Pleural fluid residue as a diagnostic tool for cytology-negative malignant pleural effusion: A proof-of-concept study

L E Nyanti, MRCPI; **N-C Huan,** FRCP; **H Y Ramarmurty,** MRCP; **T Renganathan,** MPath; **M A bin Abdul Aziz,** MPath; **J L Low,** MBBS; **K T Rosli,** MRCPI; **S Letcheminan,** MRCP; **M G Lansing,** MRCP; **K K Sivaraman Kannan,** FRCP

Medical Department, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia
larrynyanti@ums.edu.my
Department of Respiratory Medicine, Queen Elizabeth Hospital, Kota Kinabalu, Malaysia
Pathology Department, Queen Elizabeth Hospital, Kota Kinabalu, Malaysia
Department of Respiratory Medicine, Queen Elizabeth Hospital, Kota Kinabalu, Malaysia
Medical Department, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia
Department of Respiratory Medicine, Queen Elizabeth Hospital, Kota Kinabalu, Malaysia

Pleural fluid residue, or macroscopic tissue, circulating freely in the pleural fluid obtained through direct filtration, may carry diagnostic histopathological information. We aimed to determine the histopathological concordance of pleural fluid residue in diagnosing TPE and MPE, compared with conventional pleural biopsy. This was a prospective cohort study of consecutive inpatients with cytology-negative exudative effusion who underwent pleuroscopy and had their initial suctioned pleural fluid filtered for residue samples. Pleural fluid residue demonstrated malignant cells in four out of seven cases of pleural biopsy-confirmed malignancy. Pleural fluid residue has comparable cytomorphology but reduced cellularity compared with pleural biopsy. No tuberculous histological features were present in the pleural fluid residue samples. In this preliminary study pleural fluid residue provided histopathological information for malignant pleural effusion, but no incremental diagnostic information for tuberculous effusion. However larger and more definitive studies are required to clarify these findings, and to explore the utility and suitability of pleural fluid residue for mutational analysis
Keywords. Pleural fluid residue, diagnostic accuracy, clump, malignant plural effusion.

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Study synopsis
What this study adds. This study demonstrates the potential of pleural fluid residue as a non-invasive diagnostic method for confirming malignancy in cytology-negative exudative effusion.
Implications of the findings. In resource-limited settings or patients contraindicated for pleural biopsy, pleural fluid residue may provide a viable diagnostic alternative; however, this observation needs further validation.

Tuberculous pleural effusion (TPE) and malignant pleural effusion (MPE) constitute the majority of exudative pleural effusions in Malaysia.[1] Histopathological analysis is diagnostic for these conditions,[2] while pleural fluid cytology has a sensitivity of only 58.2% for MPE.[3] Clumps of tissue from chest drains are of diagnostic value in the setting of cytology-confirmed MPE,[4] but the diagnostic role of such macroscopic tissue in cytology-negative effusions remains to be elucidated. In the present study, tissue termed pleural fluid residue (PFR) was obtained through direct filtration. The objectives of the study were to determine: (*i*) histopathological correlation of PFR with thoracoscopic pleural biopsy in diagnosing TPE and MPE; and (*ii*) the cellularity, architecture and cytomorphology of PFR, and whether immunohistochemical (IHC) staining was feasible. We discuss the findings in 18 cases.

This single-centre, prospective observational cohort study was conducted on consecutive adult inpatients with cytology-negative exudative pleural effusion who underwent medical thoracoscopy in our respiratory unit from 6 September 2022 to 14 December 2022. We excluded patients with bacterial empyema and those with a pre-existing chest drain. A histopathological diagnosis of TPE in our setting is defined as histological examination identifying granulomatous inflammation, or direct visualisation of acid-fast bacilli along with a typical patient history.

During medical thoracoscopy, an initial volume of 500 mL of pleural fluid was suctioned after trocar insertion, and the drain bottle was removed for immediate filtering. The suctioned pleural fluid was extracted from the drain bottle and filtered through sterile 16-ply, 19 × 15 mesh size cotton gauze, which yielded PFR. The PFR was gently rolled into a single mass, placed on a filter paper to air-dry for 2 minutes, and finally placed into a filter-paper envelope. This was then fixed in formalin and transported to the histopathology laboratory for further processing and analysis. The act of rolling the filtered PFR into a clump pays homage to the tissue coagulum clot cell block method of transbronchial needle aspiration.[5]

Fig. 1 compares the histopathological appearance of pleural tissue obtained through pleural biopsy (A and B) and from PFR (C and

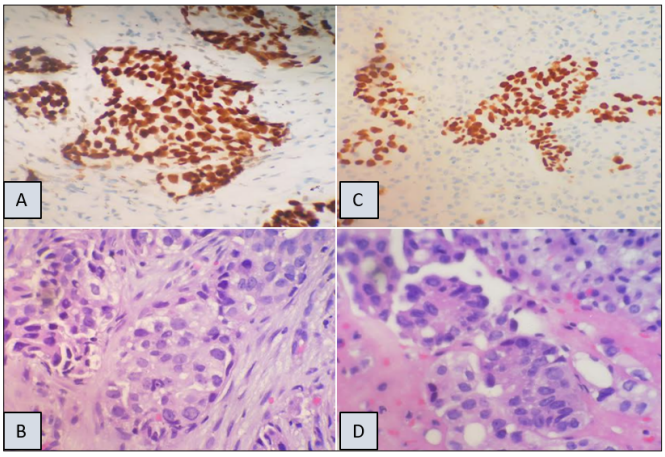

Fig. 1. Histopathological comparison between pleural tissue obtained through pleural biopsy (A and B) and from PFR (C and D). Both samples displayed strong and diffuse immunoreactivity for TTF-1 (A and C, 20×). Under H&E staining, PFR demonstrated satisfactory cellularity, albeit admixed with fibrin (D). Cytomorphological features were comparable and characterised by malignant cells arranged in cords, nests and glandular structures, with round to oval nuclei showing vesicular chromatin texture, conspicuous nucleoli and a modest amount of eosinophilic cytoplasm (B and D, H&E, 40×). (PFR = pleural fluid residue; TTF-1 = thyroid transcription factor-1; H&E = haematoxylin and eosin.)

D). Of the 18 cases, 17 yielded confirmatory pleural biopsy samples (Supplementary Table 1, available online at https://www.samedical.org/file/2143). There was positive correlation in 4 out of 7 MPEs (57.1%). Six patients were diagnosed with adenocarcinoma, of whom 4 (66.7%) had PFR consistent with malignancy; one did not have IHC staining. One patient had squamous cell carcinoma on biopsy, but PFR only demonstrated inflammatory cells. Histological examination of PFR revealed malignant columnar cells trapped within fibrin or blood clot, either arranged in a glandular pattern or singly distributed (Fig. 1D). The cytomorphology of PFR and its feasibility for IHC staining are similar to those of pleural biopsy samples, although PFR has less cellularity and poorer architectural visualisation (Fig. 1A and C). We did not do mutational analysis.

Four patients had TPE on pleural biopsy, but their PFR demonstrated only inflammatory cells. Diagnoses of uraemic pleuritis (n=1) and parapneumonic effusion (n=5) were made based on clinical history and examination, combined with absence of features of tuberculosis on pleural biopsy. The overall negative correlation was 6 out of 6 PE/TPE cases. Both PFR and thoracoscopic pleural biopsy were concordant in terms of the presence of inflammatory cells, but PFR added no further information.

In this proof-of-concept study, we demonstrated that PFR contributes valuable histopathological information in the diagnosis of cytology-negative malignant pleural effusion. Its fibrin-based morphology is comparable to that of chest drain clumps previously described.[4] PFR is morphologically comparable to pleural biopsy, and immunohistochemical staining is feasible. Despite having less cellularity, it is still theoretically sufficient for mutational analysis.

In contrast to pleural fluid cytology, in which pleural fluid is centrifuged to exclude any debris or sediments and subsequently smeared for analysis, diagnostic information from PFR is obtained from the debris found in pleural fluid. PFR also differs from a cytology cell block in that it does not require additives such as plasma and thrombin to enmesh the cellular material. Furthermore, PFR can be processed at the patient's bedside and immediately fixed in formalin. This technique could potentially negate the need for pleural biopsy in suspected malignant pleural effusion, which would be particularly helpful in patients who are not sufficiently stable for medical thoracoscopy, or those who are far from a tertiary centre with the necessary equipment to perform medical thoracoscopy.

It is likely that granulomas do not exist in TPE, as they are formed by a lymphocyte-driven, macrophage-initiated immune reaction which requires blood supply that is only present within the pleura.[6] One would not expect to see acid-fast bacilli on histopathological examination, given that tuberculous fluid is paucibacillary as a result of T-helper cell compartmentalisation and effective containment of tuberculous bacilli.[2]

The present study is not without limitations. First, inconsistent presence of PFR on filtration raises questions as to whether the filtration method could be improved, or whether fibrin-containing residue is uniformly formed in all kinds of malignant effusion; and if so, how soon it forms. It is conceivable that a smaller mesh size could improve PFR yield by capturing smaller residue, but this needs to be studied further. Second, mutational analysis was not carried out. Third, the potential role of PFR being filtered from newly inserted chest drains was not explored in our study. Fourth, a small sample size and single-centre setting reduces the generalisability of the findings. Fifth, the usefulness of PFR for other types of malignancy is unproven, as only adenocarcinoma was diagnosed in this study. These limitations will be addressed in a future pilot study.

PFR provides valuable diagnostic information to assist in the diagnosis of malignant pleural effusion, but does not carry the histopathological information necessary to diagnose pleural tuberculosis. Head-to-head studies on the usefulness of PFR and cytology cell block may elucidate PFR's diagnostic potential. Future studies are needed to explore its role in patients with newly inserted chest drains or non-adenocarcinoma malignancy, and its suitability for mutational analysis.

Declarations. Written informed consent was obtained from the patients for publication of this article and the accompanying images. A copy of the written consent is available upon request from the Editor-in-Chief of this journal. This study was approved by the Medical Research & Ethics Committee of Malaysia (NMRR ID-22-02491-IED).

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