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OFFICIAL TITLE OF THE STUDY

Stylet Slow-Pull Technique Compared To Standard Suction For Endoscopic Ultrasound-Guided Fine-Needle Biopsy In Pancreatic Solid Lesions: Data From A National Multicenter Randomized Trial.

LAST APPROVED VERSION OF THE PROTOCOL

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INTRODUCTION

Since its introduction 27 years ago, endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) has revolutionized the diagnostic approach to pancreatic masses and has become the first diagnostic test to establish a correct diagnosis in patients with suspicious pancreatic solid lesion. EUS‑FNA represents now the standard of care for the pathological diagnosis of solid pancreatic masses, and has a sensitivity and a specificity of 85%–89% and 96%–99%, respectively. Needles of different size have been used, ranging from 19 Gauge to 25 Gauge, despite the strongest evidences is for the 22 and the 25 Gauge needles. The availability of rapid on-site evaluation (ROSE) to assess specimens' adequacy by a cytopathologist during EUS‑FNA improves diagnostic accuracy of about 10 to 15%, but high costs and the lack of availability of ROSE in many centers, even the referral ones, have limited the widespread utilization of the EUS-FNA procedures. For these reasons in recent years, new needles have been designed (i.e. cutting tip or a side‑slot in the distal portion of the needle) to obtain samples suitable for histological evaluation (endoscopic ultrasound-guided fine needle biopsy [EUS-FNB]). These new needles allow the macroscopic on‑site evaluation (MOSE) of the quality of samples by the endoscopist, who can decide whether or not to perform additional biopsies. Various EUS-FNA techniques have been described, but the correct methods remain debatabl. Most of the studies have compared EUS-FNA with standard suction (10-20 ml negative suction pressure) versus the "slow-pull" EUS-FNA technique (pulling the stylet while moving the needle within the lesion), but final results were conflicting and ranged from clear advantage of the slow-pull approach to no difference between the two techniques. In the studies that showed advantages of the slow-pull technique, most of the gain was related to a significant decrease of the bloodiness of the specimens, which made the cytological reading easier to be accomplished as compared to more bloody specimens obtained with the suction technique. The interference of bloodiness with the cytological evaluation is a well-known phenomenon. On the other hand, data on the amount of blood gathered during EUS-FNB performed using the two different techniques and its interference with the performance of both slow pull and standard suction techniques in patients with pancreatic solid lesions are limited. The aim was to design a randomized controlled study to compare the amount of bloodiness of the specimens acquired by the slow-pull technique with those gathered by using the standard suction technique during EUS-FNB in patients with solid pancreatic masses. Secondary aims were the effect of blooding on the performance of the two techniques with regards to sensitivity, specificity, and diagnostic accuracy.

Materials and methods

A Randomized, multicenter study comparing the slow-pull technique with the standard suction technique during EUS-FNB in patients with pancreatic solid lesions. The study involved three referral centers for pancreatico-biliary diseases: the Gastroenterology and Endoscopy Unit. ARNAS Civico-Di Cristina-Benfratelli Hospital, Palermo, Italy; the Gastroenterology and Endoscopy Unit. Marche Polytechnic University, Ospedale A. Murri, Fermo, Italy; the Gastroenterology Unit. AUSL Bologna, Bellaria-Maggiore, Bologna, Italy.

The protocol was approved by the institutional medical ethical committee at each participating institution and written informed consent was obtained from all patients for participation in the study.

All consecutive patients with a newly diagnosed pancreatic solid lesions suitable for EUS-guided tissue acquisition were considered for enrollment. Inclusion criteria were: age >18 years old; solid pancreatic lesion on imaging MRI and/or CT-scan referred for tissue acquisition; lesion that can be visualized with EUS and needle puncturing can be technically feasible; able to sign informed consent. Patients were excluded in the presence of at least one following criteria: <18 years-old, pancreatic cystic lesions, extra-pancreatic lesions or inaccessible/non-visualizable lesions, previous gastrectomy, international normalized ratio > 1.5, impossibility to suspend anticoagulant therapy, platelet count < 50.000 cells/cubic millimeter, severe or unstable clinical conditions that contraindicated EUS procedure, pregnancy, inability to give informed consent, and refusal to participate to the study. Written informed consent was obtained from all patients before the procedure.

All the EUS procedures were carried out by A skilled endosonographer with the patients under conscious sedation or deep sedation according to the anesthesiological guidelines approved in each participating center. All EUS-FNB were done using a 20 Gauge needle (EchoTip ProCore 20G with ReCoil Stylet™, Cook Medical, Bloomington, IN, USA). The ReCoil Stylet´s has an automatic recoiling capability designed to help users to manage more easily the stylet, thus minimising the risk of contamination. Before puncturing, color doppler examination was performed to exclude the presence of interposing vessels. The needle tip was sharpened by withdrawing the stylet of approximately 2 mm, and then it was advanced into the lesion under real-time EUS guidance. In patients randomized to the stylet slow-pull techniques, 15 to-and-fro movements within the lesion were performed while gently pulling the needle stylet slowly and continuously upwards. In patients randomized to the standard suction technique, 15 to-and-fro movements within

the lesion were performed using a 10-mL suction syringe. In both sampling procedures the fanning technique was used to maximized sample acquisition. Tissue samples were expelled onto slides by reinserting the stylet and the visible core was physically lifted off the slides placing it into a formalin vial for histological evaluation. For the macroscopic adequacy, the endosonographer evaluated for the presence of a visible core defined as a worm-like material whitish/yellowish or red, not including fluid-like specimens in the formalin vial, which was judged to be adequate to perform a pathological analysis. The pathologists were "blinded" on which group the vials received were belonging to.

Randomization was performed using random sequences generated by a computer and then closed in consecutive numbered envelopes.

Data on patients' and lesions' characteristics, degree of the blood contamination of the specimen, sensitivity, specificity and diagnostic accuracy of the two techniques; adverse events related to the procedures were collected and entered in an electronic database in order to carry out the final statistical analysis.

Outcome measures

The primary end point of the study was the degree of blood contamination of the collected specimens in patients with pancreatic solid lesions who underwent EUS-FNB using the 20Gauge Procore Needle and the slow pulling or the standard aspiration techniques. Secondary outcomes were technical success, comparison of the performance of the two techniques during EUS-FNB in terms of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy, and occurrence of adverse events.

Outcome Definitions

The degree of blood contamination of the specimen was based on the pathologist evaluation of the formalin fixed and paraffin embedded (FFPE) tissue samples and defined as significant when a large amount of blood cells made pathological diagnosis difficult to be made. On the other hand, blood contamination of the specimen was defined as not significant when no or only few blood cells were present without any influence on the pathological diagnosis.

Sensitivity was defined as the true positive rate whereby the test was the final cytologic diagnosis, Specificity as the true negative rate (proportion of actual negatives that are correctly identified as such), PPV was defined as the number of true positive divided the number of positive calls (true positive and false positive), NPV was defined as the number of true negative divided the number of negative calls (true negative and false negative). Diagnostic accuracy was defined as the ratio between the sum of true positive and true negative values divided by the total number of masses.

Technical success was defined as the capability of sampling the target lesion associated with the presence of a visible core, according to endosonographer's judgment, potentially useful for the final pathological analysis.

Adverse events were defined based on the criteria expressed by Cotton et al. [Cotton PB. Gastrointest Endosc 2010;71:446-54]

Samples positive for malignancy were considered diagnostic for malignancy, while in patients with negative EUS-FNB, surgical specimen evaluation, results of other diagnostic investigations and/or a clinical follow-up of at least 6 months were used to establish the definitive diagnosis.

Statistical Analysis

The sample size was calculated based on the results of a previous study [Nakai Y. Dig Dis Sci. 2014;59:1578-85] that showed that blood contamination of the specimen was lower with the stylet slow-pull technique as compared with the standard suction technique (25% vs 70%), with a consequent increase in the diagnostic accuracy. Based on these data we calculated for a difference of 25%, at a power of 80% and an alpha of 0.05 (two-sided test), a final sample size of 110 patients (55 patients in each group). Continuous variables will be reported as mean ± standard deviation (SD). Categorical variables will be presented as numbers and percentage and will be compared either using the chi-square test (with Yates' correction when appropriate) or Fisher exact test.

Statistical was conducted using the Fisher's exact test for categorical variables and the Mann–Whitney U-test for continuous variables. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy for each arm were investigated by comparing the results with the definitive diagnosis. Statistical tests were considered

significant at a corresponding p value of <0.05. Data handling and analyses were done with SPSS 14.

RESULTS

From May 2017 to May 2018, a total of 182 consecutive patients were screened for participation in this study, of whom 72 were excluded, while 110 patients constituted the study cohort and were randomized to one of two groups: stylet slow-pull technique (55 patients) and standard suction technique (55 patients) as showed in figure 1. Overall, there were 49 male (44.5%) and 61 female (55.5%), with a mean age of 70.9±11.3 years. Patients' and lesions' characteristics are shown in Table 1 and appeared similar in both groups. The majority of lesions were located in the head of the pancreas (72 lesions corresponding to 65.5%) with similar rates in slow-pull group (69%) and in the standard suction group (62%). No difference in mean size of the lesions was found between the two groups(35.9±19.9 vs 37.6± 14.1 cm; p=ns).

Technical success was achieved in 95% of patients (slow pull technique 96% vs standard suction technique 94%, p=ns) with a not-significant blood contamination of the specimen in 85 patients (overall 77.3%: slow-pull 80% vs standard suction 74%, p=ns). A diagnosis of malignancy was obtained in 98 cases as showed in Table 2. Fifty were in the slow-pull group and 48 in the standard suction group. In 6 patients (1 in the slow-pull and 5 in the standard suction group) with a benign histological diagnosis at EUS-FNB the definitive diagnosis was chronic pancreatitis. Thus, these patients were considered true negative. Finally there were 6 false negative cases (4 in the slow pull technique group and 2 in standard suction group). Comparing the two groups (Table 3), the sensitivity, specificity, negative likelihood ratio, positive likelihood ratio, and diagnostic accuracy were 96 vs 93%, 100% vs 100%, 0.04 vs 0.07, "infinity" in both, and 96 vs 93%, respectively without any statistical difference between them. No procedure-related adverse events were observed in the two groups.