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Bronchoscopic Brushing from Central Lung Cancer—Next Generation Sequencing Results are Reliable

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Abstract

The role of bronchoscopic brushing for tumor detection and molecular testing in central lung cancer is unclear. In this study, 50 consecutive subjects with suspected central lung cancer underwent bronchoscopic brushing (31 males, median age 70, 5 never smokers). Histological results were: NSCLC/SCLC/low-grade-NET/granulation tissue in 36/8/2/4 cases. Next generation sequencing (NGS) was feasible in 62% of tumor-positive brush smear samples. In 78% of these cases, NGS displayed identical results compared to histology samples, in 22% NGS from brush smears detected specific mutations, whereas DNA quality from forceps biopsy was insufficient for NGS analysis. Sensitivity, specificity, positive predictive value, negative predictive value of brush smear analysis were 66% (95% confidence interval 50–79), 100% (40–100), 100% (85–100), and 21% (7–46). For the combined analysis of brush smear, brush tip washing and sheath tube content sensitivity was slightly elevated at 69% (53–81). In central lung cancer, bronchoscopic brushing detects tumor cells in about two-third of cases and allows a decision for or against targeted therapy in the majority of tumor-positive cases on the basis of NGS analysis.

Keywords Diagnostic bronchoscopy · Brushing cytology · Molecular genetic testing · Carcinoma · Tumor detection

Abbreviations

ALK	Anaplastic lymphoma kinase
c.	Coding deoxyribonucleotide acid sequence (mutation at nucleotide level)
DDR2	Discoidin domain receptor 2
EBUS-TBNA	Endobronchial ultrasound-guided transbronchial needle aspiration
KRAS	Kirsten rat sarcoma viral oncogene homolog
MET	Mesenchymal-epidermal transition proto-oncogene
NET	Neuroendocrine tumor
NGS	Next generation sequencing
NRAS	Neuroblastoma RAS viral oncogene homolog
NSCLC	Non-small cell lung cancer
NPV	Negative predictive value
p.	Protein (change at amino acid level)
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha
PPV	Positive predictive value
PTEN	Phosphatase and tensin homolog
RET	Rearranged during transfection proto-oncogene
ROS1	ROS1 proto-oncogene
SCLC	Small cell lung cancer
TP53	Tumor protein 53
TTF1	Thyroid transcription factor 1

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Introduction

Flexible videobronchoscopy is considered the gold standard for the evaluation of central exophytic lung cancer with endobronchial forceps biopsy being the standard sampling technique.

In some patients (e.g., under anticoagulant therapy), there may be a relevant risk of bleeding when forceps biopsies are performed. Bronchoscopic brushing is afflicted with a low risk and may be the preferred sampling tool in these cases. Albeit the risk of bleeding has been described to be about 6% after brushing in peripheral lesions, in the central airways the feasibility of careful brushing may be better and bleeding can be controlled very well in most cases [1]. However, in a large metaanalysis on central endobronchial bronchogenic carcinoma the sensitivity of brushing has shown to be lower than the sensitivity of forceps biopsy (61% versus 74%) [2].

Although next generation sequencing (NGS) is recommended in the lung cancer guidelines [3] and is broadly available, the technology is underutilized in clinical practice [4]. It has been shown that the likelihood of applying NGS testing on tumor samples was correlated with the biopsy tool which had been applied for sampling. This may be partially due to the heterogenous evidence for specific sampling procedures [4].

It has not yet been investigated whether it is possible to optimize the brushing procedure and subsequent analysis in order to maximize its impact both in terms of securing a diagnosis and providing a basis for targeted therapy.

The brush is enclosed in a sheath tube prior to deployment through the bronchoscope, and specimens are usually obtained solely from the brush bristles by performing cytological smears upon microscope slides. It is unclear whether the combined cytological analysis of brush smears, brush tip washing and sheath tube content increases the sensitivity and/or specificity of bronchoscopic brush cytology in comparison with conventional brush smears alone.

It is not known whether next generation sequencing (NGS) analysis is feasible from cytology brush samples in a sufficient proportion of cases and whether NGS results correlate well with results from histopathological samples.

Methods

Design and Patients

Fifty consecutive subjects who were admitted with the suspicion of central lung cancer between June 2014 and December 2015 were analyzed retrospectively. All subjects underwent routine bronchoscopy with cytological sampling by brushing and, where feasible, by forceps biopsy or endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), or alternatively by catheter biopsy. All studies were based on data recorded during routine clinical care.

Bronchoscopy

Samples were obtained by flexible videobronchoscopy (Olympus, BF-1TH190) from the suspicious central endobronchial lesion using a brush (Olympus, BC-202D-2010). Conventional cytological smears were prepared, and the slides were air-dried. The brush tip was transferred to a sample container filled with sterile sodium chloride solution (0.9%), and formalin solution was added. The sheath tube was flushed with sterile sodium chloride solution (0.9%), the obtained sheath tube content was transferred to a separate sample container, and formalin solution was added. All three cytological samples were sent separately for pathological evaluation. Selection of further biopsy tools depended on technical features and the nature of the endobronchial tumor but remained at the discretion of the endoscopist (Forceps: Olympus, FB 231D, EBUS-TBNA-needle: Olympus, NA-201SX-4021; catheter: Wieser, 152.12023).

Cytological Staining

All cytological specimens were processed and stained using standard cytopathological techniques (Pappenheim, Hematoxylin-Eosin, in addition immunocytochemistry at the discretion of the cytopathologist: CD56, p40, TTF1). Brush tip washings and sheath tube contents were processed as cell blocks after cytocentrifugation.

Next Generation Sequencing Analysis

Where feasible microscope slides from paraffin embedded tissue, brush smears or cytocentrifugation samples were analyzed. Areas with tumor cells were marked with a permanent thin marker on the coverslip. The tumor cell content in the relevant area of the smear was determined. Before the coverslip was placed in xylene and removed, marked areas were transferred and scratched at the back of the slide with a diamond pen. The DNA extraction from marked areas was carried out using the QiaSymphony (Qiagen, Hilden, Germany). Mutational analysis was performed by massively parallel sequencing (IonAmpliSeq-Custom-DNA-Panel and IonAmpliSeq-Library-Kit-2.0, Thermo-Fisher-Scientific, Waltham, MA, USA). After multiplex PCR, libraries were generated by adapter ligation and target enrichment using the GeneRead-DNA-Library-I-Core-Kit, the GeneRead-DNA-I-Amp-Kit (Qiagen, Hilden, Germany) and the NEXTflex-DNA-Barcodes (BioScientific, Austin, TX, USA). 12 pM of the constructed libraries were sequenced on the MiSeq (Illumina, San Diego, CA, USA) with a MiSeq-reagent-kit-V2 (300 cycles) (Illumina). Computational analysis was

performed using an in-house software tool to detect relevant somatic mutations in a quantitative manner [5].

Results from brush samples were compared with available findings from standard sampling tools (forceps, EBUS-TBNA, catheter) and were available with specimens from surgical resection.

Statistics

Descriptive statistics are provided as absolute numbers and percentages as well as median and interquartile range where appropriate. Diagnostic accuracy of brush smear, brush tip washing and sheath tube content analysis to detect tumor cells was determined based on results of forceps ($n=46$), EBUS-TBNA ($n=2$) or catheter biopsies ($n=1$) as the reference standard. Sensitivity, specificity, positive and negative predictive value were determined (PPV, NPV).

Results

Fifty consecutive subjects (31 males, 19 females) were included in the study. The median age was 70 (quartile1–quartile3: 65–75); median body mass index was 25 kg/m^2 (quartile1–quartile3: 22–29). Twenty-seven subjects were active smokers, 18 former smokers, 5 never smokers. Tumor classification according to Union internationale contre le cancer 7 was IA, IB, IIA, IIB, IIIA, IIIB, IV in 1, 2, 5, 3, 7, 6, 19 subjects, respectively. Tumor classification was not feasible due to benign diagnosis in 4 cases and due to incomplete staging in 3 cases.

Non-small cell lung cancer was proven in 36 subjects (15 squamous, 18 adeno, 3 adeno-squamous), small cell lung cancer in 8, low grade neuroendocrine tumor (typical carcinoid) in 2 subjects. Samples from surgical resections were available in 5 cases and showed identical histopathological findings as the samples obtained from bronchoscopy.

Due to cases with insufficient cell material, the cytological analysis of brush smear, brush tip washing and sheath tube content was feasible in 48, 49 and 49 cases.

Cytological analysis from brushing detected tumor cells in 29/48 subjects by brush smear analysis, in 18/49 subjects by brush tip washing analysis and in 17/49 subjects by sheath tube content analysis.

The sensitivity of brush smear analysis was 66% (95% confidence interval 50–79), the specificity was 100% (40–100), the PPV was 100% (85–100), and the NPV was 21% (7–46) (Table 1).

In one subject with histologically proven lung cancer and negative brush smears, the brush tip washing analysis was positive. The combined analysis of brush smears together with brush tip washing provided a sensitivity of 69% (53–81), a specificity of 100% (40–100), a PPV of 100% (86–100) and a NPV of 22% (7–48).

No cases of proven lung cancer with negative brush smears were detected by the additional analysis of sheath tube content samples.

Analyzing the results from brush smears compared to forceps biopsy alone, sensitivity was 71% (53–85), specificity 70% (35–92), PPV 89% (71–97) and NPV 41% (19–67).

NGS analysis from brush smear samples was feasible in 18 out of 29 subjects (62%) (Table 2).

NGS analysis from forceps biopsy samples was clinically initiated in 29 subjects and was feasible in 23 subjects (79%), in 6 subjects the tissue and/or DNA quality was insufficient for analysis.

NGS results from brush smears and forceps biopsies were compared in the 18 subjects where NGS from brush smears were feasible. Identical NGS results were found in 14 cases, although in 2 of these cases, only a limited NGS panel could be analyzed in the forceps biopsy due to low DNA coverage. In the remaining 4 subjects, NGS analysis from forceps biopsy samples was not feasible.

The mean tumor cell content in these 18 subjects was higher for the brush smears than for the forceps samples

Table 1 Diagnostic accuracy of brush smear, brush tip washing and sheath tube content analysis and combinations thereof to detect tumor cells

	Brush smear[a]	Brush tip washing[a]	Sheath tube content[a]	Brush smear + brush tip washing[a]	Brush smear + brush tip washing + sheath tube content[a]	Brush smear vs. forceps only
Cases analyzed (*n*)	48	49	49	49	49	45
Sensitivity (%)	66 (50–79)	40 (26–56)	38 (24–53)	69 (53–81)	69 (53–81)	71 (53–85)
Specificity (%)	100 (40–100)	100 (40–100)	100 (40–100)	100 (40–100)	100 (40–100)	70 (35–92)
Positive predictive value (%)	100 (85–100)	100 (78–100)	100 (77–100)	100 (86–100)	100 (86–100)	89 (71–97)
Negative predictive value (%)	21 (7–46)	13 (4–31)	13 (4–30)	22 (7–48)	22 (7–48)	41 (19–67)

[a]In these cases, the combined results of histopathological/cytological analyses of forceps, EBUS-TBNA or catheter biopsy samples were used as the reference standard. 95% confidence interval given in parentheses

Table 2 Tumor cell content and results of next generation sequencing analysis from cytology samples (brush smear) and forceps biopsy samples (individual results from 18 subjects)

Case number	Cytology from Brush smears tumor cell content (%) (tumor cell count divided by total cell count)	Histology: tumor cell content (%) (tumor cell count divided by total cell count)	Results of next generation sequencing analysis (results from brush smear samples)	Results of next generation sequencing analysis (results from histology samples)
1	20	30	TP53: c.596G > T, p.G199V; TP53: c.880G > T, p.E294*	TP53: c.596G > T, p.G199V; TP53: c.880G > T, p.E294*
2	80	70	TP53: c.517G > T, p.V173L	TP53: c.517G > T, p.V173L
3	60	70	KRAS: c.34G > C, p.G12R; TP53: c.797G > T, p.G266V	KRAS: c.34G > C, p.G12R; TP53: c.797G > T, p.G266V
4	80	20	Wild-type	Wild-type
5	50	40	TP53: c.584T > C, p.I195T	wild-type (TP53 not analyzed)
6	30	30	DDR2: c.1305G > C, p.R435S; KRAS: c.34G > T, p.G12C	DDR2: c.1305G > C, p.R435S; KRAS: c.34G > T, p.G12C
7	80	30	Wild-type	Insufficient DNA quality
8	50	80	TP53: c.892G > T, p.E298*	TP53: c.892G > T, p.E298*
9	70	30	PIK3CA: c.1624G > A, p.E542K; PTEN: c.265delC, p.P89Lfs*10; TP53: c.820G > C, p.V274L	Insufficient DNA quality
10	30	10	DDR2: c.493C > T, p.R165W; TP53: c.857A > T, p.E286V	Insufficient DNA quality
11	80	50	TP53: c.706T > G, p.Y236D	TP53: c.706T > G, p.Y236D
12	50	70	TP53: c.733G > T, p.G245C	TP53: c.733G > T, p.G245C
13	90	20	TP53: c.722C > T, p.S241F	TP53: c.722C > T, p.S241F
14	40	20	DDR2: c.360C > G, p.I120M; TP53: c.747G > T, p.R249S	DDR2: c.360C > G, p.I120M; TP53: c.747G > T, p.R249S
15	60	30	Wild-type	Wild-type (KRAS/NRAS analyzed only)
16	60	50	TP53: c.406C > T, p.Q136*	TP53: c.406C > T, p.Q136*
17	70	40	KRAS: c.35G > A, p.G12D; TP53: c.455C > T, p.P152L	Insufficient DNA quality
18	20	10	KRAS: c.34G > T, p.G12C; TP53: c.469G > T, p.V157F	KRAS: c.34G > T, p.G12C; TP53: c.469G > T, p.V157F

Tumor cell content, detected tumor protein alterations with mutations at nucleotid level and change at amino acid level given according to the standard mutation nomenclature in molecular diagnostics [6]

c. coding deoxyribonucleotide acid sequence (mutation at nucleotide level), *DDR2* discoidin domain receptor 2, *KRAS* Kirsten rat sarcoma viral oncogene homolog, *NRAS* neuroblastoma RAS viral oncogene homolog, *p*. protein (change at amino acid level), *PIK3CA* phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha, *PTEN* phosphatase and tensin homolog, *TP53* tumor protein 53

(brush smears 57% [range 20–90%] vs. forceps samples 39% [range 10–90%]).

In the single subject with negative brush smear but positive brush tip washing findings, no NGS analysis was feasible from the brush tip washing specimen due to low cell count.

Discussion

The sensitivity of bronchoscopic brushing was 66%, which is in line with the results of a previous meta-analysis where the sensitivity was approximately 61% [2].

NGS analysis was feasible in 62% of the tumor-positive brushing cytology samples. In 78% of these cases, NGS displayed identical results compared to histology samples, in 22% NGS from brush smears detected specific mutations whereas DNA quality from forceps biopsy was insufficient for NGS analysis. Overall, NGS feasibility from forceps biopsy samples is higher than from brushing samples. However, data from this study show that NGS from brush smears may represent an important alternative option in the cases where DNA quality or quantity from forceps specimens is low. It may be hypothesized that not only to increase the tumor detection but also the mutation detection rate, it may be helpful to combine bronchoscopic sampling tools.

To the best of our knowledge, this is the first study analyzing the feasibility and reliability of NGS analysis from bronchial brush smears in lung cancer.

Previous NGS studies on pulmonary cytopathology have only analyzed samples from needle aspiration cytology, pleural fluid and bronchoalveolar lavage [7]. The most important limiting factor for NGS analysis from cytology specimens is a low yield of DNA because of low overall cellularity and/or low tumor cellularity. Tumor enrichment strategies by demarcating and microdissecting tumor cell areas on the microscope slides may reduce false negative results and help to provide reliable NGS findings [7].

Bonney et al. demonstrated in their study in 75 subjects with peripheral lung lesions that in comparison with brush smears alone the supplementary analysis of brush tip washing allowed immunohistochemistry from cellblocks in an additional 9% of subjects [8]. Analyzing subjects with central lung cancer, we found that the additional cytological analysis of brush tip washing slightly elevated the sensitivity of brushing by 3%.

The results from this study show that bronchoscopic brushing represents an interesting low-risk sampling tool potentially enabling targeted therapy on the basis of cytological samples. The data may contribute to a further increase in the overall rate of molecular testing in lung cancer patients.

The study is limited due to the low number of included subjects and the retrospective design. As the patient population is well defined and as immunohistochemical results from at least one reference procedure (forceps biopsy, EBUS-TBNA, catheter biopsy or surgery) are available in all cases and in nearly all cases for NGS results, the reliability of the brushing data is well established.

Conclusion

In central lung cancer, bronchoscopic brushing detects tumor cells in about two-third of cases and allows a decision for or against targeted therapy in the majority of tumor-positive cases on the basis of NGS analysis.

Additional analysis of brush tip washing slightly increases the sensitivity of bronchoscopic brushing in central lung cancer.

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Compliance with Ethical Standards

Conflict of interest Lars Hagmeyer, Jana Fassunke, Marianne Engels, Marcel Treml, Simon Herkenrath, Sandhya Matthes, Reinhard Büttner, Winfried Randerath states that they have no conflict of interest.

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