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ORIGINAL ARTICLE

Comparison of the novel Franseen needle versus the fine-needle aspiration needle in endoscopic ultrasound-guided tissue acquisition for cancer gene panel testing: A propensity score-matching analysis

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endoscopic ultrasound-guided fine-needle aspiration, endoscopy, fine-needle biopsy, neoplasms, oncogenes.

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Abstract

Background and Aim: Reports have indicated that a surface area of 4 mm^2 or more of collected tissue sections could provide the recommended total DNA for the OncoGuide NCC Oncopanel system, which is a cancer gene panel test developed in Japan. We wished to compare the percentage of tissue sections collected by endoscopic ultrasound-assisted tissue acquisition (EUS-TA) with surface areas of ≥4 mm^2 between a conventional needle, namely the EZ Shot 3 Plus (Olympus Medical Japan, Tokyo, Japan) (EZ3), and the recent SonoTip TopGain (MediGlobe, Rohrdorf, Germany) (TopGain).
Method: From April 2010 to December 2021, among 693 EUS-TA cases, EZ3 was used in 390 cases and TopGain in 45. The EZ3 and TopGain groups were matched in a 1:1 ratio with a tolerance of 0.2, with 35 patients each matched using propensity score analysis.
Results: The TopGain group had a significantly higher percentage of cases with a tissue area of ≥4 mm^2 than the EZ3 group (42.9% *vs* 68.6%, $P = 0.030$). Multivariate analysis revealed an association between TopGain and tissue areas of ≥4 mm^2 (odds ratio 2.996, 95% confidence interval 1.068–8.403, $P = 0.037$).
Conclusions: EUS-TA using TopGain significantly collected more ≥4 mm^2 tissue area compared with EZ3, suggesting its usefulness for cancer gene panel testing.

Introduction

Recently, cancer gene panel (CGP) testing and genomic medicine have become increasingly available. There are two commercially available CGPs: FoundationOne CDx (F1CDx) and the OncoGuide NCC Oncopanel system (NOP) developed in Japan. Both these tests are performed on formalin-fixed paraffin-embedded (FFPE) tissue specimens. One of the requirements for F1CDx is that the surface area of the tissue sections collected must be at least 25 mm^2. However, the success rate of specimens collected by ultrasound endoscopy-guided tissue acquisition (EUS-TA) and submitted to F1CDx for analysis is poor, ranging between 0% and 70.4%.[1–5] The NOP recommends a surface area of approximately 16 mm^2 for tissue sections collected for reliable testing. However, an area of ≥4 mm^2 has been usually reported to be sufficient to obtain the recommended amount of total DNA,[6–8] which is less than that of F1CDx. According to previous reports, 0%, 23.8%, and 0% of the specimens collected using EUS-TA met the criteria for F1CDx, respectively, while 63.6%, 56.0%, and 39.2% met the criteria for NOP.[1,4,5] Therefore, in this novel study, we focused on whether the surface area of the tissue sections obtained by EUS-TA was >4 mm^2.

The amount of tissue collected varies depending on the type of needle used. The needles used during EUS-TA can be divided into two main types: conventional fine-needle aspiration (FNA) needles, and the recently developed fine-needle biopsy (FNB) needles. Since 2020, SonoTip TopGain (MediGlobe, Rohrdorf, Germany) (TopGain), a novel Franseen-type FNB needle designed for tissue collection with high puncture performance and minimal tissue damage has been available. However, its usefulness in cancer gene panel testing has not been fully elucidated. Therefore, we compared the surface areas of tissue sections obtained by EUS-TA using the FNA needle EZ Shot 3 Plus (Olympus Medical Systems, Tokyo, Japan) (EZ3) and TopGain.

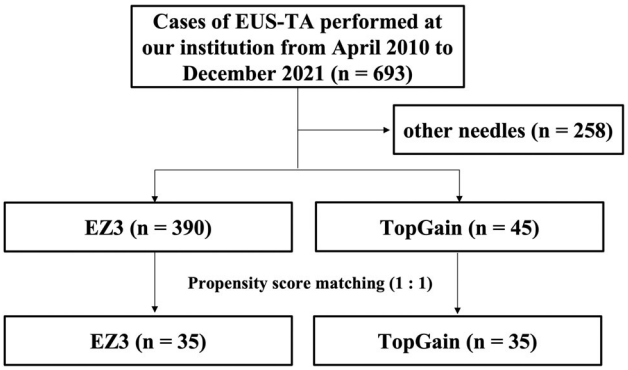

Figure 1 We used EZ3 in 390 cases, TopGain in 45 cases, and other needles in 273 cases out of 693 EUS-TA cases performed at our institution from April 2010 to December 2021. EZ3 and TopGain were matched 1:1 with a tolerance of 0.2. Propensity score-matching was performed using explanatory variables such as age, sex, clinical diagnosis of tumor, lesion site, puncture route, and needle diameter, with 35 cases matched. EUS-TA, endoscopic ultrasound-guided tissue acquisition; EZ3, EZ Shot 3 Plus (Olympus Medical Systems, Tokyo, Japan); TopGain, SonoTip TopGain (MediGlobe, Rohrdorf, Germany).

Table 1 Characteristics of patients who underwent endoscopic ultrasound-guided tissue acquisition with EZ3 and TopGain

		Before propensity score-matching			After propensity score-matching		
	EZ3 + TopGain ($n = 435$)	EZ3 ($n = 390$)	TopGain ($n = 45$)	*P*-value	EZ3 ($n = 35$)	TopGain ($n = 35$)	*P*-value
Age, median (range), years	69 (8–90)	69 (8–90)	71 (36–86)	0.450	72 (33–87)	71 (36–83)	0.400
Sex, male, *n* (%)	218 (50.1)	196 (50.3)	22 (48.9)	0.862	22 (62.8)	19 (54.2)	0.467
Lesion site, *n* (%)				**0.008**			0.485
Pancreas	288 (79.1)	263 (81.2)	25 (62.5)		24 (68.6)	21 (60.0)	
Stomach	42 (11.5)	33 (10.2)	9 (22.5)		6 (17.1)	8 (22.9)	
Lymph node	34 (9.3)	28 (8.6)	6 (15.0)		5 (14.3)	6 (17.1)	
Puncture route, *n* (%)				**0.004**			1.000
Stomach	264 (60.7)	228 (58.5)	36 (80.0)		31 (88.6)	31 (88.6)	
Duodenum	150 (34.5)	141 (36.2)	9 (20.0)		4 (11.4)	4 (11.4)	
Puncture needle diameter, *n* (%)				0.222			0.495
19 G	53 (12.3)	49 (12.7)	4 (8.9)		1 (2.9)	4 (11.4)	
22 G	331 (77.0)	297 (77.1)	34 (75.6)		28 (80.0)	25 (71.4)	
25 G	46 (10.7)	39 (10.1)	7 (15.6)		6 (17.1)	6 (17.1)	
Number of punctures, median (range)	3 (1–8)	3 (1–8)	3 (2–6)	0.373	3 (3–6)	3 (2–6)	0.530
Clinical diagnosis, *n* (%)				**0.047**			0.099
Pancreatic cancer	206 (59.0)	192 (61.9)	14 (35.9)		23 (65.7)	14 (40.0)	
Gastrointestinal stromal tumor	23 (6.6)	20 (6.5)	3 (7.7)		4 (11.4)	3 (8.6)	
Lymphoma	15 (4.3)	10 (3.2)	5 (12.8)		0 (0.0)	4 (11.4)	
Neuroendocrine neoplasm	21 (6.0)	19 (6.1)	2 (5.1)		0 (0.0)	1 (2.9)	
Autoimmune pancreatitis	10 (2.9)	4 (1.3)	6 (15.4)		1 (2.9)	5 (14.3)	
Others	160 (36.7)	145 (37.1)	15 (33.3)		7 (20.0)	8 (22.8)	

Values in bold show statistical significance at $P < 0.05$. EZ3, EZ Shot 3 Plus (Olympus Medical Japan, Tokyo); TopGain, SonoTip TopGain (MediGlobe, Rohrdorf, Germany).

Materials and methods

Study design and participants. Between April 2010 and December 2021, 693 EUS-TA procedures were performed. We used EZ3 in 390 cases, TopGain in 45, and other needles in 258 of the 693 EUS-TA cases. EZ3 and TopGain were matched at 1:1 with a tolerance of 0.2. Furthermore, propensity score-matching was performed with explanatory variables such as age, sex, clinical diagnosis of tumor, lesion site, puncture route, and needle diameter, with 35 cases matched (Fig. 1). Before matching, there was a significant difference between the two groups in terms of lesion site and puncture route. However, after matching, there was no significant difference in any of the parameters (Table 1). The tissue areas of the matched cases were measured and compared.

Procedure. A curvilinear echoendoscope (GF-UCT260; Olympus Medical Systems) and an ultrasound processor (EU-ME1 or EU-ME2, Olympus Medical Systems) were used. Additionally, we used 22-G needles as the basic type and 19- and 25-G needles as well at the discretion of the practitioner depending on the difficulty in the technique and puncture route.

EUS-TA was performed by puncturing the lesion, removing the stylet, and making 10–20 strokes within the lesion without negative pressure or with 10–20 mL of negative pressure applied with a syringe. After removing the puncture needle, a stylet was inserted to extrude the specimen, and a formalin-fixed tissue specimen was prepared. We continued the puncture until a sufficient amount of specimen could be visually be collected. After specimen preparation, hematoxylin–eosin (H&E) staining and immunohistochemical staining were performed for pathology evaluation.

All the procedures were performed by experienced endoscopists or under their direct supervision. The specimens were processed and analyzed by a cytologist and a pathologist.

Area measurement of collected tissue. The areas of the H&E-stained tissue specimens collected by EUS-TA were measured and compared between the EZ3 and TopGain groups. The area of the collected tissue was computed by summing all EUS-TA punctures. Images of the collected tissues were acquired using an all-in-one fluorescence microscope (BZ-X700; KEYENCE, Osaka, Japan), and area measurements were performed using the BZ-X Analyzer software ver 1.3.1.1 (BZ-H4A; KEYENCE). The tissue area was calculated as the area of the H&E-stained specimen, excluding blood portions.

Measurement of tumor cellularity in patients with pancreatic cancer. In collaboration with pathologists, we calculated the percentage of tumor cellularity from our EUS-TA samples from patients with pancreatic cancer using EZ3 and TopGain. We also determined the percentage of cases of tumor cellularity of at least 20% as required by NOP.

Statistical analyses. We performed a Mann–Whitney *U* test to determine whether the area of the sampled tissue was significantly different between the EZ3 and TopGain groups. Additionally, we performed a χ^2 test to observe any significant differences in the percentage of cases with a tissue area of ≥4 mm^2 between the EZ3 and TopGain groups. We calculated odds ratio (OR), 95% confidence interval (95% CI), and *P*-values for each clinical item for each event using logistic regression models with age, sex, lesion site, puncture route, EZ3 or TopGain needle, number of punctures, and clinical disease as explanatory variables. A tissue area of ≥4 mm^2 was the objective variable. The *P*-values were tested against the null hypothesis of an OR of 1.0 at a two-sided 5% significance level. We performed a multivariate analysis of the items with $P < 0.15$ from the univariate analysis. The level of significance was set at $P < 0.05$.

We used the statistical software StatFlex Ver 7.0.11 (Artech Co., Ltd., Osaka, Japan) for statistical analysis.

Ethics statement. Informed consent was obtained from all patients prior to the procedure. All researchers involved in this study complied with the "Declaration of Helsinki (revised October 2013)" and the "Ethical Guidelines for Life Sciences and Medical Research Involving Human Subjects" (Ministry of

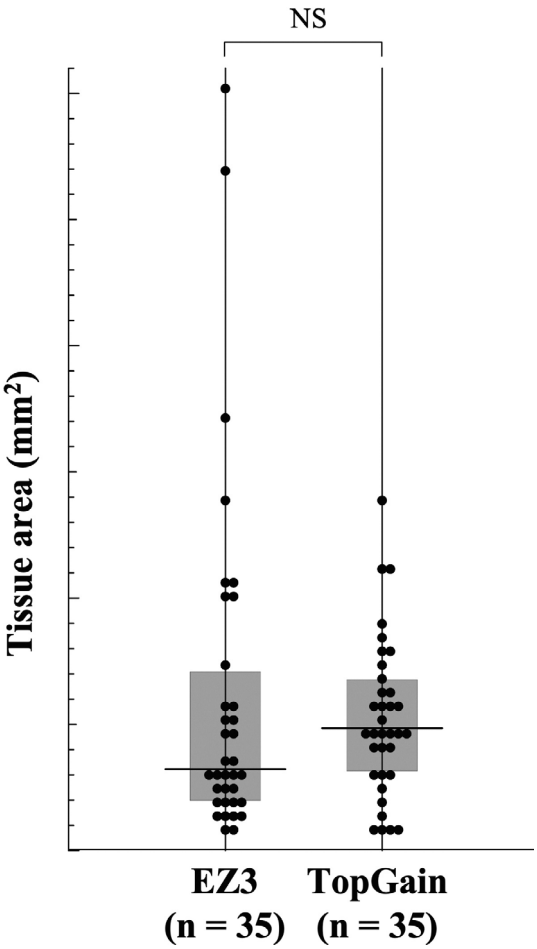

Figure 2 Tissue area collected by endoscopic ultrasound-guided tissue acquisition with EZ3 and TopGain. EZ3, EZ Shot 3 Plus (Olympus Medical Systems, Tokyo, Japan); TopGain, SonoTip TopGain (MediGlobe); NS, not significant ($P > 0.05$).

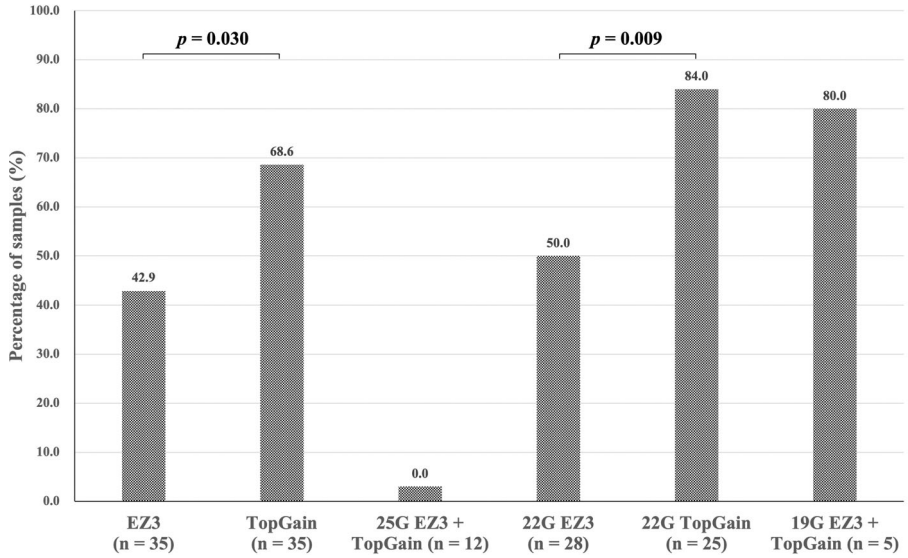

Figure 3 The proportion of cases in which the tissue area sampled by endoscopic ultrasound-guided tissue acquisition was ≥4 mm^2. EZ3, EZ Shot 3 Plus (Olympus Medical Systems, Tokyo, Japan); TopGain, SonoTip TopGain (MediGlobe).

Education, Culture, Sports, Science and Technology; Ministry of Health, Labor and Welfare; Ministry of Economy, Trade and Industry; Notification No. 1, 2021). This study was conducted with the approval of the Nagasaki University Hospital Clinical Research Ethics Committee and with permission from the head of the research institution.

Results

Tissue area collected by EUS-TA. The median tissue areas sampled were 3.22 mm^2 in the EZ3 group and 4.84 mm^2 in the TopGain group. There were no significant differences between the two groups ($P = 0.427$) (Fig. 2).

Proportion of cases in which the tissue area sampled by EUS-TA was ≥4 mm^2. The proportion of patients with a tissue area >4 mm^2 in both groups is shown in Figure 3. The TopGain group had a significantly higher percentage of cases of a tissue area of ≥4 mm^2 than the EZ3 group (42.9% *vs* 68.6%, $P = 0.030$). When EUS-TA was performed using a 25-G puncture needle, the areas of all sampled tissue in both groups were <4 mm^2. When EUS-TA was performed using a 22-G needle, the proportion of cases with tissue area >4 mm^2 in the TopGain group was significantly higher that with the EZ3 group (50.0% *vs* 84.0%, $P = 0.009$). We performed EUS-TA (five cases) using a 19-G puncture needle in both groups, and cases with a tissue area of ≥4 mm^2 sampled was 80%.

Evaluation of factors related to the collection of tissue area ≥4 mm^2 that were sampled by EUS-TA. We evaluated the factors associated with a tissue area of ≥4 mm^2 in terms of age, sex, lesion site, puncture route, needle type, number of punctures, and clinical diagnosis (Table 2). Using univariate analysis, EUS-TA collected significantly more tissue of >4 mm^2 area with the TopGain puncture needle than with the EZ3 (OR 2.909; 95% confidence interval [CI]: 1.093–7.739, $P = 0.032$). Using multivariate analysis, the type of

Table 2 Evaluation of factors related to the collection of tissue area ≥4 mm^2 that were sampled by EUS-TA

		Univariate analysis			Multivariate analysis		
Variable		Odds ratio	95% CI	*p*-value	Odds ratio	95% CI	*P*-value
Age	≥ 71	0.523	0.199–1.375	0.188			
Sex	Male	1.038	0.399–2.704	0.938			
Llesion site	Pancreas	0.450	0.162–1.253	0.126	0.416	0.140–1.237	0.114
Puncture route	Stomach	0.152	0.018–1.313	0.086	0.118	0.013–1.094	0.060
Puncture needle	TopGain	2.909	1.093–7.739	**0.032**	2.996	1.068–8.403	**0.037**
Number of punctures	>3	0.631	0.210–1.891	0.410			
Clinical diagnosis	Pancreatic cancer	0.843	0.325–2.182	0.724			

We performed a multivariate analysis of the items with P <0.15 in the univariate analysis and the level of significance at P <0.05. Values in bold indicate statistical significance at P <0.05. CI, confidence interval; SonoTip TopGain (MediGlobe, Rohrdorf, Germany).

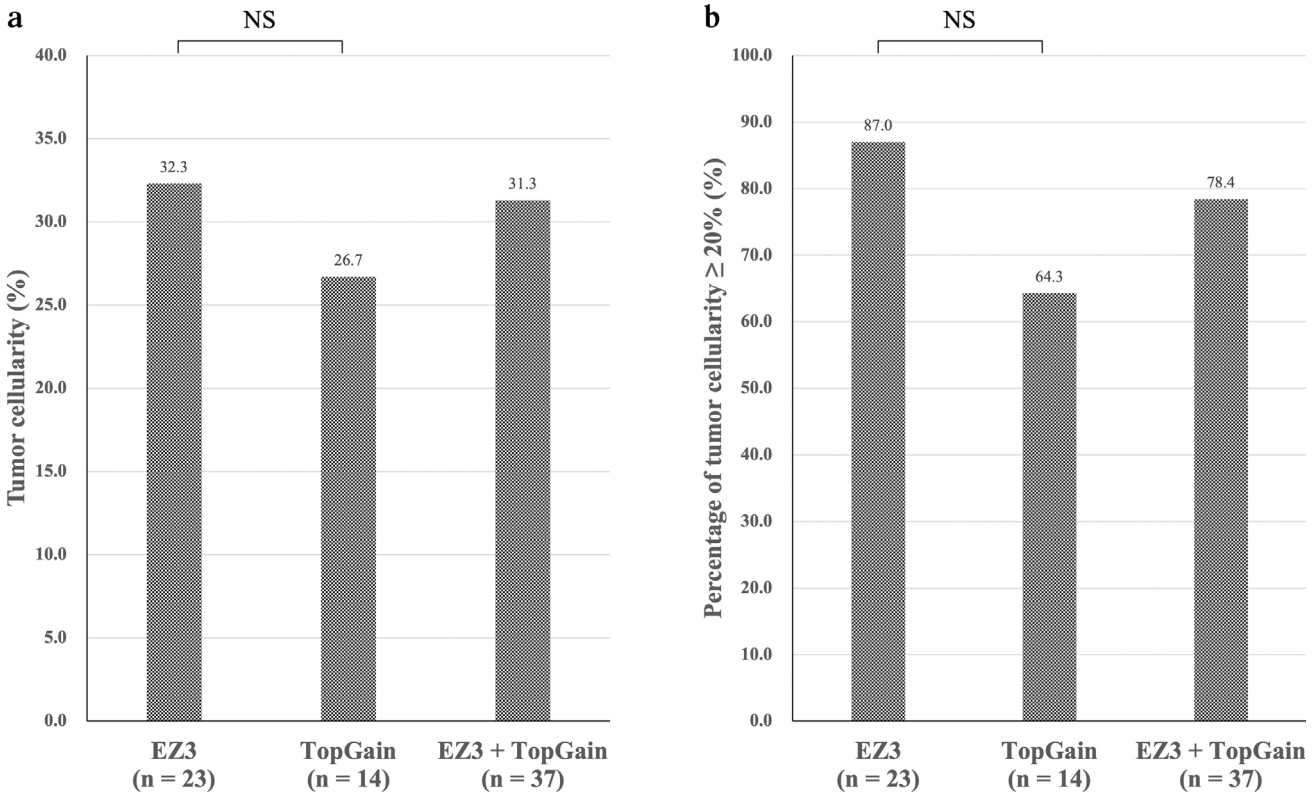

Figure 4 (a) Tumor cellularity and (b) percentage of tumor cellularity ≥20% of specimens obtained by EUS-TA in pancreatic cancer patients using EZ3 and TopGain. EZ3, EZ Shot 3 Plus (Olympus Medical Systems, Tokyo, Japan); TopGain, SonoTip TopGain (MediGlobe); NS, not significant ($P > 0.05$).

puncture needle was identified as the only independently associated factor (OR 2.996, 95% CI: 1.068–8.403, $P = 0.037$).

Tumor cellularity of specimens obtained by EUS-TA in patients with pancreatic cancer. Our EUS-TA samples from patients with pancreatic cancer showed that tumor cellularity was 32.3% (0–70.1%) in the EZ3 group and 26.7% (0–73.8%) in the TopGain group ($P = 0.188$). The percentage of cases of tumor cellularity ≥20% was 87.0% in the EZ3 group and 64.3% in the TopGain group ($P = 0.215$) (Fig. 4).

Discussion

Next-generation sequencing (NGS) of EUS-FNA/B samples for gene analysis has been reported since 2013.[2,9–15] According to a review by Imaoka et al., most reports are from custom panels from which nucleic acids are extracted at individual facilities.[16] Since the percentage of tumor cells and the amount of DNA required differed among the panels, it was impossible to evaluate the proportion of the analysis criteria met and analyze them based on the same scale. There were two commercially available CGPs: F1CDx and NOP. However, the NOP criteria could be easily satisfied. When considering CGPs for EUS-TA specimens collected in clinical practice, we focused on whether the surface area of the EUS-TA tissue sections was >4 mm^2, which may fulfill the criteria for NOP.

The results of this study revealed that the tissue area that could be sampled with EUS-TA using TopGain was not significantly different from that sampled using EZ3. The reason was attributed to an insufficient number of patients. However, EUS-TA using TopGain sampled a significantly higher percentage of tissue areas of >4 mm^2 compared with EZ3. Only a few cases used a 19-G puncture needle. However, it was not possible to compare the percentage of cases of a tissue area of ≥4 mm^2 collected by EUS-TA using only a 19-G puncture needle between the EZ3 and TopGain groups. When EUS-TA was performed using a 25-G puncture needle, all tissue areas sampled in both groups were <4 mm^2. Therefore, we compared the percentage of cases of tissue area of 4 mm^2 collected by EUS-TA using a 22-G puncture needle between the EZ3 and TopGain groups. The results were still significantly higher in the TopGain group than in the EZ3 group. In previous reports, the surface area of tissue sections that could be sampled with conventional FNA needles was often <4 mm^2.[17–20] The recently developed FNB needles could improve the quality and quantity of samples, for example, by using returned side holes or specifically processed tips.[21] Acquire (Boston Scientific Co., Natick, MA, USA), a Franseen-type FNB needle, was found to collect more tissue section surface areas than the FNA needle.[17,18,22] Although Ikeda et al. reported a higher rate of NOP criteria fulfillment with a 22-G FNB needle than with a 22-G FNA needle for EUS-TA (11.4% *vs* 32.6%, $P = 0.033$), the results were still

unsatisfactory. Furthermore, the FNB needles used were of different types rather than a single type.[5] In our hospital, we initiated FNB needle usage in 2017 for improving diagnostic performance and obtaining more tissue samples. Additionally, we have adopted TopGain, a new Franseen-type FNB needle that became available in 2020. Hisada et al. reported a 63.6% fulfillment rate of NOP criteria in EUS-TA using 19-G TopGain.[4] However, to our best knowledge, there is no report comparing TopGain and FNA needles for fulfilling the NOP criteria. Hence, we conducted this study because of its significance.

Using both univariate and multivariate analyses, TopGain was significantly superior to EZ3 in terms of puncture needle type as a factor associated with a sampling tissue area of ≥4 mm^2 in EUS-TA. Puncture needle diameter was excluded because it was not possible to analyze it, as it was a strong influencing factor. When EUS-TA was performed with a 25-G puncture needle, all tissue areas sampled were <4 mm^2. Therefore, a larger needle of at least 22 G was needed to sample >4 mm^2. In previous reports, univariate and multivariate analyses showed that FNB needle selection over the FNA needle, as well as the puncture needle diameter, was significantly related to adequacy for CGP. This is consistent with our report. However, multiple types of FNA and FNB needles were used.

There was no significant difference in the percentage of tumor cellularity or tumor cellularity >20% between the EZ3 and TopGain groups, suggesting that tumor cellularity might not change with the needle type. Previous studies have reported that a typical primary pancreatic cancer often could show only 5–20% of neoplastic cellularity.[23] However, the percentage of cases of tumor cellularity ≥20% in this study was 87.0% and 64.3% in the EZ3 and TopGain groups, respectively. Other studies that performed NOP on EUS-TA specimens obtained from patients with pancreatic cancer also reported a median tumor cellularity of 60% (7.5–85%).[4] Furthermore, the percentage of cases of a tumor cellularity of ≥20% was 77.1% and 68.6%, respectively,[1,5] similar to our report. This may be because specimens obtained by EUS-TA had higher tumor cellularity than surgical specimens.

The number of patients intended for NOP (intention-to-treat) and those actually tested (per-protocol) were both 2. The first case was a 51-year-old woman with pancreatic head cancer. We performed EUS-TA using a 25-G EZ3 and submitted the specimen to F1CDx. However, the tissue area was 0.87mm^2 and it was not suitable for examination. The second case was a 36-year-old woman with carcinoma of the head and body of the pancreas. We performed an EUS-TA using a 22-G TopGain and the tissue area was 8.05 mm^2 (≥4 mm^2). Moreover, the specimen was submitted to NOP and was available for examination. Although it is impossible to compare EZ3 and TopGain in these two cases because of the difference in needle size, we were able to experience a case in which NOP could be tested using tissue obtained with a 22-G TopGain.

This study was limited by its single-center, non-randomized, and retrospective design. Although we performed propensity score-matching, selection bias could not be eliminated. We matched the number of punctures by propensity score-matching. However, the number of punctures was not constant. The lesions for which EUS-TA was performed were not limited to single lesions. In addition, no direct comparison has been made between TopGain and existing FNB needles. Furthermore, we did not perform cancer gene panel testing on all patients. Therefore, it is necessary to conduct a multicenter, randomized, controlled trial, directly comparing between TopGain and existing FNB needles and determine whether cancer gene panel testing is feasible.

Regardless of these limitations, this is the first study to compare the TopGain and FNA needles in EUS-TA sampling of tissue areas >4 mm^2, which is one of the NOP criteria. If CGP is performed using an FNB needle on tissue collected by EUS-TA, it may enable easier cancer genomic medicine choices and improve patient prognosis.

Conclusion

In conclusion, a 22-G or larger needle was required to obtain a tissue area of 4 mm^2 or larger in EUS-TA. The possibility of obtaining a tissue area of 4 mm^2 or larger was significantly higher when TopGain was used during EUS-TA than when EZ3 was used, suggesting that TopGain may be a useful device for performing cancer gene panel testing.

Ethics approval

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Nagasaki University Hospital Clinical Research Ethics Committee (23 041 704, 18 April 2023). We obtained consent through opt-out procedure from all individual participants included in the study.

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