



# This 'Rose' Has no Thorns—Diagnostic Utility of 'Rapid On-Site Evaluation' (ROSE) in Fine Needle Aspiration Cytology

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## Abstract

Fine needle aspiration (FNA) cytology is a basic diagnostic technique used to investigate superficial and deep swellings. Rapid on-site evaluation (ROSE) using toluidine blue (TB) is easily available, cheap, cost-effective, can be used both for testing adequacy and giving provisional diagnosis. To evaluate the role of ROSE using toluidine blue staining in arriving at a diagnosis in comparison to routine stains. A total of 1500 cases of FNA of palpable swellings from sites like salivary gland, breast, thyroid, lymph node, and soft tissue lesions [non-image guided] during a 9-month period were studied. All the cases were evaluated by ROSE using toluidine blue stain and routine Giemsa/PAP staining. The results were compared in each case. Only 2% cases proved inadequate on TB, Giemsa and PAP combination, commonest site of inadequacy being lymph node. Adequate sample was obtained within two passes in 92.5% cases. The turn-around time (TAT) was 1 day in 96.4% of cases. The average time for making a provisional diagnosis on TB was 3 minutes. There was 99.2% concordance between TB and final cyto-diagnosis. Validity parameters: sensitivity 98%, specificity 100%, positive predictive value 100%, negative predictive value 99.8%, efficacy 99.2% and false negative 1.94%. ROSE using toluidine blue is a reliable means of demonstrating sample adequacy, for making a provisional diagnosis and guiding collection of diagnostic material for microbiology, immunocytochemistry (ICC), cell block and molecular testing etc. The technique is easy enough for general laboratories to incorporate into their routine practice. ROSE can be called as the "*frozen section of cytology*".

**Keywords** Rapid on site evaluation · Toluidine blue · Fine needle aspiration · Adequacy · Cell block · Cost effectiveness

## Background

FNAC is an essential, basic diagnostic technique, used to investigate superficial and deep swellings. The initial skepticism by pathologists and clinicians has gradually diminished and this simple tissue sampling technique is now practiced worldwide. It has gained importance because of its low cost, ease of performance, and safety along with fair sensitivity and specificity, especially for the diagnosis of malignancy [1].

Routinely, FNA slides are stained with Hematoxylin & Eosin (H&E), Papanicolaou, and/or Giemsa for microscopic examination. It usually takes 2–3 hours for the whole procedure. Although FNA, compared with core biopsy procedure, has a more rapid turn-around time (TAT), an adequate and representative sample is the key to correct diagnosis. Inadequate, non-representative sample would need a repeat procedure leading to delayed diagnosis, wastage of time, money, and needless anxiety to the patient [2].

ROSE of cytological material is a highly advantageous adjunct to fine-needle aspiration as it is an effective way to ensure that samples are adequate, handled properly for satisfactory

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morphological study and that they meet all pre-analytical requirements for specific diagnostic tests [3, 4]. Emerging novel molecular diagnostic technologies in current pathology practice have reinforced the essential role of ROSE in allowing pathologists to accurately divide the diagnostic cytological material into small aliquots. This procedure ensures that sufficient quantities of cells with adequate quality are obtained to permit a complete diagnostic workup. Ultimately, this will translate into an appropriate treatment plan [5].

Toluidine blue is a supra-vital stain that accentuates good nuclear detail and enables a three-dimensional view of cells in a wet mount film. It is easily available, cost-effective, and can be used for quick reporting. It also permits preservation of cytological material by destaining and restaining with permanent stains. It is especially useful in assessing the adequacy of samples in fibrotic or deep-seated lesions and minimizes false negative results. ROSE can also be used for intraoperative cytology diagnosis as an adjunct to frozen section diagnosis [6]. Very rightly said, “Rapid onsite evaluation can be called as the frozen section of cytology” [7].

## Aims and Objectives

To evaluate the role of ROSE using toluidine blue stain for (a) screening for adequacy of samples, (b) arriving at a diagnosis especially in differentiating benign from malignant lesions in comparison to routine stains, and (c) in improving TAT.

## Material and Methods

The present study was conducted in the Department of Cytopathology, of a tertiary care hospital in Mumbai. ROSE using toluidine blue was introduced as a part of our routine work flow, and we analyzed 1500 cases aspirated over a period of 9 months to study the efficacy of toluidine blue.

Detailed clinical history and all previous laboratory reports were recorded at the time of FNAC. The procedure was done using 22-25<sub>G</sub> needles with/without suction using a 10-mL disposable syringe. No local anesthesia was used. The needle was inserted into the lesion(s), 1–3 times. The aspirated material was used to make wet and dry smears and rapid on-site evaluation was done on one of the smears in each pass. The procedure was as follows: fixation in absolute alcohol for 10sec followed by 15sec in 1% aqueous toluidine blue. The excess stain was washed off with distilled water and one drop glycerin was used as a mountant to coverslip the slide. After microscopy, the toluidine blue slide was then returned to 95% ethanol for routine PAP staining.

Special stains like periodic acid Schiff (PAS), Gomori methenamine silver (GMS), Ziehl-Neelson stain, cell block with ICC were done wherever required and feasible.

## Results

Our study included 1500 cases, with an age range from 3 months to 87 years. The mean age was 33.3 years. Male/female ratio was 1:1.60. We performed aspiration in 1495 (99.6%) cases and scrape cytology in 5 (0.4%) cases. Among the various sites sampled, head and neck (cervical lymph nodes 42.2%, thyroid 3.5%, and salivary glands 3.0%) was the commonest followed by breast and axillary swellings (23.8%).

Fifty-three aspirates were from thyroid with a mean patient age of 39 years. Male to female ratio was 1:3.8. Adequate cellularity was obtained within first two passes in 49 (92.4%) cases. Forty cases (75%) were reported as benign on TB- Bethesda category (BC) II which included colloid goiter (26 patients), colloid goiter with cystic degeneration (1 patient), and thyroiditis (13 patients). This also included one case which was thought to be colloid goiter on TB but was reported as mixed thyroiditis; however, the Bethesda category remained unchanged (Fig. 1a–d). Ten cases were BC III on TB and were finally reported as BC III (atypia of undetermined significance) in nine cases and BC IV (Hurthle cell neoplasm) in one case. There were three neoplastic lesions—BC VI (papillary thyroid carcinoma—two cases) and BC IV (follicular neoplasm one case). Fifty cases (94.4%) had TAT of 1 day.

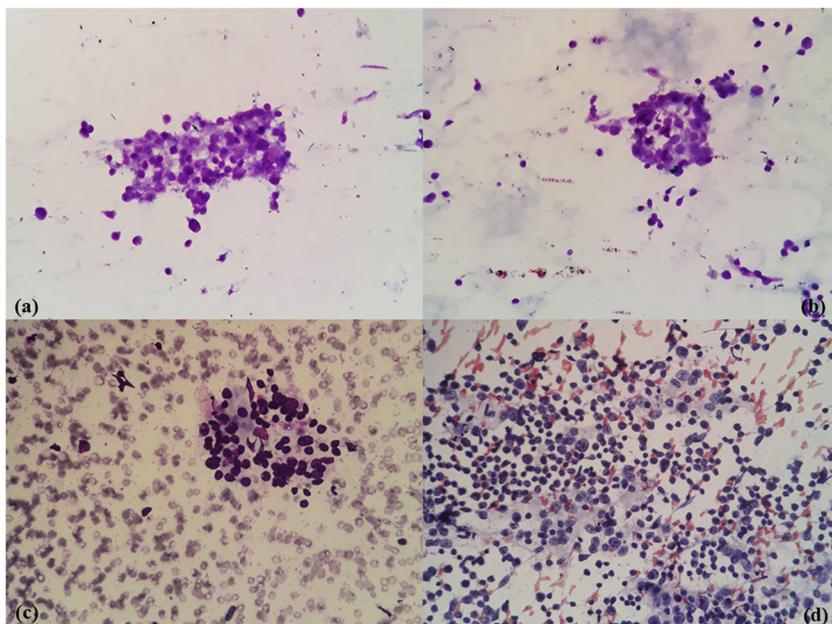
Follow-up histopathology was concordant in all available cases except for one case of BC-IV (Fig. 2a–d) which turned out to be a follicular variant of papillary carcinoma. The cause of discordance was lack of other features of papillary carcinoma on cytology.

Forty-five cases were from salivary glands; the mean age of presentation was 38.2 years and male/female ratio was 1.3:1. In 43 (98.3%) cases, adequate sample was obtained in two passes. Of the 39 benign lesions, there were 17 (37.7%) cases of pleomorphic adenoma, all being concordant on TB and final cytological diagnosis (Fig. 3a, b). All four cases of Warthin tumor were also concordant (Fig. 3c, d). We encountered two cases of mucoepidermoid carcinoma, two cases of lymphoma, and one case each of high-grade carcinoma and carcinoma ex-pleomorphic adenoma. In 42 (94.3%) cases, we had a TAT of one day. One case of mucoepidermoid carcinoma (Fig. 4a–d) on cytology showed features of secretory carcinoma on follow-up histopathology. The cause for discordance was an interpretational error due to overlapping morphologic features.

Among the breast and axillary swellings (358 cases), 53% patients were in the age group of 21–40 years with mean being 35 years. The youngest age for ductal carcinoma in our study was 26 years and the oldest was 80 years old. There were 19 (5.3%) male patients. Adequate sample was aspirated by two passes in 89.7% cases.

Eight cases among the 358 were inadequate (07 breast aspirates and 01 axillary swelling). There were 276 benign

**Fig. 1** **a, b** BC-II (Hashimoto's thyroiditis)—mixed thyroiditis—smears show thyroid follicular cells (TFCs), lymphocytes, and giant cells with scant colloid in the background. **c, d** TFCs with lymphoid cells, germinal center cells, and occasional plasma cell (**a, b** -TB, **c**- Giemsa, and **d**- PAP,  $\times 10$ )

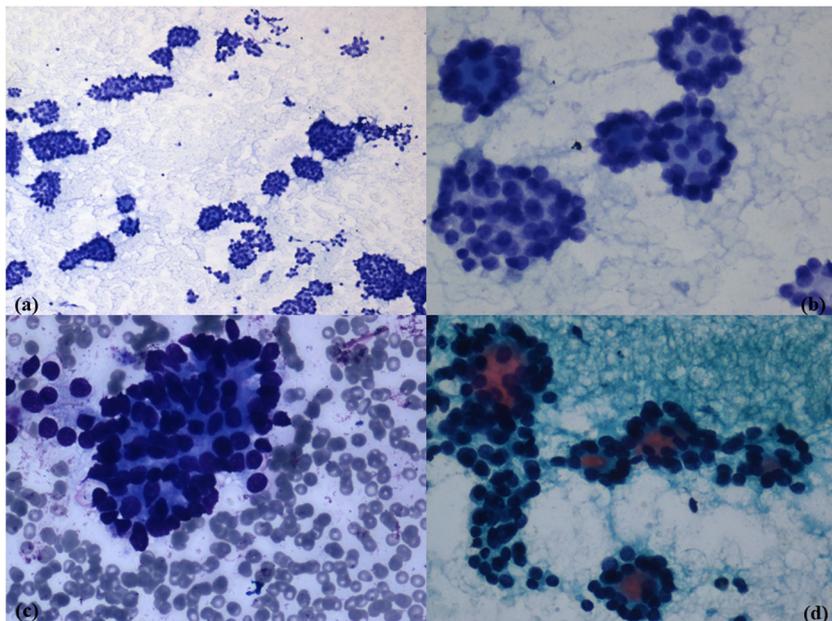


lesions, most common lesion being fibroadenoma (123 cases). Three axillary tail fibroadenoma were also seen, subsequently confirmed on histopathology. We encountered one rare and unusual case of adult filarial worms in the breast (Fig. 5a–h). Thirteen cases were suspicious for malignancy. All 59 cases of duct carcinoma (DC), 1 case each of mucinous carcinoma (Fig. 6a, b) and malignant phyllodes (Fig. 6c, d), were concordant on TB and final cytological diagnosis. Our TAT for breast FNA was 1 day in 94.7% of cases.

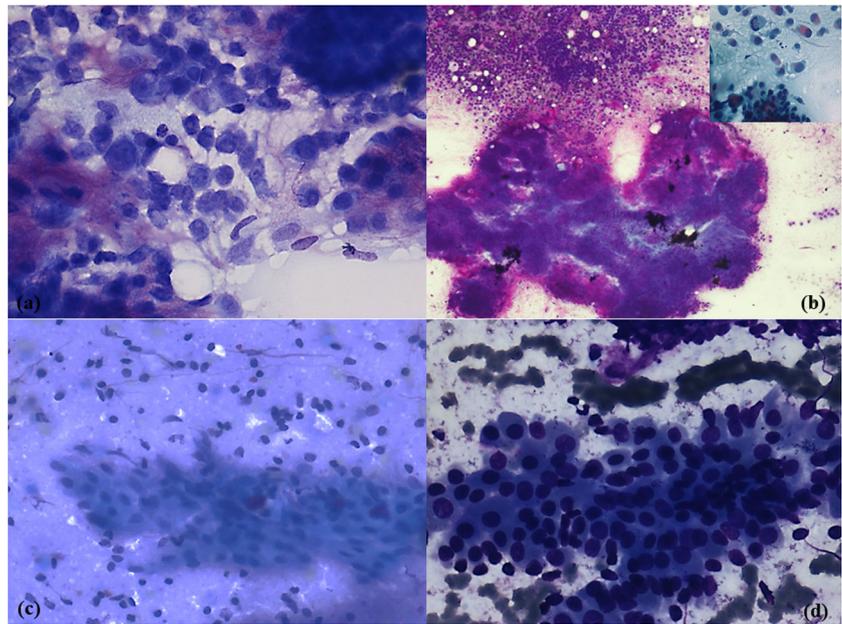
Ninety-five benign lesions had surgical follow-up and all except one were concordant on correlation. One case of fat necrosis on cytology showed infiltrating duct carcinoma

(IDC) on histopathology. An area of fat necrosis was seen adjacent to the invasive tumor on histopathology and non-representative sampling (sampling error) on FNA caused the discordance. One case each of suspicious for DC and suspicious for papillary carcinoma on cytology was reported as negative for malignancy on tru-cut biopsy. Review of the cytology confirmed the presence of lesion and the cause of discordance was a non-representative core biopsy. One case of phyllodes tumor on cytology showed chronic mastitis on histopathology. This again was a core biopsy that was not representative of the lesion, as seen on review. One case of atypical cells on scrape cytology showed Paget's disease with ductal

**Fig. 2** **a–d** BC-IV(Follicular neoplastic lesion)-Cytosmears shows TFCs in repetitive micro follicles along with few containing colloid in lumen (**a, b** -TB, Giemsa, and PAP,  $\times 10$  and  $\times 40$ )



**Fig. 3** **a, b** Pleomorphic adenoma-Cytosmears showing fibrillary chondro-myxoid material with scattered plasmacytoid cells (inset) in the background, few fragments show fibro vascular cores (**a**- TB, **b**- Giemsa, and PAP,  $\times 10$  and  $\times 40$ ) **c, d** Warthin tumor-cohesive epithelial cells few with oncocytic change with dirty proteinaceous background and lymphocytes (TB, Giemsa,  $\times 40$ )



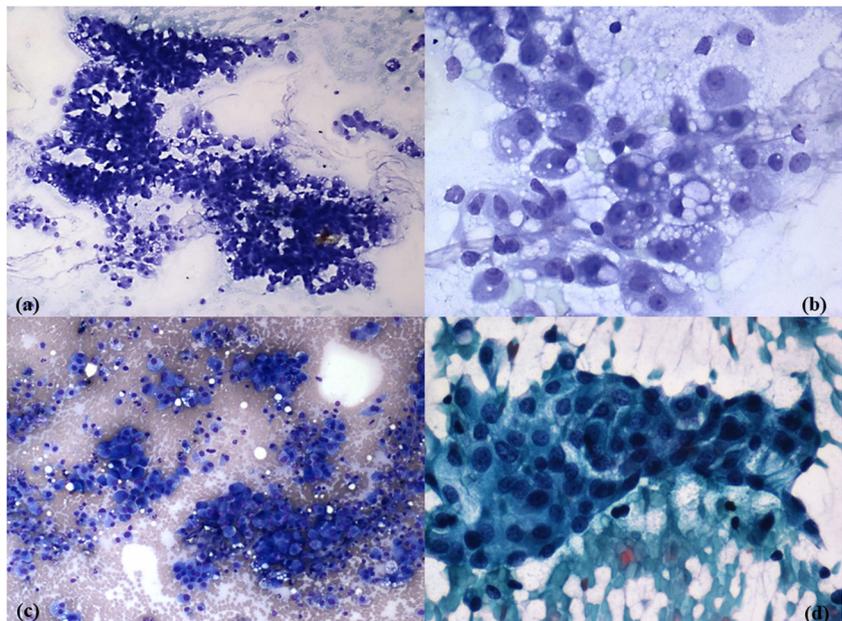
carcinoma in situ component on histology. Histopathology follow-up of malignant lesions was available in 45 patients and all were concordant.

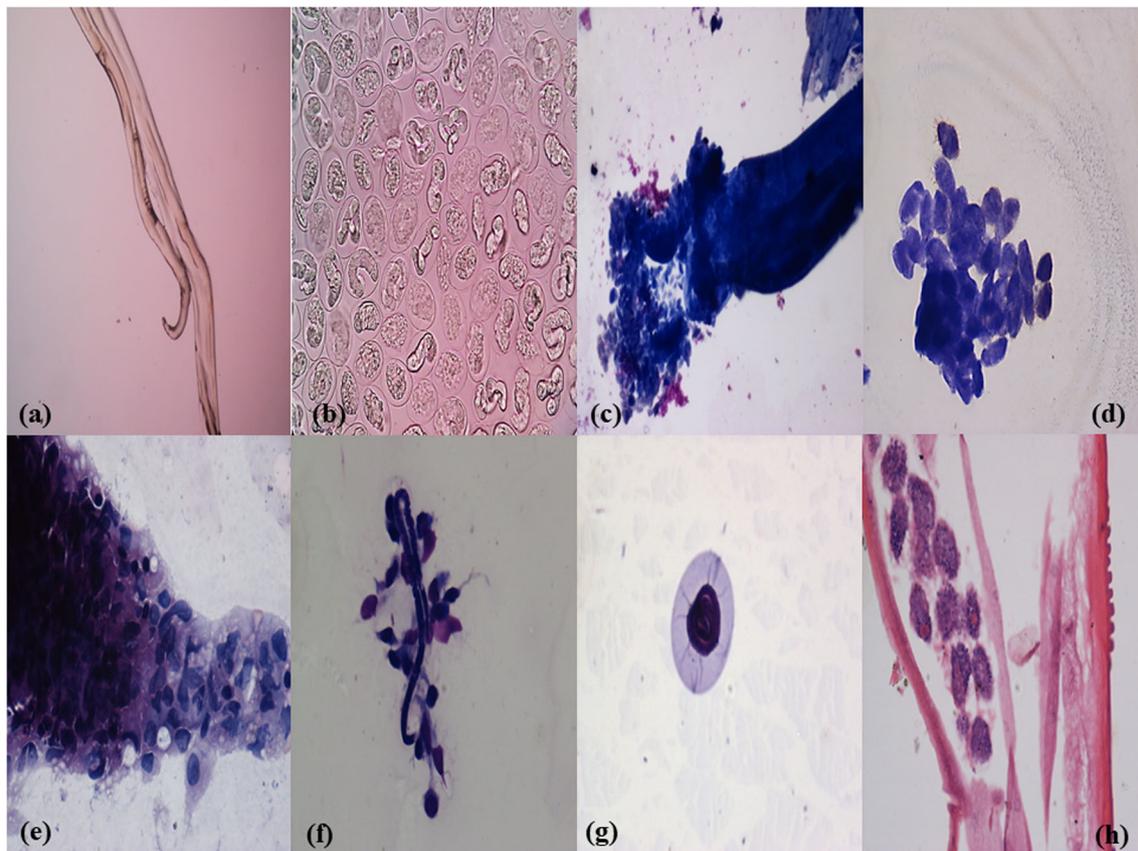
Seven hundred four cases from lymph nodes (cervical, axillary, and inguinal) were included in our study, commonest age group being 21–30 years and mean age of 29.6 years. The male-female ratio was 1:1. Adequate sample was obtained in two passes in 94.2% cases. Twelve cases out of 704 lymph node aspirates were inadequate on cytology. The spectrum of benign lesion (595 cases) included non-specific reactive lymphadenitis, dermatopathic lymphadenitis, kimura disease, granulomatous, and necrotizing lymphadenitis. Fifteen cases

were diagnosed as atypical lymphoid cells/suspicious for lymphoma on TB. There were 82 cases of malignancy which included metastases from varied primary tumors like squamous cell carcinomas, ductal carcinomas, adenocarcinomas, melanoma, seminoma, papillary carcinoma thyroid (Fig. 7a,b), neuroendocrine carcinoma (Fig. 7c,d) etc. as well as non-Hodgkin's lymphoma (NHL). All these cases were concordant on TB, Giemsa, and PAP. Lymph node TAT was 1 day in 97.3% cases.

In cases that were suspicious of lymphoma on cytology, histopathological follow-up was available in seven cases. One case each was reported as angio-immunoblastic

**Fig. 4** **a, b** Secretory carcinoma-Cytosmears showing cellular smears with cohesive and scattered tumor cells. **c, d** Cohesive and scattered tumor cells with eccentric nuclei, vacuolated cytoplasm, and prominent nucleoli (**a,b**- TB, **c**-Giemsa, and **d**- PAP,  $\times 10$  and  $\times 40$ )





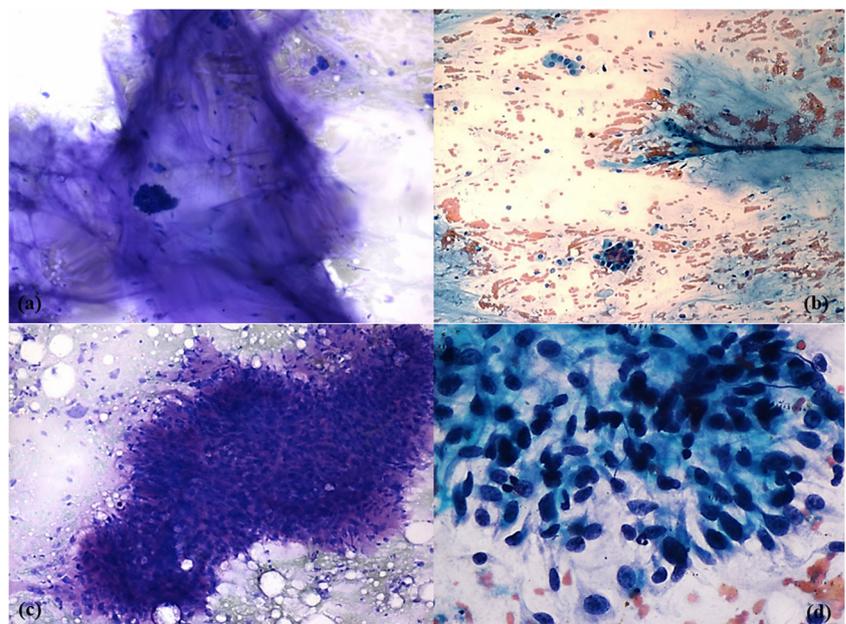
**Fig. 5** **a, b** Wet mount showing adult filaria with gravid worms and embryonated eggs. **c–g** Microfilaria with few inflammatory cells and focal granulomatous reaction in response to microfilaria. **h** Cell block

showing adult filarial worm with microfilaria (**c,d**-TB, **e,f**-PAP, **g**-Giemsa and H&E,  $\times 10$  and  $\times 40$ )

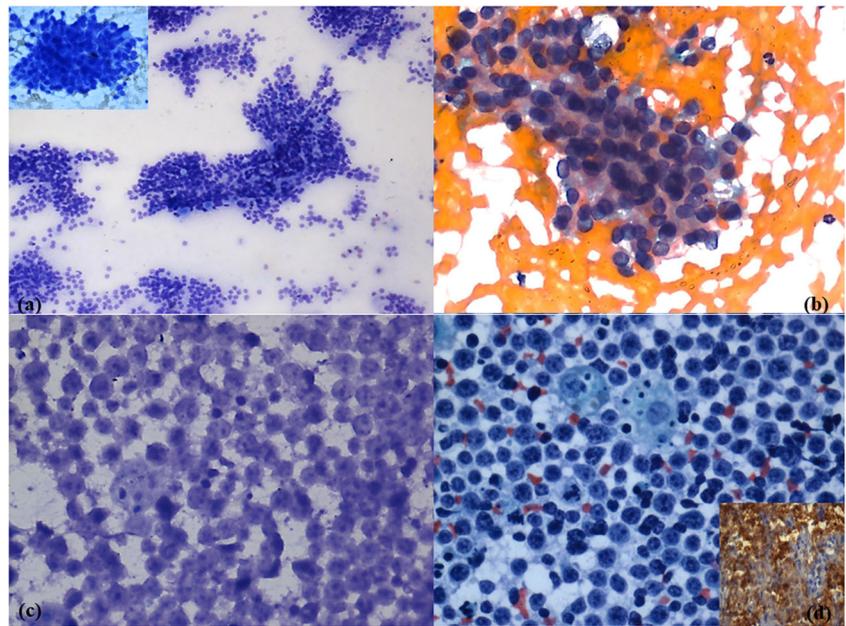
lymphoma, nodular lymphocytic predominant Hodgkin's lymphoma, and NHL (further IHC workup not available). Two cases each showed Burkitt's lymphoma and diffuse large

B cell lymphoma. Among the metastatic lymph nodes, cyto-histo correlation was available in 36 cases, all but one case was concordant. This case had been reported as

**Fig. 6** **a, b** Mucinous carcinoma breast- Cyto smears showing abundant mucin with occasional clusters of ductal epithelial cells and traversing blood vessels (**a**-TB, **b**-PAP,  $\times 40$ ). **c, d** Malignant phyllodes tumor-Cytosmears showing spindle cells in large cellular stromal fragments (**c**-TB, and **d**-PAP,  $\times 10$  and  $\times 40$ )



**Fig. 7** **a, b** Metastasis of papillary carcinoma thyroid-Cellular smears with TFCs in cohesive and branching papillaroid fragments, with micro-follicular pattern and central blob of colloid. Occasional bizarre nuclei, nuclear grooves seen (inset) (**a**-TB and **b**-PAP,  $\times 10$  and  $\times 40$ ). **c, d** Metastasis of neuroendocrine carcinoma-Highly cellular smears with dispersed cells with increased n/c ratio, stippled chromatin and prominent nucleoli. Vague rosette and apoptotic bodies seen. IHC positive for synaptophysin in cell block preparation (inset) (**c**-TB, **d**-PAP,  $\times 40$ )



adenocarcinoma on cytology but biopsy of the primary showed a squamous cell carcinoma with apocrine differentiation. This discordance was due to a combination of interpretation and sampling errors as the apocrine cells mimicked glandular cells in cytology and there was absence of unequivocal squamous cells in the aspirate.

Miscellaneous sites (340 cases) included trunk, extremities, scalp, flank back etc. with the most common age of presentation being 21–30 years (mean 37.6 years). The male-female ratio was 1.5:1. Adequate sample was obtained within two passes in 91.2% of cases. Eleven out of 340 cases were reported unsatisfactory. There was a slightly lower adequacy in PAP-stained slides as compared to TB/Giemsa. The spectrum of lesions included lipoma, keratinous cyst, fungal inflammatory lesions (Fig. 8a–f), skin scrape with histoplasmosis (Fig. 9a, b), cysticercosis (Fig. 9c–e), tumoral calcinosis, tendinous xanthoma (Fig. 10a–f), and endometriosis. One case each of sino-nasal adenoid cystic carcinoma (Fig. 11a, b), metastatic follicular carcinoma to scalp, metastatic adenocarcinoma to chest wall, renal cell carcinoma metastasis at scar site (Fig. 11c, d) were also concordant on TB and final cytology. TAT for miscellaneous site was 1st day in 97.9% cases.

Of the benign spindle cell lesions in cytology, histopathology was available in four cases, reported as neurofibroma in three cases and leiomyoma in one case. Thirty-six cases of lipomas also had a concordant histopathology follow-up of which one case showed features of chondro-lipoma. Two cases of dermatofibrosarcoma protuberans on histopathology had been reported as low to intermediate grade spindle cell neoplasm on cytology.

To further validate the utility of ROSE with TB, we compared the rate of repeat aspirations and number of slides consumed in the first 3 months after institution of ROSE with TB

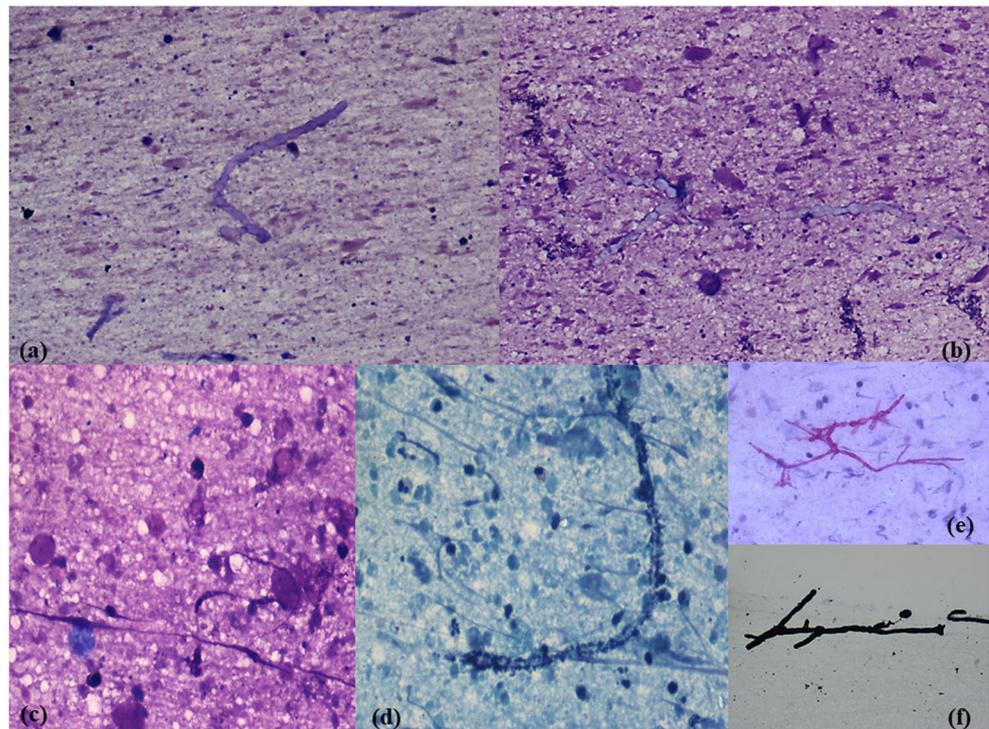
with the last 3 months before ROSE. A total of 506 non-guided cases were performed during the period of May to July (before ROSE), with 84 cases repeated at a second sitting and a total of 2946 slides consumed. Whereas during the period of September to November, there were 539 non-guided cases with repeat visit for FNA in only 25 cases and the slide consumption 2278 slides. Hence, it was noted that ROSE was helpful for better patient compliance, reduced slide consumption, and faster TAT (Fig. 12).

Overall, TB-stained smears were adequate in 1469 (98%) cases. Among the inadequate aspirates, lymph nodes were the highest (12 cases) possibly due to small size and these patients were advised ultrasound-guided FNA. Giemsa-stained smears had an adequacy rate of 97.7% cases, PAP was adequate in 96% cases and the slight low adequacy in PAP was due to the sample getting washed off during staining. Satisfactory yield was obtained in 1179 (78.7%) cases in the first pass itself. TB provisional was given as benign in 1274 (85%); suspicious 49 (3.3%); malignant 145 (9.7%) cases. Overall TAT was 1st day in 1447 (96.4%) cases. The average time taken for provisional/onsite diagnosis in our study was 3 min. We could take additional material in the 1st sitting itself for further studies like cell block and immunohistochemistry (IHC) in 3.2% cases, ICC in 2.2% cases, fungal stain in 1.9%, culture in 0.5%, fat stain in 0.06%, Ziehl-Neelsen (ZN) stain/mycobacterial culture 32% cases.

## Discussion

The success of FNA depends on five fundamental requirements: (a) samples must be representative of the lesion, (b) samples must be adequate in cellularity, (c) sample must be

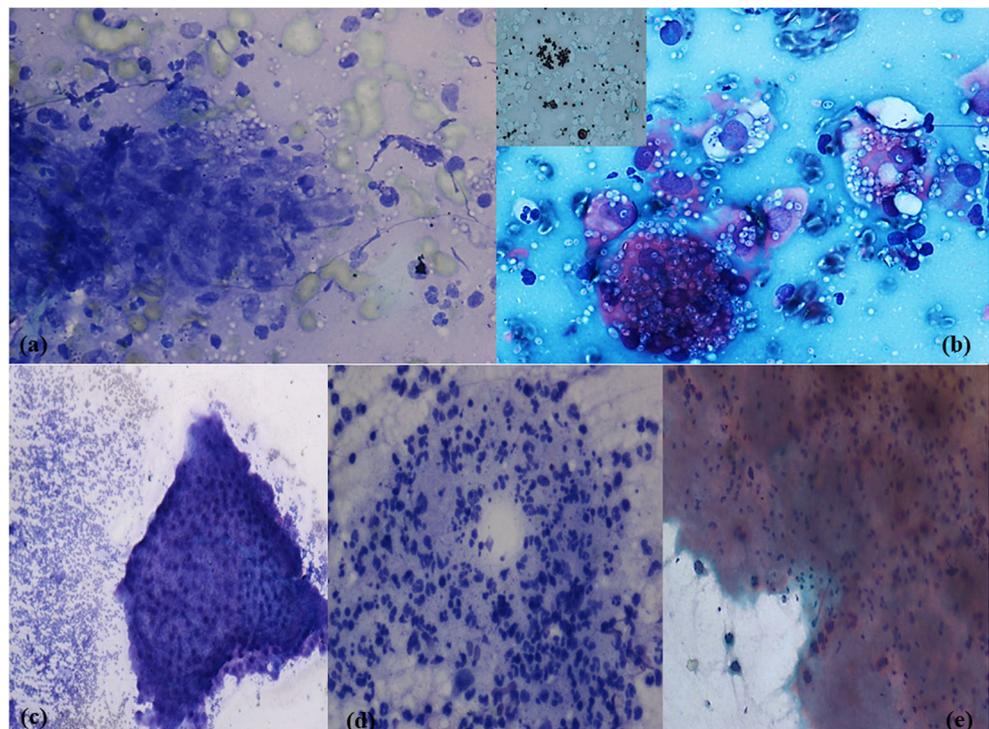
**Fig. 8** a–c Inflammatory lesion (fungal etiology)-Abundant necrotic background with SH bodies and branching fungal hyphae (a-TB and b,c-Giemsa,  $\times 40$ ). d–f Necrotic background with few inflammatory cells and branching fungal hyphae (PAP,  $\times 40$ ). e PAS positive. f GMS positive



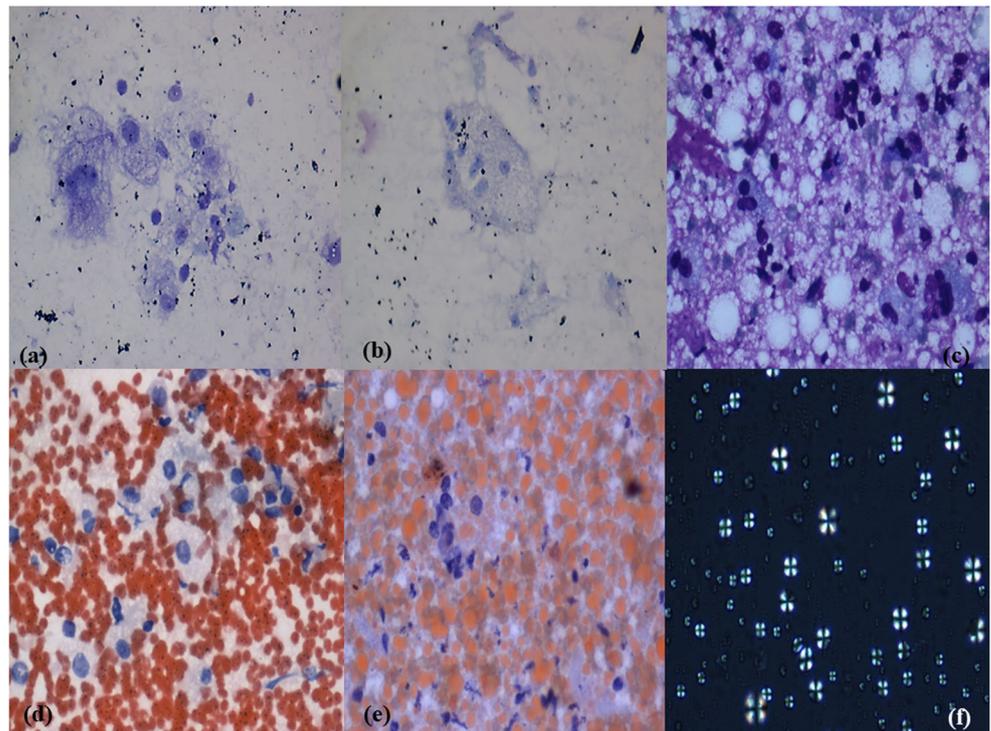
correctly smeared, (d) smear must be properly processed and stained, and (e) reporting and diagnosis by an expert in cytology [8, 9]. ROSE in cytology can be highly effective in many clinical settings. The role of ROSE has been well documented for CT-guided, endoscopic ultrasound-guided (EUS) and trans-bronchial aspiration cytology.

Numerous studies have documented an increase in adequate specimens after the implementation of ROSE compared to FNA performed before ROSE was available. The decrease in non-diagnostic specimens has been reported to drop from 15–47 to 4–23%, depending on the FNA site [10, 11]. ROSE in EBUS-TBNA has resulted in increased diagnostic yield

**Fig. 9** a Histoplasmosis-Cellular smear with epithelioid granuloma with capsulated fungal elements (TB,  $\times 40$ ). b Smear shows histiocytes with fungal elements (PAP,  $\times 40$ ). Inset- GMS fungal stain positive for capsulated elements (GMS,  $\times 10$ ). c–e Cysticercosis-Cytology showed granular bladder wall with subcuticular cells having small pyknotic looking nuclei, fibrillary parenchyma along with inflammatory cells (c,d-TB and e-PAP,  $\times 10$  and  $\times 40$ )



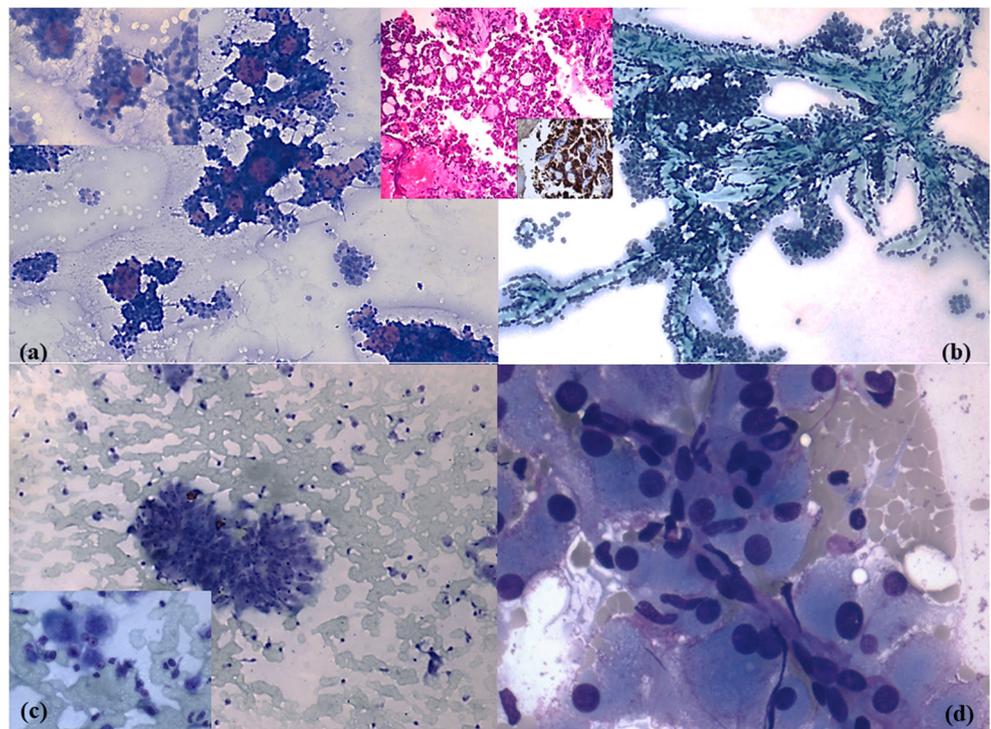
**Fig. 10 a–d** Tuberos and tendinous xanthoma-cytosmears showed lipid-rich background with many foamy histiocytes, scattered giant cells. (a,b-TB, c-Giemsa and d-PAP, × 40). **e** Oil-O-Red stain was positive in foamy histiocytes showing small and large lipid droplets. **f** Birefringent ‘maltase crosses’ in polarized microscopy.



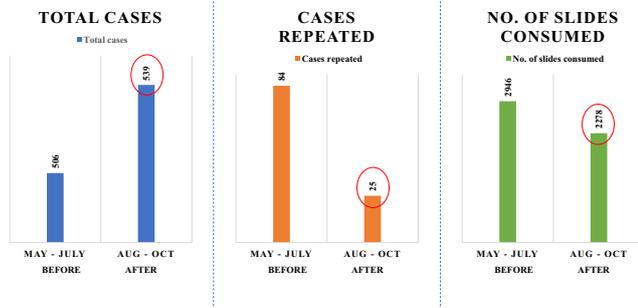
with more than 90% accuracy [12]. In contrast, following a meta-analysis, Sehgal et al. [13] concluded that EBUS with ROSE neither improved yield nor reduced the procedure time; however, it did reduce the number of needle passes required. This would be particularly important in transthoracic and trans bronchial needle aspirations (TBNA) of lung lesions, where

the number of passes is directly related to the increased risk of pneumothorax [10, 13, 14]. ROSE should be viewed in the same light as intraoperative consultation/frozen section and in some cases, may eventually preclude the need for those activities, saving the time of surgical pathologists and surgeons, and potentially decreasing the time of the surgical procedures

**Fig. 11 a, b** Sinonasal adenoid cystic carcinoma-cellular smears with clusters of small basaloid cells, round globules of hyaline material. Basaloid looking cells surrounding round hyaline globules. Cell block and IHC for CD117 positive (Inset) (a-TB, b-PAP, H&E, × 10 and × 40). **c, d** Metastasis of renal cell carcinoma-blood mixed smears with malignant epithelial cells in cohesive clusters and singly, round to polygonal appearance, eccentric nuclei, abundant granular eosinophilic cytoplasm(inset) (c-TB and d-Giemsa, × 40)



EFFECTIVENESS OF ROSE  
(Before and After implementation)



**Fig. 12** Comparison between the number of cases/number of repeats/number of slides used before and after implementation of ROSE

[15]. FNAs performed using ROSE has been demonstrated to reduce cost to the patient by as much as 35%. This is largely due to savings from FNA and/or core biopsy tools which would have been used on additional passes, as well as the cost of additional procedures after a non-diagnostic result [16]. In our study too, we found that the slide consumption and number of repeat procedures reduced after introduction of ROSE.

A wide variety of cytologic stains are employed across different laboratories, and the choice depends on the infrastructure of the hospital for on-site services, budget allocation, and the availability of trained cytology staff. Rapid staining methods like rapid H&E, ultra-fast PAP, toluidine blue, brilliant cresyl blue (BCB), and Diff-Quik have been used [17, 18]. Although Diff-Quik is the most commonly employed stain, it is relatively expensive. Toluidine blue has several advantages—it is cheaper and slides can be returned to PAP or H&E stain. It also gives better morphology in wet-fixed slides [19].

Our study had a mean patient age of 33.3 years which was lower compared to studies done by Joy et al. [2], Ruth et al. [17], and Fahad et al. [18]. We encountered a wide age range from 3 months to 87 years as was in the study of Joy et al. M/F ratio was 1:1.6 with females being slightly more, compared to studies done by Joy et al. [2] and Ruth et al. [17].

Thyroid FNA has resulted in a 70% decrease in unnecessary surgery by distinguishing benign nodular goiter, cysts, and inflammatory conditions from neoplastic lesions [20]. In our study, 70% of lesions were benign, similar to study done by Kanwal et al. [9]. A total of 3.8% of cases were malignant, which was slightly lower than the study by Sumathi et al. [21]. We had 100% adequacy rate which was in concordance with the studies done by Kanwal et al. [9], Ammangani et al. [1] while Ghofrani et al. [20] had slightly lower diagnostic rates.

The use of ROSE in thyroid lesion is controversial. O'Malley et al. [22] raised significant questions with respect to the benefit of immediate adequacy assessment of thyroid FNAs. They found that immediate adequacy assessment prolonged the average procedure time from 12.5 to 44.4 min. However, they concluded that the time was dependent on the experience of cytopathologist and radiologist.

Eedes et al. [23] reviewed the cost effectiveness of immediate specimen adequacy of thyroid FNAs and concluded that ROSE may be done in special circumstances like repeat procedures. In a study done by Jiang et al. [24], the non-diagnostic rate was 6.6% with ROSE, which was lower than 9.5% without ROSE under ultrasonography guidance, but this difference was not statistically significant. The utility of ROSE was more important in sub centimetric, mixed solid cystic, macro-calcified, and hyper vascular lesions. The number of passes and procedural time was reduced with implementation of ROSE. Sumathy C et al. [6] performed supravital stain of thyroid aspirates with 87.8% accuracy whereas the conventional H&E-stained smears gave 91.8% accuracy. The decreased accuracy by supravital stained study was probably due to difficulty in identifying Hurthle cells in supravital stains, which stand out with H&E stain.

Breast cancer is the most common malignancy among women in the developed countries. While mammographic screening and breast core biopsies are commonly used in developed countries, these methods are not uniformly available in our part of the world [25]. FNA of the breast is regarded as a safe, effective, economical, and accurate technique for diagnosing palpable breast lesions [26].

Seventy-five percent of cases in our study were benign and 18.2% were malignant, similar to studies done by Liew et al. [25] and Kanwal et al. [9]. We had a 2% non-diagnostic rate, lower than the study done by Liew et al. (9.3%).

Lymph nodes were the most common site in our study with adequacy rate being 98.3%. Sumathy C et al. [6] studied 190 cases of FNA, out of which 62 aspirates were from lymph nodes and rapid stain with TB yielded a diagnostic accuracy of 95.2%, as compared to smears stained with H&E which had an 85.5% accuracy.

Overall adequacy rate in our study was 97.8% which was in near concordance with studies done by Ammangani et al. [1] (98%), Sarode et al. [27] (99.5%), Burlingame et al. [28] (95.3%), and Kanwal et al. [9] (100%), the number of cases in their studies was much lesser than ours. We had an average of 1.2 pass attempts for obtaining adequate cellularity with time taken for making a provisional diagnosis being 3 min (avg.) which was the lowest of the studies done by Liew et al. [25], Schmidt et al. [4], Kanwal et al. [9], Ammangani et al. [1].

Concordance rate in studies done by Joy et al. [2], Liew et al. [25], Sumathy et al. [4], and Mehmood et al. [29] was 89%, 92.4%, 98%, and 98.2% which was similar to our study (99.2%). Validity parameters were sensitivity 98%, specificity 100%, positive predictive value 100%, negative predictive value 99.8%, efficacy was 99.2%, false positivity was zero, and false negative 1.9% which was in concordance with studies done by Joy et al. [2], Liew et al. [25], and Sumathi et al. [21].

Implementation of ROSE allowed proper triage of material collected in 39.8% cases, especially those of infectious

etiology wherein material for ZN staining could be collected at the same sitting. ROSE thus can be used not just for adequacy but also make a provisional diagnosis and triage the specimen appropriately [30].

Authors such as Lin et al. [31], Hassan et al. [32], Monaco SE [33], and Khurana et al. [34] have explored the possibility of tele cytology with ROSE either with static or live image transmission or whole slide scanning. This is indeed an exciting prospect because once validated, this would definitely help patient care by making all the benefits of ROSE available in settings where pathologists are unavailable.

## Conclusion

FNA with rapid on-site evaluation (ROSE) using toluidine blue staining method is a reproducible and reliable way of demonstrating the adequacy of material in FNAs from different sites. This method provides faster and easier identification of cells and can be used to make a provisional diagnosis in most of the cases. The slides can be re-stained with PAP stain without any compromise in morphological features. Planning for additional sample for ancillary testing along with cost effectiveness are some of the added benefits of ROSE. Thus, ROSE with TB provides valuable information to cytopathologists and is easy enough for general laboratories to incorporate into their routine workflow. *This ROSE truly has no thorns!*

**Consent for Publication** Written consent for publication and any additional related information was taken from all the patients involved in the study.

**Availability of Data and Materials** All the data regarding the findings are available within the manuscript.

**Authors' Contributions** TS carried out concepts and design, literature search, participated in clinical study. KK carried out data acquisition, data analysis and manuscript preparation will stand as guarantor also. MA carried out concepts and design, literature search. PS carried out concepts and design, literature search. LN participated in clinical study and manuscript review. All the authors have read and approved the final manuscript.

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