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Is determination of matrix metalloproteinases and their tissue inhibitors serum concentrations useful in patients with gastroenteropancreatic and bronchopulmonary neuroendocrine neoplasms?

Czy ocena stężeń metaloproteinaz i ich tkankowych inhibitorów może być przydatna u chorych z nowotworami neuroendokrynnymi układu pokarmowego i płuc?

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Abstract

Introduction: Gastroenteropancreatic (GEP) and bronchopulmonary (BP) neurendocrine neoplasms (NENs) are rare and slowly growing tumours. Matrix metalloproteinases (MMPs) degrade extracellular matrix and are responsible for invasion and metastasis. Tissue inhibitors of matrix metalloproteinases (TIMPs) affect the invasiveness of tumour cells and the formation of distant metastases. The aim of this study was to evaluate selected MMPs (MMP2 and MMP9) and their tissue inhibitors (TIMP1 and TIMP2) depending on the pTNM classification, grading, and the occurrence of metastases.
Material and methods: The study group consisted of 86 patients with GEP NENs. The control group consisted of 31 healthy volunteers. Serum levels of TIMP1, TIMP2, MMP2 and MMP9 were determined by ELISA (R&D Systems) in all the study subjects. The statistical calculations were performed using MedCalc.
Results: We observed significant differences in MMP2 and TIMP1 levels between the study group with NENs and the control group. TIMP1 levels were significantly higher in patients with high-grade NEN (NEC, neuroendocrine carcinoma) compared to patients with low-grade tumour (NET G1, neuroendocrine tumours G1) ($p < 0.017$). We also observed a significant correlation between TIMP1 levels and the presence of metastases in the group of patients with GEP NENs, and also higher TIMP1 levels than those in the patients without metastases ($p < 0.05$). We also found a higher likelihood of metastases in patients with GEP NENs with TIMP1 levels exceeding 206.4 ng/mL.
Conclusions: Patients with NENs secreted larger quantities of MMP2 and TIMP1. TIMP1 may be considered a marker of metastases in patients with GEP NENs. **(Endokrynol Pol 2012; 63 (6): 470–476)**

Key words: neuroendocrine neoplasms, tissue inhibitors of matrix metalloproteinases, metalloproteinases, metastases

Streszczenie

Wstęp: Nowotwory neuroendokrynne (NEN) układu pokarmowego (GEP) i płuc (BP) należą do rzadkich i wolno rosnących nowotworów. Metaloproteinazy macierzy zewnątrzkomórkowej (MMPs) mają zdolność degradacji macierzy zewnątrzkomórkowej oraz są odpowiedzialne za inwazje i powstawanie przerzutów. Tkankowe inhibitory metaloproteinaz (TIMPs) wpływają na inwazyjność komórek nowotworowych, redukcję wzrostu guza i tworzenia przerzutów odległych. Celem pracy była ocena stężeń wybranych metaloproteinaz (MMP2 i MMP9) i ich tkankowych inhibitorów (TIMP1 i TIMP2) w zależności od klasyfikacji pTNM, stopnia zróżnicowania nowotworu oraz występowania przerzutów.
Materiał i metody: Grupę badaną stanowiło 86 chorych z GEP NEN, a grupę kontrolną 31 zdrowych ochotników. U wszystkich badanych wykonano oznaczenia stężeń TIMP1, TIMP2, MMP2, MMP9 w surowicy krwi metodą ELISA (R&D System). Obliczeń statystycznych dokonano za pomocą programu MedCalc.
Wyniki: W grupie badanej z NEN zaobserwowano znamienne różnice w stężeniu MMP2 i TIMP1 w porównaniu z grupą kontrolną. Stężenie TIMP 1 było znamiennie wyższe u chorych z rakiem neuroendokrynnym (NEC) w porównaniu z grupą z NET G1 ($p < 0,017$). Zaobserwowano również istotną korelacje pomiędzy stężeniem TIMP 1 a obecnością przerzutów w grupie chorych z GEP NEN oraz wyższe stężenie TIMP1 w surowicy krwi w porównaniu z chorymi bez obecności przerzutów ($p < 0,05$). Stwierdzono również większe prawdopodobieństwo wystąpienia przerzutów u chorych z GEP NEN, gdy stężenie TIMP1 wynosiło powyżej 206,4 ng/ml.
Wnioski: U chorych z NEN występuje zwiększone wydzielanie MMP2 i TIMP1. TIMP 1 może być rozpatrywany jako wskaźnik świadczący o wystąpieniu przerzutów u chorych z GEP NEN. **(Endokrynol Pol 2012; 63 (6): 470–476)**

Słowa kluczowe: nowotwory neuroendokrynne, inhibitory tkankowe metaloproteinaz, metaloproteinazy, przerzuty

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Introduction

Gastroenteropancreatic neuroendocrine neoplasms (GEP NENs) are a heterogenous group of slow growing tumours originating in the diffuse neuroendocrine system. GEP NENs remain clinically latent for years and the diagnosis is most commonly established when the disease is already disseminated [1–3]. The histological and pathological staging of the tumours in patients with GEP NENs is based on the assessment of the tumour size (T), the presence of nodal metastases (N), and presence of distant metastases (M).

There is an ongoing search for new markers that will help to establish the diagnosis and prognosis and to monitor treatment. Most of the existing markers are used to monitor patients already diagnosed with cancer, as they do not meet the stringent criteria that would enable them to be used in the diagnostic process (i.e. low sensitivity and specificity in early disease) [4]. Non-specific markers of NENs include: chromogranin A (CgA) and neuron-specific enolase (NSE) [5, 6].

New tumour markers that are currently being investigated include matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs). MMPs are a group of proteases that are activated by zinc and calcium ions (Zn^{2+} and Ca^{2+}, respectively) [7]. Of the 22 MMPs identified in humans so far, MMP2 and MMP9 exhibit proneoplastic properties and degrade various types of collagen, such as type IV collagen, which is the principal constituent of vascular basal membranes [8]. By degrading the elements of the extracellular matrix (ECM), MMP2 and MMP9 are involved in the migration of both endothelial and tumour cells, which results in
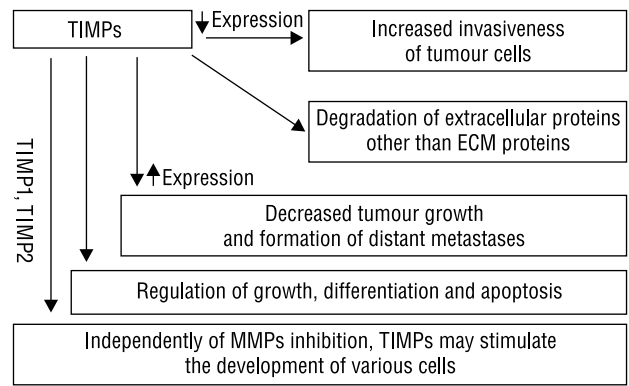

Figure 1. *The effects of tissue inhibitors of metalloproteinases (TIMPs) in tumours*

Rycina 1. *Schemat działania tkankowych inhibitorów metaloproteinaz (TIMPs) w nowotworach*

the formation of new vessels and new tumour foci and therefore metastases [9].

TIMPs are proteins that inhibit the proteolytic activity of MMPs. They affect the invasiveness of tumour cells, reduce tumour size and suppress the formation of distant metastases, regulate growth, differentiation and apoptosis and, independently of the inhibition of MMPs, are capable of stimulating the development of various cells, including tumour cells (Fig. 1). Four types of TIMPs have so far been identified (TIMP1 to TIMP4). The commonest TIMPs in tissues are TIMP1 and TIMP2 [10].

While the formation of TIMPs and MMPs in various types of tumour have been documented in numerous studies (Table I), no such data is available for NENs.

Table I. *Comparison of serum levels of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in patients with various tumours*

Tabela I. *Porównanie stężeń wybranych metaloproteinaz (MMPs) i ich tkankowych inhibitorów (TIMPs) w surowicy krwi u chorych z różnymi rodzajami nowotworów*

Tumour type	MMP2	MMP7	MMP9	TIMP1	TIMP2
Breast cancer [11, 12]	—	—	—	↑	↑
Lung cancer [13, 14]	↑	—	↑	↑	—
Colon cancer [15, 16]	—	↑	↑	—	—
Rectal cancer [17]	—	—	—	↑	—
Stomach cancer [18, 19]	—	—	—	↑	—
Pancreatic cancer [20, 21]	↑	—	↑	—	—
Ovarian cancer [22]	—	—	—	↑	—
Bladder cancer [23, 24]	↑	↑	—	—	↑
Prostate cancer [25]	↑	—	—	—	—
Neuroendocrine neoplasms	—	—	—	—	—

— no data available; ↑ elevated serum levels

Searching for new markers which would be helpful in the diagnosis and monitoring of cancer and which would translate into further patient management, offers new options for patients with NENs. The use of MMP inhibitors may be a new form of therapy for these patients, as these agents may not only affect tumour size reduction, but also the suppression of the formation of distant metastases.

In order to determine the usefulness of selected MMPs (MMP2 and MMP9) and TIMPs (TIMP1 and TIMP2) in patients with GEP NENs and BP NENs:

We evaluated serum levels of selected MMPs and TIMPs in patients with GEP NENs and BP NENs and in a control group.

We compared serum levels of selected MMPs and TIMPs depending on the pTNM classification, grading, and the presence of metastases.

Material and methods

We enrolled 86 patients with the diagnosis of GEP NEN or BP NEN aged 25 to 85 years (mean age: 57.2 years), including 52 women and 34 men. Based on their clinical and histopathological presentation, the patients were subdivided into two study groups:
— A group of patients with GEP NENs (n = 62) (Subgroup 1)
— A group of patients with BP NENs (n = 24) (Subgroup 2)

The control group consisted of 31 healthy volunteers aged 39 to 78 years (mean age: 53.7 years).

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

Inclusion criteria
Adults over the age of 18 years with a diagnosis of GEP NEN or BP NEN who provided written informed consent were included in the study.

Exclusion criteria
Patients with GEP NENs or BP NENs who refused to provide written informed consent, patients below 18 years of age, pregnant or breastfeeding women, patients with end-stage liver disease, patients with stage IV or V chronic kidney disease, patients with advanced heart failure, and patients with other tumours were excluded from the study.

Methods
Fasting blood samples for the determination of hormones were collected at 8.00am from an arm vein. The samples were centrifuged to yield serum samples, which were stored at –70°C until analysis.

Levels of the following neuroendocrine tumour markers were determined in all the patients: chromogranin A, serotonin and 5-hydroxyindoleacetic acid.

Serum levels of MMP2, MMP9, TIMP1 and TIMP2 were determined by enzyme-linked immunosorbent assay (ELISA) using the Quantikine kit from R&D Systems GmbH, Wiesbaden-Norderstadt, Germany, at analytical sensitivities of 0.16 ng/mL, 0.156 ng/mL, 0.08 ng/mL and 0.011 ng/mL, respectively.

The resulting data was subjected to statistical analysis. This involved comparing data in the study groups and assessing its correlation with the pTNM classification, grading and the presence of metastases.

The statistical calculations were performed using MedCalc. Values of measureable variables were presented as arithmetic means with standard errors of the mean (SEM). The distributions were checked for normality using the Kolmogorov-Smirnov test. The relationships between normally distributed variables were assessed using Pearson's correlation. The relationships between variables lacking normal distribution were assessed using Spearman's rank correlation coefficient, and the differences between quantitative variables and qualitative variables were assessed with non-parametric tests: the Mann-Whitney test for two groups and the Kruskal-Wallis test for three or more groups. Linear regression curves were presented for the observed correlations. The differences between individual variables in specific groups were assessed using univariate analysis of variance.

Results

In the group of patients with GEP NENs, 52% (32/62) were patients with intermediate-grade NEN (NET G2) and 35% (22/62) with low-grade tumour (NET G1) according to the 2010 WHO classification. Neuroendocrine carcinoma (NEC) was present in 13% of the patients (8/62). We assessed and compared serum levels of selected MMPs (MMP2 and MMP9) and TIMPs (TIMP1 and TIMP2) in the group of patients with NENs (n = 86) and in the control group (n = 31), as shown in Table II.

A similar assessment of serum levels of selected MMPs and TIMPs was performed in the subgroups of GEP NEN patients (Table III) and BP NEN patients (Table IV). In the group of patients with GEP NENs, higher serum levels of TIMP1 and MMP2 versus controls were found (p < 0.05 for both comparisons). In the group of patients with BP NENs on the other hand, only the levels of TIMP1 were higher compared to the control group (p = 0.024).

Serum levels of MMP2, MMP9, TIMP1 and TIMP2 in patients with GEP NENs and BP NENs were assessed depending on the pTNM classification. The

Table II. *Comparison of serum levels of matrix metalloproteinase 2 (MMP2), matrix metalloproteinase 9 (MMP9), tissue inhibitor of metalloproteinase 1 (TIMP1), and tissue inhibitor of metalloproteinase 2 (TIMP2) in patients with neuroendocrine neoplasms (NEN, n = 86) versus controls (n = 31)*

Tabela II. *Porównanie stężeń metaloproteinazy 2 (MMP2), metaloproteinazy 9 (MMP9), tkankowego inhibitora metaloproteinazy 1 (TIMP 1), tkankowego inhibitora metaloproteinazy 2 (TIMP2) w surowicy krwi u chorych z nowotworami neuroendokrynnymi (NEN, n = 86) w porównaniu z grupą kontrolną (GK, n = 31)*

	Study group NEN (n = 86)		Control group (n = 31)		p value
	Mean	SEM	Mean	SEM	
MMP2 [ng/mL]	223.35	6.77	194.77	10.17	0.03
MMP9 [ng/mL]	300.27	23.49	278.49	37.76	> 0.05
TIMP1 [ng/mL]	233.91	12.84	164.68	7.32	0.002
TIMP2 [ng/mL]	111.62	2.47	103.34	3.13	> 0.05

Table III. *Comparison of serum levels of matrix metalloproteinase 2 (MMP2), tissue inhibitor of metalloproteinase 1 (TIMP1), and tissue inhibitor of metalloproteinase 2 (TIMP2) in patients with gastroenteropancreatic neuroendocrine neoplasms (GEP NEN subgroup, n = 62) versus controls (n = 31)*

Tabela III. *Porównanie stężeń metaloproteinazy 2 (MMP2), tkankowego inhibitora metaloproteinazy 1 (TIMP1) i tkankowego inhibitora metaloproteinazy 2 (TIMP2) w surowicy krwi u chorych z nowotworami neuroendokrynnymi układu pokarmowego (GEP NEN, n = 62) w porównaniu z grupą kontrolną (GK, n = 31)*

	GEP NEN subgroup (n = 62)		Control group (n = 31)		p value
	Mean	SEM	Mean	SEM	
MMP2 [ng/mL]	228.38	8.12	194.77	10.17	< 0.05
TIMP1 [ng/mL]	246.05	16.73	164.68	7.32	< 0.05
TIMP2 [ng/mL]	112.41	3.01	103.34	3.13	0.064

Table IV. *Comparison of serum levels of tissue inhibitor of metalloproteinase1 (TIMP1) and tissue inhibitor of metalloproteinase 2 (TIMP2) in patients with bronchopulmonary neuroendocrine neoplasms (BP NEN subgroup, n = 24) versus controls (n = 31)*

Tabela IV. *Porównanie stężeń tkankowego inhibitora metaloproteinazy 1 (TIMP1) i tkankowego inhibitora metaloproteinazy 2 (TIMP2) w surowicy krwi u chorych z nowotworami neuroendokrynnymi płuc (BP NEN, n = 24) w porównaniu z grupą kontrolną (GK, n = 31)*

	BP NEN subgroup (n = 24)		Control group (n = 31)		p value
	Mean	SEM	Mean	SEM	
TIMP1 [ng/mL]	206.29	17.61	164.68	7.32	0.024
TIMP2 [ng/mL]	110.18	4.62	103.34	3.13	> 0.05

largest and the smallest primary tumours in patients with a known primary tumour size were 60 mm and 2 mm, respectively. Nearly half of the patients with GEP NENs showed lymph node involvement. In a third of the patients, it was not possible to determine whether the lymph nodes were invaded or not. Half of the patients had distant metastases. Higher values of TIMP1 were found in patients with nodal involvement (N1) compared to patients in whom it was not possible to determine nodal status (Nx) according to the pTNM classification (p = 0.031). TIMP1 levels were also higher in the group of patients with distant metastases (M1)

compared to patients without distant metastases (M0) (p = 0.016) (Fig. 2).

No statistically significant differences were shown in serum levels of the remaining MMPs (MMP2 and MMP9) and TIMP2 relative to the presence of distant metastases.

Serum levels of MMP2, MMP9, TIMP1 and TIMP2 in patients with GEP NENs and BP NENs were assessed relative to tumour grade. Elevated levels of TIMP1 were observed in patients with NEC, which showed statistical significance versus patients with G1 tumours (Fig. 3).
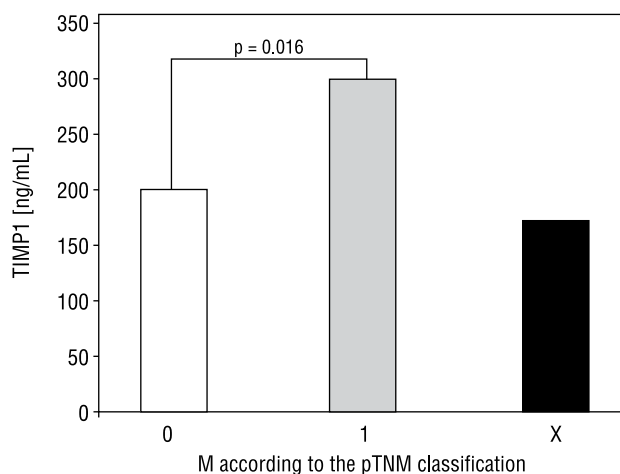
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Figure 2. *Comparison of serum levels of tissue inhibitor of metalloproteinase 1 (TIMP1) [ng/mL] relative to the presence of distant metastases (M) according to the pTNM classification in patients with gastroenteropancreatic neuroendocrine neoplasms (GEP NENs, n = 62). Mx — assessment of distant metastases not possible. M0 — absence of distant metastases; M1 — presence of distant metastases*

Rycina 2. *Porównanie stężeń tkankowego inhibitora metaloproteinazy 1 (TIMP1) [ng/ml] w zależności od obecności przerzutów odległych (M) z uwzględnieniem klasyfikacji pTNM u chorych z nowotworami neuroendokrynnymi układu pokarmowego (GEP NEN, n = 62)*

An attempt to analyse the relationship between selected MMPs and TIMPs and the pTNM classification was also made in the group of patients with BP NENs, but due to the insufficient size of the groups this statistical analysis was not performed.

Serum levels of MMP2, MMP9, TIMP1 and TIMP2 in GEP NEN patients and BP NEN patients were assessed relative to the presence and type of metastases. In more than half (36/62) of the patients with GEP NENs, metastases were present, with liver metastases being the commonest (4/5 of the patients with GEP NENs). Other locations of metastases included the lymph nodes, bone and other organs, such as the ovaries, cerebellum, and adrenal glands. In the group of BP NEN patients, metastases were present in a lower percentage of patients (21%). Each of the patients had nodal metastases. Liver metastases were observed in three out of 24 patients; in one out of 24 the disease had spread to the contralateral lung, and in one out of 24 to the scalp. Patients with GEP NENs without metastases had lower levels of TIMP1 compared to those with metastases ($p < 0.05$) (Fig. 4).

We observed a higher likelihood of metastases at TIMP1 levels exceeding 206.4 ng/mL (AUC 0.78, $p < 0.05$) (Fig. 5 and 6). No such relationships were observed between serum levels of the MMP2, MMP9 and TIMP2 in GEP NEN patients with metastases.
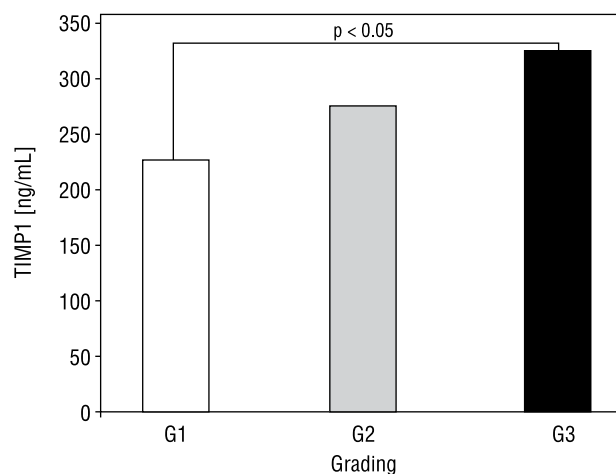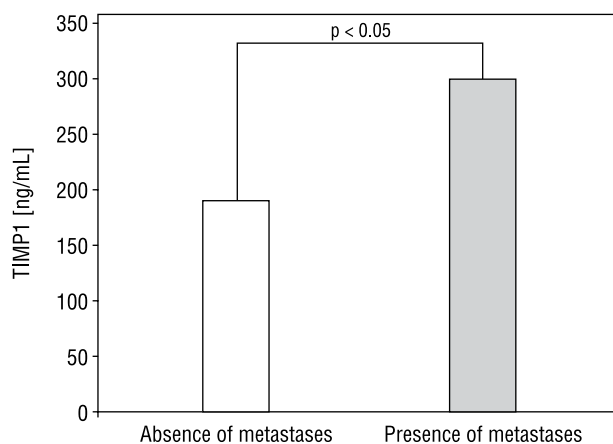

Figure 3. *Comparison of serum levels of tissue inhibitor of metalloproteinase 1 (TIMP1) [ng/mL] in patients with gastroenteropancreatic neuroendocrine neoplasms (GEP NENs subgroup, n = 62) relative to grading*

Rycina 3. *Porównanie stężenia tkankowego inhibitora metaloproteinazy 1 (TIMP1) [ng/ml] u chorych z GEP NEN w zależności od stopnia zróżnicowania nowotworu (grading)*

Figure 4. *Comparison of serum levels of tissue inhibitors of metalloproteinase 1 (TIMP1) [ng/mL] in patients with gastroenteropancreatic neuroendocrine neoplasms (GEP NENs, n = 62) relative to the presence or absence of metastases*

Rycina 4. *Porównanie stężeń tkankowego inhibitora metaloproteinazy 1 (TIMP1) [ng/ml] u chorych z nowotworami neuroendokrynnymi układu pokarmowego (GEP NEN, N=62) w stosunku do obecności przerzutów*

Due to the small size of the group of BP NEN patients with metastases (n = 5), we did not perform a statistical analysis to compare serum levels of the selected MMPs and TIMPs relative to the pTNM classification.

Discussion

The available bibliography contains data on overexpression of selected MMPs and TIMPs in many tumours. In our study, we set out to determine whether new markers
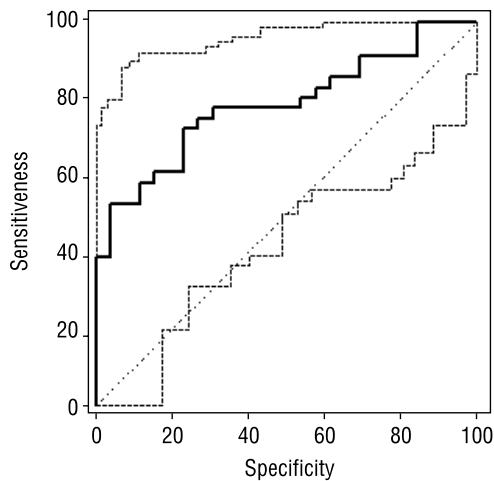
Figure 5. *ROC curve illustrating the likelihood of metastases in patients with gastroenteropancreatic neuroendocrine neoplasms (GEP NENs subgroup, n = 62) relative to serum levels of tissue inhibitor of metalloproteinase 1 (TIMP1) (AUC 0.78)*

Rycina 5. *Krzywa ROC przedstawiająca prawdopodobieństwo wystąpienia przerzutów u chorych z nowotworami neuroendokrynnymi układu pokarmowego (GEP NEN, n = 62) do stężenia tkankowego inhibitora metaloproteinazy 1 (TIMP1) (AUC = 0,78)*

Figure 6. *Comparison of serum levels of tissue inhibitor of metalloproteinase 1 (TIMP1) [ng/mL] in patients with gastroenteropancreatic neuroendocrine neoplasms (GEP NENs, n = 62) relative to the presence or absence of metastases at the cutoff point for TIMP1 level of 206.4 ng/mL*

Rycina 6. *Porównanie stężeń tkankowe inhibitora metaloproteinazy TIMP1 [ng/ml] u chorych z nowotworami neuroendokrynnymi układu pokarmowego GEP NEN (N = 62) w zależności od obecności lub braku przerzutów przy punkcie odcięcia dla stężenia TIMP1 wynoszącego 206,4 ng/ml*

of NENs could be found among MMPs and TIMPs. In the entire study group, in GEP NEN patients and BP NEN patients, we found elevated serum levels of MMP2 and TIMP1 compared to the control group. When we analysed serum levels of the selected MMPs and TIMPs in individual study groups, we found that patients with GEP NENs had elevated MMP2 and TIMP1 levels, while in BP NEN patients, only serum levels of TIMP1 were elevated compared to the control group. Similarly to our study, other studies have also shown elevated serum levels of MMP2 in patients with other tumours, including pancreatic, bladder, breast and prostate cancers [20, 24, 25] and elevated serum levels of TIMP1 in patients with stomach, rectal, ovarian and lung cancers [17, 18].

No data is, however, available on serum levels of MMPs and TIMPs in patients with NENs. There has been only one publication on the expression of MMPs and TIMPs in tissues of patients with a neuroendocrine tumour of the ileum. In that publication, Voland et al. [26] assessed, in tissues of the primary tumour, the expression of a marker of enterochromaffin (EC) cells, the so-called vesicular monoamine transporter 1 (VMAT-1), and of selected MMPs (MMP2, MMP7, MMP9, MMP11 and MMP13) and TIMPs (TIMP1, TIMP2 and TIMP3) in 25 patients with carcinoids originating in the EC cells, and compared it to the expression of these markers in nodal and liver metastases. The authors found increased expression of VMAT-1, MMP2, MMP11, TIMP1 and TIMP3 in tumour tissue compared to healthy tissues.
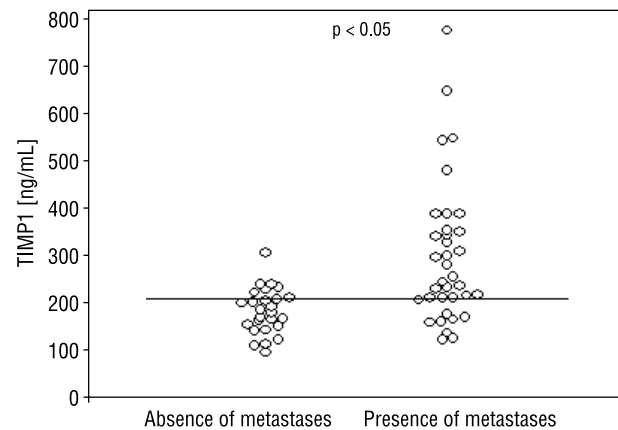
In a cancer, the formation of metastases is a complex, multistep process, determined by such factors as: proteolytic activity of tumour cells, their migratory abilities, proliferative activity and ability to neovascularisation [27, 28]. Tumour cells use MMPs to form a neoplastic infiltrate and to migrate in the process of metastasising [29]. Based on numerous, studies the role of MMPs in disseminated disease has been confirmed [30, 31]. Increased expression of TIMP1 is associated with a more advanced stage and a more aggressive course of the disease [32, 33], which has been confirmed in our study. In the group of patients with GEP NENs but without metastases, we found lower serum levels of TIMP1 compared to GEP NEN patients with metastases, which was confirmed in the assessment of TIMP1 levels relative to the pTNM classification of these patients. In the group of patients with GEP NENs, metastatic disease was present in 58% of the patients. The liver was the commonest site for metastases (83% of the patients). We observed higher levels of TIMP1 in the group of patients with GEP NENs and metastases (M1) compared to those without metastases (M0). We discovered that patients with GEP NENs were more likely to have metastases at TIMP1 levels exceeding 206.4 ng/mL. When we analysed levels of the remaining parameters in patients with GEP NENs with metastases, we did not find such relationships. This may suggest that TIMP1 could be a helpful marker for the assessment of the stage of the disease. Similar observations to these, but with respect to other tumour types, have been made by Chia-Siu Wang et

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al. [18]. In a group of 170 patients with stomach cancer, they assessed serum levels of TIMP1 and found that it correlated with nodal metastases and distant metastases to the liver and peritoneum. The authors suggested that determination of TIMP1 in the serum of patients with stomach cancer could become a promising marker for determining the progression and stage of cancer. In the already cited study by Voland et al. [26], which assessed the expression of MMPs and TIMPs in primary tumour tissue in patients with NENs, a higher expression of MMP2, MMP9, TIMP1, TIMP2 and TIMP3 was found in patients with liver metastases (M1) compared to those without metastases (M0). Mroczko et al. [34] found that serum levels of TIMP1 in patients with colorectal cancer correlated with the stage of the disease, nodal involvement and the presence of distant metastases.

Comparing our findings to those of Voland et al. [26], we showed different results, although the study above assessed expression of TIMP1 in primary focus of tumour rather than its serum levels. A lower expression of TIMP1 in primary tumour tissue does not rule out elevated serum levels of TIMP1 in these patients, which may result from the formation of TIMP1 by the cells of metastatic tumours that may show a different biological behaviour compared to the cells of the primary tumour.

Conclusions

Determination of serum levels of MMP2 and TIMP1 may be useful in NENs. Patients with NENs have increased secretion of MMP2 and TIMP1. TIMP1 may be considered a marker of metastases in patients with GEP NENs. The higher levels of TIMP1 in patients with metastases, and consequently with a more advanced stage of the disease, supports the use of this factor for prognostic purposes in this group of patients.

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