ANTICANCER RESEARCH *25*: 1773-1778 (2005)

Pro-gastrin-releasing Peptide (ProGRP) in Patients with Benign and Malignant Diseases: Comparison with CEA, SCC, CYFRA 21-1 and NSE in Patients with Lung Cancer

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Abstract. *We studied the specificity and sensitivity of pro-gastrin releasing peptide (ProGRP) in 37 healthy subjects and 195 patients with benign and 149 with malignant diseases other than lung cancer. Likewise, we compared the ProGRP with other tumor markers used in lung cancer (CEA, SCC, CYFRA and NSE) in 187 patients with NSCLC and in 66 SCLC patients. We considered 50 pg/ml, 5 ng/ml, 2 ng/ml, 3.3 ng/ml and 20 ng/ml as the upper limits of normality for ProGRP, CEA, SCC, CYFRA 21-1 and NSE, respectively. Abnormal ProGRP serum levels were found in 10% of patients with benign diseases and in 13% of patients with malignancies other than lung. Renal failure was the main source of false-positive results (51.6%). Slightly raised ProGRP serum levels, excluding renal failure, were found in 4.1% of patients with benign diseases (<80 pg/ml) and in 5% of patients with malignancies other than lung cancer or neuroendocrine tumors (<120 pg/ml). Abnormal levels of ProGRP, NSE, CEA, CYFRA and SCC were found in 30%, 22.5%, 55.6%, 65.2% and 26.7% of NSCLC and in 73%, 64%, 53%, 46% and 4.5% of SCLC, respectively. Tumor marker serum levels were related to histological type and tumor extension, with ProGRP being the most sensitive marker in SCLC, CEA in adenocarcinomas and CYFRA 21-1 in squamous tumors. The most sensitive combinations of tumor markers were ProGRP and NSE in SCLC (88%), and CEA plus CYFRA in NSCLC (82%). In summary, ProGRP is the tumor marker of choice in SCLC and NSE is a complementary tumor marker in this histological type.*

Lung cancer histological types are categorized into two groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), both with different treatments and prognosis. SCLC is an aggressive neoplasm of rapid growth, with metastatic lesions in regional lymph nodes or distant organs at the time of diagnosis, but with high sensitivity to chemotherapy and radiotherapy. NSCLC is comprised of three major histological subtypes, adenocarcinoma, squamous cell carcinoma and large cell carcinoma, in which surgery is the only treatment to achieve a possible cure.

Tumor markers have been extensively studied in lung cancer, but no specific marker has been identified for this malignancy. The carcinoembryonic antigen (CEA), SCC and cytokeratins (CYFRA 21-1, TPA and TPS) have been extensively studied in NSCLC and neuron-specific enolase (NSE) in SCLC (1-5). One of the problems with these tumor markers is the lack of lung cancer specificity with abnormal levels being found in other malignancies (6, 7). Another problem is their insufficient sensitivity and that a combination of two or three tumor markers must be used to obtain acceptable sensitivity (1-8). Furthermore, there is no clear relationship between some of these tumor markers and the histological type. CEA serum levels are significantly higher in adenocarcinomas and CYFRA concentrations are higher in squamous cell carcinoma, but it is possible to find patients with abnormal levels of these markers in other histological types, including SCLC (1-7, 9, 10). NSE is the tumor marker of choice in SCLC, being useful mainly in disease, therapy monitoring and prognosis (5, 10-13). However, its low sensitivity, particularly in patients with limited disease (LD), has led to its use in combination with other tumor markers such as CEA and CYFRA 21.1, which are not as specific for SCLC (4, 6, 9). Likewise, slightly raised NSE serum levels are found in about 10-20% of NSCLC (1-4). To improve tumor marker sensitivity and specificity, other markers have been studied in SCLC with the pro-gastrin-releasing peptide (ProGRP) being the most promising (14-16). Preliminary results have shown the utility

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Key Words: Small cell lung cancer, tumor marker, ProGRP, NSE, lung cancer, CEA, CA 125, CYFRA 21-1, SCC.

Table I. *Serum tumor marker levels in healthy subjects and patients with benign diseases.*

	No. patients	% >50 pg/ml	Mean±SD pg/ml	Range pg/ml
Healthy	37	0%	25.6±10	8-49
Non infectious lung	39	4 (8.4%)	29.2±17.7	7-90
Infectious lung	31	0%	26.0±11.1	7-47
Digestive tract	26	1 (3.8%)	22.8±19	7-96
Heart diseases	21	0%	17.5±9	7-37
Liver cirrhosis	25	0%	22.8±14	7-48
Acute hepatitis	8	1 (12.5%)	18.8±14.6	8-54
Acute renal failure	5	1 (20%)	25.0±16.1	12-52
Chronic renal failure	23	12 (51.6%)	57.4±32.6	21-145
Others	17	1 (%)	21.3±17.2	8-65
Total benign	195	20 (10%)	28.0±21.7	7-145

Table II. *ProGRP serum levels in patients with malignancies other than lung cancer.*

	No. patients	% Pro GRP >50 pg/ml	Mean±SD pg/ml	Range pg/ml
Breast	30	1 (3%)	16.2±13	7-55
Gynecological cancer	15	1 (7%)	18±14	7-61
Colorectal	17	0%	19±11	7-50
Pancreatic	12	0%	17±8	7-31
Primary liver cancer	22	2 (9%)	25.4±29	7-137
Neuroendocrine	5	2 (40%)	105±144	8-355
Prostate	10	3 (30%)	34.7±32	7-88
Other epithelial tumors	13	3 (23%)	43.7±33	10-115
Melanoma	10	1 (10%)	21±21	8-79
Hematological malignancies	15	4 (27%)	33.4±33	7-93
Total	149	17 (12%)	27±36	7-355
Total locoregional	44	3 (7%)	22±17	7-93
Total metastatic (stage IV)	105	14 (13%)	29±41	7-355

of ProGRP in the follow-up of SCLC patients (11, 12, 14, 16). Likewise, abnormal levels of ProGRP have been described in a low proportion of NSCLC, but at significantly lower concentrations (11, 12, 14). Moreover, in relation to benign or malignant diseases other than lung disease, the specificity of ProGRP has been little studied (14).

The aims of this study were: i) to evaluate ProGRP serum levels in patients with benign and malignant diseases, to determine the utility of this marker in the differential diagnosis of lung cancer, specially SCLC; ii) to compare ProGRP with other tumor markers used in patients with lung cancer (CEA, CYFRA 21-1, SCC and NSE), to evaluate their sensitivity and specificity alone or in combination and their utility in the histological diagnosis of lung cancer.

Materials and Methods

ProGRP was studied in 37 healthy subjects (39.6±10.4 years), in 195 patients with benign diseases (61.6±15.5 years) and in 402 patients with active malignant diseases (63.6±12.2 years). Patients with benign diseases included 26 patients with gastrointestinal diseases (9 pancreatitis, 5 peptic ulcer, 12 with other diseases), 25 patients with liver cirrhosis, 8 patients with acute hepatitis, 28 patients with renal failure (5 acute renal failure), 70 with respiratory tract diseases (31 infectious), 21 with cardiac diseases and 17 with other infectious benign diseases. CEA, SCC, NSE and CYFRA were studied in the subgroup of patients with lung cancer.

The staging of patients with cancer was made according to the recommendations of the UICC (17), the Veterans Administration Lung Cancer Group A Staging System (18) and the International Federation of Gynaecology and Obstetrics (19). Patients with cancer included: 187 patients with NSCLC (108 stage I-III, 79 metastatic patients), 66 patients with SCLC (26 limited disease, 40 extensive disease), 17 patients with metastatic colorectal cancer, 12 patients with pancreatic cancer (5 without metastases, 7 with metastases), 22 patients with primary liver cancer (20 without metastases, 2 with metastases), 30 patients with metastatic breast cancer, 9 patients with ovarian cancer stage IV, 6 patients with cervical uterine malignancy (2 stage I-II, 4 stage III-IV), 10 with metastatic prostatic cancer, 15 patients with hematologic malignancies (11 stage I-II, 4 stage III-IV), 10 patients with stage IV malignant melanoma, 5 with advanced neuroendocrine malignancies and 13 with other epithelial malignancies (7 with metastases).

Serum samples were obtained by venous puncture and centrifuged and stored at –70˚C until assayed. Tumor markers were measured by a commercial ELISA procedure using a Imx for SCC (ABBOTT Laboratories, Chicago, IL, USA) and a autonanalyzer Elecsys for CEA, CYFRA 21-1 and NSE (Roche Diagnostics, Germany). We considered 5 ng/ml, 3.3 ng/ml, 2 ng/ml and 20 ng/ml as the upper limits of normality for CEA, CYFRA, SCC and NSE, respectively. ProGRP was determined by a commercial sandwich ELISA (Tonen Corporation, Tokyo, Japan). We considered 50 pg/ml as the upper limit of normality.

The standard measures of diagnostic test validity such as sensitivity, specificity, and predictive values accompanied by confidence intervals of 95% were calculated for varying cut-off levels of tumor markers. The comparison of tumor marker distribution between subgroups in the study populations was based on non-parametric rank tests (the Wilcoxon-Mann-Whitney *U*-test for two groups, the Kruskal-Wallis test for three groups) and parametrial test (Student's *t*-test).

Results

Table I shows the tumor marker concentrations found in healthy subjects as well as in patients with benign diseases. None of the healthy individuals had abnormal levels (>50 pg/ml) of this tumor marker. In contrast, 10% of the patients with benign diseases showed abnormal ProGRP serum levels (>50 pg/ml). The highest proportion of false-positive results was found in patients

Table III. *Tumor marker serum levels in NSCLC patients, subdivided according to tumor extension.*

	Total Sq	Sq Mo	Sq M1	Total Adeno	Adeno Mo	Adeno M1	SCLC	NSCLC	NSCLC Mo	NSCLC M1
No. patients	84	62	22	71	31	40	9	23	9	14
CEA >5 ng/ml	45.2%	40.4%	59.1%	76.1%	67.7%	82.5%	44.4%	34.7%	33.3%	35.7%
Mean±SD ng/ml	14.6±35	10.7±17.6	25.7±62.6	108±438	26.2±50	171±577	22.4±35.3	44±93	21±47	58±113
CYFRA 21.1 >3.3 ng/ml	75%	69.3%	90.9%	60.6%	51.7%	67.5%	66.7%	43.4%	44.4%	42.8%
Mean±SD ng/ml	15.5±21.8	12.9±19	22.3±27	8.6±12.6	6.9±11.3	9.8±13.6	4.8±3.2	6.5±6.6	6±7,1	6.8±6.4
SCC >2 ng/ml	47.6%	42%	63.6%	11.3%	6.5%	15%	22.2%	0%	0%	0%
Mean±SD ng/ml	6.6±16.3	5.9±17.9	8.3±10.1	1±1	0.8±0.6	1.3±2.3	1.4±1	0.9±0.5	1±0.5	0.8±0.6
NSE >20 ng/ml	19.1%	17.7%	22.7%	26.8%	9.7%	20%	11.1%	26.1%	11.1%	35.7%
Mean±SD ng/ml	16±8	15±8	17+ 10	16±8	15±8	17±9	14+ 10	15±8	13±6	17±9
ProGRP >50 pg/ml	35.7%	33.9%	40.9%	22.5%	29%	17.5%	33.3%	30.4%	44.4%	21.4%
Mean±SD pg/ml	52.3±55	47.4±44.1	65.4±79	37+ 29.5	40.7±36	33.7±22.8	60±74	36±19	37±20	35±19

Sq: Squamous; Adeno: Adenocarcinoma; Mo: Stages I-III; M1: Stage IV

with renal failure in whom ProGRP concentrations were significantly higher than those observed in other benign diseases ($p=0.0001$). It is interesting to point out that 3 of the 7 patients with benign diseases other than renal failure and with abnormal ProGRP serum levels had abnormal creatinine values (>1.3 mg/dl).

Abnormal ProGRP serum levels were found in 13% of patients with malignancy other than lung, with no relationship to tumor extension (Table II). The highest ProGRP concentrations were observed in patients with neuroendocrine tumors. Interestingly, abnormal creatinine levels were found in 8 out of the 17 (47%) patients with ProGRP positivity and malignancies other than lung cancer. Excluding patients with renal failure or neuroendocrine tumors, slightly elevated (<120 pg/ml) ProGRP concentrations were found in only 7/136 (5%) patients.

Table III shows the tumor marker concentrations in patients with NSCLC subdivided according to tumor stage and histology. Significantly higher serum levels of CYFRA ($p=0.017$), SCC (0.002) and ProGRP ($p=0.031$) were found in squamous and CEA ($p=0.03$) in adenocarcinoma tumors. Similar results were obtained (excluding ProGRP) when comparing only patients without metastases (CYFRA $p=0.041$; SCC $p=0.031$; CEA $p=0.031$) or with metastases (CYFRA $p=0.02$; SCC $p=0.0001$; CEA $p=0.07$; ProGRP $p=0.021$).

Table IV shows the tumor marker concentrations in SCLC patients, subdivided according to tumor extension. ProGRP and NSE are the tumor markers with the highest sensitivity and serum concentrations in relation to the normal cut-off. By contrast, SCC showed the lowest sensitivity, with low (<4.3 ng/ml) and unusual positivity (only 5 patients). Tumor marker concentrations seemed to be higher in patients with extensive disease (ED) than in those with LD (excluding SCC), but these differences were only significant with NSE ($p=0.021$) and ProGRP ($p=0.045$). Higher sensitivity, as well as mean concentrations in relation to the cut-off, were found with ProGRP compared to NSE, mainly in patients with LD (Table IV). On comparing patients with NSCLC and SCLC (Tables II and III), significantly higher NSE ($p=0.001$) and ProGRP ($p=0.0001$) concentrations were found in SCLC and SCC ($p=0.005$), and CYFRA (0.041) in NSCLC.

The relationships among tumor markers and the histological type suggests their possible utility as an aid in histological diagnosis. Table V shows the probability of SCLC, using different cut-off points for ProGRP and NSE. The higher the levels of NSE and /or ProGRP, the higher the probability of SCLC. The combination of ProGRP and NSE showed abnormal levels of one tumor marker or another in 37/40 (92.5%) patients with ED and in 21/26 (80.8%) patients with LD. The inclusion of other tumor markers only slightly increased the sensitivity, because only one patient with LD had abnormal levels of CEA and CYFRA and another with ED had abnormal SCC, with ProGRP and NSE negative.

CYFRA 21-1 was the most sensitive marker in squamous tumors (69% in patients without metastases and 90.9% in patients with metastases). The addition of SCC slightly increased the sensitivity of CYFRA 21-1: 6.4% in patients without metastases and 0% in those with metastasis. The addition of CEA to these two tumor markers increased the sensitivity up to 80.6% in patients without metastases and 95.4% in those with advanced disease. CEA was the most sensitive tumor marker in adenocarcinomas (Table IV), and the addition of CYFRA increased the sensitivity up to 80.6 % in patients without metastasis and to 92.5% in those with metastases. The inclusion of SCC did not increase the sensitivity obtained using CEA and CYFRA 21-1.

Table IV. *Tumor marker serum levels in patients with SCLC.*

	Total	LD	ED
No. patients	66	26	40
CEA>5ng/ml /total	53%	42%	60%
Mean±SD	40±123	27±76	49±147
CYFRA 21.1 >3.3 ng/ml	46%	27%	58%
Mean±SD	7±15	4±5,6	9±18
SCC >2 ng/ml	4.5%	4%	5%
Mean±SD	0.8±0.6	0.7±0.7	0.8±0.6
NSE >20 ng/ml	64%	46%	73%
Mean±SD	82±151	39±60	110±184
ProGRP >50 pg/ml	73%	65%	78%
Mean±SD	598±1448	286±423	799±1809

Table V. *Probability of SCLC according to NSE and/or ProGRP serum levels*

Probability	Total lung cancer/ % of SCLC	Total lung cancer/ % of LD SCLC	Total lung cancer/ % of ED SCLC
NSE >30 ng/ml	43 (74.4%)	13 (61.5%)	30 (80%)
NSE>35 ng/ml	30 (90%)	8 (75%)	22 (96%)
NSE >40 ng/ml	26 (100%)	6 (100%)	20 (100%)
ProGRP >150 pg/ml	36 (86%)	12 (83%)	24 (87.5%)
ProGRP >200 pg/ml	31 (93.5%)	9 (100%)	22 (91%)
ProGRP >300 pg/ml	26 (100%)	8 (100%)	18 (100%)
NSE >30 and ProGRP >125	16 (100%)	3 (100%)	13 (100%)
NSE >35 and/or ProGRP >150	52 (85%)	17 (76%)	34 (91%)
NSE >40 and/or ProGRP >150	45 (89%)	15 (87%)	30 (90%)
NSE >40 and/or ProGRP >300	41 (100%)	12 (100%)	29 (100%)

Discussion

ProGRP has been studied in patients with lung cancer, and several authors have suggested that it may be useful in the differential histological diagnosis of these patients (12, 14). Likewise, the specificity of ProGRP in relation to benign lung diseases seems to be higher than 95% (12, 14, 16). However, few studies have been undertaken on the specificity of ProGRP in other benign or malignant diseases (14). This point is important because according to the data published to date, abnormal ProGRP concentrations only suggest lung cancer. Other tumor markers, with theoretically high sensitivity show abnormal false positive results in diseases not related to the organ where the tumor appears such as SCC and S-100 in renal diseases, CA 125 in endometriosis or effusions, SCC in dermatological disorders, etc (6, 8, 20-25). Likewise, tumor markers described to have organ specificity such as, for example, CA 15.3, S-100, CA 125 or CA 19.9 are produced for other malignancies (2, 6, 8, 24, 25). The knowledge of false positives with these markers will better facilitate more appropriate application.

Abnormal ProGRP concentrations were found in 13% of our patients with benign diseases, indicating that the positivity of this tumor marker is not specific for cancer. As occurs with other tumor markers, renal failure was the most frequent source of ProGRP false positive results (24, 25). These results suggest caution must be take in the evaluation of ProGRP during chemotherapy treatment and results should not be evaluated when creatinine levels are increased. ProGRP specificity is high in patients without malignancy, when renal failure is excluded. We found only slight (<80 pg/ml) increases in 4% of our patients, including 33 patients with liver diseases which are frequently associated with abnormal tumor marker levels (8, 23, 25). These results are similar to those reported by other authors in lung diseases (14, 16, 26). ProGRP specificity in relation to malignancies other than lung cancer was also high with only slightly raised levels (<120 pg/ml) in 5% of the patients, when patients with neuroendocrine tumors or with renal failure were excluded. Stieber *et al.* (26) and Inaji *et al.* (27) have also reported abnormal ProGRP levels in patients with neuroendocrine tumors. These results suggest that ProGRP may be a tumor marker for neuroendocrine tumors, as has been described with NSE. However, further studies are required to evaluate this possibility.

The use of ProGRP has been suggested in the differential diagnosis of lung cancer. Several authors have reported that abnormal ProGRP values strongly indicate SCLC (11, 12, 14, 16, 26, 27). In our experience, 30% of NSCLC and 73% of SCLC had abnormal ProGRP values. Others authors have reported similar sensitivities in SCLC, as well as its relationship to tumor stage (11, 12, 14, 16, 28). In contrast, the sensitivity of ProGRP in our NSCLC population was higher than that reported by other authors (14, 16, 26, 28). However, Takada *et al.* (28) found abnormal levels in 14% of 111 NSCLC, although with a lower cut-off of 34 pg/ml. The reasons for these discrepancies are difficult to determine when all the laboratories use the same commercial technique, with a similar cut-off value and obtain a similar sensitivity in SCLC. Our population of NSCLC included a high proportion of patients with metastatic disease or patients with locally advanced locoregional tumors (stage III) and tumor markers are habitually related to tumor extension. Most of the published articles do not indicate the tumor stage of the NSCLC patients studied, since their main goal was to evaluate ProGRP values in SCLC. Another possible explanation may

be that our patients had a higher proportion of renal failure, which is the main source of ProGRP false-positive results. In 21% (12/56) of our NSCLC patients with abnormal ProGRP values, renal failure was observed, but on exclusion of these patients, the sensitivity of ProGRP was 26% (44/170), superior to that described by other authors (14-16, 26, 28-30).This should be confirmed in additional studies. However, the interesting point is that ProGRP serum levels in NSCLC were significantly lower than in SCLC, as indicated by all the studies. It has been previously mentioned that ProGRP concentrations in other malignancies were lower than 120 pg/ml, excluding renal failure. Most NSCLC patients had slightly high ProGRP levels and only 4% (excluding renal failure) had levels higher than this cut-off point.

NSE has been the tumor marker of choice in SCLC and it is useful in the diagnosis, prognosis and follow-up of these patients (5, 12, 13, 31, 32). However, its low sensitivity, mainly in patients with LD, has led to its use in combination with other tumor markers such as CEA and CYFRA 21.1, which are not as specific for SCLC. Another problem with NSE is its presence in platelets and erythrocytes, that makes it necessary to exclude samples with hemolysis and to separate serum from the clot and maintain immediate storage at +4˚C (short-term) or –30˚C (longer term), preferably within 1 hour after sampling. Likewise, slightly raised NSE serum levels are found in about 10-20% of NSCLC (14). These problems suggest that other tumor markers may be found in SCLC. ProGRP and NSE showed the highest sensitivity in SCLC. Moreover, the sensitivity of ProGRP, as well as the mean concentrations in relation to the cut-off, were higher than those obtained with NSE. These results are similar to those found by other authors, indicating the higher sensitivity of ProGRP, particularly in patients with LD (14-16, 28, 30, 33, 34). These results, as well as the high specificity of ProGRP in other malignancies and its absence of contamination by hemolysis, indicate that ProGRP is the tumor marker of choice in SCLC. Moreover, NSE is also a good tumor marker in SCLC and, with the combination of the two tumor markers, it is possible to increase the sensitivity. In our experience, one tumor marker or another was abnormal in 37/40 (90.2%) of patients with ED and in 21/26 (80.7%) with LD. Similar results have been described by other authors, indicating an increase in sensitivity of between 14 % and 23 % with the combination of NSE plus ProGRP (14, 16, 28, 30). It should be noted that the addition of other tumor markers such as CEA or CYFRA 21-1 to ProGRP and NSE does not significantly increase the sensitivity obtained using only the latter two tumor markers.

In summary, ProGRP is the tumor marker of choice in SCLC, with a high specificity in benign diseases (excluding renal failure) and in patients with malignancies other than SCLC (levels <120 pg/ml). NSE is a complementary tumor marker to ProGRP, and the use of both markers simultaneously increases the sensitivity obtained with the use of only one alone. High levels of ProGRP (>120 pg/ml) in patients with lung cancer, excluding renal failure, indicate a high probability of SCLC.

Acknowledgements

This study was supported by Grant: Red Tematica del Cancer, Instituto de Salud Carlos III, No CO3/10, Spain.

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Received February 9, 2004
Accepted February 8, 2005