ORIGINAL PAPER

Submitted: 21.11.2021
Accepted: 22.01.2022
Early publication date: 20.05.2022

Endokrynologia Polska
DOI: 10.5603/EP.a2022.0032
ISSN 0423–104X, e-ISSN 2299–8306
Volume/Tom 73; Number/Numer 3/2022

Assessment of VEGF and VEGF R1 serum levels in patients with neuroendocrine neoplasms before and after treatment with first-generation somatostatin analogues

Violetta Rosiek, Ksenia Janas

Department of Endocrinology and Neuroendocrine Tumours, Department of Pathophysiology and Endocrinology, Medical University of Silesia, Katowice, Poland

Abstract

Introduction: Vascular endothelial growth factor (VEGF) is a known promoter of angiogenesis that can support neuroendocrine neoplasm (NEN) development. The aim of the study was to evaluate the serum VEGF and vascular endothelial growth factor receptor 1 (VEGF R1) concentration changes in patients with NEN treated with first-generation long-acting somatostatin analogues (SSA).

Material and methods: The study comprised 55 controls and 56 NEN patients before and after SSA treatment in various periods of time (months): 1–12 (n = 54), 13–24 (n = 46), 25–36 (n = 35), 37–60 (n = 26), and over 60 months (n = 22). An analysis of medical records and serum VEGF and VEGF R1 concentration measurements of NEN patients, by enzyme-linked immunosorbent assay (ELISA) were made.

Results: During SSA treatment time, a decrease of the VEGF and an increase of VEGF R1 concentrations was observed. We confirmed significant VEGF differences between 2 pairs of SSA-treated NEN patient subgroups: Group 1–12 *vs*. Group 37–60 ($p = 0.039$) and Group 1–12 *vs*. Group > 60 ($p = 0.026$). We did not note significant differences of VEGF R1 levels between SSA-treated NEN patient subgroups. Among the studied biomarkers, VEGF R1 exhibited the best performance in distinguishing between NEN patients with controls; area under the curve (AUC) = 1 ($p < 0.001$).

Conclusions: The examined angiogenesis factors (VEGF and VEGF R1) seem to have limited usage in the assessment of SSA treatment effectiveness in NEN. However, the assessment of serum levels of these factors may help in the differentiation of NEN patients and healthy controls; in particular, VEGF R1 seems to be a good diagnostic biomarker for NEN patients. **(Endokrynol Pol 2022; 73 (3): 612–618)**

Key words: *somatostatin analogues; neuroendocrine neoplasm; VEGF; VEGF R1*

Introduction

Neuroendocrine neoplasms/tumours (NEN/NET) are heterogeneous tumours arising from a diffuse neuroendocrine cell system, with a broad range of grade, pace of disease, functional status, and primary sites [1]. Their incidence in recent decades is rising and ranges between 1.33 and 2.33/100,000 population in Europe and up to 3.56/100,000 population in the USA [according to the Surveillance, Epidemiology, and End Results (SEER) database] [2], presumably because of improved diagnostic procedures and imaging techniques [1]. The majority of NET encompass well-differentiated tumours with a low proliferation rate (low Ki-67, except NET G3 with Ki-67 above 20%).

The systemic therapy of patients with NEN includes, inter alia, 1st generation somatostatin analogues (SSA) (lanreotide, octreotide) [3–5], both in functional and non-functional NEN. For functional NEN, they reduce production of hormones and secretion of biologically active substances, and control clinical symptoms, but for non-functional, well-differentiated NEN SSA also have an antiproliferative effect, which has been confirmed in 2 randomised studies: PROMID and CLARINET [2].

One of the targets for antineoplastic therapy is inhibition of angiogenesis [6], also in NEN patients. Angiogenesis involves the development of new blood vessels on the basis of already existing previous ones, which may lead to tumour growth and the dissemination of metastasis [7]. As a consequence of hypoxia [8–12], the neoplastic cells secrete vascular endothelial growth factor (VEGF), which stimulates migration and endothelial cell splitting [13], thus inducing angiogenesis of the neoplasms, and it plays important role in metastatic spread. VEGF binds to one of the three tyrosine kinase family receptors: VEGF R1, VEGF R2, and VEGF R3. VEGF has a highest affinity for binding to VEGF R1, but via VEGF R2 it strongly induces endothelial cell proliferation, mainly of blood vessels.

Violetta Rosiek, MD PhD, Department of Endocrinology and Neuroendocrine Tumours, Department of Pathophysiology and Endocrinology, Medical University of Silesia, Ceglana 35, 40–514 Katowice, Poland, tel/fax: (+48) 32 358 13 66; e-mail: vrosiek@sum.edu.pl

The discovery of antiangiogenic treatment has reduced the mortality rate in neoplasms [14, 15]. Also, in NEN patients, various strategies have been employed therapeutically to antagonize VEGF-mediated tumour angiogenesis. Lyons et al. have proven that VEGF does not stimulate neovascularization in malignant tumour fragments [16]. NEN have strong vascularization, both at the primary site and metastases, so an antiangiogenic treatment by inhibition of angiogenesis is one of the therapy lines in these patients. Moreover, on the basis of immunohistochemistry, high levels of VEGF expression were confirmed on the NEN cells.

The anti-angiogenic effects of SSA were investigated according to the presence of somatostatin receptors (SSTR) on NEN cells and the proliferating vascular endothelium. SSA may suppress angiogenesis directly through SSTR present on endothelial cells and indirectly through the inhibition of growth factor secretion, i.a. VEGF [17, 18]. For the first time, in 1986 O'Dorisio showed the inhibition effect of somatostatin analogues on angiogenesis in vitro models, and then in 1988 with Fassler et al., confirmed also the antiangiogenic effects of octreotide [19, 20]. It comprised preliminary data supporting the antiangiogenic effects of octreotide acetate in a few chicken eggs using the chicken chorioallantoic membrane model. They demonstrated that octreotide acetate could inhibit blood vessel growth. In the next study, Barrie et al. found that the angiogenesis inhibitory ability varied greatly and depended on the structure of the analogue and its amino acid sequence, implying that certain analogues bind to specific SSTR subtypes with varying degrees of affinity [21].

Our study shows the serum VEGF and VEGF R1 before and after treatment with long-acting SSA (lanreotide, octreotide) in NEN patients. Its aim was to determine whether these serum angiogenesis factors can be helpful in assessing the effectiveness of this therapy, thus selecting the appropriate group of NEN patients in whom this therapy gives the greatest benefit. We wanted to see if these tests were warranted both in the decision to start treatment with SSA and in follow-up of the response to this treatment. On the basis of recommendations of the Polish Network of Neuroendocrine Tumours experts (2017), as well as the European Neuroendocrine Tumor Society (ENETS) guidelines (2016), our NEN patients were treated with long-acting octreotide LAR (30 mg i.m. every 4 weeks), and lanreotide Autogel (120 mg *s.c.* every 4–6 weeks).

Material and methods

Patients

The study enrolled 55 healthy volunteers and 56 NEN patients before (Group 0) and after SSA treatment in various periods (months): 1–12 (Group 1–12, n = 54), 13-24 (Group 13–24, n = 46), 25–36 (Group 25–36, n = 35), 37–60 (Group 37–60, n = 26), and over 60 months (Group > 60, n = 22). The examinations were performed at the Department of Endocrinology and Neuroendocrine Tumours, ENETS Centre of Excellence, and at the Endocrinology Specialist Outpatient Clinic in Katowice. An analysis of medical records and VEGF and VEGF R1 level measurements of NEN patients, who were treated with SSA, were used to examine.

Diagnostic and analytical methods

The serum samples for VEGF and VEGF R1 measurement, both before and after SSA treatment, were collected. After centrifugation at 3000 rpm for 10 minutes, the serum was stored at a temperature of –80°C. Thereafter, serum VEGF and VEGF R1 were determined using Quantikine Human Immunoassay provided by R&D Systems (Minneapolis, MN, USA) according to the manufacturer's protocol. The results of VEGF and VEGF R1 concentrations were presented in pg/mL.

VEGF metrix: Sensitivity of the method was 9 pg/mL, and intra-assay precision and inter-assay precision was 4.4–6.7% and 6.2–8.8%, respectively.

VEGF R1 metrix: Sensitivity of the method was 3.5 pg/mL, and intra-assay precision and inter-assay precision was 2.6–3.8% and 5.5–9.8%, respectively.

VEGF and VEGF R1 values are expected to be 62–707 pg/mL and 75–179 pg/mL, respectively.

Statistical analysis

Statistical analyses were performed using STATISTICA 13.0 (StatSoft Inc., Tulsa, OK, USA). Concentrations of angiogenesis factors (VEGF and VEGF R1) were expressed as mean values ± standard deviation (median). The comparison between the 2 independent groups (NEN patients and controls) was made using the Mann-Whitney U-test. To investigate the diagnostic capacity of VEGF and VEGF R1 in detecting NEN patients, receiver operating characteristic (ROC) curves were plotted, and the area under the curve (AUC), sensitivity, and specificity were calculated. Intergroup analyses of SSA-treated NEN patients were undertaken using a 2-tailed nonparametric chi-square (Kruskal-Wallis) test and additionally by NIR Fisher's and Duncan's test. Test results were considered significant at $p < 0.05$.

Ethical issues

The study was approved by the Ethics Committee of Medical University of Silesia, Poland (KNW/0022/KB1/130/I/15 and PCN/0022/KB1/97/I/19/20). Informed written permission from all patients and healthy individuals was obtained.

Results

Patients' and controls' characteristics are presented in Table 1.

VEGF

VEGF in all NEN and controls — comparison of these groups

VEGF measurements were elevated in the NEN cohort compared to controls (Tab. 2, Fig. 1A).

AUC for VEGF levels in NEN and controls

AUC analysis could differentiate NEN from controls. Although significant, it should be noted that with

Table 1. *Clinical characteristics of study participants — patients with neuroendocrine neoplasm (NEN) and controls*

Variable	NEN (n = 56)	Controls (n = 55)
Age [years]		
Mean (range)	58 (27–80)	54 (34–77)
Gender		
Male	30	16
Female	26	39
Grade		
G1	38	N/A
G2	18	
Stage		
I	11	
II	11	N/A
III	10	
IV	24	
Disease extent — metastases		
Yes	35	N/A
No	21	
Functionality status		
NF-NEN	45	
F-NEN:	11	N/A
CS	10	
Glucagonoma	1	
Kind of treatment		
SSA		
Yes	56	N/A
No	0	
Surgery		
Yes	29	N/A
No	27	
PRRT		
Yes	0	N/A
No	56	

N/A — not applicable; NF-NEN — non-functioning NEN; F-NEN — functioning NEN; CS — carcinoid syndrome; SSA — somatostatin analogue; PRRT — peptide receptor radionuclide therapy

an AUC of 0.62, it would be considered a poor biomarker (Fig. 1C). The sensitivity and specificity for the cut-off value were calculated as 74 and 51%, respectively (Tab. 3).

VEGF in NEN patients according to treatment time groups

According to the Kruskal-Wallis test, we confirmed only 2 significant differences between SSA-treated NEN patients subgroups: Group 1–12 *vs*. Group 37–60 and Group 1–12 *vs*. Group > 60 (Fig. 2). During the SSA treatment time, a decrease of the VEGF concentration was observed, i.e. the highest VEGF level was in patients before starting SSA treatment and the lowest was in patients treated for over 60 months. On the other hand, on the basis of NIR Fisher's and Duncan's test, we found that the 3 relationships between SSA-treated subgroups were significantly different: Group 0, Group 1–12, and Group 13–24 *vs*. Group > 60 ($p = 0.019$, $p = 0.034$, and $p = 0.049$, respectively).

VEGF R1

VEGF R1 in all NEN and controls — comparison of these groups

Serum VEGF R1 levels were significantly elevated in the NEN cohort compared to controls (Tab. 2, Fig. 1B).

AUC for VEGF R1 levels in NEN and controls

The AUROC (blue line) for differentiating NEN patients from controls was 1 (95% CI: 1–1, $p < 0.001$). A maximum AUC = 1 identifies an ideal (perfect) differentiation between these groups. The diagonal red line (AUC = 0.5) in the chart corresponds to chance discrimination. VEGF R1 AUC = 1 (blue line) indicates that it is an excellent biomarker for NEN (Fig. 1D). Both the sensitivity and specificity for the cut-off value were calculated as 100% (Tab. 3).

VEGF R1 in NEN patients according to treatment-time groups

VEGF R1 levels were not significantly different between SSA-treatment NEN patient subgroups (Tab. 4). Increas-

Table 2. *Comparison of the studied factors in patients with neuroendocrine neoplasm (NEN) and controls*

Variable	NEN (n = 56) Mean ± SD (Median)	Controls (n = 55) Mean ± SD (Median)	Significance of the difference (Mann-Whitney test) p
VEGF [pg/mL]	367.46 ± 277.04 (303.35)	263.55 ± 173.87 (205.30)	0.005
VEGF R1 [pg/mL]	365.13 ± 86.99 (345.75)	96.68 ± 20.53 (92)	< 0.001

SD — standard deviation; VEGF — vascular endothelial growth factor; VEGF R1 — vascular endothelial growth factor receptor 1

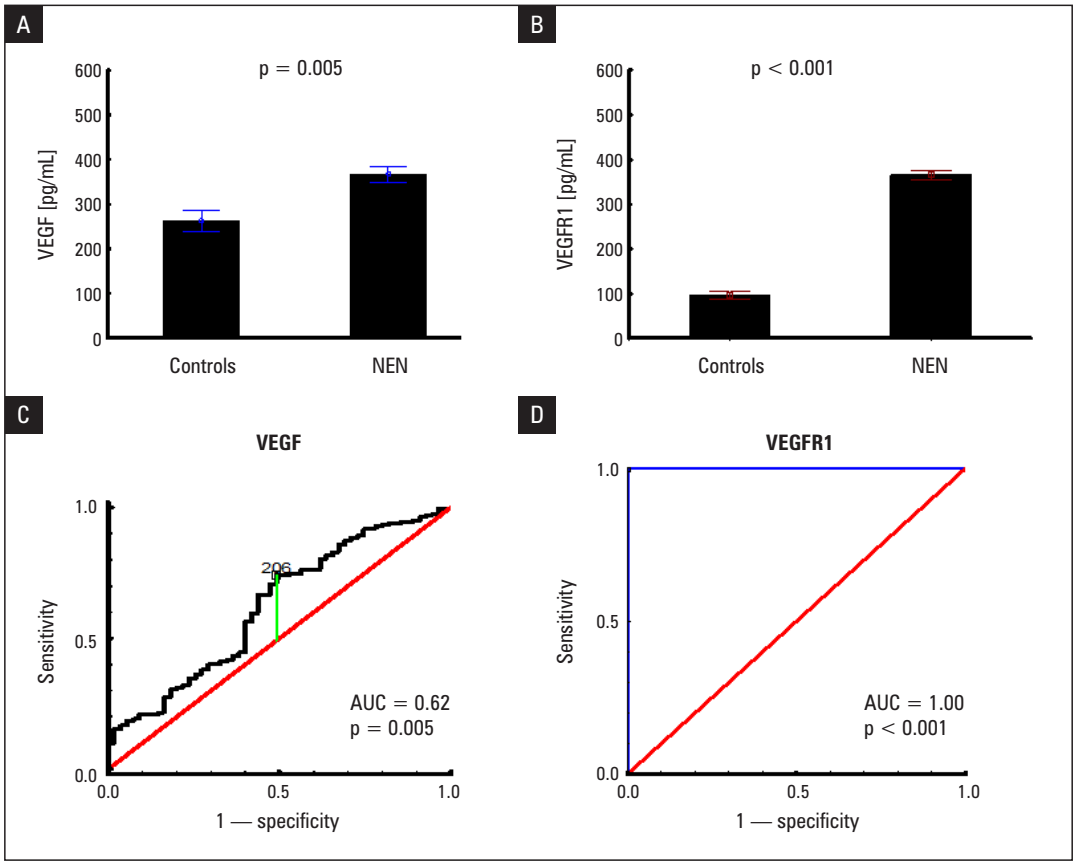

Figure 1. *Evaluation of vascular endothelial growth factor (VEGF) and its receptor (VEGF R1) in identifying groups [patients with neuroendocrine neoplasm (NEN) and controls]. Comparison of VEGF (**A**) or VEGF R1 levels (**B**) detected in NEN patients or controls. Receiver operating curve (ROC) analysis and area under the curve (AUC) were used to assess the diagnostic capacity of VEGF (**C**) or VEGF R1 (**D**) to detect NEN patients*

Table 3. *Diagnostic capacity of the studied factors*

Variable	AUC	SE	95% CI	p	Cut-off value	Sensitivity	Specificity	Accuracy
VEGF	0.62	0.04	0.54–0.71	0.005	206 pg/mL	74%	51%	70%
VEGF R1	1	0	1–1	0	190.3 pg/mL	100%	100%	100%

AUC — area under the curve; SE — standard error; CI — confidence interval; VEGF — vascular endothelial growth factor; VEGF R1 — vascular endothelial growth factor receptor 1

ing VEGF R1 concentration during SSA treatment has been noted — the lowest VEGF R1 level was observed in NEN patients before SSA treatment and the highest in the longest treated patients — for over 60 months.

Discussion

Numerous studies show that high serum and tumour tissue VEGF concentration indicates intensive development of cancer and is a poor prognostic factor. However, there have also been clinical observations that deny the importance of VEGF in neoplasms, especially its role in the progression of certain neoplasms [22, 23]. Controversy is also raised by the meaning of VEGF activity testing in the clinical evaluation of patients and in making decisions about their treatment [24–28].

Therefore, the aim of this study was to evaluate the relationship between serum VEGF and VEGF R1 and treatment with SSA treatment in NEN patients.

Treatment with SSA is the therapy of choice, both in patients with functional and non-functional NEN, in disease stabilization or progression phase, preferably in well-differentiated NEN (patients with low Ki-67 proliferation index) [5]. Some researchers hypothesized that SSA antitumour effect was i.a. the result of inhibition of angiogenesis [16]. Garcia de la Torre et al. showed that after SSA administration the synthesis and expression of VEGF in colon and rectum

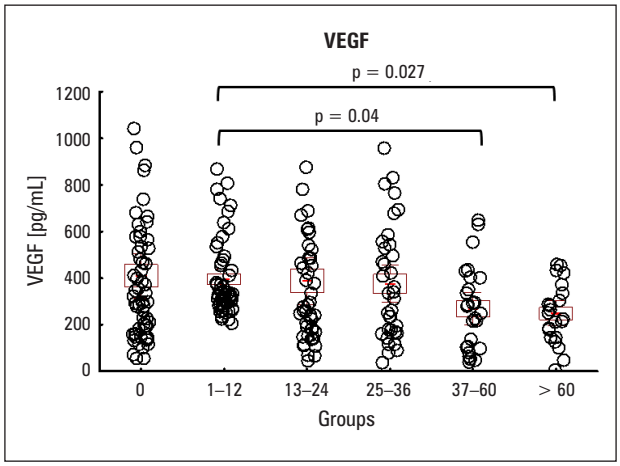

Figure 2. *Changes of vascular endothelial growth factor (VEGF) levels during somatostatin analogue (SSA) treatment (in various periods — months) in patients with neuroendocrine neoplasm (NEN)*

tumours were inhibited and serum VEGF levels were decreased [18].

Our results showed significant differences in serum VEGF and VEGF R1 levels between NEN patients and the control group. The mean VEGF concentration was higher in NEN patients than in the control group (367.46 pg/mL *vs*. 263.55 pg/mL). We also found that the serum VEGF and VEGF R1 level changes reflect the effect of SSA treatment in NEN patients. On the other hand, some treated groups did not reveal such significant VEGF level changes before and after SSA treatment.

We observed a decrease in the VEGF concentration during the time of SSA treatment. We noted the highest concentration in group 0 (before SSA treatment) and the lowest in patients treated for over 60 months (410.01 pg/mL *vs*. 247.88 pg/mL, respectively).

Perhaps SSA treatment leads to transient responses and further tumour progression because angiogenesis is regulated by various multiple pathways that are able to compensate for each other when a single pathway (VEGF/VEGF R1) is inhibited [29].

The impact of serum VEGF/VEGF R1 on oncogenesis has been a subject of research for many years. Some studies have questioned the accuracy of using serum VEGF as a marker, with the observation that VEGF is released from platelets during venipuncture [30].

Villaume et al. studied the regulation of VEGF production in gastro-entero-pancreatic NEN and the impact of drugs used in NEN therapy on VEGF secretion [31]. The study pointed out that the secretion of VEGF by 3 different endocrine cell lines is significantly decreased by octreotide. Another study [32] analysed in vitro antiangiogenic properties of octreotide. The authors showed that octreotide is able to antagonize the effects of VEGF on endothelial cell proliferation [33] but not on endothelial cell sprouting, and they concluded that the in vitro antiangiogenic effects of SSA are efficiently counterbalanced in the tumour microenvironment by the concomitant release of proangiogenic factors like VEGF. The main mechanism of angiogenesis suppression can be inhibition of endothelial nitric oxide release [34], but inhibition of circulating VEGF also plays a role in the suppression of peritumoral vessel growth [35–36].

Recently, Karpuz et al. evaluated serum VEGF levels as prognostic factors in patients with metastatic colorectal cancer [37]. The analysis included patients before and after treatment with first-line bevacizumab plus chemotherapy. There was no significant correlation between the survival and pre-treatment VEGF level.

In our study mean concentration of serum VEGF R1 was significantly higher in NEN patients than in the control group (365.13 pg/mL *vs*. 96.68 pg/mL). We also noted increasing concentration during SSA treatment — the lowest level was observed in group 0 (before SSA treatment) and the highest in patients treated for over 60 months (359.06 *vs*. 383.34 pg/mL, respectively).

Table 4. ***Angiogenesis factors — vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor 1 (VEGF R1) in patients with neuroendocrine neoplasm (NEN) treated somatostatin analogues (SSA)***

Factor	Group 0	Group 1–12	Group 13–24	Group 25–36	Group 37–60	Group > 60	Kruskal-Wallis Test (χ^2 test)
VEGF [pg/mL] Mean ± SD (Median)	410.01 ± 366.46 (321.30)	395.77 ± 165.87 (327.40)	388.19 ± 348.32 (267.75)	375.89 ± 244.11 (334.30)	268.59 ± 178.18 (264.00)	247.88 ± 128.76 (236.80)	$\chi^2 = 15.027$ p = 0.010
VEGF R1 [pg/mL] Mean ± SD (Median)	359.05 ± 76.03 (342.70)	359.38 ± 89.12 (344.10)	362.77 ± 71.23 (361.85)	375.49 ± 123.02 (361.85)	365.23 ± 60.42 (347.40)	383.34 ± 100.54 (360.30)	$\chi^2 = 1.776$ p = 0.879

SD — standard deviation

A study by Koukorakis et al. analysing serum VEGF levels and tissue activation of VEGF R2 in patients with breast and gynaecological cancer showed significantly higher serum VEGF levels in patients with breast, endometrial, and ovarian cancer compared to healthy controls and patients with benign breast/gynaecological disease in the respective organs [38]. What is more, the expression of phosphorylated VEGF R2 was higher in breast, endometrial, and ovarian cancer in patients with high VEGF serum levels; however, statistical significance was reached when all malignancies were combined.

A recent study by Behelgardi et al. analysed potential effect of new targeted drugs in the treatment of breast cancer [39]. The paper confirmed that simultaneous blockage of VEGF R1 and VEGF R2 inactivates a wider range of signalling pathways of VEGF than blockade of VEGF R1 or VEGF R2 alone, thereby more effectively suppressing tumour growth and metastasis.

Liu et al. investigated the involvement of VEGF R1 in ocular melanoma in animal models. VEGF R1 was responsible for vasculogenic mimicry network formation and was required for efficient choroidal melanoma tumour growth. The study showed VEGF R1 as a potential treatment target [40]. In a study by Enjoji et al., before surgical treatment in patients with biliary carcinoma, VEGF per platelet and VEGF R1 levels were elevated with the lapse of time [41]. Levels of both markers clearly declined as a result of surgical treatment.

A study by Sato et al. suggested that VEGF/VEGF R1 expressions could be associated with cavernous sinus invasion in pituitary neuroendocrine tumours and should be considered as a new direction for targeted therapy [42].

In summary, the studies descriptions indicate that these serum angiogenesis factors can be useful markers for gauging the clinical effect of various treatments on neoplasm patients. Some authors confirmed that in NEN patients with hypervascular tumours, immunohistochemical VEGF expression in NEN cells and serum VEGF are quantitatively correlated [43]. This discovery supports the hypothesis that VEGF production and neovascularization are required for tumour survival. In the available literature, inhibition of VEGF/VEGF R1 pathways seems to be good target for treatment of several neoplasia [44]. The antiproliferative mechanism of SSA in NEN treatment is not fully identified. SSA might suppress angiogenesis; therefore, we looked into the impact of SSA on the concentration of serum angiogenesis factors.

Conclusions

Serum VEGF and VEGF R1 levels seem to have limited usage in the assessment of SSA treatment effectiveness in NEN. Based on our observations, we can only confirm that in NEN patients, some time after treatment, the levels of VEGF increased and VEGF R1 decreased. However, serum VEGF R1 could be a potential marker for distinguishing NET patients from healthy controls.

Limitations

The main limitation of this study is the heterogeneity and different numbers of the NEN patient group. What is more, our analysis was performed on patients treated both with octreotide and lanreotide (in non-equal proportions).

Author contributions

Study conception and design: V.R. Data extraction: V.R., K.J. Analysis of data: V.R. Literature search: V.R., K.J. Writing of the manuscript: V.R., K.J. Responsibility for the paper as a whole: V.R.

Author's statement

V.R. is the first author.

Conflict of interest

The authors declare no conflict of interest.

Funding

This study was financed by the Medical University of Silesia in Katowice: grant number: KNW-1-131/N/5/0 and KNW-1-110/N/9/K.

References

1. Zhang JY, Kunz PL. Making Sense of a Complex Disease: A Practical Approach to Managing Neuroendocrine Tumors. JCO Oncol Pract. 2021 [Epub ahead of print]: OP2100240, doi: 10.1200/OP.21.00240, indexed in Pubmed: 34652954.
2. Pavel M, Öberg K, Falconi M, et al. ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. Gastroenteropancreatic neuroendocrine neoplasms: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2020; 31(7): 844–860, doi: 10.1016/j.annonc.2020.03.304, indexed in Pubmed: 32272208.
3. Faiss S, Pape UF, Böhmig M, et al. International Lanreotide and Interferon Alfa Study Group. Prospective, randomized, multicenter trial on the antiproliferative effect of lanreotide, interferon alfa, and their combination for therapy of metastatic neuroendocrine gastroenteropancreatic tumors--the International Lanreotide and Interferon Alfa Study Group. J Clin Oncol. 2003; 21(14): 2689–2696, doi: 10.1200/JCO.2003.12.142, indexed in Pubmed: 12860945.
4. Rinke A, Müller HH, Schade-Brittinger C, et al. PROMID Study Group. Placebo-controlled, double-blind, prospective, randomized study on the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors: a report from the PROMID Study Group. J Clin Oncol. 2009; 27(28): 4656–4663, doi: 10.1200/JCO.2009.22.8510, indexed in Pubmed: 19704057.
5. Kos-Kudła B, Blicharz-Dorniak J, Strzelczyk J, et al. Diagnostic and therapeutic guidelines for gastro-entero-pancreatic neuroendocrine neoplasms (recommended by the Polish Network of Neuroendocrine Tumours). Endokrynol Pol. 2017; 68(2): 79–110, doi: 10.5603/EP.2017.0015, indexed in Pubmed: 28597909.
6. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer. 2008; 8(8): 592–603, doi: 10.1038/nrc2442, indexed in Pubmed: 18650835.
7. Dvorak HF. Angiogenesis: update 2005. J Thromb Haemost. 2005; 3(8): 1835–1842, doi: 10.1111/j.1538-7836.2005.01361.x, indexed in Pubmed: 16102050.
8. Petrillo M, Patella F, Pesapane F, et al. Hypoxia and tumor angiogenesis in the era of hepatocellular carcinoma transarterial loco-regional treatments. Future Oncol. 2018; 14(28): 2957–2967, doi: 10.2217/fon-2017-0739, indexed in Pubmed: 29712486.

9. Baeriswyl V, Christofori G. The angiogenic switch in carcinogenesis. Semin Cancer Biol. 2009; 19(5): 329–337, doi: 10.1016/j.semcancer.2009.05.003, indexed in Pubmed: 19482086.
10. Ferrara N. Vascular endothelial growth factor. Arterioscler Thromb Vasc Biol. 2009; 29(6): 789–791, doi: 10.1161/ATVBAHA.108.179663, indexed in Pubmed: 19164810.
11. Ferrara N. Pathways mediating VEGF-independent tumor angiogenesis. Cytokine Growth Factor Rev. 2010; 21(1): 21–26, doi: 10.1016/j.cytogfr.2009.11.003, indexed in Pubmed: 20005148.
12. Lv X, Li J, Zhang C, et al. The role of hypoxia-inducible factors in tumor angiogenesis and cell metabolism. Genes Dis. 2017; 4(1): 19–24, doi: 10.1016/j.gendis.2016.11.003, indexed in Pubmed: 30258904.
13. Rust R, Gantner C, Schwab ME. Pro- and antiangiogenic therapies: current status and clinical implications. FASEB J. 2019; 33(1): 34–48, doi: 10.1096/fj.201800640RR, indexed in Pubmed: 30085886.
14. Ferrara N, Adamis AP. Ten years of anti-vascular endothelial growth factor therapy. Nat Rev Drug Discov. 2016; 15(6): 385–403, doi: 10.1038/nrd.2015.17, indexed in Pubmed: 26775688.
15. Mukherjee A, Madamsetty VS, Paul MK, et al. Recent Advancements of Nanomedicine towards Antiangiogenic Therapy in Cancer. Int J Mol Sci. 2020; 21(2), doi: 10.3390/ijms21020455, indexed in Pubmed: 31936832.
16. Lyons JM, Schwimer JE, Anthony CT, et al. The role of VEGF pathways in human physiologic and pathologic angiogenesis. J Surg Res. 2010; 159(1): 517–527, doi: 10.1016/j.jss.2008.12.014, indexed in Pubmed: 19577260.
17. Dasgupta P. Somatostatin analogues: multiple roles in cellular proliferation, neoplasia, and angiogenesis. Pharmacol Ther. 2004; 102(1): 61–85, doi: 10.1016/j.pharmthera.2004.02.002, indexed in Pubmed: 15056499.
18. García de la Torre N, Wass JAH, Turner HE. Antiangiogenic effects of somatostatin analogues. Clin Endocrinol (Oxf). 2002; 57(4): 425–441, doi: 10.1046/j.1365-2265.2002.01619.x, indexed in Pubmed: 12354124.
19. Fassler JE, Hughes JH, Cataland S, et al. Somatostatin analog: an inhibitor of angiogenesis: Seventh International Symposium on Gastrointestinal Hormones, Shizuoka, Japan 1988; abstract no. 2.
20. Fassler JA, O'Dorisio TM, Stevens RE, et al. Are somatostatin analogues antiangiogenic? Clin Res. 1988; 36: 869A.
21. Barrie R, Woltering EA, Hajarizadeh H, et al. Inhibition of angiogenesis by somatostatin and somatostatin-like compounds is structurally dependent. J Surg Res. 1993; 55(4): 446–450, doi: 10.1006/jsre.1993.1167, indexed in Pubmed: 7692142.
22. Chan EY, Larson AM, Fix OK, et al. Identifying risk for recurrent hepatocellular carcinoma after liver transplantation: implications for surveillance studies and new adjuvant therapies. Liver Transpl. 2008; 14(7): 956–965, doi: 10.1002/lt.21449, indexed in Pubmed: 18581511.
23. Yegin EG, Siykhymbayev A, Eren F, et al. Prognostic implication of serum vascular endothelial growth factor in advanced hepatocellular carcinoma staging. Ann Hepatol. 2013; 12(6): 915–925, indexed in Pubmed: 24114822.
24. Yu Dc, Chen J, Sun Xt, et al. Mechanism of endothelial progenitor cell recruitment into neo-vessels in adjacent non-tumor tissues in hepatocellular carcinoma. BMC Cancer. 2010; 10: 435, doi: 10.1186/1471-2407-10-435, indexed in Pubmed: 20716344.
25. Shim JuH, Park JW, Kim JiH, et al. Association between increment of serum VEGF level and prognosis after transcatheter arterial chemoembolization in hepatocellular carcinoma patients. Cancer Sci. 2008; 99(10): 2037–2044, doi: 10.1111/j.1349-7006.2008.00909.x, indexed in Pubmed: 19016764.
26. Mathonnet M, Descottes B, Valleix D, et al. VEGF in hepatocellular carcinoma and surrounding cirrhotic liver tissues. World J Gastroenterol. 2006; 12(5): 830–831, doi: 10.3748/wjg.v12.i5.830, indexed in Pubmed: 16521208.
27. Kaseb AO, Hanbali A, Cotant M, et al. Vascular endothelial growth factor in the management of hepatocellular carcinoma: a review of literature. Cancer. 2009; 115(21): 4895–4906, doi: 10.1002/cncr.24537, indexed in Pubmed: 19637355.
28. Villanueva A, Llovet JM. Targeted therapies for hepatocellular carcinoma. Gastroenterology. 2011; 140(5): 1410–1426, doi: 10.1053/j.gastro.2011.03.006, indexed in Pubmed: 21406195.
29. Zhao Y, Adjei AA. Targeting Angiogenesis in Cancer Therapy: Moving Beyond Vascular Endothelial Growth Factor. Oncologist. 2015; 20(6): 660–673, doi: 10.1634/theoncologist.2014-0465, indexed in Pubmed: 26001391.
30. Webb NJ, Bottomley MJ, Watson CJ, et al. Vascular endothelial growth factor (VEGF) is released from platelets during blood clotting: implications for measurement of circulating VEGF levels in clinical disease. Clin Sci (Lond). 1998; 94(4): 395–404, doi: 10.1042/cs0940395, indexed in Pubmed: 9640345.
31. Villaume K, Blanc M, Gouysse G, et al. VEGF secretion by neuroendocrine tumor cells is inhibited by octreotide and by inhibitors of the PI3K/AKT/mTOR pathway. Neuroendocrinology. 2010; 91(3): 268–278, doi: 10.1159/000289569, indexed in Pubmed: 20389030.
32. Walter T, Hommell-Fontaine J, Gouysse G, et al. Effects of somatostatin and octreotide on the interactions between neoplastic gastroenteropancreatic endocrine cells and endothelial cells: a comparison between in vitro and in vivo properties. Neuroendocrinology. 2011; 94(3): 200–208, doi: 10.1159/000328134, indexed in Pubmed: 21677423.
33. Adams RL, Adams IP, Lindow SW, et al. Somatostatin receptors 2 and 5 are preferentially expressed in proliferating endothelium. Br J Cancer. 2005; 92(8): 1493–1498, doi: 10.1038/sj.bjc.6602503, indexed in Pubmed: 15812556.
34. Arena S, Pattarozzi A, Corsaro A, et al. Somatostatin receptor subtype-dependent regulation of nitric oxide release: involvement of different intracellular pathways. Mol Endocrinol. 2005; 19(1): 255–267, doi: 10.1210/me.2004-0280, indexed in Pubmed: 15388796.
35. Kumar M, Liu ZR, Thapa L, et al. Anti-angiogenic effects of somatostatin receptor subtype 2 on human pancreatic cancer xenografts. Carcinogenesis. 2004; 25(11): 2075–2081, doi: 10.1093/carcin/bgh216, indexed in Pubmed: 15205362.
36. Kumar M, Liu ZR, Thapa L, et al. Antiangiogenic effect of somatostatin receptor subtype 2 on pancreatic cancer cell line: Inhibition of vascular endothelial growth factor and matrix metalloproteinase-2 expression in vitro. World J Gastroenterol. 2004; 10(3): 393–399, doi: 10.3748/wjg.v10.i3.393, indexed in Pubmed: 14760765.
37. Karpuz T, Araz M, Korkmaz L, et al. The Prognostic Value of Serum Semaphorin3A and VEGF Levels in Patients with Metastatic Colorectal Cancer. J Gastrointest Cancer. 2020; 51(2): 491–497, doi: 10.1007/s12029-019-00263-4, indexed in Pubmed: 31218581.
38. Koukourakis MI, Limberis V, Tentes I, et al. Serum VEGF levels and tissue activation of VEGFR2/KDR receptors in patients with breast and gynecologic cancer. Cytokine. 2011; 53(3): 370–375, doi: 10.1016/j.cyto.2010.12.007, indexed in Pubmed: 21208810.
39. Behelgardi MF, Zahri S, Shahvir ZG, et al. Targeting signaling pathways of VEGFR1 and VEGFR2 as a potential target in the treatment of breast cancer. Mol Biol Rep. 2020; 47(3): 2061–2071, doi: 10.1007/s11033-020-05306-9, indexed in Pubmed: 32072404.
40. Liu H, Gao M, Gu J, et al. VEGFR1-Targeted Contrast-Enhanced Ultrasound Imaging Quantification of Vasculogenic Mimicry Microcirculation in a Mouse Model of Choroidal Melanoma. Transl Vis Sci Technol. 2020; 9(3): 4, doi: 10.1167/tvst.9.3.4, indexed in Pubmed: 32704424.
41. Enjoji M, Nakamuta M, Yamaguchi K, et al. Clinical significance of serum levels of vascular endothelial growth factor and its receptor in biliary disease and carcinoma. World J Gastroenterol. 2005; 11(8): 1167–1171, doi: 10.3748/wjg.v11.i8.1167, indexed in Pubmed: 15754398.
42. Sato M, Tamura R, Tamura H, et al. Analysis of Tumor Angiogenesis and Immune Microenvironment in Non-Functional Pituitary Endocrine Tumors. J Clin Med. 2019; 8(5), doi: 10.3390/jcm8050695, indexed in Pubmed: 31100921.
43. Besig S, Voland P, Baur DM, et al. Vascular endothelial growth factors, angiogenesis, and survival in human ileal enterochromaffin cell carcinoids. Neuroendocrinology. 2009; 90(4): 402–415, doi: 10.1159/000245900, indexed in Pubmed: 19816005.
44. Kajdaniuk D, Marek B, Foltyn W, et al. Vascular endothelial growth factor (VEGF) — part 2: in endocrinology and oncology. Endokrynol Pol. 2011; 62(5): 456–464, indexed in Pubmed: 22069107.