Hepatic fine-needle aspiration cytology: The role of rapid on-site evaluation in the assessment of hepatic lesions

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ABSTRACT

Background/Aims: *Radiologically guided fine-needle aspiration cytology (FNAC) of internal organs is not cost-effective. Rapid on-site evaluation (ROSE) of smears by a cytopathologist can improve the diagnostic yield of FNACs and save time and money by reducing the need for repeat procedure/biopsy. To determine the role of ROSE in the diagnostic outcome of hepatic lesions by comparative analysis of FNAC with and without ROSE by a cytopathologist.*

Materials and Methods*: Hepatic FNACs were retrospectively analyzed over two separate time periods from January 2011 to June 2013 and from January 2015 to July 2016. Smears from 2015-2016 were subjected to ROSE by a cytopathologist after staining with toluidine blue for 1 min to assess adequacy of the material. Final report was given after hematoxylin and eosin, May Grünwald Giemsa, and Papanicolaou staining were performed. Chi-square test (non-parametric) was used to determine if there was a statistically significant increase in the diagnostic yield with ROSE.*

Results: *During 2011-2013, of the 160 radiologically guided FNACs for hepatic lesions, 22 were non-diagnostic, whereas during 2015-2016, of 142 radiologically guided hepatic FNACs, only six were non-diagnostic. With the application of ROSE, there was a statistically significant increase in the diagnostic yield of hepatic FNACs from 86.25% to 95.8% (p=0.015).*

Conclusion: *ROSE performed by a cytopathologist using toluidine blue can increase the diagnostic yield of hepatic FNACs and reduce the cost of healthcare by eliminating the need for a repeat procedure.*

Keywords: *Aspiration, liver, on-site evaluation, toluidine blue*

INTRODUCTION

Fine-needle aspiration cytology (FNAC) is a well-established, safe, non-invasive, and widely accepted technique for the evaluation of various lesions. With advancements in imaging techniques, molecular testing, and targeted therapies, FNAC can be performed under ultrasonographic, endoscopic ultrasonographic, endobronchial ultrasonographic, and computed tomographic guidance, thus making visceral lesions also amenable and accessible to FNAC, which may be used as an alternative method to more invasive biopsy procedures. This has however escalated the cost of radiologically guided FNACs due to increased requirements of trained technologists and cytopathologists. Thus, there is an emerging need to provide greater diagnostic yield for FNACs, especially those performed under radiological guidance (1). Rapid on-site evaluation (ROSE) of the aspirate smears, while performing FNAC, has been shown to increase the sensitivity and diagnostic accuracy of the procedure (2-4).

As the procedure is operator-dependent, rapid stain is performed on the smears, and adequacy of the material is checked by a cytopathologist, which not only improves the diagnostic yield but also provides material for ancillary testing at source (5).

We compared the results of FNACs performed for hepatic lesions over different time periods, i.e., from January 2011 to June 2013 and from January 2015 to July 2016. FNACs performed in the latter time period were subjected to ROSE and were found to have greater diagnostic accuracy than those performed without ROSE in the earlier time period.

MATERIALS AND METHODS

This was a retrospective analysis of radiologically guided FNACs of hepatic lesions performed at our tertiary-care institute over two separate time periods: from January 2011 to June 2013 and from January 2015 to July 2016.

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Patients with deranged prothrombin index were excluded from the procedure. The study was approved by the institutional ethics committee.

All patients who were suspected with hepatic lesions, clinically or radiologically, were subjected to percutaneous FNAC using a Franzen needle holder (Seven Star Scientific Instruments) under radiological guidance with a 22-gauge needle or long length spinal needle (Romsons) attached to a 20-mL disposable syringe (Dispovan) during suspended respiration. Informed consent for the procedure as well as permission to use their data for academic purposes was obtained. FNAC smears performed between 2015 and 2016 were subjected to ROSE by a cytopathologist to check for adequacy of the diagnostic material after staining with toluidine blue (Nice Chemicals) for 1 min. After completion of the procedure, hematoxylin and eosin (H&E; Loba Chemie), May Grünwald Giemsa (MGG; BTL), and Papanicolaou's stains (prepared in laboratory using EA 36 *LG* Reidel, Bismarck Brown BDH Glaxo Lab, OG-6 Loba Chemie Merck) were conducted as per protocol.

Statistical analysis

The diagnostic yield of FNACs in both the time periods was compared using non-parametric chi-square test with Statistical Package for Social Sciences software, version 25 (IBM Corp.; Armonk, NY, USA). The cases reported to have metastatic deposits were followed up, and the primary diagnosis was ascertained after correlating with the clinical history, histopathological findings on needle biopsy and/or cell blocks, and immunohistochemical findings, wherever possible.

RESULTS

2011-2013

The mean age at presentation was 55±12 years, with age ranging from 2 to 86 years and male-to-female ratio of 4:1. Radiologically, all cases had either single or multiple space-occupying lesions with majority of the cases suspected to have hepatocellular carcinoma (HCC; 60 cases), followed by metastasis (50 cases), abscess (10 cases), and hemangioma (4 cases). ROSE of smears was not performed during this study period. The diagnostic yield of FNACs was 86.25%.

Of the total aspirates, 22.5% (36) cases were benign and 63.75% (102) cases were malignant. Nearly 13.75% (22) cases were reported as non-diagnostic because of insufficient cellularity seen as acellular aspirates or having preparation artefacts or obscuring artefacts, which precluded the evaluation of cellular elements. A majority of such smears were hemorrhagic and contained very few scattered hepatocytes (Table 1 and Figure 1).

Benign diagnoses are listed in Table 2 (Figure 2,3). Among the malignant lesions, metastatic tumor was the most common cytomorphological diagnosis, with deposits from adenocarcinoma, squamous cell carcinoma, and melanoma being the common morphological forms. HCC was the most common primary hepatic lesion with one case being well-differentiated (Figure 4,5) and the remaining seven cases being diagnosed as poorly differentiated HCC. Four cases showed high-grade malignancy,

Table 1. Comparison of diagnosis of liver aspirates between two time periods

Diagnosis	2011-2013 (without ROSE*)	2015-2016 (with ROSE)
Benign	36 (22.5%)	39 (27.5%)
Malignant	102 (63.75%)	97 (68.3%)
Non-diagnostic	22 (13.75%)	6 (4.2%)
Total	160 (100%)	142 (100%)
p	0.015 (significant)	

*Rapid on-site evaluation (ROSE)

Table 2. Categorization of benign and malignant aspirates in two time periods

	Hepatic lesion	2011-2013 (without ROSE)	2015-2016 (with ROSE)
	Inadequate	22	6
Benign			
1	Normal liver/reactive hepatocytes	19	32
2	Regenerative nodule	9	2
3	Pyogenic abscess	6	4
4	Amoebic liver abscess	2	0
5	Aspergillus	0	1
Malignant			
1	Metastatic deposits	90	65
2	Malignant, not otherwise specified	4	7
3	Hepatocellular carcinoma	8	24
4	Hemangioendothelioma	0	1
Total		160	142

*Rapid on-site evaluation (ROSE)

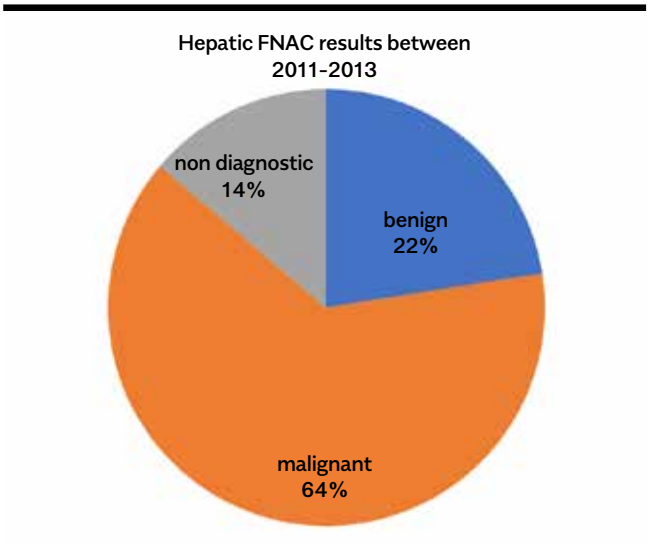

Figure 1. Results of hepatic FNAC between 2011 and 2013

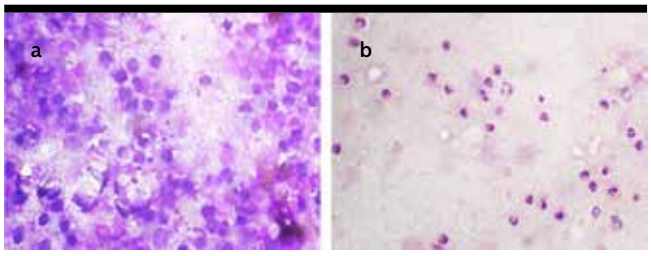

Figure 2. a, b. MGG smear of pyogenic liver abscess showing necrotic debris and degenerated neutrophils (100×) (a); Papanicolaou-stained smear showing neutrophils against necrotic background in pyogenic liver abscess (100×) (b)

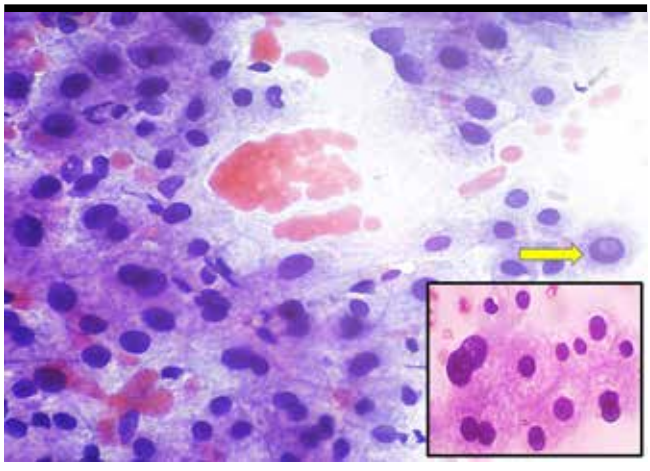

Figure 3. Smear of regenerative hepatic nodule showing sheets of hepatocytes with an occasional one exhibiting prominent nucleolus (yellow arrow) (MGG, 100×). Inset shows enlarged hepatocytes with few displaying binucleation (H&E, 400×)

which could not be further categorized as metastatic or primary (Table 2).

2015-2016

The mean age at presentation was 58±12 years, with age ranging from 21 to 76 years and male-to-female ratio of 1.9:1.

Of these cases, 97 (68.3%) were malignant and 39 (27.5%) were benign, and the number of non-diagnostic smears dropped down drastically to 6 (4.2%), which was significantly different from that of the previous study period (Table 1 and Figure 6). With the application of ROSE, the diagnostic yield of hepatic FNACs significantly increased up to 95.8%.

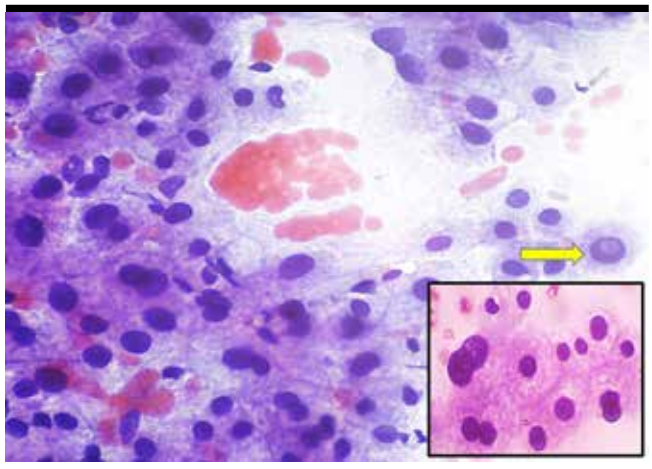

Figure 4. Smear of HCC showing stripped atypical nuclei (Papanicolaou,400×)

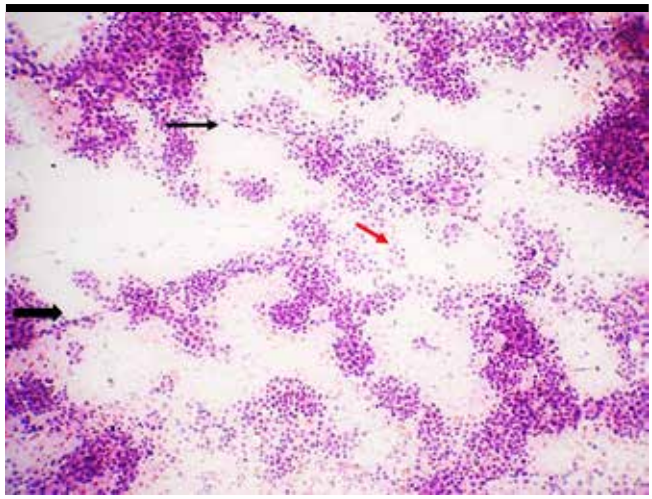

Figure 5. H&E smear of well-differentiated HCC showing tumor cells arranged in a trabecular pattern with transgressing capillaries (black arrows) and stripped atypical nuclei (red arrow) (40×)

Using the Statistical Package for Social Sciences software version 25 (IBM Corp.; Armonk, NY, USA), the chi-square statistic was 8.3453, with a p value of 0.015. The result was statistically significant as p value was less than 0.05.

The diagnoses of 39 benign cases are listed in Table 2. Among the 97 malignant lesions, metastatic tumor was the most common cytomorphological diagnosis (65 cases) with metastasis from adenocarcinoma (42 cases) [31 cases with the primary in the colon (Figure 7), 6 cases with the primary in the gall bladder, and 5 cases with the primary in the pancreaticobiliary tract], followed by metastasis from neuroendocrine carcinoma (5 cases) (Figure 8), papillary urothelial carcinoma (4 cases), small cell lung carcinoma (3 cases), hematolymphoid malignancy and squamous cell carcinoma of the esophagus (3 cases each), endometrial carcinoma (2 cases), and one case each of ovarian carcinoma, breast carcinoma, and sarcoma. The primary origin of the metastatic deposits could be ascertained after following up with the patients' clinical history, histopathology, and immunohistochemical findings.

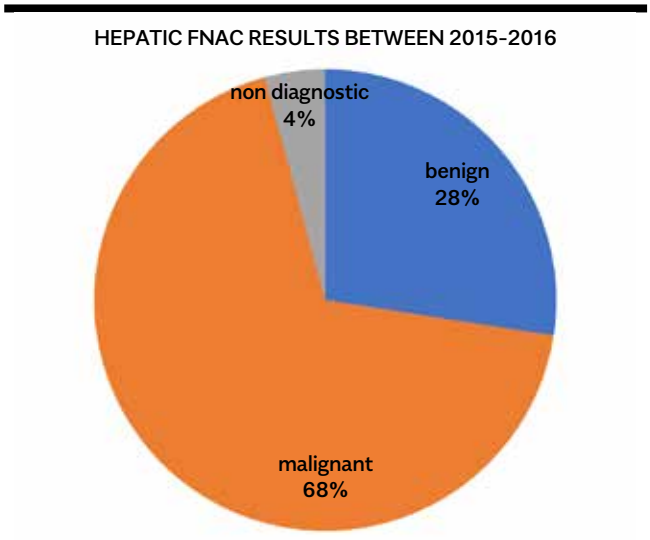

Figure 6. Results of hepatic FNAC between 2015 and 2016

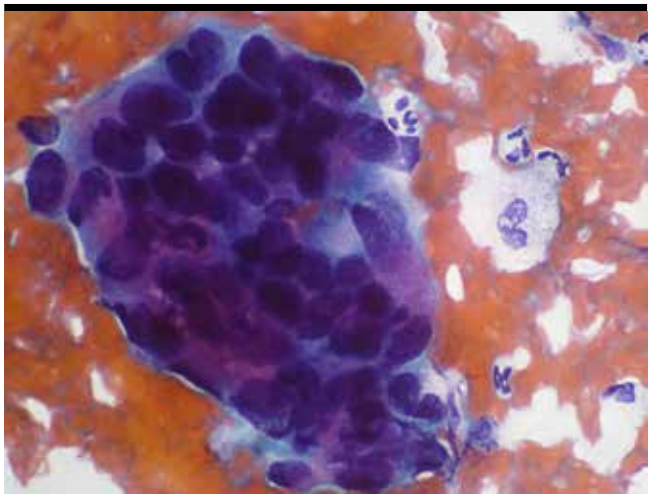

Figure 7. Papanicolaou smear showing a cohesive cluster of pleomorphic tumor cells with hyperchromatic nuclei in a case of metastatic deposits in the liver from colon adenocarcinoma (400×)

HCC was the most common primary hepatic lesion (24 cases) with 12 cases reported as moderately differentiated, seven cases as well-differentiated (Figure 2a, b), and five cases as poorly differentiated. One case was that of epithelioid hemangioendothelioma.

In seven cases, the smears could not be categorized as either HCC or metastasis, and thus, these smears were reported as malignant, not otherwise specified. In four cases, the trucut biopsy was non-representative, whereas in three cases, no trucut biopsy was performed. Hence, the exact nature of the malignancy could not be ascertained in these seven cases.

DISCUSSION

FNAC of the liver under radiological guidance is a highly operator-dependent procedure. Many factors, such as

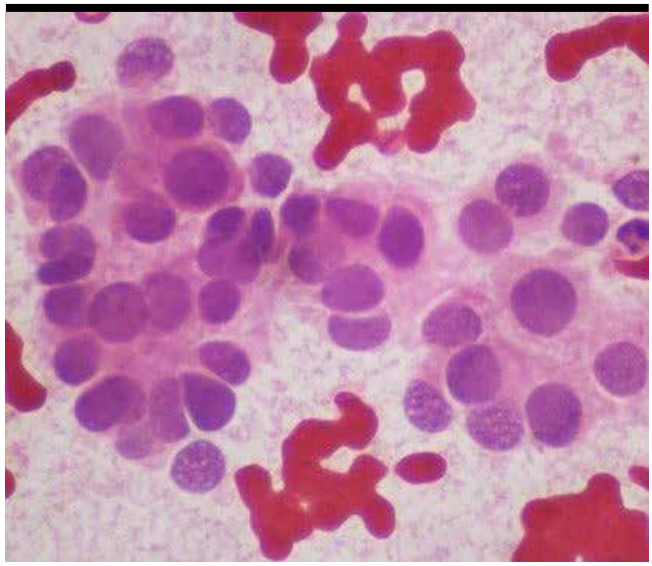

Figure 8. H&E smear showing tumor cells displaying stippled chromatin and rosettes in a case of metastatic deposits from neuroendocrine carcinoma (400×)

the nature and location of the lesion, expertise and skill of the interventional radiologist in hitting the lesion, and ROSE by the cytopathologist, decide the number of passes required to give the diagnostic yield of FNAC. Rapid on-site staining of the aspirate smears by quick stains and evaluation under light microscope by the cytopathologist provides direct feedback to the radiologist as to whether viable cells have been aspirated or not. In the case of paucicellular yield or technical issues such as clotting or obscuring artifacts, a repeat sampling in the same sitting is preferable over a next sitting, which demands more involvement of the treating physician and the operator, motivation of the patient, as well as increased expenditure (6). Saleh and Khatib (7) demonstrated that on-site evaluation of FNAC by a pathologist increases the diagnostic yield and also reduces the cost of medical care by circumventing the need for an excisional biopsy. ROSE can reduce the need of repeat procedure by 10%-30% (8). Sindhwani et al. (9) found that on combining ROSE with transbronchial needle aspiration (TBNA), 45% of cases were saved from repeat procedure due to sample inadequacy, thereby saving time and money. In one study at an institute in Pennsylvania, ROSE of FNAC saved approximately $404,525 per year by eliminating the need for repeat procedures (3). Klapman et al. (10) showed that with ROSE performed by a pathologist, only 23% of aspirates had inconclusive cytological diagnosis, whereas the number of inconclusive aspirates increased to 48% when ROSE was not available. Many other studies have shown significant increase by as much as 90% in the diagnostic yield of radiologically guided FNACs with the application of ROSE by a cytopathologist (11-14). In our study, the diagnostic yield of radiologically guided hepatic FNACs increased up to 95.8% when ROSE was applied (2015-2016) compared with 86.25% when ROSE was not applied (2011-2013), which proved to be a statistically significant improvement.

Erickson et al. (13) have suggested that with two to three needle passes, good diagnostic accuracy can be achieved for liver lesions. The role of ROSE has been explored in the endoscopic-guided FNAC of thyroid lesions, pancreatic masses, TBNA of mediastinal and lymph nodal masses, and lung cytology, while we have studied the impact of ROSE on yield of ultrasound-guided FNACs of hepatic lesions (2,8,9,11,13-15).

Comparison of patient characteristics over the two time periods revealed a male predilection throughout the study periods. Between 2011 and 2013, the number of male patients was four times that of female patients, whereas between 2013 and 2015, the number of male patients decreased to 1.9 times that of female cases. Apart from these demographical changes, there were no significant differences in radiological or serological findings of the patients in the two time periods.

According to our institute's protocol, staining the smears for 1 min with toluidine blue followed by washing in tap water gave us satisfactory results for evaluating adequacy of the smears. Toluidine blue smears are not permanent and were only used to check for adequacy of the aspirated material. These smears cannot be stored for future studies, and other cytological stains were performed for storage and further evaluation. Chandra et al. (15) also used toluidine blue for ROSE. Ramzy et al. (5) used Rapid-Diff II stain for ROSE. Most of the studies used Diff-Quik stain for ROSE (9). We did not compare results of toluidine blue with those of other quick stains, as this comparison was beyond the scope of our study.

At some centers where a pathologist is not available round the clock or during the procedure, gross inspection and ROSE of aspirates are performed by the radiologist, resulting in a diagnostic yield of 69%-72%, whereas in some places, the cytotechnician performs ROSE, resulting in a diagnostic yield of 89%. In both these scenarios, the results show that diagnostic yields are poorer than a trained cytopathologist performs the ROSE (16). Collins et al. (1) studied the efficacy of ROSE performed by cytotechnologists rather than cytopathologists to combat the time and cost logistics of using a pathologist throughout the procedure. With better training and experience, cytotechnologists can also assess the diagnostic accuracy of FNACs. Many studies have used ROSE to provide cytodiagnosis and have compared the results with those of histopathological diagnosis (4,5,9,10). However, we used ROSE only to check adequacy of the aspirated material. This was a limitation of our study. Final cytodiagnosis was given only after the evaluation of MGG, H&E, and Papanicolaou smears, similar to that in the study conducted by Chandra et al. (15). Other disadvantages associated with the use of ROSE are prolonged time for each FNAC and increased cost of using a pathologist as well as a radiologist for the same procedure (1).

In this study, we demonstrated that the use of a quick stain (toluidine blue in our case) for smears in ROSE by cytopathologists can significantly improve the diagnostic accuracy of radiologically guided FNACs of hepatic lesions. This study is an example of how evidence-based

medicine can improve patient care by introducing and adopting newer practices. The application of ROSE will have a significant impact on reducing the cost of healthcare by eliminating the need for repeat procedures or biopsy. This will also increase the confidence of clinicians on the improved outcome of FNAC as a minimally invasive diagnostic procedure that is also well-tolerated by patients.

Ethics Committee Approval: Ethics committee approval was received for this study from the Institutional Ethics Committee.

Informed Consent: Written informed consent was obtained from all the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - P.K.S., R.T.; Design - P.K.S., R.T., P.B.; Supervision - P.K.S., R.T., P.B., H.K., N.S.; Resources: P.K.S., R.T., P.B., H.K., N.S.; Materials - P.K.S., R.T., H.K.; Data Collection and/or Processing - P.K.S., R.T., P.B., H.K., N.S.; Analysis and/or Interpretation - P.K.S., R.T., P.B., H.K., N.S.; Literature Search - R.T., H.K., N.S.; Writing - R.T., H.K., N.S.; Critical Reviews - H.K., N.S.

Conflict of Interest: The authors have no conflicts of interest to declare.

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