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Selected neuroendocrine tumour markers, growth factors and their receptors in typical and atypical bronchopulmonary carcinoids

Stężenia wybranych markerów nowotworów neuroendokrynnych, czynników wzrostu i ich receptorów w rakowiakach typowych i atypowych płuc

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Abstract

Introduction: Bronchopulmonary neuroendocrine tumours (BP NET) cause many diagnostic and therapeutic problems. There is an ongoing search for biochemical markers of activity of these tumours. The use of polypeptide growth factors seems potentially feasible in establishing the diagnosis, prognosis and treatment of these tumours.

Material and methods: We included 41 patients aged 25 to 78 years with histopathologically confirmed typical and atypical bronchopulmonary carcinoid tumours and 20 healthy volunteers. We assessed the levels of specific and non-specific markers of these tumours and of selected growth factors relative to TNM classification.

Results: The levels of specific markers (serotonin and its metabolite, 5-hydroxyindoleacetic acid [5HIAA]) and non-specific markers (chromogranin A [CgA]) were significantly higher in patients with atypical carcinoid tumours. The serum levels of hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and VEGF receptor-1 (VEGFR-1) were significantly higher in patients with carcinoid tumours versus the control group. The levels of VEGFR-1 closely correlated with TNM classification. No such correlation could, however, be confirmed for the levels of HGF, VEGF or VEGFR-2.

Conclusions: Determination of CgA, serotonin and 5HIAA may be useful in the diagnosis of BP NET, particularly in atypical carcinoid tumours, and their levels depend on the presence of distant metastases. Determination of growth factors (VEGF and its receptor, VEGFR-1, and HGF) may prove useful in the clinical diagnosis of these tumours, while the assessment of VEGFR-1 expression may be helpful in tumour staging. **(Endokrynol Pol 2012; 63 (6): 477–482)**

Key words: *chromogranin A, serotonin, 5HIAA, growth factors, VEGF, VEGFR, HGF, typical and atypical lung carcinoid tumour*

Streszczenie

Wstęp: Nowotwory neuroendokrynne płuc sprawiają wiele trudności diagnostycznych i leczniczych. Trwają więc poszukiwania biochemicznych wskaźników aktywności tych nowotworów. Wykorzystanie polipeptydowych czynników wzrostu wydaje się potencjalnie możliwe w diagnostyce, rokowaniu i leczeniu.

Materiał i metody: Badaniem objęto 41 chorych w wieku 25–78 lat z potwierdzonymi histopatologicznie rakowiakami typowymi i atypowymi płuc oraz 20 zdrowych woluntariuszy, u których oceniono zależność stężeń specyficznych i niespecyficznych markerów tych nowotworów oraz wybranych czynników wzrostu w zależności od stopnia zaawansowania w skali TNM.

Wyniki: Stężenia badanych markerów specyficznych (serotonina oraz jej metabolit — kwas 5-hydroksyindolooctowy [5HIAA]) i niespecyficznych (chromogranina A [CgA]) były istotnie wyższe u chorych z rakowiakiem atypowym. Stężenia czynnika wzrostu hepatocytów (HGF), czynnika wzrostu śródbłonka naczyniowego (VEGF) i jego receptorów R1 (VEGFR-1) było istotnie większe u chorych z rakowiakami w porównaniu z grupą kontrolną. Stężenia VEGFR-1 korelowały ściśle ze stopniem zaawansowania w skali TNM, czego nie udało się potwierdzić badając stężenia HGF oraz VEGF i VEGFR-2.

Wnioski: Oznaczanie stężeń CgA, serotoniny i 5HIAA może być przydatne w diagnostyce rakowiaków atypowych płuc a wielkość tych stężeń zależy od istnienia przerzutów odległych. Potencjalną rolę oznaczeń czynników wzrostu (VEGF i jego receptorów VEGFR-1 oraz HGF) upatrujemy w diagnostyce klinicznej tych nowotworów, natomiast ocena ekspresji receptorów VEGFR-1 może być użyteczna w ocenie stopnia zaawansowania procesu nowotworowego. **(Endokrynol Pol 2012; 63 (6): 477–482)**

Słowa kluczowe: *chromogranina A, serotonina, 5HIAA, czynniki wzrostu, VEGF, VEGFR, HGF, rakowiak typowy i atypowy płuc*

Introduction

Bronchopulmonary neuroendocrine tumours (BP NET) are a group of neoplasms originating from endocrine cells disseminated throughout the human body. Neuroendocrine cells of the respiratory tract, also known as enterochromaffin cells (Kulchitsky cells), account for 0.17% of all the cells of the respiratory tract epithelium and are located in the basal layer of the bronchial epithelium and bronchial glands [1]. The aetiology and pathogenesis of

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BP NET are unclear, which to possesses difficulties in determining their clinical course and prognosis [2–4]. Due to the untypical nature of this rare group of tumours, there is an ongoing search for biochemical markers that might be helpful in establishing the diagnosis and predicting progression of the disease in various stages of the tumour. Laboratory evaluation of these tumours involves the determination of specific markers (serotonin and its metabolite 5-hydroxyindoleacetic acid [5HIAA]) and non-specific markers (chromogranin A [CgA]) [5]. There are scarce and conflicting reports on the use of polypeptide growth factors involved in the processes of neoplastic transformation, proliferation and angiogenesis as potential biochemical markers of activity of these tumours. Angiogenesis is strictly related to tumour growth and metastatic potential of tumours. Neuroendocrine tumours have long been known to be highly vascular. In the past, carcinoid tumours were believed to be characterised by a benign clinical course. However, it has been proved in the past 20 years that these are in fact malignant tumours, and the prognosis and treatment outcomes depend on cell type, differentiation and stage of the disease. Compared to other malignant tumours of the lung, carcinoid tumours tend to occur at a younger age and have a more favourable prognosis than other primary tumours of the lung, although this strictly depends on tumour type and stage. Studies have demonstrated that levels of such angiogenic factors as vascular endothelial growth factor (VEGF) [6], which exerts its actions through specific thyrosine kinase receptors (VEGFR), and hepatocyte growth factor (HGF) correlate with the aggressiveness of tumours in various organs and may be used as prognostic factors. The activity of VEGF is not limited to the vascular endothelium but may extend to certain other cell types (VEGF may, for instance, stimulate migration of monocytes or macrophages). *In vitro* experiments have shown that VEGF stimulates mitosis and migration of endothelial cells and increases the permeability of capillaries. HGF is secreted by mesenchymal cells and acts as a cytokine mainly on cells of epithelial origin, cells of mesothelial origin and on the precursors of haematopoietic cells, regulating their growth, mobility and morphogenesis. Studies have demonstrated that when released from its regulatory mechanisms, HGF causes a fulminant invasion of tumour cells into adjacent tissues and is closely linked to the metastatic potential of various types of tumours [7]. Studies in patients with non-small-lung carcinoma show a significant role in the growth of solid tumours of the lung and suggest a relationship with dissemination of the underlying disease [8, 9].

The aims of our study were to: assess the levels of specific markers (serotonin and its metabolite 5HIAA), non-specific markers (CgA) and selected growth factors (VEGF, HGF, VEGFR1, VEGFR2) in patients with typical and atypical carcinoid tumours of the lung;

— assess the relationship between CgA, serotonin, 5HIAA, VEGF, VEGFR and HGF and the TNM stage in these patients.

Material and methods

We enrolled 41 patients aged from 25 to 78 years (mean age 59.4 years) with histopathologically confirmed bronchopulmonary carcinoid tumours and 20 sex- and age-matched healthy volunteers serving as controls. Typical carcinoid tumours were present in 54% (22/41) of the patients (Group 1) and atypical ones in 46% (19/41) of the patients (Group 2). Women accounted for 72.7% (16/22) and men for 27.3% (6/22) of the patients in Group 1. The respective percentages in Group 2 were 42.1% (8/19) and 57.9% (11/19). Patients with co-existing tumours in other organs were excluded from the study. Levels of the selected parameters were determined in the serum.

All the study subjects provided informed consent to participate in the study. The study protocol was approved by the relevant ethics committee.

In both patient groups, the patients were classified according to the TNM classification published in 2009 by Travis et al. [10] (Fig. 1, 2).

Fasting blood samples for hormone determinations were collected at 8.00am from an arm vein. The serum obtained by centrifugation was stored at –70°C until analysis. The levels of the non-specific neuroendocrine tumour marker (CgA), specific neuroendocrine tumour markers (serotonin, 5HIAA), selected growth factors (VEGF, HGF) and binding proteins (VEGFR-1, VEGFR-2) were determined by enzyme-linked immunosorbent assay (ELISA). Serum levels of CgA and serotonin and 24-hour urine levels of 5HIAA were determined using the μQuant (Bio-Tek), the Serotonin ELISA (ALPCO) and the 5-HIAA ELISA (IBL International), respectively. The respective analytical sensitivities of these tests were as follows: 2.0 U/L (normal range: 2–18 U/L); 5 ng/mL (normal ranges: 80–450 ng/mL [women] and 40–400 ng/mL [men]) and 0.06 mg/L (normal range: 6–10 ng/mL). Serum levels of HGF, VEGF VEGFR-1 and VEGFR-2 were determined using the Quantikine (R&D Systems) at the analytical sensitivities of 40 pg/mL, 5 pg/mL, 3.5 pg/mL and 4.6 pg/mL, respectively.

The results were analysed using statistical methods. The analysis involved a comparison of the data in the study patient groups and an assessment of their correlations with the pTNM stage. Linear regression curves were applied to the observed correlations. The differences between the specific variables in the study patient groups were evaluated using univariate analysis of variance. P values of < 0.05 were considered statistically significant. The statistical calculations were performed

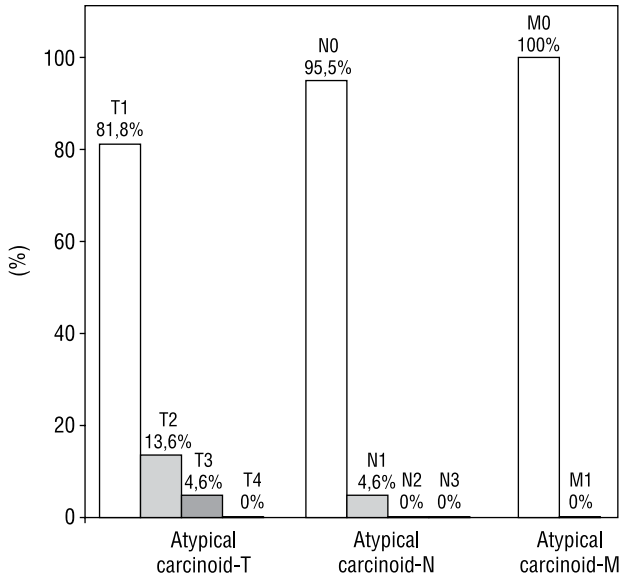

Figure 1. *Percentage distribution of patients with typical bronchopulmonary carcinoid tumours (Group 1) according to the TNM classification*

Rycina 1. *Rozkład procentowy grupy chorych z rakowiakami typowymi (grupa 1) w zależności od stopnia zaawansowania w skali TNM*

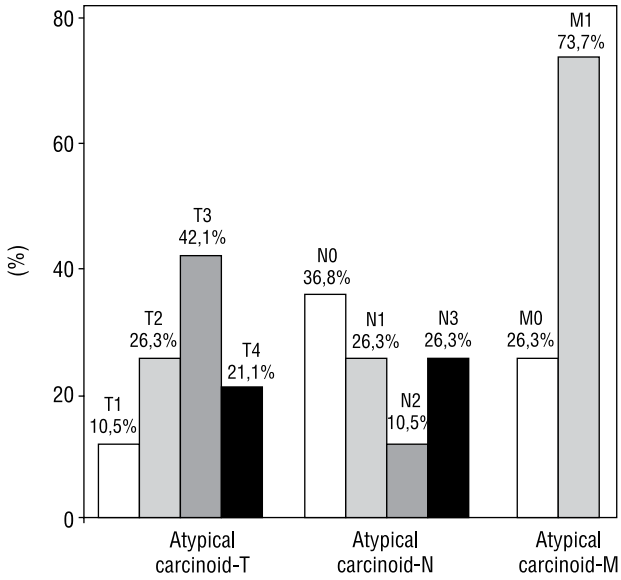

Figure 2. *Percentage distribution of patients with atypical bronchopulmonary carcinoid tumours (Group 2) according to the TNM classification*

Rycina 2. *Rozkład procentowy grupy chorych z rakowiakami atypowymi (grupa 2) w zależności od stopnia zaawansowania w skali TNM*

Table I. ***Mean levels of non-specific markers (CgA) and specific markers (serotonin and 5HIAA) in the study patient groups with BP NET and the control group***

Tabela I. ***Średnie stężenia markerów niespecyficznych (CgA) oraz specyficznych (serotoniny i 5HIAA) w grupach badanych***

	CgA [U/L] (normal range: 2–18 U/L)		Serotonin [ng/mL] (normal range: 80–450 ng/mL)		5HIAA [mg/24 h] (normal range: 2–6 mg/24 h)	
Typical carcinoid tumour group	13.02 (SD 9.10)	p = 0.026	164.18 (SD 242.00)	p = 0.014	5.75 (SD 3.48)	p = 0.048
Atypical carcinoid tumour group	1020.28 (SD 3638.46)	p = 0.018	363.94 (SD 333.98)	p = 0.021	50.32 (SD 148.90)	p = 0.032
Control group	8.09 (SD 6.23)		146.31 (SD 210.30)		4.23 (SD 2.69)	

using MedCalc. The values calculated for quantitative variables were expressed as arithmetic means.

Results

In the study patient population, serum levels of CgA and serotonin and 24-hour urine levels of 5HIAA were significantly elevated ($p < 0.02$, $p < 0.01$ and $p < 0.04$, respectively) in patients with atypical bronchopulmonary carcinoid tumours versus patients with typical carcinoid tumours and the control group (Table I).

The mean levels of CgA, serotonin and 5HIAA did not show any significant differences relative to the tumour size (T) or nodal invasion (N) but were significantly higher in patients with distant metastases (M). Summary results for patients with typical and atypical carcinoid tumours are provided in Figures 3 to 5.

Levels of selected growth factors (VEGF, HGF) were determined in the study patient groups and the control group.

Levels of VEGF (mean ± SD) in patients with BP NET were significantly higher than in the control group (359.26 ± 281.03 pg/mL v. 207.08 ± 157.84 pg/mL; $p = 0.0115$) (Fig. 6).

Levels of VEGF did not differ significantly between patients with typical lung carcinoid tumours and patients with atypical carcinoid tumours, and there

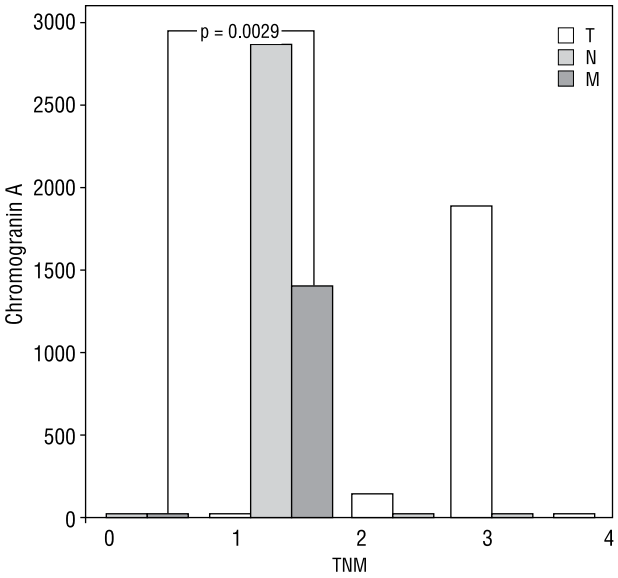

Figure 3. *CgA levels (U/L) according to the TNM classification in patients with carcinoid tumours of the lung*

Rycina 3. *Stężenia chromograniny A [U/l] w zależności od stopnia zaawansowania według skali TNM u chorych z rakowiakami płuc*

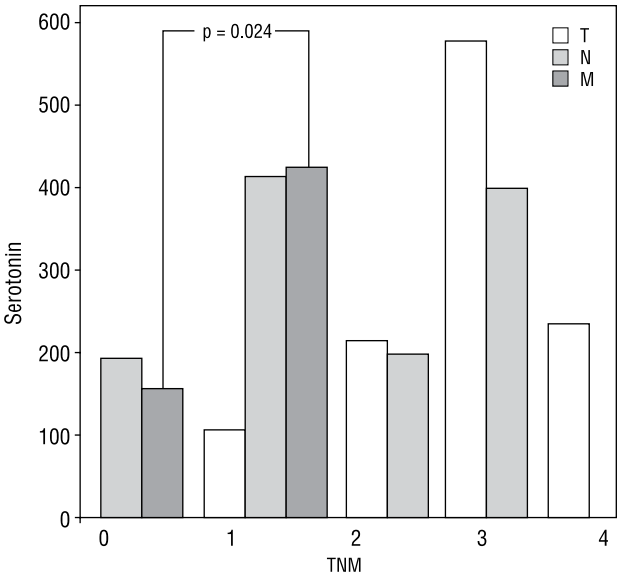

Figure 4. *Serotonin levels [ng/mL] according to the TNM classification in patients with carcinoid tumours of the lung*

Rycina 4. *Stężenia serotoniny [ng/ml] w zależności od stopnia zaawansowania według skali TNM u chorych z rakowiakami płuc*

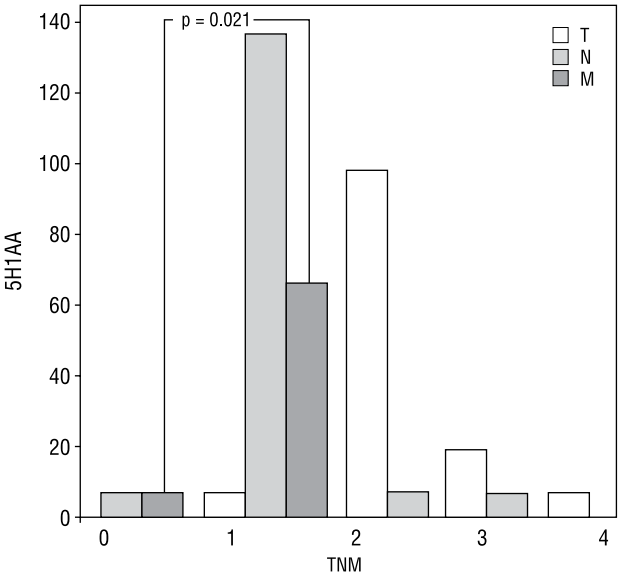

Figure 5. *5HIAA levels [mg/24 h] according to the TNM classification in patients with carcinoid tumours of the lung*

Rycina 5. *Stężenia kwasu 5HIO [mg/24 h] w zależności od stopnia zaawansowania według skali TNM u chorych z rakowiakami płuc*

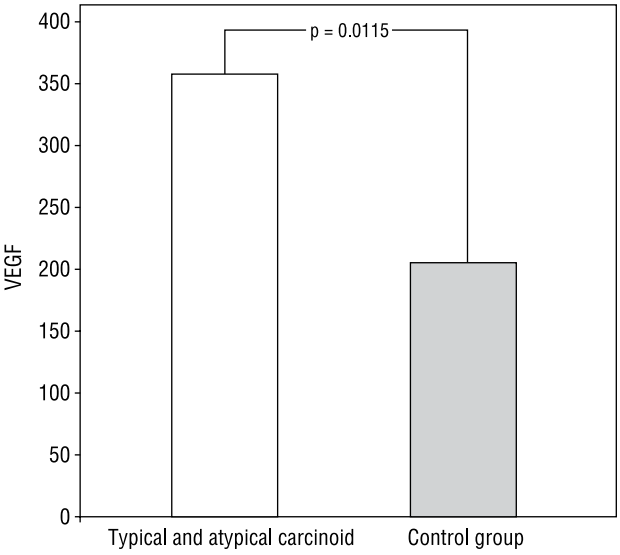

Figure 6. *Levels of VEGF (pg/mL) in patients with carcinoid tumours of the lung and in the control group*

Rycina 6. *Stężenia VEGF[pg/ml] u chorych z rakowiakami płuc oraz w grupie kontrolnej*

were no significant differences according to the TNM classification.

Assessment of VEGFR expression revealed that patients with neuroendocrine tumours of the lung showed higher expression of VEGFR1 than controls ($p = 0.002$). VEGFR1 levels were higher in the group of the most advanced tumours according to the TNM classification, taking into account the tumour size, nodal invasion and presence of distant metastases (Fig. 7). No such correlations were observed for VEGFR2.

HGF levels (mean ± SD) were significantly higher in patients with carcinoid tumours than in the control group (1,297.60 ± 362.19 pg/mL *v.* 996.04 ± 365.85;

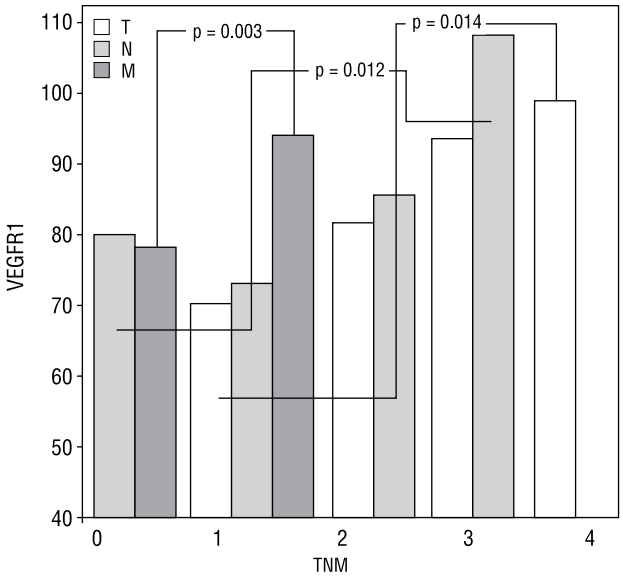

Figure 7. *Dependence between VEGFR1 expression [pg/mL] and the TNM classification in patients with bronchopulmonary carcinoid tumours*

Rycina 7. *Zależność ekspresji receptorów VEGFR1 [pg/ml] od stopnia zaawansowania w skali TNM w nowotworach neuroendokrynnych płuc*

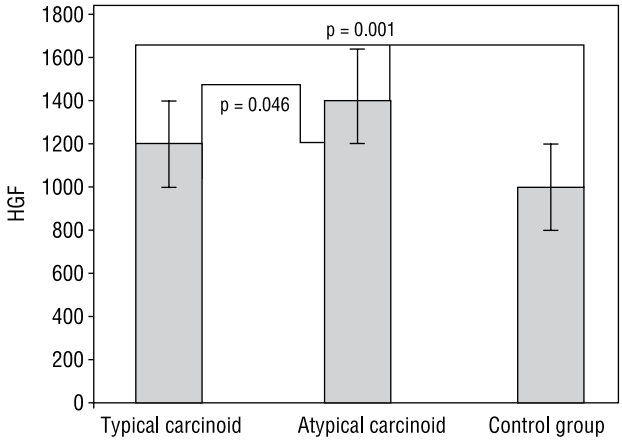

Figure 8. *Levels of HGF [pg/mL] in the two study patient groups (typical and atypical carcinoid tumours of the lung) and in the control group*

Rycina 8. *Stężenia HGF [pg/ml] w dwóch grupach badanych (rakowiak typowy i atypowy) oraz w grupie kontrolnej*

p = 0.001). HGF levels were also significantly higher in patients with atypical versus typical carcinoid tumours (p = 0.046) (Fig. 8). There was no correlation between HGF levels and TNM stage.

Discussion

Chromogranin A and serotonin are markers used in the laboratory evaluation of neuroendocrine tumours, although, as shown in other studies and confirmed in ours, their levels in bronchopulmonary neuroendocrine tumours may be within reference ranges or be slightly elevated before distant metastases appear (Fig. 3 and 4). CgA and serotonin levels were within reference ranges in Group 1 patients (none of whom had distant metastases), while patients in Group 2 had considerably elevated CgA levels (nearly 74% of these patients had distant metastases).

According to the available literature, 5HIAA levels are within reference ranges in patients with BP NET due to the lack of the enzyme aromatic amino acid decarboxylase, which we managed to confirm in the group of patients with typical bronchopulmonary carcinoid tumours. The high 5HIAA levels in the group of patients with atypical carcinoid tumours might be explained by the presence of metastases in the majority of these patients, with the metastases mostly involving the liver, where the enzyme necessary for serotonin metabolism is present (Table I). The absence of correlation between tumour size or nodal involvement and 5HIAA levels and the presence of a correlation between the presence of distant metastases and 5HIAA levels observed in our study confirms this finding (Fig. 5). These observations do suggest a role for these markers in the diagnosis of BP NET despite the many conflicting literature reports on their usefulness in these tumours.

Previous studies have shown that serum levels of VEGF and VEGFR correlate with the aggressiveness of tumours of various organs and targeted therapies that affect angiogenesis and target VEGF, among other factors, raising significant hopes among biologists, oncologists and chemotherapeutists [11–15].

Our study showed significantly higher serum levels of VEGF in patients with BP NET compared to the control group, which may suggest the potential usefulness of VEGF in the diagnosis, and a potential use of antiangiogenic agents in the treatment of these patients [16-20]. We did not observe any differences in VEGF levels relative to the primary tumour size, presence of distant metastases, or the histologic type (Fig. 6) and therefore could not confirm their usefulness as a prognostic factor. We did, however, obtain promising results when we evaluated VEGFR-1 levels. We observed significantly higher levels in patients versus controls, a close correlation between elevated levels and: primary tumour size, development of nodal involvement and development of distant metastases (Fig. 7), which — in addition to use in the diagnosis and treatment — seems to be an important prognostic factor, which obviously needs to be confirmed in multicentre randomised studies.

The available literature provides data on the significance of HGF in non-neuroendocrine small-cell and non-

small-cell lung carcinomas in the diagnosis, prediction of disease progression and assessment of response to drug treatment [16–18]. Based on the results of our study, where HGF levels were significantly higher in BP NET patients than in controls, it may be concluded that the histological type of the tumour (typical versus atypical carcinoid tumour) plays a significant role, while no correlation has been demonstrated between HGF levels and the TNM classification. It is worth noting that HGF levels were determined in serum rather than tissue samples, which might have affected the determination results in patients in various stages of the underlying illness.

It would be valuable to continue this study in a larger number of patients, as it would allow us to investigate the biology of tumours in greater detail, including the role of growth factors. This would, in turn, enable us to make better use of them in diagnostic evaluation.

In conclusion, our study has shown a potential usefulness of determining the levels of specific markers (serotonin, 5HIAA) and non-specific markers (CgA) in the diagnosis and in the evaluation for distant metastases in bronchopulmonary carcinoid tumours, particularly in atypical tumours. We see a potential role for the selected growth factors, VEGF and HGF, and of the VEGFR1 in the clinical diagnosis of these tumours, while the assessment of VEGFR-1 expression may be useful in tumour staging.

References

1. Corrin B, Nicholson AG. Pathology of the lungs. Churchill Livingstone 2006: 1–35: 527–608.
2. Lim E., Goldstraw P., Nicholson A.G. i wsp. Proceedings of the IASLC International Workshop on Advances in Pulmonary Neuroendocrine Tumors 2007. J. Thorac Oncol 2008; 3: 1194–1201.
3. Warren W.H., Welker M., Gattuso P. Well-differentiated neuroendocrine carcinomas: the spectrum of histologic subtypes and various clinical behaviors. Semin Thorac Cardiovasc Surg 2006; 18: 199–205.
4. Morandi U, Casali C, Rossi G. Bronchial typical carcinoid tumors. Semin Thorac Carciovasc Surg 2006; 18: 191–198.
5. Travis WD, Brambilla E, Muller-Hermelink HG. Pathology and genetics: tumours of the lung, pleura, thymus and heart. IARC Press, Lyon 2004.
6. Prager GW, Poettler M. Angiogenesis in cancer. Basic mechanisms and therapeutic advances. Hamostaseologie 2012; 32: 105–114.
7. Cecchi F, Rabe DC, Bottaro DP. Targeting the HGF/Met signalling pathway in cancer. Eur J Cancer. 2010; 46: 1260–1270.
8. Gumustekin M, Kargi A, Bulut G et al. HGF/c-Met Overexpressions, but not Met Mutation, Correlates with Progression of Non-small Cell Lung Cancer. Pathol Oncol Res 2012; 18: 209–218.
9. Korobko IV, Zinov'eva MV, Allakhverdiev AK et al. c-Met and HGF expression in non-small-cell lung carcinomas. Mol Gen Mikrobiol Virusol 2007; 2: 18–21.
10. William D Travis. Reporting lung cancer pathology specimens. Impact of the anticipated 7th Edition TNM Classification based on recommendations of the IASLC Staging Committee. Histopathology 2009; 54: 3–11.
11. Bich Trinh X, van Dam PA, Vermeulen PB et al. VEGF-A-independent and angiogenesis-dependent tumour growth in patients with metastatic breast cancer. Clin Transl Oncol 2011; 13: 805–808.
12. Pircher A, Hilbe W, Heidegger I et al. Biomarkers in tumor angiogenesis and anti -angiogenic therapy. Int J Mol Sci 2011; 12: 7077–7099.
13. Kajdaniuk D, Marek B, Foltyn W et al. Vascular endothelial growth factor (VEGF) — part 2: in endocrinology and oncology. Endokrynol Pol 2011; 62: 456–64.
14. Zhao S, Ma D, Dai H i wsp. Biologically inhibitory effects of VEGF siRNA on endometrial carcinoma cells. Arch Gynecol Obstet 2011; 284: 1533–1541.
15. Blakely C, Jahan T. Emerging antiangiogenic therapies for non-small-cell lung cancer. Expert Rev Anticancer Ther 2011; 11: 1607–1618.
16. Han JY, Kim JY, Lee SHet al. Association between plasma hepatocyte growth factor and gefitinib resistance in patients with advanced non-small cell lung cancer. Lung Cancer 2011; 74: 293–299.
17. Ma PC, Tretiakova MS, Nallasura V, Jagadeeswaran R et al. Downstream signalling and specific inhibition of c-MET/HGF pathway in small cell lung cancer: implications for tumour invasion. Br J Cancer 2007; 97: 368–377.
18. Ma PC, Blaszkowsky L, Bharti A et al. Circulating tumor cells and serum tumor biomarkers in small cell lung cancer. Anticancer Res 2003; 23: 49–62.
19. Kajdaniuk D, Marek B, Foltyn W et al. Vascular endothelial growth factor (VEGF) — part 1: in physiology and pathophysiology. Endokrynol Pol 2011; 62: 444–455.
20. Wiedenmann B, Pavel M, Kos-Kudla B. From targets to treatments: a review of molecular targets in pancreatic neuroendocrine tumors. Neuroendocrinology 2011; 94: 177–190.