Evaluation of selected circulating cytokines from the IL-6 family – interleukin 6, oncostatin M, and cardiotrophin-1 – in gastro-entero-pancreatic and bronchial neuroendocrine tumours

Janusz Strzelczyk[1], Ksenia Janas[1], Joanna Katarzyna Strzelczyk[2], Elżbieta Chełmecka[3], Dariusz Kajdaniuk[4], Beata Kos-Kudła[1]

[1]Department of Endocrinology and Neuroendocrine Tumors, Department of Pathophysiology and Endocrinology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Katowice, Poland
[2]Department of Medical and Molecular Biology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Katowice, Poland
[3]Department of Medical Statistics, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice, Poland
[4]Department of Pathophysiology, Chair of Pathophysiology and Endocrinology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Katowice, Poland

Introduction: The incidence of neuroendocrine tumours (NETs) increased over the last years. Most of them are non-functioning, and the course of the disease is asymptomatic for a long time. This results in late diagnosis at an advanced stage. The aim of our study was the evaluation of selected circulating cytokines of interleukin-6 family – interleukin 6 (IL-6), oncostatin M (OSM), and cardiotrophin-1 (CT1) – in NETs.

Material and methods: The study group comprised 80 patients (56%) in several subgroups, including gastroenteropancreatic (GEPNETs, $n = 64$, 80%) and bronchopulmonary neuroendocrine tumours (BPNETs, $n = 16$; 20%). Serum IL-6, OSM, and CT1 concentrations were tested using ELISA.

Results: The median concentration of IL-6 was 41.5 pg/ml in the study group and 32.6 pg/ml in the control group, and the difference was statistically significant ($p < 0.001$). The concentration of OSM was significantly lower in the study group than in the control group ($p < 0.001$), at 105.6 pg/ml and 115.5 pg/ml, respectively. There was a significant difference ($p < 0.01$) in concentration of CT1 in the study group (222.0 pg/ml) and controls (267.2 pg/ml). Our investigation into selected IL-6 family cytokines revealed differential modulation of signal transduction pathways.

Conclusions: These findings suggest that despite utilising a common signalling transducer, individual IL-6 family cytokines exert distinct biological effects on neuroendocrine tumour development. Notably, IL-6 appears to promote tumourigenesis, while OSM and CT1 exhibit inhibitory effects on gastro-entero-pancreatic and bronchial neuroendocrine tumour development. Further studies are necessary to validate the diagnostic utility of IL-6 family cytokines in NETs.

Key words: neuroendocrine tumours, cytokines, IL-6 family, IL-6, OSM, CT1, biomarker, GEPNET, BPNET.

Introduction

Neuroendocrine tumours (NETs) arise from endocrine cells located in various parts of the body. Over two-thirds of NETs derive from the gastrointestinal tract (gastro-entero-pancreatic neuroendocrine tumours, GEPNETs), followed by bronchopulmonary NETs (BPNETs) [1, 2]. The incidence of NETs has increased over the last decades. According to studies performed in the USA (the Surveilance, Epidemiology, and End Results [SEER] Program), the incidence increased from 1.09/100,000 in 1973 to 6.98/100,000 in 2012 [3].

Based on Ki-67 proliferation index, the 2019 World Health Organisation (WHO) classification divided GEP-NETs into the following categories: well differentiated NETs G1 (Ki-67 < 3%), G2 (Ki-67 from 3–20%) and G3 (Ki-67 > 20), and poorly differentiated small- and large-cell neuroendocrine cancers (NECs) (Ki-67 > 20%) [4]. According to the 2021 WHO classification of lung tumours, broncho-pulmonary neuroendocrine tumours (BPNETs) are divided into 4 groups: typical carcinoids/neuroendocrine tumours grade 1, atypical carcinoids/neuroendocrine tumours grade 2, large cell neuroendocrine carcinomas, and small cell carcinomas [5].

The unique feature of NETs is their ability to produce and secrete hormones and biogenic amines, leading to distinctive clinical symptoms (functional tumours). Nevertheless, most of the tumours are non-functioning [2, 6], which leads to late diagnosis at the advanced stage and worse prognosis [7, 8].

Therefore, there is a need to look for an efficient and accessible diagnostic tool for these tumours. Advanced imaging and molecular methods are still not generally available and generate high cost to healthcare systems. Hence, we tried to find an accessible diagnostic marker for neuroendocrine tumours. Tumour-promoting inflammation is one of the hallmarks of cancer [9]; thus, in our study we evaluated circulating interleukin 6 (IL-6), oncostatin M (OSM), and cardiotrophin-1 (CT1).

Interleukin 6 is a potent pleiotropic cytokine that regulates cell growth and differentiation and plays an important role in the immune response. Dysregulated production of IL-6 and its receptor are implicated

Evaluation of selected circulating cytokines from the IL-6 family – interleukin 6, oncostatin M, and cardiotrophin-1 –
in gastro-entero-pancreatic and bronchial neuroendocrine tumours

115

in the pathogenesis of many diseases, such as multiple myeloma, autoimmune diseases, and prostate cancer [10]. Elevated IL-6 has been considered a poor prognostic marker in patients with cholangiocarcinoma (CCA). Interleukin 6 secreted by primary cancer-associated fibroblasts is capable of inhibiting autophagy in CCA cells, thereby stimulating their proliferation [11, 12].

Oncostatin M is a member of the IL-6 family [13]. It is considered to play multiple roles *inter alia* in cell differentiation, inflammation, haematopoiesis, and metabolism [13–15]. It has been identified as a driver of protumourigenic signals and is associated with poor prognosis in some cancer types such as pancreatic [16] and gastric cancers [17], breast cancer [18], or glioblastoma [19]. Oncostatin M induces IL-6 chemokines [13, 16].

Another member of the IL-6 superfamily, CT1, is expressed in may tissues and organs, such as heart, liver, kidneys, or lungs [20]. Cardiotrophin-1 is known to be associated with cardiovascular diseases. It triggers inflammatory and atherogenic molecule expression and stimulates cell proliferation, migration, and apoptosis [21–23]. Akkoc *et al.* showed CT1 as a potent regulator of cancer-associated fibroblast autophagy in breast cancer [24].

Every member of the IL-6 family orchestrates critical responses in the physiological regulation of development, metabolism, haematopoiesis, and inflammation. Consequently, aberrant activity of these cytokines frequently contributes to the pathogenesis of cancer [14]. Knowing the utility of the mentioned cytokines in many types of cancer, in our study we tried to assess serum levels in neuroendocrine tumours originating from the digestive tract and lungs.

Material and methods

All patients were recruited at the Department of Endocrinology and Neuroendocrine Tumours, Medical University of Silesia. Informed written consent was obtained from all study participants.

Cohorts

The study assessed 142 individuals. Eighty (56%) comprised the study group, which consisted of several subgroups including gastroenteropancreatic neuroendocrine tumours (GEPNETs, $n = 64$, 80%): pancreatic (PNETs, $n = 24$, 30%), small intestine ileal/jejunal (SINETs, $n = 21$, 26%), duodenal (DNETs, $n = 4$, 5%), gastric (GNETs, $n = 8$, 10%), rectal (RNETs, $n = 7$, 9%), and lung neuroendocrine tumours (BPNETs, $n = 16$; 20%); typical carcinoids ($n = 10$, 13%); and atypical carcinoids ($n = 6$, 7%). Following a review of medical records, data pertaining to patients' neuroendocrine markers, specifically chromogranin A, serotonin, and 5-HIAA, was collected.

The control group comprised 62 healthy volunteers. All controls were asymptomatic with no history of any malig-

Table 1. Characteristics of the study participants

Parameters	Group		χ^2	*p*-value
	NET, *n* = 80	**Controls, *n* = 62**		
Age (years)	56.2 ±12.5	52.7 ±11.5		0.083
BMI (kg/m²)	26.2 ±5.3	26.3 ±4.1		0.385
Sex, *n* (%)				
Male	36 (45)	23 (37)	0.9	0.343
Female	44 (55)	39 (63)		
Diabetes, *n* (%)				
Yes	13 (16)	2 (3)	6.27	< 0.05
No	67 (84)	60 (97)		
Hypertension, *n* (%)				
Yes	30 (38)	17 (27)	1.6	0.205
No	50 (62)	45 (73)		
Smoking, *n* (%)				
Yes	12 (15)	9 (14)	0.01	0.911
No	67 (85)	53 (86)		
Mixed hyperlipidaemia, *n* (%)				
Yes	11 (14)			
No	69 (86)			
Hypercholesterolaemia, *n* (%)				
Yes	35 (44)			
No	45 (56)			
Other malignancies, *n* (%)				
Yes	13 (16)			
No	67 (84)			

BMI – body mass index, NET – neuroendocrine tumour

nancy at the time of blood draw. Table 1 shows a qualitative and quantitative comparison of the study and control groups.

The main inclusion criterion for the study group was a histologically confirmed diagnosis of neuroendocrine tumour. Exclusion criteria for both groups were pregnancy, lactation, unsure medical family history, age less than 18 years, chronic or acute heart, liver, or renal failure, and lack of informed consent to participate in the study.

Sample collection

Peripheral blood samples were collected and clotted. Sera were obtained by centrifugation (1500 rpm/15 min) and stored at –80°C until assayed. The serum concentrations of IL-6, OSM, and CT1 were measured using the Enzyme-Linked Immunosorbent Assay Kits (ELISA, Cloud-Clone Corp., TX, USA). The procedures were done according to the instruction manual Cat Nos. SEA079Hu for IL-6, SEA-110Hu for OSM, and SEA810Hu for CT1.

The analytical procedure was in accordance with the regulations given by the manufacturer in the technological manuals attached to the set. To determine the concentrations of the tested samples, a calibration curve was prepared using the standards included in the kit. Absorbance readings at 450 nm were performed using a mikroQuant reader (Bio Tek World Headquarters, California, USA), while the processing of the results was carried out using the KC-Junior program (Bio Tek, USA). Kit sensitivity – the minimum detectable dose of IL-6 is usually less than 2.9 pg/ml, OSM is usually lower than 6.3 pg/ml, and CT1 is usually lower than 6.5 pg/ml. For all kits the intra-series error was < 10% and the extra-series error was < 12%. All samples were run in duplicate. Results were reported as concentration pg/ml. All ELISAs tests were performed at the Department of Medical and Molecular Biology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice.

Radiological evaluation and clinical staging

Disease extent was assessed using anatomical computed tomography (CT) and/or magnetic resonance imaging and functional imaging ([68Ga]Ga-DOTATATE positron emission tomography [PET]/CT) in well-differentiated NETs and ([18F]FDG PET/CT) in G2/G3 NETs. Images were evaluated by an experienced specialist in radiology and/or nuclear medicine. Gastroscopy and colonoscopy were utilised for assessment of GNETs and RNETs.

Histological diagnosis

All NET patients had histologically confirmed neuroendocrine tumour disease reported by an experienced pathologist in accordance with WHO 2019 (for GEPNETs) and WHO 2015 (for BPNETs). Specimens were evaluated by H&E staining and immunohistochemistry.

Statistical analysis

The normality of distributions was assessed using the Shapiro-Wilk test. Data for normal distributions are presented as mean values ± standard deviation (m ±s), and for skewed distributions as medians (lower-upper quarters) – Me (Q_1–Q_3). Comparisons between study group and control group for patients' characteristics were performed using Student's t-test or Mann-Whitney U test. For assessing the relationship between variables, the Spearman rank correlation coefficient (r_s) was calculated.

Two-tailed tests were used, and the significance level was set at $p < 0.05$. Statistical analysis was performed using Statistica 13 (TIBCO Software Inc., 2017; http://statistica.io).

Results

Characteristics of the cohorts

Demographic and biochemical characteristics of the study participants are shown in Table 1. The study group consisted of 64 GEPNET patients (80%) and 16 BPNET patients (20%). Eighty-three of the participants were female (58%). The median age of the study group was 55 years, range 22–81 years. The median body mass index (BMI) was 25.5 kg/m², range 22.8–28.7. Forty-four per cent of the study cohort had advanced disease. The most common metastasis site was lymph nodes (34%), followed by liver (25%), and bones (9%). Fourteen per cent of the patients had functional disease. Complete clinical data of the study cohort is shown in Table 2.

There were no statistically significant differences between the study and control groups regarding sex ($p = 0.343$), age ($p = 0.083$), BMI ($p = 0.385$), and smoking habits ($p = 0.911$). A significant difference between the groups was noted in the occurrence of diabetes mellitus ($p < 0.05$). However, there were no significant differences in the occurrence of hypertension ($p = 0.205$).

Interleukin 6, oncostatin M, and cardiotrophin-1

The median (lower-upper quartile) concentration of IL-6 was 41.5 (34.8–57.9) pg/ml in the study group and 32.6 (27.9–39.2) pg/ml in the control group, and the difference was statistically significant ($p < 0.001$) (Fig. 1). The concentration of OSM was significantly lower in the study group than in the control group ($p < 0.001$): 105.6 (98.1–114.7) pg/ml and 115.5 (102.6–145.6) pg/ml, respectively (Fig. 2). There was a significant difference ($p < 0.01$) in concentration of CT1 in the study group (222.0 [186.6–258.7] pg/ml) and controls (267.2 [199.1–316.1] pg/ml) (Fig. 3). No correlation between the concentrations of IL-1, OSM, and CT1 depending on age, BMI, and Ki-67 index were observed. There was no correlation between IL-6 and OSM concentration and clinical stage; however, a weak negative correlation between CT1 concentration and clinical stage was observed ($p < 0.05$) in the study cohort (Table 3). There were no differences between IL-6, OSM, and CT1 levels at the primary site both in the BP-NET and the GEP-NET group (Table 4). In the study group there were no statistically significant differences for smokers in the levels of IL-6 ($z = 0.499$, $p = 0.618$), OSM ($z = 1.317$, $p = 0.188$), and CT1 ($z = 0.458$, $p = 0.647$). No correlation was noted between commonly used NET markers, including serum chromogranin A level, serum serotonin level, or urine 5-hydroxyindoleacetic acid (5-HIAA) concentration and the serum levels of IL-6, OSM, and CT1 (Table 5). There was no correlation between IL-6 and OSM ($r_s = 0.015$,

Evaluation of selected circulating cytokines from the IL-6 family – interleukin 6, oncostatin M, and cardiotrophin-1 –
in gastro-entero-pancreatic and bronchial neuroendocrine tumours

117

$p = 0.859$) and CT1 ($r_S = 0.179$, $p = 0.121$). Also, there was no correlation between OSM and CT1 ($r_S = -0.139$, $p = 0.220$).

Discussion

In recent years, there has been growing interest in exploring biomarkers for early diagnosis and prognostic assessment of tumours. Among these biomarkers, circulating IL-6 and its family have shown promise [10–12]. This study aimed to evaluate the potential of these factors in the diagnosis of GEPNETs and BPNETs.

Table 2. Clinical data of the study cohort

Parameters	n (%)
Origin	
GEP-NET	64 (80)
BP-NET	16 (20)
Primary site	
Pancreas	24 (30)
Small intestine	21 (26)
Duodenum	4 (5)
Rectum	7 (9)
Stomach	8 (10)
Lung	16 (20)
Grading	
1	40 (50)
2	21 (26)
3	3 (4)
Typical	10 (13)
Atypical	6 (7)
Ki-67 (%)	
< 3	45 (56)
3–20	31 (39)
> 21	4 (5)
Metastases	
Yes	35 (44)
No	45 (56)
Liver metastases	
Yes	20 (25)
No	60 (75)
Lymph nodes metastases	
Yes	27 (34)
No	53 (66)
Bone metastases	
Yes	7 (9)
No	73 (91)
Staging	
I	36 (45)
II	8 (10)
III	11 (14)
IV	25 (31)
Functional	
Yes	11 (14)
No	69 (86)
Previous surgery	
Yes	38 (47)
No	42 (53)

■ Median □ 25–75% ⊥ Not outlier range ○ Outlier ✳ Extreme
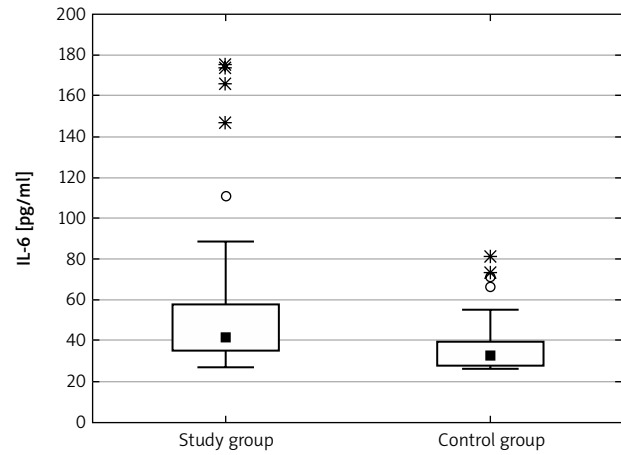
Fig. 1. Concentration of interleukin 6 [pg/ml] in the study and control group

■ Median □ 25–75% ⊥ Not outlier range ○ Outlier ✳ Extreme
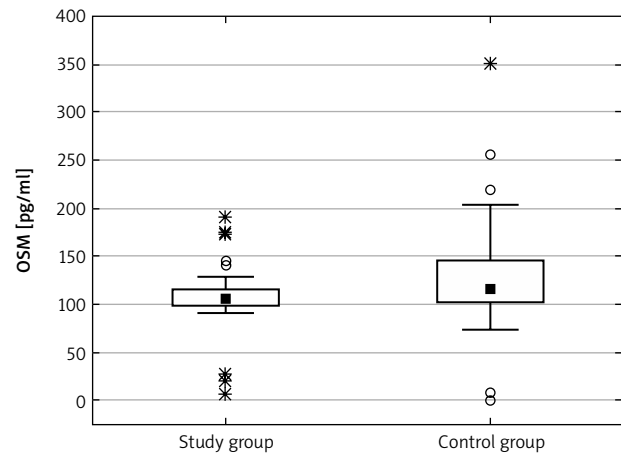
Fig. 2. Concentration of oncostatin M [pg/ml] in the study and control group

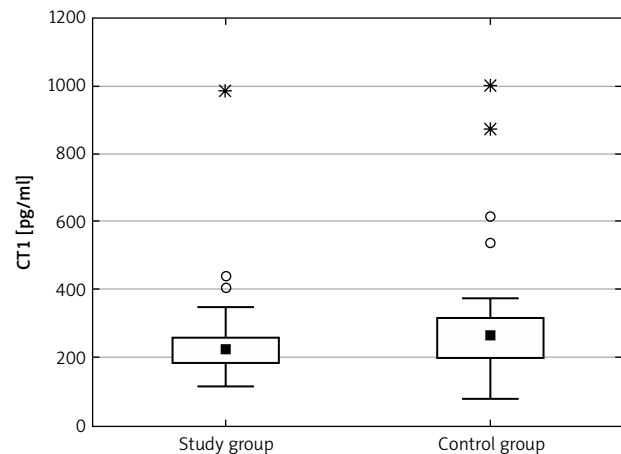■ Median □ 25–75% ⊥ Not outlier range ○ Outlier ✳ Extreme

Fig. 3. Concentration of cardiotrophin-1 [pg/ml] in the study and control group

Table 3. Correlation between body mass index, age, Ki-67, staging, and the measured parameters for the study and control groups

Parameters [pg/ml]	Control group		Study group	
	r_s	p-value	r_s	p-value
Age				
IL-6	0.167	0.188	−0.079	0.499
OSM	0.145	0.256	0.139	0.215
CT1	0.231	0.071	−0.032	0.774
BMI				
IL-6	−0.017	0.897	−0.006	0.959
OSM	0.012	0.928	−0.010	0.926
CT1	−0.155	0.229	0.021	0.850
Ki-67				
IL-6			−0.164	0.166
OSM			0.143	0.217
CT1			−0.165	0.152
Clinical stage				
IL-6			−0.160	0.166
OSM			0.060	0.595
CT1			−0.247	< 0.05

BMI – body mass index, CT1 – cardiotrophin-1, IL-6 – interleukin, OSM – oncostatin M, rS – Spearman correlation coefficient, p – statistical significance

The interleukin 6 cytokine family, including IL-6, OSM, and CT1, are critical players linking inflammation and cancer. These cytokines signal through receptors that share the glycoprotein 130 (gp130) subunit. Upon ligand binding, they activate Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathways, particularly STAT3, which promotes cancer cell proliferation, survival, and metastasis. Notably, IL-6 and OSM have been demonstrated to play pro-tumourigenic roles [13].

The study by Geisler *et al*. showed that IL-6 concentration may correspond with histopathological grading in NET – patients with G2 tumours displayed significantly lower IL-6 levels compared to patients with G1 NETs. Additionally, the results pointed out higher IL-6 levels in NET patients than in healthy controls [25], and this corresponds with our study. We observed a statistically significant difference between circulating IL-6 median concentration in the study group vs. the control group ($p < 0.001$). Another paper evaluated the influence of metabolic syndrome on inflammatory pathways in patients with well-differentiated NETs, revealing higher IL-6 expression in the peritumoural area of GEPNETs in patients with progressive disease [26].

High levels of IL-6 have been associated with poor prognosis and shorter survival in various cancer types, while lower levels are linked with better therapy response in cancers such as multiple myeloma, non-small cell lung carcinoma, oesophageal carcinoma, colorectal carcinoma, renal cell carcinoma, prostate carcinoma, breast carcinoma, and ovarian carcinoma [27–29]. Magidey-Klein *et al*. demonstrated that IL-6 influences the metastatic process through IL-6/IL-6Ra signalling, which mediates the differentiation of haematopoietic stem and progenitor cells into premetastatic monocytes-dendritic progenitors that functionally differentiate into immunosuppressive monocytes and support the metastatic switch [30]. Research indicates that IL-6 induces programmed death ligand 1 (PD-L1) expression and promotes tumour growth, e.g. in glioblastoma, hepatocellular carcinoma, lung cancer, or pancreatic cancer [13, 31–35].

All IL-6 family cytokines transmit signals through the JAK-STAT pathway, activating a diverse range of signalling pathways and transcription factors. They stimulate epithelial-mesenchymal transition processes, promoting the development of mesenchymal traits in cancer cells [31]. Inhibition of this cytokine has been shown to improve immunotherapy results. Interleukin 6-related cytokine blockage can have an antitumour effect by influencing the tumour microenvironment [13]. Oncostatin M modulates the microenvironment of the tumour and plays a role in cancer progression. Recent evidence suggests that OSM promotes tumour growth and relates to poor prognosis in several types of malignancies including pancreatic cancer, gastric cancer, breast cancer, glioblastoma, and cervical cancer [16, 17, 36–38]. Within cancer cells, OSM promotes malignancy by triggering processes such as epithelial-mesenchymal transition, the acquisition of cancer stem cell-like characteristics, migration, invasion, and the enhancement of tumourigenic and metastatic potential [19, 31, 39–41]. Studies revealed that CT1 is overexpressed in some cancer cells and can induce fibroblast autophagy, resulting in cancer cell migration and metastasis [24, 42]. It can induce ovarian carcinoma cell proliferation [43]. Another study pointed out the connection between CT1 and liver metastases in colon cancer [44]. Our analysis showed a weak negative correlation between CT1 concentration and clinical stage in the study cohort ($p < 0.05$). Nonetheless, we observed significantly lower levels of circulating OSM ($p < 0.001$) and CT1 ($p < 0.01$) in the study group compared to the control group. We assume that this outcome may relate to the stable phase of the disease with no signs of progression. These results confirm the complexity of the processes of carcinogenesis and metastasis. Growth and progression of tumours rely on a complex microenvironment comprising *inter alia* diverse cell types,

Table 4. Concentration of parameters related with primary site

Parameters	Primary site		z	p-value
	BP-NET	GEP-NET		
IL-6 [pg/ml]	40.5 (34.8–61.6)	42.6 (34.8–56.5)	0.25	0.799
OSM [pg/ml]	105.6 (96.6–117.8)	105.6 (98.1–114.7)	0.12	0.907
CT1 [pg/ml]	206.1 (171.6–250.2)	223.6 (188.2–265.3)	0.57	0.572

CT1 – cardiotrophin-1, IL-6 – interleukin, Me (Q1-Q3) – median (lower-upper quartile), OSM – oncostatin M, p – statistical significance, z – Mann-Whitney U test statistic value

Evaluation of selected circulating cytokines from the IL-6 family – interleukin 6, oncostatin M, and cardiotrophin-1 –
in gastro-entero-pancreatic and bronchial neuroendocrine tumours

119

stromal components, and a specific array of cytokines and chemokines. Despite the pivotal role of this microenvironment in tumour development, there are few detailed data on the characteristics of the tumour microenvironment in the context of neuroendocrine tumours, leaving the role of the tumour immune microenvironment in these tumours unclear [45, 46]. As shown in previous studies of other malignancies, OSM and CT1 relate to tumour growth and advancement [16, 17, 19, 24, 31, 36–44]. Xue *et al.* showed that inhibiting OSM by certain long noncoding RNA transcripts restrains the tumourigenesis and metastasis in GEPNETs [47].

Inflammation in tumourigenesis is still not fully ascertained and is still in the field of research. The progressive development of immunotherapy in recent years has led to an increase in interest in new targets. Interleukin 6 cytokines influence cancer cells mostly as tumour promoters within the tumour microenvironment [13]. We believe our study may show potential in evaluating serum cytokines in NETs; however, we know it has limitations due to its retrospective, single-centred nature and relatively small, diverse patient cohort. Furthermore, our study cohort consisted of individuals diagnosed with well-differentiated neuroendocrine tumours. Despite the presence of advanced disease in some cases, there was no evidence of disease progression. We speculate that this lack of disease progression may account for the observed concentration of OSM and CT1 in the study group.

While existing evidence suggests its association with tumour development and progression, further studies are warranted to validate diagnostic utility of IL-6 and elucidate their underlying mechanisms in NET pathogenesis. On the other hand, low levels of OSM and CT1 could be a tool for assessing and/or confirming disease stabilisation. A comprehensive understanding of the role of cytokines in NETs may facilitate early diagnosis, prognostic assessment, and personalised treatment strategies. Given that NETs have diverse phenotypes influenced by tumour origin, identifying a fair immunological biomarker presents a challenge. Therefore, we recognise the necessity for additional and more comprehensive investigations to elucidate the significance of inflammatory cytokines in the progression of neuroendocrine tumours on a broader scale.

Conclusions

In conclusion, our investigation into selected IL-6 family cytokines revealed differential modulation of signal transduction pathways. These findings suggest that despite utilising a common signalling transducer, individual IL-6 family cytokines exert distinct biological effects on NET development. Notably, IL-6 appears to promote tumourigenesis, while OSM and CT1 exhibit inhibitory effects gastro-entero-pancreatic and bronchial neuroendocrine tumour development.

Disclosures

1. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Medical University of Silesia (approval

Table 5. Correlation between parameters related with concentrations of known neuroendocrine tumour markers

Parameters [pg/ml]	Study group	
	r_s	*p*-value
Chromogranin A [μg/l]		
IL-6	0.028	0.814
OSM	−0.120	0.291
CT1	0.132	0.243
Serotonin [ng/ml]		
IL-6	−0.119	0.316
OSM	−0.168	0.146
CT1	0.079	0.496
5-hydroxyindole acetic acid (5-HIAA) [mg/24h]		
IL-6	0.032	0.783
OSM	−0.145	0.203
CT1	0.143	0.207

CT1 – cardiotrophin-1, IL-6 – interleukin, OSM – oncostatin M, p – statistical significance, rS – Spearman correlation coefficient

References

1. Kos-Kudła B, Foltyn W, Malczewska A, et al. Update of the diagnostic and therapeutic guidelines for gastro-entero-pancreatic neuroendocrine neoplasms (recommended by the Polish Network of Neuroendocrine Tumours). Endokrynol Pol 2022; 73: 387-454.
2. Pavel M, Öberg K, Falconi M, et al. ESMO Guidelines Committee. Gastroenteropancreatic neuroendocrine neoplasms: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2020; 31: 844-860.
3. Dasari A, Shen C, Halperin D, et al. Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States. JAMA Oncol 2017; 3: 1335-1342.
4. Nagtegaal ID, Odze RD, Klimstra D, et al. WHO classification of tumours editorial board. The 2019 WHO classification of tumours of the digestive system. Histopathology 2020; 76: 182-188.
5. Nicholson AG, Tsao MS, Beasley MB, et al. The 2021 WHO classification of lung tumors: impact of advances since 2015. J Thorac Oncol 2022; 17: 362-387.
6. Tsoli M, Koumarianou A, Angelousi A, Kaltsas G. Established and novel circulating biomarkers for diagnostic, predictive and prognostic use. Best Pract Res Clin Endocrinol Metab 2023; 37: 101785.
7. Pavel M, O'Toole D, Costa F, et al. Vienna Consensus Conference participants. ENETS consensus guidelines update for the management of distant metastatic disease of intestinal, pancreatic,

bronchial neuroendocrine neoplasms (NEN) and NEN of unknown primary site. Neuroendocrinology 2016; 103: 172-185.

8. Gibson WE, Gonzalez RS, Cates JMM, Liu E, Shi C. Hepatic micrometastases are associated with poor prognosis in patients with liver metastases from neuroendocrine tumors of the digestive tract. Hum Pathol 2018; 79: 109-115.

9. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.

10. Garbers C, Rose-John S. Dissecting interleukin-6 classic and trans-signaling in inflammation and cancer. Methods Mol Biol 2023; 2691: 207-224.

11. Thongchot S, Vidoni C, Ferraresi A, et al. Cancer-associated fibroblast-derived il-6 determines unfavorable prognosis in cholangiocarcinoma by affecting autophagy-associated chemoresponse. Cancers (Basel) 2021; 13: 2134.

12. Yang B, Zhang J, Wang J, et al. CSNK2A1-mediated MAX phosphorylation upregulates HMGB1 and IL-6 expression in cholangiocarcinoma progression. Hepatol Commun 2023; 7: e00144.

13. Soler MF, Abaurrea A, Azcoaga P, Araujo AM, Caffarel MM. New perspectives in cancer immunotherapy: targeting IL-6 cytokine family. J Immunother Cancer 2023; 11: e007530.

14. Jones SA, Jenkins BJ. Recent insights into targeting the IL-6 cytokine family in inflammatory diseases and cancer. Nat Rev Immunol 2018; 18: 773-789.

15. Hermanns HM. Oncostatin M and interleukin-31: cytokines, receptors, signal transduction and physiology. Cytokine Growth Factor Rev 2015; 26: 545-558.

16. Lee BY, Hogg EKJ, Below CR, et al. Heterocellular OSM-OSMR signalling reprograms fibroblasts to promote pancreatic cancer growth and metastasis. Nat Commun 2021; 12: 7336.

17. Yu Z, Li Z, Wang C, et al. Oncostatin M receptor, positively regulated by SP1, promotes gastric cancer growth and metastasis upon treatment with Oncostatin M. Gastric Cancer 2019; 22: 955-966.

18. Dinca SC, Greiner D, Weidenfeld K, Bond L, Barkan D, Jorcyk CL. Novel mechanism for OSM-promoted extracellular matrix remodeling in breast cancer: LOXL2 upregulation and subsequent ECM alignment. Breast Cancer Res 2021; 23: 56.

19. Chen M, Ren R, Lin W, Xiang L, Zhao Z, Shao B. Exploring the oncostatin M (OSM) feed-forward signaling of glioblastoma via STAT3 in pan-cancer analysis. Cancer Cell Int 2021; 21: 565.

20. López-Yoldi M, Moreno-Aliaga MJ, Bustos M. Cardiotrophin-1: a multifaceted cytokine. Cytokine Growth Factor Rev 2015; 26: 523-532.

21. Konii H, Sato K, Kikuchi S, et al. Stimulatory effects of cardiotrophin 1 on atherosclerosis. Hypertension 2013; 62: 942-950.

22. López-Andrés N, Calvier L, Labat C, et al. Absence of cardiotrophin 1 is associated with decreased age-dependent arterial stiffness and increased longevity in mice. Hypertension 2013; 61: 120-129.

23. Miteva K, Baptista D, Montecucco F, et al. Cardiotrophin-1 deficiency abrogates atherosclerosis progression. Sci Rep 2020; 10: 5791.

24. Akkoc Y, Dalci K, Karakas HE, et al. Tumor-derived CTF1 (cardiotrophin 1) is a critical mediator of stroma-assisted and autophagy-dependent breast cancer cell migration, invasion and metastasis. Autophagy 2023; 19: 306-323.

25. Geisler L, Hellberg T, Lambrecht J, et al. Inflammatory cytokines associated with diagnosis, tumor grade and prognosis in patients with neuroendocrine tumors. J Clin Med 2022; 11: 6191.

26. Pereira SS, Pereira R, Santos AP, et al. Higher IL-6 peri-tumoural expression is associated with gastro-intestinal neuroendocrine tumour progression. Pathology 2019; 51: 593-599.

27. Chen MF, Chen PT, Lu MS, Lin PY, Chen WC, Lee KD. IL-6 expression predicts treatment response and outcome in squamous cell carcinoma of the esophagus. Mol Cancer 2013; 12: 26.

28. Guo Y, Xu F, Lu T, Duan Z, Zhang Z. Interleukin-6 signaling pathway in targeted therapy for cancer. Cancer Treat Rev 2012; 38: 904-910.

29. Kumari N, Dwarakanath BS, Das A, Bhatt AN. Role of interleukin-6 in cancer progression and therapeutic resistance. Tumour Biol 2016; 37: 11553-11572.

30. Magidey-Klein K, Cooper TJ, Kveler K, et al. IL-6 contributes to metastatic switch via the differentiation of monocytic-dendritic progenitors into prometastatic immune cells. J Immunother Cancer 2021; 9: e002856.

31. Abaurrea A, Araujo AM, Caffarel MM. The role of the IL-6 cytokine family in epithelial-mesenchymal plasticity in cancer progression. Int J Mol Sci 2021; 22: 8334.

32. Lamano JB, Lamano JB, Li YD. et al. Glioblastoma-derived IL6 induces immunosuppressive peripheral myeloid cell PD-L1 and promotes tumor growth. Clin Cancer Res 2019; 25(: 3643-3657.

33. Zhang W, Liu Y, Yan Z, et al, R. IL-6 promotes PD-L1 expression in monocytes and macrophages by decreasing protein tyrosine phosphatase receptor type O expression in human hepatocellular carcinoma. J Immunother Cancer 2020; 8: e000285.

34. Kuo IY, Yang YE, Yang PS, et al. Converged Rab37/IL-6 trafficking and STAT3/PD-1 transcription axes elicit an immunosuppressive lung tumor microenvironment. Theranostics 2021; 11: 7029-7044.

35. Ware MB, Phillips M, McQuinn C, et al. Dual IL-6 and CTLA-4 blockade regresses pancreatic tumors in a T cell- and CXCR3-dependent manner. JCI Insight 2023; 8: e155006.

36. Araujo AM, Abaurrea A, Azcoaga P, et al. Stromal oncostatin M cytokine promotes breast cancer progression by reprogramming the tumor microenvironment. J Clin Invest 2022; 132: e165107.

37. Jahani-Asl A, Yin H, Soleimani VD, et al. Control of glioblastoma tumorigenesis by feed-forward cytokine signaling. Nat Neurosci 2016; 19: 798-806.

38. Caffarel MM, Coleman N. Oncostatin M receptor is a novel therapeutic target in cervical squamous cell carcinoma. J Pathol 2014; 232: 386-390.

39. Fossey SL, Bear MD, Kisseberth WC, Pennell M, London CA. Oncostatin M promotes STAT3 activation, VEGF production, and invasion in osteosarcoma cell lines. BMC Cancer 2011; 11: 125.

40. Hara T, Chanoch-Myers R, Mathewson ND, et al. Interactions between cancer cells and immune cells drive transitions to mesenchymal-like states in glioblastoma. Cancer Cell 2021; 39: 779-792.e11.

41. Geethadevi A, Nair A, Parashar D, et al. Oncostatin M receptor-targeted antibodies suppress STAT3 signaling and inhibit ovarian cancer growth. Cancer Res 2021; 81: 5336-5352.

42. Zaporowska-Stachowiak I, Springer M, Stachowiak K, et al. Interleukin-6 family of cytokines in cancers. J Interferon Cytokine Res 2024; 44: 45-59.

43. Scoles DR, Xu X, Wang H, et al. Liver X receptor agonist inhibits proliferation of ovarian carcinoma cells stimulated by oxidized low density lipoprotein. Gynecol Oncol 2010; 116: 109-116.

44. Bustos M, Dubrot J, Martinez-Anso E, et al. Cardiotrophin-1 determines liver engraftment of syngenic colon carcinoma cells through an immune system-mediated mechanism. Oncoimmunology 2012; 1: 1527-1536.

45. Cives M, Pelle' E, Quaresmini D, Rizzo FM, Tucci M, Silvestris F. The tumor microenvironment in neuroendocrine tumors: biology and therapeutic implications. Neuroendocrinology 2019; 109: 83-99.

46. Zhang WH, Wang WQ, Gao HL, Yu XJ, Liu L. The tumor immune microenvironment in gastroenteropancreatic neuroendocrine neoplasms. Biochim Biophys Acta Rev Cancer 2019; 1872: 188311.

47. Xue J, Bai J, Long Q, et al. TCF-3-mediated transcription of lncRNA HNF1A-AS1 targeting oncostatin M expression inhibits epithelial-mesenchymal transition via TGFβ signaling in gastroenteropancreatic neuroendocrine neoplasms. Aging (Albany NY) 2021; 13: 14065-14077.

Address for correspondence

Janusz Strzelczyk, PhD
Department of Endocrinology and Neuroendocrine Tumors
Department of Pathophysiology and Endocrinology
Faculty of Medical Sciences in Zabrze
Medical University of Silesia
Katowice, Poland
e-mail: janusz.strzelczyk@sum.edu.pl