

Endosonographers performing on-site evaluation of solid pancreatic specimens for EUS-guided biopsy: A formal training method and learning curves

Shi-Yu Li^{1*}, Li Gao^{2*}, Ping-Ping Zhang^{1*}, Xiao-Ju Su¹, Xiang-Yu Kong¹, Kai-Xuan Wang¹, Zhen-Dong Jin¹

¹Department of Gastroenterology, Changhai Hospital, Second Military Medical University/Naval Medical University, Shanghai, China; ²Department of Pathology, Changhai Hospital, Second Military Medical University/Naval Medical University, Shanghai, China

ABSTRACT

Objectives: This study aimed to examine the effects of a training program combining formal cytological curriculum and practical assessments on endosonographers and to determine how many operations were needed for training through learning curves. **Methods:** A formal cytological curriculum was implemented in November 2019 for four endosonographers. The competency of endosonographers before and after the curriculum was judged by quantitative scores. From December 2019 to October 2020, trainees independently examined the adequacy and atypia grade of pancreatic specimens acquired by EUS-guided-fine-needle biopsy based on specific atypical grading criteria. The accuracy of the adequacy and atypia assessment of each trainee was calculated, and learning curves were drawn to evaluate the improvement. **Results:** The median posttraining score improved by 87% from 49 to 91.5 out of 100. Ninety patients were enrolled in the practical assessments. The accuracy for specimen adequacy and atypia assessment of each trainee was 91.7%, 92.8%, 91.0%, and 89.3% and 80.0%, 82.1%, 81.0%, and 78.9%. The learning curves of each trainee showed a steady and significant improvement, and ninety cases were sufficient for satisfactory adequacy assessment. **Conclusions:** Basic knowledge of on-site cytopathologic evaluation can be gained through standardized and systematic training. Practical assessments showed that, at the completion of ninety cases, trained endosonographers reached a satisfactory level of recognizing specimen adequacy, but continue practice was needed for atypia assessment.

Keywords: EUS-guided fine needle biopsy; ROSE; endoscopy training; solid pancreatic lesions

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Li SY, Gao L, Zhang PP, Su XJ, Kong XY, Wang KX, *et al.* Endosonographers performing on-site evaluation of solid pancreatic specimens for EUS-guided biopsy: A formal training method and learning curves. *Endosc Ultrasound* 2021;10:463-71.

Access this article online

Quick Response Code:



Website:

www.eusjournal.com

DOI:

10.4103/EUS-D-21-00088

*These authors contributed equally to the manuscript.

Address for correspondence

Dr. Kai-Xuan Wang, Department of Gastroenterology, Changhai Hospital, Second Military Medical University/Naval Medical University, No. 168, Changhai Road, Yangpu District, Shanghai 200433, China. E-mail: wangkaixuan224007@163.com

Dr. Zhen-Dong Jin, Department of Gastroenterology, Changhai Hospital, Second Military Medical University/Naval Medical University, No. 168, Changhai Road, Yangpu District, Shanghai 200433, China. E-mail: zhendjin@126.com

Received: 2021-04-11; **Accepted:** 2021-09-09; **Published online:** 2021-12-27

INTRODUCTION

EUS-FNA is considered the standard method for pathological diagnosis of solid pancreatic masses.^[1-3] This is especially so for malignant or premalignant lesions including pancreatic ductal adenocarcinoma (PDAC), neuroendocrine tumor, and solid pseudopapillary neoplasm, *etc.*^[4,5] However, the procedure is technically challenging; painstaking efforts are required by endosonographers to achieve a satisfactory rate of specimen acquisition.^[6-8] Although the advent of EUS-fine-needle biopsy (FNB) might help to improve the diagnostic performance, it is still profoundly affected by the operator's experience.^[9]

Rapid on-site evaluation (ROSE) performed by an on-site cytopathologist or cytotechnician allows the immediate assessment of the acquired specimens and determines if further passes are necessary. Applying ROSE leads to improved diagnostic yield and reduces the number of unsatisfactory samples, especially for inexpert endosonographers.^[10-14] However, in a 2016 global survey, ROSE was utilized by 98% of respondents from the United States but only 48% and 55% from those in Europe and Asia, respectively.^[15] The major limitation is the shortage of on-site cytopathologists or cytotechnicians. In China, EUS training programs are being fully promoted, with a large number of endoscopists learning EUS-FNA and FNB techniques every year and more endoscopic centers equipped with the facilities. However, the number of qualified cytopathological staffs cannot satisfy the need.

Some centers have employed ROSE by endosonographers to compensate for the lack of an on-site cytotechnologist or cytopathologist.^[12] Information regarding the feasibility of this method has been inconsistent.^[16-18] We believe that conflicting results are due to nonstandardized training procedures and vague judging criteria. There have also been reports of training endosonographers in cytology.^[19-20] Such programs were usually temporary and intensive and did not include actual practice to check the results of training.

This study assessed whether endosonographers could be made competent through cytological training to perform ROSE of solid pancreatic specimens acquired by EUS-FNB and determined how many operations were needed for the endosonographers to achieve the ability to conduct cytological evaluation.

METHODS

This was a prospective self-controlled trial focusing on endosonographers' ability to gain skills through a formal cytological training program. The Ethics Committee of Changhai Hospital approved the study on 13 December 2019 (CFDA Approval Number: CHEC2019-186). The protocol was subsequently registered on Clinical Trial.gov (NCT04509687).

The training program comprised two stages [Figure 1]. A curriculum of formal cytological training sessions was organized by an expert cytopathologist (G.L.) in the first stage (November 2019). The second stage (from December 2019 to October 2020) consisted of practical assessments by the trained endosonographers during EUS-FNB procedures for solid pancreatic lesions.

Training center and study participants

This program for training on-site cytological evaluations of solid pancreatic specimens was organized by the Endoscopy Center of Changhai Hospital. The center has the highest EUS procedure volume in China, performing more than 3800 EUS and 800 EUS-FNA or FNB procedures annually, of which about 70% are for pancreatic lesions. The program was directed by an expert endosonographer (W.K.X.) and an academic cytopathologist (G.L.), both of whom had long experience in EUS-FNA and FNB for solid pancreatic lesions.

The cytology training was launched during an advanced EUS fellowship program at the center. Four endosonographers were enrolled in the study based on their resumes and an interview [Table 1]. The four endosonographers included three women and one man, median age 38 years (36–45 years), all

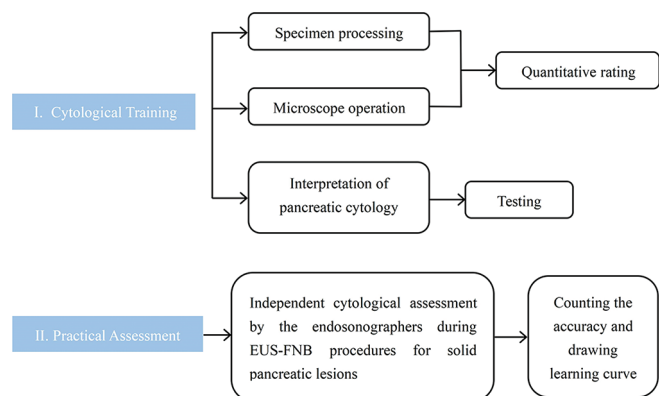


Figure 1. Flowchart illustrating the cytological training and practical assessment in the study. EUS-FNB: EUS-guided fine needle biopsy

had obtained their master's degrees and two obtained doctorate's degrees. They had become endoscopic specialists for at least 5 years and had finished the basic EUS fellowship, gained technical and cognitive EUS skills but performed fewer than 100 procedures for pancreatic lesions. None of them had experience reviewing any EUS-FNA or FNB slide or had previously participated in any formal training program or curriculum regarding cytology.

Cytopathology curriculum and assessment tools

The cytopathology curriculum, titled "What Endosonographers Need for On-site Cytological Evaluation," was hosted by the cytopathologist. The program comprised three parts: specimen processing, microscope operation, and interpretation of pancreatic cytology.

Specimen processing and microscope operation

The standard steps of specimen processing for ROSE and the microscope operation were demonstrated by the cytopathologist. For ROSE preparation, the performer is supposed to push the first drop of aspirated specimen onto a glass slide by inserting the stylet, then use another glass slide to pull parallel over the first one to spread the specimen equally. If the specimen on the slide is too thick to spread evenly, a third glass slide will be used to spread it again. For on-site evaluation, the slides need instant air-dried fixation and Diff-Quik staining. This staining method is similar to other Romanowsky-type staining in fixation method and dye, but its dye permeation requires less time, so it was used in this study. After staining, the specimen will be covered and examined under microscope.

The trainees were required to master the normative method of processing specimens and review slides proficiently under the microscope without any omission.

Cytopathology lecture

The cytopathologist gave the lecture covering the basic principles of pancreatic cytopathology. The feature introduction and atlas pictures were presented in the lecture, covering the normal morphology of pancreatic cells (ductal epithelial, acinar, and islet) and gastrointestinal epithelial cells, the background components of blood cells and mucin, and characteristics of benign pancreatic diseases such as chronic pancreatitis and autoimmune pancreatitis.

Most importantly, the lecture included the diagnostic criteria of pancreatic tumor cells including PDACs and other pancreatic tumors such as neuroendocrine tumor, solid pseudopapillary neoplasm, and mucinous neoplasms. The criteria for atypical grading taught to the endosonographers were based on the previous reported method of Hayashi *et al.*^[18] The common diagnostic cytological features of well-differentiated adenocarcinoma (WDA) were considered the following:^[21] Nuclear enlargement (more than two red blood cells as standard); anisonucleosis (nuclear size within one epithelial group varied more than 4 times); nuclear crowding/overlapping/3-dimensionality; and nuclear membrane irregularity. In the present program, aspects of the above criteria were modified. First, relatively rare features of adenocarcinoma were also taught as diagnostic bases: gap compared to confluent cell spacing; hyperchromasia; macronucleoli; mitosis; chromatin clearing; and necrosis. Second, nonadenocarcinoma pancreatic tumors were isolated from adenocarcinoma and subjected to additional diagnostic examinations such as immunohistochemical staining or flow cytometry.

The specimens were divided into three atypical grades in the modified criteria for cells: G1, without any common atypical feature; G2, with 1–2 common atypical features; G3, with 3–4 common atypical features, or 1–2 common atypical features plus any

Table 1. Background information of each trained endosonographer

	Trainee 1	Trainee 2	Trainee 3	Trainee 4
Sex	Female	Female	Male	Female
Age (years)	36	37	39	45
Education background	Master	Doctorate	Doctorate	Master
Experience of endoscopy (years)	6	5	10	12
Basic EUS fellowship	Yes	Yes	Yes	Yes
Experience of EUS-FNA/FNB (cases of solid pancreatic lesion)	≤100	≤100	≤100	≤100
Experience of cytological (evaluation or training)	No	No	No	No

rare atypical feature; or GX, with characteristics of nonadenocarcinoma pancreatic tumors.

Assessment tool

The performance of the participants (specimen processing and microscope operation) was measured by the cytopathologist using quantitative score sheets [Table 2]. A test containing ten questions about basic knowledge of pancreatic cytopathology and ten questions about specimen interpretation was administered. For specimen interpretation, the endosonographers were required to differentiate benign and malignant cells in ten cases. Each measurement of specimen processing and microscopic interpretation was awarded a maximum of 4 points. Correctly answering one question was counted as 4 points. The total possible scores of the grading and test were 100, and all pre- and post-training scores were compared to analyze the change in participants' skills.

Practical assessment during EUS-fine-needle biopsy procedures

EUS-fine-needle biopsy procedures

From December 2019 to October 2020, patients with suspected solid pancreatic lesions and with no bleeding tendency, clotting dysfunction, or other medical conditions that might contraindicate EUS-FNB were recruited for this study. All patients provided written informed consent before undergoing EUS-FNB.

The EUS-FNB procedures were performed by the experienced endosonographers (W.K.X). All patients were under standard conscious sedation with meperidine and midazolam during the procedure. A linear echoendoscope (EG-580UT, Fuji Film, Tokyo, Japan) with color Doppler guidance and a 22G or 25G needle (EchoTip ProCore, Cook Endoscopy, USA)

were used to obtain FNB specimens. The number of needle passes depended on the macroscopic evaluation of tissue specimens by the operator, characterized as whitish or yellowish tissue and free of blood and clots, with a minimum of 2 and maximum of 5 passes.

Specimen processing

The obtained specimens were processed and stained by the trainees as they were trained in turn. After each pass, the first drop of aspirated specimen from the needle was smeared by the glass slide evenly, then air-dried and stained instantly with Diff-Quik staining. The adequacy and atypical grade of the Diff-Quik stained specimens were evaluated by the four trainees independently.

After removing the stylet, the needle was flushed with saline solution to collect the remaining aspirated sample and tissue. The liquid sample was preserved in a vial containing BD CytoRich nongyn fixatives (BD SurePath, Franklin Lakes, NJ, USA) for SurePath processing. The slides derived from the liquid-based cytology preparation were examined using Papanicolaou staining. Tissue specimens were fixed in formalin solution. The liquid and tissue specimens were sent to the pathology laboratory for further staining and examination.

Diagnosis by endosonographers

The cytological diagnosis by endosonographers was conducted based on both adequacy and atypical grade. According to the atypical grade criteria, the Diff-Quik-stained specimens were classified as G1, G2, G3, or GX by the trainees, and adequate specimens were defined as containing more than 3 groups of G2, G3, or GX cells. The adequacy and atypical grade of each evaluated specimen were recorded and compared with the judgment of the cytopathologist.

Judgement by cytopathologist and final diagnosis

The cytopathologist reviewed all the Diff-Quik-stained specimens while blinded to the interpretations of the trainees or any other pathologic diagnoses and determined the adequacy and atypical grade. The diagnostic word of the cytopathologist was considered final. After comparing the diagnoses of the endosonographers with that of the cytopathologist, the specimens that were interpreted incorrectly were discussed with the trainees to encourage improvement.

The results of the Diff-Quik-stained specimens were confirmed through other pathological diagnoses and clinical follow-up.

Table 2. Quantitative score sheet used by the cytopathologist to grade the performance of endosonographers*

Items	Scores
Specimen processing	
Smear specimen equally	1, 2, 3, 4
Specimen fixation	1, 2, 3, 4
Perform Diff-Quik staining	1, 2, 3, 4
Cover the slide	1, 2, 3, 4
Microscope operation	
Display all fields clearly	1, 2, 3, 4
Total	

*Scored as follows: 1: Could not complete the operation independently and needed hands-on assistance; 2: Unable to complete the operation independently, verbal prompts were needed; 3: Could perform the operation independently, but the operation was not up to standard; 4: Could perform the operation independently and up to standard

Statistical analysis

For Stage 1, the scores of the pretraining and posttraining tests were compared to determine improvement. For Stage 2, the accuracy of the adequacy and atypia grades of each trainee was calculated, and learning curves were drawn using MATLAB software to determine how the diagnostic performance of each participant changed with the accumulation of practice. According to the study published by Petrone *et al.*,^[22] we set 90% as the effective diagnostic accuracy, which could imply the competence of the trainees for cytological evaluation.

RESULTS

All the pretraining and posttraining scores in Stage 1 were recorded and compared [Table 3]. As we can see after training, the median scores of the cytological test and performance ability of the four endosonographers had increased, respectively, from 38 (32–40) to 72 (68–76) out of 80 and 10.5 (10–12) to 19.5 (19–20) out of 20. The median total score increased from 49 (43–50) to 91.5 (88–95). This was a significant improvement of 87% (82%–105%).

For Stage 2, ninety patients with solid pancreatic lesions underwent EUS-FNB [Table 4]. The final diagnoses were confirmed by the overall cytological and histological results, surgical pathology, and clinical follow-up. There were 73 malignant lesions, 6 premalignant neoplasms, and 11 nonneoplastic lesions [Figure 2]. The 73 malignant lesions consisted of 70 PDACs, 2 adenosquamous carcinoma, and 1 neuroendocrine carcinoma. The six premalignant lesions included two mucinous neoplasms and three neuroendocrine tumors and one solid pseudopapillary neoplasm. The 11 nonneoplastic lesions comprised 2 autoimmune pancreatitis, 4 chronic pancreatitis, and 5 unknown lesions.

With regard to the diagnostic efficiency of Diff-Quik-stained specimens, 69 were consistent with the final diagnoses. The diagnostic accuracy,

sensitivity, and specificity were 76.7%, 69.9%, and 100% respectively [Table 5]. The genesis of the false-negative results lied in the inadequacy of specimens for diagnosing malignant or neoplastic disease. There was no false-positive result among the Diff-Quik-stained specimens.

The adequacy and atypical grade interpretations of the endosonographers were compared with those of the

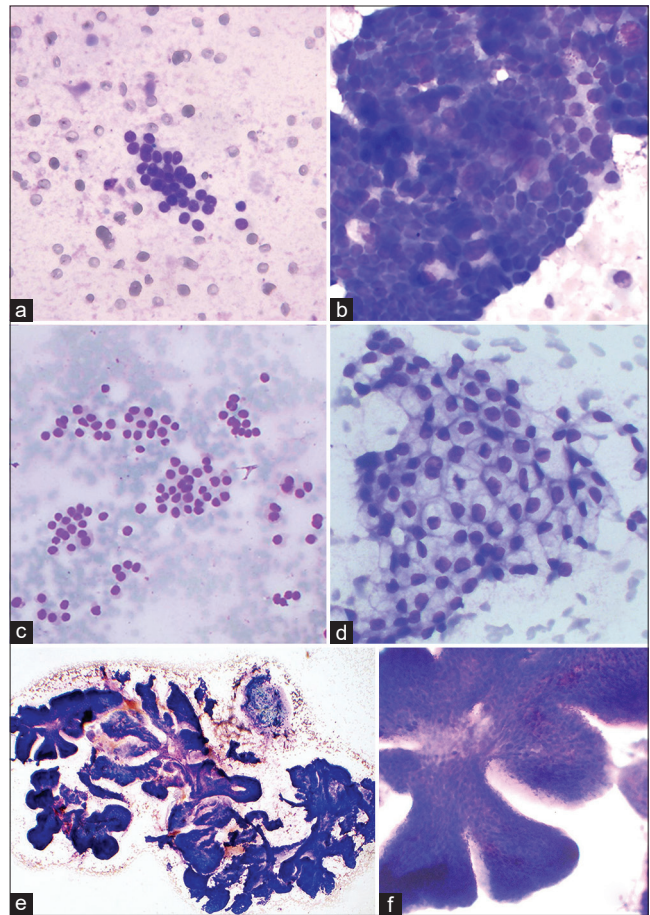


Figure 2. Benign and pre-malignant and malignant pancreatic cells, with Diff-Quik staining. (a) Normal pancreatic ductal epithelial cells; similar size to red blood cells and uniform nuclei (×400). (b) Malignant pancreatic cells; anisonucleosis, nuclear crowding, and overlapping, nuclear membrane irregularity (×400). (c) Pancreatic neuroendocrine tumor; monotonous small and loosely cohesive cells with round-oval nuclei (×400). (d) Mucinous neoplasms; abundant intracellular mucous and mildly atypical epithelial cells (×400). (e and f) High columnar pseudostratified papillary epithelium with dysplasia in solid pseudopapillary neoplasm. (e) ×100 (f) 200×

Table 3. Pre- and post- training scores of each endosonographer for technical performance and cytology assessment

	Trainee 1		Trainee 2		Trainee 3		Trainee 4	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Technical performance	11	20	10	19	10	19	12	20
Cytology	32	68	40	72	40	76	36	72
Total	43	88	50	91	50	95	48	92

cytopathologist. In this study, each patient received 2–5 passes, accordingly there were 290 Diff-Quik-stained specimens in total. The overall accuracy of the specimen adequacy assessments of the four endosonographers was 91.7%, 92.8%, 91.0%, and 89.3%. The accuracy of the atypical grade assessments was 80.0%, 82.1%, 81.0%, and 78.9%. Besides, the sensitivity and specificity of each trainee were further calculated [Table 6]. The learning curves indicated changes in diagnostic accuracy of the four endosonographers as the training progressed. All showed obvious improvement as the number of practical exercises accumulated. The curves for adequacy assessment were steeply climbing at the beginning and reached the target level of 90% at last [Figure 3]. For the atypical grade assessment, the curves among the four endosonographers had diverse starting points and tendencies but were all rising slowly near the end of training [Figure 4]. The cytopathologist graded 84.9% of the smears as high quality. Thick and uneven smearing and indistinct staining were the main causes of unsatisfactory specimens.

DISCUSSION

This is the first study to propose a cytopathologist-to-endosonographer teaching model that

combines a cytological training curriculum and practical assessments in EUS-FNB. As well, it is the first to draw learning curves for cytopathological interpretations by endosonographers. The inspection methods of each stage were standardized and reasonably gradual with one or more concrete indices: quantitative judgment of the training, diagnostic accuracy, and practical learning curves. The typical grading criteria in this study are explicit and lucid, containing the primary characteristics of pancreatic tumors, which are fit for on-site evaluation by endosonographers. Thus, this training program provides endoscopic centers, which lack adequate on-site cytopathologists to perform ROSE in EUS-FNA or FNB procedures, with a repeatable and referential method. Furthermore, this trial was successfully conducted during an advanced EUS fellowship program. The trainees could also benefit from correlating the pathological features with the manifestation of lesions under ultrasonic endoscopy so that they could better understand where to direct the needle. It therefore can be recommended as a means to unite cytopathology curriculum and conventional EUS-FNA or FNB training or supplement advanced EUS fellowship with cytological teaching.

The four endosonographers who underwent the formal cytopathology curriculum in stage one showed significant improvements, based on the changes in scores for theoretical and operational performance. To verify the effects of the training and investigate how many cases the endosonographers needed to reach an accurate diagnostic level in stage two we innovatively designed practical assessments in EUS-FNB procedures. The training curriculum

Table 4. Patients’ clinical characteristics

Characteristics	Values
Age (years)	62.3±9.4
Gender (male/female), n	54/36
Location of the mass (head/body/tail), n	41/30/19
Long axis of the mass (cm)	3.5±1.8
Puncture sites (Stomach/duodenal/both), n	51/14/25
Needle passes (n)	3.3±0.9

Table 5. The comparison between diagnoses of Diff-Quik stained sample and final diagnoses

Diagnoses of Diff-Quik	Final diagnoses		
	Nonneoplastic lesions (n=11)	Premalignant neoplasms (n=6)	Malignant lesions (n=73)
G1 (n=18)	10	0	8
G2 (n=14)	1	0	13
G3 (n=51)	0	0	51
GX (n=7)	0	6	1

Table 6. Overall diagnostic efficiency of each endosonographer in terms of adequacy and atypical grade assessments

Reviewer	Specimen adequacy			Atypical grade		
	Sensitivity (%)	Specificity (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)
Trainee 1	93.6	90.0	91.7	72.1	87.3	80.0
Trainee 2	90.0	95.3	92.8	70.7	92.8	82.1
Trainee 3	92.9	89.3	91.0	75.7	86.0	81.0
Trainee 4	87.1	91.3	89.3	72.1	85.3	78.9

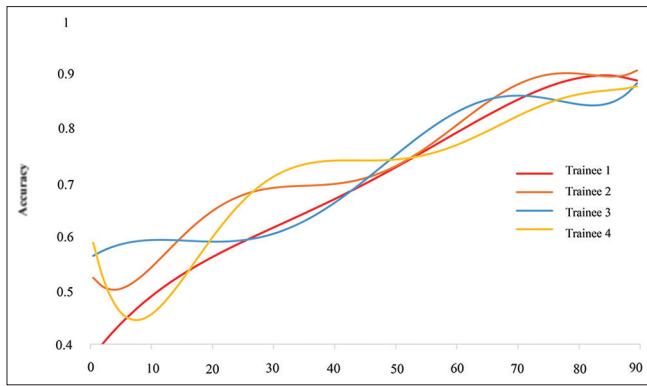


Figure 3. Graphic of the learning curve of adequacy assessments for all endosonographers

provided basic competence, while the latter stage of the program verified and consolidated the training. The demonstrated diagnostic accuracy and learning curves of the trainees were testament to the feasibility of endosonographers independently performing cytological evaluation.

The accuracy of the specimen adequacy and atypical grade assessments of each endosonographer improved over the course of the program. The adequacy assessments reached the target level at the completion of ninety cases. As is demonstrated in the previous study about training cytotechnician for performing ROSE in EUS-FNA of pancreatic lesions,^[22] the accuracy of 90% in adequacy assessments was considered sufficient. The learning curves for atypical grade assessments fluctuated among the endosonographers. By the end of 90 cases, the curves were still rising but not stabilized. This suggests the difficulty of performing the correct atypical assessment for pancreatic lesions by nonprofessional endosonographers, and the need for more than ninety cases to achieve competency. Yet, accurate recognition in adequacy assessments is sufficient in ROSE, as endosonographers are able to distinguish satisfactory specimens and thus supply appropriate feedback. However, the improvement in atypical grade assessments may contribute to adequacy interpretation. This is especially true for pancreatic tumors of low malignancy or premalignancy, which are often hard to differentiate from inflammatory changes or rarer tumors.^[23]

Compared with the final diagnoses, the accuracy, sensitivity, and specificity of Diff-Quik-stained specimens using the grading methods were 76.7%, 69.9%, and 100%. The main reason for false-negative

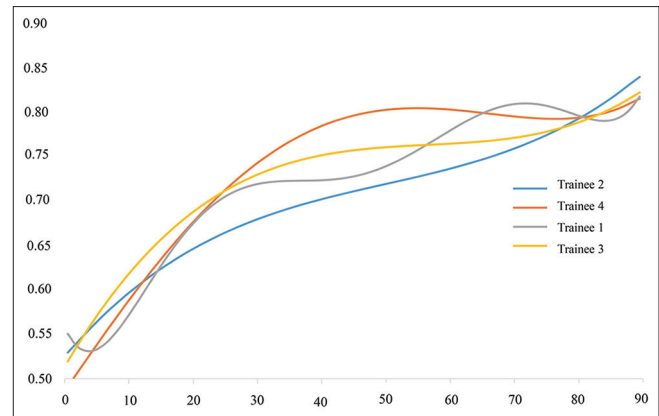


Figure 4. Graphic of the learning curve of atypical grade assessments for all endosonographers

results was that when using conventional smear cytology, only segmental liquid sample was obtained for smear specimens, which might not fully represent all pathological changes, leading to the misdiagnosis of some neoplastic lesions. According to a recent meta-analysis by Saurabh *et al.*,^[24,25] the overall diagnostic accuracy, sensitivity, and specificity of EUS-guided smear cytology in pancreatic lesions was 79.7%, 79.2%, and 99.4%, which was close to the results of this study, meaning that the diagnostic efficiency of Diff-Quik-stained specimens in this study had reached the average level of smear cytology. Notably, touch imprint cytology (TIC) is a cytological technique often applied in the frozen section of intraoperative pathology to determine whether it is benign or malignant.^[26] And the TIC technique has been introduced into the procedure of EUS-guided tissue acquisition as well. However, according to the study by Crinò *et al.*,^[27] EUS-FNB-TIC provides comparable samples to those of conventional smear cytology. EUS-FNB for pancreas is using an extremely fine needle to avoid adverse event, accordingly the acquired tissue is thinner and more brittle. Therefore, the advantage of using TIC to increase the efficiency of cytology might not compensate the costs of damaging the tissue specimen. More studies are expected to demonstrate the use of TIC in tissue core biopsy.

On-site pathological evaluation by nonprofessional endosonographers has previously been applied in some centers, but few have organized a formal training curriculum. Savoy *et al.*^[16] examined on-site cytological adequacy interpretation by endosonographers in a prospective double-blinded controlled trial, only to find endosonographers remained inferior to the

cytotechnologist in interpreting on-site cytological adequacy and diagnosis. However, a retrospective study by Hikichi *et al.*^[17] showed that the rates of specimen collection and diagnostic efficiency were comparable, whether ROSE was performed by endosonographers or cytopathologist.

We deem that the discrepancy between the two studies was due to the lack of formal cytological training and ambiguous definition of adequacy. Both studies provided brief training before the test, mainly as simple introductions by the cytopathologists and neither of them reported the effects of training. Thus, it is unknown whether the trainees were practically well trained through teaching. The participants in Savoy *et al.*^[16] made interpretation of adequacy based on the clear presence of target organ cells, with or without the presence of malignant-appearing cells. The unclear explanation regarding target organ cells might mislead the participants, all of whom had mistaken the inadequate as adequate specimens. In the Hikichi *et al.*^[17] study, adequacy was the only indicator, and atypical assessments were not required. The procedures stopped when the evaluator reckoned the cytological samples adequate, which was also an unclear determination; if they were uncertain about the adequacy, more passes were made to minimize the false-negative rate for malignant lesions. Yet, the final diagnostic accuracy might be improved by the extra passes.

Lin and Staerke^[21] believe the cytological characteristics for WDA of the pancreas in EUS-FNB specimens can be classified into two groups – common features that can be observed in 92%–99% of WDA and rare features that can be observed in 7%–38% of WDA. Moreover, they put forward that the diagnosis of pancreatic WDA could be made in FNA or FNB specimens by the observance of the common features. Then Hayashi *et al.*^[18] set up a simplified method of assessing atypical grade for PDAC based on the common features, which was proved an appropriate reference guideline for endosonographers to promptly interpret aspiration specimens: All slides graded G3/G2 were proved as malignant, suspected malignancy, atypical epithelium, or other neoplasm; and all G1 as nonneoplastic. This method made it convenient for endosonographers to interpret. Combing the previous studies and our experience, we modified the criteria, adding the rare features and the features of nonadenocarcinoma pancreatic tumors. The new grading criteria are more complicated and require more

training effort but can help the endosonographers better recognize and differentiate the neoplastic lesions.

There have been studies about training endosonographers in cytology before. Varadarajulu *et al.*^[20] conducted an intensive 2-day cytopathology training of endosonographers. It demonstrated an improvement in knowledge of the EUS-related cytopathology among the trainees, but the measurements in this training were subjective grading scores only. In addition, there was not any practical cases, and the long-term effect of this training program was not followed up, so it remained unknown whether the endosonographers could actually perform cytological interpretations independently after the program.

The main limitation of the present study is that it was an initial study exploring the endosonographers' learning ability for cytopathology. Only 4 endosonographers were recruited at a single center. Inevitably, there were some selection biases in the study subjects. For confirming the universality of the learning curves, a training program with more enrolled endosonographers is needed. Further, as a training program, this study had only 1 year to provide preliminary training to the endosonographers, the number of practical cases was insufficient for proving the stable competency at evaluating atypical grade. The trainees were expected to improve through ongoing practice, and follow-up investigation was needed to see the long-term impact. Besides, the study involved solid pancreatic masses only but no other gastrointestinal lesions, which are also the indications for EUS-FNA or EUS-FNB. As a final point, the training method and atypical grading criteria were drawn based on the experience of single institution. Though these evaluating methods were set up as objective and standardized as possible, it is still uncertain whether it will come to a similar conclusion elsewhere. Therefore, further validation is required from other endoscopy organizations.

CONCLUSIONS

This study revealed that the basic knowledge of pancreatic cytopathology could be obtained and operational performance significantly be improved through standardized and systematic training. Based on the practical assessments, the trained endosonographers were competent to recognize adequacy of pancreatic specimens from EUS-FNB. Although the number of patient cases was insufficient for training

endosonographers to judge atypical grade reliably, the results were still encouraging. Accordingly the program deserves consideration by medical centers that lack cytopathological staffs.

Financial support and sponsorship

This research was funded by Construction of Shanghai Pancreatic Diseases Medical Center (Grant No. 2017ZZ01009). Trial registration: ClinicalTrials.gov, NCT04509687.

Conflicts of interest

Zhen-Dong Jin is an Associate Editor of the journal. The article was subject to the journal's standard procedures, with peer review handled independently of this editor and his research groups.

REFERENCES

- Polkowski M, Jenssen C, Kaye P, et al. Technical aspects of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Technical Guideline – March 2017. *Endoscopy* 2017;49:989-1006.
- Cazacu IM, Luzuriaga Chavez AA, Saftoiu A, et al. A quarter century of EUS-FNA: Progress, milestones, and future directions. *Endosc Ultrasound* 2018;7:141-60.
- Wang AY, Yachinski PS. Endoscopic management of pancreatobiliary neoplasms. *Gastroenterology* 2018;154:1947-63.
- Roy A, Kim M, Hawes R, et al. Changing trends in tissue acquisition in malignant pancreatic neoplasms. *J Gastroenterol Hepatol* 2016;31:501-5.
- Artifon EL, Guedes HG, Cheng S. Maximizing the diagnostic yield of endoscopic ultrasound-guided fine-needle aspiration biopsy. *Gastroenterology* 2017;153:881-5.
- Wani S, Coté GA, Keswani R, et al. Learning curves for EUS by using cumulative sum analysis: Implications for American Society for Gastrointestinal Endoscopy recommendations for training. *Gastrointest Endosc* 2013;77:558-65.
- Shahidi N, Ou G, Lam E, et al. When trainees reach competency in performing endoscopic ultrasound: A systematic review. *Endosc Int Open* 2017;5:E239-43.
- Manfrin E, Perini C, Di Stefano S, et al. Pseudo solid-appearing pancreatic serous microcystic adenomas: Histologic diagnosis with the EUS core biopsy fork-tip needle. *Endosc Ultrasound* 2019;8:334-41.
- Kovacevic B, Vilmann P. EUS tissue acquisition: From A to B. *Endosc Ultrasound* 2020;9:225-31.
- Iglesias-Garcia J, Dominguez-Munoz JE, Abdulkader I, et al. Influence of on-site cytopathology evaluation on the diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) of solid pancreatic masses. *Am J Gastroenterol* 2011;106:1705-10.
- Hébert-Magee S, Bae S, Varadarajulu S, et al. The presence of a cytopathologist increases the diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration cytology for pancreatic adenocarcinoma: A meta-analysis. *Cytopathology* 2013;24:159-71.
- Matynia AP, Schmidt RL, Barraza G, et al. Impact of rapid on-site evaluation on the adequacy of endoscopic-ultrasound guided fine-needle aspiration of solid pancreatic lesions: A systematic review and meta-analysis. *J Gastroenterol Hepatol* 2014;29:697-705.
- Wani S, Mullady D, Early DS, et al. The clinical impact of immediate on-site cytopathology evaluation during endoscopic ultrasound-guided fine needle aspiration of pancreatic masses: A prospective multicenter randomized controlled trial. *Am J Gastroenterol* 2015;110:1429-39.
- Yang F, Liu E, Sun S. Rapid on-site evaluation (ROSE) with EUS-FNA: The ROSE looks beautiful. *Endosc Ultrasound* 2019;8:283-7.
- van Riet PA, Cahen DL, Poley JW, et al. Mapping international practice patterns in EUS-guided tissue sampling: Outcome of a global survey. *Endosc Int Open* 2016;4:E360-70.
- Savoy AD, Raimondo M, Woodward TA, et al. Can endosonographers evaluate on-site cytologic adequacy? A comparison with cytotechnologists. *Gastrointest Endosc* 2007;65:953-7.
- Hikichi T, Irisawa A, Bhutani MS, et al. Endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic masses with rapid on-site cytological evaluation by endosonographers without attendance of cytopathologists. *J Gastroenterol* 2009;44:322-8.
- Hayashi T, Ishiwatari H, Yoshida M, et al. Rapid on-site evaluation by endosonographer during endoscopic ultrasound-guided fine needle aspiration for pancreatic solid masses. *J Gastroenterol Hepatol* 2013;28:656-63.
- Harada R, Kato H, Fushimi S, et al. An expanded training program for endosonographers improved self-diagnosed accuracy of endoscopic ultrasound-guided fine-needle aspiration cytology of the pancreas. *Scand J Gastroenterol* 2014;49:1119-23.
- Varadarajulu S, Holt BA, Bang JY, et al. Training endosonographers in cytopathology: Improving the results of EUS-guided FNA. *Gastrointest Endosc* 2015;81:104-10.
- Lin F, Staerkel G. Cytologic criteria for well differentiated adenocarcinoma of the pancreas in fine-needle aspiration biopsy specimens. *Cancer* 2003;99:44-50.
- Petroni MC, Arcidiacono PG, Carrara S, et al. Does cytotechnician training influence the accuracy of EUS-guided fine-needle aspiration of pancreatic masses? *Dig Liver Dis* 2012;44:311-4.
- Grassia R, Imperatore N, Capone P, et al. EUS-guided tissue acquisition in chronic pancreatitis: Differential diagnosis between pancreatic cancer and pseudotumoral masses using EUS-FNA or core biopsy. *Endosc Ultrasound* 2020;9:122-9.
- Kong F, Kong X, Zhu J, et al. A prospective comparison of conventional cytology and digital image analysis for the identification of pancreatic malignancy in patients undergoing EUS-FNA. *Endosc Ultrasound* 2019;8:269-76.
- Chandan S, Mohan BP, Khan SR, et al. Comparison of EUS-guided conventional smear and liquid-based cytology in pancreatic lesions: A systematic review and meta-analysis. *Endosc Int Open* 2020;8:E1611-22.
- Krishnamurthy S, Meric-Bernstam F, Lucci A, et al. A prospective study comparing touch imprint cytology, frozen section analysis, and rapid cytokeratin immunostain for intraoperative evaluation of axillary sentinel lymph nodes in breast cancer. *Cancer* 2009;115:1555-62.
- Crinò Stefano Francesco, Larghi Alberto, Bernardoni Laura et al. Touch imprint cytology on endoscopic ultrasound fine-needle biopsy provides comparable sample quality and diagnostic yield to standard endoscopic ultrasound fine-needle aspiration specimens in the evaluation of solid pancreatic lesions.[J]. *Cytopathology* 2019;30:179-86.