TABLE 1. Characteristics of the enrolled patients

Characteristics	
Age, y, median (range)	67 (27–87)
Sex, male/female	52/38
ECOG performance status score, no.	
0	81
1	8
2	1
ASA score, no.	
1	86
2	4
Site of lesion, no.	
Pancreatic head	34
Pancreatic body	40
Pancreatic tail	16
Puncture route, no.	
Transgastric	56
Transduodenal	34
Size of lesion, mm, median (range)	28.2 (7.2–63.9)
Size of lesions, mm	
0-20	n = 22
21-40	n = 58
41-60	n = 8
61–	n = 2

ECOG, European Cooperative Oncology Group; *ASA*, American Society of Anesthesiologists.

diagnosed based on pathological findings in resected specimens, and 65 lesions were diagnosed by clinical course.

Adequacy score of specimen

The adequacy scores of obtained tissues for histological diagnosis are shown in Table 2 and Figure 2. The numbers of adequate and inadequate samples in the NNP and HNP groups are given in Table 3.

It was determined that 72.2% (65/90) (95% confidence interval [CI], 62.2%–80.4%) of samples obtained from the NNP group were adequate for histological diagnosis. In comparison, 90% (81/90) (95% CI, 82.0%–94.6%) of samples obtained from the HNP group were adequate for histological diagnosis. A concordance rate of 77.8% (70/90) (63 adequate and 7 inadequate for histological diagnosis) and a discordance rate of 22.2% (20/90) were determined. The samples obtained for histopathological diagnosis by using HNP were significantly superior to those obtained by using NNP (P = .0003, McNemar test) (Table 3). In 18 of these 20 patients, samples obtained by HNP were adequate for histological diagnosis, whereas samples obtained by NNP were inadequate. In the remaining 2 patients, adequate samples for histological diagnosis were obtained by NNP, but not by HNP. Therefore, it was determined that samples obtained by HNP were significantly superior to those obtained by NNP for histopathological diagnosis (P = .0003, McNemar test) (Table 3).

TABLE 2. Scores assigned to describe the adequacy of tissue obtained by EUS-FNA for histological diagnosis

		NNP						
	Score	0	1	2	3	4	5	Total
HNP	0	2	0	0	0	0	0	2
	1	0	0	2	1	0	0	3
	2	0	1	2	1	0	0	4
	3	2	1	4	11	8	1	27
	4	5	0	4	14	13	3	39
	5	2	0	0	3	3	7	15
	Total	11	2	12	30	24	11	90

NNP, Normal negative pressure; *HNP*, high negative pressure.

Accuracy

The final clinical diagnoses are listed in Table 4. Seventy-one patients ultimately had a diagnosis of pancreatic ductal adenocarcinoma, 1 had a diagnosis of acinar cell carcinoma, 1 had a diagnosis of undifferentiated carcinoma with osteoclast-like cells, and 4 had a diagnosis of carcinomas with histological types that could not be classified. Four patients had a diagnosis of neuroendocrine tumors, 1 had a diagnosis of a solid-pseudopapillary neoplasm, and 1 had a diagnosis of a secondary tumor. Seven patients had a diagnosis of pancreatitis.

A cytological diagnosis was categorized as malignancy or no malignancy. Malignancies were detected with a sensitivity of 89.2% (74/83) (95% CI, 80.7%–94.1%) and a specificity of 100% (7/7) (95% CI, 64.4%–100%).

Among the 90 samples obtained by NNP, 76 were diagnosed by using cytological and/or histological techniques. Sensitivity and specificity were 86.1% (62/72) (95% CI, 76.3%–92.3%) and 100% (4/4) (95% CI, 51.0%–100%), respectively. The total accuracy rate was 73.3% (66/90) (95% CI, 63.3%–81.3%).

Among the 90 samples obtained by HNP, 85 were diagnosed by using cytological and/or histological techniques. Sensitivity and specificity were 88.5% (69/78) (95% CI, 79.5%–93.8%) and 71.4% (5/7) (95% CI, 35.8%–91.8%),

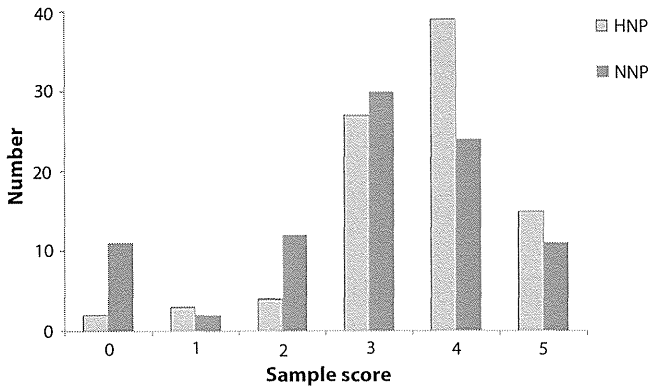

Figure 2. Scores of 0 to 5 were assigned to specimens to describe the adequacy of these samples for histological diagnosis. More samples with a score of 3 to 5 were obtained by using the high negative pressure (HNP) suction technique than normal negative pressure (NNP).

TABLE 3. A contingency table formulated to describe the adequacy of samples obtained for histological diagnosis based on the suction technique used (HNP or NNP)

		NNP		
		Adequate	Inadequate	Total
HNP	Adequate	63	18	81
	Inadequate	2	7	9
	Total	65	25	90

NNP, Normal negative pressure; *HNP*, high negative pressure.

respectively. The total accuracy rate was 82.2% (74/90) (95% CI, 73.1%–88.8%).

The accuracy of diagnoses based on the analysis of samples obtained by using EUS-FNA/HNP and EUS-FNA/NNP was equivalent (P = .06, McNemar test). It should be noted that of the 24 lesions that were not accurately diagnosed by using samples obtained by using EUS-FNA/NNP, a specimen adequate for histological diagnosis was obtained in only 10 lesions. Of these 24 cases, 16 lesions were accurately diagnosed with adequate specimens obtained by using the EUS-FNA/HNP technique. In contrast, 16 lesions that were not accurately diagnosed by using samples obtained by using EUS-FNA/HNP, 8 lesions were accurately diagnosed by using samples obtained by using the EUS-FNA/NNP technique. As such, the combined EUS-FNA/HNP technique is superior to the EUS-FNA/NNP technique for pathological diagnosis.

We analyzed the relationship between adequacy and accuracy for all specimens obtained in this study. Specimens deemed adequate for histological diagnosis had a significantly higher diagnostic accuracy than specimens deemed inadequate for histological diagnosis ($P < .001$, χ^2 test) (Table 5).

TABLE 4. Final diagnosis independently of tissue biopsies (EUS-FNA)

	Final diagnosis, no.
Ductal adenocarcinoma	71
Acinar cell carcinoma	1
Undifferentiated carcinoma with osteoclast-like cells	1
Carcinoma (unclassified)	4
Secondary tumors of the pancreas (adenocarcinoma)	1
Solid pseudopapillary neoplasm	1
Neuroendocrine tumor	4
No evidence of malignancy	7
Total	90

Tissue quality

The samples obtained by using HNP contained more blood than those obtained by using NNP (P = .0042, McNemar test). On the other hand, the degree of contamination was not significantly different between the samples obtained by using either technique (P = .0795, McNemar test) (Table 6).

Adverse events

Among the enrolled 90 patients, pancreatitis developed in 1 patient after the EUS-FNA procedure was performed. He recovered after conservative therapy. The rate of adverse events was therefore 1.1% (1/90).

DISCUSSION

Our data indicate that the use of a procedure that combines EUS-FNA with HNP provides significantly more specimens that are adequate for histological diagnosis than a procedure that combines EUS-FNA with NNP. EUS-FNA with HNP allows more cells to be acquired and preserves the tissue architecture in specimens.

A previous study showed that 25-gauge needles have a higher technical success rate, whereas more specimens adequate for histological diagnoses are obtained by using a 22- or 19-gauge needle.[4] A 25-gauge needle is therefore recommended to puncture the head of the pancreas.[4] Several studies have compared the performance characteristics of a 22-gauge needle with those of a 25-gauge FNA needle for sampling pancreatic masses, but most have failed to demonstrate superiority of either needle.[8-22] A recent systematic review and meta-analysis of EUS-FNA for solid pancreatic masses, including a large cohort of

TABLE 5. The relationship between adequacy of samples obtained for histological diagnosis and accuracy of diagnoses

		Accuracy		
		Accurate	Inaccurate	Total
Adequacy	Adequate	130	16	146
	Inadequate	10	24	34
	Total	140	40	180

$P < .001$ (χ^2 test).

TABLE 6. Quality of samples obtained by using the HNP/EUS-FNA and NNP/EUS-FNA techniques assessed based on the degree of contamination present and the amount of blood in the sample

Contamination	HNP	NNP
0: no contamination seen	70	68
1: Contamination present in <25% of the slide	19	10
2: Contamination present in 25%–50% of the slide	1	10
3: Contamination present in >50% of the slide	0	2
Amount of blood		
0: Minimal	16	28
1: Moderate	41	43
2: Significant	33	19

HNP, High negative pressure; *NNP*, normal negative pressure.

patients, revealed that a 25-gauge needle was more sensitive than a 22-gauge needle.[23] In our study, EUS-FNA by using a 25-gauge needle was successfully performed in all of the pancreatic lesions, not just lesions in the pancreatic head.

The need for suction during EUS-FNA was evaluated in previous reports, but is still controversial.[5,24,25] The European Society of Gastrointestinal Endoscopy technical guideline advocates the use of suction for EUS-FNA of solid masses/cystic lesions but for EUS-FNA of lymph nodes.[21] However, previous reports only focused on cytological examinations, not histology. The results of our study reveal that EUS-FNA with HNP enables the acquisition of more specimens adequate for histological diagnosis than what is achievable with EUS-FNA with NNP. Further study is required for the evaluation of EUS-FNA with and without HNP suction to determine whether suction is required during EUS-FNA for the purpose of histological diagnosis.

Pancreatic ductal adenocarcinoma accounts for the majority of pancreatic tumors and can be diagnosed by cell morphology and the degree of atypia. However, larger specimens are sometimes required for the histological diagnosis of other pancreatic tumors.[22,23] In fact, 90% of specimens obtained by using a 25-gauge needle and HNP were adequate for histological diagnosis. This is higher than that in previous reports describing the use of a 25-gauge needle.[4] Furthermore, greater diagnostic accuracy was achieved when specimens were adequate (Table 6), indicating that adequate specimens, optimal for histological diagnosis, can be obtained by using a 25-gauge needle. As such, the use of a 25-gauge needle with HNP improves technical performance of EUS-FNA and is the most appropriate method for pancreatic head lesions.

Diagnostic accuracy was not significantly different between the NNP and HNP groups. The majority of the enrolled patients in this study had ductal adenocarcinoma, which could be diagnosed by cell atypia alone. Our findings, however, are not limited to ductal adenocarcinoma. Pancreatic tumors with low-grade dysplasia or tumors with chronic pancreatitis, which are difficult to diagnose by only cell atypia, were also accurately diagnosed.[24] However, diagnostic accuracy differed between groups with adequate and inadequate specimens. This fact reveals that histological assessment aids the diagnosis of materials by using EUS-FNA. Suction is recommended when only a small amount of aspirate is obtained without suction.[26] One problem that we identified with the use of EUS-FNA with HNP was that the specimen obtained contained more blood. However, there was no difference between HNP and NNP in terms of diagnostic accuracy. It therefore appears that amount of blood in samples does not compromise the histological diagnosis; blood is rarely considered in the histological diagnosis of pancreatic tumors. Even if a sample contains blood, blood and cell components are visualized separately in the histological preparation.

There were some limitations in this study protocol. One limitation was the nondouble-blind clinical setting. Most patients presented with adenocarcinoma, and only a few had benign tumors or other types of malignancies. In particular, only a few patients had hypervascular tumors (n = 4, neuroendocrine tumors). This was a crossover study. In addition, our study could not compare the rates of adverse events between the 2 techniques (EUS-FNA/HNP and EUS-FHA/NNP) because the rate of adverse events was low at 1.1% and similar to the results of a previous systematic review.[25] Although this evidence suggests that EUS-FNA with HNP is feasible, additional study is required to resolve these issues.

CONCLUSION

Biopsy procedures with the EUS-FNA/HNP technique are superior to the EUS-FNA/NNP procedures in terms of tissue acquisition. This method is feasible and effective for collecting specimens for the histological diagnosis of pancreatic tumors.

ACKNOWLEDGMENTS

We thank Dr Koji Oba (Research and Clinical Trial Center, Hokkaido University Hospital, Sapporo, Japan) for conducting the statistical analysis. We also thank Dr Yoshihiro Matsuno (Department of Surgical Pathology, Hokkaido University Hospital) for advice and comments on pathological evaluation. We also express our deepest appreciation to the members of the Japan EUS-FNA Negative Pressure Suction Study Group and to their institutions. For full details, please see the Appendix (available online at www.giejournal.org).

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APPENDIX

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Number of Pages (including this page) : 9

Validation of a Nomogram for Predicting the Probability of Carcinoma in Patients With Intraductal Papillary Mucinous Neoplasm in 180 Pancreatic Resection Patients at 3 High-Volume Centers

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AQ2 **Objective:** We previously published a nomogram for prediction of carcinoma in patients with intraductal papillary mucinous neoplasm (IPMN). The objective of the current study was to validate this nomogram in an external cohort of patients at multiple institutions.

Methods: The clinical details of 180 patients with IPMN who underwent a pancreatic resection at 3 hospitals were collected. Four significant predictive factors (sex, lesion type, nodule height, and pancreatic juice cytology) were analyzed.

Results: Of the 180 patients, 66 (36.7%) had a main pancreatic duct–type IPMN and 114 (63.3%) had a branch pancreatic duct–type IPMN. The final pathological diagnosis was benign IPMN in 95 (52.8%) patients and malignant IPMN in 85 (47.2%) patients. The area under the receiver operating characteristic curve for the model was 0.760. The area under the receiver operating characteristic curve of the IPMN nomogram for prediction of malignancy was 0.747 in main pancreatic duct–type IPMN and 0.752 in branch pancreatic duct–type IPMN. The sensitivity and specificity of the model were 80.0% and 57.9%, respectively, when the predictive probability of less than 10% was used to indicate the presence of carcinoma.

Conclusions: This nomogram for predicting the probability of carcinoma in patients with IPMN was accurate in an external validation patient cohort.

Key Words: IPMN, nomogram, external validation, multicenter

(*Pancreas* 2014;00: 00–00)

In 1982, Ohashi et al[1] first described intraductal papillary mucinous neoplasms (IPMNs) of the pancreas as mucin-secreting tumors. The number of patients diagnosed with IPMN has increased with increasing awareness and advances in diagnostic imaging. In 2006, the international consensus guidelines for the management of IPMN were published.[2] However, application of these guidelines led to resection in many cases of IPMN adenoma (IPMA).[3] Many reports have attempted to identify the prognostic factors that might guide the management of patients with IPMN,[4–6] but there is no consensus with regard to the operative indications. In the revised international consensus guidelines of 2012,[7] resection is recommended for all main pancreatic duct (MPD) IPMN. In branch pancreatic duct (BPD) IPMN, the indications for resection are more conservative and "worrisome feature" that can be observed without immediate resection has been proposed.

We constructed a nomogram to predict carcinoma on the basis of a test cohort of 81 patients who had undergone IPMN resection before December 2008 at the Aichi Cancer Center Hospital (ACC). The area under the receiver operating characteristics curve (AUC) of this nomogram was 0.903 for prediction of carcinoma.[8] External validation of any diagnostic tool is important to determine whether the diagnostic accuracy reported in the original study can be reproduced outside the original cohort. In this study, we validated the IPMN nomogram in an external cohort of patients who underwent pancreatic resection at multiple institutions using standardized preoperative examination modalities, shared definitions of lesion types, and standardized pathological diagnostic criteria.

MATERIALS AND METHODS

Study Population AQ3

The study population was 281 patients with IPMN who underwent pancreatic resection at Wakayama Medical University (WMU) and Teine Keijinkai Hospital (TKH) between January 1996 and March 2011 or at ACC between January 2009 and March 2011 (Table 1). Fifty-nine cases in which endoscopic ultrasonography (EUS) was not performed preoperatively and 42 cases in which pancreatic juice cytology was not performed preoperatively were excluded. We therefore included 180 patients for validationof the IPMN nomogram. The following features were evaluated: age at the time of operation, sex, presence or absence of symptoms, preoperative laboratory values (serum amylase, carcinoembryonic antigen [CEA], and carbohydrate antigen 19-9 [CA19-9] level), imaging findings (tumor location, size of mural nodules, diameter of MPD, cyst size of BPD, type of lesion), operative procedure, and pathological findings. T1

Endoscopic ultrasonography and computed tomography (CT) were considered to be essential preoperative investigations for all patients. The height of any mural nodule(s) was determined through EUS. For MPD diameter and cyst size, CT measurement values were used.

The lesions were classified as MPD IPMN, Mix-IPMN, and BPD IPMN as per recently reported criteria.[9] With MPD IPMN, the lesions exist in the MPD and there is no cystic formation of 10 mm or greater in the surrounding branches. Cases with cystic dilatation of BPD are classified as Mix-IPMN or BPD IPMN, when the MPD diameter is 10 mm or greater or less than

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The authors declare no conflict of interest.

AQ9 **TABLE 1.** Patients of External Validation Cohort

Institute	Operation Period	No. Patients	EUS(+)	Cytology(+)
WMU	January 1996–March 2011	179	120	97
TKH	January 1996–March 2011	78	78	59
ACC	January 2009–March 2011	24	24	24
Total		281	222	180

Cytology, pancreatic juice cytology.

10 mm, respectively. In this study, both MPD IPMN and Mix-IPMN were analyzed as MPD-type IPMN (Table 2). T2

Four factors of sex, type of lesion, size of nodules, and pancreatic juice cytology were scored with the IPMN nomogram, as reported previously (Fig. 1). F1 To use the nomogram, points are assigned on a scale of 0 to 100 for each predictor and are added together for the final score. This value is located on the "total points" axis with a vertical ruler, and the ruler is followed down to read predicted cancer probability. The nomogram was used for overall prediction analysis of all 180 patients as well as the subsets of MPD type and BPD types (Table 2).

Pancreatic juice cytology was classified on levels I to V in accordance with the grade of structural and cytologic dysplasia.[10] Class I indicates completely benign and nonneoplastic epithelium of no or slight dysplasia, class II indicates regenerative or neoplastic epithelium of slight dysplasia, class III indicates neoplastic epithelium of mild dysplasia corresponding to adenoma, class IV indicates neoplastic epithelium of moderate dysplasia highly suggestive of adenocarcinoma, and class V indicates unequivocal malignant epithelium corresponding to adenocarcinoma.

According to the World Health Organization[11] (WHO) histological classification of IPMN, pathological diagnosis was classified as IPMA, borderline IPMN (IPMB), as well as noninvasive and invasive IPMN carcinoma (IPMC). *Invasive IPMC* is defined as a histological transition that is clearly present between IPMN and pancreatic ductal adenocarcinoma.[12]

Cytological and pathological diagnosis was performed by pathologists at the 3 hospitals (WMU, TKH, and ACC), and the central review of pathological diagnosis was done by A.Y. at Kyoto Prefectural University of Medicine in the cases of IPMB as well as noninvasive and invasive IPMC. All patients were categorized as benign (IPMA and IPMB) or malignant (noninvasive and invasive IPMC) on the basis of the pathological diagnosis after resection.

Statistical Analysis

Continuous variables were compared using the Student *t* test, and discrete variables were examined using the χ^2 test. All of the *P* values presented were 2 sided, and a *P* value of less than 0.05 was considered to be significant. A receiver operating characteristics curve[13,14] was used to measure the predictive accuracy of the nomogram for malignant IPMN.

On the basis of the nomogram, we selected a cutoff value for the predicted probability of malignant IPMN. The cutoff value was selected to provide high sensitivity while, at the same time, reducing the number of resections of benign IPMN. The JMP 7.0.1 statistical software (SAS Institute, Incorporation, Cary, NC) was used in the analysis.

TABLE 2. Classification of Type of Lesion in Patients With IPMN

Nomogram Lesion Type	Classification[9]	MPD	BPD Dilation
MPD	MPD IPMN	Lesions exist	None or <10 mm
	Mix-IPMN	Diameter ≥10 mm	+
BPD	BPD IPMN	Diameter <10 mm	+

RESULTS

Characteristics of Patients in External Validation Cohort

The details of the patients and their imaging, tumor location, surgical procedures, as well as pathological findings are given in Table 3. T3 Sixty-six (36.7%) patients had an MPD-type IPMN and 114 (63.3%) patients had a BPD-type IPMN. Higher grades of dysplasia in pancreatic juice cytology were found in MPD-type lesions (Table 3). The size of mural nodules was also significantly larger in MPD-type IPMN than in BPD-type IPMN. There were no significant differences in sex, presence of symptoms, preoperative laboratory values (serum amylase, CEA, and CA19-9 level), tumor location, or pathological findings between

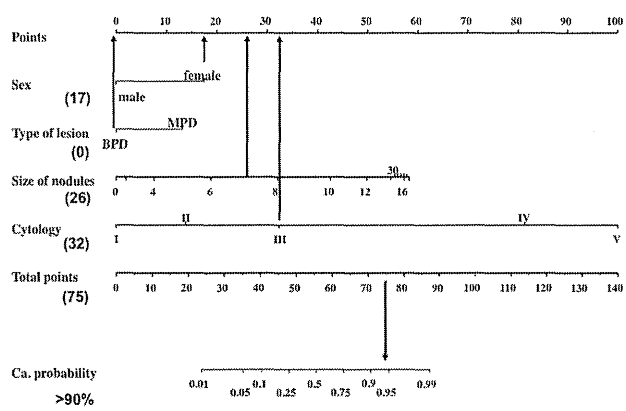

FIGURE 1. Nomogram for the detection of IPMC. Sex, BPD or MPD IPMN, size of mural nodules, and grade of pancreatic juice cytology for the individual patient were used. A line is drawn in the upward direction to indicate the number of points in each category. These points are totaled and then a line is drawn downward to indicate the patient's risk for IPMC. For example, in a case of a female with a BPD-type IPMN, a 7-mm nodule size, and a cytology class III, the patient's total score of 75 corresponds to more than a 90% likelihood of IPMC. Figure adapted from Shimizu et al.[8] Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation. AQ8

TABLE 3. Characteristics of Patients With IPMN Who Underwent Pancreatic Resection (n = 180)

No. Patients	Total (N = 180)	MPD Type (n = 66)	BPD Type (n = 114)	*P*
Background				
Age at pancreatectomy, y*	68.0 (9.2)	69.9 (7.8)	67.0 (9.2)	0.0387
Sex, n (%)				0.5578
Male	106 (58.9)	37 (56.0)	69 (60.5)	
Female	74 (41.1)	29 (44.0)	45 (39.5)	
Symptom, n (%)	57 (31.7)	20 (30.3)	37 (32.5)	0.7644
Laboratory data*				
AQ10 Amylase level, IU/la	121.0 (127.4)	124.8 (140.4)	118.8 (120.0)	0.7612
CEA level, ng/mla	2.6 (2.5)	2.7 (1.8)	2.5 (2.9)	0.7054
CA19-9 level, U/mla	40.9 (154.4)	49.5 (230.9)	35.9 (83.9)	0.5692
Pancreatic juice cytology				
I/II/III/IV/V	52/100/19/4/5	12/39/8/3/4	40/61/11/1/1	0.0236
Image findings				
Tumor location, n (%)				0.4344
Head	112 (62.2)	42 (63.6)	70 (61.4)	
Body	54 (30.0)	21 (31.8)	33 (29.0)	
Tail	14 (7.8)	3 (4.6)	11 (9.6)	
Size of mural nodules, mm*	8.3 (8.2)	10.3 (9.6)	7.2 (7.0)	0.0138
Diameter of MPD, mm*	8.8 (8.2)	15.2 (10.4)	5.0 (2.2)	<0.0001
Cyst size of BPD, mm*	25.3 (16.5)	17.7 (1.9)	29.8 (12.7)	<0.0001
Operative procedure				
AQ11 PD, PpPD/DP, MP, PR/TP, n (%)	114/56/10 (63.3/31.1/5.6)	44/13/9 (66.7/19.7/13.6)	70/43/1 (61.3/37.7/1.0)	0.0002
Pathology				
Benign IPMN, n (%)	95 (52.8)	29 (43.9)	66 (57.9)	0.0705
Malignant IPMN, n (%)	85 (47.2)	37 (56.1)	48 (42.1)	
Non./Inv.	61/24	26/11	35/13	

*Values are presented as mean (SD).

DP, distal pancreatectomy; Inv., invasive; MP, middle pancreatectomy; Non., noninvasive; PD, pancreatoduodenectomy; PpPD, pylorus-preserving pancreatoduodenectomy; PR, partial resection of the pancreas; TP, total pancreatectomy.

the patients with MPD-type IPMN and the patients with BPD-type IPMN.

Mural nodules were detected in 134 (74.4%) of the 180 patients, including 53 (80.3%) of the 66 patients with MPD-type IPMN and 81 (71.1%) of the 114 patients with BPD-type IPMN. Ten (11.8%) of the 85 patients with malignant IPMN had no nodules. In 4 patients with MPD-type IPMN and 6 patients with BPD-type IPMN, the pathological findings were noninvasive carcinoma in 9 patients and invasive carcinoma in 1 patient (data not shown).

External Validation of IPMN Nomogram

For the entire cohort of patients with IPMN, the AUC of the IPMN nomogram was 0.760 for predicting the presence of carcinoma. The AUC was similar for the patients recruited at the

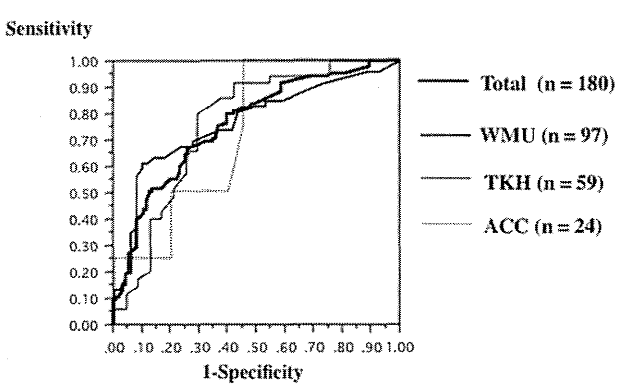

FIGURE 2. Receiver operating characteristics curve of nomogram for predicting the probability of malignant IPMN in extra validation cohort (n = 180). The AUC is 0.760. With each of the 3 centers of WMU, TKH, and ACC, the AUC was 0.768, 0.767, and 0.731, respectively.

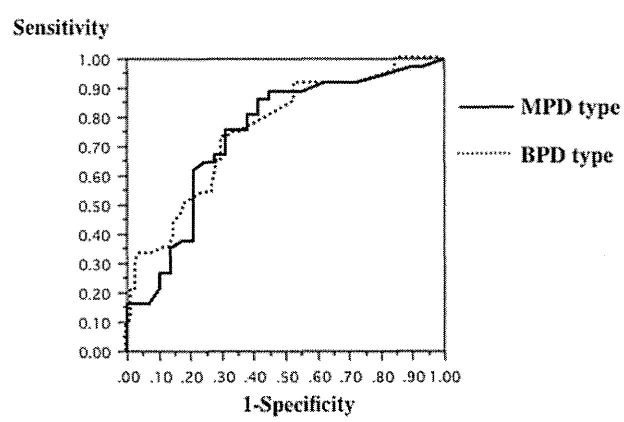

FIGURE 3. Receiver operating characteristics curve of nomogram for predicting the probability of malignant IPMN in MPD-type IPMN (n = 66) and BPD-type IPMN (n = 114). The AUC is 0.745 and 0.752, respectively.

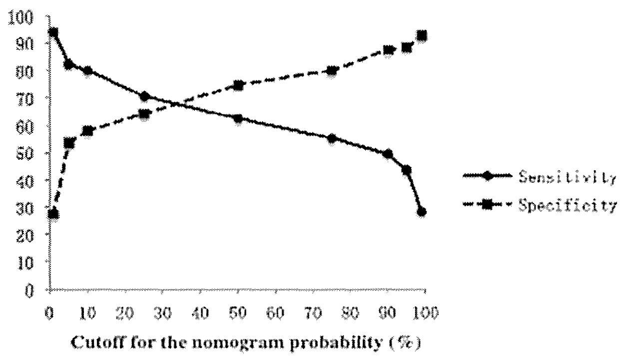

FIGURE 4. Sensitivity and specificity are estimated on the basis of the validation data set (n = 180) as a function of a cutoff point for the malignant IPMN predicted probability.

different centers (0.768, 0.767, and 0.731 for WMU, TKH, and F2 ACC, respectively; Fig. 2). For the subset of MPD- and BPD-type IPMN, the AUC of the IPMN nomogram was 0.747 and F3 0.752, respectively (Fig. 3). There was no difference in the result AQ4 between the first half period (January 1996–December 2003) AQ5 and the second half period (January 2004–March 2011), with the AUC being 0.750 and 0.749, respectively (data not shown).

Using this nomogram, if only those patients with 10% or higher predicted probability of pancreatic carcinoma underwent surgery, then the model would capture 80% (68/85) of all patients with malignant IPMN (sensitivity) while sparing 57.9% (55/95) of the patients without malignancy from undergoing an AQ6 unnecessary surgical procedure (specificity). The PPV and NPV of the nomogram were 63.0% (68/108) and 76.4% (55/72), respectively T4 F4 (Fig. 4, Table 4). There were 17 patients with malignant IPMN who had less than 10% predicted probability of pancreatic carcinoma on IPMN nomogram. In these 17 patients, the pathological findings were noninvasive carcinoma in 13 patients and invasive carcinoma in 4 patients. Three of the 4 patients with invasive carcinoma had minimally invasive carcinoma.[15,16]

The IPMN nomogram could predict carcinoma in the 66 patients with MPD-type IPMN, with a 91.9% (34/37) sensitivity, a 31.0% (9/29) specificity, a 63.0% (34/54) PPV, and a 75.0% (9/12) NPV. Applied to the 114 patients with BPD-type IPMN, the IPMN nomogram had a 70.8% (34/48) sensitivity, a 69.7% (46/66) specificity, a 63.0% (34/54) PPV, and a 76.7% (46/60) T5 T6 NPV (Tables 5, 6).

DISCUSSION

The risk for malignancy is higher in MPD IPMN and is relatively low in BPD IPMN.[2,17–20] However, there is no consensus with regard to operative indications in individual cases. In the new international consensus guidelines revised in 2012,[7] resection is recommended for all MPD IPMN, whereas, in BPD IPMN, the indications for resection are more conservative and cyst size of BPD of greater than 30 mm without "high-risk stigmata" can be observed without immediate resection. The BPD IPMN cyst size of greater than 30 mm and MPD dilation of 5 to 9 mm are classified as worrisome features, and EUS observation is recommended to decide a treatment strategy.

Nomograms have been widely used to develop treatment and follow-up strategies for various neoplasms, such as prostate and colorectal cancer.[21–25] In 2004, Brennan et al[26] reported creation of a nomogram that predicted outcome after resection of pancreatic cancer. However, there was no similar model to predict malignancy in IPMN. In response to this problem, we previously created a cancer prediction nomogram in patients with IPMN and reported its utility.[8] This nomogram is based on 4 predictive factors (sex, lesion type, nodule height, and pancreatic juice cytology data) and provides an outstanding cancer prediction capability, with an AUC of 0.903.[8]

In the present study, we validated this nomogram in an external validation cohort of patients with IPMN who underwent pancreatic resection at the 3 institutes. In this cohort of patients, we standardized preoperative examination modalities, used common definitions for the type of lesions, and conducted a central review of pathological findings, as we reported recently.[9] The newer (2010) WHO classification uses the terms *low-grade*, *intermediate-grade*, and *high-grade dysplasia* in place of adenoma, borderline, and noninvasive carcinoma. However, in this study, the subjects were 180 patients who underwent pancreatic resection at the 3 hospitals between January 1996 and March 2011. Pathologists at these 3 hospitals (WMU, ACC, and TKH) diagnosed the lesions as IPMA (mild, moderate, severe) or IPMC (noninvasive, invasive) in accordance with the classification of pancreas carcinoma of the Japan Pancreas Society.[15,16] We used the WHO (2000) histological classification of IPMN, in which pathological diagnosis is classified as IPMA, IPMB, or noninvasive and invasive IPMC.

When creating the nomogram, lesion type was classified into 2 groups: MPD type and BPD type[8] (Fig. 1). All lesions in the MPD measuring 10 mm or greater were classified as MPD-type IPMN. In this validation study, therefore, patients with Mix-IPMN of our classifications[9] were classified as MPD-type IPMN and a total of 66 patients with MPD-type IPMN and 114 patients

TABLE 4. Diagnostic Ability of Nomogram (n = 180)

	Pathological Diagnosis	
Nomogram	**Malignant IPMN (n = 85)**	**Benign IPMN (n = 95)**
Positive (n = 108)	68	40
Negative (n = 72)	17	55

Malignancy probability 10% cutoff value.

TABLE 5. Diagnostic Ability of Nomogram in MPD-Type IPMN (n = 66)

	Pathological Diagnosis	
Nomogram	**Malignant IPMN (n = 37)**	**Benign IPMN (n = 29)**
Positive (n = 54)	34	20
Negative (n = 12)	3	9

Malignancy probability 10% cutoff value.

TABLE 6. Diagnostic Ability of Nomogram in BPD-type IPMN (n = 114)

	Pathological Diagnosis	
Nomogram	**Malignant IPMN (n = 48)**	**Benign IPMN (n = 66)**
Positive (n = 54)	34	20
Negative (n = 60)	14	46

Malignancy probability 10% cutoff value.

with BPD-type IPMN were investigated (Tables 2, 3). The AUC of the receiver operating characteristics analysis was 0.760 in all 180 patients and showed good diagnostic performance even in the subset analyses of the 3 different institutions (Fig. 2). With a cutoff score of 40 points (equivalent to 10% cancer probability), we found a good diagnostic ability (sensitivity, 80.0%; specificity, 57.9% for prediction of malignancy; Fig. 4, Table 4). Invasive carcinoma with less than 10% predicted probability of pancreatic carcinoma on IPMN nomogram was present in only 4 patients. Pathologically, there was a massive invasion of the pancreatic parenchyma in only 1 patient and a minimally invasive carcinoma in 3 patients, for which prognosis seems to be comparable with that of noninvasive carcinoma.[15,16] Hence, an invasive carcinoma was missed in only 4 (2.2%) of the 180 patients.

We found good AUC values of 0.747 and 0.752 for MPD-type IPMN and BPD-type IPMN, respectively (Fig. 3). If the 66 patients with MPD-type IPMN, in whom resection is recommended based on the existing guidelines,[7] were treated on the basis of our nomogram, 9 patients without malignancy would avoid an unnecessary operation, whereas 3 patients with malignant IPMNs (noninvasive carcinomas) would have been missed (Table 5). Particularly in BPD-type IPMN, for which operative indications are controversial, using a carcinoma probability cutoff level of 10%, we are able to predict a benign IPMN by a specificity of 69.7% while maintaining a sensitivity of 70.8%, showing a high rate of diagnostic accuracy (Table 6). Although a few cancers will be missed using this approach, the nomogram seems to be a valid adjuvant tool for the clinicians to assess an individual's risk for malignant IPMN.

Sadakari et al[27] reported that, among cases of BPD IPMN with no nodules, 6 (8.2%) of 73 patients who underwent pancreatic resection had carcinoma. Recently, we reported that the size of mural nodules observed through EUS was a significant predictor of malignancy, but there were 15 patients (15/160 [9.4%]) who had carcinoma with no nodules.[9] Even in the present investigation, 10 (11.8%) of the 85 patients with cancer had no nodules. The combination of cytology and diameter of MPD[3,27] or pancreatic juice CEA measurements[28] are reported to be effective in identifying patients with carcinoma among patients with IPMN without nodules. It is difficult to predict malignant IPMN on the basis of a single parameter, and the use of a nomogram is a more reliable tool because it takes multiple factors into consideration.

Our IPMN nomogram was based on 4 significant predictive factors (sex, lesion type, nodule height, and pancreatic juice cytology data). There are some limitations to our model. Because our analysis includes the fact that pancreatic juice was obtained for cytology during endoscopic retrograde cholangiopancreatography for all patients, the nomogram may be applicable only to potential candidates for surgery rather than all patients diagnosed with IPMN. However, as for the application to a follow-up strategy in patients with IPMN, we recently reported the ability of our nomogram.[29] We recommended the risk assessment using the nomogram at the initial evaluation of IPMN and then decided follow-up schedule through CT and/or EUS. Our results indicated that annual follow-up would be appropriate for scores of less than 35, indicating an extremely low risk for cancer development within 3 years at least. Meanwhile, 3 to 6 months of close follow-up would be recommended for scores of 35 or higher; it indicates high potential for malignant transformation. Because of the retrospective nature of our study design, we plan to prospectively validate the applicability of our nomogram to management strategies for patients with IPMN.

In conclusion, we have validated this nomogram for predicting the probability of carcinoma in patients with IPMN and it may be applicable to a diverse population treated at multiple centers.

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Japanese Journal of Clinical Oncology Advance Access published October 23, 2014

JJCO Japanese Journal of Clinical Oncology

Japanese Journal of Clinical Oncology, 2014, 1–6
doi: 10.1093/jjco/hyu159
Original Article

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Prognostic value of neutrophil–lymphocyte ratio and level of C-reactive protein in a large cohort of pancreatic cancer patients: a retrospective study in a single institute in Japan

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Received 7 July 2014; Accepted 21 September 2014

Abstract

Objective: Recent studies suggest that systemic inflammatory response is closely associated with cancer patient prognosis. Although several inflammatory prognostic markers have been proposed, the data to support their validity are lacking in large Japanese cohorts.
Methods: This is a retrospective study to examine the prognostic value of inflammatory markers, such as C-reactive protein, neutrophil–lymphocyte ratio, platelet–lymphocyte ratio and modified Glasgow prognostic scale, in pancreatic cancer. Selection criteria were admittance to hospital between January 2008 and December 2012, histologically confirmed adenocarcinoma, diagnosis of invasive ductal pancreatic cancer compatible by computed tomography imaging, and followed-up until death or for 180 days or longer. The primary end point was overall survival, which was measured from the day of histological diagnosis.
Results: There were 440 patients who met the selection criteria. Of the 440 cases, 200 (45.5%) received curative resection (166 Stage I/II and 34 Stage III patients), 237 (53.9%) received chemotherapy (4 Stage I/II, 92 Stage III and 141 Stage IV patients), and the remaining 3 received palliative care. Univariate and multivariate regression analyses revealed that advanced computed tomography stage, high level of C-reactive protein (0.45 mg/dl or greater), neutrophil–lymphocyte ratio (2.0 or greater) and CA19-9 level (1000 U/ml or greater) were significantly associated with worse prognosis.
Conclusions: We verified the results of previous studies, and showed that neutrophil–lymphocyte ratio and C-reactive protein also had prognostic value in a large Japanese PC cohort.

Key words: NLR, CRP, mGPS, PLR, survival

Introduction

Pancreatic cancer (PC) has become the fifth most common cause of cancer-related mortality in Japan; it has been estimated that PC was responsible for 29 916 deaths in 2012 (1), representing ~8% of all cancer deaths. Despite recent improvements in diagnostic techniques, only a small proportion of patients are eligible for surgery, even though resection represents the only curative treatment available thus far. Accordingly, the prognosis of PC patients is extremely poor, with a 5-year survival rate after diagnosis of <5% (2).

Recent studies suggest that the systemic inflammatory response is closely associated with cancer patient prognosis (3,4). Several parameters of the systemic inflammatory response, including level of C-reactive protein (CRP), neutrophil–lymphocyte ratio (NLR), derived NLR (dNLR), platelet–lymphocyte ratio (PLR) and modified Glasgow prognostic score (mGPS), have been demonstrated in numerous reports as good prognostic indicators in lung cancer (5), hepatocellular carcinoma (6), melanoma (7), renal cell carcinoma (8), gastric cancer (9) and colorectal cancer (10). Moreover, some studies have shown that these parameters can predicted clinical outcome in regardless of the primary site (11,12).

Further, initial reports have already indicated that the inflammatory response is predictive of prognosis in patients with PC, but most of these studies included only relatively small number of cases (13–17). An Austrian group has reported the prognostic value of NLR, dNLR and CRP as useful inflammatory markers in their large cohort of PC patients (18–20). In the present study, we aimed to validate the prognostic significance of inflammatory markers in a large cohort of Japanese PC patients with reference to the Austrian studies.

Patients and Methods

This retrospective study included data from 493 consecutive patients who were diagnosed with PC at the Gastroenterology Center, Cancer Institute Hospital of Japanese Foundation for Cancer Research between January 2008 and December 2012. Among these 493 patients, we selected those for the current study if all of the following criteria were met: (i) histologically or cytologically confirmed adenocarcinoma, (ii) invasive ductal PC compatible by computed tomography (CT) imaging and (iii) followed-up until death or for 180 days or longer.

Clinical variables collected in this study were: age, gender, height, weight and performance status (PS) according to the Eastern Cooperative Oncology Group grading system; white blood cell (WBC) count; fraction of neutrophil and lymphocyte in WBC differentiation (%); levels of albumin, bilirubin, CRP and carbohydrate antigen 19-9 (CA19-9); location of the primary pancreatic tumor; clinical CT stage according to the seventh edition of TNM classification; type of therapy (i.e. tumor resection, chemotherapy or symptomatic treatment); date of surgical intervention or biopsy and date of the final follow-up or death. The baseline data were obtained within 30 days prior to surgical intervention or biopsy.

The relationship between each baseline variable and long-term survival was investigated by univariate and multivariate analyses, with special focus on the prognostic impact of systemic inflammation markers. On the basis of previous studies, CRP level of 0.45 mg/dl, NLR of 2.0, dNLR (absolute count of neutrophils divided by the absolute WBC count minus the absolute count of neutrophils) of 2.3 and PLR of 150 were selected as cutoff values for validation. The mGPS was applied by combining CRP and albumin levels: 0 was defined as normal values of CRP and albumin; 1 was defined as increased CRP (1.0 mg/dl or greater) and normal albumin; and 2 was defined as increased CRP and decreased albumin (<3.5 g/ml). Other than the five inflammatory markers, variables included in the prognostic analysis were: age (65 years or younger versus older than 65); gender; PS (0 versus 1); body mass index (>25 versus 25 or greater); location of the primary tumor (head versus body–tail); clinical CT Stage (I/II, III or IV); and CA 19-9 (>1000 U/ml versus 1000 U/ml or greater).

The primary end point of this study was overall survival (OS), defined as the time from the date of histological confirmation (the date of surgery or biopsy) to death due to any cause or to the last known date alive. All patients were assessed in December 2013. Kaplan–Meier survival plots were generated, and differences in survival among subgroups classified by each factor were evaluated by log-rank tests. Cox regression was used to determine univariate hazard ratios for OS. Age, PS and all variables with significant prognostic value in the univariate analysis were selected for further evaluation in the final multivariate Cox proportional hazard model. Multivariate Cox proportion analysis by backward elimination method was performed to determine the influence of the different variables on OS. Hazard ratios estimated by the Cox analysis were reported as relative risks with corresponding 95% confidence intervals. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the PASW Statistics 18 program (SPSS Inc., Chicago, IL, USA).

The Institutional Review Board of the Cancer Institute Hospital of the Japanese Foundation for Cancer Research approved this study, and waived the need for written informed consent from the participants because this was a retrospective non-intervention study.

Results

Of the 493 patients, 440 met the selection criteria. Of the remaining 53, 28 had other tumor histologies including neuroendocrine tumor, and 25 were transferred to a community hospital to receive palliative care within 6 months after diagnosis. Patient characteristics are summarized in Table 1. Of the 170 patients diagnosed with Stage I/II potentially resectable disease, 4 received chemotherapy because micro-metastases were found by laparotomy. Of the 127 patients diagnosed with Stage III disease, 34 underwent resection of the pancreas, 92 received chemotherapy and the remaining 1 received symptomatic treatment. Of the 143 patients diagnosed with Stage IV disease, 141 received chemotherapy and the remaining 2 received symptomatic treatment. Consequently, 200 (45.5%) patients received curative resection (166 Stage I/II and 34 Stage III cases), 237 (53.9%) received chemotherapy (4 Stage I/II, 92 Stage III and 141 Stage IV patients) and the remaining 3 received palliative care. Of the 440 selected patients, 313 (71.1%) died and the remaining 127 were still alive at the time of analysis. The median follow-up time of the 127 survivors was 18.7 months, ranging from 6.1 to 68.2 months. The median survival time of patients from the whole cohort was 11.6 months (interquartile range: 7.1–20.1 months).

Univariate Cox regression revealed that advanced CT stage, pancreatic body–tail cancer, high level of CRP, NLR, dNLR and CA19-9 level were significantly associated with worse prognosis (Table 2). We continued to analyze NLR but not dNLR in the multivariate analysis because the hazard ratio of NLR was higher than that of dNLR (1.894 versus 1.576, respectively). PLR and mGPS did not show any evident prognostic impact on survival in our cohort. In the multivariate analysis, CT stage, level of CRP, NLR and CA19-9 level were identified as independent prognostic factors in our cohort (Table 3).

Figure 1 demonstrates OS curves stratified by NLR in each CT stage, respectively. The number of patients with NLR >2.0 and those with NLR ≥2.0 were 71 (41.8%) and 99 (58.2%) in Stage I/II, 48 (37.8%) and 79 (62.2%) in Stage III and 21 (14.7%) and 122 (85.3%) in Stage IV. The prognostic value of NLR was clear especially in CT Stage I/II disease ($P = 0.014$, log-rank test). But there was no significant difference between Stages III and IV ($P = 0.079$ and $P = 0.125$).

Figure 2 demonstrates OS curves stratified by CRP in each CT stage, respectively. The number of patients with CRP <0.45 and

Table 1. Patient characteristics

Age (years)		
Median (range)	67	32–88
65 or younger	179	40.7%
Older than 65	261	59.3%
Gender		
Male	249	56.6%
Female	191	43.4%
Performance status		
0	378	83.3%
1	62	13.7%
Body mass index		
Median (range)	21.6	13.0–33.8
<25	375	85.2%
25 or greater	65	14.8%
Location of the primary tumor		
Head	220	50.0%
Body–tail	220	50.0%
Clinical CT stage		
I/II	170	38.6%
III	127	28.9%
IV	143	32.5%
C-reactive protein (mg/dl)		
Median (range)	0.12	0.01–21.9
<0.45	321	73.0%
0.45 or greater	119	27.0%
Neutrophil–lymphocyte ratio		
Median (range)	2.47	0.7–27.7
<2	140	31.8%
2 or greater	300	68.2%
Derived neutrophil–lymphocyte ratio		
Median (range)	1.77	0.5–13.3
<2.3	324	73.6%
2.3 or greater	116	26.4%
Platelet–lymphocyte ratio		
Median (range)	140.0	40.4–930.8
<150	239	54.3%
150 or greater	201	45.7%
Modified Glasgow prognostic score		
0	367	83.4%
1	49	11.1%
2	24	5.5%
Albumin (g/dl)		
Median (range)	4.0	2.4–5.0
<3.5	48	10.9%
3.5 or greater	392	89.1%
CA19-9 (U/ml)		
Median (range)	436.2	2.0–50 000
<1000	275	625%
1000 or greater	165	37.5%

those with CRP ≥0.45 were 147 (86.5%) and 23 (13.5%) in Stage I/II, 102 (80.3%) and 25 (19.7%) in Stage III and 72 (50.3%) and 71 (49.7%) in Stage IV, respectively. The prognostic value of CRP was evident in CT Stage III and IV disease ($P = 0.015$ and $P < 0.001$).

Figure 3 shows box plots of CRP and NLR in each CT stage. The dotted line means the cutoff level. The fraction of patients with NLR under the cutoff level was small especially in Stage IV, whereas most patients in Stage I/II had lower CRP level than the cutoff level.

Figure 4 demonstrates plots of the cumulative distribution function of NLR and CRP. The degree of asymmetric distribution of CRP was larger than that of NLR, with skewness coefficients of 5.568 and 4.803, respectively.

Table 2. Univariate cox regression

	HR	95% CI	*P* value
Age			
65 or younger	1		
Older than 65	0.806	0.644–1.008	0.059
Gender			
Male	0.985	0.788–1.232	0.897
Female	1		
Performance status			
0	1		
1	1.261	0.924–1.720	0.143
Body mass index			
<25	1		
25 or greater	1.192	0.883–1.609	0.252
Location of the primary tumor			
Head	1		
Body–tail	1.499	1.199–1.873	<0.001
Clinical CT stage			
I/II	1		
III	2.225	1.666–2.972	<0.001
IV	5.351	3.996–7.166	<0.001
C-reactive protein (mg/dl)			
1			
0.45 or greater	2.323	1.820–2.966	<0.001
Neutrophil–lymphocyte ratio			
<2.0	1		
2.0 or greater	1.894	1.474–2.435	<0.001
Derived neutrophil–lymphocyte ratio			
<2.3	1		
2.3 or greater	1.576	1.234–2.012	<0.001
Platelet–lymphocyte ratio			
<150	1		
150 or greater	1.048	0.838–1.309	0.683
Modified Glasgow prognostic score			
0	1		
1	2.61	1.89–3.605	<0.001
2	1.465	0.906–2.369	0.119
Albumin (g/dl)			
<3.5	1		
3.5 or greater	1.161	0.801–1.683	0.431
CA19-9 (U/ml)			
<1000	1		
1000 or greater	2.002	1.591–2.519	<0.001

HR, hazard ratio; CI, confidence interval.

Discussion

Previous studies suggest that disease progression in cancer patients is not only driven by the intrinsic properties of tumor cells, but also by systemic host reactions. Some systemic factors, in the shape of cytokines and other chemical messengers, may play an important role in cellular proliferation and metastatic ability (3,4). Although the detailed mechanisms have not been fully elucidated yet, several markers that reflect systemic inflammation have been reported to be closely associated with patient prognosis in different types of cancer (5–12). Among these inflammatory factors, we tested level of CRP, NLR, dNLR, PLR and mGPS in a large Japanese PC cohort in the current study. An Austrian group had already reported that NLR (18), dNLR (19) and CRP (20) predicted clinical outcome, and our study aimed to validate their findings. As a result, we confirmed that NLR and CRP have prognostic value in a large Japanese cohort similar to the Austrian studies. On the other hand, PLR and mGPS did not

Table 3. Multivariate cox regression

	HR	95% CI	P value
Age			
65 or younger	1		
Older than 65	0.834	0.665–1.045	0.115
Performance status			
0	1		
1	1.284	0.923–1.788	0.138
Location of the primary tumor			
Head	1		
Body–tail	1.07	0.842–1.359	0.582
Clinical CT stage			
I/II	1		
III	2.191	1.638–2.931	<0.001
IV	4.141	3.035–5.648	<0.001
C-reactive protein (mg/dl)			
<0.45	1		
0.45 or greater	1.695	1.308–2.197	<0.001
Neutrophil–lymphocyte ratio			
<2.0	1		
2.0 or greater	1.404	1.078–1.830	0.012
CA19-9 (U/ml)			
<1000	1		
1000 or greater	1.435	1.127–1.826	0.003

demonstrate any prognostic value in our cohort, possibly due to ethnic difference and/or specificity of cancer type.

As compared with the Austrian cohort, there were more patients with earlier stage disease in our cohort. The fraction of Stage IV patients was 70% in the Austrian studies and 33% in this report. The mean values of NLR and CRP were 4.75 and 2.32 mg/dl, respectively, in the Austrian reports, and 3.06 and 0.80 mg/dl, respectively, in the current one. The median survival time and interquartile range were 7 and 3–17 months, respectively, in the Austrian cohort, and 11.6 and 7.1–20.1 months, respectively, in ours. Due to a high surgeon volume in our institute, we fortunately had an advantage in recruiting many PC patients with earlier stage. In any case, the important fact was that the prognostic impacts of NLR and CRP were confirmed in resectable and unresectable PC patients, respectively, in both European and Asian cohorts.

Although we verified the prognostic value of NLR and CRP in PC patients, there were differences between the characters of NLR and CRP as prognostic markers. One important point is that NLR is a relative value. Because a neutrophil count of zero is not a realistic situation, thus, NLR cannot approach zero (Fig. 4). Figure 3 shows the distribution of NLR and CRP in each clinical stage. The level of NLR tended to become higher as the clinical stage progressed. Accordingly, the cutoff level of 2.0 was appropriate for resectable disease but

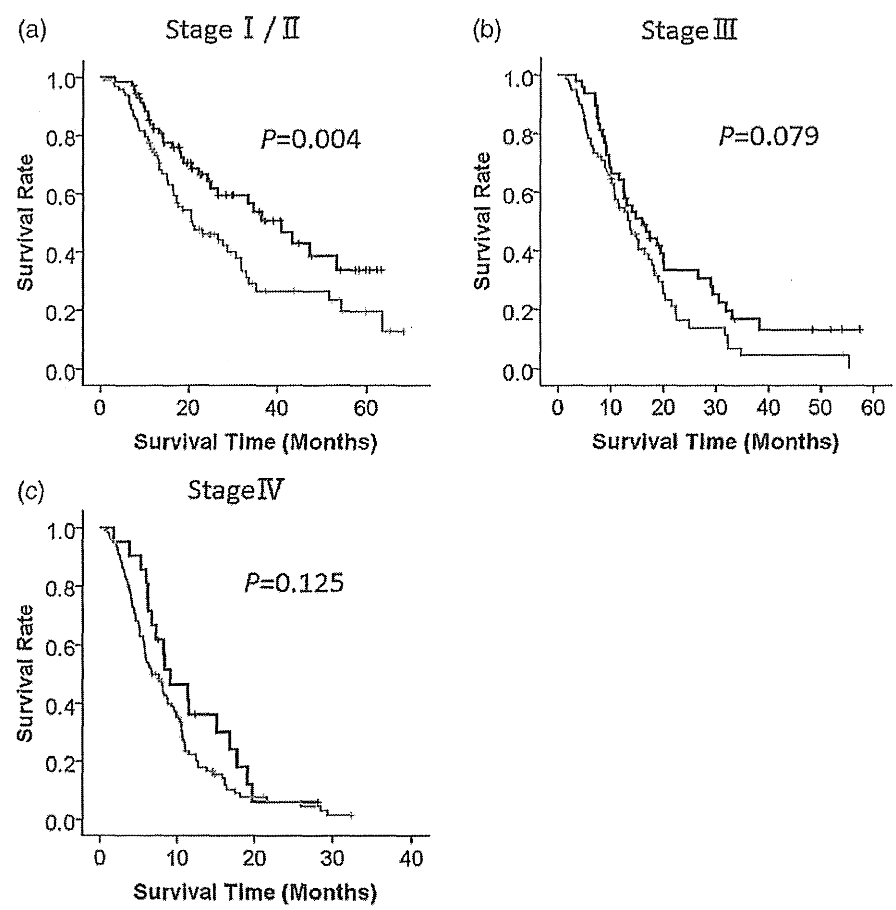

Figure 1. Overall survival curves stratified by neutrophil-lymphocyte ratio (NLR) for Stage I/II (a), Stage III (b) and Stage IV (c). Vertical lines represent censoring of data. Black and gray lines indicate subgroup of patients with NLR <2.0 and those with NLR ≥2.0, respectively. Prognosis of patients with increased NLR was significantly poorer in Stage I/II ($P = 0.004$, log-rank test).

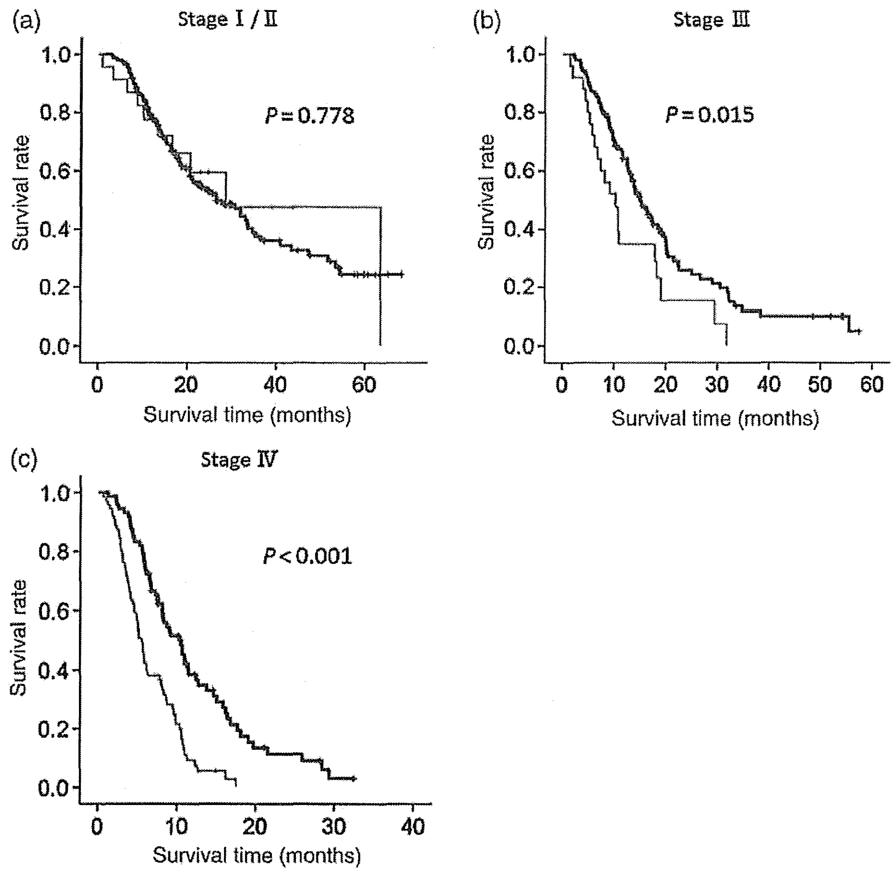

Figure 2. Overall survival curves stratified by C-reactive protein (CRP) for Stage I/II (a), Stage III (b) and Stage IV (c). Vertical lines represent censoring of data. Black and gray lines indicate subgroup of patients with CRP <0.45 and those with CRP ≥0.45, respectively. Prognosis of patients with increased CRP was significantly poorer in Stage III ($P = 0.015$) and Stage IV ($P < 0.001$, log-rank test).

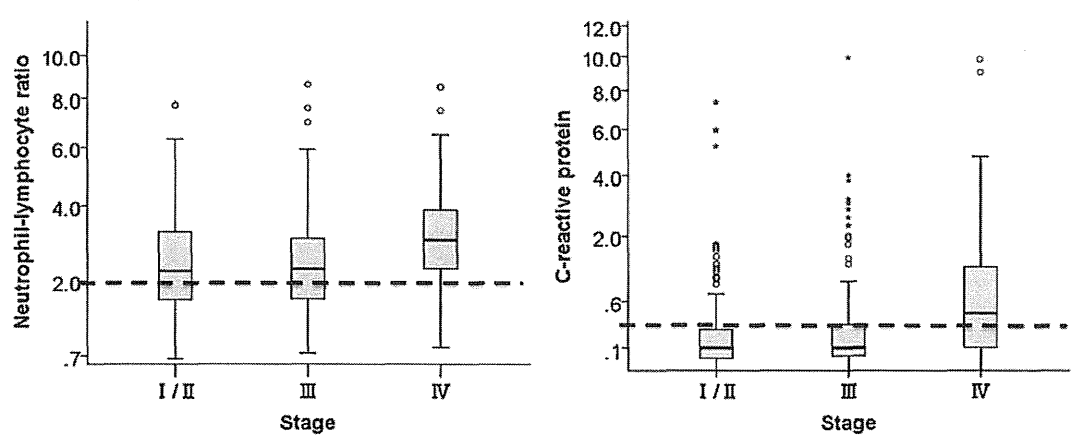

Figure 3. Box plots of CRP and NLR stratified by clinical stage. The dotted line denotes the cutoff level. The fraction of patients with NLR under the cutoff level was small especially in Stage IV, whereas most patients in Stage I/II had lower CRP level than the cutoff level.

it was too low to show the statistical significance in unresectable disease. If the cutoff level of NLR was set separately in each clinical stage, the prognostic value of NLR would be evident in both resectable and unresectable diseases. In practice, when we applied the cutoff level of 5.0 for NLR, the result was opposite from the result mentioned above, namely, the prognostic value of NLR was evident in unresectable disease, but not evident in resectable disease. On the other hand, CRP level is an absolute value, and small values close to zero represent a normal condition in general. To determine the cutoff level of CRP for patients especially in early stage was difficult because almost all

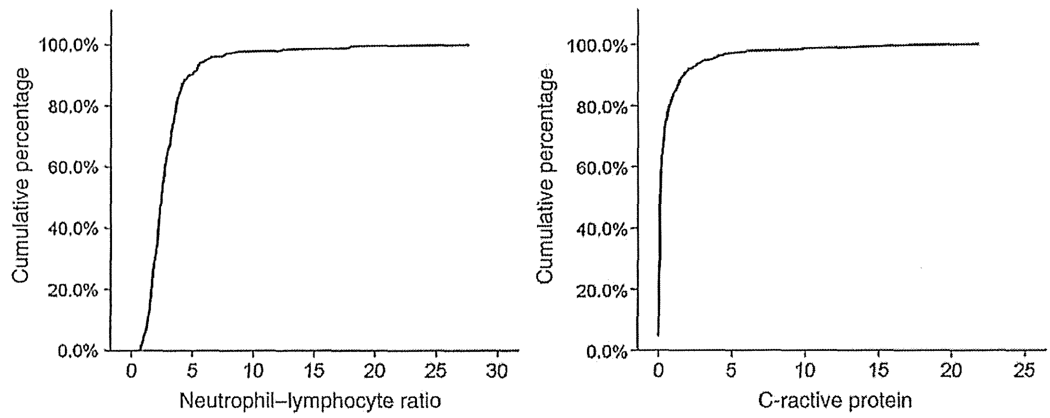

Figure 4. Cumulative distribution function plots of NLR and CRP. NLR cannot approach zero (95% of the NLR in our cohort were distributed between 1.1 and 6.2). On the contrary, small CRP values close to zero represent a normal condition. In the present study, 74% of the CRP levels were <0.5 mg/dl.

of the patients had a normal CRP level. For that reason, the prognostic value of CRP was relatively clear for advanced disease.

In conclusion, we verified the results of the Austrian studies, and revealed the prognostic value of NLR and CRP in a large PC cohort. We also found that the cutoff value of 2.0 for NLR clearly demonstrated prognostic value in potentially resectable disease, whereas CRP was a useful prognostic factor in patients who are not good candidates for curative resection. Further investigations to clarify the optimal NLR and CRP cutoff levels are warranted.

Conflict of interest statement

None declared.

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DOI: 10.3748/wjg.v20.i46.17456

World J Gastroenterol 2014 December 14; 20(46): 17456-17462
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE CONTROL STUDY

Case-control study of diabetes-related genetic variants and pancreatic cancer risk in Japan

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Supported by Grants-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare, Japan
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Received: March 4, 2014 Revised: April 18, 2014
Accepted: July 24, 2014
Published online: December 14, 2014

Abstract

AIM: To examine whether diabetes-related genetic variants are associated with pancreatic cancer risk.

METHODS: We genotyped 7 single-nucleotide polymorphisms (SNPs) in *PPARG2* (rs1801282), *ADIPOQ* (rs1501299), *ADRB3* (rs4994), *KCNQ1* (rs2237895), *KCNJ11* (rs5219), *TCF7L2* (rs7903146), and *CDKAL1* (rs2206734), and examined their associations with pancreatic cancer risk in a multi-institute case-control study including 360 cases and 400 controls in Japan. A self-administered questionnaire was used to collect detailed information on lifestyle factors. Genotyping was performed using Fluidigm SNPtype assays. Unconditional logistic regression methods were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between these diabetes-associated variants and pancreatic cancer risk.

RESULTS: With the exception of rs1501299 in the

ADIPOQ gene (P = 0.09), no apparent differences in genotype frequencies were observed between cases and controls. Rs1501299 in the *ADPIOQ* gene was positively associated with pancreatic cancer risk; compared with individuals with the AA genotype, the age- and sex-adjusted OR was 1.79 (95%CI: 0.98-3.25) among those with the AC genotype and 1.86 (95%CI: 1.03-3.38) among those with the CC genotype. The ORs remained similar after additional adjustment for body mass index and cigarette smoking. In contrast, rs2237895 in the *KCNQ1* gene was inversely related to pancreatic cancer risk, with a multivariable-adjusted OR of 0.62 (0.37-1.04) among individuals with the CC genotype compared with the AA genotype. No significant associations were noted for other 5 SNPs.

CONCLUSION: Our case-control study indicates that rs1501299 in the ADIPOQ gene may be associated with pancreatic cancer risk. These findings should be replicated in additional studies.

Key words: Single-nucleotide polymorphisms; Pancreatic cancer; Risk; Case-control study; Odds ratio

Core tip: Although it is likely that a common genetic background predisposes individuals to developing both diabetes and pancreatic cancer, very few molecular epidemiologic studies have addressed this issue. We therefore genotyped 7 diabetes-related genetic variants and found that rs1501299 in the ADIPOQ gene may be associated with pancreatic cancer risk. The role of adiponectin variants needs further study.

Kuruma S, Egawa N, Kurata M, Honda G, Kamisawa T, Ueda J, Ishii H, Ueno M, Nakao H, Mori M, Matsuo K, Hosono S, Ohkawa S, Wakai K, Nakamura K, Tamakoshi A, Nojima M, Takahashi M, Shimada K, Nishiyama T, Kikuchi S, Lin Y. Case-control study of diabetes-related genetic variants and pancreatic cancer risk in Japan. *World J Gastroenterol* 2014; 20(46): 17456-17462 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i46/17456.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i46.17456

INTRODUCTION

The etiology of sporadic pancreatic cancer remains largely unknown. Epidemiologic studies have consistently shown that pancreatic cancer is positively associated with cigarette smoking and long-standing diabetes[1,2]. A 2005 meta-analysis reported that the risk for pancreatic cancer is 82% higher among diabetics compared with those without diabetes[3], though it is unclear which factors underlying diabetes are associated with pancreatic cancer. Most epidemiological studies have been limited by self-reporting of diabetes and by the lack of objective biomarkers, such as fasting plasma glucose or insulin levels, to address the temporal relationship between diabetes and pancreatic cancer. There is increasing evidence from clinical studies that pancreatic cancer induces new-onset diabetes[4,5]. The evidence available thus far strongly suggests that the relationship between diabetes and pancreatic cancer is bi-directional.

Given the well-recognized, positive association between type 2 diabetes and pancreatic cancer risk in epidemiological studies, it may be interesting to examine whether diabetes-related genetic variants may also be associated with pancreatic cancer risk. Genome-wide association studies (GWAS) have reported that at least 30 loci are associated with susceptibility to diabetes in various populations, with the majority originating from individuals of European descent[6]. Because of the potential differences in fat distribution and genetic background between Asian and Western populations[7,8], we focused on diabetes-related genetic variants reported in studies of Japanese populations, and variants that were first reported in GWAS of other populations and then replicated in Japanese populations. Among the 7 diabetes susceptibility genes we chose for the present study, *PPARG2*, *ADIPOQ*, and *ADRB3* have been shown to be closely associated with diabetes risk in Japanese subjects[9]; *KCNQ1* was reported as a diabetes susceptibility gene simultaneously by 2 independent Japanese research groups in 2008[10,11]; *KCN11*, *TCF7L2*, and *CDKAL1* were also reported to be associated with diabetes susceptibility in GWAS of Japanese subjects[12,13].

Although it is likely that a common genetic background predisposes individuals to developing both diabetes and pancreatic cancer, very few molecular epidemiologic studies have addressed this issue. We hypothesized that diabetes susceptibility genetic variants may be associated with an increased risk of pancreatic cancer in Japanese subjects. We therefore genotyped 7 single-nucleotide polymorphisms (SNPs) in *PPARG2* (rs1801282), *ADIPOQ* (rs1501299), *ADRB3* (rs4994), *KCNQ1* (rs2237895), *KCNJ11* (rs5219), *TCF7L2* (rs7903146), and *CDKAL1* (rs2206734) and examined their associations with pancreatic cancer risk in a multi-institute, case-control study in Japan.

MATERIALS AND METHODS

Study subjects

The purpose of our case-control study was to evaluate the role of genetic polymorphisms and gene-environment interactions in the development of pancreatic cancer in Japanese subjects. The details of the study design have been described elsewhere[14]. Briefly, cases were defined as patients who were newly diagnosed with pancreatic ductal adenocarcinoma at five participating hospitals from April 1, 2010, through May 15, 2012. A diagnosis was made according to imaging modalities and further confirmed by pathology reports. Pathologically confirmed cases represented approximately 90% of all cases in this study. During the same time period, we recruited the majority of control subjects from in-