ABSTRACT

Background The aim of this prospective multicenter study was to compare a flexible 19 G needle with nitinol shaft (19 G Flex) with a standard 22 G needle for transduodenal endoscopic ultrasound (EUS)-guided sampling of pancreatic head tumors.

Methods Patients with pancreatic head tumors requiring tissue diagnosis were randomized into two arms: puncture with either a 19 G Flex needle or a 22 G needle. The primary end point was diagnostic accuracy for malignancy. The secondary end points were ergonomic scores, sample cytohistological quality, and complications. A 6-month follow-up was performed.

Results 125 patients were randomized and 122 were analyzed: 59 patients in the 19 G Flex arm and 63 patients in the 22 G arm. The final diagnosis was malignancy in 111 patients and benign condition in 11. In intention-to-treat analysis, the diagnostic accuracy for malignancy of the 19 G Flex and 22 G needles was 69.5 % (95 % confidence interval [CI] 56.1 %–80.8 %) vs. 87.3 % (95 %CI 76.5%–94.4 %), respectively (P=0.02). In per-protocol analysis excluding eight technical failures in the 19 G Flex group, the diagnostic accuracy of the 19 G Flex and 22 G needles was not statistically different: 80.4 % (95 %CI 66.9 %–92.0 %) vs. 87.3 % (95 %CI 76.5%–94.4 %; P=0.12). Technical success was higher in the 22 G arm than in the 19 G Flex arm: 100 % (95 %CI 94.3 %–100 %) vs. 86.4 % (95 %CI 75.0%–94.0 %), respectively (P=0.003). Transduodenal EUS-guided sampling was more difficult with the 19 G Flex (odds ratio 0.68, 95 %CI 0.47–0.97).

Conclusion The 19 G Flex needle was inferior to a standard 22 G needle in diagnosing pancreatic head cancer and more difficult to use in the transduodenal approach.

Clinical.Trials.gov
NCT02078232
TRIAL REGISTRATION: Multicenter, randomized, diagnostic, prospective, interventional study
NCT02078232 at clinicaltrials.gov
Introduction

Endoscopic ultrasound (EUS)-guided sampling is used to diagnose pancreatic tumors. However, the diagnostic sensitivity is not optimal for pancreatic cancer. Pooled sensitivity was estimated to be about 85% in a recent meta-analysis [1].

When the biopsy is noncontributory, a new sample must be taken, which results in delayed treatments and possible morbidity, as well as additional costs. 19 G needles may offer advantages over 22 G needles in terms of the size and quality of the tissue sample; however, these needles are stiffer and more difficult to use, and as a result, often fail, especially when biopsy is performed with the scope in a bent position, for example in the duodenum. The only study comparing 19 G with 22 G needles showed advantages of the former only when technical failures were excluded from the analysis [2]. These technical failures occurred essentially in the pancreatic head or uncinate process tumors, which were punctured via a transduodenal approach [2].

Flexible 19 G nitinol needles have been introduced to overcome these problems. Itoi et al., in their study evaluating mechanical characteristics of various 19 G needles, suggested that the 19 G Flex needle (Boston Scientific, Marlborough, Massachusetts, USA) may be more appropriate for punctures in difficult situations, such as for transduodenal approach [3]. The evidence on 19 G Flex needle performance is limited, and randomized studies comparing 19 G Flex with 22 G standard needles are lacking. Owing to this lack of evidence, no recommendations are available in European Society of Gastrointestinal Endoscopy (ESGE) guidelines [4] or ESGE EUS clinical guidelines [5].

A preliminary prospective study on 50 consecutive patients with solid tumors of the pancreas, adenopathies or submucosal lesions, showed that cytohistological analysis was possible in all cases that used the 19 G Flex needle [6]. In this study, puncture of the pancreatic lesions via a transduodenal approach was possible without any particular technical difficulty, and the diagnostic accuracy with the 19 G Flex reached 90%, without morbidity [6]. The primary objective of our study was to compare the diagnostic accuracy for malignancy of the 19 G Flex needle vs. the 22 G needle in EUS-guided sampling of solid pancreatic head tumors, accessible only via the transduodenal approach. The secondary objectives were to compare the 19 G Flex needle with the 22 G needle in terms of technical success, ergonomic scores of the needles, cytohistological quality of the obtained samples, and immediate and delayed complications.

Methods

Patients

This study was a randomized, multicenter (18 expert centers and 21 investigators), diagnostic, prospective study, promoted by the French Society of Digestive Endoscopy, and conducted between September 2013 and August 2016. All patients signed an informed consent form. The study was approved by the French ethical committee (no: 13 15) and registered in the ClinicalTrial.gov database (NCT02078232). The results were reported in accordance with the STARD and CONSORT statements.

All patients with pancreatic head tissue tumors on computed tomography or magnetic resonance imaging requiring cytohistological evidence by transduodenal EUS-guided sampling were eligible. Exclusion criteria were cystic pancreatic tumors (fluid component of >50% of the lesion), pancreatic tumors requiring transgastric puncture, hemorrhagic diseases, hemostasis and coagulation disorders (prothrombin ratio <60%, activated partial thromboplastin time >40 seconds, platelets <60 000/mm³), and long-term use of an anti-aggregant other than low-dose aspirin or an anti-coagulant treatment that could not be suspended during the endoscopic procedure.

Randomization

Patients were randomized into either the 19 G Flex needle puncture arm or the 22 G standard needle (Boston Scientific; Cook Medical, Bloomington, Indiana, USA; Olympus Medical, Tokyo, Japan) puncture arm. Randomization was balanced (1:1) and stratified by each center. The randomization lists were performed with the block method using permutation tables (one randomization list for each center).

Course of the study

An initial diagnosis was obtained from a biopsy analysis and a final diagnosis was obtained at 6-month follow-up.

In the case of puncture failure, a crossover to the other needle was performed during the same endoscopy procedure. If cytohistological analysis was noncontributory, a second puncture was scheduled. If a diagnosis could not be obtained, a puncture under scanner control in another area (liver metastases for example) was performed.

If all these investigations were noncontributory, the clinical and radiological follow-up (up to 6 months later) helped to differentiate a benign tumor from a malignant tumor (development of metastases).

All patients were followed with a consultation at 1 month and 6 months. For the patients without pancreatic cancer, the clinical and radiological surveillance was decided at the investigator’s discretion.

Puncture procedure

The examination was performed under general anesthesia without orotracheal intubation. The choice of the therapeutic or diagnostic linear echoendoscope was decided at the investigator’s discretion.

The investigator decided on the transduodenal puncture path according to the positioning of the echoendoscope: either in the first (D1), second (D2) or third (D3) portion of the duodenum.

The sampling technique was standardized for all investigators. While passing through the duodenal wall, the stylet was held in place and could be removed in case of any technical difficulty. The aspiration was activated when the needle was in place within the tumor (20 mL syringe). The tumor was always punctured using the same protocol (2 needle passes were per-
formed with 10 forward–backward movements). The fanning technique was used whenever possible.

Aspiration was stopped before the needle was removed. The tissue sample was recovered by rinsing the needle with 5 mL of preservative solution into the BD SurePath Collection Vial (Becton Dickinson, Franklin Lakes, New Jersey, USA).

**Cytohistological preparation**

There was no cytopathologist in the examination room. The material collected during the puncture was immediately suspended in a preservative solution in a BD SurePath Collection Vial and was sent to the anatomical pathology department at room temperature, without mentioning the type of needle used. Each bottle, which contained a sample from one needle, was prepared for cytological and histological analysis.

When fragments were easily identifiable within the liquid, they were directly recovered and fixed with formalin in order to carry out a standard technique (paraffin embedding, hematoxylin-eosin-safran with or without immunohistochemistry sections and stainings). Then the specimen was centrifuged, processed, and stained by Papanicolaou method, according to the manufacturer’s recommendations on a BD PrepMate and PrepStain Slide Processor (both from Becton Dickinson). The entire residual BD SurePath preservative fluid sample was then used for paraffin cell-block preparation according to the Cytoblock process. Cell blocks were prepared with the Shandon Cytohistological preparation

8 mm blocks were cut into 3–4 mm sections and stained with hematoxylin and eosin.

The pathologists who assessed the samples were blinded to the group assignment.

**Study end points and definitions**

The primary end point was the diagnostic accuracy for malignancy. Only samples being unequivocally positive for malignancy were considered as positive. Neuroendocrine neoplasms were considered malignant. Samples that were negative, indeterminate or suspicious for malignancy were considered negative. Furthermore, samples that contained inadequate material (unsatisfactory) were considered negative for malignancy. Diagnostic accuracy was defined as the ratio between the sum of true positive and true negative cases over the total number of evaluated cases.

The gold standard for the diagnosis of malignancy was cyto/histopathology of EUS-guided sampling and/or surgically resected specimens and/or follow-up at 6 months.

Secondary outcome measures were technical success, ergonomics scores of the needles, cytohistological quality of the samples, and 30-day complication rate. The technical success was defined by the rate of successful punctures of the tumor (i.e. punctures that allowed for sufficient material to be collected for analysis). Technical failure was defined as the inability to perform the procedure, including the need to change the needle device. The ergonomics of the needle (defined as comfort of use and functional design), were evaluated by three visual analog scales (VAS) allowing calculation of three ergonomic scores. The first VAS evaluated the ease of puncture (i.e. the ease of reaching and penetrating the tumor). The second VAS evaluated the ease of stylet removal, and the third VAS evaluated the ease with which the needle could be moved forward and backward.

Criteria to define cytohistological qualities of the samples were based on a reference scoring system (Mair score) [7] that evaluated the following: the amount of diagnostic cellular material present; the retention of appropriate architecture and cellular arrangement; the degree of cellular degeneration; the degree of cellular trauma; and the volume of obscuring background blood or clots (contamination). Each cytological and histological score was then reported on two VAS by the cytopathologists to assess the global cytological and histological qualities of the samples.

Complications were assessed from per-procedure up to 30 days post-procedure. All patients were called the next day after the procedure by the study nurse to assess early complications. Patients were seen either during consultation at 1 month or were recalled by the investigators. The severity of complications was graded according to the American Society for Gastrointestinal Endoscopy lexicon criteria [8].

**Statistical analysis**

In accordance with a 19 G diagnostic accuracy of 92.1 % in the study of Varadarajulu et al. [6], we hypothesized that the 19 G Flex needle would improve the diagnostic accuracy by about 20%, from 70% to 90%.

Considering a threshold \( \alpha = 0.05 \) and a statistical power of 80%, a minimum of 124 patients (62 in each arm) should be included in order to statistically demonstrate this difference.

Continuous variables were reported using medians, inter-quartile range (IQR), and categorical variables using counts and percentages; 95% confidence intervals (CI) of medians (Hahn and Meeker distribution-free method) and percentages were also reported for continuous and categorical variables, respectively [9]. Diagnostic accuracy was analyzed using intention-to-treat (ITT) analysis (modified full analysis set excluding patients who were randomized but biopsy was not attempted for unrelated reasons) and per-protocol analysis (excluding patients in whom biopsy with the assigned needle failed due to technical problems).

Independent factors discriminating 19 G Flex and 22 G groups were assessed using univariate analysis, which was followed up with a multivariate analysis. In univariate analysis, crude comparisons regarding categorical variables were carried out using the chi-squared test or the Fisher test. Normality of continuous variables was assessed using Shapiro-Wilk; as none of the continuous variables was normally distributed, crude comparisons regarding continuous variables were made using the nonparametric Wilcoxon test. Multivariate logistic regression analysis with stepwise selection was carried out on variables that were significant in the univariate analysis: stepwise selection significance level for entering variables into the model was 0.05, and significance level for the variable to be kept in the model was 0.10. The results of the multivariate analysis are presented as odds ratios (OR) with 95% CIs.
All tests were two-sided, with a significance level of 0.05. Calculations were performed using SAS V9.4 software (SAS Institute Inc., Cary, North Carolina, USA).

**Results**

A total of 128 patients were prospectively enrolled in the study. Among the 18 expert centers selected throughout France, 13 were active; the median number of cases included by participating center was 5 (IQR 3–11; 95% CI 3–16]. One patient refused to participate and two patients were excluded because of coagulation disorders. One patient did not have a puncture because of a pancreatic cystic lesion (>50%), one patient had duodenal stenosis, and one patient died from unrelated reasons (stroke or hemorrhage) 3 months after biopsy.

A total of 125 patients were randomized and 122 (59 in the 19 G Flex arm and 63 in the 22 G arm) were included in the ITT analysis (modified full analysis set excluding the 2 randomized patients in whom biopsy was not attempted for unrelated reasons such as cardiovascular event or duodenal stenosis and excluding the patient who died from stroke 3 months after biopsy because of too short follow-up and no final 6-month diagnosis). After excluding 8 patients with technical failure, 114 were included in the per-protocol analysis (Fig. 1).

There was no statistically significant difference between the groups concerning age, sex, tumor size, nature, and location, echogenicity of the suspected tumor, or intra-and peri-tumoral vascularization (Table 1).

All patients were punctured via a transduodenal approach using three different puncture paths: D1 (58.7%), D2 or D3 (41.3%). Technical success was higher in the 22 G arm compared with the 19 G Flex arm: 100% (95% CI 94.3–100%) vs. 86.4% (95% CI 75.0–94.0%), respectively (P=0.003).

---

**Fig. 1** Flowchart with immediate and 6-month follow-up results. CP, chronic pancreatitis; AIP, autoimmune pancreatitis; FN, false negative; TN, true negative; FP, false positive; PC, pancreatic carcinoma.
Final diagnosis

The final diagnosis at the 6 month follow-up included malignancy in 111 cases and benign condition in 11 cases. All malignant cases were pancreatic cancer; no cases of neuroendocrine tumors or pancreatic metastases were diagnosed.

The biopsy provided a diagnostic sample in 101 patients (44 in the 19 G Flex and 57 in the 22 G group), was nondiagnostic in 13 patients (7 and 6 patients, respectively), and failed due to technical reasons in 8 cases (all in the 19 G Flex group). The results are shown in ▶ Table 2 and ▶ Fig. 1. Among the 101 patients with diagnostic samples, the final diagnosis at 6 months was concordant with biopsy diagnosis in 96 patients; there were 4 false-negative biopsy results (2 in each group; confirmed by surgery, metastasis diagnosis or second EUS-FNA), and one false-positive result (19 G Flex group; autoimmune pancreatitis confirmed by surgery). Among the 13 patients with nondiagnostic biopsy, pancreatic cancer was eventually diagnosed in 10 cases by subsequent surgery, metastases biopsy, biliary cytology or second EUS-FNA. No malignancy was confirmed in the remaining three cases and they were categorized as benign. Among the eight patients with technically failed biopsy, pancreatic cancer was diagnosed in all cases by cross-over EUS-FNA using the 22 G needle or subsequent biopsy of liver metastasis.

Diagnostic accuracy for malignancy

The diagnostic accuracy for malignancy of the 19 G Flex needle for transduodenal puncture of a pancreatic tumor by ITT analysis was less than that of a standard 22 G needle: 69.5% (95%CI 56.1%–80.8%) vs. 87.3% (95%CI 76.5%–94.4%) respectively (P=0.02) (▶ Table 3). However, in per-protocol analysis, the diagnostic accuracies for malignancy of the two needles were not statistically different: 80.4% (95%CI 66.9%–90.2%) vs. 87.3% (95%CI 76.5%–94.4%) for 19 G Flex and 22 G, respectively (P=0.12) (▶ Table 3).

Cytohistological quality of specimens

Cytological and histological qualities were not statistically different between 19 G Flex and 22 G needles (▶ Table 4).

Furthermore, the 19 G Flex needle did not allow for a better histological diagnosis than the 22 G needle, with a 14% rate of adequate samples for histological evaluation for 19 G Flex vs. 13% for 22 G (P=0.62).
Needle ergonomic scores
The ergonomic scores of the standard 22 G needle were statistically higher than those of the 19 G Flex needle (Table 4). Median scores for ease of puncture, removal, and forward—backward movement were inferior with the 19 G Flex (P < 0.001) (Table 4).

In multivariate analysis, better scores for ease of removal and forward—backward movement were more closely associated with the 22 G needle puncture (Table 4): respective ORs for 19 G Flex needle compared with 22 G needle puncture were 0.65 (95%CI 0.47–0.91; P = 0.01) and 0.65 (95%CI 0.47–0.90; P = 0.01).

Technical difficulties (needle/scope positioning, needle removal or fanning) were observed in 17/58 patients (29.3%) with 19 G Flex vs. 7/63 (11.1%) with 22 G.

Complications
Few complications occurred within 30 days of EUS-guided sampling. In the 19 G Flex arm, nine patients experienced minor adverse event: hyperthermia, gastrointestinal hemorrhage with transient melena, acute benign pancreatitis, and transient abdominal pain. In the 22 G arm, four patients experienced minor adverse events and one patient had a major adverse event (pancreatic abscess requiring prolonged antibiotic therapy).

There was no statistically significant difference in the complication rates between the two arms (Table 4).

Discussion
This study is the first prospective randomized study to compare the performance of the 19 G Flex needle with a standard 22 G needle in transduodenal EUS-guided sampling of pancreatic tumors. Our results showed that the diagnostic accuracy of the 19 G Flex needle was inferior to the 22 G needle in ITT analysis, but comparable to the 22 G needle in per-protocol analysis. Furthermore, the ergonomic scores of the 19 G Flex needle were significantly lower than those of the standard 22 G needle.

Transduodenal EUS-guided sampling with a standard 19 G needle is technically challenging, with a large number of failures related to the stiffness of the needle [4]. It is usually recognized that this needle should not be used for the transduodenal approach. On the other hand, it has been suggested that transduodenal sampling with a 19 G Flex needle is easier because of its softer nitinol shaft. A prospective study showed a 100% rate of technically successful punctures [6]. Our study confirmed the possibility of transduodenal EUS-guided sampling with a 19 G Flex needle (86% technical success rate) but the technical difficulties remained frequent (29%); the failure rate was 14%. Puncture failures were related to the stiffness of the needle. In some cases, it was impossible to correctly position the needle or the endoscope to be able to puncture the tumor (Fig. 2, panel A) or to bypass intervening vessels. More importantly, in all cases in which the 19 G Flex needle failed, the mass could be punctured successfully with the 22 G needle (Fig. 2, panel B).
In these cases the diagnostic accuracy of the 22 G needle was 57%. Our study does not confirm the excellent results (100% technical success rate of the 19 G Flex needle) reported by Varadarajulu et al. [6]. The technical success rate in our study was 86%, which was similar to the 80% technical success rate of transduodenal punctures using a standard 19 G needle in the study by Song et al. [2].

The main advantage of using a 19 G needle is to improve the quality and quantity of the sample (a larger caliber needle can collect more tissue for cytohistological analysis) and therefore obviate the need for on-site cytopathology. In the absence of a cytopathologist, a reduction in diagnostic accuracy can be observed with EUS-FNA but not with EUS-guided fine-needle biopsy [10]. Although an immediate analysis by an on-site cytopathologist improves the quality of the sample, it can increase the time and cost of the procedure. In France, a cytopathologist is never in the room.

In our study, the sample quality collected with the 19 G Flex needle was not higher than that of the 22 G needle. In order to increase tissue quality and quantity it is important not to puncture the same area of the tumor several times, but rather to deploy the needle into different points in the tumor and to avoid necrotic areas. Needle stiffness and difficulties in needle/scope positioning, needle removal, and fanning, which were encountered in 29% of cases sampled with the 19 G Flex needle, could explain a lower quality of the sample.

It would seem that changing the shape of the needle with a core trap, could improve the quality of the sample for histological analysis [11]. However, needles with a core trap would not improve the diagnostic efficiency compared with a standard needle. The main advantage of these needles would be to establish the diagnosis with fewer punctures [12–15].

A novel fork-tip, SharkCore needle (Medtronic, Minneapolis, Minnesota, USA), could improve the quality of sampling for histological analysis with fewer punctures compared with the EUS-FNA [16]. Although the histological analysis with this needle was more often possible compared with a standard needle (59% vs. 5%; P<0.001), the diagnostic performance did not seem to be improved [17]. Recently, the Franseen needle design (Inrad Inc., Grand Rapids, Michigan, USA), which has a crown-tip with three symmetrical surfaces acting as three cutting edges, allows for a longer insertion length and wider area at the crown tip, thus facilitating greater tissue acquisition [18]. Two recent studies compared these two new needles for fine-needle biopsy, the diagnostic performance seemed comparable, with a possible histological analysis in over 90% of the cases [19, 20].

The main limitation of the current study lies in the lack of centralized pathological reading, which may have caused measurement bias in the cytohistological evaluation. Moreover, the patient distribution in the centers was heterogeneous, with two centers contributing more than half of the total study population. Finally, these two centers (the most experienced ones), had only two puncture failures with the 19 G Flex needle.

An important bias of the study is linked to the 6-month follow-up period. This monitoring period may be too short to detect pancreatic cancer in some patients without evidence of malignancy on biopsy. However, only a small number of such cases was present in each arm, and therefore the impact in case of error should be minimal.

Finally, although P values for secondary outcomes should be adjusted for multiple comparisons, no corrections were made, potentially leading to inflation of the alpha risk error.

Despite these possible biases, the study was conducted in accordance with the current practice of EUS-guided sampling in France.

In conclusion, transduodenal EUS-guided sampling of pancreatic head tumors is possible with a 19 G Flex needle. In ITT
analysis, the 19 G Flex needle was inferior to a standard 22 G needle in diagnosing pancreatic head cancer and was more difficult to use in the transduodenal approach.

Acknowledgments

We thank the patients and nurses for their important contributions to this study, Terri Galli for the English review of the article, and Laurence Curel for medical writing.

Competing interests

None

References