

Research article

Utility of rapid on-site evaluation (ROSE) using toluidine blue stain in respiratory cytology

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ABSTRACT

Introduction and Aim: Cytology has gained importance as a diagnostic test in pulmonary lesions, especially in patients presenting with advanced malignancy which are surgically non-resectable. Although cytopathological diagnosis may be limited, its role cannot be underestimated considering the minimal invasive nature of the technique. Single slide (SS) Rapid on-site evaluation (ROSE) will aid in adequacy assessment and diagnosis of pulmonary lesions on cytopathology.

Materials and Methods: We conducted a prospective study. SS ROSE was done on transbronchial fine needle aspiration and bronchial brushing specimens using toluidine blue staining. The slides were microscopically evaluated for adequacy and for diagnosis. In case of inadequate smears, a repeat was suggested. The diagnosis was compared with the diagnosis rendered on remaining slides which were processed using conventional cytology.

Results: The study includes 82 cases for ROSE. A correlation of 100% was found between ROSE diagnosis and final cytology diagnosis. Out of 82 cases, 24 cases were malignancies and 3 were granulomatous lesions on ROSE. The remaining were negative for atypical/malignant cells. 2nd pass was advised in 21 cases due to the inadequacy of the diagnostic material in 1st pass. Out of 21 cases advised for 2nd pass, 11 were found to be malignant. A statistically significant p value of 0.05 was obtained.

Conclusion: In our study, we found that ROSE using toluidine blue stain is simple, yet a very useful procedure to reduce the number of passes, the need for repeat bronchoscopy procedure and helpful in suggesting more samples for ancillary tests and rapid cytopathological diagnosis.

Keywords: Bronchoscopy; cytology; FNAC; lung; pulmonary; ROSE.

INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality worldwide. Of all the lung cancers, only 30-40% are surgically resectable at the time of diagnosis. With development of new and novel targeted therapies for the treatment of lung cancer, the pathologist's role becomes more important (1). The field of lung pathology has grown in recent years with newer diagnostic and molecular techniques and research. The International Association for the Study of Lung Cancer, the European Respiratory Society and the American Thoracic Society have given a standardized updated classification for diagnosing lung cancers using cytology samples and lung biopsies (2).

Transbronchial fine needle aspiration (TBNA) using flexible bronchoscopy (FB) is a useful sampling technique for bronchial, peribronchial, hilar and pulmonary lesions (3). Its yield ranges from 20% to 90% (4). It plays an important role in the evaluation of lung cancer in patients with mass forming lung lesions (5,6). One of the most common reasons for non-diagnostic TBNA is inadequate samples. Hence, methods like endobronchial ultrasound (EBUS) or computed tomography (CT) with rapid on-site evaluation (ROSE) preferably by a pathologist helps in improving the TBNA yield. In the event of an inadequate sample, ROSE helps by suggesting

additional passes on TBNA and changing the needling angle, site, and depth etc., Hence it helps in reducing the need for repeat bronchoscopy procedures all over again (7).

ROSE refers to the rapid stain of the cytology material smear in the bronchoscopy suite or operating room or procedure room, with evaluation by a pathologist immediately (8). TBNA uses a 21-gauge needle for procurement of material for both, cytology, and histopathology simultaneously. ROSE also provides excellent agreement with histopathology (9).

However, there are only few studies on ROSE conducted in India and this technique is underutilized in Indian scenarios. There is very sparse literature on ROSE assisted TBNA in India. It is usually due to cost problems and the non-availability of cytologists to implement ROSE. Therefore, we conducted this study to assess the utility of ROSE in lung lesions and compare it with the final cytology diagnosis.

MATERIALS AND METHODS

This was a prospective study conducted involving two departments, namely Pathology and Respiratory medicine, of Father Muller Medical College Hospital, Mangalore, Karnataka. We obtained the ethical clearance from the institution ethics committee vide letter number 136/19. A single slide (SS) ROSE was done on transbronchial fine needle aspiration and

bronchial brushings specimens using Toluidine blue staining.

Toluidine blue stain was prepared by adding and dissolving 0.5g of crystalline toluidine blue with 20mL of 95% ethyl alcohol. This was further made up to 100mL by adding distilled water. After adding distilled water, it was filtered and stored in the refrigerator. A drop of stain was put onto the smear for one minute. The excess stain was then removed. The slide was then examined under a microscope. The slides were microscopically evaluated for adequacy based on the adequacy criteria (mentioned below) and for diagnosis. The presence of at least five clusters of well-visualized epithelial cells or at least 1 cluster of lesional cells, each cluster containing at least 5 cells, was considered adequate. A turnaround time of 5 to 8 minutes was taken. In case of inadequate smears, a repeat was suggested. The remaining smears were sent for conventional cytology. The diagnosis offered on ROSE and conventional cytology were compared.

Statistical analysis was done using percentages, frequency, and chi-square test. Analysis based on p value was performed.

RESULTS

This prospective study included 82 cases with lung lesions. ROSE assessment was done for sample adequacy and to provide preliminary diagnosis. Further all the slides were diagnosed with conventional cytopathology.

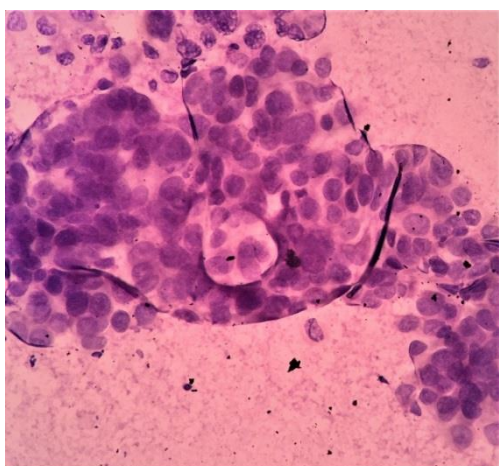


Fig. 1: 100X, Toluidine blue stain, from a case of adenocarcinoma, showing tumour cells arranged in glandular pattern and clusters. Individual tumour cells exhibit high nucleus to cytoplasm ratio, moderate degree of nuclear pleomorphism, irregular nuclear membrane and scant cytoplasm.

Out of 82 cases included in the study, many of the cases i.e., 28 (34.1 %) were of the age group of 61-70 years. With respect to the gender distribution, 59 (71.9%) cases were male and 23 (28.04%) were female.

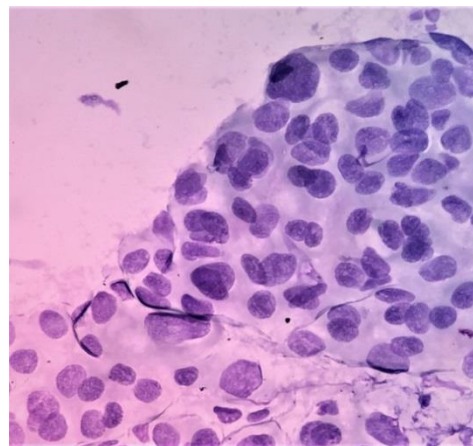


Fig. 2: 100X, Toluidine blue stain, from a case of poorly differentiated carcinoma, showing tumour cells having large, pleomorphic nucleus, nuclear membrane irregularity, and moderate amount of cytoplasm.

Out of 82 cases, 24 cases were malignancies and 3 were granulomatous pathology on ROSE. The remaining were reported as negative for atypical/malignant cells. All these cases were further reported by conventional cytopathology assessment (Fig. 1 and Fig. 2). A correlation of 100% was found between ROSE diagnosis and final cytology diagnosis.

A 2nd pass was advised in 21 (25.9%) cases due to the inadequacy of the diagnostic material on the 1st pass ROSE. Hence without ROSE assessment, roughly one out of every four cases would have required repeat setting for the entire bronchoscopy procedure. Out of 21 cases advised for 2nd pass, 11 were found to be malignant on final cytopathological diagnosis. Hence chances of missing a malignancy are reduced by using ROSE technique. In 3 cases reported as granulomatous lesions on ROSE assessment, we recommended extra specimens for Ziehl-Neelsen stain to rule out *Mycobacterium tuberculosis*. Hence, ROSE helped in specimen segregation and recommendation of additional investigations.

Comparison between ROSE diagnosis and conventional cytology diagnosis was done. Statistical analysis showed a p-value of less than 0.05 implying statistical significance.

DISCUSSION

The anatomy and physiology of the lung makes it complicated to obtain cellular material from nodular lesions by biopsy and/or needle aspiration (10). TBNA is a well tolerated diagnostic method. EBUS or CT guidance with ROSE help in increasing the yield of adequate diagnostic material and thus, providing a proper diagnosis to the patient.

Most of the studies on ROSE are conducted in other countries. In a study by Chandra *et al.*, (8) on ROSE, the smears were considered adequate in the first pass in 68.1% of the cases and those cases, which were inadequate on ROSE in the first pass were subjected to the second pass, which resulted in an increase in adequacy of yield to 93.4%. ROSE can aid in the

diagnosis. In a study by Ravaioli *et al.*, (11), involving 273 patients, they showed a diagnostic accuracy of 90 to 97% with the highest being for adenocarcinoma. In a study done by Sindhvani *et al.*, (4), involving 40 patients, the authors concluded that ROSE improved yield and helped prevent repeat procedures. Baram *et al.*, (12), in a study involving 44 cases showed no significant improvement with ROSE, however they concluded that ROSE resulted in reduction in the number of subsequent biopsies. In an observational study involving 90 cases, Diacon *et al.*, (7) concluded that utilization of ROSE allowed the procedure to be terminated early in 64% of the cases.

In a prospective study involving 80 cases, conducted by Mallya *et al.*, they concluded that ROSE would facilitate in obtaining adequate samples and aid in rapid clinical decision-making (13). In a prospective study conducted on 651 patients by Wang *et al.*, they concluded that ROSE helps pulmonologists to obtain adequate material for the histopathology diagnosis and provides them a rapid preliminary diagnosis (14). In a prospective study conducted by Jain *et al.*, they concluded that rapid on-site evaluation could make sure that targeted lesion is sampled and can also help to enable appropriate specimen triage (15). In a prospective study conducted by Sindhvani *et al.*, they concluded ROSE can increase the yield of TBNA procedure and help in reducing the number of repeat procedures. They proved ROSE as an easy, cost-effective procedure, and it is feasible in Indian settings (4).

In a prospective study by Diacon *et al.*, they concluded that Rapid on-site analysis of transbronchial aspirate sample is a greatly useful, cost-effective, and accurate in addition to routinely performed diagnostic bronchoscopy (7). In a study done by Sehgal *et al.*, they concluded that the use of ROSE was related to a lesser number of needles pass during the procedure of EBUS-TBNA and overall reduces the requirement of additional bronchoscopy for mediastinal lymph node sampling (16).

In a meta-analysis by Schmidt *et al.*, they concluded that ROSE would help to improve the adequacy rate in a wide range of tissue type sampling (16). In a retrospective chart review study conducted by Nakajima *et al.*, they concluded that use of ROSE during EBUS-TBNA helps to get a low rate of non-diagnostic sampling. And they also found higher agreement between the on-site results and final pathological diagnosis (9). In addition, Fassina *et al.*, (10) reported a good overall agreement of 71.4% in subtyping the cancer histological types on fine needle aspiration (FNA) for ROSE, and Nakajima *et al.*, (9) reported a concordance rate of 94.3% between ROSE diagnosis and final pathologic diagnosis on samples obtained by endobronchial ultrasound-guided transbronchial fine needle aspiration (EBUS-TBNA). ROSE can guide additional specimen collection for

further ancillary studies, such as immunohistochemistry (IHC) for subtyping and molecular testing. It can also guide for special stains like Ziehl-Neelsen stain for Acid-fast bacilli or stains to rule out fungal infection (17). Few studies have proved ROSE to be an effective and economical method for improvement of TBNA yield (4, 18). ROSE can also help in confirming the diagnostic tissue retrieval and early termination of the TBNA procedure (19). It also provides on-site preliminary diagnosis to the bronchoscopist and hence, it is akin to frozen section (20). In the current study, we had a relatively small sample size. However, we found ROSE using toluidine blue stain, to be a very simple, yet very helpful procedure in respiratory cytology.

CONCLUSION

TBNA and bronchial brushings are good diagnostic techniques, but its sample adequacy is variable. ROSE helps in improving the specimen yield and cellular material adequacy by TBNA. It is a useful diagnostic tool to reduce the number of needle passes during bronchoscopy procedure and reduces need for repeat bronchoscopy procedure in view of inadequate diagnostic sample. ROSE also helps in providing rapid diagnosis and guiding the pulmonologist for additional samples for ancillary testing. Hence, we conclude that ROSE must be included with TBNA and bronchial brushings to improve the sample yield and thus, helping in the right diagnosis of lung lesions. Simple stains like toluidine blue can be used for ROSE in respiratory cytology.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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