Original Article

Multi-modal tissue sampling in cone beam CT guided navigation bronchoscopy: comparative accuracy of different sampling tools and rapid on-site evaluation of cytopathology

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Background: Advanced technological aids are frequently used to improve outcome of transbronchial diagnostics for peripheral pulmonary lesions. Even when lesion access has been confirmed by 3D imaging, obtaining an accurate tissue sample however remains difficult. In this single institution study, we evaluate the comparative accuracy of different sampling methodologies and the accuracy of rapid on-site evaluation of cytopathology (ROSE) in navigation bronchoscopy cases where imaging has confirmed the catheter to have accurately accessed the lesion.

Methods: All consecutive navigation bronchoscopies in between December 2017– June 2020 performed in a room with a cone beam CT (CBCT) system where catheter position was intra-procedurally confirmed to be within or adjacent to the lesion by cone beam CT and augmented fluoroscopy were included. Individual tool outcomes were compared against one another and follow-up outcome.

Results: A mean of 11.39 samples using 2.93 tools were obtained in 225 lesions (median diameter 15 mm, 195 patients). A correct diagnosis was most often obtained by forceps (accuracy 70.6%), followed by 1.1 mm cryoprobe (68.4%), needle aspiration (46.7%), 1.9 mm cryoprobe (41.2%), brush (30.3%) and lavage (23.7%). Procedural outcome corresponded to follow-up outcome in 75.1% of lesions (80.5% of patients). Accurately diagnosed lesions were sampled significantly more often (11.91 *vs.* 9.72 samples, P=0.014). In cases where procedural outcome proved malignant, ROSE had also detected this in 47.5%.

Conclusions: Of all clinically available biopsy tools, the forceps showed most often accurate. However, extensive multi-modal sampling resulted in highest diagnostic accuracy. A hypothetical multi-modal approach of only using forceps and needle aspiration provided eventual diagnostic outcome in 91.7% of successfully diagnosed lesions. In the circumstances of our study, confirmation of malignancy on ROSE did not reduce number of biopsies taken nor biopsy time. Future research on how to improve the accuracy and effectivity of tissue sampling is needed.

Keywords: Biopsy; navigation bronchoscopy; peripheral pulmonary nodule; peripheral pulmonary lesion; rapid on-site evaluation of cytopathology

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Introduction

Despite the development and introduction of several technological platforms to help guide the physician in the past two decades, obtaining an accurate endobronchial diagnosis of small peripheral pulmonary nodules remain a challenge. These technological platforms allow for both navigation guidance towards a lesion as well as confirming accurate lesion access, which become increasingly important as lesions are small sized and located peripherally in the lung. Techniques include ultrathin bronchoscopy, virtual navigation bronchoscopy, electromagnetic navigation, radial endobronchial ultrasound probes (rEBUS), robotic assisted bronchoscopy and cone beam CT imaging (CBCT) (1-10).

Unfortunately, a confirmed successful navigation does not warrant a representative and accurate diagnosis. Several studies report the diagnostic yield and procedural sensitivity being lower than that of navigation success (2,10-12). Furthermore, a study by Coghlin *et al*. showed that the mean % area of samples obtained from visible endobronchial lung cancer on histopathology was only 33.4% and additionally found that 52% of patients had one or multiple fragments collected which contained no tumor at all (13). As lesions become smaller and are of early stage, the margin of error and the nuance become smaller, and likely even less samples will reveal the true nature of the lesion.

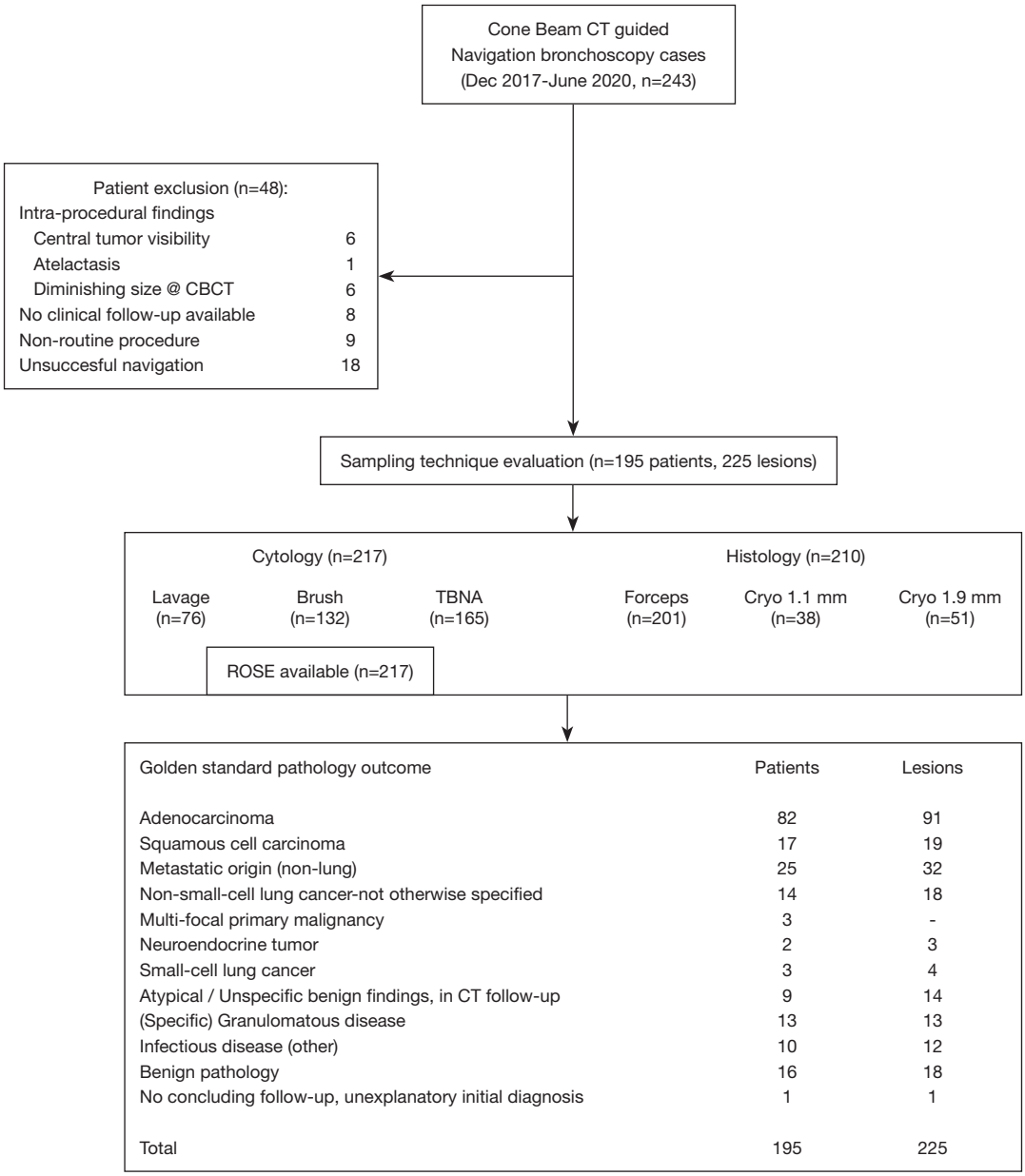
Obtaining accurate tissue samples for analysis is made difficult due to small lesion size, tumor heterogeneity, and, the ability of making a clear distinction in (early state) disease pathology in samples of limited size. The concurrent decision-making process on which tissue sampling methodology to use and deciding on when sufficient material has been collected add to this complexity. Additional general characteristics influencing the likelihood of a diagnostic outcome are the positioning relative to the bronchus, lobes involved, solidness, malignancy presence, pleural distance and patient characteristics such as emphysema or other co-morbidity (5,14-22). As two editorials recently concluded, there's however insufficient evidence to—in general—prefer one overall tissue acquisition method over the other, while several studies comparing individual technologies and concurrent methodologies have been published to date (2,23). Multiple studies suggest trans-bronchial needle aspiration (TBNA) has good outcome (15,17,18), while the brush, forceps and cryobiopsy probes are other commonly available and successfully used means (2,15,18,21,24-26). One further promising addition to the routine tissue sampling methodology is Rapid On-site Evaluation of cytopathology (ROSE), providing on-site information on cytology aspirate representativeness and the possibility of malignancy. It may enhance diagnostic yield and reduce complication rates in transbronchial and endobronchial sampling, although there is contradiction in results (27-29).

In this single center university hospital study, we comparatively assess the different tissue acquisition methodologies and the value of ROSE in a cohort of patients whom have been navigated to by CBCT guided navigation bronchoscopy for small peripheral pulmonary nodules. As the 3D CBCT image verification provides for tool analysis of only those lesions which have shown to be accurately accessed, it provides for a unique analysis. This article prospectively analyzes a cohort of consecutive patients in accordance with the STARD reporting checklist (available at https://dx.doi.org/10.21037/jtd-21-518). By relating biopsy tool outcomes per lesion, a direct paired comparison of tools is enabled whilst simultaneously incorporating clinical decision making on tool use. Combined, we report on overall diagnostic tool accuracy and agreement of ROSE with final pathology outcome in a navigation bronchoscopy setting.

Methods

Patients

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and took place in an experienced university hospital with a dedicated pulmonary pathology and interventional pulmonology team where navigation bronchoscopy is the first line diagnostic procedure for peripheral pulmonary lesions. Navigation bronchoscopy under 3D image guidance is only performed in those lesions where advanced navigation and/or confirmation is deemed necessary. The need of advanced 3D imaging in addition to r-EBUS imaging or conventional C-arm fluoroscopy was based upon physician estimation of procedure difficulty taking into account lesion size and location (>2nd order branches beyond segmental bronchi or no bronchus sign). In this study, only cases subject to the CBCT guided navigation bronchoscopy were included where 3D confirmation of lesion access was available. All patients receiving a CBCT guided navigation bronchoscopy for diagnosis of a peripheral pulmonary lesion in the period of December 2017 to June 2020 were eligible and



Golden standard pathology outcome	Patients	Lesions
Adenocarcinoma	82	91
Squamous cell carcinoma	17	19
Metastatic origin (non-lung)	25	32
Non-small-cell lung cancer-not otherwise specified	14	18
Multi-focal primary malignancy	3	-
Neuroendocrine tumor	2	3
Small-cell lung cancer	3	4
Atypical / Unspecific benign findings, in CT follow-up	9	14
(Specific) Granulomatous disease	13	13
Infectious disease (other)	10	12
Benign pathology	16	18
No concluding follow-up, unexplanatory initial diagnosis	1	1
Total	195	225

Figure 1 Flow diagram on study inclusion. Unsuccessful navigations were classified as those where imaging showed that the lesion was not successfully reached. A non-routine procedure indicated the primary reason for navigation bronchoscopy was other than diagnostics or different than normal procedural work-up due to study or clinical causes. ROSE was available only per analysis of brush and TBNA smears. TBNA, trans-bronchial needle aspiration; ROSE, rapid on-site evaluation of cytopathology.

prospectively approached for written informed study consent. Study approval was obtained by the independent local medical ethical committee (Arnhem-Nijmegen) and institutional review body (No: 2019-5148), informed consent was taken from all the patients. Patients in which a CBCT guided navigation setting was deemed unnecessary due to intra-procedural findings, or, patients in whom the lesion could not be successfully reached as verified by 3D CBCT, were excluded for analysis. After exclusion, only study subjects and concurrent peripheral lesions for which successful lesion access had been verified by means of navigation bronchoscopy remained for analysis (*Figure 1*).

Study method

Conventional bronchoscopes with 2.8 mm working channel (EB19-J10, Pentax Medical, Japan) and consecutive 2.6 mm catheters (Olympus medical guide sheath, Tokyo, Japan & Medtronic extended working channels, Minneapolis, USA) were used to navigate. Navigation was performed by one or a combination of electromagnetic navigation guidance (Medtronic SuperDimension), rEBUS (Olympus UM-S20-17S) and CBCT imaging with augmented fluoroscopy (Philips Allura/Azurion, Best, The Netherlands or Siemens Zeego, Forcheim, Germany). The procedures took place under general anesthesia. After navigation, lesion access was verified by at least CBCT and augmented fluoroscopy, but rEBUS was also often additionally used.

After confirming navigation success, the catheter remained positioned near or within the lesion throughout biopsy specimen acquisition. Biopsy specimen acquisition was performed with intermittent augmented fluoroscopy, CBCT and/or rEBUS imaging. All tool use and the amount of sampling was decided upon by the endoscopy team while taking into account factors such as safety, hypothesized efficacy, needed diagnostic testing and lesion characteristics. The intermittent ROSE outcome of individual samples was used to decide upon the need of additional sampling. With the tools that were decided upon, biopsy specimen acquisition was routinely performed in the following order; brushing, TBNA, followed by forceps and/or cryobiopsy and lavage. Sampling was routinely started using brush or TBNA. Based on the CBCT and/or rEBUS imaging outcomes, we estimated which tool had highest chance of accurately accessing the target lesion and obtaining a sample. Typically, when a bronchus sign was identified and/or rEBUS showed central access in a solid lesion, a brush was first obtained. In other cases, TBNA was the first tool of choice. Lavage was performed lastly and included injection of 10 to up to 60 mL of 0.9% saline through the catheter or endoscope. Subsequent 0.9% saline retrieval was attempted by suction until deemed sufficient for analysis. Brushing for cytopathology was performed by a pushing-pulling technique of the complete 1.8 mm brush in and out of the catheter (Olympus BC-202D-2010 brush, Medtronic SuperDimension cytology brush or Medi-globe cytology brush, Achenmühle, Germany). Needle aspiration was predominantly performed with 18G needles (Broncus FleXNeedle, San Jose, USA) by means of a similar motion as to that of the brush, with suction being applied by syringe proximally. Twenty-one gauge and 19G needles of other manufacturers were less often used, based upon commercial primary needle availability (Medtronic SuperDimension Aspirating Needle). Typically at least three aspirations were obtained with approximately ten strokes per aspiration. Serrated or non-serrated oval forceps of 1.8 mm diameter were used to acquire histology specimens (Boston Scientific Pediatric Radial Jaw 4 or Olympus FB-233D biopsy forceps). After advancement through the catheter, the forceps were opened and further pushed into the lumen until traction could be felt, after which they were closed and retracted for histology specimen collection. Cryobiopsy for histopathology was available only through a 1.9 mm cryoprobe until September 2019, after which a 1.1 mm version also became available (Erbe Elektromedizin, Tuebingen, Germany). Due to probe and sample size, the 1.9 mm cryoprobe had to be removed along with catheter and endoscope after an initial freeze of 4–7 s. Oppositely, the catheter could be left in place for repeated sampling with the 1.1 mm cryoprobe. Freezing during 5–9 s was followed by removal of the 1.1 mm probe for histopathology collection.

Individual brush and TBNA cytology samples were divided onto two slides; one Giemsa staining which was also used for ROSE and one slide for Papanicolaou smearing used only for definitive analysis. Remaining material was collected for cell block. Separate collection containers were used for every sampling tool. ROSE was available for all cases included in this study, which in our center is performed by a dedicated team of three experienced and trained cytopathology technicians who are routinely involved in all navigation bronchoscopy, EBUS, EUS and ultrasound guided TTNA procedures performed (totaling >6,000 procedures in the past decade). The ROSE procedure took place at the interventional pulmonology suite, such that two-way communication was easily maintained.

Statistical analysis

Descriptive parameters are presented as counts and percentages, along with medians, means and ranges. All individual tool outcomes and concurrent individual nodules were correlated to a procedural outcome and a golden standard follow-up outcome. Golden standard follow-up was either the final pathology diagnosis from surgical resection, additional CT guided TTNA and/or clinical follow-up with CT for at least 6 months was considered definitive. The comparative accuracy of tools was determined by pairing tool outcome in cases of individual lesions where two or more tools were used. The McNemar chi-squared test was

Table 1 Patient, lesion and procedural characteristics

Variables	Number
Patients/lesions (successfully accessed)	195/225
Age, mean [range] (years)	65.2 [36–85]
Gender (M/F)	103/92
Malignancy prevalence (pt./lesion)	76%/75%
Benign disease follow-up, median [range] (days)	491 [215–1,224]
Nodule size, median [range] (mm)	15 [5–65]
Bronchus sign (lesion)	64.4%
Diagnostic accuracy procedure	80.5% (157/195)
Diagnostic accuracy lesion	75.1% (169/225)
Lesion locations	
LUL/RUL	62/77
RML	11
LLL/RLL	32/43

LUL, left upper lobe; RUL, right upper lobe; RML, right middle lobe; LLL, left lower lobe; RLL, right lower lobe.

used to test equivalence of tool accuracy. Wilcoxon testing was performed for comparison on not-normally distributed data. Student's *t*-tests were used to test accuracy differences between unpaired data. Tests with a P value of <0.05 were considered significant. For evaluation of ROSE, all procedures where ROSE was performed were included for analysis. The concordance of procedural malignant ROSE outcome by all cytological specimens was compared with both final procedural cytology as well as procedural histology outcome. R and RStudio were used for statistical analysis (30).

Results

A total of 195 patients received successful navigation bronchoscopy as verified by CBCT and augmented fluoroscopy imaging, in which a total of 225 lesions were shown to be successfully reached (*Figure 1*, *Table 1*). Malignant disease was found in 76% of patients. Median lesion diameter was 15.0 mm (range, 5–65 mm). Lesions were pure ground glass or part solid in 7.1% and 15.1% of cases, respectively. The procedural outcome (whilst navigation had showed successful) corresponded to gold standard outcome in 80.5% of cases on a per patient basis, and 75.1% of cases on a per lesion basis.

Comparative tissue sampling method accuracy

Analysis of individual tissue sampling methods revealed forceps biopsy was most often found accurate (70.6%), followed by 1.1 mm cryoprobe findings (68.4%), TBNA (46.7%), 1.9 mm cryoprobe (41.2%) and tiers brush (30.3%) and lavage (23.7%). The pair-wise tool comparison substantiated these accuracy findings (*Figure 2*). The forceps showed superior accuracy when directly compared against all cytology techniques (brush, TBNA, lavage P<0.01), but was not significantly better than pooled 1.1 and 1.9 mm cryobiopsy accuracy (P=0.081). Pooled 1.1 mm and 1.9 mm cryobiopsy had significantly better accuracy than lavage and brush (P<0.05), but not TBNA (P=0.40). The 1.1 mm cryoprobe was more often accurate than the 1.9 mm cryoprobe (68.4% *vs.* 41.2%, respectively).

Analysis of a multi-modal sampling approach revealed sampling using forceps combined with TBNA would have provided eventual diagnostic outcome in 155 out of 169 successfully diagnosed lesions (91.7%). In the cases where these were not diagnostic (n=14), sampling by 1.1 mm and 1.9 mm cryoprobe correctly diagnosed 2 and 8 lesions whilst brushing and lavage were accurate in 4 and 3 lesions, respectively.

Cytology versus histology

The cytology findings (brush, TBNA, lavage) corresponded to gold standard follow-up outcome in 49.3% of lesions (107/217, *Table 2*). Histology findings (by forceps and cryobiopsy) were more often found accurate (72.4%), but the combination of both cytology and histology remained most accurate (75.1%). Analysis of data wherein both cytology and histology were obtained shows histology was significantly more often accurate, be it either malignant or benign (*Table 2*, P value <0.01). The histology outcome by itself was significantly more often accurate in benign lesions than in malignant lesions (P<0.01, 88.6% *vs.* 66.9%, respectively). This finding did not similarly hold for cytology outcome (48.5% accuracy in benign lesions and 51.9% accuracy in malignant lesions, *Table 2*).

Extensive multi-modal sampling

Analysis of sampling method accuracy shows lesions whom were accurately diagnosed were subjected to a significantly higher amount of sampling (*Table 3*, P=0.014). An average of 3.83 separate cytology samples (range, 0–15) and 7.56 separate

	Lavage	*Brush*	*TBNA*	*Forceps*	*Cryo 1.1 mm*	*Cryo 1.9 mm*
Lavage	23.7% (18/76)	23.9%/32.8% (n=67, P=0.21)	26.7%/37.8% (n=45, P=0.27)	24.6%/66.2% (n=65, P<0.01)	0%/100% (n=1, P=NA)	0%/46.6% (n=15, P=NA)
Brush		30.3% (40/132)	21.0%/43.2% (n=81, P<0.01)	31.1%/66.9% (n=119, P<0.01)	20%/90% (n=10, P=0.023)	25.8%/38.7% (n=31, P=0.34)
TBNA			46.7% (77/165)	48.1%/72.1% (n=154, P<0.01)	58.8%/67.6% (n=34, P=0.58)	21.9%/37.5% (n=32, P=0.27)
Forceps				70.6% (142/201)	80.6%/72.2% (n=36, P=0.51)	57.1%/42.9% (n=42, P=0.21)
Cryo 1.1 mm					68.4% (26/38)	100%/100% (n=1, P=NA)
Cryo 1.9 mm						41.2% (21/51)

Figure 2 Sampling accuracy of different instruments as used in the navigation bronchoscopy procedure. Diagonal (grey boxes): accuracy of the individual instrument, as calculated over the amount of times the instrument was used (n/N). Top right half of table: Pair-wise comparison of tool accuracy, calculated by evaluation of cases where both tools were used in the same lesion. The individual tool accuracies as calculated by every time they were pair-wise used are shown in red (rows) and blue (columns). I.e. the first row shows how often lavage was accurate versus how often other tools were accurate (in red), when both were used (in blue). In between brackets (in black) the number of times the pair-wise tool comparison was available (as both tools were used in a single lesion), followed by probability of significant accuracy differences between the two tools. NA, not applicable; TBNA, trans-bronchial needle aspiration; Cryo, cryoprobe sampling; n, amount of cases; p, probability outcome of McNemar chi-squared test for pair-wise comparison of instruments.

histology samples (range, 0–18) were acquired per procedure, equivaling an overall average of 11.39 samples (range, 1–25). In case they were used, collection of samples by forceps and 1.1 mm cryobiopsy was performed an average of 7.08 and 6.12 times per lesion, respectively. Brush and TBNA—both enabling ROSE—were used for repeat sampling a respective average of 1.64 and 3.26 times (*Table 3*). Overall outcome further showed that the forceps and TBNA sampling tools were used most of all tools, respectively having been used in 89.3% and 73.3% of lesions (*Figure 2* and *Table 3*).

Rapid on-site evaluation of cytopathology

Rapid on-site evaluation of cytopathology was available in cases where sampling by brush and/or TBNA was performed (n=217). Of 79 lesions where cytology analysis was suggestive of malignancy, ROSE concluded similarly in 57 (69.8%). Due to the discrepancy between cytology results and overall pathology results, ROSE was able to conclude malignancy in 47.5% of cases where procedural outcome showed malignancy (57/120, *Table 2*). In the procedures where ROSE was found suggestive of malignancy, significantly more samples were obtained than where it did not (respective average of 12.65 *vs*. 10.51 samples, P=0.016, *Table 3*). The biopsy time insignificantly correlated to these findings, taking an average of 25.1 minutes in ROSE findings suggestive of malignancy and 24.2 minutes in non-confirmatory ROSE findings (P=0.36).

Discussion

In navigation bronchoscopies where access of lesions with a mean 15 mm size had been verified through (repeated) 3D-imaging, we found that procedural pathology outcome corresponded to follow-outcome in 80.5% per patient (75.1% of lesions). An accurate diagnosis was most often obtained by forceps (accuracy 70.6%), followed by 1.1 mm cryoprobe (68.4%), TBNA (46.7%), 1.9 mm cryoprobe (41.2%), brush (30.3%) and lavage (23.7%). Our pair-wise comparison of tools showed a similar order of individual tool yield. By these findings, histology results alone were found representative of follow-up outcome in 72.4%, and by cytology in 49.3% of cases (*Figure 2* and *Table 3*, P value <0.01). Due to the discordance of cytology findings with overall procedural findings, and ROSE again being discordant with cytology (in 27.9% of cases), ROSE was able to help predict procedural malignant pathology outcome in only 47.5% of cases.

During the conduct of this study, (intermittent) 3D-imaging verification was frequently used both after navigation and in-between repeated sampling. We

Table 2 Sampling outcome (per lesion) versus procedural and follow-up gold standard outcome

Pathology outcome—per lesion basis	%	n
Rapid on-site evaluation of cytopathology		
ROSE correlating to malignant cytopathology findings	72.1%	57/79
ROSE correlating to procedural malignant findings (cytology & histology combined)	47.5%	57/120
Sampling accuracy in malignant lesions		
Cytopathology (procedural) accuracy	48.5%*	79/163
Histopathology (procedural) accuracy	66.9%*,**	105/157
Combined pathology (procedural) accuracy	71.0%	120/169
Molecular analysis possible†	83.9%	26/31
Sampling accuracy in benign lesions		
Cytopathology (procedural) accuracy	51.9%*	28/54
Histopathology (procedural) accuracy	88.6%*,**	47/53
Combined pathology (procedural) accuracy	87.5%	49/56
Sampling accuracy—lesions overall		
Cytopathology (procedural) accuracy	49.3%*	107/217
Histopathology (procedural) accuracy	72.4%*	152/210
Overall pathology (procedural) accuracy	75.1%	169/225

Results of rapid on-site evaluation (ROSE) of cytopathology are described specifically for malignant disease findings as found per procedural cytopathology outcomes and overall procedural outcomes (being malignant). As clinical decision making decided on tool use, only cases where cytology or histology was available were used for calculation of accuracy. *, significant differences (P value <0.01, McNemar chi-squared test) between accuracies of cytology and histology in benign, malignant and lesions overall were found when assessing paired outcomes. **, significant difference (P value <0.01, unpaired two-sided Student's *t*-test) between accuracy of histology outcomes in benign and malignant lesions. †, molecular analysis is only performed upon request (when clinically indicated), denoted here is the number of times it was possible when requested.

hypothesize the use of imaging in combination with maintained catheter position were essential in meticulous positioning and acquiring tissue at different sites of hypothesized pathology. It is likely only a minority of tissue samples contains pathologic tissue, as also indicated by Coghlin *et al.* in biopsy samples of visible lung cancers (13). The NAVIGATE study and a recent systematic review and meta-analysis substantiate these findings, finding a higher procedural success in cases of multi-modal and extensive sampling (12,31). We also found significantly more sampling was performed in lesions where an accurate diagnosis could be obtained (9.72 *vs.* 11.91 samples, P=0.014). Our repeated sampling with tools was based on clinical decision making rather than being a randomized and controlled trial. With the available tools having different properties, we deemed it relevant to tailor their use to the situation. In general, we chose our tools based upon relative positioning of the catheter to the lesion and secondary adjacent structures (e.g., pleura, vessels, cavities). When a central position in the lesion was obtained, a brush was generally the first choice (followed by secondary sampling tools). When lesion position was eccentric or trans-parenchymal access was deemed necessary, TBNA was often preferred first. The choice to not perform repeated TBNA, brush or lavage sampling more often was made consciously. EBUS-TBNA and ROSE literature has shown acquisition of more than 4 TBNA samples in EBUS is of minimal additional value and sufficient for enabling molecular analysis in the vast majority of cases (32). While we cannot perform sampling under direct ultrasonic or video guidance, the motion and straightening of the catheter after needle insertion along with ROSE findings often being consistent in outcome from first to last biopsy made us decide on reducing routine TBNA use to 3–4 times. The choice of forceps and higher

Table 3 Descriptive statistics on procedural sampling techniques

Descriptive statistics on sampling techniques	Mean (samples)	Median (samples)	Min–max (samples)	Accuracy (tool)
Cytology	3.83	4	1–15	107/217 (49.3%)
Lavage	1.18	1	1–3	18/76 (23.7%)
Brush	1.64	2	1–4	40/132 (30.3%)
TBNA	3.26	3	1–11	77/165 (46.7%)
Histology	7.56	7	0–18	152/210 (72.4%)
Forceps	7.08	7	1–18	142/201 (70.6%)
Cryobiopsy (1.1 mm)	6.12	7	1–10	26/38 (68.4%)
Cryobiopsy (1.9 mm)	1.15	1	1–2	21/51 (41.2%)
Samples total	11.39	11	1–25	169/225 (75.1%)
ROSE: malignant findings	12.65†	12	4–24	–
ROSE: benign findings	10.51†	10	1–25	–
Unsuccessful diagnosis	9.72‡	10	1–23	–
Successful diagnosis	11.91‡	12	1–25	–
Tools total	2.93	3	1–6	–

Amount of sampling obtained are computed from cases where the instrument was used to acquire at least one sample. The accuracy are furthermore given by comparing the amount of times the instrument was used and led to an accurate diagnosis as compared against the total times it was used. On the bottom half of the table, the total amount of samples obtained for the different types of procedure characteristics are furthermore given; amount of samples when ROSE concluded malignancy, did not conclude malignancy, amount of samples in undiagnostic lesions and in cases where an accurate diagnosis could be obtained. †, P value =0.016, significantly more samples were obtained when ROSE had concluded malignancy; ‡, P value =0.014, significantly more samples were obtained in accurate procedures. TBNA, trans-bronchial needle aspiration; ROSE, rapid on-site evaluation of cytopathology.

amounts of repeat biopsy when compared to other tools was partially based on the observation that precurved catheters as used in this study lost least of their curvature by forceps, consequently allowing for biopsy of different sites. This could also provide for an explanation why we find a significant cytology and histology accuracy difference in both diagnosing benign and malignant lesions (*Table 2*). But where cytology accuracy was similar in malignant and benign lesions (48.5% *vs*. 51.9%, respectively), histology outcome was of higher variation (66.9% *vs*. 88.6% for malignant and benign outcomes, respectively). We cannot clearly explain this histology accuracy difference in benign and malignant findings, as it is also contrary to generalized findings of other studies (31). As such, additional research remains needed.

Procedural ROSE is likely of highest added value if feedback and communication between the interventional pulmonology and ROSE team is continuously had and a standardization of the process is agreed upon. As previously found, the ROSE methodology may however differ among centers, with no evidence for preferring one above another smearing method (32). A meta-analysis by Mondoni *et al*. found ROSE increases the procedural yield also in diagnosis of peripheral lesions (15). Moreover, previous reports have shown that molecular analysis in EBUS-TBNA can be performed more often in a ROSE enhanced procedure (29). In this study, we uniquely use Cone beam CT imaging to verify in 3D that the lesion has been accurately accessed. Consecutively, extensive sampling was performed. Yet even after having (repeatedly) verified lesion access, a diagnosis indicative of procedural malignant outcome could be obtained by ROSE in only 47.5% of cases (*Table 2*). The discordant findings when related to procedural and cytology outcome may in part be explained by differences in available material. ROSE provides outcome on Giemsa staining, whilst additional material is harbored within Papanicolaou staining and cell block. Another cause of discordance might be related to the cytopathologist's opinion that analysis of peripheral pulmonary lesion smears is different to that of EBUS-TBNA, being more diverse.

With decreasing lesion size and less solidity, differentiating between atypical findings and malignancy becomes more difficult (2). Combined, we can conclude that ROSE was of additional value in less than 50% of cases, and, that it was not associated with a reduction of total number of samples taken nor with biopsy time. However, having ROSE, the feedback obtained from our team enabled us to adapt our sampling strategy per nodule. The results of our study propose that more sampling is performed in cases where ROSE is confirmatory of malignancy. This is opposite to one would expect, as it should logically allow for earlier abandoning of additional sampling instead. With high chance of malignancy, we wanted to be sure to have sufficient tissue for a complete analysis in the modern era. Therein, even when we had a clear confirmation on the presence of malignancy and confirmation that the sample contained sufficient cells (or estimated DNA) for full analysis, we likely still obtained additional histology samples to maximize the chance of confirmatory outcome for the patient and avoid the possibility of needing re-biopsy. As a result, in these circumstances, having ROSE to confirm malignancy did not translate into less samples taken.

To our knowledge, this is one of first clinical trials to report on 1.1 mm cryoprobes for peripheral lesion biopsy in a through-the-catheter approach. Our initial experience with the 1.9 mm cryoprobe was cumbersome, as it often dislocated initial catheter position upon insertion due to probe rigidity. This especially was problematic in cases with tight instrument angulations, and could not be compensated for completely in several cases. Consequently, we feel the probe was often not as optimally positioned when compared to other instruments. What's more, due to the probe's size, the probe had to be removed together with the catheter for a single specimen removal. As a result, only one sample could be obtained at the end of the procedure. The newer 1,1 mm cryoprobe allows for repeated sampling through the extended working channel, is much less rigid and as such; easier to use. As descriptively reported, a combination of TBNA with forceps biopsy in a curved guide sheath however deprecated the added value of also adding this tool to the inventory in 22 out of 24 cases where it was found diagnostic. It has previously been described that especially lesions non-concentrically positioned around the bronchus would be benefitted by the cryoprobe (21). Our findings show this might be less valid for the smaller sized probe, which could be caused by its difference in area and tissue penetration.

Folch *et al*. and the NAVIGATE trial report a higher degree of sampling and multi-modal sampling led to higher yield (12,31). We report routinely sampling more than 10 times per lesion in a multi-modal fashion and similarly see a higher amount of sampling correlating to finding an accurate diagnosis by procedural pathology more often (*Table 3*, P=0.014). Our high degree of repeat sampling was in part based on our clinical findings that lesion access by imaging verification was frequent, but a confirmatory diagnosis could then not always be made. The hypothetic TBNA and forceps only scenario as presented in this study however shows we would have found 91.7% of eventual diagnoses by only using these two modalities. It could be suggested that not all lesions require extensive multi-modal sampling. Yet, it can also be concluded that no commercially and readily available tool is currently a do-it-all tool even if lesion access has been confirmed. Therefore, multi-modal and repeated sampling seems to remain the recommended methodology.

Conclusions

This study evaluates the outcome of different tissue sampling tools and ROSE in a subset of navigation bronchoscopies where 3D-imaging had verified lesion access in a routine clinical setting using widely available sampling tools. With repeated multimodal sampling, pathology outcome was found corresponding to gold standard follow-up outcome in 75.1% of lesions, resulting in 80.5% of patients obtaining a representative diagnosis. In lesions where an accurate diagnosis could be obtained, significantly more sampling was performed (9.72 *vs*. 11.91 samples, P=0.014). An accurate diagnosis was most often obtained by forceps (accuracy 70.6%), followed by 1.1 mm cryoprobe (68.4%), TBNA (46.7%), 1.9 mm cryoprobe (41.2%), brush (30.3%) and lavage (23.7%). Analysis of a multi-modal sampling approach reveals sampling using only forceps and TBNA would have provided eventual diagnostic outcome in 91.7% of successfully diagnosed lesions. In cases where procedural pathology outcome proved malignant, ROSE had precedingly confirmed malignancy in 47.5%. Confirmation of malignancy from ROSE during these procedures did not translate into a reduction of the number of biopsies taken or biopsy time. In conclusion, there is currently no single tool or methodology that is a do-it-all. Future research on how to improve the accuracy and effectivity of tissue sampling in navigation bronchoscopy is needed.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study protocol was approved by the independent local medical ethical committee (Arnhem-Nijmegen) and institutional review body before start of subject inclusion (file number 2019-5148). Informed written consent was obtained from all study subjects.

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