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Determining the Cut-Off Value of Pro-Gastrin Releasing Peptide (ProGRP) in Lung Cancer According to Population Characteristics

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A B S T R A C T

The standardisation process consisted of determining tumour marker levels in all relevant population groups which are connected with the biology of the marker in normal and tumourous cells. This makes clinical application possible. ProGRP serum levels were measured in 273 healthy subjects, 176 patients with benign diseases and tumours, 200 with small cell lung cancer (SCLC), 294 with non-small cell lung cancer (NSCLC), 21 with carcinoid tumour, 93 with undifferentiated lung cancer, 35 with mixed SCLC-NSCLC, and 189 with other malignancies. ProGRP levels in patients with SCLC and SCLC-NSCLC were significantly higher than in all the other groups ($p = 5.4 \times 10^{-3}$). Moreover, in SCLC patients ProGRP levels significantly correlate with the extent of the disease and the patients' smoking habit. The cut-off level of ProGRP for SCLC is 65.89 pg/mL in the Croatian population. It is based on 96.8% specificity in benign diseases which cause problems in differential diagnosis. The sensitivity of ProGRP was 85% at the time of SCLC diagnosis.

Key words: *pro-gastrin releasing peptide (ProGRP), small cell lung cancer, tumour marker, lung cancer*

Introduction

Lung cancer is the most common cancer in the world today (12.6% of all new cancers, 17.8% cancer deaths). Almost 99% of lung tumours are carcinomas. There are two main subtypes: small cell lung carcinoma (SCLC) and non-small cell lung cancer (NSCLC), which are treated differently. According to the data of lung cancer incidence, SCLC comprises about 20% of all cases. SCLC is divided into limited and extensive stage disease, and is the most aggressive type of lung cancer[1].

The lungs of healthy individuals contain cells with different excretion products and various uptake and amidated products decarboxylation abilities. Such traits enable cells to recognise lung tumours of neuroendocrine origin that is also SCLC.

Gastrin releasing peptides (GRP) are neuroendocrine molecules present in SCLC cells. GRP stimulates growth in 20–60% of SCLC probably through autocrine growth stimulation since GRP works through GRP receptors, which belong to the superfamily of receptors linked to the G-protein[2]. These receptors are present in the cells of human lung cancer of all histological types, as well as in smokers' bronchial epithelia implying the role of GRP in early pathogenesis[3,4]. Pro-gastrin releasing peptides (ProGRP) are a stable GRP product, neuroendocrine molecules released in the sera of SCLC patients. There are three types of ProGRP molecules found in human SCLC cells having identical carboxyl-terminal regions and a different number of residues[5,6,7]. Since the half-life of GRP is only two minutes, it has been impossible to develop a clinically applicable method for its detection in sera. Instead, a recombinant ProGRP (31–98) with a region common to all the three types of previously cloned human ProGRP molecules was synthesised[5], and the ELISA immunoassay was developed[8].

It was previously considered that only the amidated forms of GRP are biologically active through either an autocrine or a paracrine pathway, but the most recent findings suggest that non-amidated forms of GRP also

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play the same role in growth stimulation and tumour cell proliferation. Non-amidated forms, such as ProGRP, are found in the tumour cells and sera of SCLC patients. The potential of ProGRP as a tumour marker in early SCLC diagnosis is enormous, but its clinical application requires standardisation according to a referent population of healthy subjects and benign diseases. Therefore, the aim of this research is to determinate the ProGRP cut-off value encompassing all relevant population data. In a broader context, high-quality direct medical care demands population specific databases, which include records on the largest possible number of diagnostic parameters. Some of the results of this study have pre -viously been reported in the form of an abstract[9] and a PhD thesis[10].

TABLE 1
DEMOGRAPHICS AND CLINICAL CHARACTERISTICS OF PATIENTS WITH A MALIGNANCY

	N	%
Small cell lung carcinoma	200	100
Disease extent(Limited/Extensive)	73/127	36.5/63.5
Risk factor(Smokers/Former smokers/Nonsmokers)	119/17/64	59.5/8.5/32
Male/female	159/41	79.5/20.5
Mean age ± SD	63 ± 9	
Non-small cell lung carcinoma	294	100
AD/SQC/LCC/LCNE	117/92/27/10	39.8/31.3/9.2/3.4
Undifferentiated NSCLC	44	15
AD-LCC	1	0.3
AD-SQC	3	1
Male/female	214/80	72.8/27.2
Mean age ± SD	63 ± 9	
Carcinoid tumour	21	100
Typical carcinoid	6	28.6
Atypical carcinoid	8	33.3
Undifferentiated carcinoid	7	38.1
Male/female	12/ 9	57.1/42.9
Mean age ± SD	63 ± 10	
Undifferentiated lung carcinoma	93	100
Male/female	54/39	58.1/41.9
Mean age ± SD		63 ± 10
Mixed small and non-small cell lung carcinoma	35	100
SCLC-AD	4	11.4
SCLC-SQC	8	22.9
SCLC-LCC	6	17.1
SCLC-NSCLC	17	48.6
Male/female	27/ 8	77.1/22.9
Mean age ± SD	64 ± 8	
Other malignant lung and pleura tumour	48	100
Tumour of the mediastinum	31	64.6
Mesothelioma	10	20.8
Lymphoproliferative tumours	7	14.6
Male/female	32/16	66.7/33.3
Mean age ± SD	57 ± 15	
Metastases to the lung	91	100
Male/female	59/32	64.8/35.2
Mean age ± SD	61 ± 11	
Malignant tumour of the other organ	50	100
Male/female	25/25	50/50
Mean age ± SD	60 ± 12	

SCLC – small cell lung carcinoma, NSCLC – non-small cell lung carcinoma, SQC – squamous cell carcinomas, AD – adenocarcinomas, LCC – large cell carcinomas, LCNE – large cell neuroendocrine carcinoma

Subjects and Methods

The sample comprised 200 SCLC patients, 294 NSCLC patients, 21 with carcinoid tumour, 93 with undifferentiated lung carcinoma, 35 with mixed SCLC and NSCLC, 48 with other malignant lung and pleura tumours, 91 with metastases to the lung, 50 with malignant tumours of other organs, 39 with benign lung and mediastinal tumours, 7 with benign tumours of other organs, and 130 with benign diseases of the lung and other organs (Tables 1, 2). Patient samples were collected consecutively. There were 273 healthy subjects. The applied standard clinical methods were reference standards for establishing the presence or absence of malignant or benign diseases of the lung. All patients with single pulmonary nodules underwent clinical examination, fiberoptic bronchoscopy and histological typing, chest X-rays, a computed tomography scan of the chest and brain, and a computed tomography scan of the upper abdomen. If in SCLC extensive disease symptoms were detected, a bone scan and biopsy of the bone marrow, liver, lymph nodes, skin and ICTP tumour marker were also conducted.

All blood samples were centrifuged after venipuncture. ProGRP levels were determined from either fresh or frozen (–20 °C) sera using an ELISA kit manufactured by Advanced Life Science Institute Inc. from Japan. The assay followed the principle of a two-step sandwich enzyme immunoassay with a 96-well microtiter plate, and was performed according to the manufacturer's instructions.

In order to compare ProGRP levels in the sera of different groups of patients and healthy control subject a Kruskal-Wallis ANOVA and Mann-Whitney U-test were also conducted using Statistika 6.0 software package (Statsoft, USA). Any value of $p<0.05$ was considered statistically significant. The receiver operating characteristic (ROC) curve was used to determine of the cut-off value using MedCalc v. 9.2.1.0 (MedCalc Software, Belgium).

Results

The patients were classified according to their histological type and the characteristics that may influence ProGRP specificity and sensitivity at the time of diagnosis. The levels of ProGRP were measured in 11 groups of benign and malignant diseases (Tables 1, 2). The subgroups were defined according to their histological type, and the clinical or pathological stage of the diseases in all the groups.

The distribution of ProGRP tumour marker values in the studied population showing the mean, median and interquartile ranges, and 95th percentile are given according to the main groups and subgroups in Tables 3 and 4. In healthy individuals or in patients suffering from benign diseases and tumours of the lung or other organs, possibly causing problems in differential diagnosis, the values of ProGRP were low.

In patients with SCLC the median and interquartile ranges, and 95th percentile of the level of ProGRP were significantly higher than in all the other groups (615.72, 130.91–1754.8, 8253.82 pg/mL). Extremely high levels of ProGRP have been detected more frequently in SCLC, especially in the extensive stage. Besides SCLC, very high levels of ProGRP were observed in the mixed tumour types SCLC-NSCLC, SCLC-large cell lung carcinoma (LCC), SCLC-squamous cell lung carcinoma (SQC) and SCLC-adenocarcinoma (AD). The 95th percentile showed higher marker levels in large cell neuroendocrine lung carcinoma (LCNE), typical and atypical carcinoid

TABLE 2
CHARACTERISTICS OF HEALTHY SUBJECTS AND PATIENTS WITH BENIGN DISEASES

	N	%
Benign lung and mediastinal tumours	39	100
Male/female	26/13	66.7/33.3
Mean age ± SD	59 ± 11	
Benign tumours of the other organs	7	100
Male/female	3/4	42.9/57.1
Mean age ± SD	59 ± 9	
Benign disease of the lung and other organs	130	100
Cronic obstructive pulmonary diseases	12	9.2
Tuberculosis	7	5.4
Pneumonia	26	20
Other benign lung diseases	79	60.8
Benign disease other organs	6	4.6
Male/female	87/43	66.9/33.1
Mean age ± SD	60 ± 14	
Healthy subjects	273	100
Male/female	150/123	54.9/45.1
Mean age ± SD	49 ± 12	

TABLE 3
DISTRIBUTION OF PROGRP SERUM LEVELS SUBDIVIDED INTO STUDIED GROUP

Group	N	Mean (pg/mL)	Median (pg/mL)	25th (pg/mL)	75th (pg/mL)	95th (pg/mL)
SCLC	200	2230.14	615.72	130.91	1754.80	8253.82
NSCLC	294	433.03	24.95	11.85	40.98	428.74
C	21	218.86	23.19	10.58	60.38	1000.00
UNDIF LC	93	56.06	43.35	21.62	64.50	119.64
SCLC-NSCLC	35	1198.46	368.89	50.67	1182.82	8253.82
OMLPT	48	24.16	23.48	12.87	32.67	47.62
ML	91	34.36	34.06	20.88	42.88	68.01
MTO	50	46.36	17.86	8.95	32.72	78.76
BTLM	39	25.22	20.02	11.22	35.11	60.95
BTO	7	33.18	36.14	0.10	60.65	84.99
BDO	6	55.69	39.62	15.24	106.89	132.69
BDL	124	29.23	27.06	12.06	39.75	63.86
H	273	18.10	15.41	0.16	25.16	55.15

SCLC – small cell lung carcinoma, NSCLC – non -small cell lung carcinoma, C – carcinoid, UNDIF LC – undifferentiated lung carcinoma, OMLPT – other malignant lung and pleura tumour, ML – metastases to the lung, MTO – malignant tumour of the other organ, BTLM – benign lung and mediastinal tumors, BTO – benign tumours of the other organs, BDO – benign disease other organs, BDL – benign disease of the lung, healthy, 25th – 25th percentile, 75th – 75th percentile, 95th – 95th percentile

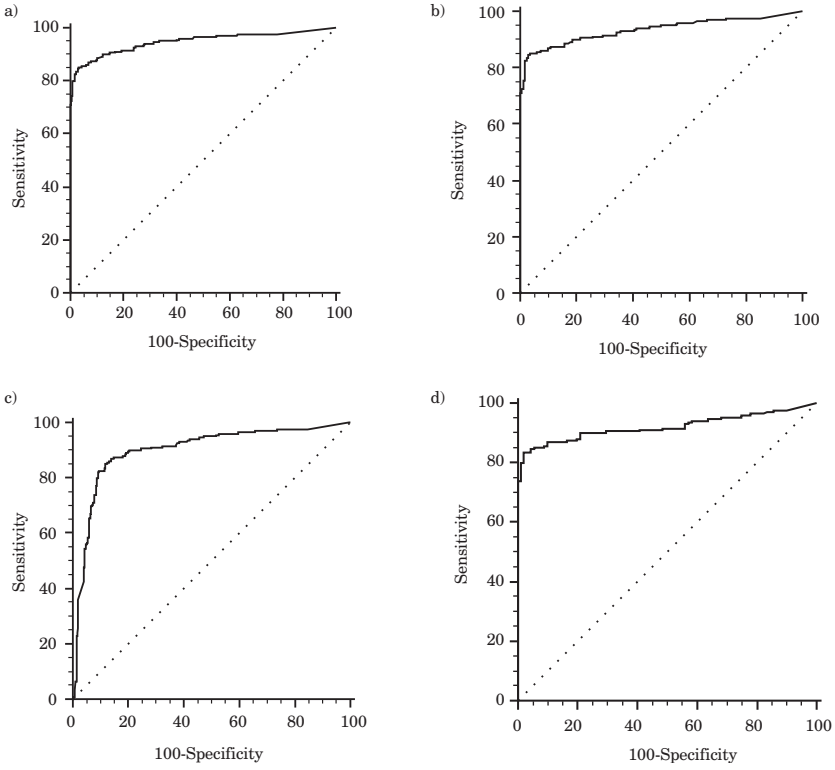


Fig. 1. Receiver operating characteristics (ROC) curves comparing the level of ProGRP in subjects without a malignant disease (specificity) and small cell lung carcinoma patients (sensitivity). a) Healthy (n=273), benign disease of the lung and other organs (n=130), and small cell lung carcinoma patients (n=200). The area under the ROC curve was 0.947, standard error 0.0113, 95% confidence interval between 0.926 and 0.964, significance level p (area=0.5) 0.0001. b) Patients with benign diseases of the lung (n=124), benign lung and mediastinal tumours (n=39) and small cell lung carcinoma patients (n=200). The area under the ROC curve was 0.934, standard error 0.0131, 95% confidence interval between 0.904 and 0.958, significance level p (area=0.5) 0.0001. c) Patients with non-small cell lung carcinoma (n=294) and small cell lung carcinoma patients (n=200). The area under the ROC curve was 0.899, standard error 0.0156, 95% confidence interval between 0.870 and 0.925, significance level p (area=0.5) 0.0001. d) Patients with metastases to the lung (n=91) and small cell lung carcinoma (n=200). The area under the ROC curve was 0.920, standard error 0.0155, 95% confidence interval between 0.882 and 0.948, significance level p (area=0.5) 0.0001.

TABLE 4
DISTRIBUTION OF PROGRP SERUM LEVELS SUBDIVIDED INTO STUDIED SUBGROUPS

Group	N	Mean (pg/mL)	Median (pg/mL)	25th (pg/mL)	75th (pg/mL)	95th (pg/mL)
LD	73	691.38	182.17	68.49	539.93	4925.63
ED	127	3114.63	1000.00	288.09	2805.81	12880.89
AD	117	37.44	25.88	11.03	36.76	140.16
SQC	92	831.35	24.49	12.11	42.42	428.74
LCC	27	88.90	16.00	4.25	38.24	57.95
LCNE	10	1835.55	58.40	33.80	1754.80	8253.82
UNDIF NSCLC	44	580.59	21.90	9.04	44.45	1000.00
AD-LCC/SQC	4	36.14	42.71	27.20	45.09	46.35
TC	6	502.09	41.79	19.37	73.78	2835.72
AC	8	185.01	39,42	9.65	190.90	1000.00
UNDIF C	7	14.78	11,89	5.38	29.83	32.72
UNDIF LC	93	56.06	43.35	21.62	64.50	119.64
SCLC-AD	4	1899.74	987.42	238.81	3560.68	5596.00
SCLC-SQC	8	419.21	305.93	19.61	861.97	978.55
SCLC-LCC	6	1857.90	638.16	146.16	1451.50	8253.82
SCLC-NSCLC	17	1167.43	304.87	53.93	1182.82	8253.82
TM	31	22.17	24.66	11.91	32.49	43.87
MES	10	24.38	22.70	14.48	39.59	57.95
LT	7	32.66	30.15	15.90	42.28	79.34
ML	91	34.36	34.06	20.88	42.88	68.01
MTO	50	46.36	17.86	8.95	32.72	78.76
BTLM	39	25.22	20.02	11.22	35.11	60.95
BTO	7	33.18	36.14	0.10	60.65	84.99
COPD	12	28.31	20.83	11.00	44.66	72.62
TB	7	21.64	27.09	0.10	35.48	47.52
P	26	33.12	24.56	14.05	49.27	80.79
OBLD	79	28.76	27.87	11.89	38.84	60.76
BDO	6	55.69	39.62	15.24	106.89	132.69

SCLC – small cell lung carcinoma, ED – extensive disease SCLC, LD – limited disease SCLC, AD – adenocarcinomas, LCC – large cell carcinomas, LCNE – large cell neuroendocrine carcinoma, UNDIF – undifferentiated, NSCLC – non-small cell lung carcinoma, SQC – squamous cell carcinomas, AC – atypical carcinoid, TC – typical carcinoid, C – carcinoid, UNDIF LC – undifferentiated lung carcinoma, TM – tumour of the mediastinum, MES – mesothelioma, LT – lymphoproliferative tumours, ML – metastases to the lung, MTO – malignant tumour of the other organ, BTLM – benign lung and mediastinal tumours, BTO – benign tumours of the other organs, COPD – cronic obstructive pulmonary diseases, TB – tuberculosis, P – pneumonia, OBLD – other benign lung disease, BDO – benign disease other organs, 25th – 25th percentile, 75th – 75th percentile, 95th – 95th percentile.

tumour (TC, AC), undifferentiated NSCLC and squamous cell lung carcinoma (SQC). In NSCLC extremely high levels were rarely measured. The median and interquartile ranges in 294 NSCLC patients were 24.95 pg/mL and 11.85–40.98 pg/mL, respectively.

There was a highly significant difference in ProGRP levels found in the sera of all the studied groups (Kruskal-Wallis ANOVA, $p=10^{-4}$). The Mann-Whitney U-test showed significant differences in the levels of ProGRP between SCLC, the mixed SCLC-NSCLC type and the patients in all the other 11 groups ($p=10^{-4}$ to $p=5.4\times10^{-3}$). Significant differences have also been found between patients with undifferentiated lung cancer and metastases to the lung in comparison to the other groups, with the exception of patients with carcinoid tumours and benign tumours. The differences are also significant between NSCLC and SCLC patients, SCLC-NSCLC, undifferentiated lung cancer and patients with metastases to the lung (Table 5). Kruskal-Wallis ANOVA showed significant differences between all the 29 histological subgroups ($p=10^{-4}$). The Mann-Whitney U-test was used for comparison between the 15 subgroups in the most significant groups (Table 6). Moreover, the comparison between SCLC extensive disease stage and the other subgroups also showed significant differences, with the only exception of patients with SCLC-AD and SCLC-LCC. The same result was also observed amongst subjects with a limited SCLC disease with the exception of 6 subgroups

TABLE 5
DIFFERENCES BETWEEN THE GROUPS ACCORDING TO PROGRP LEVELS AT THE TIME OF DIAGNOSIS USING THE MANN-WHITNEY U-TEST

Group	SCLC	NSCLC	C	SCLC-NSCLC	UNDIF LC	OMLPT	ML	MTO	BTLM	BTO	BDLO
HEALTHY	$<10^{-5}$	$<10^{-5}$	4.5×10^{-2}	$<10^{-5}$	$<10^{-5}$	4.4×10^{-3}	$<10^{-5}$	NS	2.6×10^{-2}	NS	$<10^{-5}$
SCLC	–	$<10^{-5}$	$<10^{-5}$	NS	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	2×10^{-4}	$<10^{-5}$
NSCLC		–	NS	$<10^{-5}$	$<10^{-5}$	NS	9.9×10^{-3}	NS	NS	NS	NS
C			–	4×10^{-4}	NS	NS	NS	NS	NS	NS	NS
SCLC-NSCLC				–	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	5.4×10^{-3}	$<10^{-5}$
UNDIF LC					–	$<10^{-5}$	1.4×10^{-2}	$<10^{-5}$	2×10^{-4}	NS	1×10^{-4}
OMLPT						–	3.5×10^{-3}	NS	NS	NS	NS
ML							–	4×10^{-4}	4.7×10^{-3}	NS	3.6×10^{-2}
MTO								–	NS	NS	NS
BTLM									–	NS	NS
BTO										–	NS
BDLO											–

SCLC – small cell lung carcinoma, NSCLC – non -small cell lung carcinoma, C – carcinoid, UNDIF LC – undifferentiated lung carcinoma, OMLPT – other malignant lung and pleura tumour, ML – metastases to the lung, MTO – malignant tumour of the other organ, BTLM – benign lung and mediastinal tumours, BTO – benign tumours of the other organs, BDLO – benign disease of the lung and other organs, NS – nonsignificant

(LCNE, TC, SCLC-AD, SCLC-LCC, SCLC-NSCLC, SCLC-SQC). Finally, there were no significant differences between undifferentiated lung cancer, LCNE and carcinoids.

The levels of ProGRP in SCLC patients significantly correlate with the extent of the disease and the patients' smoking habit. Once the levels of ProGRP in SCLC patients were compared according the gender and age, no significant differences were found (Table 7).

ROC curves were used to analyse the levels of ProGRP for application at the time of SCLC diagnosis, and to discriminate SCLC from NSCLC and SCLC from metastases to the lung (Figure 1). The ROC curve of ProGRP demonstrates the specificities and sensitivities not only at certain cut-off values but also over the entire range of values. Due to this, all further calculations were fixed at 95% and 96.8% specificity (the latter of which is the best selection criterion according to the ROC curve) (Table 8). The ProGRP cut-off level for benign lung diseases, and benign lung and mediastinal tumours was 62.69 pg/mL reaching 95% specificity, while the cut-off value for the reference group of healthy individuals, benign diseases of the lung and other organs was 65.89 pg/mL reaching 96.8% specificity (Figure 1).

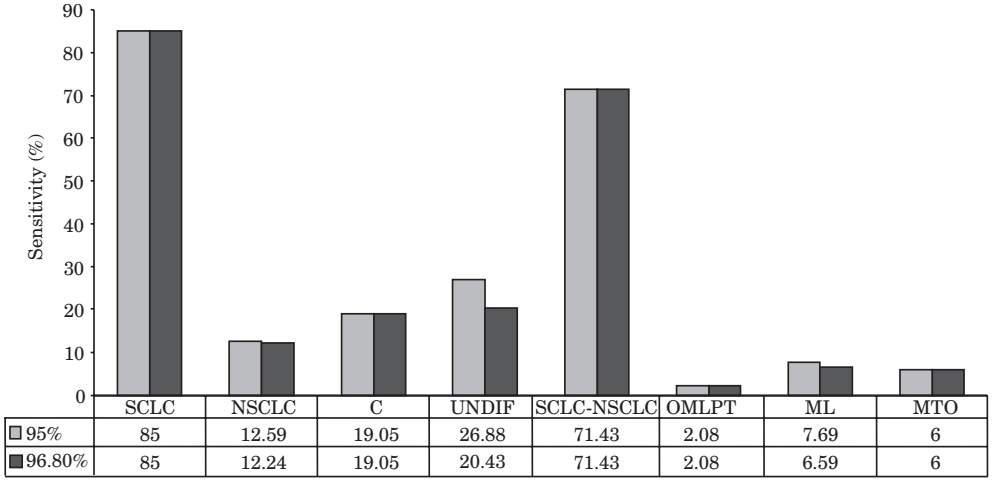


Fig. 2. Sensitivity of ProGRP by main group of malignant diseases at specificity 95% (cut-off 62.69 pg/mL) and 96,8 % (cut-off 65.89 pg/mL). SCLC – small cell lung carcinoma, NSCLC – non -small cell lung carcinoma, C – carcinoid, UNDIF – undifferentiated lung carcinoma, OMLPT – other malignant lung and pleura tumor, ML – metastases to the lung, MTO – malignant tumor of the other organ.

TABLE 6
DIFFERENCES BETWEEN THE MOST SIGNIFICANT SUBGROUPS ACCORDING TO PROGRP LEVELS AT THE TIME OF DIAGNOSIS USING THE MANN-WHITNEY U-TEST

Group	ED	LD	AD	LCC	LCNE	UNDIF NSCLC	SQC	AC	TC	SCLC-AD	SCLC-LCC	SCLC-NSCLC	SCLC-SQC	UNDIF LC	MTO
ED	–	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	3.7×10^{-2}	$<10^{-5}$	$<10^{-5}$	3×10^{-4}	6.1×10^{-3}	NS	NS	1.9×10^{-2}	5.3×10^{-3}	$<10^{-5}$	$<10^{-5}$
LD		–	$<10^{-5}$	$<10^{-5}$	NS	$<10^{-5}$	$<10^{-5}$	3.5×10^{-2}	NS	NS	NS	NS	NS	$<10^{-5}$	$<10^{-5}$
AD			–	NS	3.1×10^{-3}	NS	NS	NS	NS	8.3×10^{-3}	1.4×10^{-3}	$<10^{-5}$	3.1×10^{-2}	1×10^{-4}	NS
LCC				–	3.5×10^{-3}	NS	NS	NS	NS	1.1×10^{-2}	2.1×10^{-3}	1×10^{-4}	4.7×10^{-2}	4×10^{-4}	NS
LCNE					–	1×10^{-2}	7.4×10^{-3}	NS	NS	NS	NS	NS	NS	NS	1.5×10^{-3}
UNDIF NSCLC						–	NS	NS	NS	1.4×10^{-2}	5×10^{-3}	1×10^{-4}	NS	9.1×10^{-3}	NS
SQC							–	NS	NS	1.3×10^{-2}	2.9×10^{-3}	$<10^{-5}$	NS	1.9×10^{-3}	NS
AC								–	NS	NS	NS	6.6×10^{-2}	NS	NS	NS
TC									–	NS	NS	NS	NS	NS	NS
SCLC-AD										–	NS	NS	NS	2.3×10^{-2}	5×10^{-3}
SCLC-LCC											–	NS	NS	2.9×10^{-3}	1.1×10^{-3}
SCLC-NSCLC												–	NS	1×10^{-4}	$<10^{-5}$
SCLC-SQC													–	NS	2.2×10^{-2}
UNDIF LC														–	$<10^{-5}$
MTO															–

SCLC – small cell lung carcinoma, ED – extensive disease SCLC, LD – limited disease SCLC, AD – adenocarcinomas, LCC – large cell carcinomas, LCNE – large cell neuroendocrine carcinoma, UNDIF – undifferentiated, NSCLC – non-small cell lung carcinoma, SQC – squamous cell carcinomas, AC – atypical carcinoid, TC – typical carcinoid, UNDIF LC – undifferentiated lung carcinoma, MTO – malignant tumour of the other organ, NS – nonsignificant

TABLE 7
PROGRP LEVEL DIFFERENCES IN SCLC

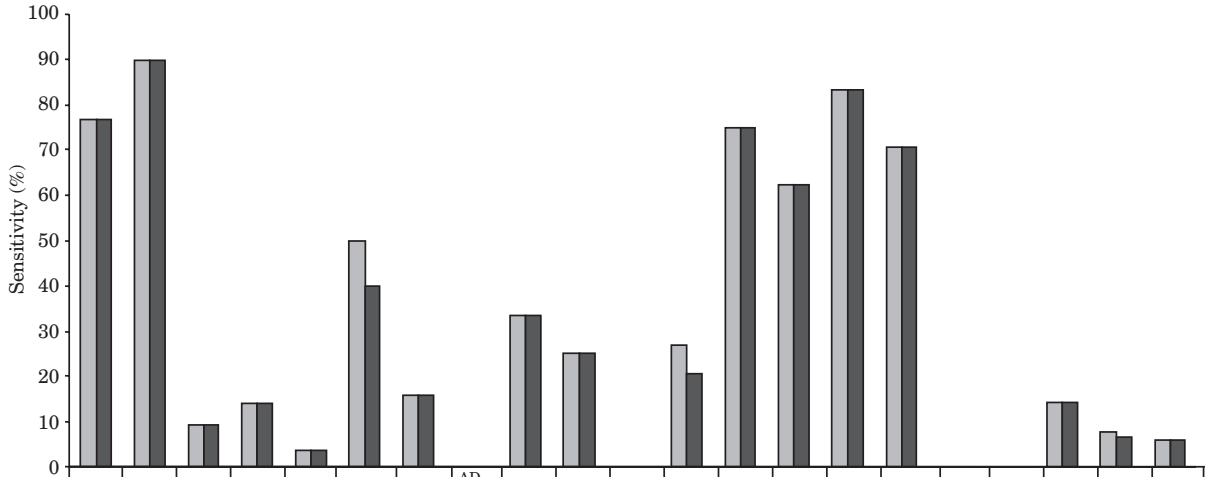
	N	Median (pg/mL)	25th (pg/mL)	75th (pg/mL)	p
Nonsmoker	64	1396.85	827.07	3781.78	$<10^{-5**}$
Former smoker	17	1000.00	145.85	2762.98	
Smoker	119	271.83	69.51	1000.00	
Male	159	617.19	134.61	1653.29	NS*
Female	41	534.51	96.39	2093.77	
Age <65	101	499.21	130.72	1756.54	NS*
Age ≥65	99	671.47	134.61	1635.14	

25th – 25th percentile, 75th – 75th percentile, * p-value determined using the Mann-Whitney U-test, ** p-value determined using the Kruskal-Wallis ANOVA, NS – nonsignificant

Using the cut-off values for the reference population, ProGRP shows to be the marker with the highest sensitivity – 85% for the detection of SCLC followed by 71.43% in the mixed SCLC-NSCLC type. In patients with a limited SCLC disease ProGRP sensitivity was 76.71%, while in the extensive disease 89.76%. However, in the other studied groups ProGRP seldom revealed a level higher than the reference range of population, and its sensitivity was much lower (Figures 2, 3).

Discussion

The clinical application of the ProGRP tumour marker to identify SCLC can be achieved through a standardisation process which depends on population characteristics, patient medical histories, the sample size researched, the time of sampling in relation to other diagnostic invasive procedures, and the clinically defined control groups expected to have a different dynamics



	LD	ED	AD	SQC	LCC	LCNE	UNDIF NSCLC	AD-LCC/SQC	TC	AC	UNDIF C	UNDIF	SCLC-AD	SCLC/SQC	SCLC-LCC	SCLC-NSCLC	TM	MES	LT	ML	MTO
95%	76.71	89.76	9.4	14.13	3.7	50	15.91	0	33.33	25.0	0	26.88	75	62.5	83.33	70.59	0	0	14.26	7.69	6
96.80%	76.71	89.76	9.4	14.13	3.7	50	15.91	0	33.33	25.0	0	20.43	75	62.5	83.33	70.59	0	0	14.26	7.69	6

Fig. 3. Sensitivity of ProGRP by main subgroups of malignant diseases at specificity 95% (cut-off 62.69 pg/mL) and 96,8 % (cut-off 65.89 pg/mL). SCLC – small cell lung carcinoma, ED – extensive disease SCLC, LD – limited disease SCLC, AD – adenocarcinomas, LCC – large cell carcinomas, LCNE – large cell neuroendocrine carcinoma, UNDIF – undifferentiated, NSCLC – non-small cell lung carcinoma, SQC – squamous cell carcinomas, AC – atypical carcinoid, TC – typical carcinoid, C – carcinoid, TM – tumor of the mediastinum, MES – mesothelioma, LT – lymphoproliferative tumors, ML – metastases to the lung, MTO – malignant tumor of the other organ.

TABLE 8
DATA FROM ROC CURVES CONSTRUCTED FOR OPTIMISING PROGRP CUT-OFF VALUES TO REACH BEST SENSITIVITY AND SPECIFICITY

Group	N	Specificity (%)	Sensitivity (%)	Cut-off (pg/mL)
H/BDLO/SCLC	273/130/200	96.8	85	65.89*
BDL/BTLM/SCLC	124/39/200	96.9	84.5	67.94*
NSCLC/SCLC	294/200	88.1	85	66.98*
ML/SCLC	91/200	97.8	83.5	73.65*
H/BDLO/SCLC	273/130/200	95	85.8	60.40
BDL/BTLM/SCLC	124/39/200	95	85	62.69
NSCLC/SCLC	294/200	95	56	428.74
ML/SCLC	91/200	95	84.5	68.01
H/BDLO/SCLC	273/130/200	100	71	165
BDL/BTLM/SCLC	124/39/200	100	71	165
NSCLC/SCLC	294/200	100	0	70128
ML/SCLC	91/200	100	74	138.4

H – healthy, BDLO – benign disease of the lung and other organs, SCLC – small cell lung carcinoma, BDL – benign disease of the lung, BTLM – benign lung and mediastinal tumours, NSCLC – non -small cell lung carcinoma, ML – metastases to the lung, * the most accurate criterion of the ROC curve

from the healthy population[11]. The approach to standardisation in determining the cut-off value of tumour markers is different from the approach to the standardisation of molecules in normal cell processes. There is no ideal marker since determining cut-off values is a process in which all the population variables influencing marker levels are evaluated. For instance, according to our research there are 79 diseases influencing CYFRA 21–1 levels[11]. The research examined all the diseases that affect the ProGRP cut-off value. The ProGRP levels measured can only be viewed as a dynamic value at >95% specificity. What matters in evaluation is not a single ProGRP cut-off value but a range of values. It is important to enable, direct and encourage clinicians to use the

marker in early SCLC diagnosis. The high sensitivity of ProGRP at 85% in our study facilitates this. It was particularly important to research the influence of all the benign and malignant diseases appearing in differential diagnosis and causing problems for the clinical application of markers. ROC curves were constructed, and at 95% specificity in the reference group of benign lung diseases with benign lung tumours and mediastinum the cut-off value of ProGRP was 62.69 pg/mL. Healthy subjects and patients with benign diseases of the lung and other organs at 96.8% specificity defined the cut-off value of ProGRP at 65.89 pg/mL as the most accurate criterion of the ROC curve indicating SCLC. Tumour markers offer some advantages in relation to other biological variables in making a diagnosis. They are specific molecules, different dosages and are reproducible in immunoassays. The better the sensitivity-specificity relationship, the sooner markers can be introduced into clinical practice, as suggested by clinicians. Our study of ProGRP shows that high values of both sensitivity and specificity point to SCLC at 65.89 pg/mL. High serum levels of ProGRP could be regarded as a value that indicates SCLC in known lung cancer phenotypic heterogeneity. In particular, according to our findings, the level of ProGRP above the cut-off value of 65.89 pg/mL and imaging findings having detected pulmonary infiltrates most probably reflect biological SCLC tumour behaviour that requires further clinical diagnostic confirmation. Regardless of the efforts to contribute to diagnosis with markers, their application remains questionable until the diagnosis made is confirmed by standard clinical methods. The application of clinical molecular biomarkers, besides markers such as ProGRP, will accelerate SCLC detection.

ProGRP provides important insight into the neuroendocrine differentiation of lung cancer, particularly in SCLC, SCLC-NSCLC, undifferentiated lung carcinoma and carcinoids. The sensitivity of ProGRP in these groups was higher than in all the other groups. This has been brought into relation with the fact that ProGRP levels and the extent of its release are very low in benign diseases and malignant lung tumours other than SCLC. Only the smallest amounts of this marker are released in these. Unknown pulmonary infiltrates without any clinical confirmation of them being SCLC and with an elevated level of ProGRP are an exception in our study. And these infiltrates are uterine cancer and larynx cancer from the group of malignant tumours of other organs, as well as dermatomes from the group of benign tumours of other organs. These two groups showed great differences in maximum levels (1219 pg/mL and 85 pg/mL). The fact that the first group were patients without SCLC having been confirmed and that the second group comprising benign tumours of other organs was made up of a very small number of patients cannot be ignored.

To support the diagnosis of SCLC for the purpose of more efficient therapeutic stratification, whether in overall, limited stage, extensive stage or mixed type lung cancer at high specificity (100%), it is essential to analyse a much larger sample of SCLC without great discrepancies in the number of subjects tested in each group.

And now ProGRP assays cost as much as molecular markers for SCLC.

This research has proven ProGRP to be a sensitive marker for 85% of SCLC patients. The rate of SCLC detection with ProGRP has been in agreement with other authors. Between 1994 and 2008 its sensitivity ranged between 41% and 100% at 90% to 99% specificity[8–24]. The results depended on the groups selected for specificity. The reference population used for specificity comprised healthy persons and patients with benign diseases of the lung and other organs. Our specificity was high (96.8%) and facilitated the detection of SCLC, SCLC-NSCLC and SCLC in undifferentiated lung cancer. Previous studies also showed that ProGRP is a superior marker for SCLC[13,25]. For the purpose of differentiating lung tumours of unknown origin the discriminative power of ProGRP was shown by a ROC curve on data on SCLC and metastasis to the lung. Although the cut-off value based on this curve is similar to our selected cut-off value, applying it in clinical practice is difficult knowing the biology of metastases including ProGRP which depends on the organ from which the metastases originated. According to different studies, cut-offs (or reference levels) have been reported to range between 16.6 and 87 pg/mL depending on the referent population[8–24]. Our reference level of 65.98 pg/mL facilitated the detection of SCLC with a positive and negative predictive value of 92.6%. Furthermore, our results prove that ProGRP correlates with the extent of the disease, which is in concordance with the findings of others authors[13,17]. Obviously, ProGRP is involved in SCLC cell growth stimulation.

The study of the further evaluation of ProGRP in longitudinal follow-ups in SCLC therapy and in cerebral neuroendocrine tumours is in progress.

If the level of ProGRP in NSCLC patients exceeds 400 pg/mL, their clinical-pathological future must be examined with regard to either SCLC (it might in fact be NSCLC-SCLC) or neuroendocrine differentiation (including the extensive stage of the disease).

It is still debatable whether a diagnosis can be made or not according to marker values, particularly in borderline cases. Accordingly, ProGRP levels are evaluated individually in each patient as a dynamic value within the context of the clinical parameters known and defined for this marker as stated in the referral. Finally, repeated standard diagnostic methods have not disproved but only confirmed our findings in terms of marker level interpretation.

Conclusion

ProGRP is a tumour marker for the detection of SCLC. Its level at the time of diagnosis indicates the presence of SCLC, later confirmed by standard clinical procedures. Its cut-off is 65.89 pg/mL at 96.8% specificity.

ProGRP is a useful marker provided it contributes to the clinical methods used for diagnosis differentiating between SCLC and NSCLC, primary tumour of the lung and metastasis to the lung from other organs.

High levels of ProGRP in undifferentiated lung cancer are most probably connected with the neuroendocrine biological activity of SCLC.

In NSCLC and carcinoids elevated ProGRP levels are most probably the consequence of neuroendocrine differentiation. This requires special attention by any future research.

Extremely high levels of ProGRP indicate a metastasised advanced disease.

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ODREĐIVANJE REFERENTNE RAZINE PRO-GASTRIN OTPUŠTAJUĆEG PEPTIDA (PROGRP) KOD RAKA PLUĆA U SKLADU S POPULACIJSKIM KARAKTERISTIKAMA

SAŽETAK

Određivanje razine tumorskog markera u grupama populacije koje su povezane s biologijom markera u normalnoj i tumorskoj stanici dio je standardizacijskog procesa koji omogućuje kliničku primjenu. Razina pro-gastrin otpuštajućeg peptida (ProGRP) je izmjerena u serumu 273 zdrava ispitanika, 176 pacijenata s benignim bolestima i tumorima, 200 pacijenata s karcinomom pluća malih stanica (SCLC), 294 s karcinomom nemalih stanica pluća (NSCLC), 21 s karcinoidom, 93 s nediferenciranim rakom pluća, 35 s mješanim SCLC-NSCLC i 189 s drugim malignitetima. Razina ProGRP je značajno viša kod pacijenata SCLC i mješanog tipa SCLC-NSCLC u odnosu na sve ostale istražene grupe ($p<5,4\times10^{-3}$). Kod SCLC pacijenata razina ProGRP značajno korelira s proširenošću bolesti i pušenjem. Referentna razina (engl. cut-off) ProGRP iznosi 65,89 pg/mL kod SCLC u Hrvatskoj populaciji. Bazira se na specifičnosti od 96,8% kod benignih bolesti koje uobičajeno predstavljaju problem u diferencijalnoj dijagnozi. U vrijeme dijagnoze SCLC senzitivnost ProGRP je 85%.