Comparison of three different tissue acquisition techniques during endoscopic ultrasound-guided fine needle biopsies of solid lesions: A randomized single blind clinical trial.

ABSTRACT

a. Endoscopic ultrasound guided fine-needle aspiration (EUS-FNA) has been used since 1990's for the diagnosis and staging of esophageal, gastric, duodenal, pancreatobiliary, rectal mediastinal lesions and intra-abdominal lymphadenopathy. Studies have shown a variable range of specimen adequacy when performing pancreatic biopsies with the standard fine needle aspiration (FNA) needles with this modality. There are several factors that affect the overall diagnostic yield of this procedure, such as endosonographer experience, presence of cytopathologist during the procedure, the needle diameter and the number of passes. In this study we will compare the yield of recently available fine biopsy needles (FNB) using three different techniques to obtain samples from solid lesions. All solid lesions will be studied with the endoscopic ultrasound. If during the procedure the characteristics of the lesion are comfortably determined and it is decided that it does not need a biopsy, then it won't be done (i.e. lipomas). These lesions are very well determined on EUS and do not need a biopsy. The three techniques to be compared in this study are: stylet slow pull (SP) vs dry suction (DS) vs wet suction (WS).

OBJECTIVES

The primary objective of this study is to compare the rate of adequacy for cytologic and/or histologic diagnosis of specimens obtained using three different techniques of tissue acquisition when performing EUS guided sampling of solid lesions with FNB needles. The three techniques to be compared are: SP, DS and WS. The secondary objectives are to compare the rate of blood contamination of the specimen obtained by each technique

BACKGROUND

Endoscopic ultrasound guided fine-needle aspiration (EUS-FNA) has been used since 1990's for the diagnosis and staging of esophageal, gastric, duodenal, pancreatobiliary, rectal and mediastinal lesions and intra-abdominal lymphadenopathy [(1)]. Studies have shown a variable range of specimen adequacy when performing pancreatic biopsies with the standard fine needle aspiration (FNA) needles with this modality [(2,3)]. When sampling the lesions, the use of a stylet and/or suction during FNA of solid lesions has been used to increase the accuracy, efficiency and quality of the specimen. Theoretically, the use of a stylet prevents obstruction of the needle lumen as it penetrates the gastrointestinal wall tissue, resulting in less contamination from GI wall cells [(4)].

In the "suction technique" the stylet of the needle can be left in place or removed before puncturing the lesion. Once the needle is inside the target, negative pressure is applied through a 10 or 20 cc syringe connected to the needle. This technique has yielded adequate specimens in

approximately 70% of cases in some studies, with no difference seen between the stylet vs no stylet modality [5, 6]. Some alterations of this technique have been used. In cases of neuroendocrine tumors especially, no suction techniques have been tried to avoid the effect of dilution by too much bleeding in such highly vascular tumors [7]. In a recent study on 52 patients [8], the accuracy was higher utilizing the suction technique compared to no suction. The bloodiness of the samples was not different in each arm. The lesions were located in the mediastinum, pancreas, adrenals and others. Another study on 100 patients in 2014 did not report any difference in the adequacy of samples obtained with or without suction [9]. The samples were more contaminated with blood in the suction group though. It has been concluded that at the expense of a bloodier sample, suction method yields a high cellular material [10]. The wet suction technique consists of flushing of the needle with 5 ml of saline solution to replace the column of air within the lumen of needle with saline solution before needle aspiration. Once the needle is flushed, negative pressure is applied with a 10 or 20 cc syringe connected to the needle. This novel technique was compared with the conventional FNA technique by using 22-gauge needles [11]. A total of 117 samples from 95 patients and 72 lesions were obtained in an alternating pattern between the two techniques. If the first pass was done by wet suction then the second one was done by the FNA conventional method. Higher cellularity scores and adequacy of specimen were achieved with wet suction (68% vs 44%, respectively) and (85% vs 75, respectively). However, the diagnostic yield difference was not specified.

In the slow pull technique, the stylet is left in place in the needle and is slightly retracted prior to puncturing the lesion. Once the needle is inside the target, the stylet is pushed completely into the needle to remove any contaminant cells and several back and forth movements are done while slowly withdrawing the stylet. One study compared slow pull technique versus suction [12]. Although scores of cellularity were lower in the slow pull group (A score of more than or equal 2 in 37.5% vs 76.7%), the contamination of samples observed was lower (25% vs 66.7%). The accuracy and sensitivity of the slow-pull technique were significantly higher than those of the suction techniques for solid pancreatic lesions [13]. Specifically for malignancy, the slow pull technique has demonstrated sensitivity for diagnosis of 90% compared to 68% with the suction technique. (12) Moreover, this technique showed less false negatives results than suction (11 vs 26%) [14 ABSTRACT].

Recently new fine needle biopsy (FNB) needles have become widely available in the US. These needles differ from FNA needles in its design and allow for long cores of tissue to obtained. Recent studies have already suggested that samples obtained with these needles are superior to the ones obtained with standard FNA needles [15]. Given the novelty of these FNB needles, literature regarding different techniques of tissue acquisition with them is scarce. Therefore, in this study we aim to compare the cellularity of specimens obtained with these needles using the three different techniques described above in a randomized fashion.

RESEARCH PLAN

Study Design

This study is planned as a single–blind, parallel group randomized clinical trial (RCT). After receiving consent from the eligible subjects, the subjects will be randomly assigned to one of the three techniques and one of two needles, stratified by the three (3) endoscopists performing EUS-FNB.

Primary endpoint

1. Compare the rate of adequate specimens obtained by slow pull vs dry suction vs wet suction techniques using the new fine needle biopsy (FNB) needles in solid lesions.

Secondary outcome

1. Compare the degree of blood contamination obtained by all three techniques.

Inclusion criteria

b. Ages 18 years and older
c. Male or female
d. Patients who require EUS and tissue sampling of solid lesions (size >1 cm) anywhere in the body: lymph nodes, stomach, esophagus, colon, small intestine, pancreas, liver, spleen or kidney.
e. Patients who are able to give consent

Exclusion criteria

a) Pregnant female
b) Coagulation disorders (platelets < 50,000/mm^3, INR > 2)
c) Patients with acute pancreatitis in the immediate 2 weeks prior to the procedure (if the lesion to be biopsied is in the pancreas). .
d) Cardiorespiratory dysfunction that precludes sedation.
e) Unable to provide informed consent
f) Previous chemotherapy or radiotherapy for pancreatic neoplasm

Patient Enrollment: Patients will be prospectively enrolled until the target sample size has been reached. This study is planned as a single-blind, parallel group randomized trial. There will be three parallel groups. The first group will be assigned to the slow pull technique, the second one to the dry suction technique and the third one to the wet suction technique. Our calculated sample size is 300 patients. In our unit, we perform approximately 10 – 15 EUS guided pancreatic mass biopsies per month. Therefore, we expect to enroll the desired number of patients in approximately 60 months.

Data Collection:

For each patient, the following data will be collected:

1. Age
2. Gender
3. Ethnicity
4. EUS characteristics of the lesion (number, location, size, echogenic pattern, invasion into other structures)
5. Number of needle passes
6. Other maneuvers if 3 passes yields an inadequate specimen
7. Immediate complications (bleeding, perforation, hemodynamic instability)

Statistical analysis

Sample size: The sample size computation was done on the basis of published data [5, 6, 11, 13]. A previous study [11] showed that Wet suction technique produced moderate to high cellularity (67.5%) compared to conventional FNA technique (44.4%). Another manuscript demonstrated that slow-pull technique had higher moderate to high cellularity (71%) as compared to suction technique with 5-mL (57.9%) [13]. Other studies [5, 6] reported cellularity between 46% and 73.6%. Based on this prior information, we assumed that one of the techniques will yield at least 25% higher cellularity compared to the baseline 45%. Based on this, a sample size of 95 per group will be sufficient to detect significant difference in at least one pair of techniques with more than 80% power and at 1% level of significance using a two-sided Z-test with pooled variance. Further, we also validated sample size computation for comparing average cellularity score across three groups. A study reported average cellularity score by dry technique as 1.85 (SD: 0.79) and by WET as 1.70 (SD:0.72) compared to average 1.45 (SD: 0.78) [11]. Using this information, a sample size of 100 per group will be sufficient to detect significant difference in average cellularity scores among groups using a one way analysis of variance (F-test) with 80% power and at 1% level of significance. The level of significance was adjusted due to multiple comparisons in both calculations. This sample size seems to be appropriate for detecting significant differences in other outcomes (such as diagnostic yield) as well. The sample size computation was carried out using PASS 14 (PASS 14 Power Analysis and Sample Size Software (2015). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass.).

Statistical Analysis: All categorical variables will be described using count and percentage whereas the quantitative variables will be described using mean and standard deviation(SD). The baseline characteristics will be compared among three groups using either one way analysis of variance (for quantitative data) or Fisher's exact tests (for categorical data). The primary outcome (cellularity score: quantitative and categorical forms and percentage positive diagnostic yield) will be compared between groups using an F-test or Fisher's exact tests. The proportion differences between two groups will be compared using two sided Z-test between two groups

while quantitative scores will be compared using t-tests with Bonferroni corrected method. The effect size will be summarized using proportion difference along with 95% confidence interval (CI) estimated using Binomial distribution. Further, relative risk regression analysis [16] will be performed for determining adjusted differences in cellularity and diagnostic yields between groups. The regression analysis will be carried out separately for each outcome. This analysis will also help to identify any factors associated with increased percentage of cellularity and diagnostic yield. The secondary outcomes will also be compared between groups using a Fisher's exact test or an F-test depending on the type of outcome. The results of relative risk regression analysis will be summarized using relative risk along with 95%CI and p-value. P-values less than 5% will be considered statistically significant results. All the statistical analyses will be processed using STATA 14 and SAS 9.4.

METHODS

Patients will be enrolled in a prospective manner at the University Medical Center of El Paso endoscopy unit. At the time of the pre-op appointment the nurse will perform the usual evaluation of the patient and will explain the EUS/FNB procedure. The day of the procedure, the endoscopist performing the procedure explain the EUS/FNB procedure and the risks of the procedure, explain the study and will consent the patients. He will randomize them using a random number list generated by a simple block randomization scheme by our study statistician, to one of three possible groups: SP, DS or WS. The randomization number will be kept in sealed opaque envelopes in numerical sequence. The random number sequence will be generated using SAS software. The patients will be asked to sign the consent only after the study has been explained to them and all their questions have been answered by the performing physician. The randomization will be via sealed envelopes that will contain cards with the technique and the needle to be used. Following randomization each patient will be assigned a code corresponding to one of the three technique arms. This code will be noted and placed in a master spreadsheet that only the study coordinator and/or assigned research personnel will handle.

All the procedures will be performed by three experienced endoscopists (Cesar Garcia, M.D., Sherif Elhanafi, M.D. and Antonio Mendoza-Ladd, M.D.) using 22 gauge SharkCore™ (MEDTRONIC) or Acquire™ (BOSTON SCIENTIFIC) FNB needles. The techniques to be used are described here:

DS: the stylet will be left in place in the needle and once the lesion has been punctured, the stylet will be withdrawn. After this, negative pressure with a 10 cc syringe will be applied to the needle. Once the lesion has been punctured, the valve between the syringe and the needle will be opened and the endoscopist will obtain the sample with multiple back and forth movements.

SP: the stylet will be left inside the needle and retracted slightly prior to puncturing the lesion in order to sharpen the needle. Once the lesion has been punctured, the stylet will be pushed completely into the needle to expel any debris from the gastrointestinal wall, then it will be

slowly withdrawn from the needle as the endoscopist is making the back and forth movements to create negative pressure in the system.

WS: Prior to puncturing the needle, the stylet will be completely withdrawn and the needle will be flushed with 10 cc of normal saline until the entire needle lumen is completely primed with fluid. After this, negative pressure with a 10 cc syringe containing 1 cc of NS will be applied in a similar manner as in the DS technique. The procedures will be performed under deep sedation according to the principles of monitored anesthesia care. The patients will be anesthetized with intravenous administration of propofol by trained anesthesists. Once the lesion is evaluated by EUS, the endoscopist will select the shortest pathway, while avoiding blood vessels, to reach the pancreatic lesion. Under real-time visualization, each lesion will be punctured with 3 needle passes.

If the tissue sample is deemed suboptimal after these 3 passes, then the endoscopist will be allowed to use a different needle and/or technique according to his preference. The specimen obtained will be assessed preliminarily by the cytology technician who will determine if more tissue is needed. Once the tissue is deemed to be sufficient, the pathologist evaluating the specimen will dictate the official cellularity and blood contamination scores according to scales previously described by Gerke et al [17] and Wee et al [18]. These will be the scores used for analysis. A score of ≥ 2 and ≥ 4 will be considered adequate for cytological and histological diagnosis respectively. The pathologists evaluating the sample will be blinded to the type of needle and technique used.

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APPENDIX

score	Explanation
0	Insufficient material for interpretation
Cytology 1-2	
1	Sufficient material for limited cytological interpretation; probably not representative
2	Sufficient material for adequate cytological interpretation
Histology 3-5	
3	Sufficient material for limited histological interpretation
4	Sufficient material for adequate histological interpretation, low quality (total material < 1 10× power field in length)
5	Sufficient material for adequate histological interpretation, high quality (> 1 10× power field in length)