EUS-guided FNA (EUS-FNA) biopsies were first reported by Vilmann et al[1] in 1992 and have a high diagnostic accuracy (range, 70%-98%).[2] In most cases, a cytological assessment is sufficient for the diagnosis of a pancreatic tumor. However, it is sometimes difficult to make a differential diagnosis by cytological data alone.[3] In such cases, evaluation of tissue architecture and morphology, namely, a histological diagnosis, is required for an accurate pathological diagnosis.

The success of puncture is important for tissue acquisition and is thus a crucial factor in EUS-FNA performance. A higher technical success rate is achievable with a 25-gauge needle than with a 22- or 19-gauge needle; however, the specimen obtained with the 25-gauge needle is less adequate for histological diagnosis compared with that obtained with the other needles.[4] Two studies have indicated that EUS-FNA approaches by using high negative pressure (HNP) suction to aspirate tissue enable acquisition of adequate tissue.[5,6] However, these studies only used the 22- and 19-gauge needles, and no studies thus far have evaluated the efficacy of 25-gauge needles for EUS-FNA in combination with HNP.

Therefore, we hypothesize that a 25-gauge needle for EUS-FNA with HNP may enable us to obtain sufficient tissue material with a high success rate. We conducted a multicenter, prospective, randomized, controlled trial to determine the accuracy of this hypothesis.

Take-home Message

- The use of the high negative pressure suction technique is superior to normal negative pressure suction in terms of the amount of sufficient material for histological diagnosis obtained via EUS-FNA.
- A high diagnostic accuracy is achievable by using a 25-gauge needle and high negative pressure suction when performing EUS-FNA on pancreatic lesions.

METHODS

Patients

Between July 2011 and April 2012, patients with solid pancreatic masses, as detected by US, CT, or magnetic resonance imaging, were consecutively enrolled in this study. Seven GI tertiary referral centers, where more than 100 EUS-FNAs are performed yearly, were considered eligible for this study. Patients with the following conditions were excluded: European Cooperative Oncology Group performance status of 4, a serious underlying disorder, American Society of Anesthesiologists classes III to IV, those taking oral anticoagulants, prothrombin time/international normalized ratio more than 1.5, platelet count less than 50,000/mm^3, pregnancy, GI obstruction, and refusal or inability to provide informed consent. The study was approved by the institutional review board of each institution and was registered with the University Hospital Medical Information Network Clinical Trials Registry (number UMIN000005939).

Procedural technique

Patients were placed in the left lateral decubitus position and were administered conscious sedation. A curvilinear echoendoscope (GF-UCT240-AL5; Olympus Medical Systems, Tokyo, Japan) was used, and EUS-FNA was performed by using a 25-gauge needle (Echo Tip Ultra; Cook Japan, Tokyo, Japan). After the needle was advanced into the target lesion, the stylet was withdrawn. A 10-mL syringe with 10-mL negative pressure (normal negative pressure [NNP]) or the Alliance II inflation system (Boston Scientific Japan, Tokyo, Japan) by using a 60-mL syringe with 50-mL HNP was attached to the proximal end of the needle, as appropriate, for the randomized protocol. The needle was then moved back and forth 10 to 20 times while performing suction. We performed EUS-FNA by using jabbing movements under continuous suction. We also used the fanning technique during EUS-FNA for pancreatic lesions if the endoscopist was able to perform the maneuver. Four EUS-FNA procedures were performed in the following order in the NNP and HNP groups, respectively: NNP-HNP-NNP-HNP and HNP-NNP-HNP-NNP. Obtained samples were categorized according to group (NNP or HNP) and fixed with formalin for histological examination. A portion of each sample, obtained by the first and second punctures, was sent for cytological examination. The remaining tissue was instantly fixed in 10% neutral-buffered formalin solution for histological examination. The EUS-FNA procedure was performed by using NNP with a 25-gauge needle or HNP with a different 25-gauge needle. On-site modified Giemsa staining (Diff-Quik; Kokusai Shiyaku, Kobe, Japan) was performed at all institutions. If an endoscopist considered samples obtained during 4 attempts at EUS-FNA insufficient for pathological diagnosis, an additional puncture was permitted. An additional puncture was performed if (1) the cytopathologist could not identify any material on the glass slide or (2) the cytopathologist could not macroscopically identify any whitish material on the glass slide. For additional punctures, any FNA procedure (needle/suction) could be performed.

Method of assignment of NNP and HNP groups

A computer-generated sequence was used to randomize patients into the NNP or HNP group. Randomized groups were stratified by institutions.

Outcome measurements

The primary outcome of this study was to determine the adequacy of tissue acquisition by the EUS-FNA/HNP combined technique and to determine the accuracy of histological diagnoses achievable by using this technique. The

secondary outcome of this study was to assess the quality and quantity of obtained tissue and the potential for adverse events arising from the use of this procedure.

Pathological assessment of samples obtained in this study

Cytological and histological analyses were performed separately. The cytological analysis was performed in on-site pathology facilities available at each hospital. Cell-block techniques were not performed for all patients in this study. The histological analysis was performed by a single expert pathologist (T.M.) based on hematoxylin and eosin staining. This pathologist evaluated the quantity and quality of each specimen and determined a histological diagnosis while blinded to clinical information, cytology, and final diagnoses.

The quantity of samples was assessed by the scoring system described by Gerke et al.[6] This scoring system is as follows: 0 indicates a sample with no material, 1 indicates that the sample contains sufficient material for limited cytological interpretation but is probably not representative, 2 indicates that the sample contains sufficient material for adequate cytological interpretation but is insufficient for histological information; 3 indicates sufficient material for limited histological interpretation; 4 indicates sufficient material for adequate histological interpretation, but a low-quality sample (total material is within a ×10 power field in length); 5 indicates sufficient material for adequate histological interpretation and a high-quality sample (total material is more than a ×10× power field in length). Figure 1 shows representative examples. In our study, a sample with a score of 3 or higher was defined as adequate for histological diagnosis. A sample with a score of 2 or lower was defined as inadequate for histological diagnosis.

The degree of contamination (eg, GI mucosa) in the specimens was categorized into 4 grades: 0, no contamination; 1, contamination present in less than 25% of the slide; 2, contamination present in 25% to 50% of the slide; 3, contamination present in more than 50% of the slide. The degree of the amount of blood in the specimens was categorized into 3 grades: 0, mild; 1, moderate; or 2, significant.

Pancreatic carcinomas, neuroendocrine tumors, lymphomas, and solid pseudopapillary neoplasms were defined as malignant diseases. Pancreatitis and non-neoplastic pancreatic tissue were defined as nonmalignant diseases. Malignancy and suspicious for malignancy were defined as positive for malignancy. Atypical cells and benign were defined as negative for malignancy. Because immunohistochemical studies could not be performed for all specimens in this study, the pathologist judged a sample to be malignant or benign based on hematoxylin and eosin staining alone. An accurate diagnosis was defined as follows: (1) positive for malignancy, with a final diagnosis of malignant disease such as carcinoma, neuroendocrine tumor, and solid pseudopapillary neoplasm (true positive); (2) negative for malignancy, with the condition ultimately being diagnosed as a nonmalignant disease, such as pancreatitis and non-neoplastic pancreatic tissue (true negative).

Diagnostic accuracy was defined as the ratio between the sum of true positive and true negative values, divided by the total number of samples. The adequacy rate was calculated by the following formula: number of adequate samples divided by total number of samples.

Clinical diagnostic methodology used for ultimate diagnosis of patients

Malignant disease was ultimately identified in patients by (1) diagnosis at autopsy after death caused by pancreatic cancer, (2) diagnosis based on histopathological analyses of surgically resected specimens, (3) radiological or clinical data indicating evidence of disease progression, or (4) diagnosis based on histopathological analyses of nodules in other organs demonstrating metastatic progression. In this study, benign disease was defined as a decrease or no change in pancreatic mass and no change in clinical data obtained for at least 6 months.

Adverse events

An adverse event was defined as any event that required the patient to stay in the hospital for a longer duration than expected or to undergo other unplanned interventions. For detailed reporting of adverse events, we referred to the Practice Committee of the American Society for Gastrointestinal Endoscopy guidelines.[7]

Sample size

The study was designed such that the sample size was large enough to obtain differences in the adequacy of samples needed for histological diagnosis.

It has been reported that a sample acquisition rate of 45.8% can be achieved by using a 25-gauge needle in pancreatic tumors.[4] We estimated that 50% and 65% of specimens obtained in the NNP and HNP groups, respectively, would be adequate for histological diagnoses. By using the McNemar test of equality of paired proportions and assuming 25% discordant pairs and a 10% dropout rate, each subject was assumed to have 1 pancreatic lesion. It was evaluated that 90 patients would be required to enable statistical analyses by using a 2-tailed test with a 5% significance level and 80% statistical power.

Statistical analysis

All statistical tests were performed by using dedicated software (JMP software version 8; SAS Institute, Cary, NC). The McNemar test was applied to adequacy, accuracy, and quality data gathered from tissue samples. $P < .05$ was considered statistically significant.

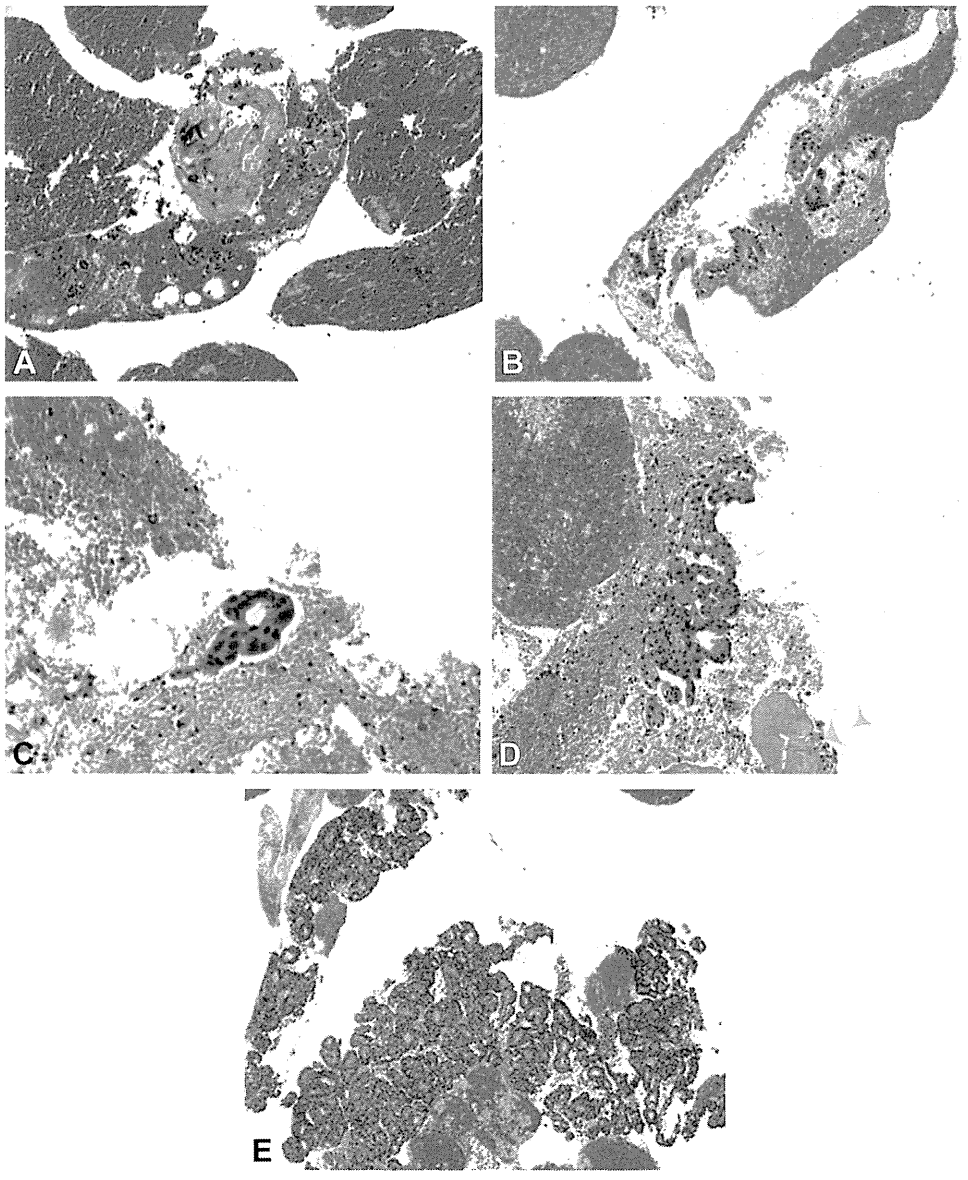

Figure 1. Representative images of specimens obtained by using EUS-guided FNA reveal differences between samples in terms of adequacy for histological diagnosis. **A**, In this sample with a score of 1, only a few cells are recognizable (hematoxylin and eosin stain, magnification ×200). This sample is inadequate for histological or cytological diagnosis. **B**, This is a sample that received a score of 2. This sample is inadequate for histological diagnosis, but might possibly be suitable for cytological diagnosis. **C**, This specimen (score of 3) is recognizable as a small tissue cluster. Evaluation of a part of tissue architecture and limited histological interpretation is possible. **D**, In this sample (score of 4), there is sufficient material for adequate histological diagnosis, and tissue architecture can be evaluated. The area of tissue on the prepared slide is within ×10 power field in length. **E**, In this sample (score of 5), there is sufficient material for adequate histological diagnosis, and tissue architecture can be evaluated. The area of tissue on the prepared slide is more than ×10 power field in length.

RESULTS

During the study period, 52 men and 38 women (90 patients) were enrolled in this study. The median age of patients was 67 years. All lesions were visible by EUS. Thirty-four patients had a lesion in the pancreas head (10 patients had lesions in the uncinate process), 40 patients in the body, and 16 patients in the tail. Fifty-six successful EUS-FNA procedures were performed through the gastric wall, whereas the remaining 34 procedures were performed through the duodenal wall. The median size of lesions was 28.2 mm (range 7.2–63.9 mm) (Table 1).

All EUS-FNA procedures were performed with on-site cytopathology evaluation. In this study, additional punctures were performed. Among these 5 patients, 2 underwent EUS-FNA with NNP by using a 22-gauge needle, 2 underwent EUS-FNA with NNP by using a 19-gauge needle, and 1 underwent EUS-FNA with HNP by using a 25-gauge needle. The definitive diagnostic procedures for a pancreatic lesion were as follows: 25 lesions were

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.

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.

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).

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%),

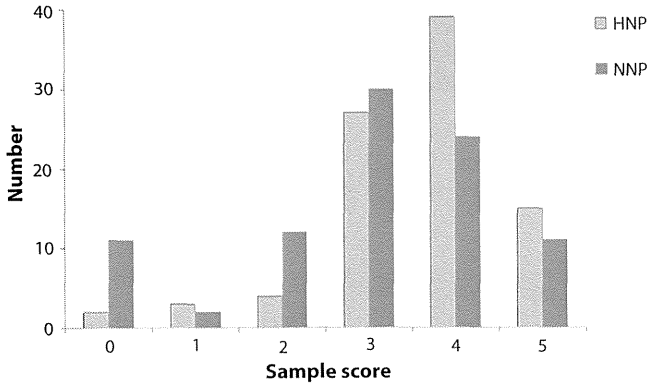

Figure 2. Scores of 0 to 5 were assigned to specimens to describe the adequacy of these samples for histological diagnosis. More samples with a score of 3 to 5 were obtained by using the high negative pressure (HNP) suction technique than normal negative pressure (NNP).

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).

REFERENCES

1. Vilmann P, Jacobsen GK, Henriksen FW, et al. Endoscopic ultrasonography with guided fine needle aspiration biopsy in pancreatic disease. Gastrointest Endosc 1992;38:172-3.
2. Wani S, Early D, Kunkel J, et al. Diagnostic yield of malignancy during EUS-guided FNA of solid lesions with and without a stylet: a prospective, single blind, randomized, controlled trial. Gastrointest Endosc 2012;76:328-35.
3. Levy MJ, Wiersema MJ. EUS-guided Trucut biopsy. Gastrointest Endosc 2005;62:417-26.
4. Sakamoto H, Kitano M, Komaki T, et al. Prospective comparative study of the EUS guided 25-gauge FNA needle with the 19-gauge Trucut needle and 22-gauge FNA needle in patients with solid pancreatic masses. J Gastroenterol Hepatol 2009;24:384-90.
5. Larghi A, Noffsinger A, Dye CE, et al. EUS-guided fine needle tissue acquisition by using high negative pressure suction for the evaluation of solid masses: a pilot study. Gastrointest Endosc 2005;62:768-74.
6. Gerke H, Rizk MK, Vanderheyden AD, et al. Randomized study comparing endoscopic ultrasound-guided Trucut biopsy and fine needle aspiration with high suction. Cytopathology 2010;21:44-51.
7. Cotton PB, Eisen GM, Aabakken L, et al. A lexicon for endoscopic adverse events: report of an ASGE workshop. Gastrointest Endosc 2010;71:446-54.
8. Imazu H, Uchiyama Y, Kakutani H, et al. A prospective comparison of EUS-guided FNA using 25-gauge and 22-gauge needles. Gastroenterol Res Pract 2009;2009:546390.
9. Lee JH, Stewart J, Ross WA, et al. Blinded prospective comparison of the performance of 22-gauge and 25-gauge needles in endoscopic ultrasound-guided fine needle aspiration of the pancreas and peripancreatic lesions. Dig Dis Sci 2009;54:2274-81.
10. Siddiqui UD, Rossi F, Rosenthal LS, et al. EUS-guided FNA of solid pancreatic masses: a prospective, randomized trial comparing 22-gauge and 25-gauge needles. Gastrointest Endosc 2009;70:1093-7.
11. Yusuf TE, Ho S, Pavey DA, et al. Retrospective analysis of the utility of endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) in pancreatic masses, using a 22-gauge or 25-gauge needle system: a multicenter experience. Endoscopy 2009;41:445-8.
12. Siddiqui AA, Lyles T, Avula H, et al. Endoscopic ultrasound-guided fine needle aspiration of pancreatic masses in a veteran population: comparison of results with 22- and 25-gauge needles. Pancreas 2010;39:685-6.
13. Camellini L, Carlinfante G, Azzolini F, et al. A randomized clinical trial comparing 22G and 25G needles in endoscopic ultrasound-guided fine-needle aspiration of solid lesions. Endoscopy 2011;43:709-15.
14. Uehara H, Ikezawa K, Kawada N, et al. Diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration for suspected pancreatic malignancy in relation to the size of lesions. J Gastroenterol Hepatol 2011;26:1256-61.
15. Fabbri C, Polifemo AM, Luigiano C, et al. Endoscopic ultrasound-guided fine needle aspiration with 22- and 25-gauge needles in solid pancreatic masses: a prospective comparative study with randomisation of needle sequence. Dig Liver Dis 2011;43:647-52.
16. Lee JK, Lee KT, Choi ER, et al. A prospective, randomized trial comparing 25-gauge and 22-gauge needles for endoscopic ultrasound-guided fine needle aspiration of pancreatic masses. Scand J Gastroenterol 2013;48:752-7.
17. Vilmann P, Săftoiu A, Hollerbach S, et al. Multicenter randomized controlled trial comparing the performance of 22 gauge versus 25 gauge EUS-FNA needles in solid masses. Scand J Gastroenterol 2013;48:877-83.
18. Madhoun MF, Wani SB, Rastogi A, et al. The diagnostic accuracy of 22-gauge and 25-gauge needles in endoscopic ultrasound-guided fine needle aspiration of solid pancreatic lesions: a meta-analysis. Endoscopy 2013;45:86-92.
19. Iglesias-Garcia J, Dominguez-Munoz E, Lozano-Leon A, et al. Impact of endoscopic ultrasound-guided fine needle biopsy for diagnosis of pancreatic masses. World J Gastroenterol 2007;13:289-93.
20. Iglesias-Garcia J, Poley JW, Larghi A, et al. Feasibility and yield of a new EUS histology needle: results from a multicenter, pooled, cohort study. Gastrointest Endosc 2011;73:1189-96.
21. Puri R, Vilmann P, Săftoiu A, et al. Randomized controlled trial of endoscopic ultrasound-guided fine-needle sampling with or without suction for better cytological diagnosis. Scand J Gastroenterol 2009;44:499-504.
22. Wallace MB, Kennedy T, Durkalski V, et al. Randomized controlled trial of EUS-guided fine needle aspiration techniques for the detection of malignant lymphadenopathy. Gastrointest Endosc 2001;54:441-7.
23. Polkowski M, Larghi A, Weynand B, et al. Learning, techniques, and complications of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Technical Guideline. Endoscopy 2012;44:190-206.
24. Haba S, Yamao K, Bhatia V, et al. Diagnostic ability and factors affecting accuracy of endoscopic ultrasound-guided fine needle aspiration for pancreatic solid lesions: Japanese large single center experience. J Gastroenterol 2012;48:973-81.
25. Wang KX, Ben QW, Jin ZD, et al. Assessment of morbidity and mortality associated with EUS-guided FNA: a systematic review. Gastrointest Endosc 2011;73:283-90.
26. Varadarajulu S, Fockens P, Hawes RH. Best practices in endoscopic ultrasound-guided fine-needle aspiration. Clin Gastroenterol Hepatol 2012;10:697-703.

Department of Medical Oncology and Hematology, Sapporo Medical University, Sapporo (2), The First Department of Internal Medicine, Gifu University Hospital, Gifu (3), Department of Gastroenterology, Gifu Municipal Hospital, Gifu (4), Department of Gastroenterology and Hepatology, Mie University, Mie (5), Center for Gastroenterology, Teine-Keijinkai Hospital, Sapporo (6), Department of Gastroenterology, The University of Tokyo, Tokyo (7), Department of Surgical Pathology, Hokkaido University Hospital, Sapporo (8), Japan.

Reprint requests: Hiroshi Kawakami, MD, PhD, Department of Gastroenterology and Hepatology, Hokkaido University Graduate School of Medicine, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan.

If you would like to chat with an author of this article, you may contact Dr Kawakami at hiropon@med.hokudai.ac.jp.

APPENDIX

Japan EUS-FNA Negative Pressure Suction Study Group consists of H. Kawakami, MD, PhD, T. Kudo, MD, M. Kuwatani, MD, PhD, K. Eto, MD, PhD, Y. Abe, MD, S. Kawahata, MD, N. Sakamoto, MD, PhD, Hokkaido University Hospital (Department of Gastroenterology and Hepatology); T. Mitsuhashi, MD, PhD, Y. Matsuno, MD, PhD, K. Marukawa, CT (IAC), J. Moriya, CT (IAC), Hokkaido University Hospital (Department of Surgical Pathology); K. Oba, PhD, Hokkaido University Hospital (Research and Clinical Trial Center); T. Hayashi, MD, PhD, Y. Ishiwatari, MD, PhD, M. Ono, MD, Sapporo Medical University School of Medicine (Department of Medical Oncology and Hematology); T. Hasegawa, MD, PhD, K. Nakanishi, MD, PhD, J. Ogino, MD, PhD, H. Sanuma, PhD, CT (IAC), Sapporo Medical University School of Medicine (Department of Surgical Pathology); I. Yasuda, MD, PhD, S. Doi, MD, PhD, K. Toda, MD, PhD, T. Yamauchi, MD, PhD, J. Kawaguchi, MD, PhD, S. Uemura, MD, PhD, Gifu University Hospital (First Department of Internal Medicine); Y Hirose, MD, PhD, Gifu University Hospital (Department of Tumor Pathology); T. Mukai, MD, PhD, M. Nakashima, MD, PhD, Gifu Municipal Hospital (Department of Gastroenterology); T. Yamada, MD, PhD, M. Etori, CT (IAC), Gifu Municipal Hospital (Department of Pathology); T. Inoue, MD, PhD, R. Yamada, MD, PhD, Y. Takei, MD, PhD, Mie University (Department of Gastroenterology and Hepatology); T. Shiraishi, MD, PhD, M. Yoneda, CT (IAC), Mie University Graduate School of Medicine (Department of Pathologic Oncology); A. Katanuma, MD, H. Maguchi, MD, PhD, K. Yane, MD, Teine-Keijinkai Hospital (Center for Gastroenterology); T. Shinohara, MD, PhD, T. Sugimura, CT (IAC), Y. Nakajima, CT (IAC), Teine-Keijinkai Hospital (Department of Pathology); K. Kawakubo, MD, PhD, H. Isayama, MD, PhD, Y. Nakai, MD, PhD, N. Yamamoto, MD, PhD, The University of Tokyo (Department of Gastroenterology); M. Tanaka, MD, PhD, The University of Tokyo (Department of Pathology).

Dig Dis Sci
DOI 10.1007/s10620-013-2590-4

ORIGINAL ARTICLE

Factors Predictive of Adverse Events Associated with Endoscopic Ultrasound-Guided Fine Needle Aspiration of Pancreatic Solid Lesions

Akio Katanuma · Hiroyuki Maguchi · Kei Yane · Shunpei Hashigo · Toshihumi Kin · Maki Kaneko · Shin Kato · Ryusuke Kato · Ryo Harada · Manabu Osanai · Kuniyuki Takahashi · Masanori Nojima

Received: 29 October 2012 / Accepted: 24 January 2013

Abstract
Background Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) provides high diagnostic accuracy with a low incidence of procedural complications. However, it occasionally causes serious complications, and factors that increase the susceptibility to such adverse events remain unknown.
Aims We aimed to examine post-procedural events and determine risk factors associated with EUS-FNA of pancreatic solid lesions.
Methods This single-center retrospective study included 316 consecutive patients with pancreatic solid lesions who underwent 327 EUS-FNA procedures from April 2003 to September 2011. We registered all patients undergoing EUS-FNA in the database and retrospectively ascertained the presence/absence of post-procedural adverse events.
Results The incidence of post-procedural adverse events, including moderate to mild pancreatitis, mild abdominal pain, and mild bleeding, was 3.4 %. Univariate analysis showed that the incidence of post-procedural events was significantly increased in patients with tumors less than or equal to 20 mm in diameter ($P < 0.001$), those with pancreatic neuroendocrine tumors (PNET) ($P = 0.012$), and patients who had intervening normal pancreas for accessing the lesion ($P = 0.048$). Multivariate analysis identified tumors measuring less than or equal to 20 mm in diameter (OR 18.48; 95 % CI 3.55–96.17) and case of PNETs (OR 36.50; 95 % CI 1.73–771.83) were an independent risk factors.
Conclusions EUS-FNA of pancreatic solid lesions is a safe procedure. However, pancreatic lesions with small diameters and pancreatic neuroendocrine tumors are important factors associated with adverse events after EUS-FNA.

Keywords EUS-FNA · Adverse events · Pancreatitis · Risk factor · Pancreatic cancer · Pancreatic neuroendocrine tumor

Introduction

Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is a valuable tool for obtaining histological diagnoses and has been widely used since it was first reported [1]. EUS-FNA provides high histological diagnostic accuracy for pancreatic lesions, submucosal tumors, and lymph nodes [2–10]. Although adverse events such as pancreatitis, bleeding, and perforation are known to be associated with EUS-FNA, the reported complication rate is extremely low [11–16]. However, adverse events associated with EUS-FNA have not yet been clearly defined and nor has their severity been classified. Moreover, the risk factors for adverse events associated with EUS-FNA procedures have not yet been determined. The purpose of this study was to determine the incidence of adverse events in patients undergoing EUS-FNA who were registered in our database and to identify risk factors for the development of post-procedural adverse events in patients who undergo FNA of pancreatic solid lesions.

A. Katanuma (✉) · H. Maguchi · K. Yane · S. Hashigo · T. Kin · M. Kaneko · S. Kato · R. Kato · R. Harada · M. Osanai · K. Takahashi
Center for Gastroenterology, Teine-Keijinkai Hospital, 1-40-1-12 Maeda, Teine-ku, Sapporo, Hokkaido 006-8555, Japan
e-mail: akio-ka@ta2.so-net.ne.jp

M. Nojima
Department of Public Health, Sapporo Medical University School of Medicine, Sapporo, Japan

Published online: 20 February 2013

Patients and Methods

Patients

A total of 316 consecutive patients with pancreatic solid lesions who underwent 327 EUS-FNA procedures from April 2003 to September 2011 were included in the study (Table 1). We registered all patients undergoing EUS-FNA in the database and retrospectively ascertained the presence/absence of complications. We performed EUS-FNA on pancreatic solid lesions because cystic tumors were a contraindication at our center. Patients with hemorrhagic tendencies were not included as candidates for EUS-FNA, and those on anticoagulant therapy were instructed to discontinue the medication prior to the procedure. Prior to undergoing EUS-FNA, all patients provided written informed consent.

EUS-FNA Procedures

All patients who were scheduled to undergo FNA were hospitalized for the procedure. EUS-FNA procedures were performed by physicians who perform an average of 150 patients per year and have more than 10 years of experience. Blood analyses were performed less than 48 h before EUS-FNA. All the patients were placed in the left lateral position, and sedation was accomplished using either intravenous diazepam (5 mg) or pethidine hydrochloride (35 mg) along with intravenous midazolam (5 mg). The patients were kept fasting after the procedure and given an antibiotic twice after the examination.

Table 1 Baseline characteristics of pts who underwent EUS-FNA of pancreatic solid lesions

Characteristics	Values
Age, years, mean + SD (range)	66.5 ± 11.5 (23–92)
Sex, M:F	178:149
Diagnosis	
Pancreatic cancer	275 (84.1 %)
Chr. pancreatitis/TFP	24 (7.3 %)
PNET	13 (4.0 %)
AIP	4 (1.2 %)
Metastatic tumor	2 (0.6 %)
SPN	2 (0.6 %)
Accessary spleen	2 (0.6 %)
Others	5 (1.5 %)
Needle size	
19-guage	31 (9.5 %)
22-gauge	268 (82 %)
25-gauge	28 (8.6 %)

TFP tumor forming pancreatitis, *PNET* pancreatic neuroendocrine tumor, *AIP* autoimmune pancreatitis, *SPN* solid-pseudopapillary neoplasm

All FNA procedures were completed using a curved linear echo endoscope (GF-UCT240, GF-UCT260; Olympus Medical Systems, Tokyo, Japan). Basically, a 22-G needle (EZ-shot; Olympus Medical Systems; and EchoTip Ultra; Cook Medical, Winston-Salem, NC, USA) was used; however, a 19- or 25-G needle (EchoTip Ultra; Cook Medical) was selected when necessary. Immediately after the tissue samples were obtained, they were stained using the Diff-Quik method in the presence of a cytologist to confirm the adequacy of the sample for cytological diagnosis.

All ultrasonography images obtained during the procedure were stored on a computer as electronic images. Using these images, we retrospectively confirmed whether the needle pass site as via normal pancreatic tissue (Fig. 1a, b). In cases where the needle pass was via normal pancreatic tissue, the length of the needle penetration was measured (Fig. 1c). The length was measured using a distance marker on the ultrasonography images and assigned to one of 3 categories: <1, 1–2, and >2 cm.

Assessment of Adverse Events and Variables

Physicians and/or nurses confirmed the subjective symptoms and physical findings on the day following the procedure and at least 1 week later. Blood biochemical tests were also performed for all patients to detect any abnormalities in laboratory data. In patients with suspected complications, diagnostic imaging, including computed tomography (CT), was performed as needed. Adverse events and severity grading were defined according a report from a workshop held by the American Society for Gastrointestinal Endoscopy (ASGE) [17]. Acute pancreatitis was defined as upper abdominal pain associated with nausea or vomiting and accompanied by at least a three-fold elevation of serum amylase or lipase. Significant gastrointestinal bleeding was defined as a drop in the hemoglobin level by >2 g/dl as compared with the pre-procedure baseline levels together with clinical evidence of bleeding. Abdominal pain was defined as pain not caused by pancreatitis or perforation. Because all EUS-FNA procedures were performed in the hospitalized, the period until oral intake was used as a basis for evaluating severity instead of the length of hospital stay. Severity was classified as mild, moderate, or severe if the patient required less than 3 days of fasting, 4–10 days of fasting, or more than 10 days of fasting, respectively.

Statistical Analysis

Differences and linear trends in the proportions of the categorical variables were analyzed using Fisher's exact

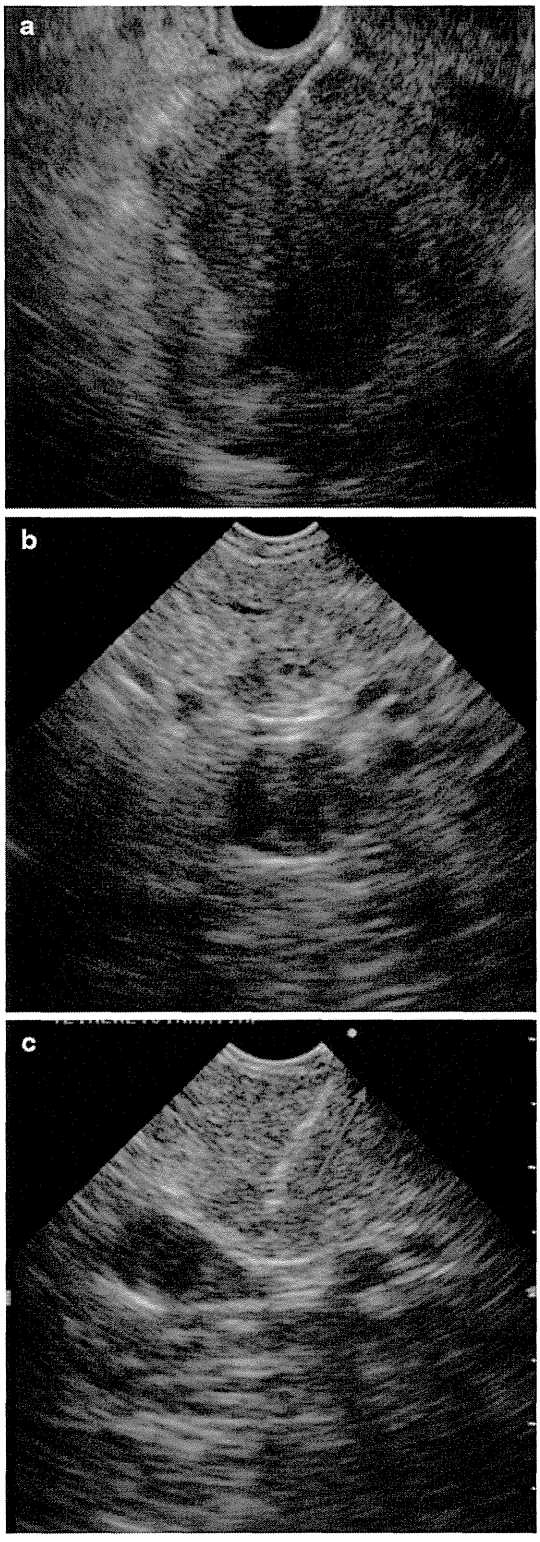

Fig. 1 Using ultrasound images, needle pass site was confirmed as via normal pancreatic tissue. **a** Needle pass was not via normal pancreas. **b** Needle pass via normal pancreas. **c** In cases where the needle pass was via normal pancreatic tissue, the length of the needle penetration was measured. The length was measured using a distance marker on the ultrasonography images and assigned to one of 3 categories: <1, 1–2, and >2 cm

test or the Chi square (χ^2) test for trend. Student's *t* test was used to compare continuous variables. Multivariate analysis using a logistic regression model was performed using the forward method. The odds ratios (ORs) and 95 % confidence intervals (95 % CI) were calculated to evaluate the predictors of complications. Two-tailed *P* values of less than 0.05 were considered statistically significant.

Results

Adverse Events and Severity Grading After EUS-FNA

Needle passes were performed for a mean of 2.78 times using 19-, 22-, and 25-G needles for 31 (9.5 %), 268 (82.0 %), and 28 (8.6 %) procedures, respectively (Table 1). The needle pass site was the stomach in 198 cases (60.6 %) and the duodenum in 129 cases (39.4 %). The incidence of adverse events was 3.4 % (11 patients): pancreatitis was noted in 6 patients (moderate in 1 case and mild in 5 cases); mild abdominal pain in 4 patients; and mild bleeding in 1 patient (Table 2). The underlying disease was pancreatic cancer in 7 cases, pancreatic neuroendocrine tumor (PNET) in 3 cases, and chronic pancreatitis in 1 case. Eight cases (73 %) involved small lesions, less than or equal to 20 mm in diameter. A 19-G needle was used in 2 cases, and a 22-G needle was used in the other 9 cases. Needle passes were performed 2 times in 2 cases, 3 times in 4 cases, 4 times in 3 cases, and 5 times in 2 cases. In 9 cases (82 %), the number of needle passes was 3 or more. The needle pass site was the via normal pancreas in 8 cases (73 %) (Table 3). All cases were managed by conservative therapy only.

Risk Factors for Adverse Events

The following variables were examined in the 327 patients who underwent FNA of the pancreas: age, sex, location of target, tumor size, tumor type (benign, PNET, or other pancreatic tumor), site of needle pass, size of the needle used, mean number of needle passes, whether or not the needle pass was via the normal pancreas, and length of needle penetration into normal pancreas tissue. The results of univariate analysis showed that the incidence of procedural complications was significantly increased in cases

Table 2 Incidence of post-procedure events after EUS-FNA

	No of cases, %	Severity grading
Pancreatitis	6, 1.8	Moderate 1, mild 5
Abdominal pain	4, 1.2	Mild 4
Bleeding	1, 0.3	Mild 1
Total	11, 3.4	Moderate 1, mild 10

Table 3 Characteristics in case of adverse events

No.	Age	Sex	Diagnosis	Location	Size (mm)	Site of needle pass	Needle size (gauge)	No. of passes	Presence or absence of normal pancreas	Length of the needle penetration	Adverse events	Severity
1	64	F	PC	Head	20	Duodenum	22	2	Presence	1–2 cm	Pancreatitis	Moderate
2	60	M	PNET	Tail	26	Stomach	19	4	Absence		Pancreatitis	Mild
3	83	F	PC	Head	18	Duodenum	22	3	Presence	1–2 cm	Pancreatitis	Mild
4	78	F	PNET	Tail	8	Stomach	22	4	Absence		Pancreatitis	Mild
5	61	M	PC	Head	32	Duodenum	22	2	Presence	<1 cm	Pancreatitis	Mild
6	71	M	PC	Head	20	Duodenum	22	5	Presence	1–2 cm	Pancreatitis	Mild
7	79	F	PC	Tail	54	Stomach	22	4	Presence	<1 cm	Bleeding	Mild
8	67	M	PC	Head	15	Duodenum	22	3	Absence		Abdominal pain	Mild
9	75	M	CP	Tail	5	Stomach	22	3	Presence	>2 cm	Abdominal pain	Mild
10	58	F	PC	Head	15	Duodenum	19	3	Presence	<1 cm	Abdominal pain	Mild
11	85	M	PNET	Head	15	Duodenum	22	5	Presence	<1 cm	Abdominal pain	Mild

PC pancreatic cancer, *PNET* pancreas neuroendocrine tumor, *CP* chronic pancreatitis

involving tumors measuring $\leq$20 mm in diameter ($P < 0.001$), cases of PNETS ($P = 0.012$), and cases with an increased length of needle penetration (those in which the puncture needle had to traverse normal pancreas tissue) ($P = 0.048$). Because statistical significance was observed among the 3 disease categories (benign, PNET, and other tumors), we performed paired comparisons. The P values for the paired comparisons were as follows: $P = 0.065$ for benign versus PNET, $P = 1.000$ for benign versus other tumors, and $P = 0.009$ for PNET versus other tumors (Table 4). Multivariate analysis identified tumors measuring less than or equal to 20 mm in diameter (OR 18.48; 95 % CI 3.55–96.17) and PNETs (OR 36.50; 95 % CI 1.73–771.83) as independent risk factors (Table 5).

Discussion

In this study, the incidence of post-procedural adverse events was 3.4 %, which is slightly higher than that reported in previous studies. However, a prospective study conducted by Sendino et al. [18] reported a complication rate of 3.1 % and a severe complication rate of 1.2 % in 219 patients undergoing EUS-FNA. Although our study was retrospective, a certain level of accuracy was ensured because the patients undergoing FNA were registered in a database and were admitted to the hospital for the procedure. Additionally, the clinical findings, including the results of blood tests performed on the day after EUS-FNA, were recorded precisely. Furthermore, we were able to properly assess not only the patients with severe complications but also those who experienced mild adverse events. Of these 11 cases, all except 1 experienced mild adverse events. Thus, EUS-FNA appears to be a safe procedure for patients with pancreatic solid lesions.

The relationship between the incidence of adverse events after EUS-FNA and needle size is an important issue, and a 22-G needle was our first choice. Siddiqui et al. [19] reported that no adverse events were observed after the procedure using either 22- or 25-G needles. In this study, the results of both univariate and multivariate analyses suggested that needle size was not a factor that was significantly associated with an elevated risk of adverse events. In theory, the incidence of adverse events after EUS-FNA with a larger diameter needle was expected to be higher than that after procedures using needles that were smaller in diameter. Needle pass with a larger diameter needle may increase the risk of both tissue damage and adverse events. However, in our study, moderate pancreatitis occurred in 1 patient with pancreatic cancer when we used a 22-G needle and a needle pass in the duodenum (Fig. 2a). After EUS-FNA, the patient experienced abdominal pain. Abdominal CT revealed fluid collection around the pancreas head (Fig. 2b). We performed a surgery to remove the pancreatic cancer 19 days after EUS-FNA. The intraoperative findings revealed a blood clot around the pancreas head lesion, and adhesion was confirmed between the pancreas head and the duodenal wall (Fig. 2c). However, the resection was completely successful.

In this study, univariate analysis revealed the following statistically significant risk factors for post procedural adverse events: tumors measuring less than or equal to 20 mm in diameter, 3 or more punctures, and a greater

Table 4 Analysis of risk factors for the complications in patients who underwent FNA of the pancreas

	With complications $n = 11$	Without complications $n = 316$	P value[a]
Age	71.0 ± 9.6	66.1 ± 11.4	0.163[b]
Sex			
M:F	7:4	171:145	0.760
Location			
Head	6	129	0.371
Body/tail	5	187	
Tumor size (mm)			
≤20	3	268	<0.001
>20	8	48	
Benign	1	34	0.012[c]
PNETs	3	11	
Other tumors (PK, sarcoma, SPN)	7	271	
Site of needle pass			
Stomach	5	193	0.353
Duodenum	6	123	
Needle size (gauge)			
25	0	28	0.263[d]
22	9	259	
19	2	29	
Number of needle passes			
<2	2	156	0.063
>3	9	160	
Needle pass via the normal pancreas			
Yes	8	143	0.121
No	3	173	
Length of the needle penetration			
Absence	3	173	0.048[d]
<1 cm	4	89	
1–2 cm	3	43	
>2 cm	1	11	

[a] Fisher's exact test

[b] t test

[c] P values for paired comparisons are as follows: $P = 0.065$ for benign versus PNETs, $P = 1.000$ for benign versus other tumor, and $P = 0.009$ for PNETs versus tumor

[d] Chi-square test for trend

Table 5 Results of logistic regression on complications after EUS-FNA with regard to variables

Factor	OR (95 % CI)	P value
Sex (M)	1.87 (0.40–8.70)	0.424
Age	1.07 (0.99–1.16)	0.088
Tumor location (body/tail)	0.71 (0.02–21.35)	0.845
Tumor size (≦20 mm)	18.48 (3.55–96.17)	<0.001
Benign	Ref.	
PNETs	36.50 (1.73–771.83)	0.021
Other tumor	6.76 (0.47–96.38)	0.159
Site of needle pass (Duodenum)	1.77 (0.06–53.06)	0.742
Needle size (22/25-gauge)	0.20 (0.03–1.63)	0.134
Number of needle passes (>3 time	3.56 (0.56–22.50)	0.178
Length of the needle penetration		
Absence	Ref.	
<1 cm	1.49 (0.24–9.43)	0.669
1–2 cm	3.20 (0.44–23.02)	0.249
<2 cm	9.71 (0.63–148.57)	0.103

length of penetration in cases where the needle pass had to traverse normal pancreas tissue. Furthermore, in our multivariate analysis, small tumor size and PNETs were found to be significant independent risk factors. One of the potential causes of adverse events may be the difficulty in performing needle passes for small lesions. Needle passes for small tumors may be more difficult to execute than those for large tumor masses. Occasionally, many needle passes are required because the target is too small. In addition, back-and-forth movement is difficult. This may lead to pancreatic damage and adverse effects such as pancreatitis and bleeding. Moreover, the possible causes of pancreatitis include injuries to the main pancreatic duct or its branches. Although penetration through the normal pancreas was not found to be a risk factor in our multivariate analysis, a needle pass through the normal pancreas was more likely to be necessary for accessing small lesions, and the possibility of injuries to the normal pancreas and the main pancreatic duct or its branches cannot be ignored. Vascularity of the target lesion is another factor. Our data demonstrate that PNETs are a risk factor for adverse events. In general, PNETs are hypervascular tumors and the risk of bleeding from these tumors is increased compared with other tumors. When bleeding occurs around the pancreatic parenchyma after EUS-FNA, it may cause inflammation and lead to adverse events. These factors may have helped identify a small tumor size and PNETs as risk factors. Because this was a retrospective study, a prospective study involving a larger number of patients is needed to precisely determine the risk of adverse events.

The limitations of this study included the fact that it was a retrospective analysis performed at a single center, and only pancreatic solid lesions were included. Cystic lesions of the pancreas, especially intraductal papillary neoplasms (IPMN), are contraindications for EUS-FNA not only in our center but also in many other Japanese institutions because of the risk of tumor dissemination due to leakage of cystic fluid. For this reason, we could only evaluate pancreatic solid lesions. The frequency of complications associated with pancreatic solid lesions after EUS-FNA is low compared with that associated with cystic lesions [12,

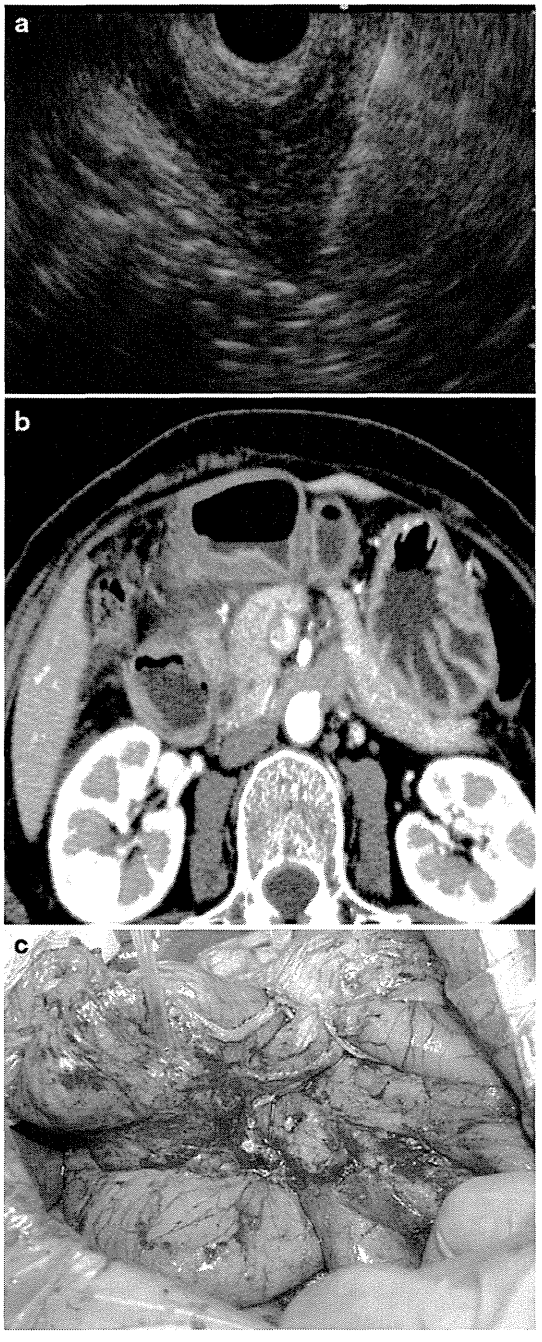

Fig. 2 A case of pancreatitis after EUS-FNA. **a** FNA was performed using 22-gauge needle. **b** Abdominal CT revealed fluid collection around the pancreas head. **c** The intraoperative findings revealed a blood clot around the pancreas head lesion, and adhesion was confirmed between the pancreas head and the duodenal wall

20]. However, in our study, the rate of complications was 3.4 %, including mild cases. The possible mechanisms for the development of adverse events differ depending on whether the lesion is solid or cystic. Therefore, determining the risk factors for adverse events after EUS-FNA in patients with pancreatic solid lesions is very important. Moreover, owing to improvements in echoendoscope and needles, we can now visualize smaller lesions and attempt to perform EUS-FNA of these lesions. For these reasons, a clarification of the risk factors associated with EUS-FNA is necessary.

In conclusion, EUS-FNA of pancreatic solid lesions is a safe procedure. However, pancreatic lesions with small diameters and PNETs are important factors associated with adverse events following EUS-FNA.

Conflict of interest None.

References

1. Vilmann P, Jacobson GK, Henriksen FW, et al. Endoscopic ultrasonography with guided fine needle aspiration biopsy in pancreatic disease. *Gastrointest Endosc.* 1992;38:172–173.
2. Eloubeidi MA, Chen VK, Eltoum IA, et al. Endoscopic ultrasound-guided fine needle aspiration biopsy of patients with suspected pancreatic cancer: diagnostic accuracy and acute and 30-day complications. *Am J Gastroenterol.* 2003;98:2663–2668.
3. Gress F, Gottlieb K, Sherman S, et al. Endoscopic ultrasonographyguided fine-needle aspiration biopsy of suspected pancreatic cancer. *Ann Intern Med.* 2001;134:459–464.
4. Harewood GC, Wiersema MJ. Endosonography-guided fine needle aspiration biopsy in the evaluation of pancreatic masses. *Am J Gastroenterol.* 2002;97:1386–1391.
5. Savides TJ, Donohue M, Hunt G, et al. EUS-guided FNA diagnostic yield of malignancy in solid pancreatic masses: a benchmark for quality performance measurement. *Gastrointest Endosc.* 2007;66:277–282.
6. Hoda KM, Rodriguez SA, Faigel DO. EUS-guided sampling of suspected GI stromal tumors. *Gastrointest Endosc.* 2009;69:1218–1223.
7. Mekky MA, Yamao K, Sawaki A, et al. Diagnostic utility of EUS-guided FNA in patients with gastric submucosal tumors. *Gastrointest Endosc.* 2010;71:913–919.
8. Philipper M, Hollerbach S, Gabbert HE, et al. Prospective comparison of endoscopic ultrasound-guided fine needle aspiration and surgical histology in upper gastrointestinal submucosal tumors. *Endoscopy.* 2010;42:300–305.
9. Yasuda I, Tsurumi H, Omar S, et al. Endoscopic ultrasound-guided fine needle aspiration biopsy for lymphadenopathy of unknown origin. *Endoscopy.* 2006;38:919–924.
10. Krishna NB, Gardner L, Collins BT, et al. Periportal lymphadenopathy in patients without identifiable pancreatobiliary or hepatic malignancy. *Clin Gastroenterol Hepatol.* 2006;4:1373–1377.
11. O'Toole D, Plazzo L, Arotçarena R, et al. Assessment of complications of EUS-guided fine-needle aspiration. *Gastrointest Endosc.* 2001;53:470–474.
12. Mortensen MB, Fristrup C, Holem FS, et al. Prospective evaluation of patients tolerability, satisfaction with patient information, and complications in endoscopic ultrasonography. *Endoscopy.* 2005;37:146–153.
13. Lee LS, Saltzman JR, Bounds BC, et al. EUS-guided fine needle aspiration of pancreatic cysts: a retrospective analysis of complications and their predictors. *Clinical Gastroenterol and Hepatol.* 2005;3:231–236.

14. Al-Haddad M, Wallace MB, Woodward TA, et al. The safety of fine-needle aspiration guided by endoscopic ultrasound: a prospective study. *Endoscopy*. 2008;40:204–208.
15. Eloubeidi MA, Tamhane A. Prospective assessment of diagnostic utility and complications of endoscopic ultrasound-guided fine needle aspiration. Results from a newly developed academic endoscopic ultrasound program. *Dig Dis*. 2008;26:356–363.
16. Fabbri C, Luigiano C, Cennamo V, et al. Complications of endoscopic ultrasonography. *Minerva Gastroentero Dietol*. 2011; 57:159–166.
17. Cotton PB, Eisen GM, Aabakken L, et al. A lexicon for endoscopic adverse events: report of an ASGE workshop. *Gastrointest Endosc*. 2010;71:446–454.
18. Sendino O, Garcia P, Gimeno-Garcia AZ, et al. Complications of endoscopic ultrasonography (EUS) and EUS-guided fine needle aspiration (EUS-FNA): a prospective investigation in a large series of patients. *Gastrointest Endosc*. 2007; 65:AB199.
19. Siddiqui UD, Rossi F, Rosenthal LS, et al. EUS-guided FNA of solid pancreatic masses: a prospective, randomized trial comparing 22-gauge and 25-gauge needles. *Gastrointest Endosc*. 2009;70:1093–1097.
20. Bournet B, Migueres I, Delacroix M, et al. Early morbidity of endoscopic ultrasound: 13 years' experience at a referral center. *Endoscopy*. 2006;38:349–354.

Asahara et al. Journal of Translational Medicine 2013, 11:291
http://www.translational-medicine.com/content/11/1/291

JOURNAL OF TRANSLATIONAL MEDICINE

RESEARCH Open Access

Phase I/II clinical trial using HLA-A24-restricted peptide vaccine derived from KIF20A for patients with advanced pancreatic cancer

Shingo Asahara[1*], Kazuyoshi Takeda[2], Kenji Yamao[3], Hiroyuki Maguchi[4] and Hiroki Yamaue[5]

Abstract

Background: We previously developed an immunotherapy treatment utilizing a cancer vaccine reagent KIF20A-66 in order to treat pancreatic cancer. KIF20A-66 is HLA-A24-restricted epitope peptide derived from KIF20A, a member of kinesin super family protein 20A that is significantly transactivated in pancreatic cancer. In this report, we further demonstrated non-randomized, open-label, single centered phase I/II clinical trial of immunotherapy using the KIF20A-66 peptide for the patients with advanced pancreatic cancer.

Methods: Vaccination was performed to the patients with metastatic pancreatic cancer, in whom gemcitabine-based therapy had failed. In phase I study, KIF20A-66 peptide was subcutaneously injected weekly in a dose-escalation manner (doses of 1.0 and 3.0 mg/body, 6 patients/1 cohort). After safety was assessed, phase II study was conducted using 3.0 mg of KIF20A-66 peptide.

Results: KIF20A-66 peptide vaccination was well tolerated in the doses we examined and tumor responses after 1 month of the treatment were evaluated. Among 29 patients who completed one course of the treatment at least, stable disease (SD) was found in 21 cases, while progressive disease (PD) was found in 8 cases, indicating that the disease control rate was 72%. Objective tumor shrinkage was observed in 8 cases, including 1 case of complete response (CR). The median survival time (MST) and progression free survival time (PFS) were 142 days and 56 days, respectively. These results clearly demonstrate that overall survival of the patients was significantly prolonged, compared to the historical controls of 9 cases with unmatched HLA in the same hospital (MST: 83 days), as well as 81 cases in our and other hospitals (MST: 63 days).

Conclusion: The patients vaccinated with KIF20A-66 peptide had better prognosis than the control group with best supportive care (BSC). Thus, we concluded that KIF20A-66 vaccination is significantly effective as an immunotherapy against advanced pancreatic cancer. KIF20A-66 peptide was well tolerable in the dose of either 1.0 mg or 3.0 mg/body, and effectively induced peptide-specific response of cytotoxic T lymphocyte (CTL). Further clinical study using this peptide is a promising approach for advanced pancreatic cancer to achieve high potential benefit for better prognosis.

Clinical trial registration: UMIN-CTR, number UMIN000004919

Keywords: KIF20A, Peptide vaccine, Pancreatic cancer

Introduction

Pancreatic cancer remains one of the most challenging conditions to treat, due to extremely poor prognosis with the overall five-year survival of less than 10% [1-3]. During the last decades, gemcitabine has been the standard single-agent chemotherapy for unresectable pancreatic cancer [4,5]. Regarding combination chemotherapy, several phase III trials of gemcitabine-based multi-drug regimens have been attempted, whereas significant improvement in survival has not been observed [6-14]. Although TS-1, a prodrug of 5-FU, has been employed as a major alternative approach in a variety of solid tumors, the single-agent treatment of TS-1 yielded non-inferiority result against the gemcitabine treatment [15]. After all, once pancreatic cancer became

* Correspondence: s.asahara@chibatoku.or.jp
[1]Department of Internal Medicine, Chiba Tokushukai Hospital, Chiba, Japan
Full list of author information is available at the end of the article

refractory to gemcitabine, there is virtually no effective treatment for the patients. Hence, novel strategy providing better survival benefit is urgently required, in particular, for the patients with advanced pancreatic cancer.

Cancer immunotherapy is a promising approach to fight against cancer, and thus we have conducted research and development of peptide vaccines targeting tumor-specific antigens [16-19]. Briefly, we identified dozens of cancer-testis or oncofetal proteins from more than 1,000 clinical cancer tissues using cDNA microarray including 32,000 genes or ESTs [20]. Utilizing the result of this genome-wide expression profile analysis, we tried to establish an epitope peptide derived from the tumor-associated antigen mentioned above, which is applicable for cancer peptide vaccination [21,22]. KIF20A, kinesin family member 20A, is one of the candidates of such target antigen, as it was up-regulated in the majority of pancreatic cancer [23]. Therefore, we developed an epitope peptide, namely KIF20A-66, restricted to HLA-A*2402 that is the most common HLA-A allele in a Japanese population [24]. We here report the results of a phase I/II clinical trial using KIF20A-66 mono peptide as cancer immunotherapy for the patients with advanced pancreatic cancer.

Methods

Patient eligibility

Patients with unresectable or metastatic pancreatic cancer, who were resistant to gemcitabine and TS-1 treatments or unable to continue the treatment of gemcitabine or TS-1 because of severe adverse events, were enrolled in this trial from March 2009 to February 2010 at Chiba Tokushukai Hospital. The eligibility criteria are as follows: unresectable pancreatic cancer with metastatic, recurrent and/or locally advanced disease based on diagnostic imaging using computed tomography (CT) and histological examinations. Other entry criteria included the HLA-A*2402-positive status, an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, age of 20–85 years, life expectancy of at least 2 months, adequate respiratory, and liver and kidney functions for vaccination treatment. The exclusion criteria are as follows: pregnancy or lactation, active infection, other active malignancy, non-recovered injury, and treatment with immunosuppressive agents or steroid. Written informed consent was obtained from each individual patient, and the study was approved by Tokushukai Group Ethical Committee. The study was registered at University Hospital Medical Information Network (UMIN) Center with the Clinical Trial Registration number UMIN000004919.

Control group

Clinical data used as the control group (BSC, multicenter, n = 81) in this study were obtained from our and other hospitals where written informed consent was obtained at each institution. Clinical information of each patient utilized in our statistical analysis includes age at diagnosis, sex, performance status at the endpoint of the Standard Chemotherapy, treatment status at primary lesion, median survival time, and mean survival time. This study was approved by the institutional review board at each institution.

Study design and end points

This study is a non-randomized, open-label phase I/II clinical trial with dose escalation of KIF20A-66 peptide mono-therapy. The primary end point of phase I part was safety of peptide vaccination and tolerance for phase II part. The primary end point of phase II part was antitumor effects assessed by CT scan in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1. The secondary end points were overall survival (OS), progression free survival (PFS), immunological responses assessed by CTL induction specific to the KIF20A-66 peptide and the injection site reactions (ISRs). In phase II part, the information of 9 patients with best supportive care in the Chiba Tokushukai Hospital from January 2007 to January 2009 was used as a historical control.

Treatment protocol

After emulsified with Incomplete Freund's adjuvant (Montanide ISA51VG, SEPPIC, France), KIF20A-66 peptide in the amount of 1.0 or 3.0 mg/body was subcutaneously administered on days 1, 8, 15 and 22 in a 28 days-treatment cycle. After two cycles of the vaccination, the peptide was administrated once in every two weeks until tumor progression was observed in the patient.

Toxicity assessment

The toxicity was assessed based on the Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0).

Peptides

The KIF20A-66 peptide (KVYLRVRPLL) was synthesized and its quality was analyzed by American Peptide Company Inc. (Sunnyvale, CA). The epitope peptide derived from HIV-Env peptide (RYLRDQQLL), restricted to HLA-A*2402, was used as a control to evaluate CTL response.

Enzyme-linked immunospot (ELISPOT) assay

To evaluate the peptide-specific CTL response, ELISPOT assay was performed after *in vitro* sensitization [16]. Briefly, frozen Peripheral Blood Mononuclear Cells (PBMC) derived from the same patient were thawed, cultured with respective peptide and IL-2 (Novartis, Emeryville, CA) (IVS), and harvested after two weeks. Followed by

$CD4^+$ cell depletion, IFN-γ ELISPOT assay was performed utilizing HLA-A*2402-positive TISI cells (IHWG Cell and Gene Bank, Seattle, WA) stimulated by either vaccinated peptide or HIV-Env peptide (as control). Reaction in a MultiScreen-IP 96-plate (Millipore, Bedford, MA) was measured by an automated ELISPOT reader, Immuno-SPOT S4 (Cellular Technology Ltd, Cleveland, OH) with ImmunoSpot Professional Software Version 5.0 (Cellular Technology Ltd). All ELISPOT assays were performed in triplicate. The number of peptide-specific spots was calculated by subtracting the number of the spots of control cells from that of the cells stimulated by vaccinated peptide. The peptide-specific T cell response was classified into four grades (–, +, ++, and +++), according to the algorithm flow chart described in our previous report (+++ : the content rate of CTL is more than 0.2% , ++ : 0.02 - 0.2%, + : 0.01 - 0.02%, –: less than 0.01%) [25]. Sensitivity of ELISPOT assay was estimated as approximate average level utilizing proficiency panels conducted by Cancer Immunotherapy Consortium (CIC) in 2009 and 2011 [26].

Flow cytometry

Expression of peptide specific T cell receptor (TCR) was examined by FACS-CantoII (Becton Dickinson, San Jose, CA) using KIF20A-66/HLA-A*2402 dextramer-PE (KI F20A-dextramer) according to the manufacturer's instruction (Immudex, Copenhagen, Denmark). HIV-A24 epitope peptide (RYLRDQQLL)/MHC-dextramer (HIV-dextramer) was used as negative control. Briefly, cells were incubated with peptide-HLA-A*2402 dextramer-PE for 10 minutes at room temperature, then treated with FITC-conjugated anti-human CD8 monoclonal antibody (mAb), APC-conjugated anti-human CD3 mAb, PE-Cy7-conjugated anti-human CD4 mAb, and 7-AAD (BD Biosciences, San Jose, CA) at 4°C for 20 minutes. Analysis gate was set on the staining profiles using HIV-dextramer, and positive cell percentage ($dextramer^+$ cells/$CD3^+$ $CD4^-$ $CD8^+$ cells) was calculated by subtracting the percentage of HIV-$dextramer^+$ from that of KIF20A-$dextramer^+$.

Statistical analysis

StatView version 5.0 (SAS Institute Japan Ltd., Japan) was used for statistical analysis. TTP and OS curves were estimated using the Kaplan-Meier methodology and analyzed with a log-rank test. Mann–Whitney U test and Chi-square test were used to compare patient characteristics.

Results

The peptide vaccine treatment

A total of 31 patients with chemotherapy-refractory pancreatic cancer were enrolled in this trial. 16 patients had unresectable tumor and 15 had recurrent one after surgery. Tables 1 and 2 indicate clinicopathological information of the 31 patients, as well as the patients in control group, who received best supportive care in our and other hospitals (Table 1). The peptide in the amount of either 1.0 mg or 3.0 mg per body was examined in this phase I/II study. These dosages were well tolerated in the 31 patients with advanced pancreatic cancer. There is no severe adverse event (SAE) related to the peptide vaccine in the 1.0 mg/body-injected group, except the immunological response at injection sites. As well, no SAE was observed in the first 6 patients in the 3.0 mg/body-injected group during the first cycle in the treatment. Hence, we determined that 3.0 mg per body is an appropriate dose for phase II part in this study.

Immunological injection site reactions (ISRs) of all the 31 patients were evaluated. Clinical responses of 29 patients out of 31, who received at least one treatment cycle (4 injections), were evaluated by immuno-monitoring. ISRs, including adverse reactions on the skin in grades 1–3, was observed in 23 patients out of 29. It should be noted that there were two patients who were incompatible with further vaccination treatment due to the exclusion criteria, such as autoimmune hepatitis and interstitial pneumonia. The patient, who experienced grade 3 autoimmune hepatitis after 11 months of vaccination, was recovered after drug withdrawal. Another patient with the interstitial pneumonia was well recovered by hospital treatment without any steroid therapy. In these cases, we could not rule out the possibility whether these adverse events were related to vaccine treatments or not.

Clinical outcomes of eligible patients

Among the 29 patients examined in this trial, 21 patients yielded the status of "stable disease" (SD), while 8 resulted in "progressive disease" (PD) after one cycle of the treatment (injections of the peptide vaccine for 4 times) (Table 2). The rate of disease control at the time of one cycle was calculated to be 72%. 8 patients showed objective tumor response at target lesions (Figure 1). On the other hand, according to RECIST criteria, the other patients were not classified as partial response (PR), since the ratio of tumor shrinkage was insufficient. One patient (case 9) achieved "complete response" (CR) after SD over the long term (Table 2, Figures 1a, 2, and 3). The rate of objective response to the total was calculated to be 25.8%.

Case 9 describes a 33-year-old female ended up with CR after 25 months including a long period of SD (Figure 1a). This patient underwent pancreatoduodenectomy in November 2008 and was diagnosed with giant cell pancreatic cancer. Adjuvant chemotherapy utilizing gemcitabine was discontinued at the one course

Table 1 Clinical status and profile of the patients

	KIF20A peptide vaccine treatment	Best supportive care	
	Chiba (n = 31) *	Chiba (n = 9) *	Multi-center (n = 81) **
Age (average, (range))	61.3 (33–80)	64 (53–82)	64.5 (41–85)
Sex (Male: Female)	17:14	5:4	49:32
Performance status (0:1:2:3)	11:8:12:0	1:3:3:2	13:28:36:0 ***
Status of primary lesion (Resected: Unresected)	15:16	1:8	23:58
Median survival time (days)	142.0 ± 23.7	83.0 ± 33.5	62.0 ± 6.5
Mean survival time (days)	171.8 ± 23.8	93.3 ± 14.8	91.1 ± 11.6

*, Clinical data obtained at our institution, Chiba Tokushukai Hospital.
**, Clinical data of Multi-center (n = 81) include those obtained from Chiba and other three hospitals.
***, 4 cases were excluded, since Performance Status was not determined.

of drug administration, due to severe adverse reactions including hematopoietic toxicity. In February 2009, a progressive solitary liver metastasis was diagnosed (Figure 1a). There was no clinical sign of inflammation at the time of April 13th, 2009. White blood cell count ($2.8 \times 10^3/\mu$-l) and CRP level (0.02 mg/dl) were within normal limits. Vaccination started on April 23rd, 2009, and the tumor kept stable condition during the administration. After 8 months, shrinkage of the tumor size was observed. Vaccination was discontinued after 11 months, because the level of liver enzyme was increased and thus autoimmune hepatitis was suspected. Nonetheless, the tumor continued to shrink and became undetectable by CT 25 months after the start of administration. At the time of the submission of this manuscript, there is no sign of relapse or metastasis, and the general condition of the patient has been kept well with the performance status (PS) of zero.

Case 14 reports a 60-year-old male who showed objective response (Figure 1b). After pancreatoduodenectomy, gemcitabine treatment started in October 2008 and liver metastasis was found 3 months later. Followed by TS-1 chemotherapy, we found that metastatic lesions in the liver progressed after the condition of SD during 3 cycles of TS-1 treatment. After 1 cycle of the peptide vaccine, one target lesion of liver metastases located at S8 was shrunken. This lesion kept shrinking until September 2009, and became hardly detectable by CT scan. Similarly, a metastatic lesion in the lymph node was significantly shrunken until September 2009. However, the other target lesion (S4) in the liver showed no response to the vaccine treatment and the tumor progression was promoted after 2 cycles. Finally, the patient died at 220 days after the start of the vaccination.

In case 24, a 74-year-old male also showed objective response (Figure 1c). After distal pancreatectomy in August 2007, adjuvant chemotherapy utilizing gemcitabine was performed for 6 months and then switched to TS-1 because of the side effect. Bone metastasis was found in the xiphoid process by CT scan in April 2009. Radiation therapy was performed to the xiphoid process in May 2009, but the tumor did not respond well. The patient was enrolled into the peptide vaccine trial in July 2009 after one month of cooling off period. Bone metastasis started to shrink after one cycle of the peptide vaccine treatment. The precordial pain was rapidly diminished and well controlled without opioid treatment. After the 5th shot of the peptide, Grade 3 interstitial pneumonia was observed and the treatment was discontinued. The patient was hospitalized in one week of treatment without any steroid therapy and then well recovered. Even without the vaccination, pain was well controlled and tumor markers kept decreasing for the next two months. After the re-progression of the disease, gemcitabine was administered and no clinical effect was observed. Since the patient desired to receive the peptide vaccine again, we obtained an approval of the re-entry of this case from the Ethical committee. The vaccine treatment was restarted with careful monitoring, while neither adverse events nor clinical effect was observed in this second round of drug administration. His overall survival period from the first day of administration was 495 days.

The median overall survival time of 31 patients was 142 days, and the progression free survival period was 56 days (Figures 4a and 4b). In comparison with the control group without the vaccine treatment, who are the patients visited Chiba Tokushukai Hospital in the period between January 2007 and January 2009 (MST: 83 days), overall survival of the patients with the KIF20A-peptide vaccination was statistically significant ($p = 0.0468$, MST: 142 vs. 83 days) (Figure 4c). Moreover, MST of the patients who received BSC was 63 days. Compared to the control group in multi-center, Overall Survival of the vaccinated patients was significantly improved ($p = 0.0020$, MST: 142 vs. 63 days) (Figure 4c). Taken together, we concluded that the cancer vaccination utilizing KIF20A-derived peptide was significantly effective as immunotherapy against advanced pancreatic cancer.

Table 2 Patient characteristics and clinical responses

No.	Age	Sex	Target lesion	Dose of peptide (mg)	Number of injection	Clinical response*	Objective Response	Response lesion	Injection site reaction(Grade)	CTL response	
										Pre-vaccination	Post-vaccination**
1	75	M	Local LNs	1	4	PD			0	N.A.	+
2	57	F	Local	1	11	PD			1	++	++
3	72	M	Liver	1	3	-			0	N.T.	N.T.
4	60	M	Lung, local LNs	1	19	SD	Yes	Lung metastasis	2	+	-
5	72	F	Primary , liver	1	12	PD			1	+	+++
6	65	F	Liver	1	4	PD			0	+	+
7	61	F	Local , liver	3	14	SD			2	+	+++
8	57	F	Primary, liver	3	10	SD			2	++	+++
9	33	F	Paraaortic LNs	3	29	SD	Yes(CR)	Liver metastasis	3	N.A.	+++
10	76	M	Liver	3	12	PD			2	-	++
11	55	F	Primary, lung	3	17	SD			1	+	-
12	58	M	Primary	3	5	PD			0	-	-
13	58	F	Live, lung, LNs	3	10	SD			1	-	++
14	60	M	Liver, LNs	3	17	SD	Yes	Liver metastasis, LNs	2	+++	+++
15	80	F	Liver, LNs, lung	3	5	PD			0	-	+
16	58	M	Primary, liver, lung	3	13	PD			1	-	++
17	49	M	Parimary	3	17	SD			2	+	+++
18	62	M	Primary, liver, LNs	3	7	SD			1	-	+++
19	61	M	Primarym, liver, lung, LNs	3	11	SD			2	-	+
20	58	M	LNs, lung	3	25	SD			2	+	+++
21	47	M	Primary, liver	3	13	SD			1	-	+
22	71	F	Liver, local LNs	3	7	SD	Yes	Liver metastasis	2	N.A.	++
23	50	M	Local, LNs	3	6	SD			0	N.A.	-
24	74	M	Bone	3	21	SD	Yes	Bone metastasis	2	N.A.	+++
25	69	F	Primary	3	2	-			0	N.T.	N.T.
26	80	M	Liver, lung	3	18	SD			1	+	+++
27	44	M	Liver, lung, local LNs	3	24	SD	Yes	Lung and liver metastasis	1	+	-
28	61	F	Peritoneal, local LNs	3	9	SD	Yes	Peritoneal metastasis	0	-	-
29	46	M	Liver	3	10	SD			2	-	+++
30	64	F	Liver	3	9	SD			2	-	+++
31	68	F	Liver	3	9	SD	Yes	Liver metastasis	2	+	+++