Serum *N*-Glycan Profiles in Patients with Intraductal Papillary Mucinous Neoplasms of the Pancreas

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Short title: Serum *N*-glycans in IPMNs

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Abstract

Background/Objectives: Diagnosing the invasiveness of intraductal papillary mucinous neoplasms (IPMNs) is difficult, especially by blood test. Alterations in serum glycan profiles have been reported for several cancers, but changes in serum glycan profiles have not been investigated in patients with IPMNs. The objectives of this study were to determine the serum *N*-glycan profile and to investigate its clinical utility in patients with IPMNs.

Methods: We measured serum *N*-glycan profiles in 79 patients with IPMNs, including 13 invasive IPMNs, by performing comprehensive glycome analysis and assessed the relationship between *N*-glycan changes and clinical parameters.

Results: Seventy glycans were identified and their expression profiles were significantly different depending on the cyst size, the presence of an enhancing solid component, and the histological grade of the IPMN. Nine glycans were highly expressed in patients with invasive IPMNs. The glycan m/z 3195, which is a fucosylated tri-antennary glycan, had the highest diagnostic value for distinguishing invasive IPMNs from non-invasive IPMNs (area under the receiver operating characteristic curve = 0.803). Multivariate analyses revealed high levels of m/z 3195 [odds ratio (OR), 20.5; 95% confidence interval (CI) 2.60–486.4] and the presence of enhancing solid components (OR, 35.8; 95% CI, 5.39–409.6) were significant risk factors for invasive IPMNs.

Conclusions: We performed a comprehensive evaluation of the changes in serum *N*-glycan profiles in

patients with IPMNs for the first time. We determined that increased expression of fucosylated complex-type glycans, especially m/z 3195, is a potential marker for invasive IPMNs.

Keywords: biological markers; fucosylation; glycomics; intraductal papillary mucinous neoplasm

Introduction

Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas are potentially malignant mucin-producing intraductal epithelial neoplasms. The true incidence of IPMNs is unknown because many IPMNs are small and asymptomatic. Recent studies have reported that the prevalence of unsuspected pancreatic cysts, including IPMNs identified on computed tomography (CT) or magnetic resonance imaging (MRI), is ~2.6–20% in adults [1-3].

Clinical guidelines recommend that main duct-type IPMNs (MD-IPMNs) and branch duct-type IPMNs (BD-IPMNs) with high-risk stigmata (obstructive jaundice with cystic lesion of the head of the pancreas, enhancing solid component within the cyst, and main pancreatic duct cyst size ≥ 10 mm) be treated by resection because of the high risk of malignancy [4, 5]. Pancreatic resection is associated with high rates of perioperative morbidity and mortality. Several reports indicate that malignancy rates of resected MD-IPMNs and BD-IPMNs are not sufficiently high (36–100% and 6–47%, respectively), to justify performing high-risk pancreatic resection for certain cases [5]. The new International consensus guidelines 2012 and the European experts consensus statement on cystic tumours of the pancreas 2013 attempt to improve the specificity of the recommendations, but their value for patient outcomes is still controversial [5, 6]. Therefore, a new serum biomarker for invasive IPMNs is required to improve the accuracy of preoperative diagnosis.

Glycan-based serological assays are useful to detect serum biomarkers for cancer. Carbohydrate

antigen 19-9 (CA19-9) and lens culinaris agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3) are commonly used as tumor markers [7, 8]. Aberrant glycosylation of serum proteins is observed in sera of patients with various types of cancer, including colon cancer, ovarian cancer, and pancreatic cancer [9-12]. We reported previously that multi-branch antennary and fucosylated glycan was elevated in pancreatic cancer and hepatocellular carcinoma [13, 14]. Although these changes might occur in patients with invasive IPMNs, the serum glycome profile of these patients has not been investigated.

In the present study, we analyzed changes in serum *N*-glycan profiles in patients with various stages of IPMNs and evaluated the potential use of glycans as new clinical markers for invasive IPMNs.

Methods

Patients and Diagnosis

We enrolled 146 consecutive patients who were diagnosed with IPMNs via imaging modalities and were admitted to Okayama University Hospital between May 2004 and August 2013. The diagnostic criteria were as follows: (i) dilation of the main pancreatic duct (MPD) and/or a cystic dilation of the branch duct and (ii) secretion of mucin from the major or minor papilla identified by endoscopic retrograde cholangiopancreatography or duodenoscopy. We excluded 67 patients from which serum samples were not obtained or histocytological examinations were not performed. The study subjects included 79 patients with IPMNs who were diagnosed using both radiographic imaging and histocytological examination. All patients were evaluated with CT or MRI and determined to have the maximum diameter of MPD and cyst size, the presence of mural nodules, and an enhancing solid component. In this study, we expressed the size of MD-IPMNs as 0 mm, and we defined mural nodules that were enhanced by contrast-enhanced CT or MRI as “enhancing solid component”. Serum tumor markers, including carcinoembryonic antigen (CEA), CA 19-9, s-pancreas-1 antigen (Span-1), and duke pancreatic monoclonal antigen type 2 (DUPAN-2) were measured at initial diagnosis. CEA and CA19-9 were analyzed by an electrochemiluminescence immunoassay. Span-1 was analyzed by an immunoradiometric assay. DUPAN-2 was analyzed by an enzyme immunoassay. A total of 40 patients underwent surgery. We confirmed the pathological diagnosis of the resected tissues according to the following World Health Organization 2010 IPMN grade classification [15]: low-grade dysplasia (n = 13), intermediate-grade

dysplasia (n = 8), high-grade dysplasia (n = 9), and invasive carcinoma (n = 10) including minimal invasion (n = 2). Thirty-nine patients did not require surgery. These patients were evaluated by radiological, cytological, or histological examination of specimens obtained by fine needle aspiration (FNA) or brushing, and classified into the following three subgroups: low-intermediate grade dysplasia (n = 35; cytology = class II–III, without radiographic invasion, without progression during a median observation period of 5 years; range 1–10 years), high-grade dysplasia (n = 1; cytology = class V; without radiographic invasion, without progression during 3 years), and invasive carcinoma (n = 3; cytology = class V; with radiographic invasion and/or metastasis). We excluded the patients whose diagnoses were changed from dysplasia to invasive carcinoma within a year. Classification into MD-IPMNs, BD-IPMNs, and mixed-type was performed according to the 2012 international consensus guidelines [5].

Written informed consent for collecting serum and using clinical data was obtained from all patients. The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki and was approved by the institutional review board (authorization number #667).

Glycoblotting

We collected serum samples from all patients at the time of hospital admission. The blood samples were centrifuged for 10 minutes at 15,000×*g*, the supernatant was removed and immediately frozen, and the samples were stored below −30°C until use. Glycoblotting was performed according to a procedure

described previously [16]. In brief, 10 μL serum samples were applied to an automated instrument (Sweetblot prototype 7, System Instruments Co.) for pre-treatment and for glycoblotting. Glycans were enzymatically cleaved from proteins, captured on BlotGlyco H beads (Sumitomo Bakelite Co.), and sialic acids were methyl-esterified. The processed glycans were tagged with benzyloxyamine (BOA), released from the beads, and analyzed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Ultraflex 3, Bruker, Germany). Sweetblot takes 11 hours to analyze 96 serum samples.

Statistical Analysis

Fisher's exact test was used to compare categorical data. Wilcoxon's rank sum test was used to compare continuous data. The diagnostic value of potential markers was assessed using an area under the receiver operating characteristic curve (AUROC). A multivariate logistic regression model was used to identify risk factors for invasive IPMNs. The cutoff values for the glycans were determined by receiver operating characteristic (ROC) analysis, with the best combination of sensitivity/specificity values used to assign the patients into invasive IPMN and non-invasive IPMN groups. For statistical analysis, $p < 0.05$ was considered significant. JMP (version 10.0.2) software packages (SAS Institute, Cary, North Carolina, USA) were used for statistical analyses.

Results*Patient Population Characteristics*

This study included 79 patients. The median age of the patients was 69 years (range, 43–83). Forty-six (58%) patients were male and 33 (42%) patients were female (Table 1). Thirteen patients had invasive carcinoma. Of these, ten (77%) patients were confirmed by surgical histology, and three (23%) patients were diagnosed by histocytological malignancy and imaging of tumor invasion or metastasis. Forty-three (54%) patients were classified as having BD-IPMNs, including four patients with invasive IPMNs. Thirty-six (46%) patients were classified as MD-IPMNs or mixed-type IPMNs, including nine patients with invasive IPMNs. Enhancing solid components were observed in 10 out of 13 (77%) patients with invasive IPMNs and 7 out of 66 (11%) patients with non-invasive IPMNs.

Glycan Expression Correlates with Clinicopathological Features

Glycans were classified into six groups according to structure, including the state of fucosylation. Table 2 presents the glycan characteristics and expression patterns that correlated with specific clinicopathological features. For all glycan subtypes, excluding high-mannose glycans and bi-antennary glycans with fucose residues, glycan expression was significantly higher in patients with larger cystic lesions than in those with smaller lesions. The glycan expression fold-change differed depending on the group (1.1–1.8). The expression of complex-type glycans with fucose residues (including bi-, tri-, and tetra-antennary degrees of branching) was significantly higher in patients with enhancing solid

components and invasive carcinomas. The expression of tri- and tetra-antennary glycans with fucose residues correlated well with the histocytological grade. In patients with invasive carcinoma, the expression of tri- and tetra-antennary glycans with fucose residues was approximately two-fold higher than that in patients with low-intermediate grade dysplasia. There were no significant differences in glycan expression with respect to gender, age, classification, and MPD size, except for the expression of high-mannose type glycans, which correlated with gender.

Diagnostic Value of Specific N-Glycan Expression for Detecting Invasive Carcinoma

We examined 70 glycans and found that the expression of nine glycans was significantly higher in patients with invasive IPMNs than in patients with non-invasive IPMNs (Table 3). These nine glycans were complex-type glycans; seven (78%) had fucose residues and six (67%) were tri- or tetra-antennary glycans.

The glycan *m*/*z* 3195 is a tri-antennary glycan with fucose residues. This glycan exhibited the highest diagnostic value for distinguishing invasive IPMNs from non-invasive IPMNs (AUROC = 0.803). Expression of glycan m/z 3195 was significantly higher in invasive IPMNs (median concentration = 6.749 μM) than in non-invasive IPMNs (median concentration = 4.017 μM, p = 0.0006) (Figure 1). The sensitivity and specificity of glycan m/z 3195 for diagnosing invasive IPMNs were 92.3% and 66.7%,

respectively. The diagnostic value of glycan m/z 3195 was higher than those of existing tumor markers, including CA19-9 (AUROC = 0.756), CEA (AUROC = 0.533), and DUPAN-2 (AUROC = 0.611).

Logistic regression analysis of clinical variables and glycan expression was performed to identify risk factors for invasive IPMNs (Table 4). Clinical variables that might affect the prognosis were selected based on the 2012 international consensus guidelines [5], and included classification (main and mixed-duct type), cyst size (≥ 30 mm), main pancreatic duct size (≥ 10 mm), and presence of enhancing solid component. Potential biomarker glycans for diagnosing invasive IPMNs were selected for further analysis (Table 3).

Univariate analyses revealed that the presence of enhancing solid components and high levels of CA19-9, SPAN-1, m/z 2890, m/z 3195, and m/z 3341 were risk factors for invasive IPMNs. Multivariate analyses revealed that the presence of enhancing solid components [odds ratio (OR), 35.8; 95% confidence interval (CI), 5.39–409.6] and high levels of m/z 3195 (OR, 20.5; 95% CI 2.60–486.4), m/z 2890 (OR, 7.67; 95% CI 1.04–160.9), and m/z 3341 (OR, 6.12; 95% CI 1.06–54.1) were risk factors for invasive IPMNs.

We also evaluated the utility of m/z 3195 in resected cases to identify risk factors for invasive IPMNs more strictly (Table 5). Similar results were obtained from the multivariate analyses. High levels of m/z 3195 (OR, 17.5; 95% CI, 2.1–423.8) and the presence of enhancing solid components (OR, 13.5; 95% CI, 2.2–126.4) were significant risk factors for invasive IPMNs.

We also analyzed the expression of glycans in the high-grade dysplasia group, including the minimal invasion (n = 11) and low-intermediate dysplasia groups (n = 21) in resected cases. None of the glycans exhibited significant statistical differences between the two groups.

Discussion

This is the first study to investigate serum *N*-glycan profiles in patients with IPMNs. We found that expression of tri- and tetra-antennary glycans with fucose residues correlated with cyst size, the presence of mural nodules, the presence of enhancing solid components, and histological and/or cytological malignancy. The glycan m/z 3195 is a tri-antennary glycan with fucose residues and had one of the highest diagnostic values for diagnosing invasive IPMNs, which was comparable to the diagnostic value of the presence of enhancing solid components. While the OR and specificity of high serum m/z 3195 was lower than that of the presence of enhancing solid component for diagnosing invasive IPMNs, the sensitivity of glycan m/z 3195 was higher than that of the presence of enhancing solid component (92.3% and 76.9%, respectively). Therefore, glycan analysis might not be a gold standard for indicating surgical resection but might be a good auxiliary method for monitoring the invasiveness of IPMNs during patient follow-up, although prospective studies are needed to confirm m/z 3195's usefulness for this purpose. Moreover, the measurement of serum glycans is less invasive than performing contrast-enhanced imaging analyses making glycan measurement especially suitable for use during the follow-up period.

Increased fucosylation of glycoproteins is a common modification in patients with various types of cancer, including AFP in hepatocellular carcinoma [8], prostate-specific antigen in prostate cancer [17], and haptoglobin in pancreatic ductal cancer [12]. Three of four fucosylated oligosaccharide structures of haptoglobin, which are elevated in pancreatic ductal cancer [12], coincided with m/z 2525, m/z 2890, and

m/z 3195. These same glycans were elevated in invasive IPMNs in the present study. Similarly, m/z 2525 (bi-antennary glycan with fucose residues), m/z 2890, m/z 3195, m/z 3341 (tri-antennary glycan with fucose residues), m/z 3560, and m/z 3865 (tetra-antennary glycan with fucose residues) were also increased in pancreatic ductal cancer as we reported previously [14]. In addition, we did not detect elevated expression of tri- and tetra- antennary glycans with fucose residues in other cystic tumors of the pancreas (two mucinous cystic adenoma, two serous cystic neoplasm, and one lymphoepithelial cyst) in a preliminary study. These results suggest that glycome profile changes in IPMN with carcinogenesis are similar to those in pancreatic ductal cancer.

The mechanism of up-regulation of circulating glycoproteins in patients with pancreatic cancer is unknown. Okuyama et al. reported that haptoglobin mRNA was not strongly expressed in a pancreatic cancer cell line [18], but expression of haptoglobin mRNA in a hepatoma cell line was higher when the cell line was co-cultured with the pancreatic cancer cell line PSN-1 that strongly expresses interleukin 6 mRNA [19]. Based on these data, Miyoshi et al. speculated that fucosylated proteins might be produced in the liver after stimulation with cytokines such as interleukin 6; however, there is no direct evidence in humans to support this hypothesis [18-20].

In a recent international consensus guideline, the decision for surgical intervention relied on imaging features, symptoms, and cytological examination [5]. Although no serum biomarker was recommended as an indicator for resection, two reports described the efficacy of serum biomarkers [21,

22]. CA19-9 may help differentiate between invasive IPMNs and non-invasive IPMNs [21]. Although the international consensus guideline does not adopt CA19-9 as a biomarker for invasiveness, the European experts consensus statement considers elevated CA19-9 serum levels to be a relative indication for surgical resection [6]. However, the sensitivity of CA19-9 was low, and we could not demonstrate its value using multivariate analysis in the current study. MUC5AC is another biomarker that may be used to differentiate high-grade dysplasia or carcinoma from lesser degrees of dysplasia [22]. MUC5AC is a secretory mucin that is expressed in the gastric mucosa and cystic fluid. It has similar expression levels in the cystic fluid of both high-risk and low-risk cysts, but is differentially expressed in serum. While the mechanism of differential expression in serum is unclear, MUC5AC may prove to be a useful biomarker.

Several studies have reported on the utility of cytological and molecular analyses of cyst fluid to identify malignancy [22-28]. However, a cyst fluid sample has to be obtained by endoscopic ultrasound and fine needle aspiration or endoscopic retrograde cholangiopancreatography, which carry risks of peritoneal dissemination or complication [29]. Therefore, our results have clinical benefits with respect to safety and usefulness.

Although several reports indicated that MPD size was a risk factor for invasive IPMNs [30-32], we did not observe a statistically significant difference in invasiveness based on the size of the MPD. This discrepancy may be due to the small sample size in our study. Actually, the prevalence of invasive IPMNs in cases with MPD $\geq$ 10 mm was higher than those cases with MPD < 10 mm (27% and 14%,

respectively).

There are four limitations in our study. First, this is a retrospective study with a small sample size. Second, this study included 39 patients who did not require surgery. In these 39 patients, invasiveness was radiologically evaluated and we confirmed the diagnosis by patient follow-up for at least one year. Third, we do not know the physiological roles for the changes in the expression of these glycans due to limited methodology for analyzing the functions of glycans. Fourth, the glycan m/z 3195 had low accuracy for detecting invasive IPMNs, and ~25% of the patients were not correctly diagnosed by this glycan. Additional contrast-enhanced imaging might compensate for this weakness.

To our knowledge, this is the first report demonstrating specific serum *N*-glycan profiles in patients with invasive IPMNs. High expression levels of fucosylated glycans with tri- and tetra-antennary structures may serve as biomarkers that predict invasive IPMNs. Although the comprehensive detection system used in this study is costly, future development of a simple detection system would make clinical application feasible. A future prospective study will confirm the clinical usefulness of glycans as biomarkers.

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Figure legends

Figure 1. Box-plot figure of glycan m/z 3195 expression in patients with non-invasive IPMNs and invasive IPMNs. Expression of glycan m/z 3195 was significantly higher in patients with invasive IPMNs (median concentration = 6.749 μM) than in patients with non-invasive IPMNs (median concentration = 4.017 μM, p = 0.0006).

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TABLE 1. Characteristics of Patients with Intraductal Papillary Mucinous Neoplasm.

Variable	Total	(*n* = 79)
Age, year	69	(43-83)
Sex, *n* (%)		
Male	46	(58%)
Female	33	(42%)
Classification†, *n* (%)		
Main and mixed duct	36	(46%)
Branch duct	43	(54%)
MPD size (mm)	5	(1.0-19.0)
Cyst size (mm)	20	(0-86.0)
Mural nodule, *n* (%)	32	(41%)
Mural nodule size (mm)	6.5	(1.0-25.0)
Enhanced solid component, *n* (%)	17	(22%)
Laboratory data		
CEA (ng/mL)	2.38	(0.67-106.3)
CA19-9 (U/mL)	12.7	($0.50\text{-}2.04\times10^4$)
Span-1 (U/mL)	12.7	(5.00-656.1)
DUPAN-2 (U/ml)	25	($12.5\text{-}1.05\times10^4$)
Cytological grade, *n* (%)		
Class I	0	(0%)
Class II	18	(46%)
Class III	17	(44%)
Class IV	0	(0%)
Class V	4	(10%)
Histological and/or cytological grade, *n* (%)		
Low-intermediate grade dysplasia	56	(71%)
High grade dysplasia	10	(13%)
Invasive carcinoma	13	(16%)

Abbreviations: MPD, main pancreatic duct; CEA, carcino-embryonic antigen; CA19-9, carbohydrate antigen 19-9; Span-1, s-pancreas-1 antigen; DUPAN-2, duke pancreatic monoclonal antigen type 2.

NOTE: Values are median (range) unless noted otherwise. †classification was performed according to the 2012 international consensus guideline.

TABLE 2. Characteristics of Expressed Glycans that Correlate with Clinicopathological Features.

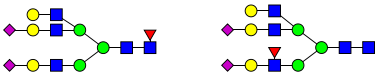
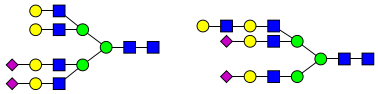
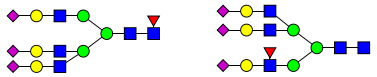
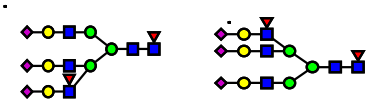
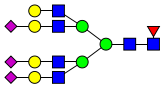
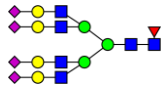
	n	High mannose type		Hybrid type		Complex type							
						Bi-antennary				Tri- and Tetra-antennary			
						with fucose residue		without fucose residue		with fucose residue		without fucose residue	
		Glycan expression (μM)	p	Glycan expression (μM)	p	Glycan expression (μM)	p	Glycan expression (μM)	p	Glycan expression (μM)	p	Glycan expression (μM)	p
Age													
< 70 years	40	10.2	0.20	6.8	0.66	63.7	0.33	103.7	0.67	6.0	0.07	20.9	0.26
≥ 70 years	39	9.8		7.0		67.1		102.8		10.3		18.5	
Gender													
Male	46	9.3	0.01*	7.0	0.31	67.7	0.91	102.3	0.98	9.3	0.22	17.4	0.09
Female	33	10.4		6.7		61.2		103.3		6.7		20.6	
Classification													
Branch duct	43	9.7	0.35	6.7	0.18	61.7	0.34	102.5	0.69	5.7	0.15	17.7	0.15
Main & mixed	36	10.1		7.1		68.3		104.3		8.9		21.0	
MPD size													
< 10 mm	64	9.7	0.48	6.8	0.12	61.5	0.07	102.6	0.47	7.4	0.18	19.2	0.48
≥ 10 mm	15	10.6		7.2		73.4		104.6		11.4		21.1	
Cyst size													
< 30 mm	57	9.8	0.72	6.7	0.002*	59.2	0.10	100.7	0.004*	6.6	0.002*	17.7	0.04*
≥ 30 mm	22	10.2		8.1		70.5		113.8		11.7		22.6	

Mural nodule													
No	47	9.5	0.04*	6.7	0.17	61.7	0.05	102.5	0.37	6.6	0.03*	17.9	0.42
Yes	32	10.2		7.0		70.5		103.8		10.4		20.7	
Enhancing solid component													
No	62	9.5	0.02*	6.9	0.05	62.0	0.046*	102.6	0.26	6.7	0.007*	19.0	0.14
Yes	17	10.4		7.5		76.1		104.6		11.3		22.5	
Histological and/or Cytologyical grade													
LGD or IGD	56	9.8	0.41	6.8	0.20	60.5	0.03*	102.4	0.07	6.6	0.002*	17.8	0.36
HGD	10	10.3		6.7		77.7		98.3		8.5		23.0	
Invasive	13	10.1		7.5		76.5		111.4		12.4		20.5	

Abbreviations: *n*, number; MPD, main pancreatic duct; LGD, low-grade dysplasia; IGD, intermediate-grade dysplasia; HGD, high-grade dysplasia; Invasive, invasive carcinoma.

NOTE: Values of glycan expression are median; *$p < 0.05$.

TABLE 3. Diagnostic Values of N-Glycans for Invasive IPMN.

Glycan, *m/z*	Proposed monosaccharide compositions‡	Glycan Expression (μM)			
		Non-invasive IPMN (*n* = 66)	Invasive IPMN (*n* = 13)	p	AUC
1591		10.06 (3.220-23.42)	12.27 (7.046-25.86)	0.04	0.679
2074		16.69 (3.220-26.56)	19.15 (16.25-29.50)	0.01	0.725
2525		8.427 (3.220-15.73)	10.78 (7.480-14.04)	0.002	0.776
2890		1.202 (0.568-4.852)	2.079 (1.074-6.101)	0.001	0.786
3109		0.964 (0-3.220)	1.166 (0.719-2.655)	0.04	0.683
3195		4.017 (1.284-13.04)	6.749 (3.171-19.36)	0.0006	0.803
3341		0 (0-3.330)	1.513 (0-6.294)	0.0007	0.784
3560		0.623 (0-3.220)	0.977 (0.510-4.919)	0.002	0.770
3865		0.634 (0-3.220)	1.257 (0.485-3.972)	0.004	0.756

NOTE: Values of glycan expression are median (range). ‡Rombus, sialic acid; triangles, fucose; square, N-acetyl glucosamine; yellow circles, galactose; green circles, mannose.

TABLE 4. Univariate and Multivariate Analyses of IPMN Patients to Identify Risk Factors for Invasive IPMN.

	n	Univariate			Multivariate		
Variable		OR	95% CI	p	OR	95% CI	p
Age (< 70 year)	40	1.17	0.35–3.98	0.80	–	–	–
Gender (Male)	46	1.18	0.35–4.26	0.79	–	–	–
Classification (Main and mixed)	36	3.25	0.95–13.0	0.06	–	–	–
Cyst size (≥ 30 mm)	22	1.80	0.49–6.19	0.36	–	–	–
MPD size (≥ 10 mm)	15	2.22	0.53–8.26	0.26	–	–	–
Enhancing solid component (present)	17	28.1	6.87–150.8	<0.0001*	35.8	5.39–409.6	0.0001*
CEA (≥ 5 ng/ml)	13	1.68	0.33–6.72	0.50	–	–	–
CA19-9 (> 37 U/ml)	11	10.5	2.56–46.1	0.001*	8.07	0.56–125.2	0.12
DUPAN-2 (> 150 U/ml)	7	4.65	0.82–24.4	0.08	–	–	–
Span-1 (> 30 U/ml)	12	8.57	2.17–35.5	0.003*	0.74	0.04–9.85	0.82
m/*z* 2890 (≥ 1.366 μM)	41	15.3	2.77–287.0	0.0006*	7.67	1.04–160.9	0.04*
m/*z* 3195 (≥ 4.935 μM)	34	24.0	4.31–451.4	<0.0001*	20.5	2.60–486.4	0.003*
m/*z* 3341 (≥ 0.797 μM)	36	9.02	2.19–61.5	0.001*	6.12	1.06–54.1	0.04*

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; other abbreviations are the same as those used in Table 1.

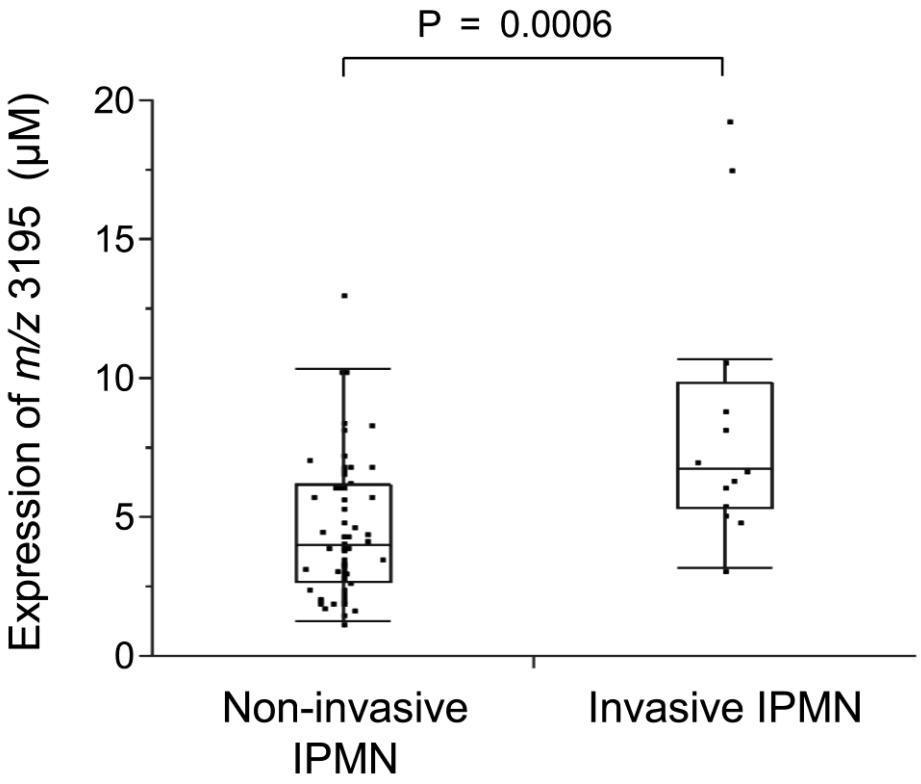
NOTE: Only variables with calculated $p < 0.05$ during univariate analysis were analyzed in the multiple logistic regression models. *$p < 0.05$.

TABLE 5. Risk factors for invasive IPMN in surgically treated patients.

	n	Univariate			Multivariate		
Variable		OR	95% CI	p	OR	95% CI	p
Age (< 70 year)	24	1.00	0.23–4.62	1.00	–	–	–
Gender (Male)	25	0.87	0.20–4.03	0.85	–	–	–
Classification (Main and mixed)	30	1.45	0.28–11.0	0.67	–	–	–
Cyst size (≥ 30 mm)	15	1.15	0.25–4.97	0.85	–	–	–
MPD size (≥ 10 mm)	12	1.00	0.18–4.56	1.00	–	–	–
Enhancing solid component (present)	13	9.33	1.99–54.8	0.004*	13.5	2.25–126.4	0.004*
CEA (≥ 5 ng/ml)	6	0.56	0.03–4.11	0.60	–	–	–
CA19-9 (> 37 U/ml)	6	3.86	0.60–25.3	0.15	–	–	–
DUPAN-2 (> 150 U/ml)	2	3.22	0.12–87.2	0.43	–	–	–
Span-1 (> 30 U/ml)	5	6.00	0.84–52.9	0.07	–	–	–
m/z 2890 (≥ 1.366 μM)	24	9.00	1.43–176.6	0.02*	7.14	0.97–149.8	0.05
m/z 3195 (≥ 4.935 μM)	22	11.8	1.86–231.3	0.006*	17.5	2.13–423.8	0.005*
m/z 3341 (≥ 0.797 μM)	24	3.50	0.73–25.8	0.12	–	–	–

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; other abbreviations are the same as those used in Table 1 and 4.

NOTE: Only variables with calculated $p < 0.05$ during univariate analysis were analyzed in the multiple logistic regression models. *$p < 0.05$.


P = 0.0006
Expression of m/z 3195 (μM)
20
15
10
5
0
Non-invasive IPMN
Invasive IPMN