Thoracic Cancer ISSN 1759-7706

BRIEF REPORT

Application of liquid-based cytology test of bronchial lavage fluid in lung cancer diagnosis

Yang Yang[1], Xiuwei Zhang[1], Jianbao Lu[2], Paul Zarogoulidis[3], Xin'an Wang[4] & Haidong Huang[5]

1 Department of Respiratory Medicine, Jiangning Hospital, Nanjing Medical University, Nanjing, China
2 Department of Respiratory Medicine, People's Hospital of Liyang, Liyang, China
3 Pulmonary Department-Oncology Unit, "G.Papanikolaou" General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece
4 Department of Respiratory Medicine, People's Hospital of Binzhou, Binzhou, China
5 Department of Respiratory Medicine, Changhai Hospital, Second Military Medical University, Shanghai, China

Keywords
bronchial lavage fluid; diagnosis; liquid-based cytologic test; lung cancer.

Correspondence
Haidong Huang, Department of Respiratory Medicine, Changhai Hospital, Second Military Medical University, 168 Changhai Road, Shanghai 200433, China.
Tel: +86 21 31161322
Fax: +86 21 51190920
Email: hhdongbs@126.com

Received: 20 November 2012; accepted 25 February 2013.

doi: 10.1111/1759-7714.12036

Yang Yang and Xiuwei Zhang contributed equally to the work.

Abstract

The aim of this study was to compare the diagnostic significance of the liquid-based cytologic test (LCT) and conventional smear (CS) in bronchial lavage fluid (BLF) in lung cancer patients, as well as the evaluation of LCT applications in BLF for different types of lung cancer. A total of 210 patients were divided into two groups of LCT and CS. The positive rates of the two groups were compared by stratified analysis of different bronchoscopic appearances. The positive rate of LCT and CS groups was 35.84% and 11.835%, respectively, which indicated a statistical significance between the two groups ($P < 0.001$). Furthermore, the detection rate of squamous carcinoma in the LCT group was (72.7%), which was higher than that of the CS group (41.7%) ($P = 0.041$). However, there was no difference between the biopsy and biopsy combined with LCT groups ($P = 0.417$), in terms of direct bronchoscopic appearance. We concluded that LCT was superior to CS in BLF that was acquired by bronchoscopy from lung cancer patients. Moreover, LCT was better than CS in diagnosis of squamous cell carcinoma. LCT could be used as an important complement of bronchoscope biopsy and could have the potential to be widely applied.

Introduction

As an improved cytological smear technique, the liquid-based cytologic test (LCT) has been widely and successfully used in gynecologic cell pathology.[1–4] Cells spread in a thin layer, along with the removal of mucus, blood, and inflammatory exudates, have been the main advantages of LCT.[5] It has also been applied in bronchial brushing as a reliable method, which increased the positive rate of bronchial brushing as a result.[6–9] Currently, there are few reports in China about its application in BLF from lung cancer, especially after biopsy. This study was designed to evaluate the clinical value of LCT in lung cancer diagnosis by comparing the positive rates of the two methods. Furthermore, we intended to apply stratified analysis according to different bronchoscopic types of lung cancer to discuss the outlook of LCT application in different types of lung cancer.

Materials and methods

A retrospective study was conducted with a total of 210 inpatient or outpatient cases with lung cancer in our hospital between February 2009 and May 2012. Chest computed tomography (CT) was performed, which showed the occupying lesion and conformed to the radiographic feature of the tumor. Bronchoscopy biopsy and bronchial lavage were applied to all cases to obtain histological and cytological specimens, and were performed by the same qualified doctor. For those cases that could not be clearly diagnosed by bronchoscopy, histological specimens were acquired by CT guided percutaneous lung puncture biopsy. A number of improvements in cellular detection technology were applied in the department of pathology of our hospital in October 2010 that were of benefit for this study. Conventional smears were conducted on 104 cases of bronchial lavage fluid (BLF) before October 2010; LCT was used on the remaining 106 cases after

October 2010. There were a total of 168 men (80%) and 42 women (20%), with the youngest patient 38 years old and the eldest 83 years old (average age 65.8 ± 9.1).

The Guidelines on Diagnostic Flexible Bronchoscopy (2008 Edition)[10] and Bronchial Lavage Fluid Cytology in The Detection of Technical Specifications (Draft) were followed during this study.[11] All cases accepted to undergo a chest CT scan to ascertain the lesion site, as well as electrocardiogram. Furthermore, routine blood, blood type, and coagulation function tests were applied. The patients received a 10-ml injection of 2% lidocaine, 20 minutes before the operation. The Pentax EB-1570K electronic bronchoscope was used with nasal intubation to conduct a biopsy with the help of disposable biopsy forceps under the guidance of endoscopy, combined with a CT scan. Five specimens were taken according to the conditions of bleeding. Biopsies were not taken from unclear operation fields after continuous negative stress suction for two seconds. The tip of the bronchoscope was inserted into the bronchial branch orifice after the biopsy was performed. A total of 100 mL physiological saline was injected into the biopsy orifice through the working channel of the bronchoscope, 20 mL at a time. A total of 50 mL bloody lavage fluid was recovered with a vacuum extractor under the suction pressure of 80 mmHg and the lavage bottle was taken down. In group A, the BLF was conducted with conventional smear, while LCT was applied in group B.

The BLF of patients in group A was sent to pathology department within 30 minutes. The samples were centrifuged in 2000 RPM after filtration. The sediment was collected after discarding the supernatant. The sediment was suctioned and placed on slides. It was then smeared on few more slides, were fixed with 95% ethanol for 10 minutes, hematoxylin and eosin (H&E) stained, sealed, and examined under microscope.

The BLF of patients in group B was uniformly mixed, oscillated, and added into 30 mL of Cytolyt solution (Hologic Inc., USA). The samples were then sent to the pathology department. The rest of the samples were centrifuged at 2000 RPM after filtration. The supernatant was discarded and the sediment was collected. Thinprep (TP) preservation solution was added to it, mixed and oscillated for 15 minutes. Ultrathin cell smears were then prepared by TCT microcomputer processing system. The smears were fixed with 95% ethanol for 10 minutes, H&E stained, sealed, and examined under the microscope.

All biopsy specimens from the two groups were fixed in 4% formalin and embedded in paraffin. Finally, 5-μm thick serial sections were made, H&E stained, sealed, and observed under the microscope. The positive rate of the two methods was compared by the Chi Square test using the SPSS 13.0 software. A P of less than 0.05 was considered statistically significant.

Results

In the CS group, invasive appearance was 40.4%, proliferative appearance 49%, external compressive appearance 3.8%, and inflammatory appearance 7%. In the LCT Group invasive appearance was 34%, proliferative appearance 58.5%, external compressive appearance 0.9%, and inflammatory appearance 6.6%. The constituent ratio of bronchoscopic appearance was fine in the two groups ($P = 0.165$).

The positive rate of BLF after biopsy in the CS group was 11.5%, while the positive rate of BLF after biopsy in LCT group was 35.8%. The positive rate in the LCT group was significantly higher than in the CS group, therefore, there was statistical significance ($P < 0.001$).

The total positive rate of bronchoscopic biopsy in the LCT group was 78.3%, while the positive rate of biopsy combined with LCT was 83.0%. The positive rate of different operating methods had no significant difference ($P = 0.491$). In the subgroup analysis, the positive rate of direct bronchoscopic appearance was 84.7%, while the positive rate of biopsy combined with LCT was 88.8% ($P = 0.417$).

The detection rate of adenocarcinoma in the CS and LCT groups was 50.0% and 21.1%, respectively. Although the adenocarcinoma detection rate in the CS group was higher than that of the LCT group, there was no statistical difference between the two groups. The detection rate of squamous carcinoma was 41.7% in the CS group, while it was 72.7% in the LCT group. Furthermore, the LCT group had an apparently higher detection rate than the CS group ($P = 0.041$).

Discussion

This study predominantly compared the BLF LCT testing and CS detection, where the main variable was different types of bronchoscopic appearance. Table 1 shows that the constituent ratio of bronchoscopic appearance in both groups was good ($P = 0.165$).

Conventional smear had a low positive rate in patients with lung cancer in this study (11.5%). This could be attributed to a too thick and uneven mucus sample, along with blood or inflammatory cells that covered the actual cells.[12] The cytologic diagnostic accuracy was significantly improved (35% improvement) by the application of LCT through automatic single independent staining.[13–15] There have been few reports available documenting the advantages and disadvantages of LCT and CS for non-gynecological specimen preparation and diagnosis, which subsequently prove that LCT is superior to CS and could increase the positive rate of tumor diagnosis.[16] In view of the lack of this data, we attempted to apply LCT into BLF, expecting that it might improve lung cancer diagnosis. In our study, the positive diagnostic rate of LCT

Table 1 Constituent ratio of different bronchoscopic appearance in the two groups

Variables	Group	CS Group (A)	Constituent ratio (%)	LCT Group (B)	Constituent ratio (%)	*P*
Bronchoscopic appearance	Invasive	42	40.4	36	34.0	0.165
	Proliferative	51	49.0	62	58.5	
	External Compressive	4	3.8	1	0.9	
	Inflammatory	7	6.7	7	6.6	

CS, conventional smear; LCT, liquid-based cytologic test.

with BLF was higher than that of CS, which indicated a manifestly statistical difference, as shown in Table 2 ($P = 0.000$). The LCT provided a higher diagnostic yield of pulmonary malignancy in bronchoalveolar lavage (BAL) specimens when compared with conventional direct smear techniques and can be an alternative method of diagnosis in this setting.[17] There have been no reports in this respect in China and abroad. Applying bronchial lavage after the biopsy could collect the maximum number of tumor cells, which could then be stored in the preservation solution in order to preserve their integrity and to effectively decompose mucus, blood cells, and other impurities. Clean background, programmed dyeing, and sectioning made smear cells clear and strong in the three-dimensional sense, so that abnormal cells could be more easily observed.[18–20] Potential advantages of TP include better utilization of skilled cytotechnologists and streamlining the workflow in the laboratory.[5]

The different bronchoscopic appearances were divided into direct and indirect signs.[21] Direct signs included invasive and proliferative lesions. Indirect signs included external compressive and inflammatory lesions. In the LCT group, there were 98 cases with direct signs that were diagnosed with lung cancer by bronchoscopy, with the bronchoscopic appearances as infiltrative and proliferative lesions (Table 3). The direct features were easier to biopsy than the indirect, which might be the most important reason as to why most of the lung cancer patients that were diagnosed by bronchoscopy had direct signs.

In this study, there were four patients that showed neoplastic proliferative squamous carcinoma in the LCT group. We failed to get histopathological findings in these patients because hemorrhage occurred after biopsy, but we managed to obtain cell pathology results from the BLF. There was one case with mild inflammatory adenocarcinoma that we failed to biopsy under bronchoscope, but we were again able to obtain the cell pathology results from BLF instead. There was no statistically significant difference in the total positive rate between biopsy and biopsy combined with LCT groups ($P = 0.491$). Likewise, there was no statistically significance difference in the total positive rate between biopsy and biopsy combined with LCT groups, in terms of the direct signs under bronchoscopy in subgroup analysis ($P = 0.417$) – which did not confirm the report by Gang Li *et al*.[22] However, the positive rate was significantly higher in the LCT combined with biopsy group, as compared to that of the biopsy group, indicating that LCT was an important supplement to the tissue biopsy.

There has been a study[23] showing that invasive and proliferative lesions were the main types of bronchoscopic appearances in lung cancer, with the primary type being squamous carcinoma. In Table 4, the detection rates of squamous carcinoma in the CS and LCT groups are 41.7% and 72.7%, respectively ($P = 0.041$). The reason behind this might be that proliferative squamous carcinoma often grows as an intracavity cauliflower-like or nodular hyperplasic mass with a rich vascular supply or yellow-white sphacelus on the surface.

Table 2 Positive rate of the two groups

Group	Positive (constituent ratio %)	Negative (constituent ratio %)	*P*
CS	12 (11.5)	92 (88.5)	0.000
LCT	38 (35.8)	68 (64.1)	

Positive rate of BLF after biopsy in CS group: 11.5%; positive rate of BLF after biopsy in LCT group: 35.8%; positive rate in the LCT group was significantly higher than in the CS group ($P < 0.001$). BLF, bronchial lavage fluid; CS, conventional smear; LCT, liquid-based cytologic test.

Table 3 Positive rate of different bronchoscopic appearance and operating methods in LCT group

Group	Direct appearance	Indirect appearance	Total positive rate
Biopsy	84.7% (83/98)	0% (0/8)	78.3% (83/106)
Biopsy combined with LCT	88.8% (87/98)	12.5% (1/8)	83.0% (88/106)

LCT, liquid-based cytologic test.

Table 4 Detection rate of different types of lung cancer

Group	Adenocarcinoma	Squamous Carcinoma	Small Cell Carcinoma	Other Types	Total
CS	6	5	1	0	12
LCT	8	28	2	0	38

Note: Other types included adeno-squamous carcinoma and large cell carcinoma. CS, conventional smear; LCT, liquid-based cytologic test.

The first forceps biopsy often caused a moderate hemorrhage, which made it hard to carry out the second biopsy and brush biopsy. On such occasions, the operator mainly focused on endoscopic hemostasis. Furthermore, it happened so frequently that we could not acquire the histopathological findings because there were not enough biopsy specimens or the biopsy tissues were sphacelus. However, in such cases, bronchial lavage was collected after biopsy and we managed to obtain enough of the tumor cells for LCT. Based on these results, bronchial lavage and biopsy could complement each other to provide valuable results and to increase the diagnostic positive rate. Moreover, bronchial lavage technology is a flexible operation and well tolerated by patients. It did not increase the risk of bleeding and could significantly improve operation success, especially for inexperienced surgeons. The detection rate of adenocarcinoma between the LCT and CS groups was not statistically different ($P = 0.051$). Some of the cases that were underrepresented might account for this result. Therefore, further studies are needed to address this issue.

In conclusion, LCT with BLF after bronchoscopic biopsy is clearly superior to CS. Moreover, for the proliferative squamous cases, LCT is more advantageous and could be used as an important complement of the bronchoscopic biopsy. Therefore, it has the potential to be widely applied in clinical settings.

Acknowledgments

The authors are grateful to the Pathology Department of Jiangning Hospital, Nanjing Medical University for their cooperation.

Disclosure

The authors declare no conflict of interest.

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