If the concentration of proneurotensin and proenkephalin show that there is a higher risk of breast cancer, close check-ups are always recommended: Early detection buys time in the tireless battle against breast cancer and usually increases chances of successful treatment.

Proneurotensin (pro-NT) and proenkephalin (pro-ENK) are the only predictive plasma biomarkers which give evidence on a change in risk when it comes to the development of breast cancer. This has been presented with significant data in various clinical studies. The sphingotest® pro-NT and sphingotest® pro-ENK enable the concentration of proneurotensin and proenkephalin respectively to be determined reliably and quickly.

The detection of pro-NT and pro-ENK takes place in the laboratory using a conventional laboratory immuno-diagnostic practice (Immunoassay). A fasting blood EDTA sample is needed which is simple and easy to handle in the clinical setting.

sphingotec GmbH aims to develop diagnostic methods for prediction, prevention, intervention strategies and early treatment of diseases in the fields of cancer, cardiovascular diseases and acute care. In order to realize this mission we provide plasma biomarkers indicating susceptibility for a specific disease which enable monitoring, prevention and intervention strategies. The company, B.R.A.H.M.S. AG, which was founded by Dr. Andreas Bergmann, developed several innovative biomarkers. Bergmann went on to establish sphingotec which is located in Hennigsdorf. sphingotec has facilities in the Technology Campus, one of the largest industrial parks, populated with several biotechnology companies.

Further information can be obtained here:

sphingotec GmbH
Neuendorfstraße 15A, 16761 Hennigsdorf
Germany
Phone +49 33 02/2 05 65-0

sphingotec, LLC
245 First Street, 18th Floor Cambridge, MA 02142
USA
Phone +1 617-444-8640.

info@sphingotec.com, www.sphingotec.com