Diagnosis of Small Cell Lung Cancer by Pro Gastrin Releasing Peptide (ProGRP)

Diagnostik des kleinzelligen Bronchialkarzinoms mittels Pro Gastrin Releasing Peptide (ProGRP)

K. Yamaguchi[1], Petra Stieber[2]

Summary: ProGRP (Pro Gastrin Releasing Peptide), the mammalian counterpart to the amphibian bombesin, is the more stable precursor of the gut hormone gastrin releasing peptide (GRP) originally isolated from porcine stomach.
When compared to other oncological biomarkers of relevance for lung cancer like CEA, CYFRA 21-1 and NSE, the relatively new marker ProGRP has meanwhile proved not only to be more frequently released by small cell lung cancer (SCLC) cells but also more specific for both the tumor and the organ. The high discriminatory power of ProGRP is based on the facts that ProGRP release and subsequently ProGRP levels are very low in various benign disorders and that even malignant tumors other than SCLC (with the exception of medullary thyroid carcinoma) release minimal amounts of ProGRP, if at all.
Depending on the extent of the disease, 47 to 80 % of small cell lung tumors release ProGRP. Thus, in most publications, the diagnostic sensitivity of ProGRP has been reported to be higher than that of NSE. However, as can be expected from the different pathophysiologic background of ProGRP and NSE, these two analytes have a clear additive sensitivity in SCLC and play complementary roles in the diagnosis of small cell lung cancer.

Keywords: tumor marker; ProGRP; lung cancer; small cell; SCLC.

Zusammenfassung: Bei ProGRP handelt es sich um eine biologische Vorstufe des GRP (Gastrin Releasing Peptide), welches das Korrelat der Säugetiere zum Bombesin der Amphibien darstellt und ursprünglich aus Schweinemägen isoliert wurde.
Beim Vergleich mit anderen, für das Bronchialkarzinom relevanten, onkologischen Biomarkern wie CEA, CYFRA 21-1 und NSE stellte sich heraus, dass ProGRP nicht nur häufiger von Zellen kleinzelliger Bronchialkarzinome freigesetzt wird, sondern auch im Hinblick auf die Tumor- und Organspezifität den anderen Markern überlegen ist. Das hohe Diskriminationsvermögen von ProGRP liegt darin begründet, dass ProGRP im Rahmen der verschiedensten benignen Erkrankungen sowie auch von anderen malignen Tumoren (mit der Ausnahme des medullären Schilddrüsenkarzinoms) höchstens in sehr geringen Mengen freigesetzt wird.
Je nach Ausdehnung der Tumorerkrankung setzen 47 bis 80 % der kleinzelligen Bronchialkarzinome ProGRP frei. Somit geht aus den meisten Publikationen hervor, dass ProGRP der Neuronspezifischen Enolase NSE überlegen ist, aber wie schon der unterschiedliche pathophysiologische Hintergrund vermuten lässt, haben diese beiden Teste eine klare additive Empfindlichkeit und ergänzen sich in der Diagnostik des kleinzelligen Bronchialkarzinoms.

Schlüsselwörter: Tumormarker; ProGRP; Bronchialkarzinom; kleinzellig; SCLC.

On a worldwide basis, lung cancer is the most frequent and most deadly malignancy with continuously growing incidence. Despite many efforts to improve diagnosis and therapy, the 5-year survival rate of lung cancer patients increased only slightly over the last decades and is now around 13 %. The prognosis and therapeutical concept in lung cancer depends mainly on the extension of the tumor and its histological type. Lung cancer is classified as small cell lung cancer (SCLC), squamous cell carcinoma, primary adenocarcinoma and large-cell carcinoma. Small cell lung cancer accounts for 20 to 25 % of the new cases of bronchogenic carcinoma and differs clinically and biologically due to its neuroendocrine differentiation from the other histological types, which behave similarly concerning prognosis and therapy and are thus pooled into one group as non-small-cell lung cancer (NSCLC). Since the incidence of distant metastases at the time of primary diagnosis is high in SCLC and these tumors are, in contrast to non small cell lung carcinomas (NSCLC), very sensitive to chemotherapeutic reagents and radiotherapy, primary systemic therapy plays an important role in the management of these patients.

Neuroendocrine markers like Neuronspecific Enolase (NSE), Chromogranin A (CGA), Synaptophysin,

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and Neural Cell Adhesion Molecule (NCAM) are helpful when used in immunohistochemistry in characterising malignant lung tumors. The diagnostic accuracy for SCLC is different for each of these molecules. Similarly, NCAM, NSE and Synaptophysin are also expressed in non small cell lung tumors and other primary tumors and Chromogranin has a higher specificity but a low sensitivity for SCLC. Neuropeptides like bombesin, which is the amphibian counterpart of the mammalian gut hormone Gastrin-releasing peptide (GRP) are known to have a superior specificity for the lung and to be often produced by cells of SCLC [1]. In addition, GRP is described to stimulate the growth of SCLC cells and to support the metastatic process by cell to cell interactions.

When these neuroendocrine molecules and neuropeptides are released into the blood of patients suffering from SCLC, they might serve as serum markers of this malignant disease. Thus, NSE has been regarded for many years as the marker of first choice for monitoring the course of this disease and also supporting diagnosis at time of primary presentation. Chromogranin A and NCAM were found to have a low sensitivity in serum as compared to NSE. Although GRP was already described and isolated for the first time in 1978 [2], it was not possible to measure this molecule in blood in a fast and reproducible way due to its extreme instability (half life period, about 2 minutes). Therefore, recombinant ProGRP (31–98), a carboxy-terminal region common to three types of previously cloned human ProGRP-molecules, was synthesized, so that first a radioimmunoassay [3] and finally an ELISA [4] could be developed for the measurement of this more stable precursor of GRP in serum. Initial investigations [3–6] revealed a high specificity and sensitivity of ProGRP in serum for small cell lung cancer, although GRP was described to be present in tumor tissue extracts of NSCLC at a frequency of 17 %. These findings point to a diagnostic capacity of ProGRP at the time of primary diagnosis of SCLC which can only be performed on the basis of a high overall specificity. To obtain an impression of the general pattern of release of ProGRP, we investigated – following the recommendations of the EGTM (European Group on Tumor Markers) – this new marker in a broad variety of benign disorders including influencing factors and other malignant diseases than lung cancer.

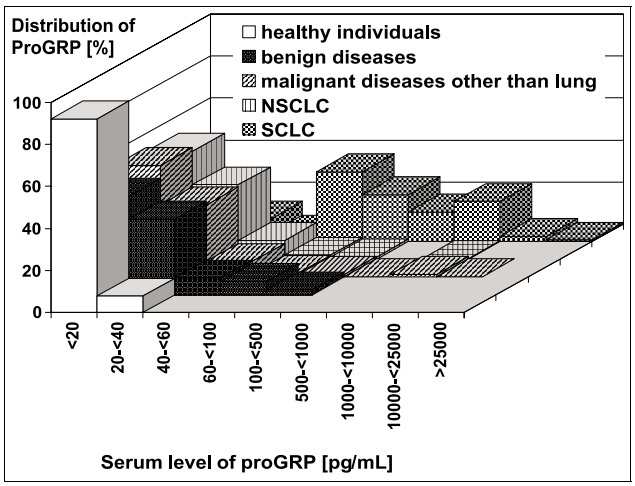

Figure 1 Frequency distribution of ProGRP in healthy individuals, various benign diseases, malignant diseases other than lung cancer, non small cell lung cancer, small cell lung cancer.

Diagnostic specificity of ProGRP (Table 1, Fig. 1)

Tumorspecificity

The values of ProGRP in *healthy individuals* are described to range from 1 to 75 pg/mL. The median of our own investigations is around 10 pg/mL, the 95th percentile about 20 pg/mL.

In *benign diseases* (n = 477), the medians vary between 10 and 68 pg/mL, we observed the highest single values up to 310 pg/mL in patients with renal failure. This influencing factor has already been described earlier and must be taken into consideration when ProGRP is used for diagnostic purposes in patients suspected of tumor disease and suffering from chronic or acute renal failure.

Patients suffering from various benign gynecological disorders (endometriosis, ovarian cysts) have similar levels like healthy individuals. Benign disorders of the breast (mastopathy, benign tumors, mastitis), the lung (sarcoidosis, TB, chronic obstructive pulmonary disease, hamartoma, chronic and acute pneumonia, benign pleural effusions, fibrothorax, pleurisy) as well as autoimmune diseases (without renal involvement) cause only very slightly elevated levels, up to 80 pg/mL. Benign gastrointesinal disorders (liver cirrhosis, acute or chronic hepatitis, primary biliary cirrhosis, cholelithiasis, cholangitis, acute or chronic pancreatitis, pancreatic cysts, gastritis, colitis ulcerosa and Crohn's disease), urological diseases (stones, urinary tract infections, bleedings) and infectious diseases (mainly bacterial infections with significant CRP-release before therapy) showed somewhat higher 95th percentiles and reached ProGRP-values up to 120 pg/mL.

The respective cut-off values at 95 % specificity were calculated as follows: healthy individuals: 22 pg/mL; benign lung dis. 55 pg/mL; benign gynecological dis. 33 pg/mL; benign gastrointestinal dis. 95 pg/mL, benign urological dis. 103 pg/mL; benign breast dis. 38 pg/mL.

Organspecificity

ProGRP is obviously not released into the circulation by other malignant tumors (all at time of primary diagnosis, without therapy) than lung cancer. The medians of the different groups investigated are almost comparable to those of healthy individuals, the highest values

Table 1 Distribution of ProGRP in healthy individuals, various benign and malignant diseases including lung cancer

Group	N =	mean [pg/mL]	median [pg/mL]	95 % percentile	range [pg/mL]	sensitivity at 95 % specificity
healthy individuals	90	10.3	10.1	22	1.0–30	
ben. lung dis.	209	21.0	19.0	55	1.0–83	
ben. urol. dis.	51	43.4	31.5	103	1.0–139	
renal insufficiency	16	87.9	68.0	313	2.0–313	
BPH	49	41.3	36.3	88	3.7–96	
ben. gyn. dis.	49	10.5	10.6	23	1.0–33	
ben. gastro. dis.	65	22.5	20.4	52	2.7–119	
ben. breast dis.	31	24.1	23.9	39	8.2–45	
autoimmune dis.	30	24.2	22.3	52	7.4–57	
infectious dis.	23	41.9	31.3	119	9.4–119	
colorectal cancer	51	18.1	12.9	54	1.0–101	8
pancreatic cancer	51	12.8	11.7	26	1.0–55	2
stomach cancer	51	14.6	12.3	38	1.0–58	2
hepatocell. cancer	50	17.3	12.2	48	1.7–82	6
breast cancer	53	17.8	15.2	47	1.4–64	6
ovarial cancer	50	17.9	12.5	35	1.0–236	18
prostate cancer	51	30.2	29.0	58	5.7–93	2
bladder cancer	34	28.5	29.1	44	4.1–48	0
renal cancer	41	27.7	27.5	52	2.3–72	0
med. thyroid gland Ca	24	1461	27.7	17939	2 –21655	25
all lung cancer	638	321	20.0	1153	1.0–33099	17
NSCLC	493	63	19.0	52	1.0–14432	5
SCLC	145	1304	179	7489	1.0–33099	64

reached (about 240 pg/ml) are even lower than those in benign diseases. But there is one important exception that has to be noted: ProGRP might – in rare cases – reach very high levels in medullary thyroid carcinomas. This fact, announced in 1984 by Ken Yamaguchi et al. on the basis of tissue investigations, was published in 2000 [7] and can be confirmed by us based on single experiences in routine application of ProGRP.

In non small cell lung cancer, the median and 95th percentile of ProGRP is the same as for benign lung diseases, confirming that there is almost no release in these tumors. But in single cases of NSCLC, there might be a significant release of ProGRP up to 10.000 pg/mL. One has to be aware of the fact that the „gold standard“ histology does certainly not always reach 100 % and that the diagnostic accuracy of histology depends on the number of tumor sections made. In small cell lung cancer, the median (179 pg/mL) and 95th percentile (7498 pg/mL) are significantly elevated, more than 20 % of SCLC patients exhibit serum ProGRP values exceeding 10-fold the cut off for benign lung diseases.

Diagnostic sensitivity and specificity/ sensitivity profile of ProGRP

Depending on the cut-off value and the distribution of tumor stages, ProGRP is described in the literature to possess a sensitivity between 47 % and 86 % [1, 5, 6, 8, 9, 10] for small cell lung cancer. In the present investigation, sensitivity is 64 % based on a specificity of 95 % for benign lung diseases. The ProGRP-release seems not to correlate with tumor stage, ProGRP is already released in „limited“ small cell lung cancer with almost the same high sensitivity as in extended disease. The corresponding „false positive“ rates in non small cell lung cancer are between 0 and 5 %.

The overall profile of specificity and sensitivity for all cancers (Fig. 2) as compared to the organ-related corresponding benign diseases shows a high diagnostic capacity for ProGRP in small cell lung cancer: at a specificity of 100 % (for benign lung diseases) a sensitivity of almost 60 % for SCLC remains.

The diagonal ROC curves, obtained for all other cancers investigated, demonstrate again the high specificity

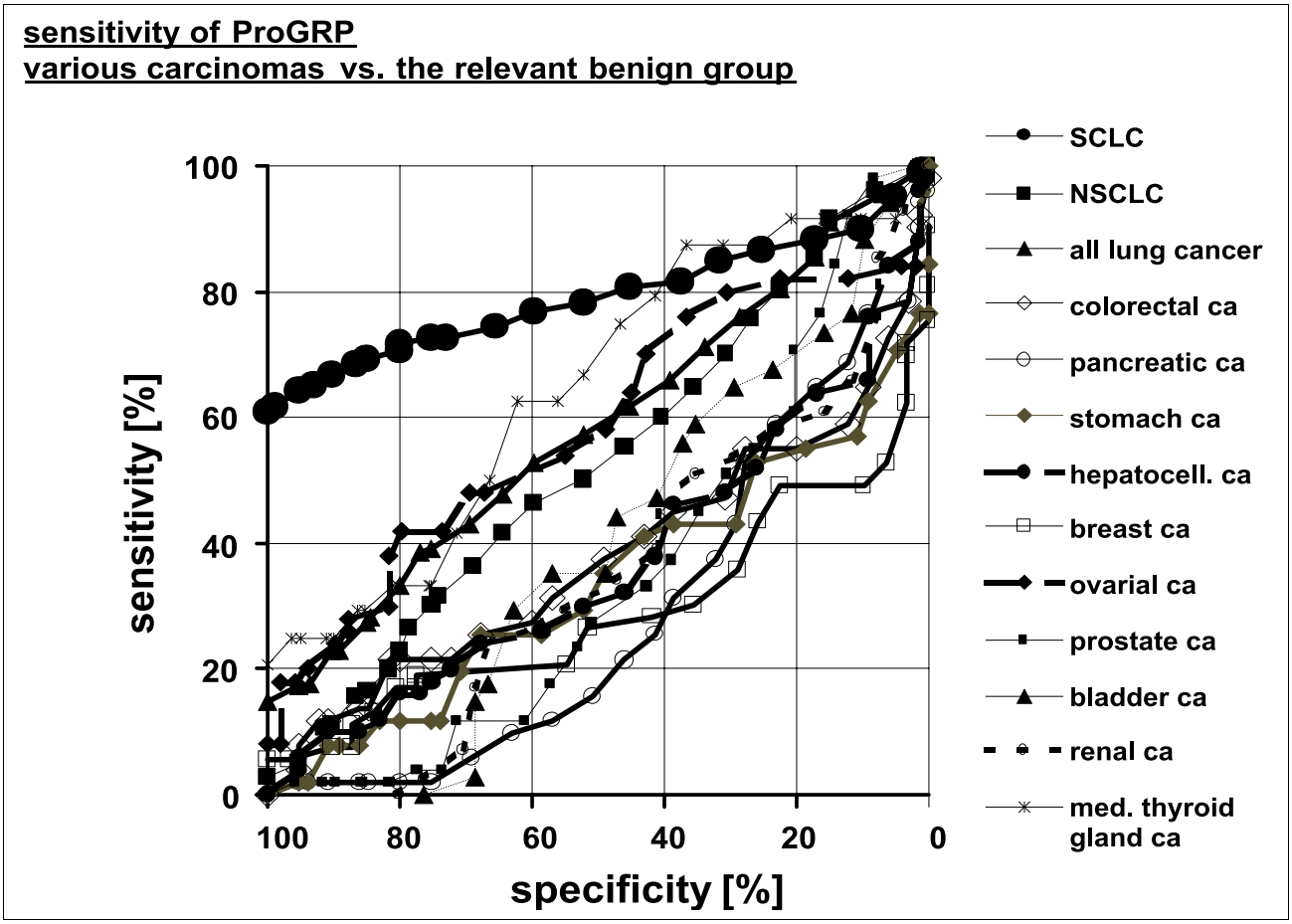

Figure 2 ROC-curves for various cancers and their corresponding organ related benign diseases (N = 1843).

of ProGRP for small cell lung cancer and its negligible release by other malignant tumors.

Comparison with other oncological biomarkers

ProGRP as a single marker has a high sensitivity in small cell lung cancer as compared to benign diseases of the lung. When compared to CEA, CYFRA 21-1, NSE and Chromogranin A, ProGRP has meanwhile proved to be superior in tumor as well as organ specificity [6, 9]. The high discrimination of ProGRP is due to the fact that ProGRP levels and extent of release are very low in various benign disorders and that even malignant tumors other than SCLC (with the exception of medullary thyroid carcinoma) release only smallest amounts of ProGRP, if at all.

The sensitivities reported by several authors for small cell lung cancer range from 47 % to 86 %, these different results depend, at least partly, on the composition of the patient group investigated and on the different cut-off values used. The diagnostic sensitivity of ProGRP is in most of the publications higher than (65 % versus 43 %) and in a few publications comparable to NSE (47 % to 45 %). But, as expected due to the completely different pathophysiologic background of ProGRP and NSE, these two analytes have a clear additive sensitivity of 10 to 20 % in SCLC and play complementary role in the diagnosis and management of small cell lung cancer [6, 8].

Differentiation of lung tumors of unknown origin by ProGRP (Fig. 3)

In those cases where, for various reasons, biopsy cannot be performed or histology fails to differentiate between the different types of lung cancer, ProGRP might support diagnose finding.

According to our investigations in NSCLC, the release of ProGRP is reduced to a minimum of 3 % (NSE up to 26 %). Also, in serum of patients with lung metastases due to other primaries, there is only a minor ProGRP release, up to 100 pg/mL. In small cell lung cancer, high serum levels of ProGRP are often measured which can considerably exceed NSE values. A ProGRP-release > 500 pg/mL is indicative for primary lung cancer and is due with a probability of 99 % to small cell lung cancer. A ProGRP-release of > 200 pg/ml in primary lung tumors is, regardless of the pathohistological classification, highly suspicious of at least a mixed histology with a small cell component. Lamy et a.l confirmed our findings

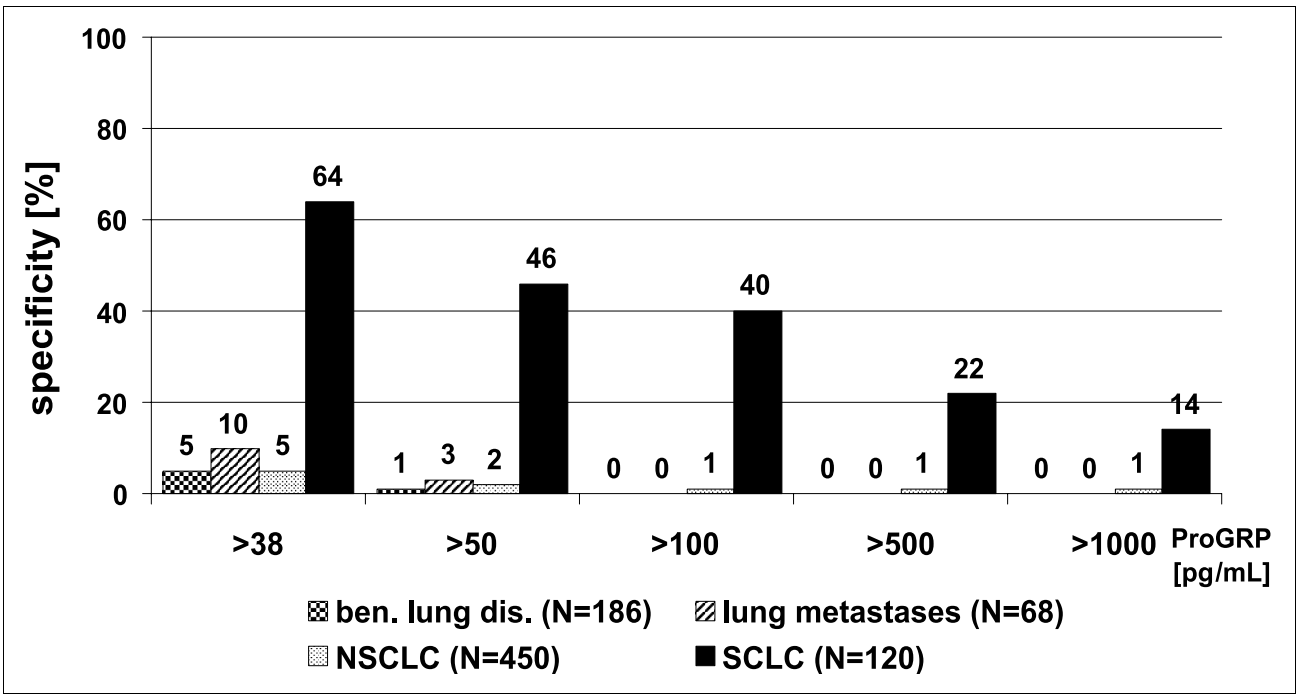

Figure 3 Optimizing the ProGRP cut-off values to reach higher specificity for SCLC.

concerning the high diagnostic ability to discriminate between NSCLC and SCLC, he found an AUC of 0.97 for ProGRP and 0.95 for NSE [9].

ProGRP in follow-up care and therapy control

Few data exist to date regarding this important medical indication. In SCLC, the relevance of NSE, CEA and ProGRP in recurrent disease has very recently been reported [10]. It could be stated that ProGRP revealed with 74 % sensitivity the highest detection rate of recurrent disease (NSE: 32 %, CEA: 56 %). It is especially important to note that in none of the patients with ProGRP-release before first treatment was ProGRP negative at time of recurrent disease (12 % NSE and 6 % CEA). ProGRP reflected with 67 % the disease course of patients with SCLC most accurately of these three markers (NSE: 20 %, CEA: 38 %). Nevertheless, there was a clear additive effect up to 79 % sensitivity combining ProGRP and NSE. The median lead time for the detection of recurrent disease was 35 days for ProGRP, no lead time could be found for NSE.

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