ABSTRACT

Background and objective: The liquid-based cytological test (LCT) is successfully and widely used to assess cervical cytology. This study aimed to compare the cytological findings and diagnostic sensitivity of LCT with those of the pick-and-smear (PS) method for diagnosing lung cancer.

Methods: Sputum specimens from 101 patients diagnosed with lung cancer were studied.

Results: LCT slides showed decreased areas of cell monolayers, a clearer background and distinct, stereoscopic cytological features. The LCT had a significantly higher diagnostic sensitivity for lung cancer (80.2%) than the PS method (63.4%, P < 0.05), particularly for small cell lung carcinoma (P < 0.05). Combination of the LCT with the conventional PS method showed significantly higher diagnostic sensitivity for the detection of adenocarcinoma (80.6%) compared with the PS method alone (55.6%, P < 0.05).

Conclusions: LCT is a useful and easily performed technique that can be widely applied, and is suitable for mass screening for the early diagnosis of lung cancer.

Key words: cytopathology, liquid-based cytological test, lung neoplasm, sputum.

INTRODUCTION

Sputum cytology is regarded by many clinicians as a non-invasive, cheap and simple test for the diagnosis of lung cancer. It complements radiology in the diagnostic workup of lung cancer patients and can be useful for identifying lung carcinoma, especially at the early and occult stages. However, it has shown a low yield in prospective screening trials and is of little or no value in the identification of peripheral cancers. The major limitation of the sputum smear method has been variable quality due to contamination with blood, saliva or inflammatory cells. The liquid-based cytological test (LCT) is a useful cytological technique and its clinical application was approved by the Food and Drug Administration in 1999. It is successfully used in cervical cytology for the preparation of clear cell monolayers through the removal of mucus, necrotic material and inflammatory cells. This study aimed to directly compare the LCT with the conventional pick-and-smear (PS) method, with respect to the quality of the slides and diagnostic sensitivity. The use of the LCT for the diagnosis of lung cancer was also evaluated.

METHODS

The study was conducted in accordance with the regulations of the institutional review boards at China Medical University. From January to October 2004, 708 outpatients and inpatients with histories of respiratory symptoms were recruited at The First Affiliated Hospital, China Medical University. The patients were instructed to rinse their mouths and brush their teeth before coughing sputum, to reduce contamination with food dregs and bacteria. They were also instructed to increase their intake of liquids and to expectorate into a plastic cup following a deep cough upon awakening. This procedure was to be repeated on at least three consecutive days. Of these 708 patients, 101 were diagnosed with lung cancer on the basis of histopathology and/or cytological findings at bronchoscopy. These 101 patients, as well as 40 randomly selected patients without lung cancer, were included as study subjects and control subjects, respectively. In the study group there were 68 men and 33 women, ranging in age from 30 to 93 years. Histological examination confirmed that there were 48 cases of squamous cell carcinoma (SCC), 36 cases of adenocarcinoma (ADC) and 17 cases of small cell lung carcinoma (SCLC).

After macroscopic examination, four smears were prepared from fresh, unfixed sputum and immediately fixed in 95% ethanol and stained with...
Papanicolaou. The residual sputum was transferred to a small bottle with the same volume of CytoRich liquid (Tripath Imaging Inc., Burlington, NC, USA). One millilitre of mucolytic agent (Tripath Imaging) was added for every 10 mL of sputum, incubated at room temperature for 30 min and vortexed for 10 s. Additional mucolytic agent was added to the mixture until the mucus was completely lysed. The mixed liquid was then transferred to a 50-mL tube and centrifuged at 2000 g for 10 min. The supernatant was removed and the pellet was resuspended in 7.5 mL of distilled water. This suspension was vortexed again and centrifuged at 2000 g for 5 min. The supernatant was removed and the pellet was vortexed and transferred to the AutoCyte PREP system (Tripath Imaging), in which slides were automatically prepared and stained. Two slides were prepared from each tube and were stained with Papanicolaou.

For all sputum samples, slides prepared by both the LCT and conventional PS methods were screened and assessed independently by two cytologists.

The sensitivities of the two methods were compared by the chi-squared test using the SPSS 10.0 software package. Statistical significance was defined as \( P < 0.05 \).

RESULTS

In comparison with the conventional PS method, LCT demonstrated a number of advantages. First, screening time was reduced and screening efficiency was increased due to the decreased areas of cell monolayers. Second, the slides had a clearer background due to the dissolution of mucous material, the destruction of most of the red blood cells and significantly reduced numbers of inflammatory cells. Thus, abnormal and tumour cells were more readily discernible. Regardless of cell type, the cells were clearly stained and their morphology was well preserved. The microscopic fine structure of the nuclear envelope, nucleoli and chromatin was also clearly discernible and stereoscopic.

In the LCT slides, SCC cells were evenly distributed in a perfect monolayer pattern, without mucus (Fig. 1). Single ADC cells showed globular and stereoscopic morphology in the LCT slides and small aggregates of ADC cells were arranged in an acinic pattern (Fig. 2). SCLC cells showed mulberry structures (Fig. 3). A comparison of slide quality between the PS method and the LCT is shown in Table 1.

Table 2 shows comparisons of the LCT, PS and combination of the LCT and PS methods for detecting lung cancer. Of the 101 sputum samples from patients with lung cancer examined by the conventional PS method, only 64 were found to have cancer cells, due to obscuring by mucous material, inflammatory cells and necrotic debris. The diagnostic sensitivity was only 63.4% (64/101). However, the LCT showed 80.2% (81/101) diagnostic sensitivity due to a clearer background and well-preserved cell morphology. This difference in diagnostic sensitivity between the two methods was significant \( (P < 0.05) \). When the PS method and the LCT were evaluated in
combination, the diagnostic sensitivity for detecting lung cancer was 85.1%, significantly higher than the PS method alone (63.4%, \( P < 0.01 \)). No cancer cells were found in the 40 samples from patients without lung cancer.

Of the 38 positive SCC cases as determined by LCT, five were negative by the PS method, and 33 were positive by either the LCT or the PS method. On the other hand, of the 37 positive SCC cases as determined by the PS method, four were positive by the PS method but negative by LCT, and 33 were positive by either LCT or the PS method. The diagnostic sensitivity for the detection of SCC was 87.5% when the LCT and PS methods were combined. There was no significant difference when compared with either method alone (\( P > 0.05 \)).

Of the 28 positive ADC cases as determined by LCT, nine were negative by the PS method and 19 were positive by either LCT or the PS method. Of the 20 positive ADC cases as determined by the PS method, one was positive by the PS method but negative by LCT, and 19 were positive by either LCT or the PS method. Although there was no significant difference in diagnostic sensitivity between the two methods (\( P > 0.05 \)), when LCT and the PS method were combined, the diagnostic sensitivity for ADC was 80.6%, significantly higher than that of the PS method alone (55.6%, \( P < 0.05 \)).

Of the 15 positive SCLC cases as determined by the LCT, seven were positive by the PS method and the remaining eight were negative. The diagnostic sensitivity of the LCT for the detection of SCLC was 88.2%,
significantly higher than that of the PS method alone (41.2%, \( P < 0.05 \)).

**DISCUSSION**

The detection of cancer cells by sputum smear has been used to diagnose lung cancer for over 100 years. Diagnostic sensitivity is reportedly quite variable due to different preparation methods in different institutions. Recently, the diagnostic sensitivity of sputum cytology was reported to be 84.4% and 31%, whereas the sensitivity of LCT was 80.2% in this study. There was a significant difference in diagnostic sensitivity between the LCT and the PS method, and when the two methods were combined, the diagnostic sensitivity for the detection of lung cancer was 85.1%, significantly higher than that of the PS method alone (63.4%, \( P < 0.01 \)).

The well-known Saccomanno method was used to collect specimens in a fixative, blend the material to liquefy the mucus and disperse the cells in an evenly distributed monolayer on slides. This method provided more information for the diagnosis of lung cancer and decreased false negative results. However, Perlman et al. reported that this method was not better than the fresh smear method, particularly for the diagnosis of SCLC. Recently, Tang et al. used dithiothreitol as a mucolytic agent and found this approach to be more sensitive than the conventional PS method for the diagnosis of lung cancer. The LCT in cytological screening of sputum has been used to diagnose lung cancer for over 100 years. The detection of cancer cells by sputum smear has been used to diagnose lung cancer for over 100 years. Diagnostic sensitivity is reportedly quite variable due to different preparation methods in different institutions. Recently, the diagnostic sensitivity of sputum cytology was reported to be 84.4% and 31%, whereas the sensitivity of LCT was 80.2% in this study. There was a significant difference in diagnostic sensitivity between the LCT and the PS method, and when the two methods were combined, the diagnostic sensitivity for the detection of lung cancer was 85.1%, significantly higher than that of the PS method alone (63.4%, \( P < 0.01 \)).

The area of a conventional smear is generally 1375 mm\(^2\) and the time needed for screening is 7 min, whereas the area of the LCT monolayer is only 134 mm\(^2\), and the time needed for screening is reduced to 2.5 min. Forty-eight samples could be processed simultaneously and the cells stained automatically using standard procedures in one hour. The cell monolayers showed a clearer background and stronger contrast qualities due to the programming of specimen processing, slide preparation and staining. Therefore, the cytologists were more easily able to concentrate on screening every field of vision with a notable increase in screening efficiency. This is in agreement with a previous report by Rana et al.

With the conventional sputum smear method, SCLC slides contained necrotic material, and showed small cells in loose arrangement. The malignant cells were ‘flooded’ with mucus, epithelial cells and other impurities in the sputum, and as a result the diagnostic sensitivity was lower. Wang et al. reported that the diagnostic sensitivity for SCLC was 50% when using the sputum processing method. However, the present study showed that with the LCT the diagnostic sensitivity for SCLC increased to 88.2% from 41.2% observed with the conventional PS method. The LCT therefore represents a useful method of sputum processing not previously used in diagnostic cytology, and could be a potentially powerful tool for the diagnosis of SCLC.

In this study, the epithelial cells were deposited naturally on glass slides coated with poly-L-lysine in an automatic procedure. There was no smearing or distortion, the cells were distinctly stereoscopic and cellular structures were well preserved, especially in the ADC slides. Twenty specimens were positive for ADC when examined using the conventional PS method, whereas 28 specimens were positive with the LCT. Although there was no significant difference in diagnostic sensitivity between the two methods, the diagnostic sensitivity for ADC was 80.6% when the PS method and LCT were combined, which was significantly higher than that for the PS method alone (55.6%, \( P < 0.05 \)).

On the other hand, the diagnostic sensitivity of the LCT for SCC was not as good. The number of cases positively diagnosed using the PS method was 37, whereas with the LCT only 38 cases were positively diagnosed. This may have been due to the large number of squamous cells obtained with the PS method, and the fact that the number of monolayer slides prepared for each specimen may not have been sufficient. In the case of the seven SCC and one ADC specimen examined using the LCT, the first slide showed no cancer cells, although carcinoma cells were found on the second. This is likely to be due to the non-globular morphology of SCC cells, which are not readily sedimented and therefore lost during centrifugation.

Motherby et al. reported a diagnostic sensitivity of 82.6% for the diagnosis of lung tumours if one slide was prepared from the bronchial secretions, 88.8% with two slides and 94.0% with seven to eight slides. This indicates that a single slide may not adequately represent all the cellular elements from a sputum specimen. We suggest that at least four slides be prepared for each sample. Further studies are needed to use all residual material and investigate the diagnostic accuracy of LCT in detecting cancer cells in sputum from patients with lung cancer.

The LCT provides automatic preparation of cell monolayers from sputum that are highly representative of the cellular content of sputum samples and are superior to those obtained by the PS method. LCT results in a clearer background, smaller areas to be screened and well-preserved, distinctly stereoscopic original cellular structures. The method is therefore suitable for mass screening for the early diagnosis of lung cancer.

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**REFERENCES**

3 Kennedy TC, Miller Y, Prindiville S. Screening for lung cancer revisited and the role of sputum cytology and fluorescence bronchoscopy in a high-risk group. *Chest* 2000; **117**: 72S–79S.


