Pro-Gastrin-Releasing Peptide (ProGRP) – A Diagnostic Biomarker for Small-Cell Lung Cancer

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1 Background

Lung cancer is the most frequent malignant tumour in men worldwide (Fig 1). In the US, lung cancer is the most frequent cause of death from cancer in men and women. Due to women’s changing smoking habits, lung cancer has now overtaken breast cancer with regard to cancer-related mortality and accounts for around 25% of overall cancer mortality rates in women. In spite of worldwide efforts to improve the diagnosis and therapy of lung cancer, the 5-year survival rate has only improved marginally and, standing at 13%, it is virtually as low as it was 25 years ago.

The prognosis of and the therapeutic approach to lung cancer depends, first and foremost, on the spread of the tumour at the time of initial diagnosis and on the histological classification. Based on their different clinical picture as well as on their sensitivity to chemotherapy and radiology treatments, lung cancers are divided into two large histological subtypes: the non-small cell lung cancers (NSCLC) and the small-cell lung cancers (SCLC). The non-small cell lung cancers make up 75% of all lung cancers and consist primarily of adenocarcinoma, squamous epithelial carcinoma and large-cell carcinoma that are treated in an identical way, which tends to be surgery. Small-cell lung cancers make up 25% of all lung cancers and, due to their frequently occurring neuroendocrine characteristics but also due to their often more advanced and, at times, metastatic stage, they mostly tend to be treated with chemoor radiotherapy. Hence, the histological classification of lung cancers is an essential basis for any therapeutic procedure.

In immuno-histochemistry, neuroendocrine markers such as neuron-specific enolase (NSE), chromogranin A (CGA) and...
Synaptophysin are useful to characterise malignant tumours in a more in-depth way. However, there are differences in the diagnostic effectiveness of each one of these markers. For example, NSE and synaptophysin are also expressed in the tissue of non-small cell lung cancers and other cancer types. Whilst chromogranin A has a higher specificity for the small-cell lung cancer, it is associated with lower sensitivity. For many years, neuropeptides such as bombesin, which is found in amphibians and correlates to the gastrin-releasing peptide (GRP) of mammals, have been known to have a high specificity for lung tissue and are often produced by small-cell lung cancer cells.

In mammals, the immune reactivity of GRP, which is similar to bombesin, has primarily been found in the brain, the lungs, the colon and the neuroendocrine cells of the prostate. Gastrin-releasing peptide was extracted as a peptide with 27 amino acids from the gastrointestinal tract of a pig, and the cDNA of this peptide was cloned on the basis of a human carcinoid tumour of the lungs. The physiological function of GRP is the stimulation of the release of gastrin in the human gastrointestinal tract as well as the vasodilation of the respiratory tract. The expression and release of GRP in small-cell lung cancer has been described by many researchers and has ultimately led to the use of GRP for the immunohistochemical classification of lung tumours. However, the extraction of GRP turned out to be a very complex and labour-intensive affair and appeared too difficult for a routine application in the blood, also and in particular due to the very short half-life of two minutes. It turned out that the pro-gastrin-releasing peptide (ProGRP) was a much more stable precursor of GRP, which can be established in the serum using an ELISA (ALSI, Japan or IBL, Hamburg) based on recombinant ProGRP (31-98).

In comparison with other oncological biomarkers that are relevant for lung cancer such as CEA, CYFRA 21-1 and NSE, ProGRP has not only been found to be released more frequently by the cells of small-cell lung cancers but it has also been found to be superior to other biomarkers with regard to tumour and organ specificity. The great discrimination capacity of ProGRP is based on the fact that ProGRP is, at most, only released in very small quantities in people who suffer from various benign conditions as well as from other malignant tumours (with the exception of medullary thyroid cancer). In addition, the release of ProGRP does not appear to be dependent on the stage of the small-cell lung cancer, which emphasises the diagnostic value of this marker also for the early stages of the tumour. The following data describes the diagnostic significance of ProGRP for small-cell lung cancer.

2 Diagnostic specificity of ProGRP

2.1 Tumor specificity ProGRP is a peptide, which, physiologically, is found in low concentrations in the blood of every human being. Hence, it is not a tumour-specific protein. The concentration of ProGRP is measured in pg/mL and amounts to between 2 pg/mL and 50 pg/mL in healthy people. The median is 20 pg/mL and the 95th percentile amounts to 35 pg/mL.

In people with benign conditions, the median values are between 15 pg/mL and 70 pg/mL while the highest values (up to 350 pg/mL) can be observed in patients with restricted kidney function. This influencing variable must be taken into consideration, in particular if, upon a suspicion of lung cancer, ProGRP is used on patients with renal insufficiency for the purpose of differential diagnosis.

In people with benign gynaecological illnesses (endometriosis, ovarian cysts) the ProGRP levels are comparable with healthy people. Benign conditions of the mammary gland (mastopathy, benign breast tumours, mastitis), the lungs (TB, sarcoidosis, chronic obstructive lung disease, hamartoma, chronic and acute pneumonia, benign pleural effusion, fibrothorax, pleurisy) as well as autoimmune diseases (without involvement of the kidneys) can occasionally lead to a slightly increased ProGRP release of up to 80 pg/mL. Benign gastrointestinal diseases (cirrhosis of the liver, acute and chronic hepatitis, primary biliary cirrhosis, cholelithiasis, cholangitis, acute or chronic pancreatitis, pancreatic cysts, gastritis, ulcerative colitis, Cronh's disease), urological conditions (stones in the urinary tract, infections of the urinary tract, bleeding) and infectious processes (untreated bacterial infections with a clear release of CRP) can lead to ProGRP values of up to 150 pg/mL.

The corresponding threshold values at a specificity of 95% are as follows: healthy people: 35 pg/mL; benign lung conditions: 45 pg/mL; benign gynaecological diseases: 35 pg/mL; benign gastrointestinal conditions: 95 pg/mL; benign urological conditions: 103 pg/mL, benign diseases of the mammary land: 38 pg/mL.

Overall, and as long as kidney function is normal, the tumour specificity of ProGRP at levels of around 150 pg/mL is 100%. (Fig 2)

2.2 Organ specificity and/or diagnostic sensitivity The largest amounts of ProGRP are released in people with small-cell lung cancer. The median value is 250 pg/mL and the 95th percentile is 11 500 pg/mL and over 20% of SCLC patients have ProGRP values that are more than 10 times higher than the reference range for benign lung conditions. According to the relevant literature and depending on the threshold value used and the composition of the patient collectives, the sensitivity of ProGRP for small-cell lung cancer is between 47% and 86%.

In this context, the release of ProGRP is not correlated to the tumour stage. ProGRP is released with a similarly high sensitivity during the "limited disease" stage as during the advanced stage—which represents a clear diagnostic advantage for potential screening tests.
In non-small-cell lung cancer (NSCLC), the median value and the 95th percentile of ProGRP resemble the ones measured in people with benign lung conditions, which confirms the not very pronounced and rare release in people with non-small-cell lung cancer. In spite of this, it is possible that sometimes, even in cases of NSCLC, high ProGRP concentrations of up to >10 000 pg/mL are recorded. In such cases, one needs to take into account that most lung cancers are mixed-cell cancers and that the diagnostic safety of histology depends on the tissue sample as well as the number and localisation of the examined tissue cuts. The release of large amounts of ProGRP indicates at least a partially small-cell component of the tumour suggesting other and better treatment options.

In these cases, it is essential that, in cooperation with the respective pathology unit, a re-evaluation of the relevant tumour tissue is carried out taking into consideration further immunohistochemical approaches for neuroendocrine tumours. The ProGRP medians of all other examined carcinoma are comparable with those of healthy people with the highest measured concentrations (up to 250 pg/mL) being only slightly higher than in the benign control collectives.

If ProGRP release is high, there are a few exceptions, which need to be considered for the purpose of differential diagnosis: In individual cases, it is possible that people with medullary thyroid cancer release large amounts of ProGRP with this release being accompanied by the known CEA or calcitonin release (Fig 2 under "cancers with the exception of lung cancers", Fig 3).

In addition, further neuroendocrinologically active primary tumours such as prostate cancer, ovarian cancer and cancer of the oesophagus can produce a release of the neuropeptide ProGRP. Tests have shown that increased metastazation and androgen independence of prostate cancer cells can lead to a rise in ProGRP concentrations. Furthermore, elevated ProGRP values in metastized prostate cancer have been found to be independent predictors for a shortened response to hormone therapy.

Overall, ProGRP with a specificity of 100% reaches a sensitivity of 53% for the occurrence of small-cell lung cancer. (Fig 3)

3 ProGRP in the differential diagnosis of coin lesions

Due to its great diagnostic significance, a determination of ProGRP levels is always indicated in cases of lung masses—indeed of whether a histological classification based on a biopsy has been carried out or not.

In NSCLC, small amounts of ProGRP are released. With an area under the curve (AUC) of 97%, ProGRP achieves an extraordinarily high discrimination capacity between small-cell and non-small-cell lung cancers. Lung metastases of other, non-small-cell primary tumours lead at most to a small release of ProGRP <100 pg/mL.

A ProGRP release of >200 pg/mL is a strong indicator of the existence of a primary lung cancer and the likelihood of it being small-cell lung cancer is >99%. A ProGRP release of >100 pg/mL in primary lung cancers is independently of the histopathological classification—a clear sign of at least a mixed histology with a small-cell component.

If on the basis of intact kidney function, the ProGRP concentration is >100 pg/mL and the tumour has been histologically classified as NSCLC, the histological findings should be reviewed to ensure that a small-cell component or a neuroendocrine differentiation has not been overlooked. (Fig 4)

4 Comparison with other tumour markers
Of all currently known oncological biomarkers, ProGRP not only has the highest sensitivity but also the highest specificity for small-cell lung cancer. Compared with CEA, CYFRA 21-1, NSE and chromogranin A, ProGRP was found to be superior with regard to released quantity as well as tumour and organ specificity. The great discrimination capacity of ProGRP is due to the fact that the extent of the ProGRP release is very low in benign conditions and that other (non-small-cell and non-neuroendocrine) cancers too lead to either no or only a small release of ProGRP.

The sensitivity of ProGRP for SCLC, which has been described by various authors, varies between 47% and 86%. This means that, in most tests, the sensitivity of ProGRP is higher than for NSE (65% vs 43%) and is comparable with NSE in other publications (47% vs 45%). Neuron-specific enolase is also frequently released in increased quantities in small-cell lung cancers but it is dependent on the stage of the tumour and it also occurs in other cancers, in particular in cancers with liver metastases whatever their histology. Hence, NSE is inferior to ProGRP with regard to organ specificity. On the other hand, due to their different pathophysiological background, ProGRP and NSE have a clear additive effect of 10%–20% as predictive markers of small-cell lung cancer and play a complementary part in the diagnosis and assessment of disease progression.

5 ProGRP for progression monitoring and therapy control

So far, there have only been a few studies on progression monitoring with ProGRP based on large patient collectives. Recently, the relevance of NSE, CEA and ProGRP at the time of recurrence of small-cell lung cancer has been described. According to these findings, at 74% ProGRP has the highest sensitivity with regard to relapse detections (NSE: 32%, CEA: 56%). What is particularly notable, is the fact that all patients where ProGRP was released due to the primary tumour, showed once more an increase in ProGRP release (12% false negative NSE findings, 6% for CEA) at the time of tumour recurrence.

In addition, due to a sensitivity of 67%, ProGRP is the clearest indicator of the progression of small-cell lung cancer (NSE: 20%, CEA: 38%). However, based on a sensitivity of 79%, the combination of ProGRP with NSE has been found to have a significant additive effect. The median of the lead time for the early detection of disease progression was 35 days for ProGRP whilst no lead time was observed for NSE.

6 A short summary of the most important details

Due to its release pattern, ProGRP presents a very rare oncological biomarker with a great diagnostic potential. As the ProGRP release is independent of the tumour stage, this marker could undoubtedly be useful for screening high-risk patients such as smokers. However, if ProGRP is used for this screening application, one should not base its use on the threshold value in comparison with healthy people but one should at least choose a threshold value of 200 pg/mL in order to avoid false positive test results in this asymptomatic situation of supposedly healthy persons. In addition, this test should only be conducted on patients with intact kidney function.

The currently most important field of indication for ProGRP is its determination within the context of the primary diagnosis of lung cancer to support the histological diagnosis or to review the histology or, at an inoperable stage, to establish a diagnosis.

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