ORIGINAL ARTICLE

The Role of the Selective Arterial Secretagogue Injection Test for Non-Functional Pancreatic Neuroendocrine Tumor

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ABSTRACT

Context The selective arterial secretagogue injection test is a functional examination to determine localization of functional neuroendocrine tumor. Since the selective arterial secretagogue injection test capitalizes on the hormone-secreting ability of neuroendocrine tumor, no reports on this test in non-functional neuroendocrine tumors have yet been published. **Objective** In this study, we evaluate the utility of the selective arterial secretagogue injection test for non-functional neuroendocrine tumors. **Methods** The selective arterial secretagogue injection test was conducted in 8 non-functional neuroendocrine tumors cases. Results were compared to 10 functional neuroendocrine tumor (5 insulinoma, 5 gastrinoma) cases, and reactivity of insulin and gastrin was retrospectively considered. Insulin and gastrin immunostaining was conducted, and immunostaining results were compared with selective arterial secretagogue injection test reactivity. **Results:** The insulin-positive rate following vasa vasorum stimulation was 75% (6/8), and that for distant vessels was 25% (2/8). Selective arterial secretagogue injection test reactivity and tumor localization were related. Insulin immunostaining was positive in 50% of cases (4/8). The increase in insulin in the selective arterial secretagogue injection test in negative immunostaining cases was 8.9 μU/mL, and that in positive immunostaining cases was 31.4 μU/mL. Positive immunostaining cases tended to have greater increases in insulin than negative immunostaining cases (P=0.089). Positive gastrin response rates were 75% (6/8), 100% (8/8), 0%, and 0% in the superior mesenteric artery, gastroduodenal artery, dorsal pancreatic artery, and proximal splenic artery, respectively. Gastrin immunostaining was negative in all cases, and no relationship between tumor localization and selective arterial secretagogue injection test reactivity was observed. **Conclusion** An increase in insulin response in non-functional neuroendocrine tumors suggests that tumors may have latent insulin secretory ability.

INTRODUCTION

Pancreatic neuroendocrine tumor (PNET) is a rare tumor, representing 1-2% of all pancreatic tumors [1]. According to Yao *et al*. the incidence of newly diagnosed PNET patients in the United States is 0.32 people per 100,000 [2]. In 2005, an epidemiological study reported the incidence of newly diagnosed patients with PNET in Japan to be 1.01 people per 100,000, which is higher than that in the United States; this breaks down to 0.50 people with functional neuroendocrine tumors (F-NET) and 0.51 people with non-functional neuroendocrine tumors NET (NF-NET) [3]. Although F-NET is a minute tumor, hormones released from the tumor are associated with specific clinical symptoms, such as low blood sugar, diarrhea, and intractable ulcers, so tumor diameter at the time of diagnosis is relatively smaller than that of NF-NET. In contrast, NF-NET is not associated with any hormone-specific symptoms, and is usually only detected after symptoms such as epigastric pain, back pain, and jaundice have progressed. NET typically appears as a circumscribed oval-shaped mass with contrast enhancement, as reflected by it receiving nutrition through abundant blood flow and expansive growth. However, a small NET can often not be indicated, and a diagnosis of localization is sometimes difficult.

The selective arterial secretagogue injection (SASI) test is a functional examination to determine localization of gastrinoma, and was initially reported by Imamura *et al*. [4]. Hormones reactively released from the tumor are measured from the hepatic vein by injecting an irritant (calcium or secretin) from the pancreatic or duodenal artery that provides nutrition. The tumor location can then be identified since it occurs in the vasa vasorum region,

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Abbreviations F-NET functional neuroendocrine tumor; NF-NET non-functional neuroendocrine tumors; SASI selective arterial secretagogue injection
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where hormones are produced. The SASI test is also useful in insulinoma, glucagonoma, and VIPoma, as previously reported [5, 6], and has been a useful test for surgical method selection in F-NET. Since the SASI test capitalizes on the hormone-secreting ability of NET, no reports on this test in NF-NET have yet been published. In this study, we evaluated the SASI test for NF-NET. In addition, we have defined F-NET as a tumor that exhibits specific clinical symptoms by a hormone secreted from the tumor, and NF-NET as a tumor that exhibits no specific clinical symptoms.

METHODS

Subjects and Evaluation Items

SASI tests were conducted at Hiroshima University Hospital from April 2007 to April 2013 in 8 NF-NET cases in which surgery was performed. Five cases of insulinoma and five cases of gastrinoma in which the SASI test was performed during the same period were used for comparison. The reactivity of insulin and gastrin in the SASI tests conducted in NF-NET cases was compared. Furthermore, insulin and gastrin immunostaining of excised samples was performed, and these results were compared with the SASI test reactivity results. This research was carried out after obtaining the approval of the ethics committee of this facility.

SASI Test

The SASI test was conducted by inserting a catheter through the superior mesenteric artery (SMA), gastroduodenal artery (GDA), dorsal pancreatic artery (DPA), proximal splenic artery (SA), and distal SA, and 0.025 mEq/kg calcium gluconate was administered selectively. A blood sample was taken from the right hepatic vein just before administration as well as 30 seconds, 60 seconds, and 120 seconds after administration, and insulin and gastrin were measured. A >100% increase in insulin level over the previous value and a >80 pg/mL and ≥20% increase in gastrin over the previous value was deemed to be a positive response. The vasa vasorum was used to observe tumor staining via angiography; when it could not be detected, the vasa vasorum was anatomically estimated.

Immunostaining

Excised samples were fixated with formalin, and 3-μm paraffin-embedded thin sections were prepared. Immunostaining was conducted using automatic staining equipment (DAKO AUTOSTAINER: DAKO JAPAN Co., Tokyo, Japan) following the manufacturer's protocol. Primary antibodies were anti-insulin mouse monoclonal antibody (Histofine SAB-PO(M) KIT: NICHIREI BIOSCIENCES INC., Tokyo, Japan) used as an undiluted solution, and anti-gastrin rabbit polyclonal antibody (Novocastra™ Lyophilized Rabbit Polyclonal Antibody Gastrin: Leica Biosystems Newcastle Ltd., Newcastle Upon Tyne, UK) diluted to 1:100. The En Vision Kit (DAKO JAPAN Co., Tokyo, Japan) was used for visualization. No antigen activation treatment was conducted. For immunostaining, samples that were >10% stained were noted as (2+), those stained 0-10% were noted as (+), and those not stained were noted as (-).

Statistical Analysis

The Mann-Whitney U test was used for verification and a *P*-value <0.05 was determined as statistically significant.

RESULTS

Patient Disposition and Baseline Characteristics

Patient backgrounds are shown in **Table 1**. Subjects included 2 men and 6 women; the average age was 62.4 years (range, 44-74 years). Regarding tumor localization, 2 cases were in the pancreatic head, 5 in the pancreatic body, and 1 in the pancreatic tail. Regarding the vasa vasorum, there were 2 cases each of SMA, GDA, and DPA, and 1 case each of proximal SA and distal SA. The average tumor size was 8.8 mm (range, 5.5-12 mm); 6 cases had G1 tumors and 2 cases had G2 tumors using the 2010 WHO classification. None of the cases showed symptoms.

The insulinoma subjects used for comparison were all women, with an average age of 62.0 years (range, 47-81 years). Tumor localization included 1 case in the pancreatic head and 2 each in the pancreatic body and pancreatic tail. The average tumor size was 15.0 mm (range, 12-19 mm); 3 cases had G1 tumors and 2 had G2 tumors by the 2010 WHO classification. Gastrinoma subjects included 3 men and 2 women, with an average age of 40.8 years (range, 19-59 years). All gastrinomas were located in the duodenum. The average tumor size was 9.8 mm (range, 1-16 mm); 3 cases had G1 tumors and 2 had G2 tumors according to the 2010 WHO classification.

Immunostaining

Immunostaining results are shown in **Figure 1.** Positive insulin staining was observed in 4 out of 8 cases. Focal responses were observed in 3 out of the 4 positive cases,

Table1. Patient characteristics.

	NF-NET	Insulinoma	Gastrinoma
Patients, n	8	5	5
Sex, male/female	2/6	0/5	3/2
Average age, years(range)	62.4(44-74)	62.0(47-81)	40.8(19-59)
Location, n duodenum/head/body/tail	0/2/5/1	0/1/2/2	5/0/0/0
Average tumor size, mm(range)	8.8(5.5-12)	15.0(12-19)	9.8(1-16)
WHO2010, n G1/G2	6/2	3/2	3/2

and a diffuse response was observed in the remaining case. No gastrin immunostaining positivity was observed in any of the cases **(Table 2).**

Insulin Reactivity in the SASI Test

The insulin-positive rate in NF-NET cases when the vasa vasorum was stimulated was 75% (6/8). The median of increase in insulin in positive response cases was 19.1 μU/mL, and the average value was 63.1 μU/mL (range, 8.6-291.4 μU/mL). A case in which a positive response was observed in the vasa vasorum (Case No. 5) is shown in **Figure 2**. The SASI test was conducted on a 5.5-mm pancreatic body NF-NET. An insulin response was observed in the DPA, which is part of the vasa vasorum (25.8 μU/mL → 319.9 μU/mL); however, no insulin responses were observed in the SMA, GDA, or SA. Positive insulin responses were observed in Case No. 1 and Case No. 4 following stimulation of a vessel far away from the tumor. Imaging of the vessel region was conducted again for these two cases; however, the regions were determined to be outside the range of resection since a tumor could not be identified. Five to six years have passed since surgery, with no detectable tumors being observed.

The SASI test positive response rate for insulin-negative immunostaining cases was 75% (3/4), that for insulin-positive immunostaining cases was 75% (3/4), and that for insulinoma was 100% (5/5), respectively. The mid-value and average values of the increase in insulin before and after calcium load for insulin-negative immunostaining

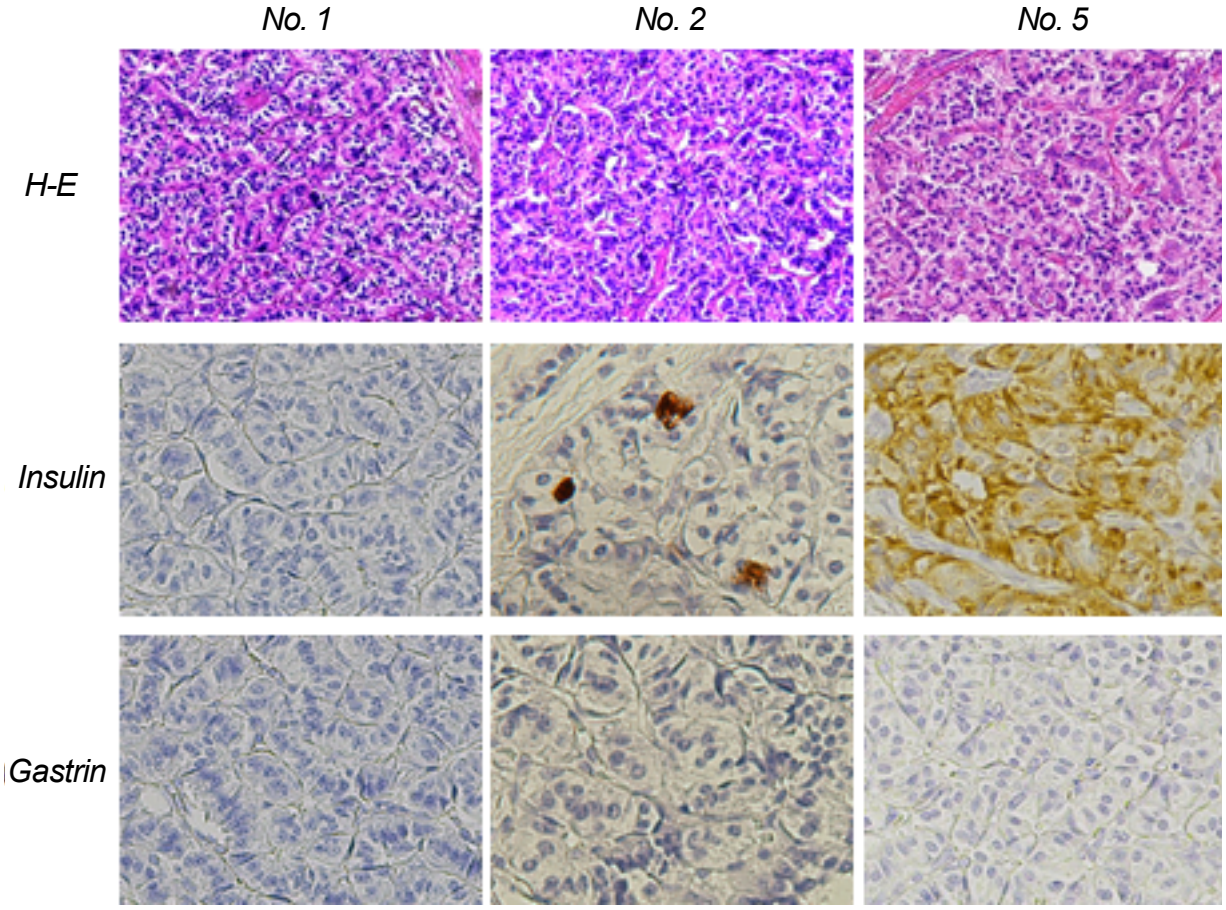

Figure 1. Immunohistological test: From the top, H-E staining (×100), insulin immunostaining (×200), gastrin immunostaining (×200). For insulin immunostaining, Case No. 1 was negative, 1% of tumor cells in Case No. 2 were positive, and many tumor cells were positive in Case No. 5. Gastrin immunostaining was negative in all cases.

Table 2. SASI test and immunostaining of NF-NET.

No.	Location	Feeding Artery	Insulin Reaction		Immuno-Reaction for Insulin	Gastrin Reaction		Immuno-Reaction for Gastrin
			Feeding Artery	Distant Artery		Feeding Artery	Distant Artery	
1	P-body	SMA	-	+(SA)	-	-	+(GDA)	-
2	P-tail	SA	+	-	+	-	+(GDA)	-
3	P-body	DPA	+	-	-	-	+(GDA)	-
4	P-head	GDA	-	+(SA)	+	+	-	-
5	P-body	DPA	+	-	2+	-	+(GDA, SMA)	-
6	P-body	SMA, CA	+	-	-	+	+(GDA)	-
7	P-head	GDA	+	-	-	+	-	-
8	P-body	SA	+	-	+	-	+(GDA, SMA)	-

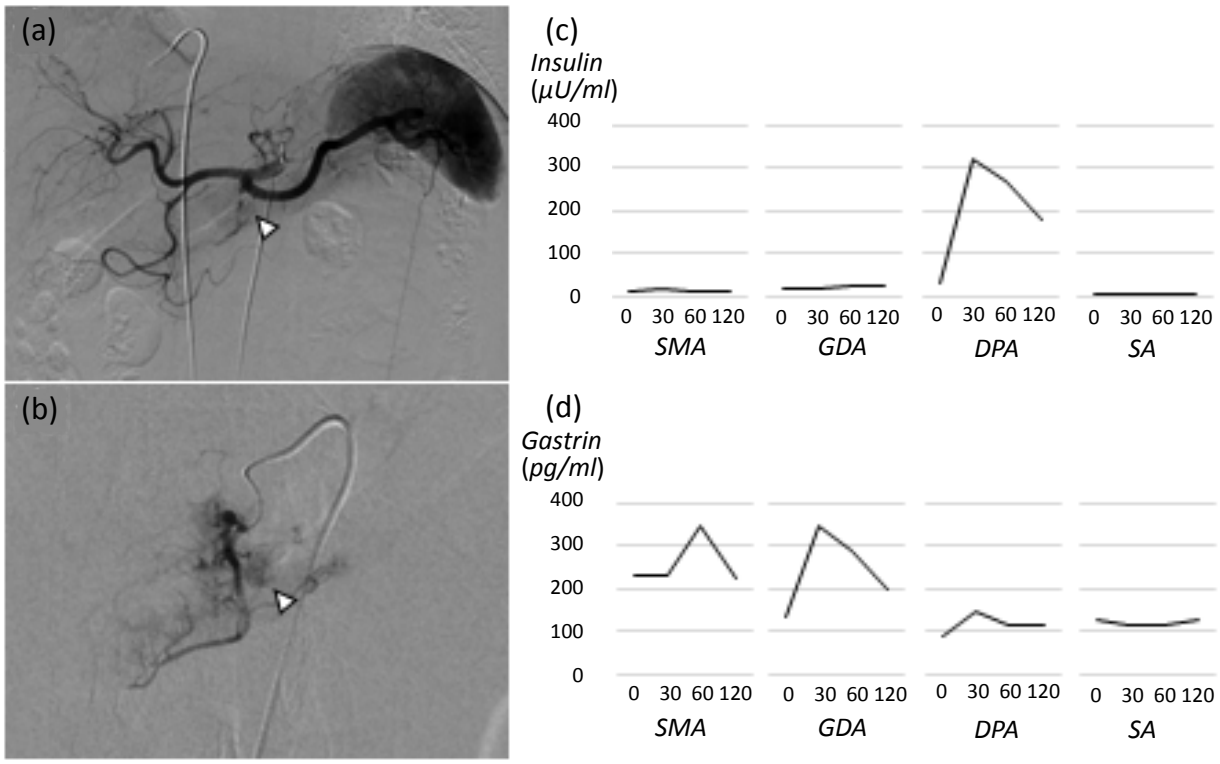

Figure 2. Selective arterial secretagogue injection (SASI) test: **(a.).** Celiac arteriography. **(b.).** Dorsal pancreatic arteriography. A tumor stain is visualized in the body of the pancreas. **(c.).** A rapid increase of insulin was observed following calcium injection into the DPA. **(d.).** A rapid increase in gastrin was observed following calcium injection into the SMA and GDA.

cases were 8.9 μU/mL and 13.7 μU/mL (range, 8.6-23.6 μU/mL), those for insulin-positive immunostaining cases were 31.4 μU/mL and 112.5 μU/mL (range, 14.6-291.4 μU/mL), and those for insulinoma were 155.4 μU/mL and 716.3 μU/mL (range, 23.3-2736.9 μU/mL), respectively.

Gastrin Reactivity in the SASI Test

For gastrin, the positive response rates in NF-NET cases were 75% for SMA (6/8), 100% for GDA (8/8), and 0% for both PSA and SA. No correlation between tumor localization and these responses was observed. For increase in gastrin before and after calcium load, the mid-value was 136 pg/mL and the average value was 291.4 pg/mL (range, 110-622 pg/mL) for SMA, and for GDA the mid-value was 367 pg/mL and the average value was 970.5 pg/mL (range 159-5.493 pg/mL). The positive response rate for comparison subjects with gastrinoma was 100% (5/5), and for increase in gastrin before and after calcium load, the mid-value was 2.801 pg/mL and the average value was 8.503.6 pg/mL (range, 450-23,182 pg/mL).

DISCUSSION

The SASI test is a diagnostic method based on differences in functional responsiveness between origin cells and tumor cells. Secretin and calcium receptors (CaR) are generated in both gastrinoma and insulinoma [7-9]. Secretin in G-cells suppresses gastrin secretion, while in gastrinoma, a completely different response occurs as contact with secretin causes G-cells to immediately release gastrin [10]. Furthermore, specific hormones are secreted in intrapancreatic insulinoma due to the lack of generation of secretin receptors in β cells. In contrast, when calcium is used as an irritant, intracytoplasmic calcium concentration increases through CaR on the tumor cell membrane in response to the increase in extracellular calcium concentration, resulting in hormone secretion by tumor cells [11, 12]. Although CaR is also generated by β cells, there is a difference in responsiveness by β cells to extracellular calcium concentration and that of insulinoma [8, 11]. These differences in responsiveness form the principle of the SASI test. CaR is also generated by G-cells, and promotes gastrin secretion in response to increased extracellular calcium concentration. As described above, the SASI test evaluates the release of hormones by tumors. SASI test results have been useful for the diagnosis of localization in F-NET. In our experience, the SASI test could be used to diagnose 100% of localized insulinoma and gastrinoma cases. Cases of 1-mm gastrinoma that cannot be observed by any other imaging test other than the SASI test have been observed; diagnosis of such localized tumors was possible only by SASI test [13]. However, the authors could not find any reports about use of the SASI test in NF-NET.

A 75% (6/8) insulin-positive response was observed following calcium injection into the vasa vasorum. Insulin release may cause a response in β cells due to CaR generation, as mentioned above. However, since an insulin response was observed in 2 out of 8 cases when blood vessels located far from the tumor were stimulated, secretion is considered to have occurred in response to NF-NET. In comparison to insulin responsiveness with the SASI test and insulin immunostaining, a positive response rate of 75% (3/4) among both negative

and positive immunostaining samples was observed. However, an increase of 8.9 μU/mL (range, 8.6-23.6 μU/mL) of insulin (δ Ins) was obtained among the negative immunostaining samples, and an increase of 31.4 μU/mL (range, 14.6-291.4 μU/mL) was obtained among the positive immunostaining samples. Thus, higher insulin responsiveness among positive samples was observed (*P*=0.089) **(Figure 3).** In particular, strongly positive insulin staining was observed in Case No. 5, and a significant increase of δ Ins (291.4 μU/mL) was observed. Our comprehensive analysis of these results suggests that insulin responses observed using the SASI test reflects the potential insulin secretion capacity of NF-NET tumors.

In contrast, for gastrin, frequent positive responses induced by stimulation of the SMA and GDA were observed, and negative gastrin immunostaining results were obtained for all NF-NET samples. There was no correlation between response and tumor localization. We think one of the reasons for this inconsistency results, an increase in gastrin have occurred due to stimulation of G-cells in the pyloric region of the stomach and the upper part of the small intestine by calcium injected into the SMA and GDA. However, when comparing the increase in gastrin (δ Gas) in G-cells to that of gastrinoma, an average δ Gas of 136 pg/mL was obtained in G-cells in the SMA, that for the GDA was 367 pg/mL, and that for gastrinoma was 2,801 pg/mL. Thus, a significantly higher response was observed in gastrinoma (*P*=0.024) **(Figure 4).** When determining gastrin responsiveness in the pancreatic duodenal region, the possibility of false-positive results must be considered.

The sensitivity of insulin responsiveness in NF-NET is 75%, and efficacy cannot be established in all cases.

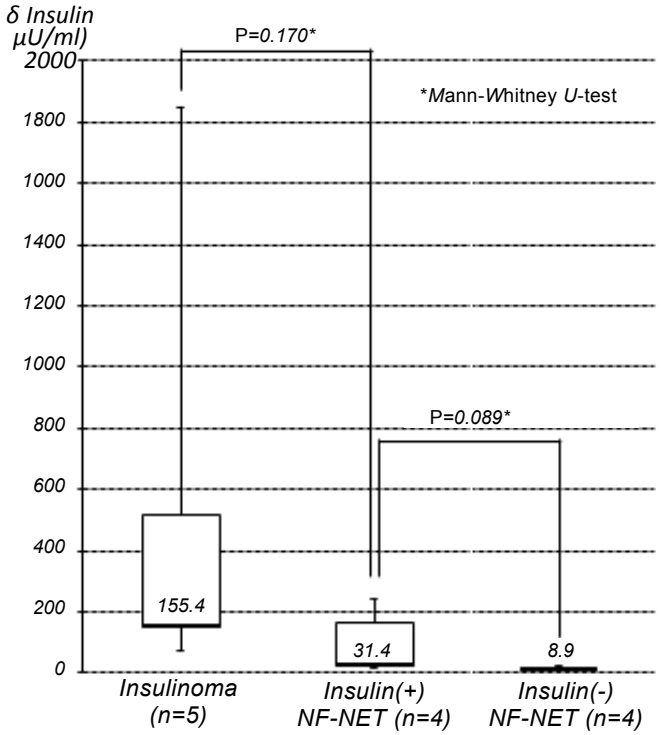

Figure 3. Increase in insulin in the SASI test (δ Ins): Insulin immunostaining-positive cases showed higher insulin responsiveness (*P*=0.089) compared to insulin immunostaining-negative cases.

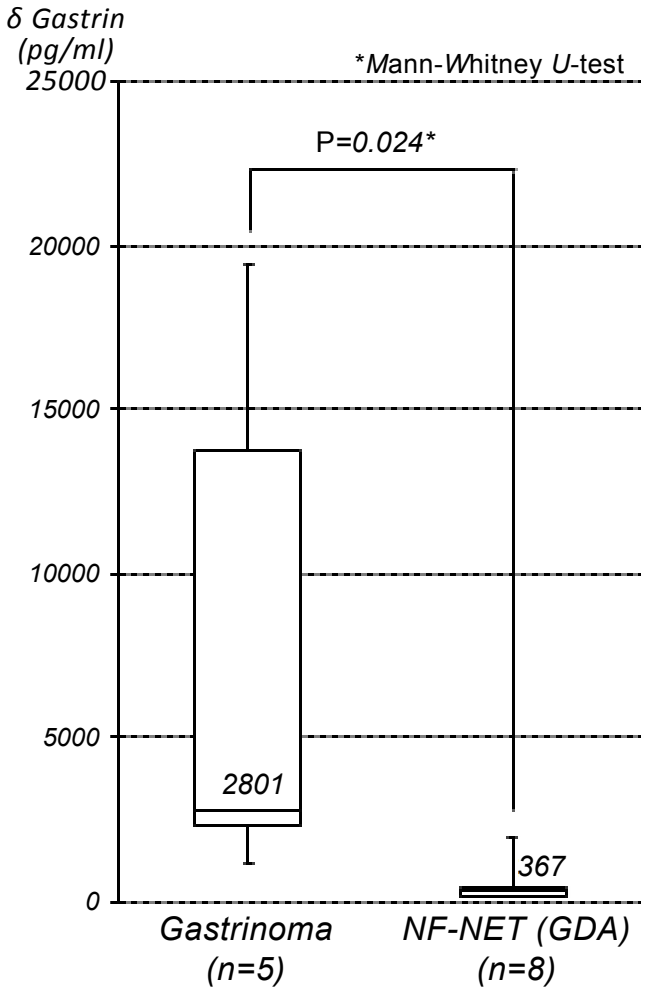

Figure 4. Increase in gastrin in the SASI test (δ Gas): δ Gas of gastrinoma was significantly higher than δ Gas when the GDA of a NF-NET case was stimulated (*P*=0.024).

However, in NET with atypical imaging, such as cases in which the tumors are small, endoscopic ultrasound-guided fine needle aspiration cannot be used due to difficulty in ensuring puncture lines, the presence of cystic degeneration, and poor imaging effects. Hence, diagnosis of NET by insulin responsiveness using the SASI test can be valuable. In addition, this can be performed as a screening process for cases with multiple lesions, since 18% of NET cases are reported to involve multiple tumors [3]. If an insulin response is observed, re-examination by imaging may facilitate identification of small lesions.

Limitations of this study include the fact that it was implemented at a single facility and involved only a small number of cases. To resolve these issues, a larger sampling of cases from multiple facilities should be collected and evaluated.

CONCLUSION

An increase in insulin in NF-NET was observed using the SASI test, suggesting the possibility of insulin secretion by tumors. Caution is required to avoid false-positive results of gastrin with respect to stimulation from the SMA and GDA.

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Conflicts of Interest

The authors have no conflict of interests to declare regarding this study

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