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Brief Communication

Baseline plasma chromogranin A levels in patients with well-differentiated neuroendocrine tumors of the pancreas: a potential predictor of postoperative recurrence

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Short title: Prognostic value of plasma CgA in PNETs

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ABSTRACT

Background: The present study aimed to elucidate prognostic values of baseline plasma chromogranin A (CgA) concentrations in patients with resectable, well-differentiated pancreatic neuroendocrine tumors (PNETs).

Methods: Preoperative CgA levels in 21 patients with PNET were correlated with clinicopathological factors and patients' survival.

Results: Plasma CgA levels ranged 2.9–30.8 pmol/mL (median 6.0), and were significantly elevated in patients with post-operative recurrence ($P = 0.004$). Using the receiver operating characteristic curve, the optimal cutoff value to predict tumor recurrence was determined as 17.0 pmol/mL. This threshold identified patients with recurrence with 60% sensitivity, 100% specificity, and 90% overall accuracy. Patients with higher CgA levels showed worse recurrence-free survival than those with low CgA levels, both in total ($P < 0.001$) and in G2 patients ($P = 0.020$).

Conclusions: Combined plasma CgA concentrations and WHO grading may assist in better stratification of PNET patients in terms of the risk of recurrence.

Key Words: Chromogranin A; Pancreatic neuroendocrine tumor; Pancreatic cancer; Recurrence; Tumor markers

INTRODUCTION

The plasma concentration of chromogranin A (CgA) is a known diagnostic tumor marker for neuroendocrine tumors (NETs) [1,2]. CgA is an acidic glycoprotein with a molecular mass of 49 kDa and is widely expressed by both non-neoplastic and neoplastic neuroendocrine cells [3]. CgA matures in intracytoplasmic dense core granules and is secreted by exocytosis [4]. Previous studies have shown that elevated baseline CgA levels were associated with poor clinical outcomes; suggesting that plasma CgA has both diagnostic and prognostic values for patients with NETs [4-6]. However, some of those studies have involved mainly patients with unresectable NETs. Various tumors, such as gastrointestinal and pancreatic NETs or G1/2 and G3 tumors, were also simultaneously examined. Therefore, prognostic values of plasma CgA in patients with pancreatic NET (PNET) remain unclear.

In the present study, we examined baseline plasma CgA levels in Japanese patients with resectable, well-differentiated PNET and determined their correlations with other clinicopathological features, including recurrence-free survival. This is a retrospective study on a non-consecutive cohort of PNETs.

MATERIALS AND METHODS

Patient selection

The study protocol was approved by the Ethics Committee at Kobe University, and written informed consent was obtained from all patients. We retrospectively reviewed 52 patients with PNETs who were treated at our institute from January 2008 to April 2013. Among the 52 patients, 28 (54%) were randomly tested for plasma CgA, and 21 (40%) of them were enrolled in this study. The remaining 7 patients were excluded because of the use of proton-pump inhibitors (PPIs; n = 3), unresectable disease (n = 3), or refusal to undergo surgery (n = 1). The following surgical procedures were performed: subtotal stomach-preserving pancreatoduodenectomy (n = 6), pylorus-preserving pancreatoduodenectomy (n = 1), distal pancreatectomy (n = 6), spleen-preserving distal pancreatectomy (n = 1), central pancreatectomy (n = 2), and enucleation (n = 5). An additional liver resection was simultaneously carried out in 1 of 2 patients with liver metastasis. The other patient with hepatic involvement was thought to have resectable disease based on pre-operative imaging, but was found to have multiple small metastatic nodules in the liver, leading to incomplete resection. Therefore, complete resection was achieved in 20 patients, while small metastatic deposits remained in 1 patient.

Examination of plasma CgA levels

All blood samples were collected after an overnight fast, and plasma samples were stored as aliquots at -80°C until analysis by a commercial laboratory (SRL, Inc., Tokyo, Japan). Measurement of plasma CgA levels was performed with an EIA kit (YK070 Human Chromogranin A EIA kit; Yanaihara Institute, Inc., Shizuoka,

Japan). Plasma CgA levels were measured within the range from 2.4 to 92.5 pmol/mL. This radioimmunoassay system utilizes a synthetic peptide corresponding human CgA (344-374) and an antibody raised against the synthetic peptide. The kit can measure CgA levels in a highly sensitive and specific manner with no crossreactivity with other CgA-derived peptides. The specific normal range for humans is not available for this kit.

Pathological examination

One patient underwent serological and imaging examinations prior to surgery in our institute, but surgery was carried out in another hospital; a tissue sample was not available for this case. Histology slides for the remaining 20 cases were reviewed by two experienced pathologists (KO and YZ) with additional immunohistochemical stainings for endothelial markers as previous described [7]. For 1 patient who had multiple PNETs, the largest tumor was chosen for the histologic examination.

Follow-up data

Two patients with G1 insulinoma (12 mm, both) and another patient with non-functioning tumor (15 mm) who underwent surgery in another hospital were followed at our outpatient clinic with repeated computed tomography (CT) scans every 12 months. The remaining patients were followed at our outpatient clinic with repeated CT scans every 3–6 months during the first 2–3 years and yearly thereafter. The postoperative follow-up period ranged from 5 to 84 months (median: 53 months). During follow-up, 3 patients, including 1 patient with incomplete resection, died of PNETs, and another patient died of hepatocellular carcinoma. The 1-, 3-, and 5-year overall survival rates were 96%, 87%, and 75%, respectively.

Statistics

Categorical variables were compared with Fisher's exact test, and continuous variables were compared with the Mann-Whitney tests for samples with normal distribution or with Student's t tests for samples with asymmetrical distribution. A two-sided *P* value of less than 0.05 was considered statistically significant. To describe the best cutoff value for baseline plasma CgA levels to predict tumor recurrence, receiver operating characteristic (ROC) analysis was performed, and the area under the ROC curve (AUC-ROC) was calculated. Survival curves were estimated using the Kaplan-Meier method and compared by the generalized Wilcoxon test. *P* values of less than 0.05 were considered statistically significant. All analyses were performed with JMP 12.2 for Macintosh (SAS Institute, Inc., Cary, NC).

RESULTS

Plasma CgA levels

The distribution of baseline plasma CgA levels in the 21 patients is shown in Figure 1. The median baseline plasma CgA level was 6.0 pmol/mL (range: 2.9–30.9 pmol/mL).

Clinicopathological features and their correlations with plasma CgA levels

The study cohort consisted of 11 men and 10 women with a median age of 59 years (range: 29–87 years). The median tumor size was 17 mm (range 10–50 mm). Serum creatinin levels of all patients were within the normal range (0.46–0.98 mg/dL; normal range 0.5–1.3 mg/dL). No patients had clinical evidence of pancreatitis, liver failure, or heart failure. The serum gastrin level evaluated in 17 patients (81%) ranged between 58 and 824 pg/mL (median 117 pg/mL; normal range 42–200 pg/mL) and was elevated above the normal range in 4 patients. One patient had a history of a NET in the lung, and another patient had a history of a NET in the duodenum. The diagnosis of von Hippel-Lindau disease was confirmed in 2 patients, while multiple endocrine neoplasia type 1 was diagnosed in 1 patient. Among various clinical parameters, only the age of patients was correlated with plasma CgA levels ($P = 0.018$; Table 1).

In a comparison of CgA levels with histological findings, higher CgA levels were associated with the presence of venous invasion ($P = 0.046$) and perineural infiltration ($P = 0.008$). No other pathological parameters including tumor size and histologic grades were correlated with plasma CgA levels (Table 1).

Association between plasma CgA levels and tumor recurrence

Clinical outcomes were examined in 20 patients who had curative resection. During follow-up (until April 2015), 5 patients (25%) were found to have recurrence in the liver, bone, brain, and/or lymph nodes. The median interval from surgery to recurrence was 14 months (range: 4–30 months). In a comparison of clinicopathological parameters between patients with and without recurrence (Table 1, right), potential predictive factors for tumor recurrence were tumor size ($P = 0.033$), venous invasion ($P = 0.005$), perineural infiltration ($P = 0.017$), Ki-67 index ($P = 0.011$), and mitotic count ($P = 0.038$).

Baseline CgA levels in patients with tumor recurrence were also significantly higher than those in patients without recurrent disease ($P = 0.004$). The ROC curve showed that 17.0 pmol/mL was the optimal cutoff point (AUC-ROC = 0.793) to predict tumor recurrence with 60% sensitivity, 100% specificity, and 90% overall accuracy. Using this threshold, patients with higher CgA levels (n = 3) showed significantly worse recurrence-free survival than those with lower CgA levels (n = 17; Figure 2a). Because all patients with G1 tumors did not experience recurrence, an additional analysis was carried out using only G2 neoplasms. High CgA levels (≥17.0 pmol/mL) remained a significant predictive factor for tumor recurrence in patients with G2 PNETs (Figure 2b).

DISCUSSION

This is the novel clinical study demonstrating that baseline plasma CgA levels could be a potential prognostic marker for patients with resectable, well-differentiated PNETs. Interestingly, plasma CgA elevation helped us to predict tumor prognosis, even when the analysis was restricted to G2 NETs (Fig. 2b). Therefore, combined CgA concentrations and WHO grading may be useful for establishing an improved stratification scheme for patients with PNETs. Although CgA levels were measured before surgery in this study, CgA monitoring after surgery may also be useful.

The specific factors mediating the observed elevation in plasma CgA concentrations in patients with PNETs remain unclear. Given that CgA expression is increased as neuroendocrine cells mature, it is possible that plasma CgA levels may be affected by the degree of tumor differentiation [8]. This may be the reason that CgA levels are highest in small bowel NETs, where tumor cells are highly matured with many intracytoplasmic granules. Another factor that potentially affects CgA concentrations is tumor burden [2]. Although no significant correlation was found between tumor size and CgA levels in the current study, such a correlation may be observed in more advanced tumors. Interestingly, a previous study on functioning NETs showed that plasma CgA levels and tumor-related symptoms are reduced in response to somatostatin analogs, despite the lack of change in tumor size, suggesting that CgA levels may reflect the biological activities of the tumor [9].

Other basic studies also revealed that CgA or its cleavage products may be involved in cell adhesion and proliferation [10,11]. These previous observations may explain our findings that higher plasma CgA levels were associated with vascular or perineural infiltration. Given that histological grade and neural invasion are known risk factors for recurrence in patients with PNETs [12], plasma CgA levels may mirror the microscopic local invasiveness of PNETs and therefore could predict tumor recurrence.

Several medical conditions and drugs that affect plasma CgA concentrations are known. Examples include renal insufficiency and hypergastrinemia, both of which increase plasma CgA levels. In the present study, patients taking PPIs were excluded, but four patients remained to have hypergastrinemia. It might be due to other undetermined endocrinal stimuli or gastrin produced by the tumor. Another issue that influences the plasma CgA level is its analytical system. Levels of plasma CgA are known to vary substantially depending on which assay is used. Therefore, although the optimal cut-off for plasma CgA concentration was determined as 17.0 pmol/mL in the present study, the cut-off point requires validation for other assays.

This study has several limitations. First, this is a retrospective study on selected cases in which plasma CgA concentrations were measured before surgery. Second, the follow-up period (median: 53 months) may not have

been long enough to examine slow-growing tumors, like PNETs. Third, multivariate analyses were not attempted because of the small sample size.

In conclusion, the current study suggested that elevation of plasma CgA may serve as a predictive factor for tumor recurrence in patients with resectable, well-differentiated PNETs. Although this study could not examine consecutive cases with potential selection bias remaining, patients with elevated baseline CgA levels may be at high risk of recurrence, and careful monitoring with repeated imaging studies is necessary, especially in the several years post-surgery.

ACKNOWLEDGMENTS

The authors declare that they have no conflict of interest.

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FIGURE LEGENDS

Figure 1. Distribution of baseline plasma chromogranin A levels in the 21 patients examined in this study.

Figure 2. Survival analyses of patients with PNETs based on plasma CgA concentrations.

a Patients with high baseline plasma CgA levels (≥17.0 pmol/mL) show significantly worse recurrence-free survival than those with low plasma CgA levels (<17.0 pmol/mL).

b In a subanalysis using only patients with G2 PNETs, plasma CgA elevation (≥17.0 pmol/mL) remained a predictive factor for recurrence-free survival.

CgA chromogranin A, *PNET* pancreatic neuroendocrine tumor

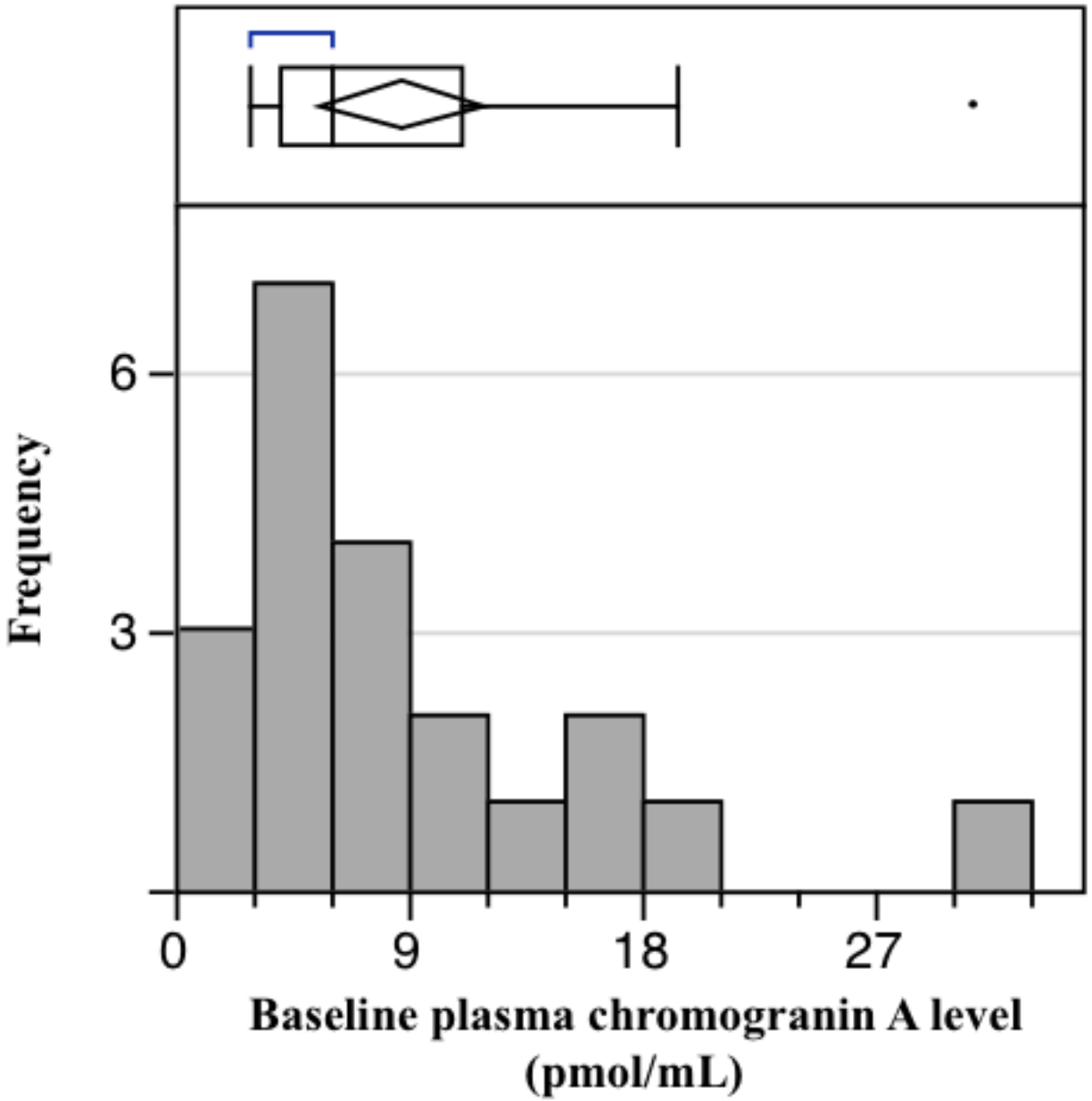
TABLE 1. Patient characteristics and those during follow-up after curative resection

Variable	Total		*P* value	Recurrence		*P* value
	n = 21	Plasma CgA, pmol/mL Median (range)		Positive (n = 5)	Negative (n = 15)	
Age, n						
≥65 years	8	11 (2.9–30.8)	0.018*	3 (38)	5 (63)	0.347
<65 years	13	5.6 (2.9–15.7)		2 (17)	10 (83)	
Sex, n						
Male	11	5.6 (2.9–30.8)	0.646	4 (40)	6 (60)	0.303
Female	10	6.0 (2.9–19.4)		1 (10)	9 (90)	
Tumor size, n						
≥20 mm	10	6.1 (2.9–30.8)	0.438	5 (50)	5 (50)	0.033*
<20 mm	11	6.0 (2.9–15.7)		0	10 (100)	
No. of pancreatic masses, n						
Single	20	6.0 (2.9–30.8)	0.679	5 (26)	14 (74)	1.000
Multiple	1	5.6		0	1 (100)	
Tumor location, n						
Head	11	5.6 (2.9–30.8)	0.751	4 (36)	7 (64)	0.319
Body–Tail	10	7.2 (2.9–17)		1 (11)	8 (89)	
Type of hormone production, n						
Non-functioning	17	5.7 (2.9–30.8)	0.893	5 (31)	11 (69)	0.530
Insulinoma	4	7.2 (2.9–9.2)		0	4 (100)	
Lymph node metastasis, n						
Positive	6	7.8 (2.9–19.4)	0.585	2 (33)	4 (67)	0.613
Negative	15	5.7 (2.9–30.8)		3 (21)	11 (79)	
Liver metastasis, n						
Positive	2	17.6 (15.7–19.4)	0.055	1 (100)	0	0.250
Negative	19	5.7 (2.9–30.8)		4 (21)	15 (79)	
Lymphatic invasion[a], n						
Positive	2	2.9	0.211	0	2 (100)	1.000
Negative	18	6.3 (2.9–30.8)		5 (29)	12 (71)	
Venous invasion[a], n						
Positive	9	9.6 (3.9–30.8)	0.046*	5 (63)	3 (38)	0.005*
Negative	11	5.7 (2.9–12.4)		0	11 (100)	
Perineural invasion[a], n						
Positive	7	15.7 (3.9–30.8)	0.008*	4 (67)	2 (33)	0.017*
Negative	13	5.7 (2.9–12.4)		1 (8)	12 (92)	
Ki-67 index[a], n						
>2 %	10	8.1 (2.9–30.8)	0.119	5 (56)	4 (44)	0.011*
≤2 %	10	5.9 (2.9–12.4)		0	10 (100)	
Mitotic count[a], n						
≥2 per 10 HPF	8	7.4 (3.9–30.8)	0.136	4 (57)	3 (43)	0.038*
<2 per 10 HPF	12	5.8 (2.9–17.0)		1 (8)	11 (92)	
Histological classification[a], n						
G1	9	6.0 (2.9–12.4)	0.381	0	9 (100)	0.033*
G2	11	6.5 (2.9–30.8)		5 (50)	5 (50)	
Baseline plasma CgA level						
Median (range), pmol/mL	NA	NA	NA	17.0 (3.9–30.8)	5.6 (2.9–12.4)	0.004*

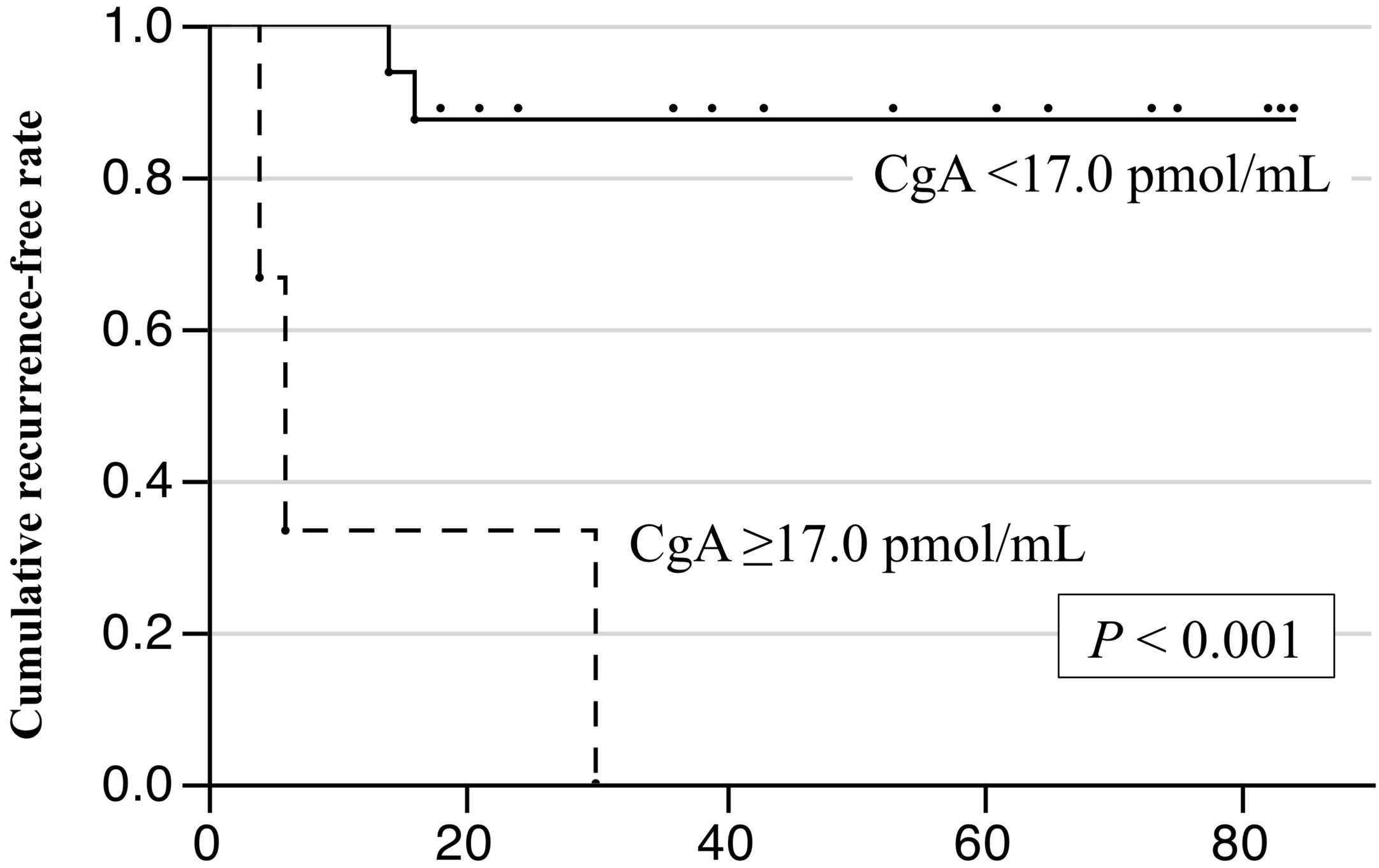
CgA chromogranin A, *HPF* high power field, *NA* not applicable

* indicates statistical significance at a *P* value of less than 0.05

[a] 20 patients were assessed for total cohort and 19 patients were assessed for recurrence


Frequency
6
3
0
9
18
27
Baseline plasma chromogranin A level
(pmol/mL)

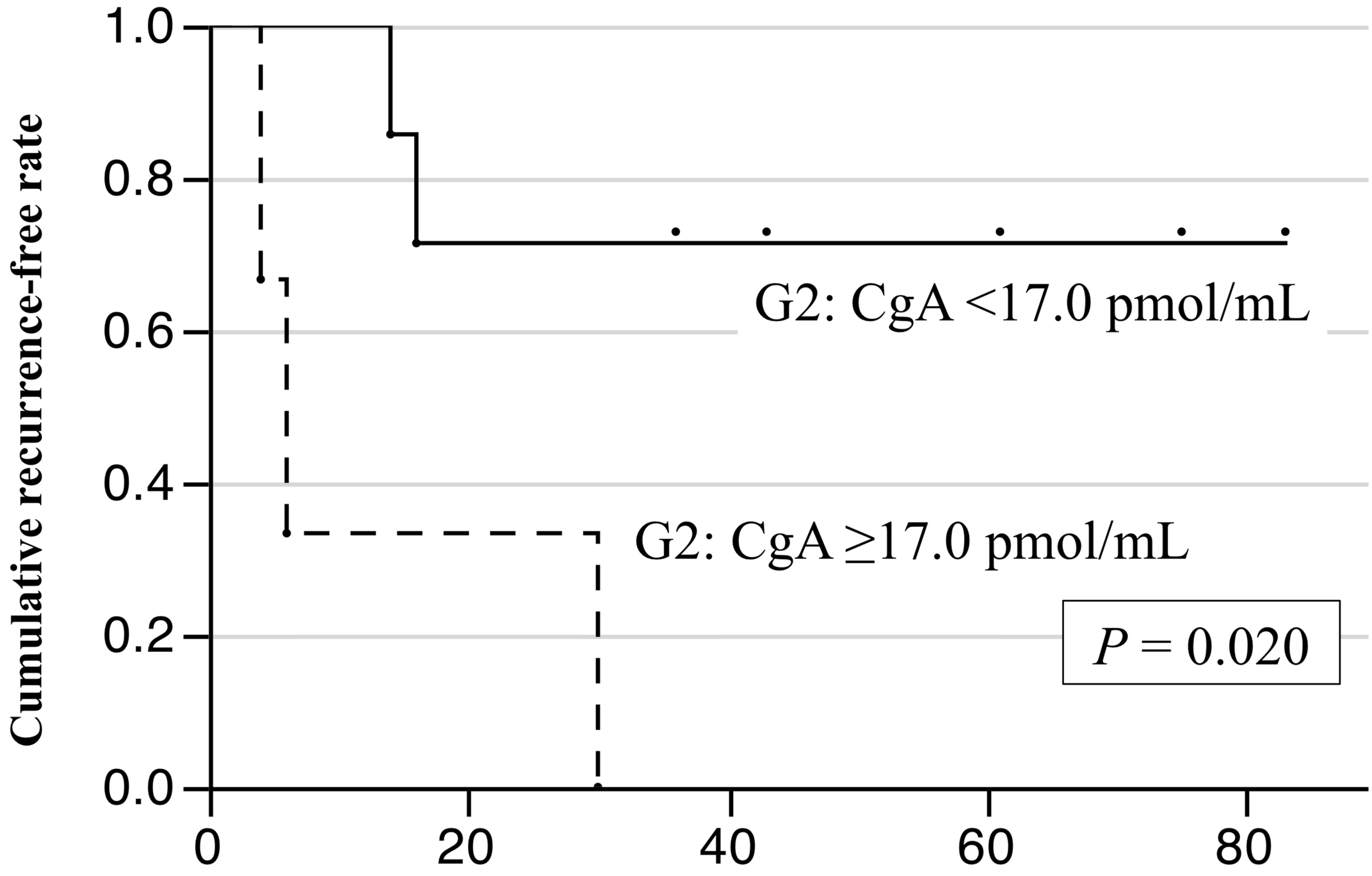
a



Number at risk

CgA <17	**17**	**13**	**9**	**7**	**3**
CgA ≥17	**3**	**1**	**0**	**0**	**0**

b



Number at risk

CgA <17	**7**	**5**	**4**	**3**	**1**
CgA ≥17	**3**	**1**	**0**	**0**	**0**