RESEARCH

Serum levels of neuron-specific enolase as a prognostic factor for disease progression in patients with GET/NEN in the pancreas and the small intestine

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Abstract

The aim of this study was to assess the usefulness of neuron-specific enolase (NSE) concentrations as a prognostic factor in patients with neuroendocrine neoplasms and to determine the relationship between NSE and clinicopathological features. Serum NSE levels were measured in 179 NEN patients before treatment. It was found that NSE levels in patients with a primary pancreatic location were higher compared to patients with a small intestine lesion ($P = 0.015$). NSE levels were significantly higher in patients with primary pancreatic location with histological grade G2 compared with the group with low-grade G1 ($P = 0.047$). Patients with initial liver involvement showed significantly higher NSE levels compared to patients with tumour location in the pancreas ($P = 0.009$). Statistical analysis confirmed that higher NSE levels were associated with disease progression ($P = 0.001$) in both the overall study group and in patients with tumours in the pancreas and small intestine. During treatment monitoring, an increase in median NSE concentrations was observed in patients with persistent progression with subsequent blood draws, and a decrease in NSE concentrations was observed in patients with disease stabilisation. We showed that NSE concentrations have prognostic value for progression-free survival in addition to primary liver involvement. In conclusion, the most important results of the study include the demonstration of an association between NSE concentrations and clinical status, which confirms its usefulness in patient monitoring and as a potential predictive indicator for progression-free survival in patients with NENs.

Key Words

- neuron-specific enolase
- neuroendocrine neoplasms
- prognostic factor
- pancreas
- small intestine

Endocrine Connections (2022) **11**, **e210647**

Introduction

Neuron-specific enolase (NSE) is a dimer characterised by the presence of a neuron-specific γ subunit. NSE is one of the isoenzymes of enolase, classified as a glycolytic enzyme. The biological half-life of this isoenzyme is approximately 24 h. NSE is found in the cytoplasm of neurons and neuroendocrine cells. Mechanisms leading to the destruction of these cells cause an increase in NSE concentration in body fluids. Hence, numerous studies have evaluated its usefulness in predicting the degree of brain damage, for example, in patients after cardiac arrest (1, 2).

Increased NSE concentrations are also observed in the course of neoplastic diseases. Increasing concentrations of the marker in patients with initially diagnosed

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prostate cancer may be associated with a component of neuroendocrine cells in adenocarcinoma or with the initiated process of transformation of cancer cells in the neuroendocrine direction. Elevated NSE levels are among the unfavourable prognostic factors because the neuroendocrine subtype of prostate cancer is characterised by greater aggressiveness, more rapid metastasis and resistance to treatment. Of particular interest to researchers are patients with hormone-refractory prostate cancer, in whom the treatment used is most likely to stimulate the differentiation of adenocarcinoma cells in a neuroendocrine direction (3, 4, 5).

Literature data indicate that serum NSE concentrations are also useful in patients with non-small cell lung cancer. Elevated levels of a marker of neuroendocrine differentiation have been shown to be a poor prognostic factor in NSCLC patients treated with EGF receptor tyrosine kinase inhibitors. Elevated NSE levels in patients with adenocarcinoma of the lung with chromosomal rearrangements in the anaplastic lymphoma kinase gene show similar properties (6, 7). The literature mainly presents the results of work on the clinical utility of NSE in patients with small cell lung cancer (8, 9). The first studies conducted as early as in the 1980s showed a correlation between its levels and the stage of the disease and response to treatment. Further studies also confirmed the usefulness of NSE determinations in predicting the course of the disease (10, 11, 12).

However, in clinical practice, the determination of NSE levels is dedicated mainly to a specific group of patients with neuroendocrine tumours, particularly those of the gastrointestinal tract – gastroenteropancreatic (GEP) neuroendocrine neoplasms (NENs) (13). Neuroendocrine tumours are a heterogeneous group of tumours originating from cells of the diffuse neuroendocrine system. These tumours are classified as rare diseases, although a steady increase in incidence has been observed. The analysis of data obtained in the framework of the Surveillance, Epidemiology and End Results (SEER) Program of the National Cancer Institute associates the increase in the incidence with an improvement in the diagnosis of neuroendocrine tumours (14). In this diagnosis, the determination of NSE, as well as chromogranin A (CgA), assists in the diagnosis and treatment monitoring of patients (15, 16, 17).

Both markers are classified as so-called non-specific markers, released by different tumours regardless of their hormonal activity. These markers show varying degrees of sensitivity, which depends primarily on the degree of differentiation of the tumour cells. The highest sensitivity of NSE has been demonstrated in patients with neuroendocrine carcinoma, whose cells are characterised by low degree of differentiation. Therefore, its measurements have found application in clinical practice primarily in patients diagnosed with neuroendocrine carcinoma (low differentiated cells – G3 with a high proliferative index Ki67 > 55%) (18). In contrast, the utility of NSE assays in patients with highly and intermediately differentiated neuroendocrine tumours (G1–G3 and Ki67 < 55%) remains an open question.

The primary aim of this study was to evaluate the utility of NSE concentrations as a prognostic factor for progression-free survival (PFS) in patients with NENs. We were also interested in determining the relationship between NSE concentrations and the location of the primary lesion and selected clinicopathological features.

Materials and methods

Serum NSE levels were determined in 179 patients with NENs of the gastrointestinal tract treated in the Oncology Department at Wawelska Street. The study group consisted of 109 women and 70 men aged 28–88 years (median age, 65 years).

Lesions were located in the pancreas in 82 patients, in the small intestine in 75 patients, in the rectum in 14 patients and in the stomach in 8 patients.

According to the current 2019 World Health Organization (WHO) classification, based on histological malignancy grade (G) and Ki-67% proliferation index, the study group included 112 patients with high-grade tumour: NETG1, Ki-67 < 3%, 55 with intermediate-grade tumour: G2, Ki-67 = 3–20% and 12 patients with low-grade NET G3, Ki-67 > 20% (19).

Clinical status was assessed during patient monitoring based on WHO performance status, physical and subject history and biochemical parameters. In the assessment of clinical status, stabilisation (SD) was observed in 118 patients and progression (PD) in 49 patients.

In the study group, NSE levels were determined in 96 patients during treatment monitoring at 3 months and at 6 months after the first follow-up. The characteristics of the study group are presented in Table 1.

NSE levels were determined by electrochemiluminescence with Roche kits in the Cobas E601 system. The cut-off point of the marker was adopted according to the recommendations of the manufacturer of the reagents – 16.3 ng/mL. STATISTICA 9.0 (StatSoft) package was used for statistical calculations, and the

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Table 1 Clinico-pathological characteristics of patients.

Parameters	Number of patients (%)
Gender	
Female	109/179 (61)
Male	70/179 (39)
Primary lesion	
Pancreas	82/179 (46)
Small intestine	75/179 (42)
Colon	14/1179 (8)
Stomach	8/179 (4)
Histological grade (G)	
NET G1	112/179 (63)
NET G2	55/179 (30)
NET G3	12/179 (7)
Pancreas and G	
NET G1	42/82 (51)
NET G2	33/82 (40)
NET G3	7/82 (9)
Small intestine and G	
NET G1	61/75 (81)
NET G2	11/75 (15)
NET G3	3/75 (4)
Baseline liver involvement	
Yes	113/177 (64)
No	63/177 (36)
NSE	
<16.3 ng/mL	95/179 (55)
>16.3 ng/mL	81/179 (45)

Mann–Whitney *U* test, chi-square test and Spearman's rank correlation coefficient were used. The disease-free survival (DFS) analyses were performed using Kaplan–Meier method, applying one-way analysis with the use of log-rank test to compare survival curves. For multivariate analysis, Cox regression was used.

The authors state that approval to conduct this study was obtained from the Ethics Committee of Maria Sklodowska-Curie National Research Institute of Oncology; the ID of the approvals: 23/2022.

Results

In the whole study group, elevated NSE concentrations were observed in 81 patients, representing 45% of the study population. Median marker concentration was 15.5 ng/mL (range, 8.5–370 ng/mL). In the group of patients with lesion location in the pancreas, NSE levels were elevated by 54%, in patients with a primary lesion in the small intestine by 42%, in 4 patients with location in the stomach and in 5 in the rectum.

Only two locations, pancreas and small intestine, were included in the evaluation of the relationship between NSE concentrations and the site of the primary lesion. The Mann–Whitney test revealed statistical differences between the concentrations of the marker and the studied groups of patients. Higher NSE levels were observed in patients with pancreatic neuroendocrine tumour, with a median concentration of 17.2 ng/mL, compared with marker levels in patients with a lesion in the small intestine, median 14.3 ng/mL, (P=0.015) (Fig. 1).

NSE concentrations and clinicopathological parameters

We analysed the association of concentrations of the determined biomarker with clinicopathological features: tumour cell differentiation grade (G) and mitotic activity (Ki-67 index – MIB1 antibody) according to 2019 WHO classification and primary liver involvement, gender and age. There was no correlation between NSE concentrations and gender as well as no correlation with age. The Kruskal–Wallis test, confirmed by the Spearman test, showed a statistical correlation between NSE concentrations and patient groups formed according to the 2019 WHO classification. Marker concentrations increased with increasing histological grade and Ki-67 values (R=0.33; P=0.001). The demonstrated relationship is shown in Fig. 2. Due to the observed similar distribution of marker concentrations between patients with low and intermediate histological grades, the Mann–Whitney test was applied to determine the differences in marker concentrations between G1 and G2. The analysis showed no statistical differences between NSE levels and patients in low and intermediate histological grades.

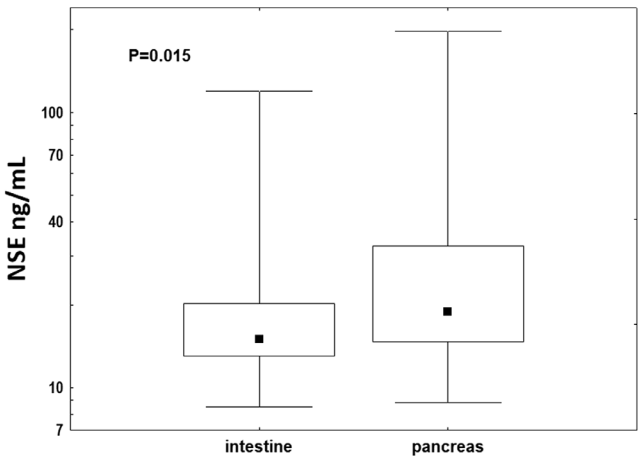

Figure 1
Medians and NSE concentrations depending on primary tumour location in NENs patients.

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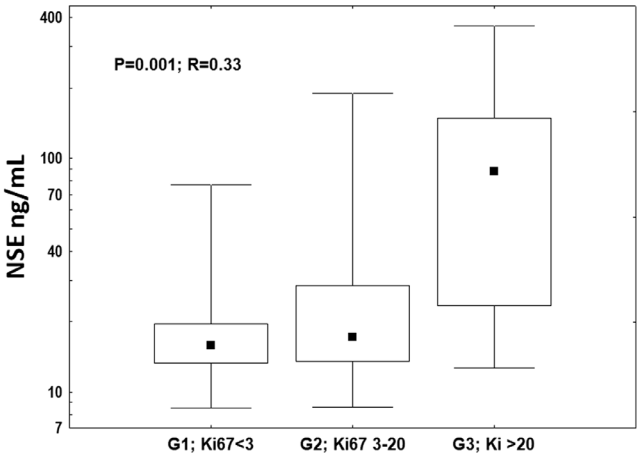

Figure 2
Medians and NSE concentrations depending on clinicopathological features: tumour cell differentiation grade (G) and mitotic activity (Ki-67 index – MIB1 antibody) in NENs patients.

The differential group was patients with high histological grade – G3.

The relationship between NSE concentrations and histological grade was examined in patients with a specific location of the primary lesion. It was found that in patients with the primary location of the tumour in the pancreas, NSE levels were significantly higher in the group with G2 compared with the group with G1 ($P = 0.047$). In contrast, in patients with localization of the lesion in the small intestine, there was no correlation between marker levels and the histological grade, G1 vs G2.

The effect of the presence of liver metastases on NSE levels was also analysed. Patients with initial liver involvement showed significantly higher NSE levels in the whole study group ($P=0.004$), as well as in patients with tumour localisation in the pancreas ($P = 0.009$). No such relationship was found in patients with tumour localization in the small intestine.

NSE concentrations and clinical status

In the study group, the relationship between marker concentrations and clinical status was assessed during patient monitoring. Disease stabilisation was confirmed in 118 patients and progression was observed in 49 patients. In patients with disease stabilisation, the proportion of patients with elevated NSE levels was similar regardless of the location of the primary lesion. Disease progression was associated with a high proportion of patients with elevated marker levels in the analysed patient groups.

Statistical analysis confirmed that significantly higher NSE levels were associated with disease progression ($P=0.001$) both in the entire study group and in patients with tumours in the pancreas ($P=0.001$) and small intestine ($P=0.001$) (Fig. 3 and Table 2).

NSE in treatment monitoring

The effect of treatment on the behaviour of NSE concentrations was analysed in 96 patients. The study group consisted of 96 patients, including 27 with progression (28%): 16 with primary lesion in the pancreas, 8 in the small intestine, 2 in the stomach and 1 in the rectum and 69 with stabilization of disease (72%). Further marker determinations were performed after 3–4 months (second collection) and after 6 months of treatment (third collection).

In patients with confirmed progression of the disease, the median NSE concentration was 35.0 ng/mL, and under the influence of applied treatment, in some patients, stabilisation of the disease was observed, which was reflected by a decrease in NSE concentration as early as in the second sampling (median NSE, 15.1 ng/mL), maintained in subsequent sampling (median NSE, 12.6 ng/mL). In contrast, patients with persistent progression at the second blood draw showed an increase in median NSE concentrations (41.6 ng/mL) and only a slight decrease in concentrations, which still remained above normal after 6months of treatment (25.0 ng/mL).

Prognostic value of NSE

The most important step of the study was to assess the prognostic value of determining NSE concentrations.

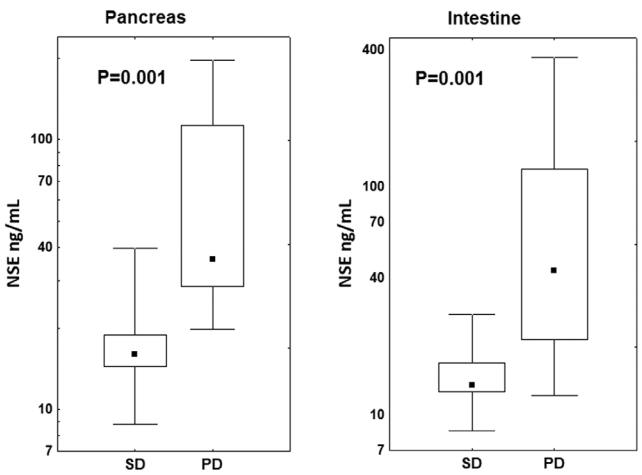

Figure 3
Medians and NSE concentrations in NENs patients with primay location in the pancreas and in the intestine depending on clinical status: PD, progression; SD,stabilisation.

Table 2 Medians and ranges of NSE concentrations in GEP-NEN patients according to clinical status.

	Clinical status	NSE, ng/mL			*P*
		Median	Range	%*	
All patients	Stabilisation	14.6	7.0–39.6	25	0.001
	Progression	26.6	12.2–370	86	
Pancreas	Stabilisation	14.7	7.0–39.6	26	0.001
	Progression	31.7	15.2–197.5	88	
Small intestine	Stabilisation	13.5	8.2–22.2	28	0.001
	Progression	22.1	12.0–370	86	

*The percentage of patients with elevated levels of NSE.

In our study, a 2-year follow-up period allowed us to assess the prognostic value of NSE concentrations in relation to time to PFS. A total of 153 GEP-NEN patients were included in the 2-year follow-up; progression was observed in 52 patients representing 34% of the study group, including 67% with pancreatic and 42% with intestinal localization. Univariate log-rank analysis showed no significant relationship between NSE levels and PFS separately in patients with GEP-NEN location in the pancreas and in the small intestine. In the entire study group, a trend was observed for NSE concentrations at $P = 0.052$ and a significant relationship between primary liver involvement and PFS ($P = 0.001$). Multivariate Cox analysis showed that NSE concentrations ($P = 0.038$; 95% CI: 0.213–1.067) were an independent prognostic factor for PFS in NEN patients and, among clinical parameters, only primary liver involvement ($P = 0.001$; 95% CI: 0.044–0.796) (Fig. 4). In addition, the chi-square test showed a significantly higher proportion of patients with elevated NSE levels in the group of patients with progression after 2 years of follow-up (53%) than in those with stabilisation (37%), $P < 0.023$.

Discussion

In the study group of patients with gastrointestinal neuroendocrine tumours NEN/GET, the pancreas and the small intestine were among the most common locations of the primary lesion. The percentage of patients with elevated NSE levels was similar in both locations. However, significantly higher levels of the marker were observed in patients with a pancreatic lesion. Similar to our results, van Adrichem *et al*. observed higher NSE levels in patients with pancreatic lesions in relation to the other locations. It should be emphasised that van Adrichem's study concerned patients in ENETS TNM stage IV GEP-NETs (20).

The subject of most available studies is the evaluation of the usefulness of NSE concentrations in estimating the degree of cell differentiation of neuroendocrine tumours. The degree of malignancy is among the prognostic and predictive factors, together with the proliferative index (Ki67) used in the 2019 WHO staging classification. This most important parameter is determined only in material obtained by invasive methods, and hence, studies are being undertaken to use non-invasive methods to assist in estimating this factor. In our study, we observed an increase in marker concentrations with stage, which was confirmed by a statistically significant correlation coefficient. Patients with high-grade tumours, although

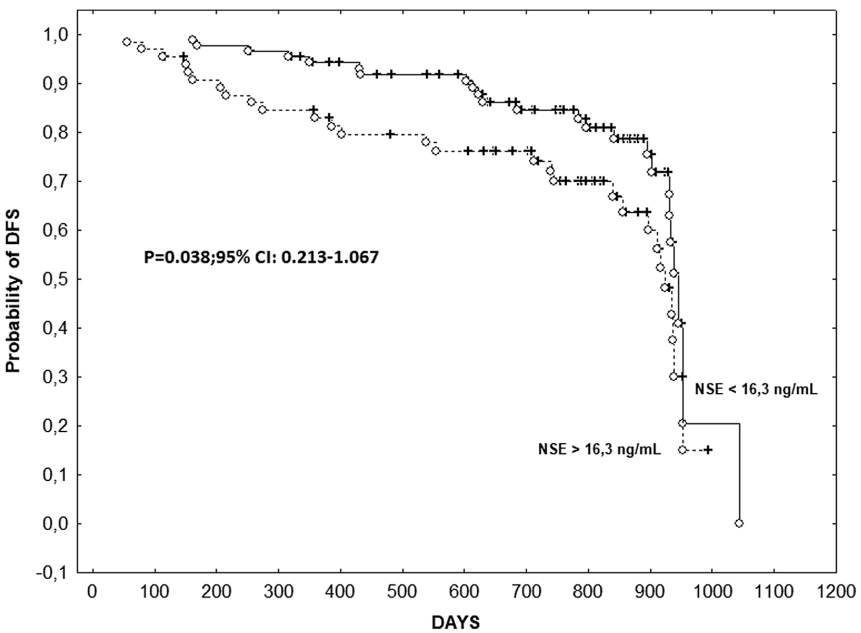

Figure 4
Kaplan–Meier curve estimates of PFS in NEN patients stratified by serum NSE levels.

constituting a small group, were distinguished from the studied group by the highest values of NSE concentrations. This result was expected, as the marker is dedicated to neuroendocrine carcinomas characterised by a high degree of malignancy. However, the object of our interest was patients with lesions of low and intermediate degree of malignancy, who constitute the most numerous group of patients with gastrointestinal neuroendocrine tumours. Graphically presented results of the relationship between NSE concentrations and malignancy grade suggested that there were no differences in concentrations between G1 and G2 grades. Statistical analyses confirmed that in the whole group of patients, NSE concentrations did not differentiate between these two degrees of malignancy. To date, studies investigating the relationship between NSE concentrations and degree of malignancy have produced an exceptional variety of results. Authors report differences in NSE concentrations between grades or show similar results to our study, as well as report no association of marker concentrations with malignancy grade (16, 21, 22, 23).

When pointing out the differences between the results presented in the literature on the relationship between NSE levels and the degree of malignancy, special attention should be paid to the location of the primary lesion in patients. The reason for discrepancies in literature reports may be the fact that neuroendocrine tumours are localized in different organs, and therefore, we speak about the results of analysis in heterogeneous groups of patients, for example, patients with diagnosed endocrine pancreatic tumour, with lesion in the small intestine or a group of patients defined as neuroendocrine tumours of the gastrointestinal tract. In our study, although we did not show a correlation between NSE concentrations in the whole group, we found significantly higher levels of the marker in the group with pancreatic lesions of intermediate malignancy (G2) compared to patients with low malignancy (G1). In a study by Yang Lv *et al*. concerning a group of patients with a similar diagnosis, no statistical differences in NSE levels were observed between G1 and G2 patients (21). On the other hand, in our study, the analysis of concentrations in patients with small intestine lesions did not show a correlation between the degree of malignancy and the values of the marker determined. The results of our study did not confirm literature reports in which the authors indicate statistical differences already between low and intermediate degree of malignancy and NSE in patients with small intestine lesions (22).

NSE concentrations were not associated with gender or age. A similar lack of correlation between these parameters is confirmed by other authors (16).

Unusual symptoms accompanying NEN/GET neuroendocrine tumours are the reason for the diagnosis of the disease at an advanced stage, often with the presence of liver metastases. In our study group, up to 64% of patients had confirmed liver metastases. This group of patients was characterised by significantly higher concentrations of the marker in relation to patients without liver metastases. The results of our study confirmed previous reports on the influence of liver lesions on NSE concentrations (21, 22). These results are difficult to relate to the study reporting no effect of the presence of liver metastases on NSE levels because the authors do not specify the site of metastasis and the group of patients without metastases included only three patients (16).

The results presented above characterise the usefulness of NSE determinations at the initial diagnostic stage. It is known that markers belong to the group of tests supporting the diagnosis. An unquestionable advantage is a short time of waiting for the result, and when NSE concentrations are high, we can confirm the presence and extent of the disease process. The clinical usefulness of markers is primarily determined by their ability to be used in monitoring treatment, i.e. to demonstrate the relationship between concentrations and clinical condition confirmed by standard tests. Knowing the difference in concentrations between the two main locations in the study group, the whole group of patients was analysed, as well as patients with the location of the primary lesion in the pancreas and small intestine were considered separately. A significant result was the demonstration that regardless of the location of the primary lesion, disease progression was always signalled by statistically higher NSE concentrations. Similar observations regarding the relationship between NSE concentrations and clinical status are confirmed by data published by other authors (21, 22).

At the same time, the results suggested the need for further analyses aimed at assessing the usefulness of NSE determinations in predicting the course of the disease. In our study group, it was possible to adopt an endpoint defined as time free from progression. At the cut-off value of the marker adopted according to the manufacturer's recommendations, i.e., 16.3 ng/mL, the prognostic value of NSE for time free from disease progression after 2 years of follow-up was demonstrated. In our study, primary liver involvement also had prognostic value for PFS. It was also confirmed that disease progression was statistically more frequent in patients with elevated NSE levels. The prognostic value of NSE in patients with neuroendocrine tumours of the gastrointestinal tract is rarely evaluated in the literature; studies have focused mainly on the

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diagnostic part, concerning the possibility of using NSE determinations for estimating the degree of malignancy and the presence or absence of liver metastases. In the available publications, long-term follow-up of patients allowed the researchers to take overall survival time as the endpoint. Both studies demonstrated the prognostic value of NSE assays in a group of patients with confirmed non-endocrine pancreatic neuroendocrine tumours and in patients with ENETS TNM stage IV gastrointestinal neuroendocrine tumours (20, 21).

Significant findings included the demonstration of differences in NSE concentrations between patients with the location of the lesion in the pancreas and those with the tumour in the small intestine. The highest NSE concentrations in patients with a pancreatic lesion also differentiated between patients with low and intermediate grades of malignancy. The demonstrated relationships indicate the necessity of conducting studies in homogeneous groups of patients in terms of the location of the primary lesion.

In conclusion, the most important results of the study include the demonstration of an association between NSE concentrations and clinical status, which confirms its usefulness in patient monitoring, especially in patients with pancreatic location and as a potential predictive indicator for PFS in all patients with NENs. Studies on the prognostic value of NSE need to be continued to definitively verify the utility of NSE concentrations in predicting time to progression.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This work did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Ethical approval
Written informed consent was obtained from all patients before the treatment. No ethical approval was required for this study, since the data used are publicly available and the identified.

Author contribution statement
M F, M K and B K conceived the research and took overall supervision in the study. M F, M K and A K-C performed experiments and collected data. M F, M K performed data analysis. M F, M K wrote the manuscript. M F, M K and B K contributed to the discussion of results and to the review of the manuscript.

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Received in final form 27 June 2022
Accepted 27 July 2022
Accepted Manuscript published online 27 July 2022