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Original Article

Surepath liquid-based cytology combined with conventional bronchial brushing smears in the diagnosis of primary and secondary pulmonary malignant tumors

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Abstract: Objective: To discuss the value of Surepath liquid-based cytology (SP) combined with conventional bronchial brushing smears (CS) in the diagnosis of pulmonary malignant tumors. Methods: 872 cases which were suspected as pulmonary malignant tumors undergoing fiberoptic bronchoscopy from a single institution were included in this study. Conventional bronchial brushing smears and Surepath liquid-based cytology slide were prepared for each case. The diagnostic value of CS and SP were discussed for pulmonary primary and secondary malignant tumors. Results: Totally 645 cases were confirmed as malignant tumors by biopsy, which included 630 cases of bronchogenic carcinomas, 3 cases of non-Hodgkin lymphoma and 12 cases of secondary malignancy. For these malignant lung tumors, 559 cases were detected by CS and 563 cases were showed positive by SP, the sensitivities of CS and SP were 86.7% (559/645) and 87.3% (563/645), respectively. If these two methods were combined, the sensitivity could reach 90.4% (583/645). For the 633 of primary malignant tumor, 547 cases were positive by CS, the sensitivity was 86.4% (547/633), 552 cases were positive by SP, the sensitivity was 87.2% (552/633), the sensitivity of CS combined with SP in the diagnosis of primary malignant tumors reached 90.2% (571/633). For the 12 cases of secondary malignancies, the sensitivity of CS was 100% (12/12) while the sensitivity of SP was 91.7% (11/12). Conclusions: Bronchial brushing cytology is a good complementary examination for fiberoptic bronchoscopic biopsy. The combination of CS and SP in diagnosis of pulmonary malignant tumors was superior to CS alone. It is suggested that these two methods should be combined together to improve the diagnostic value of fiberoptic bronchoscopy for primary and secondary malignant lung tumors.

Keywords: Liquid-based cytology, conventional bronchial brushing smear, pulmonary malignant tumors

Introduction

Bronchogenic carcinoma is the most common malignant tumor worldwide and it is the leading cause of cancer mortality [1-4]. The bronchial brushing cytology is a kind of very useful method for detecting pulmonary malignant tumors including bronchogenic carcinomas. It also can be used to detect the secondary malignancies involved the lung. Conventional smear (CS) was the major method of cytological preparation in China for many years. Liquid-based cytology (LBC) which emerged in 1990s is a kind of relatively new cytological preparation method, its applications in screening and diagnosis of cervical cancer have been well recognized. LBC is also increasing applied for the non-gynecological cytology in China these years [5, 6]. This study retrospectively analyzed Surepath liquid-based cytology combined with conventional bronchial brushing smears in the diagnosis of pulmonary malignant tumors.

Materials and methods

Patients

A total of 872 cases suspected of pulmonary malignant tumors were taken fiberoptic bronchoscopy in West China Hospital of Sichuan University since January to October, 2014. There were 623 males and 249 females. The ages were from 19 to 87 years old, and the median age was 61. Conventional smears and Surepath liquid-based cytology slide were prepared for each case.

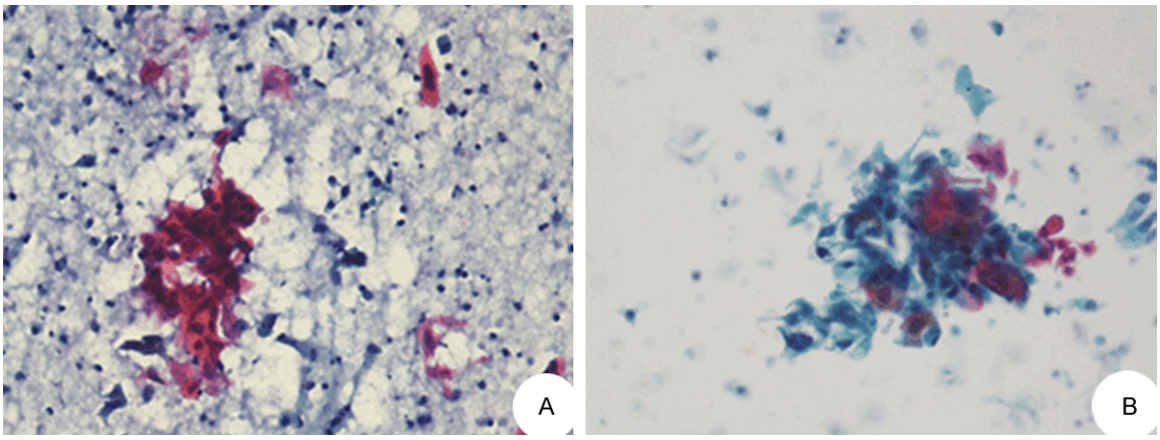

Figure 1. Squamous carcinoma CS (A) and SP (B) from the same patient. The keratinized tumor cells showed hyperchromatic nuclei, the background of SP was cleaner than CS (Papanicolaou stain, ×400).

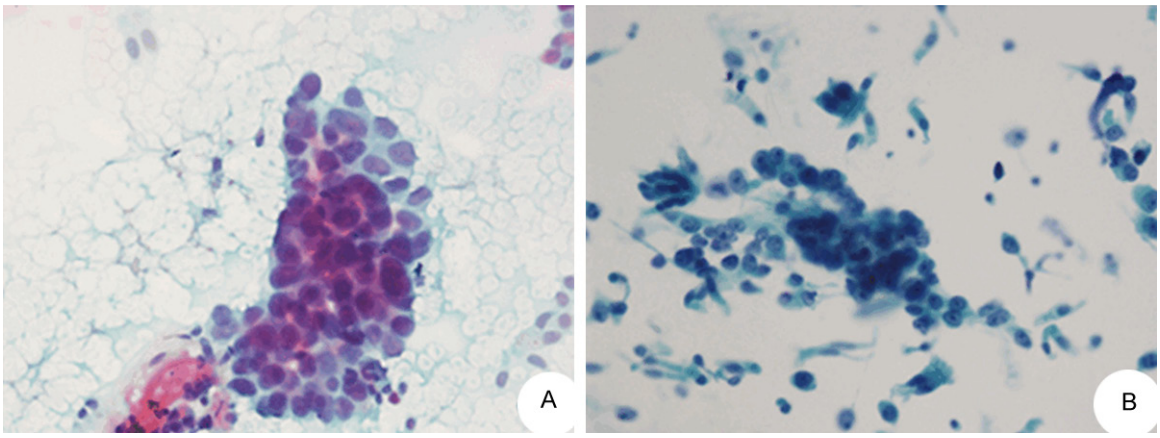

Figure 2. Adenocarcinoma. CS (A) and SP (B) from the same patient. Glandular structure formed by tumor cells could be identified in both preparations, the background of SP was cleaner than CS (Papanicolaou stain, ×400).

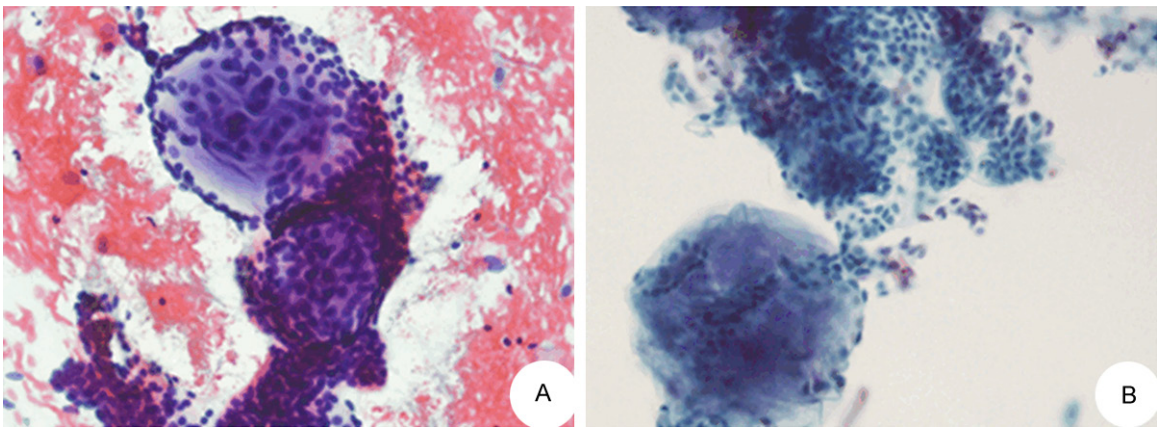

Figure 3. Adenocystic carcinoma. CS (A) and SP (B) from the same patient. Extracellular hyaline globules, basement membrane-like material and basaloid tumor cells cloud be identified; the background of SP was cleaner, and the detail structure of tumor cells was clearer than CS (Papanicolaou stain, ×400).

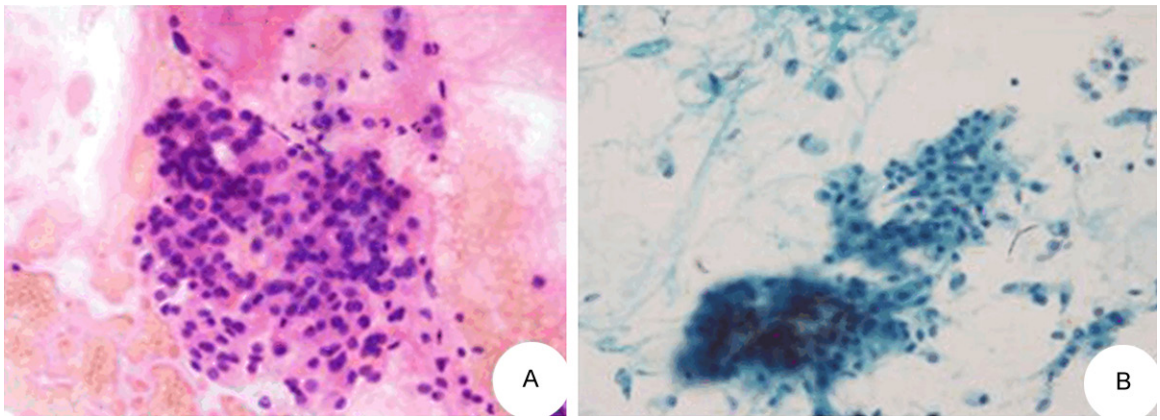

Figure 4. Mucoepidermoid carcinoma. CS (A) and SP (B) from the same patient. Squamous cells, mucous cells and intermediate cells could be found in the mucoid background; the detail cellular structures of SP were clearer than CS (Papanicolaou stain, ×400).

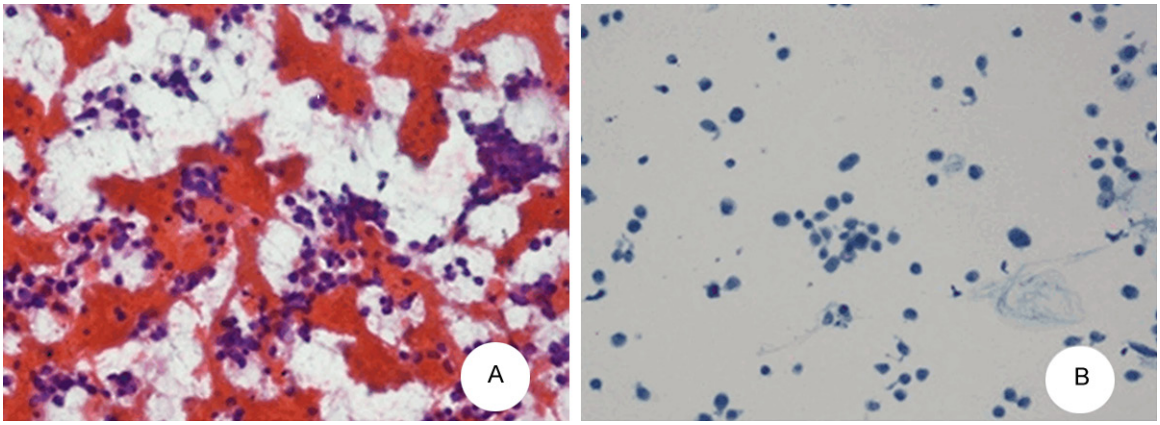

Figure 5. Small cell carcinoma. CS (A) and SP (B) from the same patient. The tumor cells showed nuclear molding and finely granular chromatin; the background of SP was cleaner than CS and the tumor cells showed degenerative changes in CS (Papanicolaou stain, ×400).

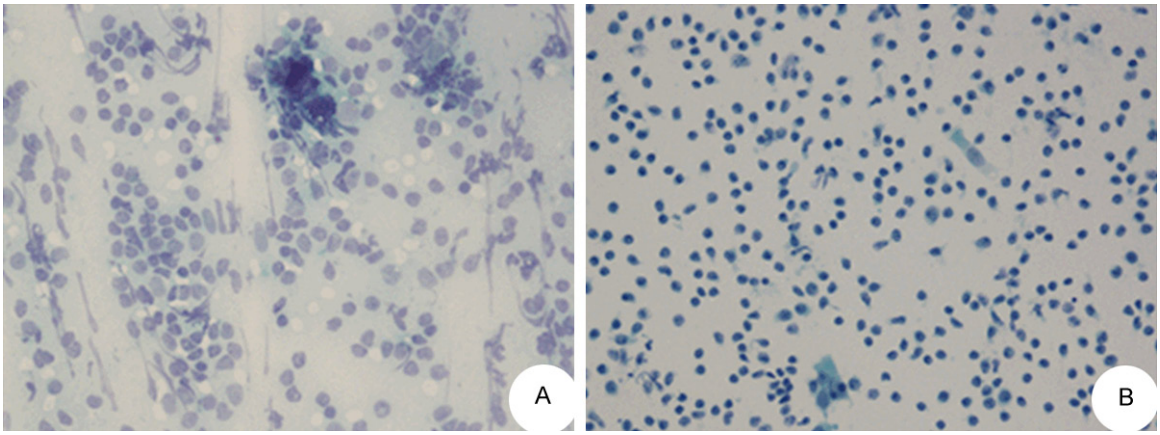

Figure 6. MALT lymphoma. Scattered immature lymphocytes presented in CS (A) and SP (B) of the same patient. The tumor cells in CS obviously degenerated (Papanicolaou stain, ×400).

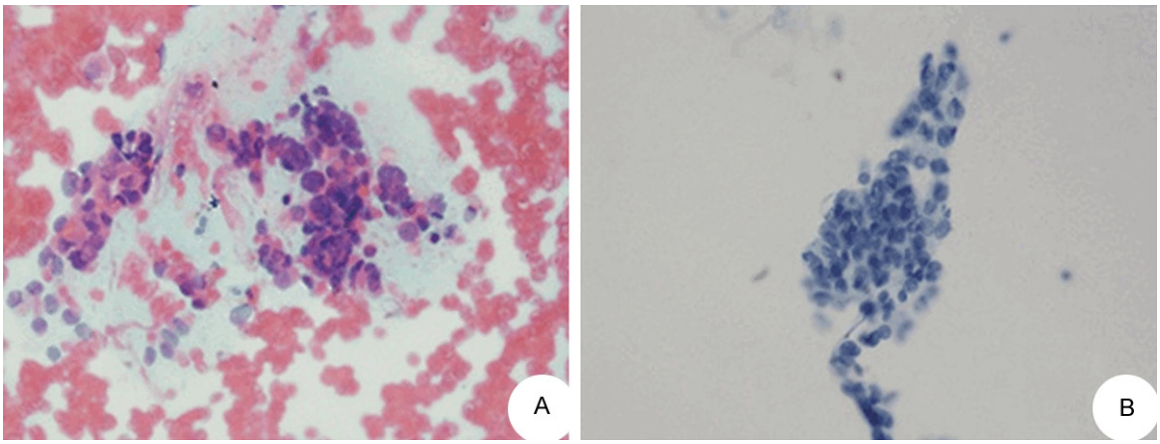

Figure 7. Alveolar rhabdomyosarcoma. Oval tumor cells with large and hyperchromatic nuclei presented in both CS (A) and SP (B) of the same patient; there were less red blood cells in the background of SP (Papanicolaou stain, ×400).

Table 1. Comparison of CS and SP in the diagnosis of pulmonary malignant tumors

Histological diagnosis	N	CS		SP		CS+SP	
		Positive	Sensitivity	Positive	Sensitivity	Positive	Sensitivity
Squamous carcinoma	264	230	87.1%	225	85.2%*	238	90.2%*
Adenocarcinoma	197	161	81.7%	167	84.8%*	169	85.8%*
Small cell carcinoma	130	121	93.1%	124	95.4%*	125	96.2%*
Other NSCLC	39	33	84.6%	35	89.7%*	37	94.9%*
NHL	3	2	66.7%	1	33.3%	2	66.7%
Secondary	12	12	100%	11	91.7%	12	100%
Total	645	559	86.7%	563	87.3%	583	90.4%

*P>0.05, when compared with CS. CS = Conventional smear; SP = Surepath liquid-based preparation; NSCLC = Non-Small Cell Lung Carcinoma; NHL = Non-Hodgkin Lymphoma; Secondary = Secondary Malignancy; N = Number.

Conventional bronchial brushing smears

After routine fiberoptic bronchoscopy, the suspected lesion was brushed and smeared. Usually two smears were prepared for each case. Then the smears were fixed in 95% alcohol immediately for 15 to 30 minutes, and performed Papanicolaou stain.

Surepath liquid-based cytology

After making direct smears, the brush was put into a vial containing SurePath™ preservative fluid (BD), then washed for 10 times and removed. Let the solution stand for at least 15 minutes in room temperature and then transfer it to a 12 ml centrifugal tube. After adding 4 ml density gradient solution, the sample were centrifuge for 5 minutes (600 g), the supernatant was discarded, and the cell aggregates at the bottom of centrifugal tube was mixed. The sediments were transferred to the AutoCyte PREP system, in which slides were automatically prepared and stained. One slide was prepared for each case and then performed Papanicolaou stain.

Criteria for diagnosis

The result was taken as positive if tumor cells or atypical cells were detected in CS or SP; otherwise, the result was negative. The final diagnosis was confirmed by bronchial biopsy and the classifications of tumors were according to the WHO classifications of lung.

Statistical analysis

χ^2 test was performed using SPSS 13.0 software package for data analysis. $P<0.05$ was defined as statistical significance.

Results

For the 872 patients suspected pulmonary malignant tumors clinically, 645 cases were

Table 2. The comparison of CS and SP for diagnosing secondarypulmonary malignant tumors

Primary site	Biopsy	CS	SP
Colon	MT adeno	Sus of adeno	Atypia
Colon	MT adeno	Sus of adeno	Atypia
Colon	MT adeno	Sus of adeno	Atypia
Colon	MT adeno	Sus of adeno	Atypia
Colon	MT adeno	Adeno	Adeno
Colon	MT adeno	Adeno	Adeno
Colon	MT adeno	NSCC	NSCC
Kidney	MT RCC	NSCC	NSCC
Breast	MT IDC	Adeno	Adeno
Ovary	MT adeno	MT adeno	MT adeno
Mediastinum	DLBCL	Atypia	Neg
Groin	MT Rhabdo	Malignant tumor	Malignant tumor

CS = Conventional smear; SP = Surepath liquid-based preparation; MT = metastatic; Adeno = adenocarcinom; Sus of adeno = suspicious of adenocarcinom; NSCC = Non-Small Cell Carcinoma; Neg = negative for malignancy; RCC = renal cell carcinoma; IDC = invasive ductal carcinoma; DLBCL = diffuse large B-cell lymphoma; Rhabdo = Rhabdomyosarcoma.

Table 3. The comparison of cytology and forceps biopsy of fiberoptic bronchoscopy for diagnosing lung tumors

Biopsy	CS	SP	Final diagnosis	Method
Neg	Suspious	Suspious	SCLC	Repeat
Neg	Neg	Suspious	DLBCL	Surgical
Suspious	NSCLC	NSCLC	SCC	Repeat
Neg	Suspious	Suspious	Harmatoma	Surgical
Neg	Suspious	Suspious	Adeno	Surgical
Neg	Suspious	Neg	Adeno	Surgical

CS = Conventional smear; SP = Surepath liquid-based preparation; NSCLC = Non-Small Cell Lung Carcinoma; DLBCL = diffuse large B-cell lymphoma; Adeno = adenocarcinom; Suspious = suspicious of tumors; Neg = negative for malignancy; SCC = Squamous cell carcinoma; Repeat = repeat bronchial biopsy; Surgical = surgical pathology diagnosis.

confirmed as malignant tumors by fiberoptic bronchoscopic biopsy. There were 500 cases of non-small cell carcinoma (NSCLC, 77.5%, 500/645) which include 264 cases of squamous cell carcinoma (**Figure 1**), 197 cases of adenocarcinoma (**Figure 2**), 3 cases of adenosquamous carcinoma, 3 cases of adenocystic carcinoma (**Figure 3**), 1 of mucoepidermoid carcinoma (**Figure 4**), 1 of lymphoepithelioma-like carcinoma and 31 cases of unspecified non-small cell carcinoma. In addition to NSCLC, the primary malignant lung tumors in this study also included 130 cases of small cell lung carcinoma (SCLC, 20.2%, 130/645) (**Figure 5**), 3 cases of primary non-Hodgkin's lymphoma (2 extramedullary plasmacytomas and 1 extranodal marginal zone lymphoma of mucosa associated lymphoid tissue, MALT lymphoma, 0.5%, 3/645) (**Figure 6**). Besides primary malignant lung tumors, there were 12 cases of secondary malignancy (0.19%, 12/645) including 7 metastatic adenocacinomas from colon, each 1 metastatic carcinoma from breast, kidney and ovary, 1 metastatic alveolar rhabdomyosarcoma from groin (**Figure 7**), and 1 diffuse large B-cell lymphoma from mediastinum.

For the 645 malignant lung tumors confirmed by bronchial biopsy, 559 cases were detected by CS and 563 cases were showed positive by SP, the sensitivities of CS and SP were 86.7% (559/645) and 87.3% (563/645), respectively. If these two methods were combined, the sensitivity could reach 90.4% (583/645). For the 633 of primary malignant tumor, 547 cases were positive by CS, the sensitivity was 86.4% (547/633), 552 cases were positive by SP, the sensitivity was 87.2% (552/633), the sensitivity of CS combined with SP in the diagnosis of primary malignant tumors reached 90.2% (571/633). For the 12 cases of secondary malignancies, 12 patients were positive by CS, the sensitivity was 100% (12/12), 11 patients were positive by SP, the sensitivity was 91.7% (11/12), the sensitivity of CS combined with SP in the diagnosis of secondary malignancies was also 100% (12/12). The comparison of CS and SP in diagnosis of pulmonary malignant tumors was shown in **Table 1**. The sensitivity of SP was higher than CS in diagnosis of adenocacinomas, small cell carcinomas and unspecified NSCLCs while the sensitivity of CS was higher than SP in diagnosis of squamous carcinomas, non-Hodgkin's lymphomas (NHL) and secondary malignancies. If all the malignant tumors were added together, the SP showed better sensitivity than CS. However, it couldn't conclude that the sensitivities of SP and CS had statistically significant difference ($P>0.05$).

For the 12 secondary malignant tumors, only 1 metastatic ovary adenocarcinoma was diag-

nosed by both CS and SP because of the available clinical information. 3 metastatic adenocarcinomas were diagnosed as adenocarcinomas by both CS and SP without conforming the tumor origination; each 1 metastatic colon adenocarcinoma and renal cell carcinoma was diagnosed as non-small cell carcinoma by both CS and SP, 1 metastatic rhabdosarcoma from groin was diagnosed as malignant tumor by both CS and SP without conforming the tumor type. 4 metastatic adenocarcinomas were suspicious of adenocarcinomas by CS while only atypical cells were detected by SP, 1 diffuse large B-cell lymphoma from mediastinum was diagnosed as atypia by CS while it was negative by SP.

The comparison of bronchial brushing cytology smears and Surepath liquid-based preparation for diagnosing secondary malignant tumors was listed in **Table 2**.

In this study, 44 cases were suspected of malignant tumors and 183 cases were negative for malignancy by the forceps biopsy though fiberoptic bronchoscopy. When we followed these patients, we found 6 patients were diagnosed as tumors by repeat fiberoptic bronchoscopic biopsy or using surgical specimen. The cytological diagnoses of these patients were all positive or suspicious of tumors. The detail information of these patients was listed in **Table 3**.

Discussions

The bronchial brushing cytology is an effective complementary examination of bronchial biopsy for detecting primary and secondary pulmonary malignant tumors [7]. However, the excessive mucus, red blood cells or inflammatory cells in the conventional bronchial brushing cytological smears might cover abnormal cells. Besides these, the dry artifacts of conventional smears due to delayed fixation are very common. Liquid-based preparation has been applied to the cytological diagnosis since 1990s, which ThinPrep and AutoCyte Prep Cytology Test (also known as Surepath Cytology Test) are most widely used and approved by FDA. The basic principles of Surepath technology discussed in this study are gradient centrifuge and spontaneously cell sedimentation. It is one of the best liquid-based preparations.

Our study showed the sensitivity of Surepath preparation was higher than conventional bronchial brushing smears in diagnosis of adenocarcinoma, small cell carcinoma and unspecified NSCLC while the sensitivity of CS was higher than SP in diagnosis of squamous cell carcinoma, NHL and secondary malignancy. The sensitivities of CS and SP in the diagnosis of all pulmonary malignant tumors were 86.7% and 87.3% respectively; if these two methods were combined, the sensitivity could reach 90.4%. For primary malignant tumors, the sensitivity of CS and SP were 86.4% and 87.2% respectively; the sensitivity of SP combined with CS reached 90.2%. For the 12 cases of secondary malignancies, only 1 case was missed by SP, all patients were diagnosed or suspected as malignant tumors by CS. In our study, 5 cases which the original fiberoptic bronchoscopic biopsies were negative were diagnosed as various tumors by repeat bronchial biopsy or using surgical specimen. For these cases, the cytological diagnoses were all suspicious of tumors by CS or SP. 1 patient was confirmed as squamous cell carcinoma by repeat bronchial biopsy, which the original forceps biopsy was suspicious of malignancy while the original cytological diagnosis was NSCLC. We can see the combination of SP, CS and forceps biopsy will improve the diagnostic value of fiberoptic bronchoscopy for the pulmonary tumors.

In comparison with conventional bronchial brushing smear, liquid-based cytology has the following advantages: (1) Samples are transferred to special preservative solution immediately after brushing and making direct smears. As a result, almost all samples are retained, and the dry artifacts are avoided [8]. (2) Mucus, blood and inflammatory cells are almost separated from epithelial cells or tumor cells after processing which can make the abnormal cells easy to be detected. (3) The slides are easier to review due to smaller screen area because the cells of Surepath technology are concentrated on an area 13 mm in diameter, which can reduce the screening time, and may avoid false negative diagnosis. (4) The automated operation of Surepath technology makes it possible to process a batch of samples together, and several slides for special staining or immunocytochemical staining could be prepared as needed [9]. It will be helpful to determine the types of primary malignant tumors and the origination of the secondary malignant tumors if liquid-based preparation was combined with immunocytochemical tests, which can make the cytological diagnosis more accurate.

The studies which compared conventional smears with liquid-based preparation in respiratory cytology had different results. Higher diagnostic accuracy for LBC was reported by some authors [5, 6, 10-12], whereas other studies showed no statistical difference [9, 13, 14]. Most studies of LBC about pulmonary cytology were performed with ThinPrep [10, 12, 15], while much fewer researches used SurePath [16]. Moreover, most of these studies only involved bronchogenic carcinomas such as adenocarcinomas, squamous cell carcinomas and small cell carcinomas. Our present study not only discussed these three types bronchogenic carcinoma but also included primary adenosquamous carcinomas, adenocystic carcinomas, mucoepidermoid carcinoma and lymphoepithelioma-like carcinoma. 3 cases of primary non-Hodgkin's lymphoma were also involved. Besides the primary malignant lung tumors, there were 12 cases of secondary malignancies such as metastatic carcinomas and sarcoma. As far as I know, the tumor types in this study are the most abundant compared with other researches which discussed the diagnostic accuracy of SP and CS for pulmonary malignancies.

In conclusion, liquid-based cytology, such as Surepath cytology test should be applied to the cytological diagnosis of pulmonary malignant tumors. Bronchial brushing cytology is an effective complementary examination of bronchial biopsy. The combination of conventional bronchial brushing smears and Surepath liquid-based cytology can improve the diagnosis value of fiberoptic bronchoscopy for pulmonary primary and secondary malignant tumors.

Disclosure of conflict of interest

None.

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