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Rapid On-Site Digital Histopatholocal Evaluation (RODE) on Endobronchial Ultrasound (EBUS) Mediastinal Lymph Node Cryobiopsy Sampling Using Confocal Laser Microscopy—A Proof-Of-Concept Study

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ABSTRACT

The current standard for determining sample adequacy during Endobronchial Ultrasound guided Mediastinal Sampling is ROSE (Rapid on-site Evaluation) on samples obtained by Fine needle Aspiration. There is an increasing interest in Mediastinal Lymph node cryobiopsy, where tissue samples are obtained using a 1.1 mm cryoprobe. Rapid onsite evaluation on cryobiopsy samples can also be performed by imprint cytology. This proof-of-concept study explores the use of Viva Scope 2500 for rapid on-site digital histopathological evaluation of Endobronchial Ultrasound (EBUS) guided mediastinal lymph node cryobiopsy samples. Confocal Laser Microscopy allows for high-resolution imaging and expedites diagnosis. The study involved four cases of mediastinal lymph node cryobiopsy, and the samples obtained were subjected to rapid digital histopathological examination by Viva Scope 2500 (confocal laser microscopy), enabling lesion in tool confirmation and the results obtained were compared with the final conventional histopathology results. Confocal Laser microscopy by Viva Scope 2500 was found to be comparable to conventional histopathology, superior to conventional histopathology in providing rapid on-site diagnosis minimising the time to diagnosis, with added advantage of better sampled tissue preservation for downstream molecular analysis. RODE reduces procedural time and increases confidence in the operator during mediastinal lymph node sampling enabling lesion in tool confirmation. RODE is a better alternative to ROSE and conventional histopathology decreasing the time to diagnosis.

1 | Introduction

Endobronchial Ultrasound-guided Transbronchial Cryo-Nodal biopsy (EBUS-TBCNB) has become increasingly adapted in clinical practice after the initial feasibility report published by Gonuguntla et al. [1]. The samples obtained are larger with architectural preservation, facilitating more extensive immunohistochemical staining, which can be crucial for diagnosing specific subset of diseases like Lymphoma and Granulomatous disorders like sarcoidosis. Conventional EBUS TBNA involves needle aspirations, which are then subjected to Rapid On-site Evaluation (ROSE) in centres where the facility is available. In this study we describe a novel technique of Rapid On-site Digital Histopathological Evaluation (RODE)

using Confocal Laser microscopy (CFM) with the new Viva Scope 2500 (2500M-G4; Viva Scope, Munich, Germany). This is a diagnostic tool providing high-resolution histopathology like images of desired tissue samples. It allows rapid virtual microscopic tissue analysis and directly yields digital images ready for telepathology applications [2]. This tool expedites the process, minimising the duration needed for diagnosis.

The confocal microscopic technique has been employed in different fields of medicine. To the best of our knowledge, this is the first study describing the utility of Viva Scope 2500 for Rapid Onsite Digital Histopathological evaluation of tissue sample mediastinal cryo-nodal biopsy. The samples obtained by nodal cryo-biopsy in all the cases were adequate for a histopathological diagnosis and Immunohistochemistry. The results obtained with the help of Viva Scope were superior in quality and compatible in nature with the conventional microscopic image, with the advantage of rapid diagnosis in less than 5 min.

2 | Case Series

2.1 | Materials and Methods

2.1.1 | Steps of Mediastinal EBUS-TBCNB

For EBUS-TBCNB, we use a 1.1 mm flexible cryoprobe (Erbe Cryo 20402-401, Tübingen, Germany).

For transbronchial mediastinal Cryo biopsy, a small incision is made on the endobronchial mucosa, using a high-frequency needle cautery knife thereafter replaced by the cryoprobe. The cryoprobe is advanced slowly towards the puncture site and pushed gently into the lymph node. Under real-time ultrasound guidance, the cryo-probe position is confirmed within the lymph node. The cryoprobe is then activated for about 3–4 s, and the scope is pulled out. The specimens are thawed in saline and later transferred to the cytomatrix of the Viva Scope for further staining and processing (Figure 1).

2.1.2 | Staining Procedure—Viva Scope 2500

The Cytomatrix, a positively charged mesh, must be pre-charged before placing a biopsy sample on it. To charge the mesh, 5–7 drops of 70% ethanol is applied directly onto the surface. Once charged, the biopsy sample is placed on the mesh (Figure 1). The next step is to fix the sample using 70% Ethanol, approximately for 10 s. Following fixation, the sample is stained with Acridine Orange for around 30 s, then with Fast Green for about 20 s. The above steps are repeated for 2–3 times in the same order. The sample is to be rinsed very carefully using 0.9% NS to wash off excess stains. The biopsy sample along with the Cytomatrix is loaded between two glass slides equipped with strong magnets. The magnets are used to flatten the tissue and preserve the sample's integrity. Finally, the sandwiched Cytomatrix with the biopsy sample is ready to be loaded onto the Viva Scope 2500 for scanning.

In all the cases, the sample scanned with Viva Scope were later sent to the laboratory for routine histopathological examination with conventional microscopy to compare the results. The same tissue sample were subjected to immunohistochemistry and necessary markers for guiding further treatment.

FIGURE 1 | Biopsy sample placed on positively charged mesh Cytomatrix.

The Viva Scope 2500 employs dual lasers operating at 488 nm (blue) and 638 nm (red). Before imaging, a fluorescent dye is administered to the tissue, which responds to the blue laser, effectively illuminating cellular structures such as nuclei. Simultaneously, the red laser generates a reflectance signal providing detailed structural information about the sample. These reflectance and fluorescence signals are acquired simultaneously and seamlessly correlated in real-time during the imaging process.

An integrated algorithm transforms these signals into pseudo-coloured images reminiscent of H&E staining. These images capture comparable information to traditional histology, offering flexibility in examination from an overview of the entire sample to detailed views at magnifications up to 550-fold.

3 | Results

3.1 | Case 1

50-year-old female patient with a history of hypertension and Diabetes mellitus presented with complaints of streaky haemoptysis for 1 month. CT thorax of the patient showed a heterogeneous soft tissue density mass lesion in the right hilar region causing extrinsic compression and enlarged mediastinal lymph nodes including the pre-tracheal, and right paratracheal lymph nodes (Figure 2). After pre-anaesthetic evaluation, the patient was posted for Endobronchial ultrasound (EBUS). EBUS-guided TBCNB was taken from the right lower paratracheal lymph node (station 4R) with no post-procedural complications. Histopathology images obtained from the Viva Scope showed tumour-forming glands and acini suggestive of Adenocarcinoma (Figure 2).

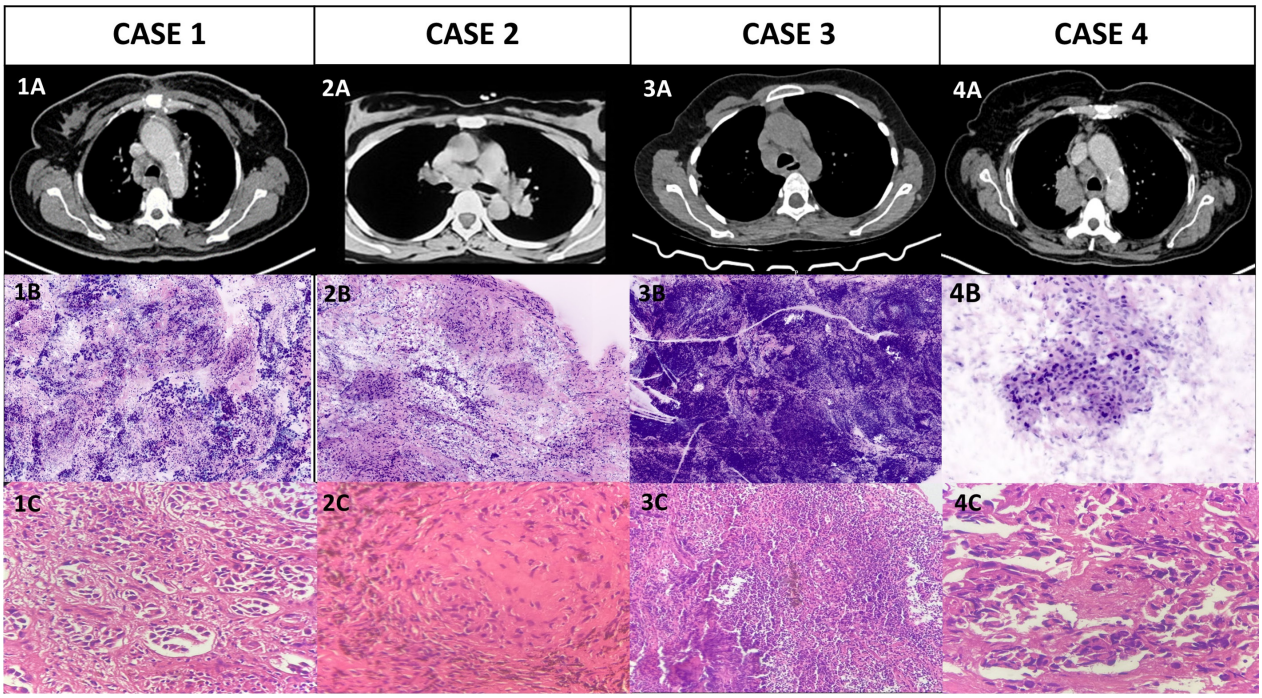

FIGURE 2 | (1A) CT image, (1B) VivaScope image, and (1C) conventional microscopic image: Mediastinal cryo-biopsy from lymph node station 4R in case 1 showing tumour forming glands and acini suggestive of Adenocarcinoma. (2A) (CT image), (2B) VivaScope image and (2C) conventional microscopic image: Mediastinal lymph node station 7 cryo-biopsy in case 2 showing non-necrotizing granuloma with fibrosis. (3A) CT image, (3B) VivaScope image, and (3C) conventional microscopic image: Mediastinal lymph node station 4R cryo-biopsy in case 3 showing maintaining architecture with prominent germinal centers—Reactive lymph node. (4A) CT image, (4B) VivaScope image, and (4C) conventional microscopic image: Cryo-nodal biopsy from mediastinal lymph node station 4R in case 4-malignant cells forming clusters infiltrating stroma suggestive of non-small cell lung carcinoma.

3.2 | Case 2

Forty-year-old female, presented with complaints of low-grade fever and dry cough for 5 months. CT thorax showed multiple heterogeneous mediastinal lymph nodes in the right paratracheal, subcarinal, pre-vascular, and hilar region (Figure 2). EBUS-guided cryo-nodal biopsy was performed from lymph node station 7 (subcarinal). Histopathology imaging with the help of a Viva Scope showed non-necrotising granuloma with fibrosis (Figure 2).

3.3 | Case 3

Thirty-four-year-old female patient, with a history of pulmonary tuberculosis 1 year ago, presented with history of dry cough for 1 month. CT thorax showed enlarged mediastinal lymph nodes, the largest in the right lower paratracheal region (Figure 2). The patient underwent EBUS-guided cryo-nodal biopsy of the right lower paratracheal lymph node (4R). Rapid Histopathology showed well-maintained architecture with prominent germinal centres suggestive of Reactive lymph node (Figure 2).

3.4 | Case 4

Seventy-eight-year-old lady with no known comorbid illnesses, presented with chronic cough, low-grade fever, loss of appetite and weight. Chest CT showed a spiculated soft tissue density mass lesion in the right upper lobe with enlarged mediastinal lymph nodes (Figure 2). After the Pre-anaesthetic work-up, EBUS-guided cryo-nodal lymph node biopsy was obtained from the mediastinal lymph node in the right lower paratracheal region (4R). Histopathology imaging using Viva Scope showed malignant cells forming nests and clusters infiltrating the stroma suggestive of non-small cell lung carcinoma (Figure 2).

4 | Discussion

EBUS guided Mediastinal Cryobiopsy is shown to be superior in diagnostic yield compared conventional EBUS-TBNA, especially in diagnosing benign mediastinal pathologies like sarcoidosis and other Lymphoproliferative diseases [3].

In the last few decades, confocal laser scanning microscopy (CLSM), also known as confocal microscopy, has been used in clinical practice to enable a quasi-histologic resolution of a given tissue in a few minutes [4]. It allows non-invasive, high-resolution imaging of fresh tissue in its native state [5, 6]. Confocal microscopy allows rapid on-site evaluation of various samples, with minimal preparation and the advantage of preservation of tissue architecture. This process omits the requirement of a freezing apparatus or microtome for slide preparation.

In contrast to the probe based confocal microscopy, this ex vivo confocal fluorescence microscopy is very much comparable to conventional Microscopy and does not need any further reference

images as pathologists are familiar to the stains used in this technology, and hence can easily adopt to the image interpretation. The Vivascope CLM offers real-time, non-invasive imaging for tissue analysis, fitting well with traditional pathology methods. The CellVizio confocal microscope is probe based and provides high-resolution, in vivo imaging of the alveolar component based on autofluorescence and is done during bronchoscopy.

In the study, all the samples obtained with cryo-nodal biopsy were taken into saline and later transferred into the Viva Scope cytomatrix with positively charged mesh.

We studied the feasibility of using Confocal microscopy to quickly scan the tissue obtained by cryo-nodal biopsy from mediastinal lymph node and accurately interpret the images. After processing the sample using Viva Scope 2500, we transferred the same tissue samples for the conventional histopathological staining process to compare the results. Cytomatrix is compatible with formalin fixation followed by paraffin embedding. After completion of a fluorescence confocal microscopy examination, the scaffolds were retrieved from the Viva Scope slot. To create a lasting cellblock, they underwent formalin fixation and paraffin embedding following alcohol dehydration and xylene clearing, akin to standard histological procedures. The sample obtained by cryonodal biopsy was adequate for further assessment of molecular markers.

Confocal microscopy using the Viva Scope 2500 ex vivo microscope is a novel technology offering H&E-like images. We have used acridine orange in our study as the fluorescent agent for staining. Other fluorescent dyes used in ex vivo CFM imaging by other investigators include cresyl violet, indocyanine green, methylene blue, toluidine blue O, and proflavine [7]. The contrast agents lead to at least a 1000-fold improvement in nucleus-to-cytoplasm contrast and aid in the recognition of tissues [8]. The lasers used in confocal microscopy (Viva Scope) are of two different wavelengths (488 nm- blue, 638 nm-red) thereby creating two distinct images, a fluorescence image, and a reflectance image. Both signals are scanned simultaneously and are used to develop pseudo-coloured images. The device software employs an algorithm to convert the acquired image data into colours that closely mimic those seen in H&E staining. The resulting images convey information comparable to traditional histology and can be examined at any magnification level.

All the patients in the study successfully obtained instant and accurate diagnoses within minutes of procuring the sample. Using confocal microscopy, rapid on-site evaluation of tissue samples was performed with precision, compatible with conventional histopathology. This is similar to a large multicentric study conducted by Amendoeira et al. [9] on the role of confocal microscopy on the diagnosis of pancreatic solid tumours obtained by EUS-FNB which confirmed the role of instant confocal microscopy by Viva Scope as a valid alternative to traditional microscopy.

Ex vivo confocal microscopy allows rapid analysis, sparing the time-consuming and expensive tissue fixation, cutting, and staining procedure [10].

Although the Vivascope is more expensive, its cost can be offset by sharing the machine across multiple departments, allowing for collaborative use and shared expenses, making it a valuable investment for comprehensive diagnostic capabilities.

In conclusion, confocal microscopy is a valuable tool for rapid onsite evaluation, offering real-time, high-resolution imaging that enhances the speed and accuracy of diagnoses.

We propose the conventional Rapid On-site evaluation (ROSE) will be replaced by Rapid Onsite Digital Histopathological evaluation (RODE) for mediastinal lymph node sampling providing a quick diagnosis in less than 5 min.

Author Contributions

Conceptualisation: Dr. Harikishan. Methodology: Dr. Harikishan, Dr. Preeti and Dr. Milap. Writing original draft and data curation: Dr. Sejal and Dr. Preeti. Review and editing done by all authors.

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Ethics Statement

The authors declare that appropriate written informed consent was obtained for the publication of this manuscript and accompanying images. This study was approved by Institutional ethics committee, Yashoda academy of medical education and research (Reg. no. ECR/49/INST/AP/2013/RR-22). Ethics approval number—RP/PP/08-2024.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the finding of the study are available on request from the corresponding author. The data of this study are not publicly available due to privacy or ethical restrictions.

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