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When high viscosity of pancreatic cysts precludes effective EUS-FNA: a benchtop comparison of negative pressure devices

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ABSTRACT

Background and study aims Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) of pancreatic cystic lesions (PCLs) is an important diagnostic tool; however, it is often unsuccessful due to high viscosity of cystic fluid. In an effort to improve FNA, we objectively compared eight vacuum device configurations to determine the most effective method for aspirating viscous fluid collections. We also tested a high-frequency oscillation (HFO) technique that could be employed in FNA.

Materials and methods Maximum gauge pressures of four vacuum devices were measured: two standard EUS-FNA syringes, a 50-cc Alliance II device, and a nonmedical hand vacuum pump. To aspirate a viscous stock solution, 19-gauge and 22-gauge needles were used and flow rates were calculated. HFO was also applied to the needle during aspiration to determine effect on aspiration rate.

Results Aspiration devices generated maximum gauge pressures ranging from –21.5 to –27.5 inHg. The 19-gauge FNA needle aspirated viscous fluid 11.3 × faster on average than a 22-gauge needle. HFO increased average flow rates by 29.7% in 19G and 124.6% in 22G configurations.

Conclusion EUS-FNA of viscous fluid can be optimized by using the lowest possible gauge needle and connecting a vacuum device capable of generating and sustaining near perfect vacuum. This can be accomplished by maximizing syringe volume. In addition, connector-tubing length between the syringe and needle should be minimized, and tubing wall should be sufficiently strong to resist collapse under vacuum. Other novel techniques to increase fluid yield include a hand vacuum pump and application of HFO to FNA.

Introduction

One strategy for early detection of pancreatic cancer may be through identification and treatment of pancreatic cystic lesions (PCLs) that have malignant potential. PCLs have become increasingly identified as incidental lesions on imaging in recent years, and their evaluation is important to identify premalignant lesions, including mucinous PCLs, solid pseudopapillary tumors, and cystic neuroendocrine tumors [1]. PCLs have a reported prevalence of 1.2% to 25% with prevalence significantly increasing with age, thus rendering them a significant public health issue [2 – 4].

PCLs are often first discovered incidentally on abdominal ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI); however, these imaging modalities cannot adequately distinguish lesions that have malignant potential.

Endoscopic ultrasound (EUS) has proven to be more beneficial in classifying PCLs not only because of its higher resolution but also its ability to allow for direct cystic fluid sampling through fine needle aspiration (FNA) for biochemical, cytological, DNA, and tumor marker analysis. Malignancy within a cystic neoplasm can be identified by cytology with 83% to 99% specificity, although reported sensitivities vary from 25% to 88% [3]. Because of the limited sensitivity of cytology, cyst fluid can be biochemically analyzed for amylase, lipase, and tumor markers such as carcinoembryonic antigen (CEA). When morphologic criteria on EUS are combined with cytology and CEA levels (cutoff 192 ng/mL), EUS-FNA could differentiate mucinous from nonmucinous lesions with 91% sensitivity and 31% specificity. A meta-analysis of aspirates from EUS-FNA found CEA to have a sensitivity of 63% (95% CI, 59–67%) and specificity of 88% (95% CI, 83–91%) for identification of mucinous cystic tumors [4]. Unfortunately, direct fluid sampling for analysis is not obtainable in a significant percentage of cases due to the high viscosity of cystic fluid.

This not uncommon issue faced by endoscopists today prevents EUS-FNA from being used to obtain adequate PCL fluid for cytologic, biochemical, DNA, and tumor marker analysis. In a prospective series study by Hong et al., adequate amounts of fluid could be aspirated for analysis and cytology in only 81% of patients, and 67% of cysts unable to be aspirated were found to be either premalignant or malignant [5]. To mitigate this issue, we chose to investigate what limitations exist that preclude viscous fluid aspiration, and furthermore what variables can be modified to increase cystic fluid yield.

Passage of fluid through an FNA needle is governed by the Hagen-Poiseuille Law, that is:

$$V = \frac{\pi PR^4}{8\mu l}$$

V = velocity of fluid aspiration
P = pressure difference across the pipe
R = radius of the pipe
μ = dynamic viscosity of the fluid
L = length of the pipe

The Hagen-Poiseuille Law indicates that the velocity of cystic fluid in a needle is directly proportional to the pressure difference across both ends of the needle, inversely proportional to the length of the needle, and most importantly, exponentially proportional (a power of 4) to the radius of the needle [6]. This equation assumes laminar flow of an incompressible, Newtonian fluid in a long pipe of constant cross section. Manipulation of the variables leads to increased fluid velocity and in effect volumetric yield.

In this side-by-side comparison of aspiration device configurations, we objectively evaluated various methods available to aspirate viscous fluid in EUS-FNA, notably: needle gauge, vacuum pressure and extension tubing as well as application of a novel technique – high-frequency oscillation (HFO) to the needle – to determine which configurations are most capable of aspirating viscous fluid.

Materials and methods

Maximum gauge pressures of four vacuum devices: 10-cc EUS-FNA syringe (B. Braun Medical Inc.), 20-cc EUS-FNA syringe (Boston Scientific), 50-cc Alliance II System, and a nonmedical hand vacuum pump were calculated (▶ Fig. 1).

Aspiration rates of the vacuum devices connected to both 19-gauge and 22-gauge EUS needles (Boston Scientific Expect Slimline) were calculated using a corn syrup stock solution with a dilution factor of 2.25. All runs were completed in a temperature-controlled environment to ensure consistent solution viscosity.

The EUS needle tip was secured in a 10-mL, ±0.2 mL graduated cylinder containing the stock solution, ensuring that the needle tip remained below the meniscus and sheath above the meniscus during each run so as to not skew true volume aspirated. Duration to aspirate 3 mL of solution was recorded and runs were performed five times for each configuration to ensure statistical significance. P values generated from t-tests comparing aspiration rates were compared, and values of P< 0.05 were considered statistically significant results.

The effect of high-frequency oscillation on aspiration rate was measured by application of a vibrating transducer to several FNA configurations: 10-cc, 20-cc, and 50-cc syringes connected to both 19-gauge and 22-gauge needles. A Honda HEC-3020-P2B transducer was applied to the needle-syringe interface via a two-way stopcock to provide oscillation between 19.5 kHz and 20.5 kHz using a Micromechatronics Inc PDUS200 ultrasonic driver. Duration to aspirate 3 mL of solution was recorded and runs were performed five times for each configuration to ensure statistical significance. P values generated from t-tests comparing aspiration rates were compared, and values of P< 0.05 were considered statistically significant results.

Results

The four aspiration devices generated maximum gauge pressures ranging from –21.5 inHg to –27.5 inHg, with the 10-cc syringe generating the least pressure and the hand vacuum pump generating the most pressure (▶ Table 1).

The 19G needle yielded on average 11.3 × faster aspiration rates versus the 22-gauge needle when connected to identical aspiration devices.

The hand vacuum pump, which generated the greatest negative pressure, aspirated viscous fluid fastest when connected to both 19-gauge and 22-gauge needle configurations. The 20-cc syringe aspirated fluid faster than the 10-cc syringe connected to the 19-gauge needle; however, there was no significant difference in aspiration rates between the 10-cc and 20-cc syringe connected to the 22-gauge needle (▶ Fig. 2).

The Alliance II system with 50-cc syringe and connected tubing aspirated viscous fluid slower than either the 10-cc or 20-cc syringe. Yet, we found that removing the 30-cm extension tubing from the Alliance II device increased aspiration rates in both 19-gauge and 22-gauge needles compared to using the device with the tubing connected (P= 0.014 and P= 0.018, respectively).

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► **Fig. 1** **a** Aspiration devices pictured left to right: 10-cc EUS-FNA syringe (B. Braun Medical Inc.), 20-cc EUS-FNA syringe (Boston Scientific), 50-cc Alliance II Syringe System (Boston Scientific), and a nonmedical hand vacuum pump connected to a syringe barrel via rubber adapter. **b** Application of high-frequency oscillation to the needle syringe interface in FNA using a Honda transducer.

► **Table 1** Maximum negative pressure generated by each vacuum device.

Vacuum device	Pressure (inHg)
10-cc EUS-FNA syringe (B. Braun Medical Inc.)	–21.5
20-cc EUS-FNA syringe (Boston Scientific)	–25
50-cc Alliance II System (Syringe and Inflation/Litho Handle) with connected tubing (Boston Scientific)	–26
50-cc Alliance II System (Syringe and Inflation/Litho Handle) without connected tubing (Boston Scientific)	–26
Nonmedical hand vacuum pump (Mityvac)	–27.5

EUS-FNA, endoscopic ultrasound-guided fine-need aspiration

When HFO was applied to 19-gauge and 22-gauge needle configurations, aspiration rates increased among all device configurations compared to no HFO (► **Fig. 3**). When using the 19-gauge needle, average flow rate increased 24.1 % with a 10-cc syringe ($P < 0.001$), 35.8 % with a 20-cc syringe ($P < 0.0001$), and 29.4 % using a 50-cc syringe ($P < 0.01$). Overall, HFO applied to the 19-gauge needle yielded an average rate increase of 29.7 % compared to no HFO. When using the 22-gauge needle, average flow rate increased 114.8 % using a 10-cc syringe ($P < 10^{-5}$), 133.7 % using a 20-cc syringe ($P < 10^{-6}$), and 125.3 % using a 50-cc syringe ($P < 10^{-5}$). Overall, HFO applied to the 22-gauge needle yielded an average rate increase of 124.6 % compared to no HFO.
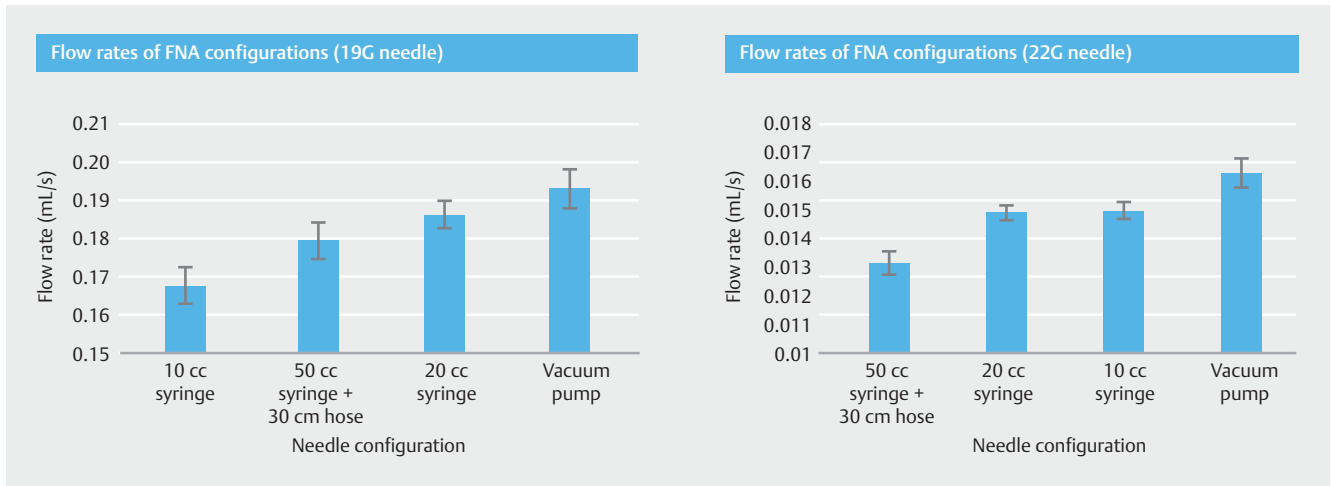
Discussion

Aspiration of PCLs is essential for evaluation and identification of incidental lesions found on imaging that are indeterminate as they may have malignant potential. Despite highly viscous fluid typically being mucinous in origin, fluid analysis is still important as current guidelines recommend evaluation with EUS-FNA of any cystic lesion over 3 cm in diameter or when cross-sectional or EUS imaging confirms an epithelial nodule, dilated main pancreatic duct, or suspicious mass lesion [3]. Diagnostic criteria and cyst classification rely on chemical and cytologic analysis, and lack of this information introduces significant clinical uncertainty into patient management with many patients potentially unwilling to undergo therapy without a definitive diagnosis.

The high viscosity of some PCLs makes it difficult for clinicians to aspirate adequate fluid for analysis, so much so that it may not be possible to extract a mere 1 mL of fluid in a reasonable amount of time, or at all. For that reason, it is important to understand optimal parameters of an FNA configuration to maximize fluid yield when highly viscous fluid precludes FNA.

In this study, we have shown how changing various parameters can increase aspiration rate and increase fluid yield of a given sample of fluid. We demonstrate how modifying variables of the Hagen-Poiseuille Law (pipe diameter, pipe length, and vacuum pressure) as well as adding HFO to the needle affected aspiration rates.

We demonstrated that lower-gauge needles exponentially increased aspiration rates. The Hagen-Poiseuille Law indicates that flow rate is proportional to R^4, where R is the radius of the pipe. Therefore, the 19-gauge needle, with a diameter of 0.912 mm, should flow approximately four times faster than the 22-gauge needle, with a diameter of 0.644 mm. However, our results showed that the 19-gauge needle flowed approxi-

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Fig. 2 Comparison of flow rates among four aspiration devices: 10-cc EUS-FNA syringe (B. Braun Medical Inc.), 20-cc EUS-FNA syringe (Boston Scientific), 50-cc Alliance II Syringe System (Boston Scientific), and a nonmedical hand vacuum pump using both 19-gauge and 22-gauge EUS needles (Boston Scientific Expect Slimline). **a** *P* values in comparing mean flow rates were <0.05. **b** There was no statistically significant difference in aspiration rates in the 22-G needle between a 10-cc syringe (B. Braun Medical Inc.) and 20-cc syringe (Boston Scientific).
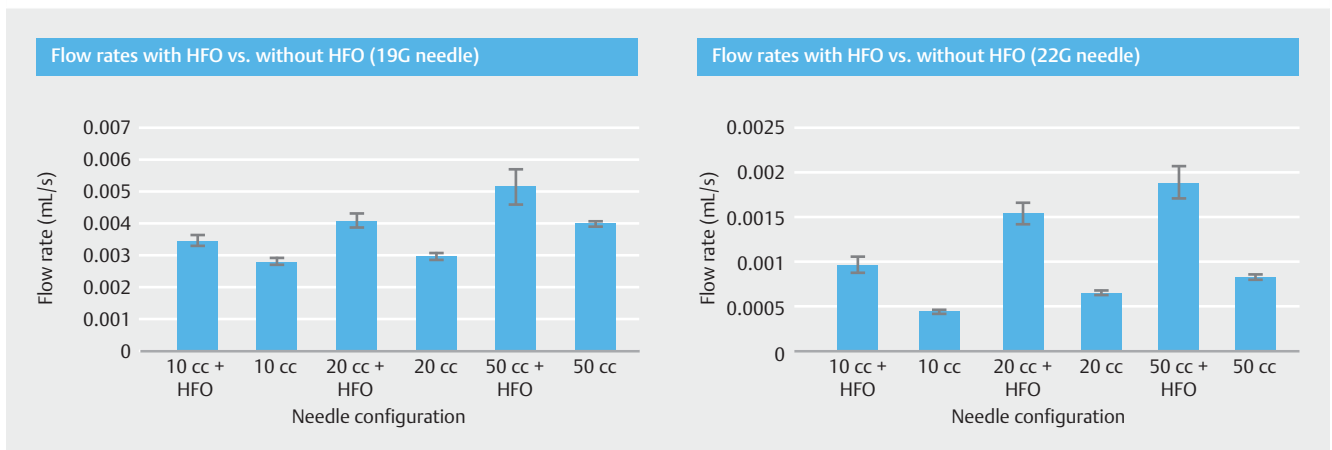
Fig. 3 Comparison of aspiration rates among 10-cc, 20-cc, and 50-cc syringes connected to both 19-gauge and 22-gauge EUS needles (Boston Scientific Expect Slimline) with and without application of high-frequency oscillation (HFO). HFO increased aspiration rates significantly in all groups compared to controls.

mately 12 times faster than the 22-gauge needle for each test configuration. This is evident in ▶ **Fig. 2**, in which the 19-gauge needle resulted in faster aspiration rates for the 10-cc syringe, 20-cc syringe, vacuum pump, and 50-cc syringe groups by a whole order of magnitude. In a clinical setting, it is important to note that increasing needle size is the single most effective way to increase aspiration rate.

The positive correlation between syringe volume and aspiration rate can be explained by Boyle's Law. For a closed system, P_1V_1 is equal to P_2V_2, where P is pressure and V is volume. In FNA, the closed system is created by a syringe chamber, EUS needle, and attached tubing between the syringe and needle. The system is bounded by a plunger on the vacuum end, and cystic fluid on the needle end. Based on $P_1V_1 = P_2V_2$, volume of the closed system is inversely proportional to pressure, such that an increase in volume decreases pressure (increases va-

cuum). Since volume is created in the closed system when a plunger is pulled, larger syringes can effectively create more volume and thus more vacuum. It is important to note, however, that vacuum is limited by atmospheric pressure, with maximum theoretical vacuum level being −29 inHg.

The hand vacuum pump, which generated the greatest pressure of all configurations tested, had the fastest aspiration rates when connected to both 19-gauge and 22-gauge needles (▶ **Fig. 1**). Becuase the hand vacuum pump is capable of generating and sustaining near theoretical maximum vacuum (−27.5 inHg), fluid can be aspirated faster versus the other fixed-volume syringes tested. The hand vacuum pump has the ability to sustain maximum pressure when fluid is aspirated, because air can be continuously extracted as fluid enters the collection chamber, whereas a syringe chamber filling with fluid consistently loses vacuum pressure.

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In terms of comparing syringe volume, we found that the 20-cc syringe was able to aspirate fluid faster than the 10-cc syringe when using a 19-gauge needle (▶ **Fig. 2**), which is in accord with Boyle's law. It follows that the 20-cc syringe also can generate more vacuum than the 10-cc syringe.

To the contrary, we found that there was no statistical significance in aspiration rates between the 10-cc and 20-cc syringe when connected to the 22-gauge needle. Despite the 20-cc syringe generating more pressure, it is suspected that the much slower rate of aspiration leads to greater pressure leak in the 20-cc syringe than the 10-cc syringe because the seal has a greater surface area. In addition, the extra 3.5 inHg generated by the 20-cc syringe versus the 10-cc syringe may not be adequate enough to create a significantly faster aspiration rate given the 22-gauge needle's high resistance to flow.

In a clinical setting, it is important to note two points regarding vacuum pressure in FNA. First, larger syringe volumes are capable of generating more negative pressure, thus yielding faster aspiration rates and in effect greater fluid yield. However, this phenomenon is less evident with larger-gauge needles. Second, 10-cc and 20-cc EUS-FNA syringes are not ideal vacuum devices when attempting to extract viscous fluid from cysts. Unlike fixed-volume syringes, a hand vacuum pump is capable of both generating and sustaining near theoretical maximum vacuum, and therefore can aspirate viscous cysts faster and more effectively.

The 50-cc Alliance II system is essentially a 50-cc syringe with 30-cm tubing attached, and we found that this device generated less vacuum and yielded slower aspiration rates compared to smaller syringes. Despite the device having a larger volume syringe, the 30-cm tubing negatively impacted the device's ability to aspirate fluid. When removing the hose from the apparatus, we demonstrated that aspiration rates were 5.13 % faster.

The reason why tubing leads to slower aspiration rates is twofold. First, the hose adds an extra area of resistance in the vacuum chamber. The increased resistance slows fluid movement through the needle, thus decreasing aspiration rate. This phenomenon is supported by the Hagen-Poiseuille Law, in which the length of a pipe is inversely proportional to flow rate. In addition, the extra tubing introduces more air that must be extracted by the syringe, resulting in diminished vacuum pressure. An endoscopist attempting to aspirate a viscous fluid collection should remove all extension tubing from the EUS-FNA apparatus to increase aspiration rate and fluid yield.

When HFO was applied to the syringe-needle interface, flow rates of fluid significantly increased. In 19-gauge needle configurations, average flow rates increased by 29.7 % and in 22-gauge needle configurations, rates increased by 124.6 %. This statistically significant increase in flow rate with introduction of HFO is due to a reduction in static friction forces. It is assumed that flow remains laminar in the presence of HFO, and therefore the Hagen-Poiseuille Law applies.

We considered testing other variables of FNA, including adding a 25-gauge needle, a needle group with and without a side hole, and comparison of various company needles of the same gauge. However, we believe that these would have added unnecessary complexity to the study. FNA needles that are 25-gauge are extremely small in diameter and almost never used for pancreatic cyst fluid analysis and comparison of equal gauge needles from various companies should not yield results that are statistically significant.

Although we have established a robust proof-of-concept experiment for parameters that can increase fluid aspiration rate and yield, there are some limitations to our study. First, we aspirated corn syrup and glycerin solutions rather than viscous fluid collections in vivo. The different viscosities and heterogeneities of cystic fluid may negatively affect their aspiration despite adjusting certain aspiration device parameters. This may be especially true with fluids that are more viscous than what was simulated in the lab, such that removing extraneous tubing, increasing vacuum pressure, or adding HFO may not consistently produce a significant increase in aspiration rate and yield. However, we do expect significant increases in aspiration rate and fluid yield from viscous PCLs and other fluid collections that are of the same or lower viscosity.

In terms of future directions to improve FNA, an additional method would involve heating viscous fluid in a PCL via an endoscopic probe. Heat would decrease fluid viscosity and thus increase aspiration rate in accordance with the Hagen-Poiseuille Law, in which viscosity is inversely proportional to flow rate.

Conclusion

This experiment has proven that there are several modifiable parameters of FNA that can increase aspiration rate and yield of viscous PCLs and other fluid collections. Because flow rate increased exponentially with decreasing needle gauge, this is by far the quickest and most efficacious way of increasing aspiration rate and viscous fluid yield. Other techniques include increasing vacuum pressure by using a larger-volume syringe or ideally a hand vacuum pump, as well as removing extraneous tubing from the apparatus and applying HFO to the needle syringe interface.

Competing interests

Dr. Matthew Moyer is a consultant for Boston Scientific.

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