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Gross visual inspection by endosonographers during endoscopic ultrasound-guided fine needle aspiration

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ABSTRACT

Background/objectives: A clear criterion for terminating endoscopic ultrasound fine needle aspiration (EUS-FNA) without rapid on-site evaluation (ROSE) has not been established. However, a possible solution includes gross visual inspection (GVI) of the sample obtained with EUS-FNA. We performed a retrospective study to elucidate the efficacy of GVI for the diagnostic yield of EUS-FNA.
Methods: Patients who underwent EUS-FNA of a pancreatic mass using a standard 22-G needle from January 2017 to December 2017 were included in the study. At least two punctures were performed for each patient, and GVI was performed for each pass by endoscopists. The correlation between GVI and pathological findings were investigated per needle pass for the first two passes. Regarding GVI, we evaluated the presence of a visible core (with or without) and the sample quantity (large or small).
Results: We evaluated 126 EUS-FNA specimens and analyzed 252 needle passes. A final diagnosis of malignancy was made for 119 patients (94%). Accuracy rates were 92.5% with a visible core and 70.0% without a visible core ($p < 0.01$), and 85.2% for large sample quantities and 70.2% for small sample quantities ($p < 0.01$). Univariate analysis indicated that the presence of a visible core and large sample quantity were associated with accuracy. Multivariate analysis indicated that only the presence of a visible core was significant.
Conclusions: GVI can predict the correct diagnosis when ROSE is unavailable. Evaluating the presence of a visible core is more sensitive than assessing the quantity of the sample obtained.

Introduction

Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) has become a well-established procedure for determining diagnoses of patients with suspected pancreatic masses because of its high diagnostic yield [1–4]. Routine EUS-FNA for pancreatic masses uses 22-gauge (G) and 25-G needles; however, a single pass with these needles is associated with suboptimal performance [3]. Consequently, multiple passes are necessary to achieve a satisfactory diagnostic yield [3].

Diagnosis by rapid on-site evaluation (ROSE) is highly reliable and reduces the number of passes [5–9]. However, ROSE is not implemented in many institutions, particularly in Europe and Asia, because of the limited pathology staffing, cost, and additional procedure time [3,10]. Without ROSE, there is no clear criterion for determining the number of FNA passes; therefore, unnecessary passes are performed.

A possible solution for this problem is gross visual inspection (GVI) of samples obtained during EUS-FNA. Several researchers regard the presence of a visible core in the specimen as an indicator for terminating EUS-FNA; however, little is known about the influence of GVI of samples obtained by standard 22-G needles on the diagnostic yield [3,11,12]. Therefore, in this study, we aimed to elucidate the clinical benefit of GVI of samples obtained with EUS-FNA.

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Methods

Patients

This retrospective study was conducted at Shizuoka Cancer Center, which is a tertiary referral cancer center for pancreatobiliary diseases. We annually perform more than 600 pancreatobiliary EUS procedures. EUS-FNA was performed using a linear array echoendoscope (GF-UCT260; Olympus Medical Systems Corp, Tokyo, Japan) under conscious sedation. In April 2016, standardized EUS-FNA procedures regarding the number of FNA passes, GVI, sample preparation, and assessment of the quantity of histological samples were introduced at our institution. Consecutive patients who underwent EUS-FNA at our center were retrospectively studied between January 2017 and December 2017. The inclusion criterion was undergoing the first EUS-FNA for a pancreatic solid mass using a standard 22-G needle. The exclusion criterion was insufficient data in the endoscopic and pathological reports in our database. All patients provided informed consent to undergo the procedures. The study was approved by the Institutional Review Board of our center and was conducted in accordance with the principles of the Declaration of Helsinki.

EUS-FNA

After excluding the presence of a regional or collateral vasculature, the pancreatic mass was punctured using a standard 22-G needle (Echo Tip Ultra; Wilson Cook Medical Inc., Winston-Salem, NC, USA). After the needle was advanced in the target lesion, the stylet was removed. Then, either 10 mL or 50 mL of suction was applied using a syringe chosen according to the preference of the endosonographer. When the endosonographer considered that sufficient material had not been obtained during the first pass using 10 mL of suction, 50 mL suction was applied during additional passes. To apply 50 mL of suction, a 50-mL syringe with a three-way stopcock and an extension tube was attached to the proximal end of the FNA needle. After pulling the plunger of the syringe until a scale of 50 mL, the handle of the three-way stopcock was rotated to lock the pressure. Under negative pressure, 10 to 20 to-and-fro movements of the needle inside the lesion were performed. The needle was withdrawn from the lesion after the release of negative pressure.

Gross visual inspection and sample preparation

GVI was carefully performed by two experienced endoscopists (H.I. and H.M.) using two indexes, namely, the presence of a visible core and sample quantity. When there was a discrepancy between opinions, a decision was reached after a discussion between them. The specimen was entirely expressed onto a plate by reinserting the stylet in the needle. The presence of a visible core, which was defined as white pieces of tissue, was carefully investigated (Fig. 1). Subsequently, all specimens were placed in a saline or formalin bottle. After swinging the bottle slowly to unwind the blood clot and leaving it on a table, the sample quantity was examined regardless of the presence of a visible core. A large or small quantity of samples was defined as more or less than one circle of blood clot in the bottle (Fig. 1). Regarding the number of FNA passes, at least two punctures were performed for each patient. If a visible core was confirmed after two consecutive punctures, then the procedure was terminated. Otherwise, additional punctures were performed until the endoscopist decided that a sufficient sample had been obtained for pathological analysis. Samples obtained during the first pass were placed in saline, and a portion of the samples was smeared onto two glass slides for cytological evaluation using hematoxylin and eosin (HE) staining and Papanicolaou staining. If residual material was present, then it was used for histological evaluation with HE staining. Samples obtained during the second pass and the following passes were used for histological evaluation. Formalin-fixed tissue specimens were embedded in paraffin, and sections stained with HE were examined. Immunohistochemical staining was performed if necessary. All samples were processed by the pathology department. No on-site pathological examination was performed.

Pathological assessment

The sample in the bottle was carried to a pathology department. Then, processing of the specimen and creation of the slides were performed by a cytoscreener. A pathological evaluation was performed for each pass. Samples that were considered suspicious or positive for malignancy, neuroendocrine neoplasm (NEN), or lymphoma were categorized as positive for malignancy; however, samples that were considered negative or atypical were categorized as negative for malignancy. After only the first pass, the pathological diagnosis was based on a combination of cytological and histological diagnoses. The quantity of the histological sample was routinely evaluated using HE staining and a scoring system (scale, 0–5) for the sample obtained during the second pass [13]. Briefly, a score of 0 indicated no sample material; higher scores indicated larger samples. Scores of 3–5 indicated sufficient material for limited histological interpretation and a sample $>10\times$ power fields in length. In the current study, we evaluated the number of samples with scores of 3–5, which were defined as adequate specimens for histological interpretation, and the number of samples with a score of 5, which was defined as a histological core specimen (Fig. 2). Pathological assessment was performed by a pathologist with ample experience with FNA interpretation (K.S.).

Final diagnosis

The final diagnosis was based on the following: (1) surgical diagnosis based on a resected specimen; (2) positive for malignancy at the time of the FNA diagnosis with a compatible clinical course; and (3) negative for malignancy at the time of the FNA diagnosis with a lack of deterioration or spontaneous resolution on imaging findings for a minimum clinical follow-up time of 6 months.

Main outcome measures

We evaluated the first two passes of each patient in the study. Endoscopic and pathological reports were retrospectively reviewed to evaluate the following: (i) per-pass analysis of diagnostic accuracy according to GVI; (ii) multivariate logistic regression analysis for accuracy; and (iii) rate of obtaining a sample with a histological core according to GVI for tumor patients. An accurate diagnosis using EUS-FNA was defined as positive malignancy with a final diagnosis of a malignant disease (true positive) and negative malignancy with a final diagnosis of a benign disease (true negative). Accuracy was defined as the sum of the true positive and true negative results divided by the total number of analyzed lesions.

Statistical analysis

Continuous variables are presented as median and range. Categorical variables summarized by absolute frequency and percentage were compared using Fisher's exact test. We calculated odds ratios using a cross table during univariate analysis. In addition, a multivariate logistic regression analysis for accuracy was

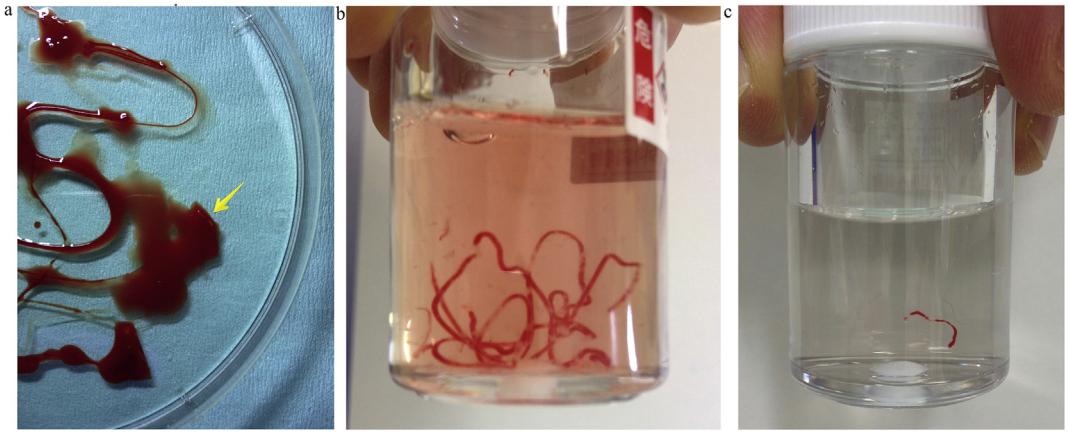

Fig. 1. Gross visual inspection. **A.** Sample expressed on the plate is presented and a visible core can be seen (→).**B.** Sample in a formalin bottle judged as a large quantity of samples, of which the blood clot formed several circles.**C.** Sample in a formalin bottle judged as a small quantity of samples, of which the blood clot did not form a circle.

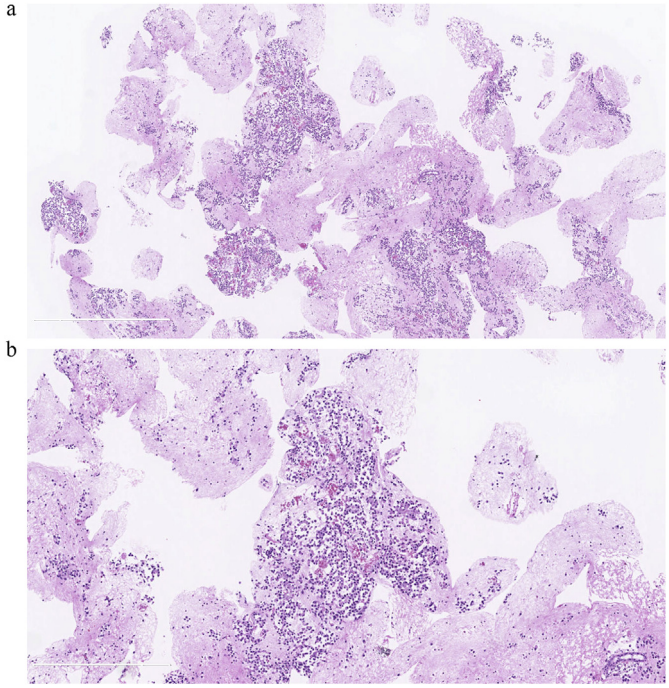

Fig. 2. Histological evaluation of specimens obtained using the standard 22-G needle (hematoxylin and eosin staining). **A.** In this sample with a score of 5 for the quantity of tissue, the tissue architecture can be evaluated. The area of tissue on the prepared slide is more than >10 × power fields in length.**B.** Magnified image of (A), indicating neuroendocrine neoplasm (magnification × 200).

performed using tumor size (<25 vs. ≥25 mm), puncture site (duodenum or stomach), negative pressure during FNA (10 mL or 50 mL), the presence of a visible core (yes or no), and sample quantity (large or small) as potential predictive factors; $p < 0.05$ was considered significant for all tests. Statistical analyses were performed with JMP statistical software version 13.2.0 (SAS Institute, Cary, NC, USA).

Results

During the test period, 152 patients underwent their first EUS-FNA for a pancreatic mass. The standard 22-G needle was used for 142 patients of these patients. A total of 16 patients were excluded because of insufficient data in the endoscopic and pathological reports. Therefore, 126 patients were analyzed. Patient characteristics are shown in Table 1. A median of two FNA passes (range, 2–7) per patient were performed in the pancreatic mass. We analyzed 252 pairs of passes (the first two passes) performed for each patient. Regarding negative pressure, 10 mL and 50 mL of suction were applied during 80 and 172 passes, respectively. The final diagnosis was malignancy for 119 patients (94.4%). The diagnosis was based on surgical pathology and EUS-FNA results and the clinical course of 33 and 93 patients, respectively. Seven patients had benign disease, which was determined based on EUS-FNA results and the clinical course. The accuracy rate per procedure was 97.6%.

Regarding GVI, a visible core was observed in 185 passes (73.4%) and blood without a visible core was observed in only 67 passes (33.5%); 213 (84.5%) and 39 (15.5%) passes were categorized as large and small quantities of samples, respectively. Visible cores in the large quantities and small quantities of samples were seen in 81.2% (173/213) and 30.8% (12/39), respectively. Regarding the first pass, a direct smear could be prepared for all cases, although paraffin-embedded tissue for histological analysis was created for 112

Table 1
Patient characteristics.

Median age, years (range)	69 (36–89)
Sex, male:female, *n*	62:64
Median tumor size, mm (range)	26 (4–77)
Lesion location, head:body or tail, n	75:51
Puncture site, duodenum:stomach, n	72:54
Median number of passes, (range)	2 (2–7)
Final diagnosis, n (%)	
Malignant	119 (94.4)
Adenocarcinoma	105
Neuroendocrine neoplasm	8
Malignant lymphoma	1
Metastatic pancreatic cancer	5
Benign	7 (5.6)
Focal pancreatitis	6
Accessory spleen	1
Diagnostic yield per procedure, %	
Accuracy	97.6
Sensitivity	97.5
Specificity	100.0
PPV	100.0
NPV	70.0

EUS-FNA, endoscopic ultrasound fine needle aspiration; PPV, positive predictive value; NPV, negative predictive value.

Table 2
Per-pass analysis of diagnostic accuracy according to gross visual inspection.

Diagnostic yield	All	Presence of visible core			Quantity of sample		
		Yes	No	*p*	Large	Small	*p*
Accuracy, % (n/N)	88.5 (223/252)	95.1 (176/185)	70.1 (47/67)	<0.01	90.6 (193/213)	76.9 (30/39)	0.03
Sensitivity, % (n/N)	87.8 (209/238)	95.0 (171/180)	65.5 (38/58)	<0.01	90.1 (183/203)	74.3 (26/35)	0.02
Specificity, % (n/N)	100.0 (14/14)	100 (5/5)	100 (9/9)	1.0	100 (10/10)	100 (4/4)	1.0
PPV, % (n/N)	100.0 (209/209)	100 (171/171)	100 (38/38)	1.0	100 (183/183)	100 (26/26)	1.0
NPV, % (n/N)	32.6 (14/43)	35.7 (5/14)	31.0 (9/29)	1.0	33.3 (10/30)	30.8 (4/13)	1.0

PPV, positive predictive value; NPV, negative predictive value.

cases (88.9%). A per-pass analysis of diagnostic accuracy according to GVI is shown in Table 2. Accuracy and sensitivity were significantly higher for samples with a visible core and large quantities of samples than for samples without a visible core and small quantities of samples.

Univariate and multivariate analyses of factors affecting the accuracy of EUS-FNA were also performed (Table 3). The univariate analysis indicated that the presence of a visible core and a large quantity of samples were associated with correct FNA results. The multivariate analysis indicated that only the presence of a visible core remained significant.

The quantity of histological samples was evaluated for tumor patients according to GVI (Table 4). Rate of samples with an adequate specimen for histological interpretation and a histological core specimen were higher for samples with a visible core and a large quantity of samples than for samples without a visible core and a small quantity of samples. However, a significant difference was found only for the method of distinguishing the presence of a visible core.

Discussion

This study demonstrated the association between GVI and the diagnostic yield of EUS-FNA for pancreatic masses using a standard 22-G needle. Our results indicated that the visible core of the samples obtained was likely to lead to a correct diagnosis and could predict the adequacy of histological samples of tumor patients.

A strength of the study was that it investigated the effect of GVI on the diagnostic yield of a commonly used standard 22-G needle, which has not been studied previously. Our results suggested that the presence of a visible core had a positive influence on the diagnostic yield. Nguyen et al. conducted a prospective study to assess the GVI of obtained specimens to predict the adequacy of cytological samples obtained using EUS-FNA for pancreatic masses using a standard 22-G needle [14]. Consequently, neither trained EUS technologists nor cytotechnologists were able to provide a reliable assessment of FNA adequacy using GVI of specimens, and both tended to overestimate the amount of cytologic material present [14]. Our results revealed that the visible core in the obtained sample could predict the adequacy of the histological sample because an adequate specimen for histological interpretation was found in 95.7% of the samples with a visible core. The difference between the present and the previous study may be attributed to the difference in the GVI methods. We directly observed specimens expressed on a plate, but the other study examined an unstained smear. Assessing the adequacy of specimens using GVI of an unstained smear may be difficult because visible core tissue was crushed into smaller pieces on the glass slides.

The European Society of Gastrointestinal Endoscopy recommends three to four passes to obtain a high number of diagnostic samples and high sensitivity for malignancy when ROSE is unavailable [3]. Our policy of determining the number of FNA passes

Table 3
Univariate and multivariate analyses of factors affecting the accuracy of EUS-FNA.

		Univariate analysis		Multivariate analysis	
		OR (95% CI)	*p*	OR (95% CI)	*p*
Tumor size	≥25 mm	1.84 (0.84–4.03)	0.16	1.39 (0.46–3.32)	0.46
	<25 mm	1		1	
Puncture site	Stomach	1.54 (0.69–3.47)	0.33	1.62 (0.67–3.90)	0.28
	Duodenum	1		1	
Negative pressure	50 mL	2.22 (1.01–4.86)	0.06	1.71 (0.71–4.09)	0.23
	10 mL	1		1	
Presence of visible core	Yes	8.32 (3.56–19.5)	<0.0001	7.48 (2.96–18.9)	<0.0001
	No	1		1	
Quantity of sample	Large	2.90 (1.21–6.95)	0.03	0.97 (0.34–2.72)	0.95
	Small	1		1	

EUS-FNA, endoscopic ultrasound fine needle aspiration.

Table 4
Quantity of histological samples according to gross visual inspection for tumor patients.

	Adequate specimen for histological interpretation	*p*	Histological core specimen	*p*
Overall, % (n/N)	92.4 (110/119)		73.9 (88/119)	
Presence of visible core				
Yes, % (n/N)	95.7 (89/93)	0.02	80.6 (75/93)	0.004
No, % (n/N)	80.8 (21/26)		50.0 (13/26)	
Quantity of sample				
Large, % (n/N)	94.2 (98/104)	0.09	76.0 (79/104)	0.21
Small, % (n/N)	80.0 (12/15)		60.0 (9/15)	

involved terminating EUS-FNA when a visible core was confirmed for two consecutive punctures. Two punctures were performed because we considered that the visible core was not 100% adequate for pathological examination. However, the policy resulted in a 97.6% accuracy rate per procedure with a median number of two passes, indicating the possibility of reducing the number of passes. Furthermore, judgment of the sample quantity also became an indicator of accuracy to some extent, but it did not remain a significant factor affecting accuracy in the multivariate analysis. Therefore, when a visible core was not recognized, repeat puncture until confirmation of a visible core may not be necessary if a large quantity of blood samples is obtained. We recommend our method for all cases because it is very simple. However, considering the possibility of reducing the number of passes, surgery candidates with pancreatic body and tail tumors and those with small tumors will benefit because the former patients are at potential risk of tumor seeding after EUS-FNA via the stomach and the latter patients tend to undergo more passes [15]. In fact, in our study, the median number of passes was three for tumor sizes smaller than 26 mm and two for tumor sizes 26 mm or larger ($p < 0.001$).

Similar results were reported by a recent prospective study evaluating the efficacy of GVI of the specimen obtained by standard 19-G needles during EUS-FNA for various lesions including those of the pancreas [16]. In that study, GVI was used for the visible core and revealed that the presence of a visible core 4 mm or longer was associated with significantly higher diagnostic performance during histologic and cytologic evaluations [16]. GVI of the sample obtained with the 19-G needle might be easier because the visible core might be larger than that obtained with the 22-G needle; however, the results provided supportive evidence.

Because superior biopsy needles, such as a Franseen needle and a fork-tip needle, have become available recently, the frequency of using standard needles is decreasing [17]. However, biopsy needles have some drawbacks. The handling of these needles is more difficult than the handling of standard needles due to the shape of the needle tip and the rigidity of the needle. Therefore, in some situations when acute angulation of the endoscope is needed to puncture, a standard needle is considered preferable. This tendency is probably seen when less experienced endoscopists perform the procedure. Moreover, one pass of a biopsy needle does not guarantee an adequate sample for pathological interpretation. Only a small number of samples can be obtained occasionally. Our results may be applied to this situation because GVI can predict the correct diagnosis of the sample obtained by a biopsy needle, although this should be verified in a future study.

This study had several limitations. First, selection bias was inevitable because of the retrospective nature of the study. However, the use of a standardized method for EUS-FNA including GVI and pathological assessment was another strength of the current study. Therefore, we investigated the diagnostic yield and quantity of samples according to GVI under similar conditions. Nevertheless, a prospective study is mandatory to obtain a firm conclusion. Second, 50 mL of suction was used for approximately half of all passes, although 10 mL of suction is commonly applied during EUS-FNA. Because samples obtained using a 25-G needle with 50 mL of suction were more likely to be adequate for histological diagnoses compared with 10 mL of suction in a randomized control trial, we used 50 mL of suction in the clinical setting [18]. Consequently, negative pressure was not a factor affecting accuracy in the multivariate analysis. However, the use of 50 mL of suction might affect GVI. Third, we only targeted pancreatic masses; therefore, further study is needed to evaluate the efficacy of GVI of other lesions such as lymph nodes and submucosal tumors. Finally, the quantity of histological samples was assessed only for tumor patients because evaluating the quantity of samples obtained from benign disease was difficult.

Conclusion

GVI is practical for predicting correct diagnoses when ROSE is unavailable. When performing GVI, evaluating the presence of a visible core rather than assessing the quantity of samples obtained is the superior method. Confirmation of a visible core of the specimen can be an indicator to terminate EUS-FNA using a standard 22-G needle. In addition, a specimen with a visible core can predict the adequacy of the histological sample.

Acknowledgments

The authors declare no conflicts of interest related to this article.

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