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# The diagnostic utility of squash smear cytology in lung malignancies

Bhanita Baro<sup>1</sup> , Shilpa K. Lad<sup>1\*</sup>, Ketaki Utpat<sup>2</sup> and Anurag Saha<sup>1</sup>

## Abstract

**Background:** Lung malignancy is the most common cause of death worldwide. The main role of pathologist is to differentiate small cell from other cell types as treatment and prognosis varies. Although histopathology is routinely done, squash smears yield better cytomorphology.

**Aims and objectives:** To study the clinical history, evaluate the diagnostic yield by means of cytology and histopathology correlation followed by determination of sensitivity and specificity of squash smear cytology for detection of lung malignancies.

**Materials and methods:** The present study was an observational analytical study conducted over a period of 3.5 years. Total 60 squash smears were obtained by computed tomography-/bronchoscopy-guided biopsy and stained with Papanicolaou and May-Grunwald stain. Squash smears were correlated with histopathological findings.

**Statistical analysis:** Statistics in the form of sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio were done.

**Results:** Out of total 60 cases, 71.67% cases were males and 65% cases were smokers. Right lung was mostly involved (65%). The major symptom noted was dyspnea on exertion (90%). In 66.7% cases, malignancy was reported while in 10% suspicious and in 23.3% negative for malignancy. The most common malignancy diagnosed was squamous cell carcinoma (18.3%). The sensitivity and specificity of 92.1% and 83.3% were calculated respectively for squash smear, while the positive predictive value (PPV) was 94.6% and the negative predictive value (NPV) was 76.9%.

**Conclusion:** Squash smear cytology utilization in diagnosis of lung malignancy is a simple, fast, and reliable procedure with good sensitivity and specificity. It can be used as complementary to histopathology when need arises.

**Keywords:** Bronchoscopy-guided biopsy, CT-guided biopsy, Lung malignancies, Squash smear cytology

## Background

Lung malignancy is the leading cause of death in men and also among women. There has been an increase in incidence of lung malignancies throughout the world, although the treatment and prognosis of all subtypes of lung cancer is distinct, a precise cytological and histological typing of the cancer is required. The squamous cell carcinoma was the commonest subtype (40–60%) [1–3]

earlier followed by adenocarcinoma (20–30%), small cell carcinoma (5–10%), and large cell carcinoma (1–2%), but adenocarcinoma [4–6] has outdone squamous cell carcinoma by becoming the commonest type of lung carcinoma as per literature.

The main role is to distinguish between small cell and non-small cell carcinoma as the treatment varies for both the subtypes [7]. Bronchoscopic-guided biopsy was obtained through transbronchial route, while CT (computed tomography)-guided biopsy was recommended for peripherally located tumors [8]. Squash smear provides good preservation of cytomorphology of cells

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while the only disadvantages are drying and crushing artifacts [9, 10].

Although squash smear cytology is widely used for diagnosis of central nervous system tumors. However, literature search shows very few publications of its use in the respiratory tract malignancies [9, 10]. We planned to undertake this study to evaluate the diagnostic efficacy of squash smear cytology in lung malignancies.

### Aims and objectives

To study the clinical history, evaluate the diagnostic yield by means of cytology and histopathology correlation followed by determination of sensitivity and specificity of squash smear cytology for detection of lung malignancies.

### Materials and methods

The present study was an observational analytical study (i.e., retrospective study) done in a tertiary care hospital. A total of 60 cases were studied over a period of 3.5 years, i.e., from 2016 to 2019 as per the cases sent to our department by the pulmonologist.

### Inclusion criteria

Patients who had undergone fiberoptic bronchoscopy-guided and computed topography-guided biopsy for lung malignancy and in whom squash smear cytology was sent were included in the study.

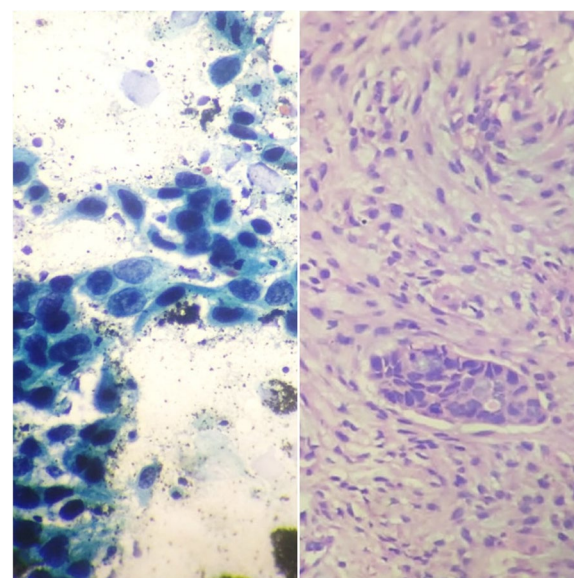
### Exclusion criteria

We excluded cases where two bits of tissues could not be obtained for squash smear cytology.

The clinical and radiologic findings, i.e., bronchoscopy and CT findings, were noted from the patient's records. Squash smear was prepared by the bronchoscopist and the slide was sent to cytology section. Half of the total air-dried slides sent were stained with MGG (May-Grunwald Giemsa) stain and remaining slides after rehydration were stained with Papanicolaou stain (pap stain). Slides were assessed in terms of adequacy of the sample, diagnostic yield, and diagnostic efficacy of the sample. Biopsy correlation was obtained by keeping histopathology as the gold standard, from the findings recorded in the patient's file. Retrospective data was collected from the records maintained in cytology section and slides were reviewed.

### Statistical analysis

Diagnostic efficacy in terms of sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio were done. Numbers were by mean, standard deviation, median,

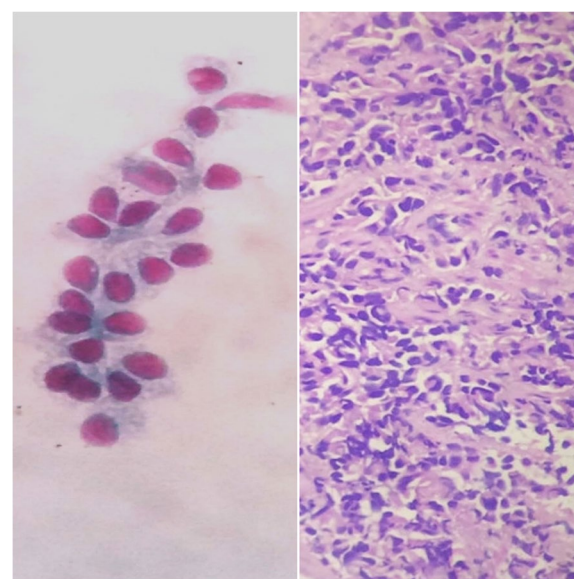


**Fig. 1** Squamous cell carcinoma lung (MGG stained-squash smear cytology and H&E-stained histology slide); 100× magnification

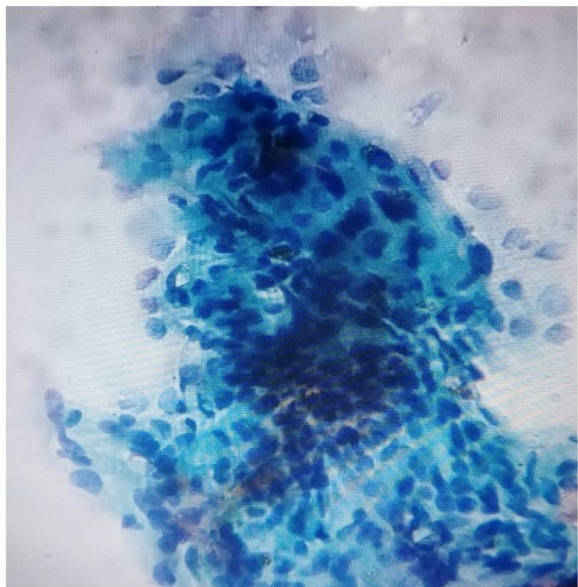
and interquartile range. Frequency was in number and percentage.

### Results

In our study, patients' age ranged from 20 to 80 years, with a mean age of 58.2 years, and 40% of patients belonged to the age group (51–60) years. There was a male preponderance with male to female ratio of 2.5:1.65% patients were smokers of which 97.4% were



**Fig. 2** Lung adenocarcinoma (PAP stained-squash smear cytology and H&E-stained histology slide); 100× magnification



**Fig. 3** Metastatic renal cell carcinoma on MGG stained-squash smear cytology; 100× magnification

males and 2.56% cases were females. There was no history of occupational exposure to any carcinogens.

The right lung was most commonly involved in 66.67% cases followed by the left lung, upper lobe of the right lung being the most commonly involved. Out of total 60 cases, 45 cases were obtained by CT-guided and rest 15 cases by bronchoscopy-guided biopsy.

Out of total 60 cases, 66.7% were malignant, 10% cases as suspicious of malignancy, and 23.3% cases were reported as no evidence of malignancy on squash smear cytology. The squamous cell carcinoma (Fig. 1) was the most commonly reported (18.3%) cases followed by adenocarcinoma (10%) (Fig. 2), non-small cell lung carcinoma (8.33%), small cell carcinoma (6.67%), and metastases (5%) cases (Fig. 3 and Table 1). However, in 18.3% cases, it was not possible to sub classify the malignancy; henceforth, it was reported as positive for malignancy on cytology report. Squash smear diagnosis was correlated with histopathology diagnosis wherever possible. Out of total 60 cases, histopathological correlation was obtained in 50 cases, whereas 10 cases were lost to follow-up.

Our study revealed a diagnostic yield of 66.7% and diagnostic efficacy of 48.3% for squash smear cytology. The concordance rate between smear cytology and

**Table 1** Sub classification of cases as per squash smear cytology diagnosis (n=60)

Squash smear cases	No. of cases	Percentage
Squamous cell carcinoma	11	18.33%
Adenocarcinoma	6	10%
NSCLC	5	8.33%
Small cell carcinoma	4	6.67%
Metastases	3	5%
Positive for malignancy	11	18.3%
Suspicion for malignancy	6	10%
Negative for malignancy	14	23.3%
Total	60	100%

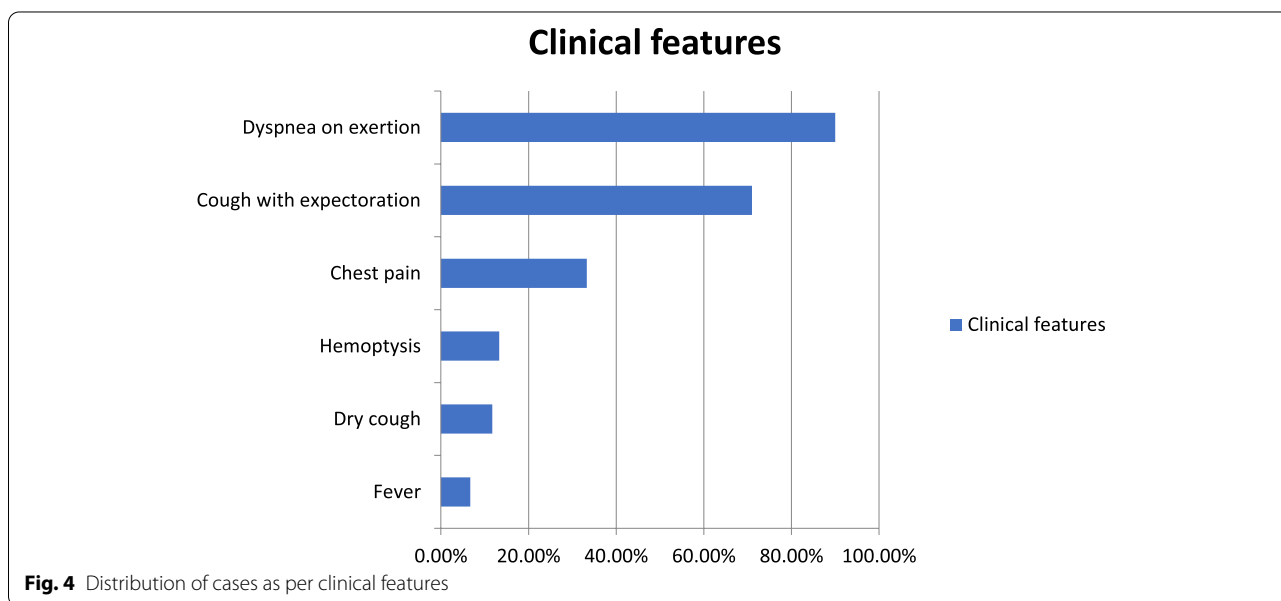
Malignant cases  
40 cases  
(66.67%)

**Table 2** Typing of lung carcinomas with respect to smoking habit (n=60)

Type of carcinoma	Smoker( cases)	Non-smoker (cases)
Squamous cell carcinoma	8	3
Adenocarcinoma	4	2
NSCLC	2	3
Small cell carcinoma	2	2
Positive for malignancy	8	3
Metastases cases	1	2
Suspicious cases	5	1
Negative cases	8	6

coherence of squash cytology with biopsy diagnosis in clinically diagnosed cases of lung carcinoma.

In the present study, age ranged from 20 to 80 years; all were adults which are in comparison to other studies. The youngest patient in our study was a 20-year-old male. The highest incidence of lung carcinoma belonged to age group (51–60 years) which was also shown by studies like Saha et al., Das et al., Mehta et al., and Mondal et al. [1, 2, 11, 12]. This age group being more vulnerable to increased incidence of malignancy due to late presentation by patients, lack of proper knowledge, and unavailability of proper health care system. There was male preponderance with male to female ratio of 2.5:1; male preponderance was also shown by various other studies. Male preponderance can be due to increased smoking habits, chronic obstructive lung disease (COPD),



histopathology was calculated to be 90%. We considered only the positive cases in which subtyping could be done on cytology. The sensitivity and specificity of squash smear was 92.1% and 83.3% respectively. Positive predictive value, negative predictive value, positive ratio, and negative ratio were 94.6%, 76.9%, 5.41, and 0.096 respectively.

## Discussion

This was an observational analytical study carried out over a period of 3.5 years (i.e., retrospective study). This study was conducted to assess the diagnostic ability and

and occupational exposure as well as increased susceptibility to malignancy [13]. Most common lobe involved was the right upper lobe in 36.3%, which was also seen in the study by Shrivastava et al. [14]. Studies by Makde et al. showed the left upper lobe of lung to be most commonly involved [15]. Konjengbam et al. showed the right lung to be involved more than the left lung but the left upper lobe being most commonly involved than the right upper lobe [16]. As compared with the study by Mehta et al. in the present study, 75% cases were obtained by CT-guided and 25% by bronchoscopic-guided technique [11]. In the international study by Yang et al. where he chose two methods, CT-guided and ultrasound guided,



bronchoscopy guided was not done. According to his study, CT-guided was done in 79.71% cases [17]. The present study had reported 65% smokers, also seen in studies by Modi et al. with 64.2% cases and Makde et al. that reported 64% cases [15, 18]. Majority of smokers were males in 38 cases (97.4%) and only in 1 case (2.5%) female gave history of smoking. Study by Giri et al. had 75.9% male smokers [19] whereas study by Gangopadhyay et al. showed 80% males were smokers and 1.5% females were smokers [20]. In all the subtypes of malignant cases, there is interrelationship of smoking seen, as maximum patients were smokers. Interrelationship of smoking with subtyping was also shown by various studies in literature (Table 2). Similar study by Sareen et al. showed squamous cell carcinoma to be most commonly seen in relation with smoking habits [21], while studies by Mehta et al., Mondal et al., and Modi et al. show adenocarcinoma to be most commonly seen in smokers [11, 12, 18].

In the present study, dyspnea on exertion was the commonest symptom (90%) followed by cough with expectoration (71%), chest pain (33.3%), hemoptysis (13.3%), dry cough (11.7%), and fever (6.7%) (Fig. 4). The study by Shrivastava et al., Giri et al., and Yang et al. also showed cough as the commonest symptom. This discrepancy can be explained by the fact that symptom develops as per site of involvement and size of lesion [14, 17, 19].

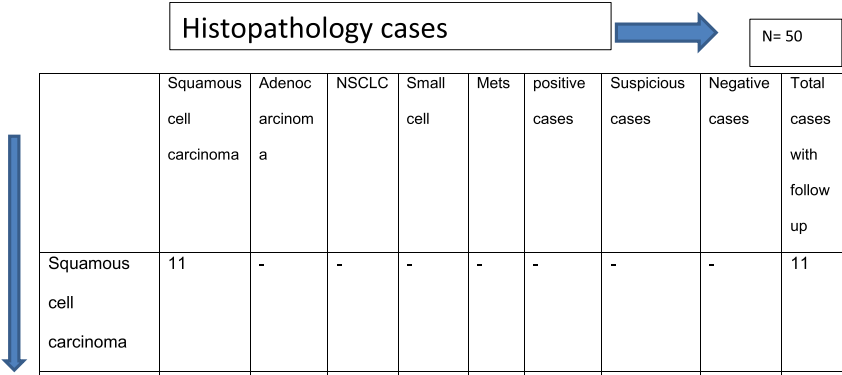
In the present study, smear cytology cases were broadly classified into three categories: malignant 40 cases (66.7%), negative for malignancy 14 cases (23.3%), and suspicious of malignancy 6 cases (10%). Study by Shrivastava et al. (68.7%) [14] shows similar values but study by Sengupta et al. (51.4%) which showed low percentage of malignancy compared to the present study [22]. Saha et al. (94.7%), Das et al. (89.17%), Giri et al. (75%), and Konjengbam et al. (71.4%) showed high incidence of malignancy compared to the present study [1, 2, 16, 19]. Another international study by Yang et al. showed 72.4% malignant cases in his study done on 69 patients clinically diagnosed with lung malignancy [17]. Maximum cases of lung malignancy were primary, while 3 cases were diagnosed as metastatic carcinoma on squash smear; their primary being from renal cell carcinoma, carcinoma cervix, and metastases of high-grade neoplasm. Study by Saha et al. reported 1 case of metastases to lung in known cases of renal cell carcinoma [1]. Das et al. also reported 1 case of metastatic adenocarcinoma in their study [2].

The present study calculated diagnostic yield of 66.7% ( $n=40$ ), i.e., total malignant cases calculated on squash smear cytology. Diagnostic efficacy was 48.3% ( $n=29$ ), i.e., the cases in which subtyping was possible on squash smear cytology. Study by Srivastava et al., Konjengbam et al., Giri et al., and Yang et al. showed 68.7%, 71.4%,

**Table 3** Showing distribution of histopathological cases ( $n=50$ )

Histopathology diagnosis	Number of cases	Percentage
Squamous cell carcinoma	12	24%
Adenocarcinoma	7	14%
Small cell carcinoma	7	14%
NSCLC	7	14%
Carcinoid	1	2%
Metastases	4	8%
Negative for malignancy	10	20%
Spindle cell lesion(benign lesions labeled as negative cases)	2	4%
Total cases	50	100%

Malignant cases  
38 cases

**Table 4** Table showing correlation of squash smear cytology with histopathology


	Squamous cell carcinoma	Adenocarcinoma	NSCLC	Small cell	Mets	positive cases	Suspicious cases	Negative cases	Total cases	Follow up not obtained
Squamous cell carcinoma	11	-	-	-	-	-	-	-	11	0
Adenocarcinoma	-	5	1	-	-	-	-	-	6	0
NSCLC	-	-	5	-	-	-	-	-	5	0
Small cell	-	-	-	4	-	-	-	-	4	0
Mets	-	-	-	-	2	-	-	-	2	1
Positive cases	-	1	1	3	2	-	-	2	9	2
Suspicious cases	-	1	-	-	-	-	-	4	5	1
Negative cases	1	-	-	-	-	1	-	6	8	6
Total	12	7	7	7	4	1	0	12	50	10

**Table 5** Showing sensitivity and specificity of cytology in various studies

Study	Sensitivity ( in percentage)	Specificity ( in percentage)
<b>Present study</b>	<b>92.1%</b>	<b>83.3%</b>
Dong et al.(2017)	94.5%	95.12%
Biancosino et al.(2016)	94.8%	98.8%
Gangopadhyay et al.(2011)	96%	100%
Mukherjee et al.(2010)	97.7%	100%
Modi et al.(2016)	91.5%	72.5%

75%, and 72.46% diagnostic yield rate respectively which is comparable to our study [14, 16, 17, 19]. Our study had reported squamous cell carcinoma to be the commonest tumor accounting for 18.33%. Similarly study by Shrivastava et al. (50%) [14], Makde et al. (28%) [15], Reema Kouli et al. (56.75%) [3], Sharma RK et al. (62%) [23], Das et al. (60.74%) [2], and Konjengbam et al. (35%) [16] also showed squamous cell carcinoma to be commonest

tumor. Whereas study by Mondal et al. showed adenocarcinoma of lung to be most commonly occurring tumor (52.63%) [12] and study by Sengupta et al. showed non-small cell lung carcinoma to be commonest tumor (13.5%) [22]. The discrepancy was because our study had maximum cases of male smokers.

In the present study, histopathology correlation was obtained in 50 cases; in the rest of the 10 cases, follow-up was not obtained. Histopathology was helpful in cases where squash smear was given as suspicious, non-small cell lung carcinoma, or positive for malignancy. Out of 50 cases on histopathology, 38 (76%) cases were malignant, 10 (20%) cases were negative for malignancy, and 2 (4%) cases were diagnosed as spindle cell lesion (Table 3). Out of total 38 malignant cases, squamous cell carcinoma was the commonest accounting for 24% of cases (12 out of 50) followed by adenocarcinoma in 14% cases (7 out of 50), small cell carcinoma in 14% cases (7 out of 50), NSCLC in 14% cases (7 out of 50), metastases to lung in 8% (4 out of 50) cases, and carcinoid in 2% (1 out of 50) cases. Concordance between squash smear cytology and histopathology was obtained in 45 cases (i.e., 90%). Study by

Modi et al. also showed concordance rate of 88.57% in their study [18] (Table 4). On subtyping squamous cell carcinoma, NSCLC and small cell carcinoma showed 100% concordance rate in the present study. Our study had a discordant rate of 10% (5 out of 50). Discordant cases were noted in the form of false negative on cytology in 3 cases (6%) and false positive on cytology in 2 cases (4%). Out of 2 discordant cases, which were negative for malignancy on squash smear, 1 case was reported as squamous cell carcinoma and another 1 case was reported as carcinoid on histopathology. The reason for cases positive on squash smear and negative on histopathology were:

- 1) Sampling issues, as very tiny squash smear was being provided.
- 2) Drying artifact, crush artifact, clusters not separated.
- 3) The cells were not optimally preserved.
- 4) Desmoplastic reaction.

Negative cases on squash and positive on biopsy may be due to low cellularity and improper fixation of smears. The cases where subtyping could not be done were reported as NSCLC due to poor fixation, the cells could not be identified, and in cases of high-grade tumor with marked pleomorphism which were non-distinguishable from tumor of origin. The tumor sizes varies greatly and this may affect the results of squash smear cytology and histopathology due to tumor inhomogeneity. In spite of these limitations, this squash smear cytology indeed shows excellent results [17].

Our study had sensitivity rate of 92.1% with specificity of 83.3%, PPV is 94.6% and NPV is 76.9%. The values were comparable to various studies (Table 5). Hence, it justifies that squash smear cytology is a highly sensitive and specific tool in diagnosis and subtyping of lung malignancy. The above studies used FNA cytology as a diagnostic modality which is very similar to squash smear preparation; hence, we compared our study with these studies [4, 5, 18, 20, 24]. International study by Yang et al. which is a smear cytology study over a period of 1.2 years included 69 patients showed pathological classification rate to be 89.19% [17]. The present study had calculated diagnostic accuracy of 90%, which is comparable to other studies done by Modi et al. (88.5%), Konjengbam et al. (91.4%), Roy et al. (84.2%), and Singh et al. (85.3%) [16, 18, 25, 26]. Also, the positive likelihood ratio was calculated to be 5.41 and negative likelihood ratio was 0.09. Hence, squash smear cytology can be used as complementary tool to histopathology to select treatment modalities and

to avoid unnecessary surgeries in patients with lung malignancies.

The limitations in our study were related to availability of very few references in literature, and also, we could not perform immunohistochemistry in our cases due to poor fixation problem.

## Conclusion

We conclude that Squash smear cytology is a safe, reliable diagnostic tool and provides early diagnosis in lung malignancies. It has high concordance rate (90%) with histological diagnosis and can be used for choosing treatment options. Discordance rate was low, i.e., in 10%, the reason was sampling issues, drying artifact, crushing artifact, and desmoplastic reaction obscuring cytomorphology of cells. It can be used as a complementary tool with histopathology to arrive at an accurate diagnosis as it provides better cytomorphology of tumor cells.

## Abbreviations

CT: Computed tomography; MGG stain: May Grunwald Giemsa stain; PAP stain: Papanicolaou stain; NSCLC: Non-small cell lung carcinoma; COPD: Chronic obstructive lung disease.

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## Authors' contributions

I/we certify that I/we have participated sufficiently in the intellectual content, conception and design of this work or the analysis and interpretation of the data (when applicable), as well as the writing of the manuscript, to take public responsibility for it and have agreed to have my/our name listed as a contributor. I/we believe the manuscript represents valid work. Neither this manuscript nor one with substantially similar content under my/our authorship has been published or is being considered for publication elsewhere, except as described in the covering letter. I/we certify that all the data collected during the study is presented in this manuscript and no data from the study has been or will be published separately. I/we attest that, if requested by the editors, I/we will provide the data/information or will cooperate fully in obtaining and providing the data/information on which the manuscript is based, for examination by the editors or their assignees. Financial interests, direct or indirect, that exist or may be perceived to exist for individual contributors in connection with the content of this paper have been disclosed in the cover letter. Sources of outside support of the project are named in the cover letter. I/We hereby transfer(s), assign(s), or otherwise convey(s) all copyright ownership, including any and all rights incidental thereto, exclusively to the Journal, in the event that such work is published by the Journal. The Journal shall own the work, including 1) copyright; 2) the right to grant permission to republish the article in whole or in part, with or without fee; 3) the right to produce preprints or reprints and translate into languages other than English for sale or free distribution; and 4) the right to republish the work in a collection of articles in any other mechanical or electronic format. We give the rights to the corresponding author to make necessary changes as per the request of the journal, do the rest of the correspondence on our behalf and he/she will act as the guarantor for the manuscript on our behalf. All persons who have made substantial contributions to the work reported in the manuscript, but who are not contributors, are named in the Acknowledgment and have given me/us their written permission to be named. If I/we do not include an Acknowledgment that means I/we have not received substantial contributions from non-contributors and no contributor has been omitted. Dr. Bhanita Baro (First and corresponding author). Dr. Shilpa K Lad (Second author). Dr. Ketaki Utpat (Third author). Dr. Anurag Saha (Fourth author). The authors read and approved the final manuscript.

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