Serum Biomarker Status with a Distinctive Pattern in Prognosis of Gastroenteropancreatic Neuroendocrine Carcinoma

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Keywords

Neuron-specific enolase · Carcinoembryonic antigen · Carbohydrate antigen 19-9 · Gastroenteropancreatic neuroendocrine carcinoma · Biomarkers · Overall survival · Subtype

Abstract

Objective: Gastroenteropancreatic neuroendocrine carcinoma (GEPNEC) is a major research focus, but the application of biomarkers to guide its prognostication and management is unsatisfying. Clinical values of conventional serum biomarkers, neuron-specific enolase (NSE), carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA199) warrant scrutiny. **Methods:** Patients diagnosed with GEPNEC with baseline NSE, CEA, and CA199 levels provided in Peking University Cancer Hospital were retrospectively studied. Relationships between biomarkers and prognosis were investigated by the χ^2 test, Kaplan-Meier analysis, and univariate and multivariate Cox regression analyses. **Results:** A total of 640 GEPNEC patients were enrolled. NSE, CEA, and CA199 were elevated in 59.5%, 28.5%, and 21.3% of the population, respectively. Higher NSE had worse median overall survival (OS) (17.0 months vs. not reached, hazard ratio = 2.77 [2.06, 3.73], $p < 0.001$), and so did patients with higher CEA and CA199. Multivariable analysis confirmed that NSE and CA199

correlated with OS independently. Baseline NSE level and NSE remission predicted OS and the response of patients with first-line etoposide plus cisplatin (EP) treatment. Furthermore, we combined NSE/CEA/CA199 to segregate GEPNEC into novel subgroups, namely, adenocarcinoma-like NEC (ALN), neuroendocrine-like NEC (NLN), and triple-normal NEC (TNN). The groups shared distinctive clinicopathologic features and prognosis (21.0 months vs. 17.1 months vs. not reached, $p < 0.001$). The EP regimen remained the priority treatment option in NLN/TNN, while ALN was predisposed to “adenocarcinoma-like chemotherapy.” **Conclusions:** Elevation of NSE, CEA, or CA199 was common and independently indicates poor prognosis in GEPNEC patients. Serum biomarker-based subtypes suggest meaningful clinical implications and appropriate therapeutic approaches, illuminating promising ways to characterize the prognosis of GEPNEC.

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Introduction

With the incidence of gastroenteropancreatic neuroendocrine neoplasms soaring recently, management of these diseases has been provoking more attention. In particular, the poorly differentiated entity, gastroenteropan-

creatic neuroendocrine carcinoma (GEPNEC), has a median survival duration of 5–37 months [1–3]. Current staging systems to characterize the prognosis of GEPNEC are complicated and impractical in some situations, especially when primary sites and pathological factors are taken into consideration. Thus, simple and practical tools to discriminate survival and indicate treatment options across heterogeneous populations are urgently required [1–4]. However, the clinical value of biomarkers in NEC has long been underestimated [5]. Recently, identifying serum biomarkers of NEC has been conducive to clinical use, which warrants further reinspection.

Circulating biomarkers are classified as either diagnostic (can be used to discriminate and diagnose disease, e.g., chromogranin A [CgA] [6]), prognostic (can forecast disease course, e.g., CgA [7], neuron-specific enolase [NSE] [8, 9]), or predictive (can predict response to treatment [10, 11]). Currently, CgA is established in many aspects of neuroendocrine tumours (NETs) in guidelines, but ideal serum prognostic/predictive biomarkers for GEPNEC have long been undetermined, especially in large-sample studies [4, 8, 10–12].

NSE, which is instrumental in aerobic glycolysis [13–15], is elevated in 30–50% of GEPNET [16]. NSE is considered an independent marker for neuroendocrine origin tumours, exemplified in indicating poorer survival of advanced pancreatic NET (pNET), bladder-origin NEC, GEPNET, Merkel cell carcinoma, and small cell lung cancer (SCLC) [8, 9, 13, 17–25]. In addition, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA199), which are associated with physiopathologic processes of tumorigenesis and applied in other digestive systems, have shown value in NEN [20, 26–29]. There is, however, no robust evidence of clinical implications of NSE/CEA/CA199 in GEPNEC.

We proposed to investigate the roles of serum NSE/CEA/CA199 in patients with GEPNEC, assessing their distribution and comprehensively analysing clinical-pathologic characteristics and prognosis. Further, we attempted to divide NEC into subgroups based on these markers, anticipating uncovering more clinical traits and therapy implications, and finally determining their clinical applicability in practice.

Methods

In this study, we collected data consecutively from patients who were histologically diagnosed with GEPNEC, including pure NEC and mixed neuroendocrine non-neuroendocrine carcinoma (MiNEC) from August 1st, 2014 to August 1st, 2019 in Peking Uni-

versity Cancer Hospital. All patients underwent baseline evaluations that included demographic information, clinical manifestations, pathologic characteristics, and therapeutics. The stage was evaluated by the eighth edition of the American Joint Committee on Cancer (AJCC) Tumour-Node-Metastasis system, while pathology was evaluated by the 2019 WHO classification.

Eligibility criteria included

- Patients diagnosed by experienced pathologists as poorly differentiated NEC or MiNEC.
- Baseline levels of NSE, CA199, or CEA were available.
- Patients with complete survival data.

Serum tumour biomarkers were measured at the first consultation using immunoassay, with the upper limit of normal value set as follows: serum NSE of 0–15.2 ng/mL, CA199 of 0–37 U/mL, and CEA of 0–5 ng/mL. We re-assessed each biomarker after the second cycle of first-line regimens and defined biochemical remission as over 50% decrease than its baseline level. All patients were regularly followed up through outpatient clinics or phone calls. The first follow-up was performed within 3 months with subsequent follow-up cycles ranging from 6 to 12 months. The primary outcome was overall survival (OS), calculated from date of diagnosis to death by any cause or date of last follow-up. Therapy was divided into 3 categories: etoposide-platinum, irinotecan-platinum (IP), and “adenocarcinoma-like chemotherapy” (other non-EP/IP chemotherapy which focus mainly on adenocarcinoma of corresponding primary locations, namely, FOLFOX, XELOX, etc.). Response was assessed every 6 weeks for the first 6 months using Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1, according to which the objective response rate (ORR; defined as the percentage of patients with complete or partial response), and the disease control rate (DCR; defined as the percentage of patients with objective response and stable disease) or progressive disease rate were calculated.

Pearson χ^2 tests (Fisher’s test, when necessary) and one-way analysis of variance (2-sided *t*-tests, when 2 groups) were implemented to show the differences between the groups for categorical variables and continuous variables, respectively. Tumour biomarkers were assessed as categorical (normal vs. elevated) variables for associating with the OS and adjusted for confounding. Survival probabilities were revealed using the Kaplan-Meier method with log-rank test to examine the association. Univariate and multivariate analyses employing the Cox proportional hazards model identified independent factors statistically relevant to survival. All *p* values were two-sided, and *p* < 0.05 was considered significant. We utilized R version 3.5.2 (The R Foundation, Vienna, Austria) to perform these procedures.

Results

Baseline Information of GEPNEC

Among 2,048 patients recruited in our centre, 640 GEPNEC patients, including 463 pure NEC and 177 MiNEC, were determined to be eligible for the study (online suppl. Fig. 1; see www.karger.com/doi/10.1159/000519948 for all online suppl. material). The average age was 58.4 ± 11.3 and the sex ratio was 2.64 (M/F). The top primary

Table 1. Baseline information for clinicopathological characteristics of GEPNEC (N = 640) patients

Level	Overall	NSE		CEA [§]		CA199 [§]		p value
		Normal	Elevated	Normal	Elevated	Normal	Elevated	
N	640	259	381	443	177	487	132	
Sex (%)								
F	175 (27.3)	72 (27.8)	103 (27.0)	132 (29.8)	40 (22.6)	138 (28.3)	33 (25.0)	0.515
Age (mean [SD])	58.36 (11.30)	58.81 (10.89)	58.05 (11.57)	57.87 (11.48)	59.83 (10.39)	58.59 (11.24)	57.53 (11.23)	0.335
Ki67 (%) (mean [SD])	67 (23)	65 (24)	68 (22)	66 (23)	69 (22)	66 (24)	69 (20)	0.162
Primary site (%)								
Oesophagus	90 (14.1)	27 (10.4)	63 (16.5)	62 (14.0)	27 (15.3)	76 (15.6)	12 (9.1)	
Stomach	241 (37.7)	125 (48.3)	116 (30.4)	172 (38.8)	66 (37.3)	201 (41.3)	37 (28.0)	
Duodenum	24 (3.8)	9 (3.5)	15 (3.9)	18 (4.1)	6 (3.4)	16 (3.3)	7 (5.3)	
Small intestine	11 (1.7)	5 (1.9)	6 (1.6)	7 (1.6)	3 (1.7)	7 (1.4)	3 (2.3)	
Colon	35 (5.5)	13 (5.0)	22 (5.8)	22 (5.0)	12 (6.8)	21 (4.3)	12 (9.1)	0.003
Rectum	57 (8.9)	17 (6.6)	40 (10.5)	37 (8.4)	18 (10.2)	47 (9.7)	9 (6.8)	
Pancreas	59 (9.2)	23 (8.9)	36 (9.4)	40 (9.0)	16 (9.0)	39 (8.0)	17 (12.9)	
Liver	19 (3.0)	6 (2.3)	13 (3.4)	15 (3.4)	2 (1.1)	12 (2.5)	6 (4.5)	
Others [†]	104 (16.2)	34 (13.1)	70 (18.4)	70 (15.8)	27 (15.3)	63 (12.9)	29 (21.7)	
Histology [‡] (%)								
SCNEC	294 (69.0)	93 (64.6)	231 (71.3)	215 (71.1)	72 (64.0)	226 (70.2)	60 (66.7)	
LCNEC	120 (28.2)	46 (32.3)	74 (26.1)	79 (26.4)	36 (32.0)	87 (27.0)	27 (30.0)	0.866
MNEC	12 (2.8)	5 (3.1)	8 (2.7)	8 (2.5)	5 (4.0)	9 (2.8)	3 (3.3)	
Stage (%)								
I	30 (4.7)	16 (6.2)	14 (3.7)	25 (5.7)	5 (2.8)	27 (5.6)	3 (2.3)	
II	45 (7.0)	29 (11.2)	16 (4.2)	37 (8.4)	7 (4.0)	40 (8.2)	4 (3.0)	
III	206 (32.2)	105 (40.7)	101 (26.5)	155 (35.1)	46 (26.0)	168 (34.6)	33 (25.0)	0.004
IV	307 (48.0)	83 (32.2)	224 (58.5)	189 (42.7)	106 (59.3)	213 (43.8)	81 (60.6)	
N [‡]	52 (8.1)	25 (9.7)	27 (7.1)	36 (8.1)	14 (7.9)	38 (7.8)	12 (9.1)	
Lymph-node metastases [‡] (%)								
Yes	210 (41.4)	99 (40.0)	111 (42.8)	155 (41.4)	54 (43.8)	165 (41.5)	38 (41.0)	0.395
Distant metastases (%)								
Yes	307 (48.0)	83 (32.2)	224 (58.5)	189 (42.7)	106 (59.3)	213 (43.8)	81 (60.6)	0.001
Hepatic metastases (%)								
Yes	197 (30.9)	53 (20.6)	144 (37.8)	125 (28.3)	64 (36.2)	129 (26.6)	62 (47.0)	<0.001
Extrahepatic metastases (%)								
Yes	238 (37.3)	59 (23.0)	179 (47.0)	140 (31.7)	89 (50.3)	165 (34.0)	63 (47.7)	0.005
NSE (%)								
>1*ULN	381 (59.5)	–	–	228 (51.5)	135 (76.3)	261 (53.6)	100 (75.8)	<0.001
CEA (%)								
>1*ULN	177 (28.5)	42 (16.3)	135 (37.2)	–	–	108 (22.3)	66 (50.0)	<0.001
CA199 (%)								
>1*ULN	132 (21.3)	32 (12.4)	100 (27.7)	66 (14.9)	66 (37.9)	–	–	–

MNEC, mixed-cell type (containing both small cell and large cell components); ULN, upper limit of normal; NSE, neuron-specific enolase; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen 19-9. [†] Others included NEC originated from other sites of digestive systems or peritoneal cavity. [‡] There are 20 and 21 patients who have no CEA and CA199 data, respectively. [§] N referred to undefined stage data due to lack of clinical information. [‡] Histologic type data were missed in 214 patients. [‡] Lymph-node metastases data were missed in 133 patients.

(For legend see next page.)

site was stomach (37.7%), followed by oesophagus (14.1%), pancreas (9.2%), and rectum (8.9%). Baseline metastatic diseases were observed in 48.0% of patients. The median follow-up time was 32.8 months, and the median survival duration was 21.3 months. Elevated NSE, CEA, and CA199 had prevalence values in GEPNEC of 381 (59.5%), 177 (28.5%), and 132 (21.3%), respectively. The stage and distant metastases in elevated biomarkers were all significantly different, with p values <0.05 (Table 1). We also compared elevation of biomarkers with different anatomical sites. NSE elevation was mostly seen in patients with oesophagus and rectum NEC, while colon NEC patients had the highest CEA elevation rate, and pancreatic NEC patients had the highest CA199 elevation. Gastric NEC (gNEC), on the other hand, had relatively low proportions of NSE elevation, significantly lower than oesophagus and rectum counterparts (online suppl. Fig. 3). For pathologic type, we observed that only NSE in small cell GEPNEC (GEPSCNEC) patients was relatively higher than large cell GEPNEC (GEPLCNEC), though differences were not significant (online suppl. Fig. 4).

Biomarkers in Prognosis

We specifically reviewed the clinical-pathologic characteristics of subgroups, given the distinctions between pure NEC and MiNEC (online suppl. Table 1). Among 463 pure NEC patients (72.3%), up to 291 (62.9%) patients had elevated NSE, 127 (28.5%) had elevated CEA, and 95 (21.3%) had elevated CA199 ($p < 0.001$). All markers were linked with advanced stage (all $p < 0.001$). Elevated NSE group had higher rates of metastatic disease (59.1% vs. 34.9%), CA199 associated with hepatic metastases (51.6% vs. 27.6%), while CEA associated with extra-hepatic metastases (52.0% vs. 33.5%) (all $p < 0.001$). Conversely, histologic types and Ki67 did not correlate with biomarker levels. KM survival analysis revealed significant differences between elevated versus normal NSE (17.0 months vs. not reached) (Fig. 1), as well as CA199 (15.9 vs. 29.0 months) and CEA (16.6 vs. 25.5 months) (all $p < 0.0001$). MiNEC ($n = 177$, 27.7%) was also investigated (online suppl. Table 1). We found that 85.5% of mixed components were adenocarcinoma, while the rest were squamous carcinoma (10.5%) and mixed non-neu-

roendocrine components. NSE (19.3 months vs. not reached, $p < 0.0001$), CEA (17.7 months vs. 60.0, $p = 0.00013$), and CA199 (17.7 vs. 55.3 months, $p = 0.00044$) levels all showed significance for OS of MiNEC (Fig. 1). Significantly increased hazard ratios of NSE (HR = 2.47 [1.56–3.9], $p = 0.001$ for pure NEC and HR = 5.64 [2.39–13.3], $p = 0.001$ for MiNEC) were observed in multivariate analysis, while CA199 (HR = 2.29 [1.38–3.79], $p = 0.001$) had significantly worse survival in pure NEC (Table 2). We specifically analysed histologic subgroups of GEPNEC as a reference. We found that in both GEPSCNEC and GEPLCNEC, the HRs increased in the NSE-elevated group, and elevated CEA indicated a worse survival in GEPSCNEC (online suppl. Table 2).

Biomarkers in Predicting Therapeutic Efficacy

We also analysed associations between biomarkers and advanced NEC patients with first-line EP regimen. Only the baseline NSE elevation group was significantly distinguished from their counterparts in OS (10.7 months vs. not reached, $p = 0.0021$) (Fig. 2). The baseline NSE elevation group was also significantly different in multivariate analysis (HR = 3.4 [1.44, 8.03], $p = 0.005$) (online suppl. Table 3). To some extent, biomarker remission had associations with clinical events, as CEA remission predicted a better OS (12.8 months vs. 31.8 months, $p = 0.077$) (online suppl. Fig. 2), and NSE remission correlated with a better ORR ($p = 0.009$) (online suppl. Table 4).

Biomarker-Based Subtypes

To better characterize therapy response, GEPNEC was classified into 3 categories via biomarkers. We defined the NSE-elevated group as “neuroendocrine-like NEC” (NLN, $n = 381$). “Adenocarcinoma-like NEC” (ALN, $n = 63$) was deemed as either elevated CEA or CA199 with normal NSE; those with no elevations of all biomarkers were defined as “triple-normal NEC” (TNN, $n = 194$). For distribution of these subtypes among all sites, NLN was most common in oesophagus and rectum, while ALN was relatively more prevalent in the intestine and TNN in the stomach. Concerning clinical-pathologic features, NLN was predisposed to advanced disease (58.8%), compared with TNN (28.6%) and ALN (41.3%) (Table 3). Significant differences were observed in OS of TNN, ALN, and NLN (not reached vs. 21.0 vs. 17.1 months, $p < 0.001$) (Fig. 3). Multivariable analysis reaffirmed that NLN achieved significantly far worse OS (HR = 3.92 [2.55–6], $p < 0.001$) than TNN (online suppl. Table 5). This was in line with treatment response, as

Fig. 1. OS regarding the biomarkers elevation in NEC: pure NEC with NSE (a), CEA (b), CA199 (c) and MiNEC with NSE (d), CEA (e), CA199 (f). MiNEC, mixed neuroendocrine non-neuroendocrine carcinoma; OS, overall survival; NSE, neuron-specific enolase; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen 19-9.

Table 2. Univariate analysis applying log-rank test and multivariate analysis estimated through Cox proportional hazards model in pure NEC and MiNEC patients

Factors	Univariate			Multivariate		
	HR	CI 95	<i>p</i> value	HR	CI 95	<i>p</i> value
<i>Pure NEC</i>						
Sex (M)	1.04	0.78–1.37	0.802	–	–	–
Age	1	0.99–1.01	0.994	–	–	–
Ki67	0.72	0.38–1.37	0.317	–	–	–
Distant metastases	3.05	2.36–3.95	0.001	2.78	2.02–4.28	0.001
Lymph-node metastases	1.74	1.1–2.73	0.017	1.62	0.97–2.69	0.063
Hepatic metastases	2.41	1.87–3.12	0.001	0.78	0.37–1.63	0.505
NSE >1*ULN	2.77	2.06–3.73	0.001	2.47	1.56–3.9	0.001
CEA >1*ULN	1.83	1.4–2.38	0.001	0.97	0.61–1.56	0.912
CA199 >1*ULN	2.33	1.75–3.11	0.001	2.29	1.38–3.79	0.001
<i>MiNEC</i>						
Sex (M)	1.03	1.01–1.06	0.006	1.04	1–1.09	0.063
Age	1.64	0.92–2.93	0.092	1.23	0.49–3.09	0.657
Ki67	5.43	2.2–13.41	0.001	0.85	0.18–3.95	0.835
Distant metastases	14.04	3.34–58.96	0.001	11.96	2.49–57.41	0.002
Lymph-node metastases	1.42	0.78–2.56	0.251	–	–	–
Hepatic metastases	3.73	1.82–7.64	0.001	1.22	0.56–2.64	0.614
NSE >1*ULN	3.92	2.36–6.5	0.001	5.64	2.39–13.3	0.001
CEA >1*ULN	2.47	1.54–3.97	0.001	2.08	0.91–4.77	0.083
CA199 >1*ULN	2.41	1.45–4	0.001	1.79	0.75–4.3	0.19

Values are *n* (%). *p* values determined with likelihood ratio test for HRs in Cox proportional hazards regression 95% CI. Blank values (–) are too insignificant (*p* > 0.1) in univariate analysis to be included in multivariate analysis. MiNEC, mixed neuroendocrine non-neuroendocrine carcinoma; CI 95, 95% confidence interval; ULN, upper limit of normal; NSE, neuron-specific enolase; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen 19-9; HR, hazard ratio.

NLN had a higher progressive disease rate (32.5%) than ALN (32.5%) or TNN (34.9%) (Table 3). We specifically illustrated that ALN had the best ORR (50%) and DCR (75%) to “adenocarcinoma-like chemotherapy,” separating it as a superior entity for non-EP regimens. EP proved to be an optimal choice in NLN patients (DCR [67%], ORR [35%]) and in TNN (DCR [72%], ORR [20%]) (Table 4).

Discussion

Compared to comprehensive biochemical indicators in SCLC, the role of circulating tumour markers to predict and monitor outcome has not been properly assessed in extra-pulmonary NEC [30]. The traditional serum marker CgA is limited because it is hard to assess and has unsatisfying power in high-grade NEN [15]. We thus examined the predictive abilities of routinely assessed NSE/CEA/CA199 and established them as excellent serum

subtypes reflecting clinical features and treatment response of GEPNEC.

Epidemiologically, we confirmed that the prevalence of NSE-elevated patients was much higher in GEPNEC than in GEPNET, and the same was true for elevated CA199 and CEA. In general, primary sites of GEPNEC correlated with NSE levels, when oesophagus and rectal NEC were significantly higher than gNEC; the reasons why these locations had higher frequency remain undetermined but have clinical implications. CEA and CA199 mainly increased in NEC of adenocyte-enriched primary sites (colon and pancreas) and the proportions were relatively low in the oesophagus, which may reflect an adenocarcinoma-like feature in these locations. Additionally, the levels of biomarkers had no pathologic specificity.

We propose that these biomarkers are favoured in evaluating prognosis during management of GEPNEC. It is widely acknowledged that NSE reflects systemic cancer burden and manifests metastases [2]. Moreover, CEA/CA199 can also identify advanced disease, indicating

their value in assessing tumour burden. Higher NSE is relevant to worse progression free survival/OS in advanced pNET (RADIANT-1/RADIANT-3), IV GEPNET, and SCLC [8, 9, 17–20, 31]. These results are consistent with much higher elevated CEA/CA199 proportions in metastatic SCLC [13, 32, 33]. We confirmed that NSE level was predisposed to undesirable prognosis, and the same was true for CEA and CA199. We upgraded the prognostic values of CEA/CA199. CEA was previously reported to be associated with survival of SCLC, disease progression, and post-operative/treatment monitoring [32–34]. Recent studies also revealed its capability of predicting survival for patients with gNEC and gNET [26, 35]. The mechanisms underlying CEA/CA199 and aggressiveness of NEC are undefined but are possibly due to tumour hypoxia and vessel invasion [29]. Accumulating evidence suggests that higher NSE can be applied in different histologic types, as it is independently correlated with a poorer OS in SCLC [24, 36–38]. Nonetheless, clinical implications of NSE in LCNEC survival are disputed [39, 40]. But we demonstrated that NSE can be extended to GEPLCNEC. We recommend applying CEA to evaluate prognosis of GEPSCNEC, and applying CA199 to evaluate prognosis of GEPLCNEC.

We suggested that tumour biomarkers were endowed with predictive meanings: baseline NSE elevation may be correlated with resistance to first-line EP regimen, while NSE remission may forecast response in GEPNEC, in accordance with its predictive strength in first-line therapy in SCLC [26, 30, 37]. Responders to CEA in first-line therapy were more likely to experience longer OS. Although no significance was detected in CA199, it was reported to predict first-line treatment response of NEN [41–43]. Further investigations may validate these discoveries.

Traditional tumour-node-metastasis staging and histopathological categories still have flaws and are not concise enough in prognosis and management of NEC [44]. Biomarker combinations may shed light on the problem, as they were previously identified to describe survival in advanced pNET and generate a predictive modelling framework (NSE and lactate dehydrogenase) for guiding SCLC therapy [8, 17, 45]. NSE is commonly acknowledged as a major neuroendocrine-marker, and CEA/CA199 may represent “adeno-markers,” so we determined whether subtypes could be constructed based on biomarkers to delineate different NEC features. NLN (with higher neuroendocrine biomarkers) usually behaves more aggressively than ALN (with higher “adeno-markers”) and TNN, manifesting higher frequency of metastases and worse survival. Thus, the proportions of

Fig. 2. Predictive values of biomarkers remission in the advanced NEC with first-line EP regimens: NSE (**a**), CEA (**b**), CA199 (**c**). NSE, neuron-specific enolase; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen 19-9; EP, etoposide plus cisplatin; ULN, upper limit of normal value.

Table 3. Baseline information for subtype-related clinic-pathological characteristics in NEC

	Level	TNN	ALN	NLN	<i>p</i> value
<i>N</i>	638*	194	63	381	
Age (mean [SD])		58.84 (10.97)	58.94 (10.85)	58.05 (11.57)	0.676
Sex (%)	F M	60 (30.9) 134 (69.1)	12 (19.0) 51 (81.0)	103 (27.0) 278 (73.0)	0.179
Site (%)	Oesophagus Stomach Intestine Rectum Pancreas Others	24 (12.4) 94 (48.5) 20 (10.3) 11 (5.7) 17 (8.8) 28 (14.4)	3 (4.8) 31 (49.2) 10 (15.9) 6 (9.5) 6 (9.5) 7 (11.1)	63 (16.5) 116 (30.4) 43 (11.3) 40 (10.5) 36 (9.4) 83 (21.8)	0.001
Component (%)	Small cell Large cell Mixed/unknown	60 (60.6) 34 (34.3) 5 (5.1)	23 (54.8) 17 (40.5) 2 (4.8)	169 (67.9) 69 (27.7) 11 (4.4)	0.433
Ki67% (mean [SD])		63 (25)	70 (19)	68 (22)	0.032
Lymph-node metastases (%)	Yes	120 (65.6)	39 (76.5)	196 (77.5)	0.069
Distant metastases (%)	Yes	55 (28.6)	26 (41.3)	224 (58.8)	<0.001
Hepatic metastases (%)	Yes	34 (17.7)	17 (27.0)	144 (37.8)	<0.001
Extrahepatic metastases (%)	Yes	38 (19.8)	19 (30.2)	179 (47.0)	<0.001
Therapy (%)	CT EP/IP	43 (32.6) 89 (67.4)	15 (31.2) 33 (68.8)	98 (29.1) 239 (70.9)	0.355
Efficacy (%)	PR SD PD	22 (20.2) 49 (45.0) 38 (34.9)	15 (37.5) 12 (30.0) 13 (32.5)	91 (29.2) 94 (30.1) 127 (40.7)	0.027
DCR rate (%)	DCR PD	71 (65.1) 38 (34.9)	27 (67.5) 13 (32.5)	185 (59.3) 127 (40.7)	0.396

TNN, triple-normal NEC; ALN, adenocarcinoma-like NEC; NLN, neuroendocrine-like NEC; Intestine, duodenum, small intestine, and colon; CT, chemotherapy on corresponding adenocarcinoma of same location; EP/IP, etoposide/irinotecan plus cisplatin regimens; DCR, disease control rate; CR, complete response; PR, partial response; SD, stable disease; PD, progression disease; NSE, neuron-specific enolase; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen 19-9. * There are 2 patients without enough information for classifications (normal NSE level with lack of both CEA and CA199 information).

different-primary site NLN may account for the disparity in prognosis, for example, poorer outcome in oesophagus NEC [3, 18, 46].

We also illustrated that contrary to the common EP regimen used in advanced NEC, ALN responded better to “adenocarcinoma-like chemotherapy,” which may be conducive to shifting first-line therapy in this entity. The EP regimen was still a better option than IP in NLN and TNN, consistent with current guidelines [47]. But NLN showed an inconsistent lower DCR and shorter OS than TNN. It may be associated with innately more aggressive

behaviours of NLN, and possibly related to secondary drug resistance of NLN in next-line treatment, arousing controversy on optimal sequential treatment. This association required future prospective validation on second-line therapy. Our subtypes provide novel evidence when incorporating comprehensive clinical features and are more informative in first-line therapeutic options. We recommend that our findings are utilized as alternative guidance to traditional histologic subtypes, as the subtypes may exhibit broader clinical implications when integrated with other discriminatory tools.

Fig. 3. OS regarding the serum-based subtypes of the NEC. There are 2 patients without enough information (normal NSE level with lack of both CEA and CA199 information). NLN, neuroendocrine-like NEC; ALN, adenocarcinoma-like NEC; TNN, triple-normal NEC; OS, overall survival; NSE, neuron-specific enolase; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen 19-9.

Consequently, our study provides strong evidence of the value of biomarkers in GEPNEC, which have been ignored by previous studies. Firstly, contrary to previous reports with tremendous heterogeneity and small samples of biomarkers [48], this is a large-sample study concentrating on GEPNEC. Moreover, NSE/CA199/CEA is easily detected and had promising monitoring strength similar to traditional CgA. Novel progression of biomarkers, including circulating cell-free DNA, circulating tumour cells, microRNAs, or long non-coding RNAs, and blood transcripts (e.g., NETest) are underway for GEPNET [49, 50]. Most discoveries are based on a small number of samples and have not reached a consistent standard [51, 52]. Our biomarkers are economical with higher performance. These classical serum biomarkers are superior in repeatability, accessibility, and intuitive interpretation compared to the high cost and immaturity of forthcoming biomarkers.

There are some limitations of this study. Firstly, it is a retrospective analysis from a single centre. To mitigate this, we carried out strict screening to enrol eligible patients with convincing data and monitor any inconsistency during the process to confirm the quality of the data. There may also be false-positive values of serum biomark-

Table 4. Therapy response to subtypes of NEC

Types	First-line	PR	SD	PD	ORR, %	DCR, %	p value
TNN	CT	4	14	16	12	53	0.15
	EP	11	28	15	20	72	
	IP	2	3	6	18	45	
ALN	CT	6	3	3	50	75	0.26
	EP	3	6	7	19	56	
	IP	3	3	5	27	55	
NLN	CT	15	25	43	18	48	0.0073*
	EP	47	44	44	35	67	
	IP	26	20	31	34	60	

TNN, triple-normal NEC; ALN, adenocarcinoma-like NEC; NLN, neuroendocrine-like NEC; EP, etoposide plus cisplatin; IP, irinotecan plus cisplatin; CT, "adenocarcinoma-like therapy" on adenocarcinoma of same locations; DCR, disease control rate; PR, partial response; SD, stable disease; PD, progression disease; ORR, objective response rate. * p value < 0.05 as significance.

ers (especially in CEA/CA199), which is led by confounding factors, such as tobacco use, diabetes, and inflammatory conditions. Thus, there are still no optimized cut-off values. Inconsistency of the reference range of the utilized assay in different studies also hampers interpretation. We employed Cox regression models to adjust all available parameters to lower the biases. In addition, a small number of patients were pre-treated before referral to our institute or their treatment had lapsed. This accounted for missing baseline values. Various pre-referral or post-progression treatment modalities might expand heterogeneity, so we strictly enrolled and analysed 3 major regimens. Despite the day-to-day variation of all serum biomarkers, combining them with radiographic tools in evaluating treatment response may be more applicable and worth exploring.

In conclusion, our cohort allowed us to identify meaningful biomarkers (NSE/CEA/CA199) with respect to their predictive values in GEPNEC. We demonstrated that elevation of NSE, CEA, and CA199 had poorer survival. NSE was affirmed as significant (both pure NEC and MiNEC) and CA199 in NEC by multivariable analysis. NSE independently correlated with response to first-line therapy in advanced GEPNEC. We recapitulated serum biomarker pattern-based subgroups of NEC, revealing valid clinical characteristics and prognosis. Notably, the distinctive entity ALN was recommended for adenocarcinoma-like chemotherapy. Further prospective and randomized studies are warranted to validate the clinical utility of traditional tumour biomarkers in GEPNEC.

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Statement of Ethics

We declare that the research is conducted ethically in accordance with the World Medical Association Declaration of Helsinki. We state that all subjects have given their written informed consent and that the study protocol was approved by the Ethics Committee of the Peking University School of Oncology with a reference number of 2017YJZ36.

Conflict of Interest Statement

We have read and understood the policy on disclosing conflicts of interest and have no conflicts of interest to declare.

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Author Contributions

Ming Lu, Jie Li, and Lin Shen made substantial contributions to conception and design. Ming Lu, Yu Sun, Jian Li, Jun Zhou, Xicheng Wang, and Zhi Peng helped acquire the data. Jianwei Zhang drafted the article and analysed and interpreted the data. Ming Lu, Xiaotian Zhang, Yanshuo Cao, and Panpan Zhang participated in drafting the article and revising it critically for important intellectual content.

Data Availability Statement

All data generated or analysed during this study are included in this article and its supplementary material files. Further enquiries can be directed to the corresponding author.

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