Original Article

Agreement on endoscopic ultrasonography-guided tissue specimens: Comparing a 20-G fine-needle biopsy to a 25-G fine-needle aspiration needle among academic and non-academic pathologists

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Background and Aim: A recently carried out randomized controlled trial showed the benefit of a novel 20-G fine-needle biopsy (FNB) over a 25-G fine-needle aspiration (FNA) needle. The current study evaluated the reproducibility of these findings among expert academic and non-academic pathologists.

Methods: This study was a side-study of the ASPRO (ASpiration versus PROcore) study. Five centers retrieved 74 (59%) consecutive FNB and 51 (41%) FNA samples from the ASPRO study according to randomization; 64 (51%) pancreatic and 61 (49%) lymph node specimens. Samples were re-reviewed by five expert academic and five non-academic pathologists and rated in terms of sample quality and diagnosis. Ratings were compared between needles, expert academic and non-academic pathologists, target lesions, and cytology versus histological specimens.

Results: Besides a higher diagnostic accuracy, FNB also provided for a better agreement on diagnosing malignancy ($\kappa = 0.59$ vs $\kappa = 0.76$, $P < 0.001$) and classification according to Bethesda ($\kappa = 0.45$ vs $\kappa = 0.61$, $P < 0.001$). This equally applied for expert academic and non-academic pathologists and for pancreatic and lymph node specimens. Sample quality was also rated higher for FNB, but agreement ranged from poor ($\kappa = 0.04$) to fair ($\kappa = 0.55$). Histology provided better agreement than cytology, but only when a core specimen was obtained with FNB ($P = 0.004$ vs $P = 0.432$).

Conclusion: This study shows that the 20-G FNB outperforms the 25-G FNA needle in terms of diagnostic agreement, independent of the background and experience of the pathologist. This endorses use of the 20-G FNB needle in both expert and lower volume EUS centers.

Key words: FNA, FNB, interobserver agreement, pathology

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INTRODUCTION

TRADITIONALLY, ENDOSCOPIC ULTRASOUND (EUS)-guided tissue sampling has been carried out using a thin and flexible fine-needle aspiration (FNA) needle, which mainly yields individual cells (cytology) rather than histologically intact tissue fragments. Although diagnostic accuracy rates of FNA are fair, intact tissue fragments are preferred to enable identification of tumor invasion and allow for ancillary immunological and molecular testing; for example, in submucosal and neuroendocrine tumors.1–9 Furthermore, histology enables genetic profiling and a patient-tailored approach, which is becoming increasingly relevant in this era of personalized medicine.10–14 The growing need for histology resulted in the introduction of fine-needle biopsy (FNB) needles.

So far, most studies reported an equal performance of FNA and FNB needles,6–9,15 but, recently, two large randomized trials showed a significant diagnostic benefit of FNB.16,17 One of these studies, the randomized controlled ASPRO (ASpiration vs ProCore) trial, was carried out in 13 EUS clinics, worldwide.17 This study showed a diagnostic benefit of a novel 20-G FNB needle (ProCore; Cook Medical, Bloomington, IN, USA) over the widely used 25-G FNA needle (EchoTip Ultra; Cook Medical), irrespective of lesion type, size, and the number of passes carried out. However, general applicability of these findings cannot be warranted, as study participation was confined to expert centers only.

Ideally, the superiority of a diagnostic device is reproducible in expert and non-expert hands. Therefore, the present study compares the diagnostic agreement on samples obtained with the novel 20-G FNB to the 25-G FNA needle among expert academic pathologists and non-academic pathologists.

METHODS

Study design

IN THE COURSE of the ASPRO trial (ClinicalTrials.gov: NCT02167074), 13 EUS centers randomized 608 consecutive patients with a solid pancreatic lesion, lymph node, or submucosal or other solid lesion to sampling with a 20-G FNB (ProCore; Cook Medical) or with a 25-G FNA needle (EchoTip Ultra; Cook Medical), between February 2015 and September 2016. Parameters regarding specimen characteristics and diagnostic accuracy were compared. Gold standard diagnosis was based on the prior ASPRO study17 either on pathological evaluation of the surgical resection specimens or on clinical follow up for at least 9 months when surgical resection was not indicated. Gold standard diagnosis was recorded by the principal investigator of each of the participating centers.

For the present side-study, the first 125 pancreatic and lymph node cases that were enrolled in the ASPRO study were included. Samples from these cases were reassessed by five expert academic and five non-academic pathologists. Diagnosis of malignancy and quality scores were assessed, and agreement on these outcome measures was compared between the two needles and between academic and non-academic pathologists.

As our study was a clinical trial, all authors could access the study data and have reviewed and approved the final manuscript.

Center, pathologist and case selection

ASpiration versus ProCore study centers were invited to contribute to this study if they had collected at least 20 solid pancreatic and lymph node samples by April 2016, and their pathologist was trained to read both cytology and histology. Five ASPRO study centers fulfilled these criteria (Milan, Osaka-Sayama, Rome, Rotterdam, and Santiago de Compostela). Each center was represented by the specialized ‘academic’ pathologist who was also involved in the original ASPRO study. This academic pathologist invited a ‘non-academic’ colleague from a local community practice hospital with a general clinical profile to participate. Expert academic pathologists had reviewed between 3000 and 40 000 EUS samples, including both FNA and FNB during their career, whereas the non-academics had a sample review track record of between 50 and 1000. Per case, the academic pathologists selected the minimum number of slides required to obtain a tissue diagnosis, including immunohistochemically stained slides, if available.

Endoscopic ultrasonography-guided tissue sampling

Endoscopic ultrasonography procedures were carried out with a convex array echoendoscope (either Pentax EG-3870 UTK or EG-3270UK; Pentax, Tokyo, Japan, or Olympus UTC 140/180/260; Olympus, Tokyo, Japan) as described in the ASPRO study.17 Three study sites had on-site pathological evaluation at their disposal (Milan, Rotterdam, and Santiago de Compostela).

Specimen processing

Tissue samples were preserved according to local practice. Cytological tissue samples were smeared onto glass slides and stained with (Diff Quick, RAL diagnostics) or hematoxylin

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and eosin (HE) staining (Milan, Osaka-Sayama, Rome). Remainder of the cytological specimens were collected in (CytoLyt, ThinPrep® CytoLyt Solution, Marlborough, MA, USA), saline (Osaka-Sayama), or formalin (Milan). Cell suspensions were processed into cell blocks using the (Cellient automated cell block system Hologic, Toronto, Canada) or agar technique (Milan, Rome, Santiago de Compostela). Osaka-Sayama did not further process cytology. Histology was collected in CytoLyt (Santiago de Compostela and Rotterdam) or formalin (Milan, Rome, Rotterdam, Osaka-Sayama). Samples collected in formalin were processed as paraffin blocks, sectioned at 3–4 microns, and stained with HE for morphological evaluation.

Review session

Cases were reviewed during a 2-day session at the Erasmus MC University Medical Center Rotterdam, the Netherlands in April 2016. Each expert academic pathologist presented the selected cases providing information on the patient’s gender, age and relevant medical history, type of target lesion (lymph node or solid pancreatic lesion) and a minimal deviation from the acceptable interrater reliability of 0.6 for agreement on the presence of malignancy, and a minimal deviation from the interrater reliability of 0.2 between the two needles, n = 10. Interobserver agreement was calculated by the use of kappa statistics Fleiss’ κ-statistic and 95% confidence intervals (CI). Kappa-statistics were interpreted according to the convention of Landis and Koch: <0, no agreement; 0–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; and 0.81–1.0, almost perfect agreement. The chi-squared test was used to compare the diagnostic agreement between the two study needles, academic and non-academic pathologists, target lesion types, and cytological and histological samples. Although all 10 observers assessed the samples for each of the outcome parameters, we reported only the average outcome per parameter. Last, univariate logistic regression analysis was applied to assess whether a pathologist’s expertise and sample quality influenced diagnostic accuracy. Outcomes of this analysis are expressed as odds ratio (OR) with 95% CI. Statistical significance was defined as P < 0.05 (two-tailed). Analyses were carried out using SAS version 9.4 (SAS Institute, Cary, NC, USA), and SPSS version 22, Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA).

RESULTS

Target lesion and procedure characteristics

A total of 125 samples were reviewed, of which 74 were collected by FNB (59%) and 51 by FNA (41%), with a mean of 2.8 needle passes. Sixty-four were solid...
pancreatic lesions (51%) and 61 were lymph nodes (49%) with a mean size of 30.4 ± 1.3 mm. Table 1 shows the case and sampling specifics. Techniques intended to increase the sample yield were applied in 94% of cases; suction with a syringe in 74 (63%), the slow-pull technique in 50 (37%), and a combination of the two in five (4%). The gold standard diagnosis comprised 26 (21%) non-malignant cases and 99 (79%) malignant cases (Table 1). The gold-standard diagnosis was based on surgical resection specimens in 31 cases (25%).

### Diagnostic accuracy and agreement

In line with the ASPRO study results, FNB samples provided higher accuracy than FNA for malignancy (88% vs 77%, \( P = 0.002 \)) and classification according to Bethesda (76% vs 61%, \( P = 0.002 \)). Regarding the primary question of diagnostic agreement, FNB samples provided better agreement on the presence of malignancy (\( \kappa = 0.76 \) vs 0.59, \( P < 0.001 \)) and classification according to Bethesda (\( \kappa = 0.61 \) vs 0.45, \( P < 0.001 \), Table 2). This was true for both expert academic and non-academic pathologists (Table 2).

Assessment per target lesion showed that for lymph nodes, FNB provided higher agreement on the presence of malignancy and classification according to Bethesda. However, in pancreatic lesions, FNB only outperformed FNA for agreement on the Bethesda classification, not for the presence of malignancy (Table 3). When comparing histology to cytology, agreement on the presence of malignancy was better for histological samples, but agreement on the Bethesda classification was better for histological samples only if they had been obtained with the FNB needle (Table 4).

### Specimen quality and agreement

Compared to FNA, FNB samples contained fewer artifacts (52% vs 45%, \( P = 0.007 \), Table 2), but agreement was low for both FNB (\( \kappa = 0.10; 95\% \text{ CI} 0.07–0.14 \)) and FNA samples (\( \kappa = 0.17; 95\% \text{ CI} 0.13–0.21 \)). Agreement did not differ between expert academic and non-academic pathologists for FNA (\( P = 0.132 \)) or FNB (\( P = 0.212 \)). Sample sufficiency for diagnosis, percentage of target cells, presence of tissue cores, and suitability for additional analysis were all better for FNB than for FNA, but, again, agreement on these parameters was poor (\( \kappa = 0.04 \)) to fair (\( \kappa = 0.55 \), Table 2). As for the collection of histology, use of FNB provided histological samples more often than did FNA (70% vs 36%, \( P < 0.001 \), Table 2). Agreement on all of the above-mentioned quality parameters was highest for the expert academic pathologists. Furthermore, agreement among the expert academic pathologists was higher for FNB than for FNA specimens. In non-academic pathologists, however, FNB provided for better agreement than FNA only for the identification of tissue cores (\( \kappa = 0.26 \) vs 0.04, \( P < 0.001 \)).
Factors affecting diagnostic accuracy

Besides the type of needle, other factors affecting EUS-sample diagnosis are shown in Table 5. A pathologist's background (expert academic or non-academic) did not influence the diagnostic accuracy of either needle \((P = 0.250)\). Presence of artifacts did have an effect, as this resulted in a lower diagnostic accuracy \((P = 0.030)\). Last,

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the presence of tissue cores significantly improved diagnostic accuracy ($P = 0.003$).

**DISCUSSION**

In addition to the previously reported diagnostic benefit of a novel 20-G FNB over a commonly used 25-G FNA needle, the present study shows that diagnostic agreement is also higher for FNB than for FNA samples. More importantly, agreement on FNB samples was higher among pathologists from different backgrounds (academic vs community practice) and with different levels of experience (high vs lower volume). The benefit of FNB equably applies to pancreatic and lymphatic target lesions. The finding that FNB samples were of better quality and harbored histology more often likely contributed to their superior diagnostic performance.

Most studies on EUS-needle devices have been carried out in expert high-volume centers. However, EUS-guided tissue sampling is increasingly applied in lower-volume centers. So far, few studies have evaluated the reproducibility of EUS-FNA/FNB results. Moreover, most of these studies had a limited number of observers, concerned one type of target lesion, or were carried out in an academic practice only. Previous studies reported diagnostic agreement rates ranging from moderate to excellent for FNA ($\kappa = 0.45–0.89$) and FNB ($\kappa = 0.61–0.94$). Recently, a promising study aimed to validate a novel scoring system to further optimize diagnostic agreement among cytopathologists. Unfortunately, despite the fact that observers were selected from tertiary centers, diagnostic agreement for pancreatic FNA specimens was still suboptimal ($\kappa = 0.56$). Compared to these agreement rates, the 20-G FNB needle performed well, especially when we take into account that pathologists from all over the world were included, academics and non-academics alike. The 20-G FNB needle may thus contribute to improve reproducibility of EUS-FNA/B diagnosis.

The first explanation for better agreement on FNB samples is its high tissue core rate, as the collection of histology rather than cytology was positively associated with higher agreement. This is supported by the finding that the cytological yield of FNB was also higher than for FNA, but only availability of tissue cores for histology, and not cytology, contributed to a better diagnostic accuracy. The importance of tissue core samples over cytological samples to reach a correct diagnosis when using an FNB needle has been previously described by others. Compared to other FNB needles, the cytological yield of the current 20-G FNB needle was also high. Whereas previous studies reported sufficient cellularity in 19%–52%, in the current study, this was 68%. The only device that provides higher histology and cytology rates is the 19-G needle, which obtains cores in 88% of samples and an adequate amount of loose target cells in 91%. However, the reported clinical applicability of being

| Table 4 Diagnostic agreement on cytological and histological specimens per needle type |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Agreement κ (95% CI)            | Cytology        | Histology       | P-value         |
| Bethesda classification         |                 |                 |                 |
| All samples                     | 0.51 (0.49–0.52)| 0.60 (0.59–0.61)| <0.001          |
| FNA ($n = 47$)                  | 0.49 (0.46–0.50)| 0.52 (0.49–0.55)| 0.432           |
| FNB ($n = 74$)                  | 0.52 (0.49–0.54)| 0.62 (0.61–0.63)| <0.001          |
| Presence of malignancy          |                 |                 |                 |
| All samples                     | 0.76 (0.74–0.78)| 0.97 (0.95–0.99)| <0.001          |
| FNA ($n = 47$)                  | 0.73 (0.71–0.76)| 0.89 (0.86–0.92)| 0.002           |
| FNB ($n = 74$)                  | 0.78 (0.75–0.81)| 0.99 (0.79–1.00)| <0.001          |

CI, confidence interval; FNA, fine-needle aspiration; FNB, fine-needle biopsy.

| Table 5 Factors affecting diagnostic accuracy by univariate analysis |
|---------------------------------------------------------------|
| Diagnostic accuracy (95% CI) | Univariate OR (95% CI) | Pathologist experience |
|--------------------------------|------------------------|------------------------|
| Pathologist experience         | Univariate OR (95% CI) | Pathologist experience |
| Expert academic                 | 0.96 (0.82–1.12)       | 0.587                  |
| Non-academic                    |                        |                        |
| Presence of artifacts           | 1.45 (1.22–1.74)       | <0.001                 |
| No                              |                        |                        |
| Yes                             |                        |                        |
| Type of tissue                  | 0.55 (0.32–0.94)       | 0.030                  |
| Histology                       |                        |                        |
| Cytology                        |                        |                        |

CI, confidence interval; OR, odds ratio.
able to obtain tissue with the 19-G FNB needle (81%) is much lower than with the 20-G FNB needle (99%). Although the increased flexibility of the 20-G FNB needle is likely a major contributor to its better performance, other needle design adjustments may also have improved the tissue acquisition rate.22,24

Another quality parameter that may have contributed to the high diagnostic agreement on samples obtained with FNB is a low artifact rate. Although artifacts do not necessarily decrease accuracy when abundant tissue is collected, previous studies have shown that they may hamper advanced genetic testing, for example.34 Interestingly, agreement on the presence of artifacts was low for both needles (although slightly better for FNB than for FNA). This is in line with the fact that agreement on all sample quality parameters was rather low, similar to reports from others.21,24 This may result from a lack of EUS-sample quality definitions. In the current study, we tried to minimize this limitation by using the predefined scoring system, as proposed by the Papanicolaou Society of Cytopathology in 2014.18

There are several limitations to our study. First, each academic pathologist brought and presented his or her own slides. Although they too were blinded for the final outcome, we cannot exclude recall bias. However, this only applied to a few cases per pathologist. Second, pathologists assessed samples individually, whereas, in daily practice, difficult cases are often discussed among colleagues. Therefore, interobserver agreements reported in the current study may underestimate real-life reproducibility. Third, our study involved pathologists from 10 centers from around the world, whereas previous studies were confined to no more than five centers from the same geographical region. In the absence of uniform guidelines for EUS-guided tissue sampling and processing, it is inevitable that there are geographical and institutional differences in the work-up of specimens. These differences may have resulted in slight differences in the appearance of specimens, which may have hampered interpretation by pathologists not familiar with certain preparation techniques. Last, it must be considered that all samples were collected by expert endononographers.

In conclusion, the present study demonstrates that the novel 20-G FNB needle outperforms the 25-G FNA needle in terms of diagnostic agreement, as its diagnostic superiority is not limited by the expertise and experience of the reviewing pathologist. Better sample quality and presence of histology seem to be the determinants responsible for the better diagnostic performance of the 20-G FNB needle. Together with the favorable accuracy rates from the previous ASPRO study, current findings advocate the use of the novel 20-G FNB needle in high- as well as in lower-volume EUS centers.

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CONFLICTS OF INTEREST

PRISCILLA VAN RIET is a consultant for Cook Medical. Marco Bruno is a consultant and lectures for Cook Medical and a consultant and lectures for Boston Scientific. Kenneth Chang is a consultant for Cook Medical and Olympus. Masayuki Kitano received speaking honoraria from Olympus.

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