

Utilization of High-pressure Suction for EBUS-TBNA Sampling in Suspected Lung Cancer

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Background: Sample adequacy for immediate molecular testing is paramount in lung cancer. To date, several endobronchial ultrasound with transbronchial needle aspiration (EBUS-TBNA) sampling setups have been evaluated, however, the utilization of high-pressure suction (HPS) has not yet been reported. The aim of this study was to evaluate the utilization of HPS onto the needle and its effect on sample volume and adequacy for molecular testing in patients with suspected lung cancer.

Methods: We retrospectively analyzed 128 consecutive EBUS-TBNA performed for suspected lung cancer. This was confirmed in 109 patients. Other diagnoses confirmed in 12, and 7 referred for surgery. Sixty-three patients (89 targets) had HPS (May to September 2020), and compared with 46 (72 targets) who had standard vacuum syringe suction (October 2019 to March 2020). Several parameters and outcomes evaluated, such as number of needle passes, needle strokes, needle size, target size, positron emission tomography avidity, procedure time, blood content score, sample volume, adequacy for molecular testing, as well as baseline patient characteristics and complication rate.

Results: There was no difference between the 2 groups in all baseline parameters and characteristics. In multivariable

analysis, HPS was associated with significantly higher sample volume (11.2 vs. 9.1 mm³, $P=0.036$) and less additional procedures to achieve full molecular profiling (2/52 vs. 7/40, $P=0.042$), in necrotic targets of non-small cell lung cancer. Diagnostic yields were comparable.

Conclusion: HPS appears to be simple, no-cost, and safe, promising higher sample volume compared with vacuum syringe suction, and also appears to be associated with higher success of full molecular testing with less additional procedures, in non-small cell lung cancer necrotic targets.

Key Words: EBUS TBNA, lung cancer, lymph nodes, needle biopsy, molecular test

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Lung cancer remains one of the biggest challenges for health care, with ~50,000 cases annually in the United Kingdom. Molecular marker “reflex testing” has become the “gold standard” following any tissue biopsy in suspected cases, allowing for patient stratification to targeted therapies. With the diagnosis and treatment pathways becoming shorter, such as the NHS England approved National Optimal Lung Cancer Pathway (49 d to definitive treatment),¹ as well as the implementation of national lung cancer screening programmes, it is paramount that patients are given the best possible opportunity to get full results with 1 procedure. Endobronchial ultrasound with transbronchial needle aspiration (EBUS-TBNA) has been the cornerstone for diagnosis and mediastinal lymph node staging in suspected lung cancer (depending on disease stage) for almost a decade. Various techniques of sampling have been evaluated, including syringe suction/no suction,^{2–4} stylet/no stylet,⁵ as well as rapid on-site evaluation,⁶ in an effort to increase the sample size, diagnostic yield, and enable molecular testing. However, to our knowledge, the use of a high-pressure suction setup connecting directly onto the EBUS-TBNA needle has not been investigated.

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OBJECTIVES

In this retrospective study, we aimed to evaluate the feasibility of immediate molecular testing, sample volume, and diagnostic yield of our local practice with EBUS-TBNA utilizing high-pressure suction (HPS) directly onto the needle, compared with the conventional standard vacuum syringe suction technique (VSS), in patients with suspected lung cancer undergoing the procedure for either diagnosis, staging, or both.

METHODS

We retrospectively screened 145 consecutive EBUS-TBNA procedures performed under conscious sedation (intravenous midazolam and alfentanil) in our unit between October 1, 2019 and September 30, 2020 (Fig. 1). Of those, 17 were excluded as the indication for the procedure was benign disease, infection, or other primary malignancy. One hundred twenty-eight patients had EBUS-TBNA for suspected lung cancer (diagnosis, staging, or both). Of those, 4 confirmed benign disease and 5 confirmed metastases

from a different primary cancer, and excluded, as no molecular profiling specific for lung cancer was done. Seven patients referred directly for curative surgery according to their disease stage, hence excluded as the lack of complete molecular profiling would not change their management. We finally analyzed 109 EBUS-TBNA confirmed lung cancer cases. Due to a change in our local protocol based on quality improvement project results, in the latest 63 consecutive procedures performed for suspected lung cancer (89 targets sampled) between May 2020 and September 2020 we used HPS, and compared it to a group of 46 consecutive procedures performed for suspected lung cancer (72 targets sampled) in which we used VSS, between October 2019 and March 2020. No EBUS-TBNA procedures performed during April 2020 due to the “peak” of the coronavirus disease 2019 pandemic and associated restrictions. All procedures were performed by 2 experienced operators (> 500 procedures combined experience), assisted by 3 experienced endoscopy nurses (A.C., J.H., and J.M.). For all procedures we used

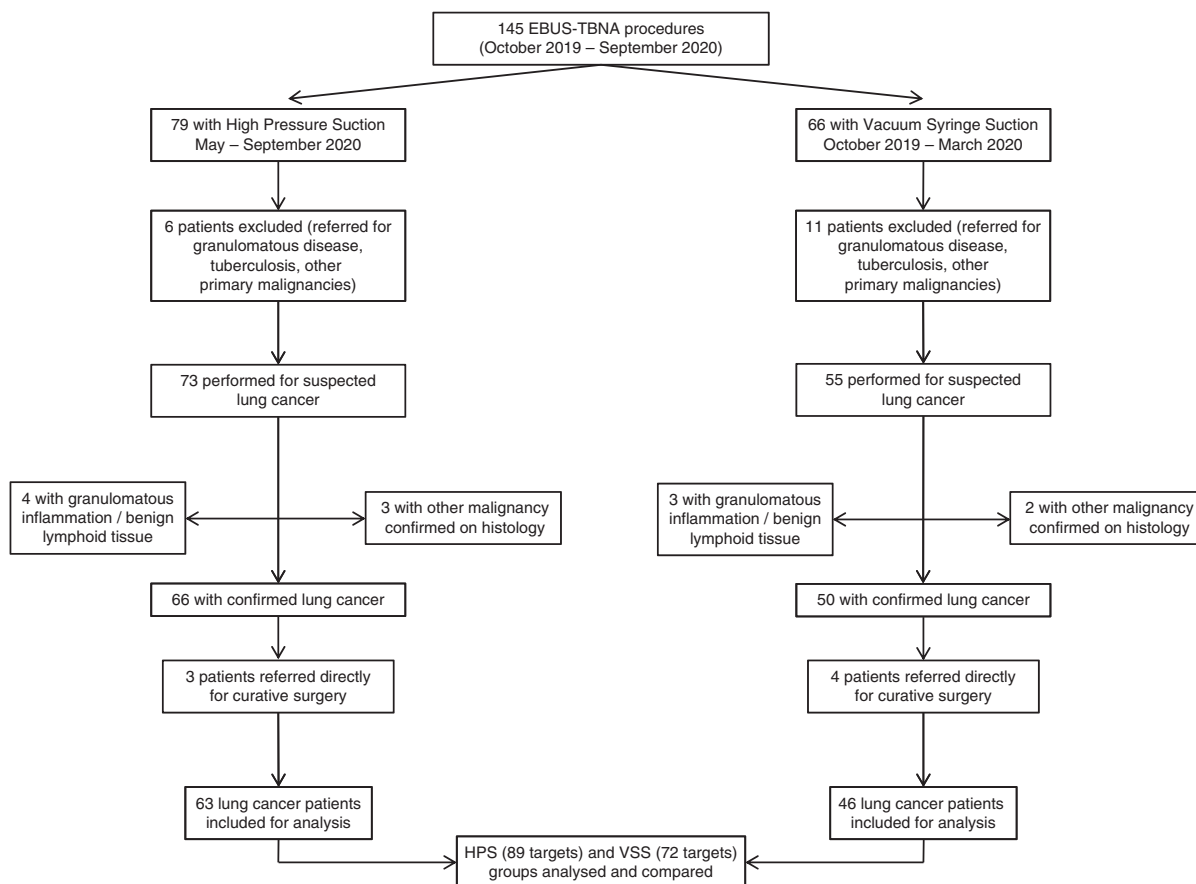


FIGURE 1. Study flow diagram. EBUS-TBNA indicates endobronchial ultrasound with transbronchial needle aspiration; HPS, high-pressure suction; VSS, vacuum syringe suction.

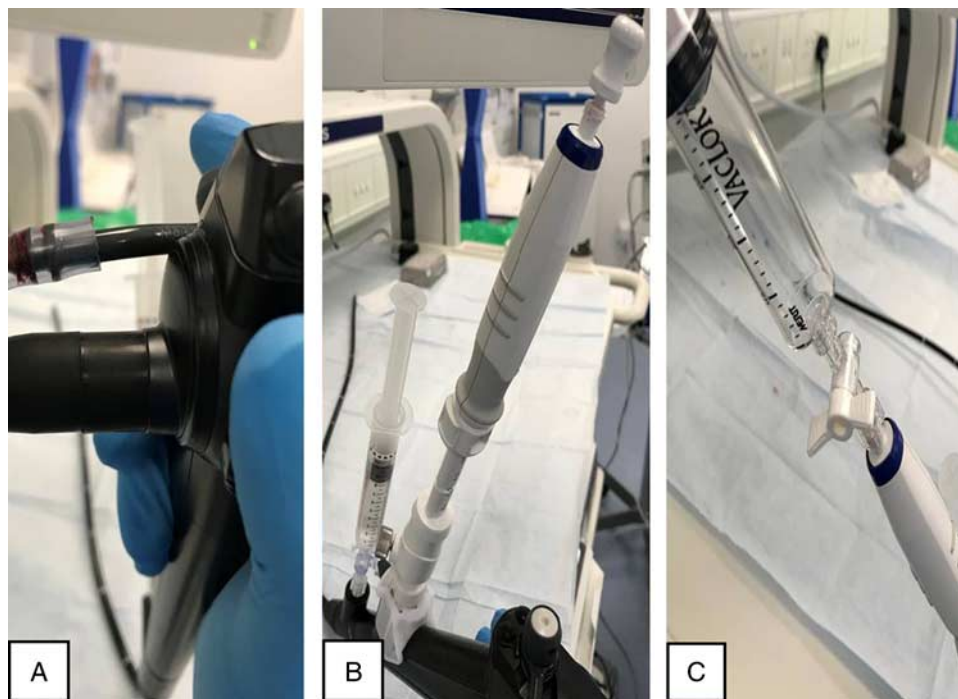


FIGURE 2. Standard vacuum syringe sampling setup. A, Wall suction tubing connected to the suction channel outlet of endobronchial ultrasound scope. B, Endobronchial ultrasound with transbronchial needle aspiration needle and balloon syringe connected. C, Standard VacLok vacuum syringe connected on endobronchial ultrasound with transbronchial needle aspiration needle, set at 20 mL. *++*

the Olympus Slim EBUS bronchoscope (BF-UC290F), with Olympus dedicated needles. We utilized B-Mode in combination with the “built-in” function of elastography (ELST) to assess for necrosis in all 161 targets. We aimed for an optimum number of ≥ 3 needle passes per target, with ≥ 50 needle strokes per pass, taking into account patient comfort, safety and feasibility. Complication rate was monitored and reported as per standard operating procedure of our unit.

Procedural Technique

Standard VSS Setup

In this standard technique, after successful puncture of the target, the stylet is removed after a few strokes to expel any possible bronchial epithelial plug, and then the 20 mL VacLoc (Merit Medical Systems Inc., South Jordan, UT) vacuum syringe included in the needle pack is attached securely on the needle driver. The pre-set syringe suction is applied and sampling begins (Fig. 2). When the standard number of needle strokes within the target is completed, the needle is removed and the sample retrieval process begins. The process is repeated for ≥ 3 passes and ≥ 50 strokes per pass at each target.

HPS Setup

In our technique variation, the standard 20 mL “VacLoc” syringe is not being used. Instead, the standard suction tubing (1.5 m length, 6mm inner diameter), which normally during the procedure connects the suction channel of the bronchoscope with the wall-mounted suction for airway clearance, is tightly connected on the needle driver. This connection is only brief (for the duration of the needle sampling), and at a set suction pressure of 40 kPa. The inner diameter of the suction tube allows it to stay tightly fitted on the needle driver throughout the sampling process, without extra input required from the operator (Fig. 3). When the standard number of needle strokes is completed, the suction tubing is connected back on the channel of the bronchoscope to allow airway clearance and bleeding control if needed, the needle is removed and the sample retrieval process begins. The process is repeated for ≥ 3 passes and ≥ 50 strokes per pass at each target.

Histopathology

All EBUS-TBNA histology samples (needle cores) were expelled, by using the stylet, from the needle directly into 10% neutral buffered



FIGURE 3. High-pressure suction setup. The wall suction tubing is disconnected from the suction outlet of the endobronchial ultrasound scope, and connected directly onto the endobronchial ultrasound with transbronchial needle aspiration needle instead. *u+*

formalin pots (CellStor). We do not have rapid on-site evaluation capacity in our endoscopy. A “needle rinse” was then obtained by expelling any remainder of the sample into Cytolyt (methanol-based buffered preservative) for cytologic cell block preparation. On histopathologic examination, a blood content score was assigned to each sample pot by the receiving histopathologist who was blinded to the sampling technique used, based on a preagreed visual score (0 to 2, 0 = no visible blood, 1 = light red shading, 2 = red shading).⁷ The “needle core” biopsies were then filtered through a cassette, the sample volume was measured by calipers,⁸ and embedded in paraffin blocks. For anaplastic lymphoma kinase (ALK) testing, immunohistochemistry (IHC) was performed using the Ventana ALK (D5F3) IHC test (CE-IVD), “Ultra” platform. For epidermal growth factor receptor (EGFR) testing, mutation analysis performed by real-time polymerase chain reaction using the Roche COBAS EGFR mutation test (CE-IVD) (42 mutations in exons 18 to 21). For ROS1, IHC was performed using the “Cell Signaling Technology” ROS1 D4D6 Rabbit monoclonal antibody, on the Ventana

Benchmark “Ultra” system. For programmed death ligand 1 (PD-L1) testing, tumor material was tested with the Dako PD-L1 IHC 22C3 pharmDx test. All 161 histopathology samples were processed by the same technicians, and reports were generated by 2 experienced lung histopathologists. Success of molecular testing was defined as the completion of all appropriate testing that would affect patient management, as agreed in the multidisciplinary team meeting.

Statistical Analysis

Descriptive analysis was used according to the type of variables. Categorical variables were compared as the count and percentage using χ^2 tests. A diagnostic procedure was defined as “achieving specific diagnosis via EBUS-TBNA,” and diagnostic yield was calculated for both groups. We used 1-way multivariate analysis of covariance to compare the HPS with the VSS group, with “sample volume” as the outcome, adjusting for 4 variables [target size, number of needle strokes, number of needle passes, and positron emission tomography (PET) avidity]. To avoid the very common “overfitting” issue with such models, we set with a conservative variable number of “1/20” for the 86 overall necrotic targets analyzed. As the initial 1-way multivariate analysis of covariance showed statistical significance with satisfactory Wilks’ lambda, we proceeded with individual 1-way analysis of covariances for variables, and Bonferroni correction, before reporting final *P*-values. We used a Mantel-Haenszel model to test for differences in number of additional procedures needed to complete molecular analysis in appropriate cases. Statistical analysis carried out using IBM SPSS Statistics, version 25.0 (SPSS, Chicago, IL). The analysis was approved as a service evaluation from the hospital committee (no. 236.19-20).

RESULTS

There was no statistically significant difference between the HPS and VSS baseline patient group characteristics (Table 1). Intravenous sedation doses used in both groups were similar. The mean number of needle passes performed was no different between the 2 groups either ($P=0.141$), and the same applies to the overall needle strokes per target ($P=0.461$). We did not observe a statistical difference in the mean procedure time, with 28.4 minutes for the HPS group, compared with 26.7 minutes for the VSS group ($P=0.526$). The 90-day mortality observed in both groups was 0%, there were no

TABLE 1. Baseline Cohort Characteristics of Patients Who Had EBUS-TBNA With a Suspicion of Lung Cancer, and Their Diagnoses

	HPS Group (N = 73)	VSS Group (N = 55)	P
Age	59 (± 11.9)	62 (± 9.2)	0.156
Female [n (%)]	32 (50.8)	21 (45.6)	0.593
WHO performance status	1.8 (± 0.52)	1.7 (± 0.46)	0.30
Current smokers [n (%)]	51 (69.8)	39 (84.8)	0.597
Indication of EBUS-TBNA [n/N (%)]			
Diagnosis	36/73 (49.3)	30/55 (54.5)	0.916
Staging	4/73 (5.4)	3/55 (5.4)	0.989
Both	33/73 (45.3)	22/55 (40.1)	0.962
Other malignancy confirmed [n/N (%)]	3/73 (4.1)	2/55 (3.6)	0.921
1 breast		1 breast	
1 colorectal		1 colorectal	
1 esophageal		1 b-cell lymphoma	
Granulomatous changes/lymphoid tissue [n/N (%)]	4/73 (5.4)	3/55 (5.4)	0.99
Lung cancer confirmed [n/N (%)]	66/73 (90.4)	50/55 (90.9)	0.988
Non-small cell lung cancer [n/N (%)]	52/63 (82.5)	40/46 (86.9)	0.957
Adenocarcinoma [n/N (%)]	37/52 (71.1)	29/40 (72.5)	0.651
Squamous [n/N (%)]	15/52 (28.8)	11/40 (27.5)	0.99
Small cell lung cancer [n/N (%)]	8/63 (12.6)	4/46 (8.7)	0.510
PET positive targets [n (%)]	74 (83.1)	64 (88.9)	0.297
Target FDG (SUV _{max} ± SD)	4.3 (± 2.6)	4.8 (± 2.2)	0.357
Target size (mm)	16.3 (± 6.4)	14.7 (± 5.9)	0.197
Midazolam (mg)	2.04 (± 0.38)	2.08 (± 0.34)	0.571
Alfentanil (mcg)	591.67 (± 180.14)	583.54 (± 167.74)	0.811
Procedure time (min)	28.4 (± 13.1)	26.7 (± 14.7)	0.526
Needle size 19 G [n (%)]	1 (1.1)	3 (4.2)	0.210
Needle size 21 G [n (%)]	84 (94.3)	68 (94.4)	0.978
Needle size 22 G [n (%)]	4 (4.5)	1 (1.4)	0.261
No. needle passes	3.82 (± 0.71)	3.67 (± 0.54)	0.141
No. needle strokes	48.6 (± 3.2)	49.2 (± 6.8)	0.461
Target necrosis present overall [n (%)]	52 (58.4)	34 (47.2)	0.157
Target necrosis present in NSCLC [n (%)]	35 (67.3)	18 (45)	0.032*

*Statistically significant P values.

χ² Test was used for comparison, with P < 0.05 considered statistically significant.

EBUS-TBNA indicates endobronchial ultrasound with transbronchial needle aspiration; FDG, fluorodeoxyglucose; HPS, high-pressure suction; NSCLC, non-small cell lung cancer; PET, positron emission tomography; SUV_{max}, maximum standardized uptake value; VSS, vacuum syringe suction; WHO, World Health Organization.

pneumothoraces overall (0%), with comparable bleeding (minor) rates of 0.3% and 0.4% (in HPS and VSS groups, respectively). The median blood content score did not differ between the 2 groups (1.42 ± 0.34 vs. 1.36 ± 0.23, HPS and VSS, respectively, P = 0.202) (Fig. 4). From the 161 targets sampled, necrosis was confirmed (by B-Mode and ELST combined), in 58.4% (HPS group) and 47.2% (VSS group) of targets, which was not statistically significant (P = 0.157).

Histopathologic diagnoses were similar, with non-small cell lung cancer (NSCLC) being the most common [82.5% in HPS (52) and 86.9% (40) in VSS group]. Within those 92 NSCLC cases, there was an even distribution of subtypes between the 2 groups, with 67 cases of “adenocarcinoma” (37 in HPS, 29 in VSS), and 26 with “squamous differentiation” (15 in HPS, 11 in

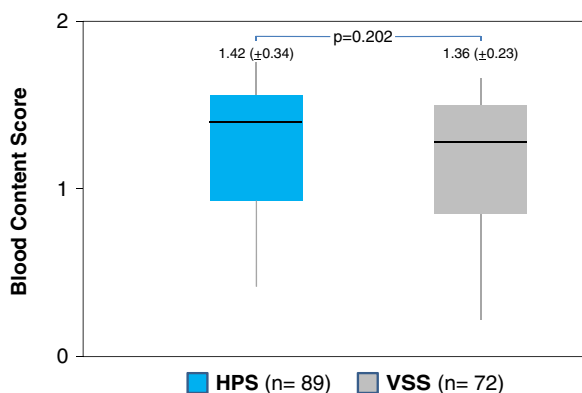


FIGURE 4. Blood content score allocated based on sample appearance in formalin pot by using a preagreed scale (0 = no visible blood, 1 = light red shading, 2 = red shading). The histopathologists were blinded to the suction method used. HPS indicates high-pressure suction; VSS, vacuum syringe suction.

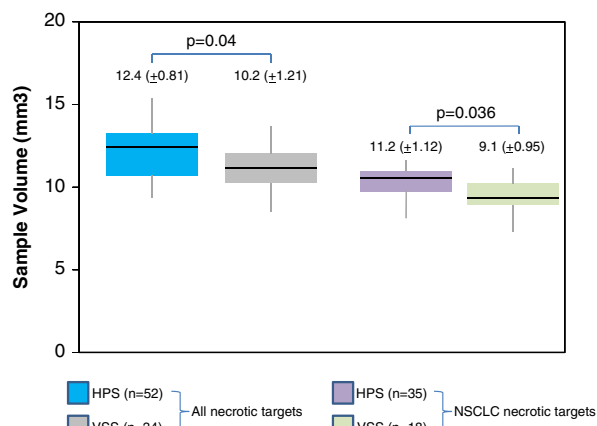


FIGURE 5. Box plots showing the sample volume differences observed between the 2 groups (HPS vs. VSS) in all necrotic targets and the subgroup of NSCLC necrotic targets. HPS indicates high-pressure suction; NSCLC, non-small cell lung cancer; VSS, vacuum syringe suction. $\mu \pm$

VSS). There were 8 cases of small cell lung cancer in the HPS group and 4 in the VSS group. A “ViziShot 2” 21G EBUS-TBNA needle was used in 94.3% of targets in the HPS group and 94.4% in the VSS group ($P=0.978$).

After multivariable analysis, adjusting for 4 confounders (target size, number of needle passes/strokes, PET avidity), HPS was associated with significantly higher sample volume (mm^3) compared with VSS in all necrotic targets [12.4 ± 0.34 vs. 10.2 ± 1.21 , respectively, $P=0.04$, Wilks’ lambda 0.514, 95% confidence interval (CI): 0.53-0.95] (Fig. 5, Table 2). This significant difference was not observed when analyzed all targets including non-necrotic ones ($n=161$) with $P=0.068$ (Wilks’ lambda .973, 95% CI: 0.65-1.01). Interestingly, when looked at the NSCLC subgroup, necrosis (B-Mode and ELST) was present in 67.3% (35) of targets having HPS, and 45% (18) of those having VSS, which was significantly different

($P=0.032$). In this subgroup, there was also a statistically significant difference in the sample volume obtained (11.2 ± 1.12 vs. 9.1 ± 0.95 , for HPS and VSS, respectively) with $P=0.036$ (Wilks’ lambda 0.427, 95% CI: 0.55-0.98) (Fig. 5, Table 2). In addition, after a Mantel-Haenszel analysis of this subgroup, we found that the number of cases requiring additional procedures to allow complete molecular profiling (when required) were significantly different, with 7 cases in the VSS group (17.5%) and 2 cases in the HPS group (3.84%) ($P=0.042$, 95% CI: 0.62-0.99, odds ratio 0.8). Adequacy for both EGFR and PD-L1 was 98% in the HPS group (100% for ALK and ROS1). In the VSS group, the success rate for EGFR and PD-L1 was 92.5%, for ALK 100%, and 97.5% for ROS1. The mean size of targets did not differ, and the same applies to PET avidity, number of needle passes and needle strokes (Table 1). After completing the multivariable analysis and proceeding with separate univariate analyses for variables, there were no significant univariate effects found in terms of target size, number of needle passes, needle strokes or PET avidity.

Finally, accounting for all 145 procedures screened (October 2019 to September 2020), the overall yield for HPS and VSS was comparable, with values of 95% and 94%, respectively.

DISCUSSION

Study Limitations

This was a single-center, retrospective study, with a small number of cases, carrying inevitable biases. The consecutive nature of the analysis within an established EBUS-TBNA service, and the fact that all procedures and histopathologic analyses were done by the same operators reduces some risks, with the exposures and cointerventions having a higher (but by no means ideal) likelihood of similarity

TABLE 2. Statistical Significance Set at $P < 0.05$

	HPS Group	VSS Group	95% CI	P	Wilks’ Lambda
Sample volume in all targets ($n = 161$) (mm^3)	12.1 (± 1.43)	10.6 (± 1.68)	0.92 (0.88-1.02)	0.068	0.973
Sample volume in all necrotic targets ($n = 86$) (mm^3)	12.4 (± 0.81)	10.2 (± 1.21)	0.73 (0.53-0.95)	0.04*	0.514
Sample volume in necrotic NSCLC targets ($n = 53$) (mm^3)	11.2 (± 1.12)	9.1 (± 0.95)	0.76 (0.55-0.98)	0.036*	0.427

*Statistically significant P values.

Sample volume is reported as mean \pm SD, and the P -values reported are following Bonferroni correction via the SPSS software process. Model used was 1-way MANCOVA for “sample volume” between the 2 groups, after adjusting for 4 confounders (target size, number of needle passes, number of needle strokes, PET avidity), followed by separate 1-way ANCOVAs for variables, as the initial analysis showed statistical significance. There were no significant univariate effects found in terms of target size, number of needle passes, needle strokes or PET avidity.

ANCOVA indicates analysis of covariance; CI, confidence intervals; HPS, high-pressure suction; NSCLC, non-small cell lung cancer; MANCOVA, multivariate analysis of covariance; PET, positron emission tomography; VSS, vacuum syringe suction.

between the 2 groups. Also, unavoidably in such interventions there is always residual bias from potential confounders, which we attempted to address by multivariable analysis.

Histopathology

Our histopathology department does not routinely report “total tissue sample area” for EBUS-TBNA (no software) hence we did not assess this. Unfortunately, we have no means to accurately weigh the sample with precision (0.00001 g) scales either, as other groups have previously reported, which would be ideal. Therefore, we decided to use “sample volume,” as an objective alternative, also used by other groups previously.⁸ For sample “bloodiness,” we used the semiquantitative score previously described by Wolters et al.⁷ This is a preagreed visual “red shading scale” quantifying the blood content in the formalin pot upon receipt of the sample by the laboratory (0=no visible blood, 1=light red shading, 2=red shading). We do agree that this score is not validated and subjective, however several published studies evaluating the EBUS-TBNA sample quality with various needle sizes do not seem to use any relevant set criteria to assess blood content at all. In our study, all samples were scored by the same histopathologists, blinded to the suction technique used. Furthermore, it is technically not feasible to document the exact amount of blood in milliliters, as the sample is collected in a vessel prefilled with formalin (or on a slide in other studies).^{8–10}

Assessment of Tissue Necrosis

The combined use of B-Mode and ELST to assess for target necrosis might also appear controversial. Although widely used and very useful in trained hands, ELST has not yet been extensively validated. It is a dynamic colored ultrasound feature, noninvasively assessing the tissues for “stiffness,” by measuring their deformation in response to external compression or internal pulsations and movements.¹¹ There is some evidence that ELST can help in distinguishing malignant from benign lymph nodes, and in guiding target sampling to avoid as much as possible the necrotic (softer) areas within targets.¹² Necrotic targets tend to be challenging in providing a decent tissue sample, which in turn can affect further processing, as in a study by Jurado et al.¹³ where 18% of NSCLC patients did not have sufficient material to complete molecular testing. The European Federation of Societies for Ultrasound in Medicine and Biology recommend the use of real-time ELST in tumor

staging, to identify particular areas within the lymph node.¹⁴ We need to stress that assessing the utility of ELST was not an aim of our study, and it certainly has its limitations. However, our study seems to be the first to report a systematic assessment of all targets for necrosis before sampling, with B-Mode and ELST, in an effort to minimize sampling selection bias (necrosis avoidance).

Other Known Confounders

One would also argue that many technical confounders might have affected the sampling adequacy, such as needle size, number of passes, and percentage of tumor cells. However, all of those factors have been previously evaluated in a meta-analysis and no such association was demonstrated.¹⁰ Moreover, our standard process of ≥ 50 needle strokes per target might seem controversial to the reader. The BTS 2011 Guideline for Advanced Bronchoscopy does not mention an optimum number of needle passes or strokes.¹⁵ This maybe partly explained by the lack of evidence at the time of the guideline composition. There have been various studies more recently, showing an increasing yield with more passes, with the curve reaching “plateau” $>95\%$ after the third pass. In our study, we decided to continue with ≥ 3 passes per target as per our usual practice. The optimum number of needle strokes is still unknown, despite the ATS/CHEST 2016 Guideline recommending 5 to 15 strokes per target.¹⁶ This seems to have been based on only 1 previous small study (102 patients) by Lee et al.¹⁷ This study population predates by almost a decade the era of routine molecular testing in NSCLC, frequently requiring more tissue for processing, which would explain the authors’ impression that 5 to 15 strokes were probably enough. It would be very interesting to compare groups with various needle stroke numbers in NSCLC, in terms of adequacy for molecular testing, as to our knowledge this has not been done yet. In our unit, we have been using ≥ 3 passes and ≥ 50 needle strokes per target as a standard operating procedure, to avoid (as much as possible) any sampling bias. We also used 21 G needles for the vast majority of the sampling (94.5%), making it unlikely that this could influence the result, as demonstrated previously by other research groups.^{9,10,18,19}

On PET imaging, we did not observe any difference in avidity of the sampled targets, with a maximum standardized uptake ratio of 4.3 (SD \pm 2.6) and 4.8 (SD \pm 2.2) in the HPS and VSS

groups, respectively (interquartile range was 1.5 to 22.3 and 1.8 to 19.5, respectively). This is unlikely to have contributed at all in the sample volume results, as in the multivariable analysis there was a negative *P*-value with a high Wilks' lambda.

In terms of safety and complexity of the technique, one might argue that disconnecting the main bronchoscope suction during sampling might be complicated, however realistically the operator does not use any suction during the needle passes. On the contrary, the assistant does not need to prepare the vacuum syringe for every pass and the operator needs to only briefly unplug the suction tubing from the bronchoscope channel and plug it onto the needle, which makes the process simpler.

Our study has some interesting findings which build on existing work around the robustness of EBUS-TBNA in diagnosis and staging of lung cancer. To our knowledge, this is the first study evaluating the effectiveness of HPS applied directly onto the EBUS-TBNA needle, in terms of sample volume and adequacy to perform molecular testing. Other lower vacuum suction levels and sampling setups have been previously investigated. In a study by Harris et al,² the use of VSS versus no suction did not demonstrate any difference in diagnostic yield, but the adequacy for molecular testing was not specifically evaluated. Various small differences in vacuum have also been tested (0, 10, 20, 30 mL). Shiroyama et al³ investigated the efficacy of EBUS-TBNA suction using a 30 mL syringe (instead of the standard 20 mL), in a single-center, retrospective, and nonrandomized study. However the operator had been previously trained in EBUS-TBNA techniques only on training mannequins and animal models. In our study, operators were highly experienced, eliminating the "learning curve" bias and the possibility of inadequate sampling associated with poor technique. Iyer et al⁴ did not find a difference between "no suction," 10 and 20 mL syringes. We would argue that all the previously investigated levels of suction are probably not "high" enough to demonstrate any actual differences between them, hence results so far have been mostly of "noninferiority." In our study, the 40 kPa of suction used is certainly much higher compared with a 20 or 30 mL syringe. Scholten et al,⁵ reported that omitting the stylet does not affect the diagnostic yield either, however, the group did not assess for adequacy for molecular profiling.

In an effort to clarify if the actual vacuum generated via HPS was indeed much higher

compared to VSS at the needle tip, we performed further in vitro work. We could only perform in vitro measurements, as we would need an entirely new study population with further "ethics approval for use of devices" to perform this in vivo. This of course has its limitations as we were not dealing with "perfect laboratory conditions," but nevertheless provides some insight regarding the vacuum generated at the tip of the needle. A digital Gas Flow Multi-Meter (TSI, 5200 Series) was used, attached in-line to the 21 G needle setup, with the needle penetrating a plastic chamber of set volume (10 mL silicone tubing, blocked from either side). The only difference was the pressure setup used (VSS vs. HPS). Multiple test measurements were obtained, with vacuum values recorded (Torr). The VSS setup was consistently generating a vacuum around -520 Torr ($SD \pm 26$), with the HPS demonstrating vacuum values consistently above -999 Torr (device limit was 999, so no $\pm SD$ available). This means that the vacuum generated by using HPS is most likely " $\times 2$ greater" compared with VSS (likely even higher, as the device limit was 999 Torr). Interestingly, a further search in the literature revealed a study by Haseler et al²⁰ in which the authors tested the vacuum generated by using 10 and 20 mL syringes with 21 G needles, for fine needle aspiration tissue biopsies. They reported a vacuum of -517 Torr with the 20 mL setup, which is indeed very close to what we measured.

The needle size itself has also been a subject of debate. In a recent study by Walters et al,⁷ 19 and 22 G size needles were evaluated for sample size and diagnostic yield, however, no data on feasibility of molecular testing was provided. In addition, the use of 19 G needle was investigated by Dooms et al,⁹ and there was no superiority in the success of next generation sequencing. One might argue that by using a size 21 G needle for the majority of the sampling (94.4%) in our study, we might have jeopardized the sample adequacy, as it has been shown previously that a 19 G can provide more tissue.⁹ However, our diagnostic yield was comparable to the existing literature. It is also important to mention that, in 3 of 7 cases in the VSS NSCLC subgroup that required an additional procedure to achieve a complete molecular testing, we used a 19 G needle. A search in the literature evaluating the adequacy of EBUS-TBNA for molecular testing reveals a mix of prospective and retrospective studies

covering a period of 11 years (2007 to 2018), mainly around EGFR, and to a lesser extent ALK, ROS1 and PD-L1. In the only systematic review and meta-analysis available to date,²¹ Labarca and colleagues, reported a pooled probability of sufficient sample for EGFR and ALK of 94.5% and 94.9%, respectively. We found that in the HPS group the sample was adequate for EGFR and ALK in 98% of cases (92.5% in VSS group). For ALK, we had 100% adequacy in both groups (compared with 94.9% from meta-analysis data). For PD-L1, the meta-analysis reported a rate of 100%, however, only 2 small studies were analyzed. In our study, adequacy for PD-L1 was 98% and 92.5%, for the HPS and VSS group, respectively. ROS1 was successful in 100% of cases having HPS, and 97.5% in the VSS group. Overall, it seems that the HPS group showed overall higher adequacy for molecular testing than the existing data, however, our small study size is limiting us from drawing concrete conclusions.

Complication Rate

Since complications are so rare during EBUS-TBNA, a much larger sample size would be required to be able to draw solid conclusions about the extent of bleeding or other associated complications with the technique.

CONCLUSIONS

The novel use of HPS directly onto the EBUS-TBNA needle appears to be a promising, easy, no-cost, and safe technique variation. Our single-center retrospective study has several limitations as described herein, however HPS was still associated with significantly higher sample volume and success of immediate molecular testing, without the need of additional procedures, in necrotic lesions of NSCLC patients. Our results, although thought-provoking, certainly warrant further thorough investigation through a well-designed, pragmatic, multicentre randomized-controlled trial.

REFERENCES

1. NHS England and Cancer Research UK. National Optimal Lung Cancer Pathway. Update version 3. 2020. Available at: www.cancerresearchuk.org/sites/default/files/lung_cancer_implementation_guide_august_2017.pdf. Accessed September 29, 2020.
2. Harris K, Maroun R, Attwood K, et al. Comparison of cytologic accuracy of endobronchial ultrasound transbronchial needle aspiration using needle suction versus no suction. *Endosc Ultrasound*. 2015;4:115–119.
3. Shiroyama T, Okamoto N, Suzuki H, et al. Usefulness of high suction pressure for sufficient tissue collection during endobronchial ultrasound guided transbronchial needle aspiration. *PLoS One*. 2013;8:e82787.
4. Iyer H, Mohan A, Jain D, et al. Comparison of sample adequacy of endobronchial ultrasound guided transbronchial needle aspiration using 0 ML, 10 ML and 20 ML suction pressures in malignant and benign diseases. *Am J Respir Crit Care Med*. 2019;199:A1392.
5. Scholten EL, Semaan R, Illei P, et al. Stylet use does not improve diagnostic outcomes in endobronchial ultrasonographic transbronchial needle aspiration: a randomized clinical trial. *Chest*. 2017;151:636–642.
6. Sehgal IS, Dhoria S, Aggarwal AN, et al. Impact of rapid on-site cytological evaluation (ROSE) on the diagnostic yield of transbronchial needle aspiration during mediastinal lymph node sampling: systematic review and meta-analysis. *Chest*. 2018;153:929–938.
7. Wolters C, Darwiche K, Franzen D, et al. A prospective, randomized trial for the comparison of 19-G and 22-G endobronchial ultrasound-guided transbronchial aspiration needles; introducing a novel end point of sample weight corrected for blood content. *Clin Lung Cancer*. 2019;20:e265–e273.
8. Kinoshita T, Ujiie H, Schwock J, et al. Clinical evaluation of the utility of a flexible 19-gauge EBUS-TBNA needle. *J Thorac Dis*. 2018;10:2388–2396.
9. Doods C, Vander Borgh S, Yserbyt J, et al. A randomized clinical trial of flex 19G needles versus 22G needles for endobronchial ultrasonography in suspected lung cancer. *Respiration*. 2018;96:275–282.
10. Colella S, Scarlata S, Bonifazi M, et al. Biopsy needles for mediastinal lymph node sampling by endosonography: current knowledge and future perspectives. *J Thorac Dis*. 2018;10:6960–6968.
11. Dietrich CF, Jenssen C, Herth FJ. Endobronchial ultrasound elastography. *Endosc Ultrasound*. 2016;5:233–238.
12. He H, Lu X, Ma H, et al. Value of endobronchial ultrasound elastography in the diagnosis of mediastinal and hilar lymph node metastasis in lung cancer. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*. 2016;41:30–36.
13. Jurado J, Saqi A, Maxfield R, et al. The efficacy of EBUS-guided transbronchial needle aspiration for molecular testing in lung adenocarcinoma. *Ann Thorac Surg*. 2013;96:1196–1202.
14. Bamber J, Cosgrove D, Dietrich CF, et al. “EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 1: Basic principles and technology.” *Ultraschall Med*. 2013;34:169–184.
15. Du Rand IA, Barber PV, Goldring J, et al. BTS Interventional Bronchoscopy Guideline Group. Summary of the British Thoracic Society guidelines for advanced diagnostic and therapeutic flexible bronchoscopy in adults. *Thorax*. 2011;66:1014–1015.
16. Wahidi MM, Herth F, Yasufuku K, et al. Technical aspects of endobronchial ultrasound-guided transbronchial needle aspiration: CHEST Guideline and Expert Panel Report. *Chest*. 2016;149:816–835.
17. Lee HS, Lee GK, Lee HS, et al. Real-time endobronchial ultrasound-guided transbronchial needle aspiration in

- mediastinal staging of non-small cell lung cancer: how many aspirations per target lymph node station? *Chest*. 2008;134:368–374.
18. Pickering EM, Holden VK, Heath JE, et al. Tissue acquisition during EBUS-TBNA: comparison of cell blocks obtained from a 19G versus 21G needle. *J Bronchol Interv Pulmonol*. 2019;26:237–244.
 19. Nakajima T, Yasufuku K, Takahashi R, et al. Comparison of 21-gauge and 22-gauge aspiration needle during endobronchial ultrasound-guided transbronchial needle aspiration. *Respirology*. 2011;16:90–94.
 20. Haseler LJ, Sibbitt RR, Sibbitt WL Jr, et al. Syringe and needle size, syringe type, vacuum generation, and needle control in aspiration procedures. *Cardiovasc Intervent Radiol*. 2011;34:590–600.
 21. Labarca G, Folch E, Jantz M, et al. Adequacy of samples obtained by endobronchial ultrasound with transbronchial needle aspiration for molecular analysis in patients with non-small cell lung cancer. Systematic review and meta-analysis. *Ann Am Thorac Soc*. 2018;15:1205–1216.