Optimizing tissue handling of EUS-FNA of solid pancreatic lesions: a pilot study to the effect of a tissue preparation training for endoscopy personnel on sample quality and diagnostic accuracy

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ABSTRACT

BACKGROUND

In the absence of rapid on-side pathological evaluation (ROSE), endoscopy staff generally 'smears' endoscopic ultrasound guided (EUS) fine-needle aspiration (FNA) specimens on a glass slide. As this technique is vulnerable to preparation artifacts, we assessed if its quality could be improved through a tissue-preparation-training for endoscopy staff.

METHODS

In this prospective pilot study, 10 endosonographers and 12 endoscopy nurses from 7 regional EUS-centers in the Netherlands were invited to participate in a EUS-FNA smear-preparation-training. Subsequently, post training slides derived from solid pancreatic lesions were compared to pre-training 'control' slides. Primary outcome was to assess if the training positively affects smear quality and, consequently, diagnostic accuracy of EUS-FNA of solid pancreatic lesions.

RESULTS

Participants collected and prepared 71 cases, mostly pancreatic head lesions (48%). 68 controls were selected from the pre-training period. The presence of artifacts was comparable for smears performed before and after training (76% versus 82%, p=0.363). Likewise, smear cellularity (≥50% target cells) before and after training did not differ (44% (30/68) versus 49% (35/71), p=0.480). Similar, no difference in diagnostic accuracy for malignancy was detected (p=0.998).

CONCLUSION

In this pilot EUS-FNA smear-preparation-training for endoscopy personnel, smear quality and diagnostic accuracy were not improved after the training. Based on these results, we plan to further study other training programs and possibilities.

INTRODUCTION

Since its introduction in 1992, endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) is increasingly popular, due to its ability to sample difficult-to-reach target lesions at a low complication rate. Although the technique has gained global ground, diagnostic accuracy rates still vary from 68% to 98% [1-5], depending on patient characteristics, sampling techniques, and tissue handling and processing [6-14].

Historically, EUS-FNA tissue has been collected by spreading material on a glass slide, the so called 'smear technique'. Although this technique is fast and cheap, its diagnostic value is easily hampered by contamination and preparation artifacts [15, 16]. In the absence of (cyto) pathological assistance in the room (ROSE), specimens are prepared by the endoscopy staff, generally without formal training. There is limited data on their performance as compared to a specialized (cyto)pathologist. Although it seems that endoscopy staff is capable of assessing smear adequacy for diagnostic purposes [17-21], reports on their ability to prepare the smears themselves are conflicting [22-25]. We hypothesized that a tissue-preparation-training for endoscopy staff can improve smear quality and, thus, diagnostic accuracy of EUS-FNA.

METHODS

Study design

In this prospective pilot study, endosonographers and endoscopy nurses of seven regional EUS-centers in the Netherlands were invited to participate in a one-day EUS-FNA-specimen preparation training, if they had not undergone formal tissue preparation training before. To assess the impact of the training, quality and diagnostic accuracy of smears were compared before and after the training. For this, all study samples were sent to the Erasmus MC University Medical Center Rotterdam for expert review. As the study did not intervene with routine patient care, the Medical Ethics Committee of the Erasmus University Medical Center of Rotterdam waived the need to comply to the Medical Research Involving Human Subjects Act (MEC-2016-022). This committee also specifically approved for the use of any tissue and fluid samples as a model, as the training location was restricted to a controlled area (biohazard) at the department of Pathology in the Erasmus University Medical Center in Rotterdam.

Training program

The specifically designed training program comprised of a 2-hour theoretical and 2-hour practical 'hands-on' part. The training was provided by an expert pathologist and a group of cytotechnicians from the Erasmus University Medical Center in Rotterdam. During the theoretical part, participants were educated on pancreas pathology, including solid and cystic pancreatic neoplasms, chronic pancreatitis, and focal inflammation. Furthermore, several examples of

normal pancreas cytology and histology were discussed, as was the Bethesda classification and common diagnostic pitfalls in pancreas (cyto)pathology. Next, participants were lectured on the different FNA tissue preparation techniques, including smears, and commonly encountered pitfalls. The main focus of the training was optimal smear preparation. To prepare a good smear, participants were taught to apply the collected specimens 1cm from the edge of the glass slide. Then, they were told to place a second glass slide on top of the first glass slide that contained the drop of FNA material, and try to evenly distribute the tissue using the so-called sandwhich method. In addition, participants were explained to limit the amount of tissue per glass slide (only 1 drop!) to prevent thick cells layers or overlapping cells, and to avoid crushing artefacts by pressing the two glass slides too firmly. Last, they were instructed on the importance and timing of on-site fixation, staining and drying of the material. During the hands-on workshop, participants learned how to optimally smear and stain FNA-specimens, and how to avoid common pitfalls during preparation. Porcine pancreatic tissue was used as training specimens.

FNA-sample selection

After the training day, each participating center prospectively included all consecutive cases, scheduled for EUS-FNA of solid pancreatic lesions between April 2016 and September 2017. Subsequently, an equal number of historical controls (prior to the training date) was selected for each center. We did not match our controls based on needle type or size or the sampling technique used, as there is there is limited evidence on the impact of these variables on diagnostic accuracy of EUS-FNA. Samples that were prepared by (cyto)pathologists and/or cytotechnicians were excluded.

EUS-guided tissue sampling and specimens handling

EUS-guided tissue sampling was performed according to a standard protocol, using a convex array echoendoscope (Pentax EG-3870 UTK, Pentax EG-3270 UK; Pentax, Tokyo, Japan, Olympus UTC 140/180, Olympus linear GF-UCT180; Olympus, Tokyo, Japan). Tissue sampling was done by endosonographers, who performed between 25 and 100 EUS-guided tissue sampling procedures annually. The optimal sampling position was determined by scanning the target lesion and its environment with color and pulsed Doppler. Patients were punctured using a 19, 22- or 25-gauge FNA needle (EchoTip; Cook Medical, or Expect; Boston Scientific). Per target lesion, the trainees performed two smears from a single pass. All residual material was processed according to the standard protocols of the laboratories involved (Table 1). Furthermore, the number of passes, sampling strategy, and use of additional sampling techniques (e.g. applying negative suction with a syringe) was left at the discretion of the endosonographers. If available, ROSE, but only after the trainee had performed the study smears. In that case, the on-site (cyto)pathologist was not allowed to comment on in the glass slide preparation of the trainee.

Table 1. EUS-guided tissue sampling and tissue processing specifics per center

Center	EUS-scope type	Annual EUS-FNA per endosonographer	ROSE available	Additional techniques	SMEAR preparation	Liquid cytology medium	Thin-layer cytology technique	cell block technique
Albert Schweitzer Hospital, Dordrecht	Olympus linear GF-UCT180	25	Yes	Slow pull or Suction	Air dry, Hemocolor	CytoLyt	ThinPrep	Cellient Hologic
Reinier de Graaf Hosptial, Delft	Olympus linear GF-UCT180	30	No	Slow pull	Air dry No stain	CytoLyt, or Polytransportbuffer*	ThinPrep	Agar
Erasmus MC University Medical Center Rotterdam	Pentax EG-3870 UTK Olympus UTC 140/180	50	Yes	Slow pull or Suction	Air dry Diffquick	CytoLyt	ThinPrep	Cellient Hologic
Haga Hospital, The Hague	Olympus linear GF-UCT180	25	Yes	Slow pull	Air dry Diffquick	Formalin	None	Paraffin cell block
Ijsselland Hospital, Rotterdam	Olympus linear GF-UCT180	25	Yes	Slow pull	Air dry Diffquick Giemsa	CytoRichRed	None	Agar
Maasstad Hospital, Rotterdam	Pentax EG-3270 UK Olympus linear GF-UCT180	30	No	Slow pull	Air dry Diffquick	CytoRichRed	None	Aalfix cell block*
Sint Franciscus Hospital, Rotterdam	Pentax EUS-scope	20	No	Slow pull or Suction	Air dry No stain	CytoRichRed	None	Agar

FNA: fine needle aspiration, ROSE: rapid on-site pathological evaluation,
**medium/technique developed locally.*

Outcome measures and definitions

The primary outcome measure was to assess if this one day 'hands-on' EUS-specimens-preparation-training improved the diagnostic accuracy of smears, in the absence of an on-site (cyto) pathologist. Diagnostic accuracy for malignancy was calculated from the correct number of cases that were defined as atypical/suspect for malignancy or malignant. In addition, accuracy for the Bethesda classification was calculated from the number of cases that were correctly classified into the categories; non-diagnostic, benign, atypical/suspect for malignancy or malignancy, according to the formula: (true positive + true negative) / all patients. Gold-standard diagnosis was based on surgical resection specimens, or a clinical follow-up period of at least 1 year for non-operated patients.

Secondly, we assessed if the training improved sample quality, which was defined as sample artifacts (fixation, thick smear/clots, obscuring blood or inflammation, cytolysis, contamination, other) and cellularity (presence of </≥50% target cells).

Statistics

Outcome measures were expressed as means ± standard deviations (SD) or as medians with interquartile ranges (IQR). Statistical significance was assessed with the use of Student's t-test for normally distributed continuous data; either the chi-square test for categorical data (with Yates' correction when appropriate) or Fisher exact test for categorical data; and the median test for non-normally distributed continuous data. Sample quality and diagnostic accuracy were compared between cases and controls using a logistic mixed effect model with a random intercept for participating center [26]. The latter has been done to take into account the clustering structure of this multi-center trial, i.e., that observations from the same site may be correlated. Statistical significance was established as $p<0.05$ (two-tailed). Analyses were carried out using SPSS version 21, Statistical Package for the Social Sciences, SPSS Inc., Chicago, Illinois, and R (version 3.4.2).

Power calculation

To determine the power needed for this study, we assessed the impact of the introduction of ROSE in one of the participating centers as a substitute intervention for our smear-preparation-training. To determine if smear accuracy had improved, an expert pathologist reviewed 20 smears from the period before and 18 smears from the period after ROSE was introduced in that center. Smear accuracy improved with 30% since the implementation of ROSE. Based on this assumption, a two-group continuity corrected chi-squared test with a 0.05 two-sided significance level will have 80% power to detect the difference between a Group 1 proportion (results before training), $\pi 1$ of 0.400 and a Group 2 proportion (results after training), $\pi 2$, of 0.670 (odds ratio of 3.045) when the sample size in each group is 60 cases [27].

Table 2. Characteristics of EUS-tissue training participants

Hospital	Profession	Age (years)	Female	Experience with EUS-FNA (years)	No. of EUS-FNA procedures performed annually
1	Doctor	42	No	12	100
1	Doctor	39	Yes	4	30
2	Nurse	24	Yes	2	300
2	Nurse	33	Yes	6	300
2	Nurse	22	Yes	2	300
2	Nurse	23	Yes	0	25
2	Nurse	30	Yes	0	30
3	Doctor	38	No	3	10
3	Doctor	35	Yes	1	25
3	Nurse	48	Yes	3	25
4	Doctor	44	Yes	10	50
4	Doctor	42	Yes	8	50
4	Nurse	48	Yes	11	92
5	Nurse	37	Yes	8	60
5	Doctor	49	No	7	50
5	Nurse	31	Yes	7	50
6	Nurse	29	Yes	5	40
6	Nurse	29	Yes	5	40
6	Nurse	47	Yes	0	45
6	Doctor	36	Yes	2	50
7	Doctor	39	No	1	60
7	Doctor	44	Yes	10	25

FNA: fine needle aspiration, no.: number.

RESULTS

Endoscopy staff characteristics

A total of 10 endosonographers and 12 endoscopy nurses attended the EUS-specimens-preparation-training. Participants were selected by the principal investigators of the participating centers, during a meeting in February 2016. If they had not received a formal EUS-sample-preparation-training previously, the study coordinator invited the participants by e-mail. Table 2 demonstrates the participants' characteristics. Majority of the trainees was female, with a median age of 38 (range 22-49). As only one of the centers was an academic hospital, most were working at a community hospital (77%). Experience with EUS-FNA ranged from several months to years. We consider our study population to be representative for, at least, the other regions in the Netherlands, since most regions in the Netherlands comprise an academic and several smaller hospitals. Furthermore, majority of today's medical staff comprises young to

Table 3. Characteristics of included cases and controls.

Variables	Controls (n=68)	Cases (n=71)	p-value
Center of inclusion, n (%)			
Albert Schweitzer	6 (9)	6 (9)	n.s.
Reinier de Graaf	12 (18)	15 (22)	
Erasmus MC	28 (41)	28 (39)	
Haga Hospital	3 (4)	3 (4)	
Ijsselland Hospital	6 (9)	6 (9)	
Maasstad Hospital	6 (9)	6 (9)	
Sint Franciscus Hospital	7 (10)	7 (10)	
Target lesion location, n (%)			
Head	39 (57)	34 (48)	0.003
Uncinate process	5 (7)	6 (9)	
Neck	9 (13)	4 (6)	
Corpus	9 (13)	14 (20)	
Tail	0 (0)	13 (18)	
Missing	6 (8)	0 (0)	
Target lesion size (mm), mean ± SD	28.7 ± 9.63	31.0 ± 1.37	n.s.
FNA needle size, n (%)			
19-gauge	3 (6)	1 (1)	0.016
22-gauge	31 (57)	27 (38)	
25-gauge	20 (37)	43 (61)	
Number of passes, median (IQR)	3.00 (2.00-3.00)	3.00 (2.00-3.00)	n.s.

N: number, mm: millimeter, SD: standard deviation, FNA: fine needle aspiration, n.s: non significant.

Table 4. Diagnostic outcome of SMEAR samples from cases versus controls.

Variables, n (%)	Cases (n=71)	Controls (n=68)	p-value**
Presence of artifacts	54 (76)	56 (82)	0.363
Type of artifacts*			
Poor fixation	3 (6)	3 (5)	1
Thick smear/clots	45 (83)	42 (75)	0.351
Cytolysis	25 (46)	30 (54)	0.567
Cellularity			
<50%	36 (51)	38 (56)	0.480
≥50%	35 (49)	30 (44)	
Sample diagnosis			
Impossible to determine	21 (30)	21 (31)	0.998
Benign	1 (1)	1 (1)	
Atypical/suspect for malignancy	13 (18)	12 (18)	
Malignant	36 (51)	34 (50)	
Gold standard diagnosis			
Benign	4 (6)	2 (3)	0.556
Atypical (NET, pancreatitis)	3 (4)	5 (7)	
Malignant	64 (90)	61 (90)	
Diagnostic accuracy for diagnostic classification Bethesda	51 (36)	47 (32)	0.667
Diagnostic accuracy for malignancy % (n/n)	66 (47/71)	66 (45/68)	0.998

*NET: neuroendocrine tumor, n: number, *more than one option possible, **generalized linear mixed model.*

middle-aged women, and exposure to EUS-FNA varied greatly, which corresponds well with exposure in the academic and non-academic centers.

Target lesion characteristics

71 cases and 68 controls were assessed (Table 3), with a mean lesion size of 31mm (SD± 1.37mm). Pancreatic corpus and tail lesions were somewhat over-represented in the control group (p=0.003, table 3). Most case lesions were sampled with a 25G needle (61%), while controls were mostly targeted with a 22G needle.

Smear quality

The presence of artifacts was comparable for smears prepared before and after the training session (76% versus 82%, p=0.363, table 4), as were individual types of artifacts. Also, for smear cellularity, there was no difference between cases and controls (p=0.480).

Smear diagnosis and accuracy

After a median follow-up time of 24 months (range 21-32), 70 (50%) of the smears were scored as malignant, 25 (18%) as atypical or suspect for malignancy, and 2 (1%) as benign. Smears were considered non-diagnostic in 42 lesions (30%). Gold standard diagnosis revealed 125 (90%) malignant lesions, 8 (6%) atypical lesions or suspect for malignancy (one IgG-mediated pancreatitis, two pancreatitis, five neuroendocrine tumors), and 6 (4%) benign lesions (three chronic pancreatitis, one fibrotic lesion, two non-specified benign lesions). Similar to FNA sample quality, tissue-preparation-training did not result in a significant increase in the diagnostic accuracy for malignancy (p=0.998) or the Bethesda classification (p=0.667, table 4).

DISCUSSION

With this pilot study, we aimed to evaluate the efficacy of an EUS-FNA-smear-preparation-training for endoscopy staff in centers lacking ROSE. Unfortunately, our training did not improve the smear quality or diagnostic accuracy in our regional EUS-working group. For this, several reasons may be found.

First of all, our training program may have been inadequate to achieve a significant improvement in the performance of the trainees. As official EUS-sample preparation-courses do not exist, we had to design our own program. We chose a comprehensive training, combining theoretical and practical hands-on elements. However, this program may have fallen short. It is, for example, well known that practical skills are better achieved after extensive training, and tend to grow with exposure. Therefore, it may have be more effective to intensify or repeat the training by one or more refresh sessions. In addition to this, the specimen collection period

may have been too short to allow trainees to gain sufficient experience, thereby improving their skills.

Secondly, it has been demonstrated that self-assessment and standardized feedback improves the learning curve for colonoscopy of Gastroenterologists in training [28]. Therefore, implementing standardized self-assessment forms could have increased the training effect. In addition, we could have implemented frequent multidisciplinary meetings of the trained endosonographers with the (cyto)pathologists. Such off-site feedback moments may further improve the learning curve for smear preparation.

Thirdly, our results might be inherent to the nature of the smear technique itself, since it is a manual method that is sensitive to artifacts and is prone to heterogeneous preparations. In contrast, cytological examination using a liquid-based medium (LBC), such as ThinPrep or cell block, has several advantages including less contamination by red blood cells, less drying artifacts [8].

A limitation of our study is that our power calculation was based on the training effect on our regional EUS-working group. Therefore, we could not assess the impact of the training on an individual basis. This prevents us from identifying trainees who did benefit from the training. It is known, that a learning curve can vary greatly between trainees. This has been shown for endoscopy and endoscopic retrograde cholangiopancreatography (ERCP) learning [29], and seems to have led to a more competence-based training schedule rather than a threshold number-based training for Gastroenterology residents [30, 31]. As our group comprised of endoscopy staff (both physicians and nurses) from high, medium and low volume centers with different levels of experience, differences in learning curves seem inevitable. Previous studies found that endosonographers performed equally well as compared to cytopathologists, but endoscopy nurses did not [22-25]. We did not power our study to compare the smear quality and accuracy between doctors and nurses.

Another limitation, one that hampers most EUS-FNA studies, is the inter-center variability in practice protocols. As we report in table 1, our centers use a variety of sampling and tissue preparation techniques. Although this may introduce a bias, today, this is inevitable in multicenter studies, as no consensus exists on the optimal sampling and tissue handling technique [15, 30, 32].

Furthermore, the endpoints that we used to measure EUS-FNA quality are not globally harmonized. The most important problem is that there are no uniform guidelines that advise on how to mark FNA sample diagnosis [33], and there is no consensus on how to describe sample quality. Therefore, quality definitions used in the current study were jointly created by the study group.

Taken all together, this pilot EUS-FNA smear-preparation-training for endoscopy personnel did not improve EUS-FNA smear quality or accuracy. Nevertheless, it stands to reason that endoscopy staff could benefit from some form of specimens-preparation-training, and perhaps an adjusted, more elaborate program will be more effective. However, optimization of smear

quality is only one link in the chain towards a higher diagnostic accuracy for EUS-FNA. Therefore, we also need to explore other strategies to achieve this.

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