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Original Article

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The Role of Plasma Chromogranin A as Assessment of Treatment Response in Non-functioning Gastroenteropancreatic Neuroendocrine Tumors

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Purpose

Chromogranin A (CgA) has been considered to be valuable not only in the diagnosis but also in monitoring the disease response to treatment. However, only a few studies have been published on this issue. We purposed to evaluate whether biochemical response using plasma CgA level is reliable in concordance with the clinical response of grade 1-3 nonfunctiong gastroenteropancreatic neuroendocrine tumors (GEP-NETs).

Materials and Methods

Between March 2011 and September 2013, a total of 27 cases in 18 patients were analysed, clinically and radiologically while serial CgA tests were also conducted during treatment. Tumor responses were defined by both Response Evaluation Criteria in Solid Tumors (RECIST) criteria ver. 1.1 and biochemical criteria based on the CgA level.

Results

Among the 27 cases analysed, no difference in the basal CgA level was observed with regard to gender, primary tumor site, tumor grade (World Health Organization classification), liver metastasis, number of metastatic site, and line of chemotherapy. The overall response rate (RR) by RECIST criteria ver. 1.1 was six out of the 27 cases (22.2%) and eight out of the 27 cases (29.6%) for biochemical RR. The overall concordance rates of the response based on RECIST and biochemical criteria were 74%. In grades 1 and 2 GEP-NETs (n=17), the concordance rate of the disease control was 94.1%. There was a significant difference for progression-free survival (PFS) between responders and non-responder in accordance to biochemical criteria (35.73 months vs. 5.93 months, p=0.05).

Conclusion

This study revealed that changes of the plasma CgA levels were associated with tumour response. Additionally, biochemical response based on serial CgA may be a predictive marker for PFS in GEP-NETs.

Key words
Chromogranin A, Gastro-enteropancreatic neuroendocrine tumor, Predictive

Introduction

Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are rare malignant neoplasm with an incidence ranging from two to five cases/100,000/yr. Because it originates from the enterochromaffin serotonin-producing cell, it has a unique feature of hormone secretion and expression of distinctive differentiation markers [1-6].

Clinical features of GEP-NET are very heterogeneous and nonspecific. Some patients remain asymptomatic for several years, or complain of episodic flushing, abdominal pain, nausea, vomiting, and diarrhea. In most cases, due to the vagueness of symptoms, a diagnosis is delayed (3-10 years on average), with an increased risk of developing metastases. The ability of the imaging method to localize primary or

metastatic site of GEP-NET also has some limitations. Most GEP-NETs are highly vascular, thus could easily be detected by a contrast enhanced computed tomography (CT); however, approximately 6% to 20% are hypovascular and often difficult to be evaluated by a CT scan or other imaging methods. GEP-NETs originating from jejunum and ileum are also often difficult to identify on an image due to their small size [7]. Therefore, non-invasive parameters, indicating GEP-PET, are needed for diagnosis, following-ups and prognosis.

Chromogranin A (CgA), a glycoprotein contained in secretion granules of neuroendocrine cells, is the most abundant granin in GEP-NETs and widely used as a circulating tumor marker, but only few studies have been published on the role of CgA in patients with GEP-PET, and the range of sensitivity were variously reported [8-10].

We investigated to evaluate whether biochemical response using serial plasma CgA is reliable in concordance with the tumor response based on Response Evaluation Criteria in Solid Tumors (RECIST) criteria in GEP-NETs irrespective of chemotherapeutic agents. Simultaneously, we analyzed the relationships between the CgA level and clinicopathological characteristics.

Materials and Methods

1. Patient

A total of 27 cases in 18 patients, who were pathologically diagnosed in GEP-NETs, were analysed between March 2011 and September 2013. For all cases, serial CgA was checked during the course of treatment. Non-functioning was defined to the absence of clinical syndromes of hormonal hypersecretion, such as hypoglycemia, peptic ulcer, diarrhea, steatorrhea, acromegaly, cushing's syndrome, and gallstone. The following clinicopathological characteristics of all 18 patients

Table 1. Baseline CgA level according to clinical characteristics in 27 neuroendocrine tumor-cases (18 patients)

Factor	No.	CgA levels (U/L)	p-value
Sex			
Male	12	85.25 (42.65-740.00)	0.232
Female	15	201.15 (33.18-895.00)	
Primary tumor site			
Stomach	2	714.41 (688.83-740.00)	0.187
Duodenum	3	53.62 (51.70-70.87)	
Ampulla of Vater	1	142.00	
Colon	2	100.08 (80.19-119.97)	
Rectum	6	105.95 (33.18-253.59)	
Pancreas	11	391.86 (42.65-895.00)	
Liver	1	244.09	
Unknown	1	221.86	
WHO classification			
Grade I neuroendocrine tumor	9	221.86 (33.18-548.32)	0.759
Grade II neuroendocrine tumor	8	107.89 (42.65-770.00)	
Neuroendocrine carcinoma	10	100.08 (51.70-895.00)	
Liver metastasis			
Yes	18	134.47 (33.18-895.00)	0.662
No	9	142.00 (80.19-740.00)	
No. of metastatic sites			
1	17	148.98 (42.65-770.00)	0.581
≥ 2	10	105.95 (33.18-895.00)	
Line of chemotherapy with serial CgA monitoring			
First-line	15	128.59 (33.18-895.00)	0.725
Second-line	7	83.31 (51.70-770.00)	
Third-line	4	400.95 (53.62-740.00)	
Fourth-line	1	391.86	

CgA, chromogranin A; WHO, World Health Organization.

Table 2. The treatment-evaluation by RECIST criteria and by biochemical (CgA level) response criteria

Response	RECIST criteria	Biochemical criteria
Complete	-	-
Partial	6 (22.2)	8 (29.6)
Stable disease	17 (63.0)	16 (59.3)
Progressive disease	4 (14.8)	3 (11.1)

Values are presented as number (%). RECIST, Response Evaluation Criteria in Solid Tumors; CgA, chromogranin A.

were collected: age, sex, primary site, tumor grade in accordance to the 2010 World Health Organization (WHO) classification, liver metastasis, number of metastatic site, site of metastasis, and information of chemotherapy. Systemic chemotherapies for GEP-NET included octreotide, VIP (vincristine, ifosfamide, cisplatin), XELOX (capecitabine, oxaliplatin), EP (etoposide, cisplatin), pazopanib, sunitinib, everolimus, and XELIRI (capecitabine, irinotecan).

2. Efficacy assessment

Biochemical efficacy was estimated according to the criteria proposed by the Italian Trials in Medical Oncology (ITMO) Group [1] for evaluating markers (biochemical response). Partial response (PR) was defined as ≥ 50% decrease in plasma CgA compared to the baseline CgA; stable disease (SD) was defined as a decrease < 50% or as an increase < 25%; and progressive disease (PD) was defined as an increase ≥ 25%. The level of CgA was determined from venous blood samples drawn into EDTA-containing tube after overnight fasting and collected before systemic treatment. The plasma CgA level was measured by CgA-RIA (Chromoa assay, CIS Bio International, Saclay, France) with a normal range of 27-94 ng/mL. Chromoa is based on sandwich enzyme-linked immunosorbent assay, using two monoclonal antibodies (the same antibodies as CGA-RIACT) directed against the central domain of the molecule (145-245), which is less sensitive to proteolysis.

The tumor size was measured by using a CT or magnetic resonance imaging by RECIST criteria ver. 1.1.

3. Statistical analysis

The CgA level is reported as the median value and the range. Group comparisons were performed using a nonparametric test of Mann-Whitney or Kruskal-Wallis, followed by a Dunn multiple comparison test, as appropriate. Comparisons of paired values were performed using a nonparametric test of Wilcoxon. The chi-square test and the Fisher exact test (for value less than 5) were employed to compare the sensitivity and rate of concordance in different groups. In all statistical tests, a 5% level of significance was used.

Progression-free survival (PFS) was measured as the time from the date of chemotherapy to the date of first documented disease progression or death. The PFS were estimated using the Kaplan-Meier method with log-rank analysis. A two-sided p-value of less than 0.05 was considered significant. All analyses were performed using SPSS ver. 19.0 (SPSS Inc., Chicago, IL).

Results

1. Charateristics of cases

The baseline characteristics of 27 cases of 18 patients are listed in Table 1. The median age was 56 years (range, 38 to 76 years). Pancreatic NETs account for 40.5% of GEP-NETs. The rectum was the second most common site of GEP-NET (22.2%) in this study. According to WHO classification, ten of the 27 cases (37%) were grade 3 neuroendocrine carcinoma. Grades 1 and 2 NETs were nine (33.3%) and eight (29.6%), respectively. Eighteen of the 27 patients had liver metastasis. Measurable lesions were found on CT images in 18 out of the 27 cases.

Table 3. Comparison of response by RECIST criteria and biochemical criteria (CgA level) in 27 cases

Factor		RECIST criteria		Total
		Responder	Non-responder	
Biochemical criteria	Responder	3	5	8
	Non-responder	3	16	19
Total		6	21	27

RECIST, Response Evaluation Criteria in Solid Tumors; CgA, chromogranin A.

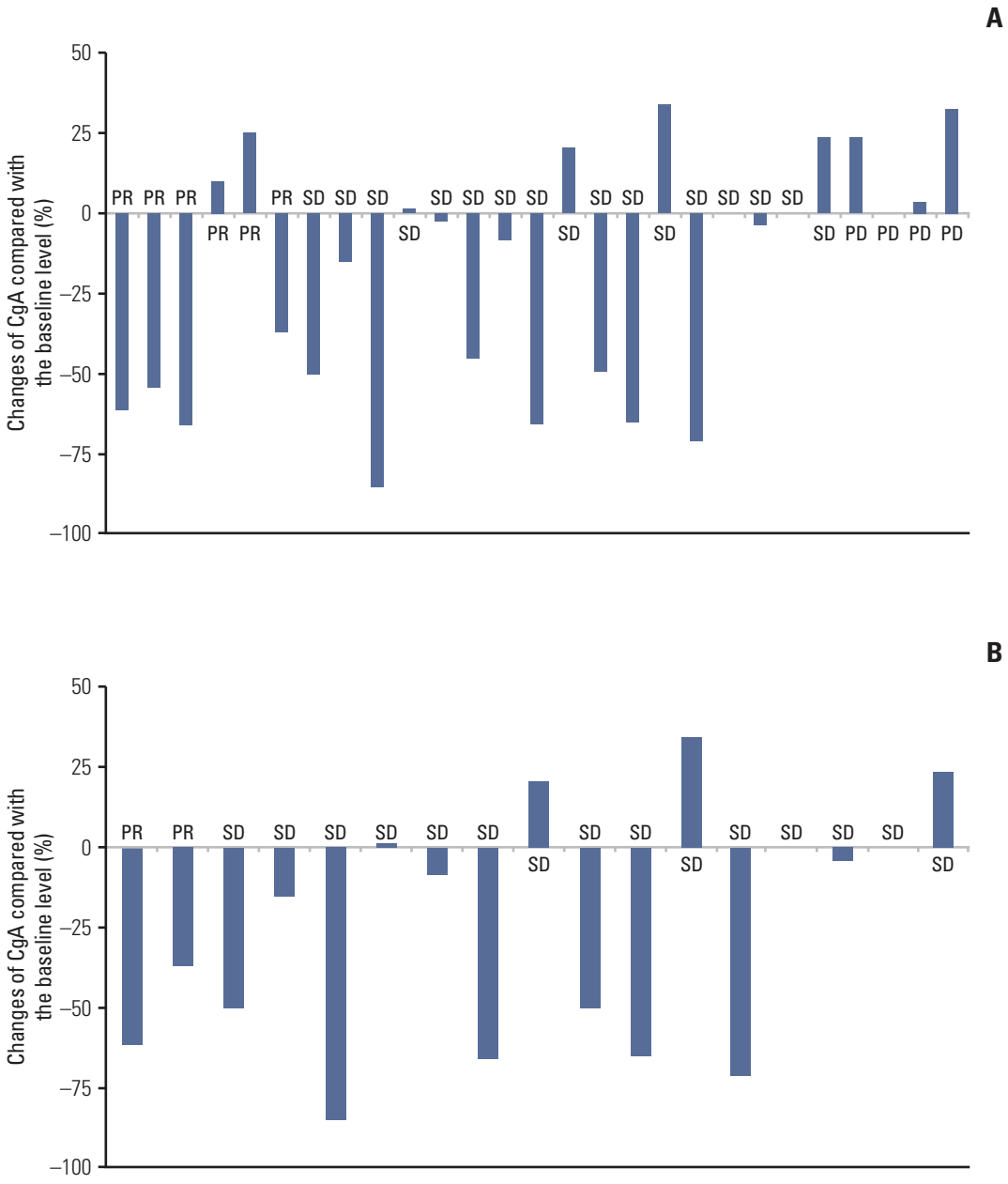

Fig. 1. Association of treatment responses with percentage changes in the chromogranin A (CgA) levels compared to the baseline levels in grades 1-3 (A) and grades 1-2 (B) cases.

2. CgA measurement at baseline

Among the 27 cases included in this study, no difference in the basal CgA level was observed in terms of gender, primary tumor site, tumor grade (WHO classification), liver metastasis, number of metastatic site, and line of chemotherapy with serial CgA monitoring. The plasma CgA level ranged from 33.18 to 895 ng/mL (Table 1).

3. Correlation of treatment response and survival with changes of CgA levels

Biochemical and tumor responses to systemic therapy are shown in Tables 2 and 3. The overall response rate (RR) by RECIST criteria was six out of the 27 cases (22.2%) and eight out of the 27 cases (29.6%) for biochemical RR. The concordance of response between RECIST criteria and biochemical

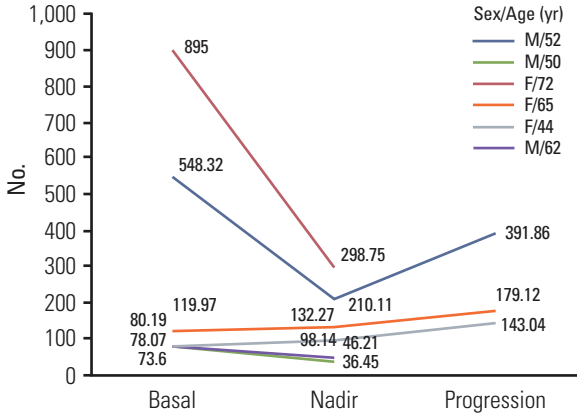

Fig. 2. Changes in the chromogranin A levels in patients who achieved the tumor response.

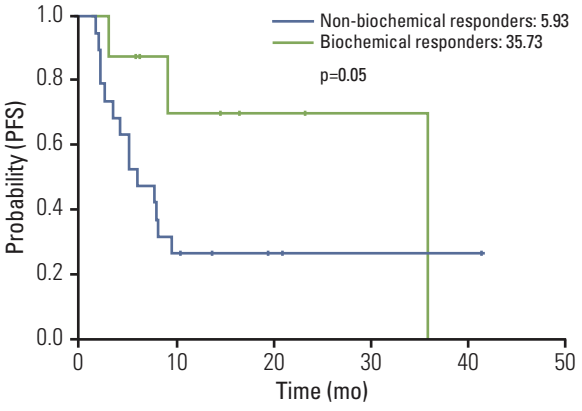

Fig. 3. Progression free survival (PFS) according to the status of the biochemical response.

Table 4. Comparison of CgA change according to treatment response in other studies

		Serum CgA determination	CgA increase	CgA stable	CgA decrease	Concordance between event and CgA-change
Welin et al. [11]	Recurred midgut carcinoid tumors	CgA > 4 nmol/L	28/33 (85)	-	-	-
Sondenaa et al. [12]	After surgical resection of midgut carcinoid tumors	CgA > 30 nmol/L	7/7 (100)	-	-	-
Baudin et al. [13]	After disease progression	CgA > 100 U/I	5/6 (85)	-	-	-
Jensen et al. [14]	Median F/U: 12 mo	SD: within ± 25% PD > +25% PR < –25%	83/97 (85)	204/279 (73)	39/50 (78)	76%
Walter et al. [15]	Median F/U: 27 mo	SD: within ± 50% PD > +50% PR < –50%	28/50 (56)	22/47 (47)	7/15 (46)	51%
Nehar et al. [16]	Median F/U: 33 mo	SD: within ± 25% PD > +25% PR < –25%	89%	78%	79%	80%
Chou et al. [17]	Asian patients, median F/U: 12 mo	Responder: decrease ≥ 20% Non-responder: decrease < 20%	6/6 (100)		5/5 (100)	100%

Values are presented as number (%) unless otherwise indicated. CgA, chromogranin A; SD, stable disease; PD, progressive disease; PR, partial response.

criteria were 74% (Table 3). Compared with the baseline values, a decrease of ≥ 50% in the CgA level were observed in three out of six cases (50%) with PR by RECIST criteria (Figs. 1A, 2). In only grades 1 and 2 GEP-NETs cases (n=17), the concordance of disease control between RECIST criteria and biochemical criteria were 94.1% (Fig. 1B). There was a significant difference for PFS between responders and non-responders (35.73 months vs. 5.93 months, p=0.05) based on the biochemical criteria (Fig. 3). A subgroup analysis of PFS between responders and non-responders, in accordance to RECIST response, tumor grade, and primary site, were not statistically significant, but showed longer PFS in the biochemical responder group (Fig. 4).

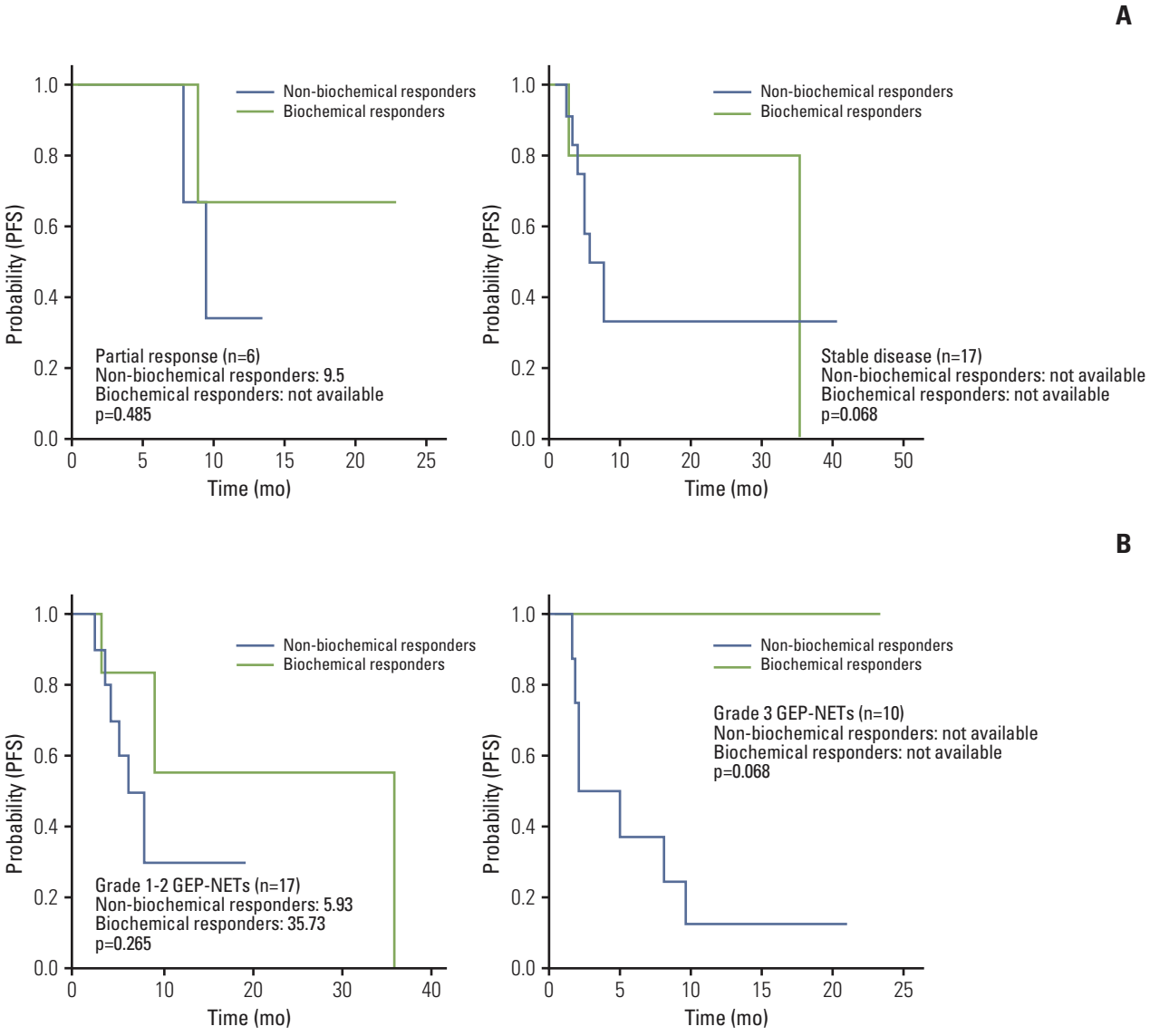

Fig. 4. Subgroup analysis of progression-free survival (PFS) between responders and non-responders of chromogranin A according to Response Evaluation Criteria in Solid Tumors criteria (A), tumor grade (B), *(Continued to the next page)*

Discussion

CgA is the most abundant granin in GEP-NETs and represents the best general marker in the tissue and blood. CgA expression generally correlates with the number of dense core granules in the neuroendocrine cells. The neuroendocrine cells secrete CgA and hormones during the secretory granule exocytosis process. The CgA level has been used to indicate neuroendocrine cell activity. Thus, CgA monitoring may be helpful in assessing the response to the different therapeutic options. In some studies, CgA was considered as a biomarker of response. However, it is still controversial whether serial CgA changes reflect tumor response for treatment. This study showed that the change of the CgA level was correlated with tumor response in nonfunctioning GEP-NETs. Additionally, biochemical response based on serial CgA may be a predictive marker for PFS in GEP-NETs.

Changes in circulating CgA have been reported to represent tumor burden and treatment response. To our knowledge, there were no definite measuring criteria of plasma CgA for tumor response and conflicting result of sensitivity

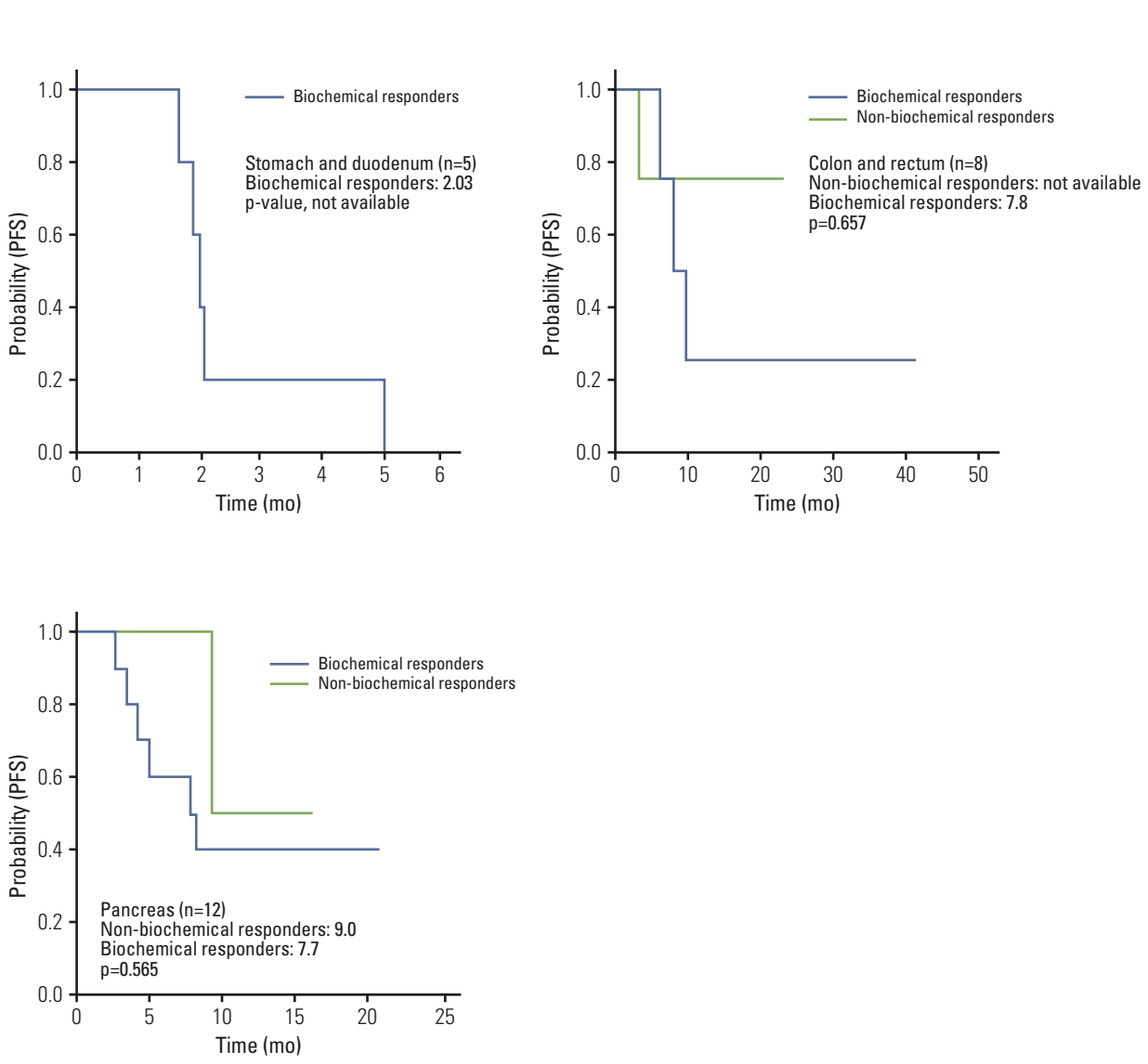

Fig. 4. *(Continued from the previous page)* and primary sites (C). GEP-NETs, gastroenteropancreatic neuroendocrine tumors.

and specificity in accordance to treatment response were reported in the literature. Previous studies [2-10,18] showed acceptable sensitivity (54%-78%) and specificity (60%-86%) for regression and SD; Jensen et al. [14] also reported that plasma CgA concentration is important to disclose tumor progression with specificity and sensitivity, 86% and 86%, respectively [16,19,20]. In our study, the concordance of response between RECIST criteria and biochemical criteria was 74%. Compared with the baseline values, a decrease of ≥ 25% in the CgA level was observed in four out of six patients (66.7%) with PR based on RECIST criteria and an increased CgA levels was shown in all 4 patients with PD (100%). Our result is similar to the report of Chou et al. [17]. Among 11 patients available for serial CgA levels, all five patients with SD or partial remission had a more than 20% decrease in the CgA levels compared to the baseline value. All six patients with PD showed a less than 20% decrease or increase in the CgA levels. Several other studies also showed the possibility of CgA as a biochemical marker of treatment response (Table 4).

High-baseline CgA value has been considered to be an independent poor prognostic marker for GEP-NETs in

previous studies [21-25]. However, whether changes of the CgA levels compared to the baseline values could predict the prognosis has not been established. In this study, there was a significant difference for PFS between the responders and non-responders for biochemical criteria (p=0.05). It suggested that a decrease in the CgA levels from the baseline levels may be an important predictive marker for survival in GEP-NETs.

This study was a retrospective analysis with small sample size, and heterogeneous patient population. The CgA levels have been affected from a diverse array of diseases. Moreover, a recognized international standard for CgA assay is not available and variations in assay types may influence results. Nevertheless, this analysis identified the usefulness of serial CgA monitoring as a biomarker that predicts treatment response and survival.

Conclusion

This study revealed that the changes of the plasma CgA levels were associated with tumour response. Additionally, biochemical response based on serial CgA may be a predictive marker for PFS in GEP-NETs.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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