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ORIGINAL ARTICLE

Diagnostic flex-rigid pleuroscopic biopsy of parietal pleura for exudative pleural effusions in suspected malignant and tuberculosis cases: a retrospective study of 219 cases

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Abstract

Objectives: This study was carried out to ascertain the aetiology of exudative pleural effusions when other diagnostic investigations such as pleural fluid and sputum examination for cytology and acid fast bacilli fail to yield a definitive diagnosis and to differentiate between tuberculosis and malignancy in cases suspicious of malignancy. *Methods:* Pleuroscopic biopsies were obtained in 219 cases by Chest Physicians in the endoscopy suite using flexi-rigid fiber-optic pleuroscopes. Histological sections were stained with H&E and microscopic examination performed. Ziehl-Nielsen stain for acid fast bacilli was performed in all suspected tuberculosis cases and immunohistochemistry for Thyroid transcription factor 1 and other markers were carried out for all cases suspicious of malignancy. *Results:* Adequate biopsy material for interpretation was obtained in 210 (95.9%) of 219 cases. Histopathology revealed 79 (37.6%) cases were tuberculosis, 64 (30.5%) were malignant (primary from lung and other sites), 62 (29.5%) were non-specific inflammation and 5 (2.4%) were empyema. A definitive diagnosis of tuberculosis, malignancy and empyema was obtained in 70.5% of cases. Tuberculosis was encountered in a younger age-group than malignancy. Mean age for tuberculosis patient was 49 years while for malignant patients was 63 years. The majority (79.6%) of malignances encountered were metastatic lung adenocarcinoma.

Keywords: flex-rigid pleuroscopy, tuberculous pleural effusion, malignant pleural effusion, pleural biopsy, exudative pleural effusion

INTRODUCTION

Pleuroscopy is a visualization of the pleura and contents of the pleural cavity using an endoscope. This procedure provides the physician a window into the pleural space to perform biopsy of the parietal pleura under visual guidance, particularly in cases of exudative effusions of unknown origin. During the last decade, advances in video technology and improved endoscopic instrumentation have prompted a resurgence of interest in minimally invasive chest procedures among interventional pulmonologists.[1] Pleuroscopy is a safe, well-tolerated procedure with minimal discomfort and risk. The present communication is to elaborate on the importance of pleuroscopic biopsy when routine pleural fluid and sputum examination for cytology and acid fast bacilli (AFB) and other investigations fail to differentiate between tuberculosis and malignancy especially in the places like Sabah, Malaysia, where the prevalence of tuberculosis (TB) is very high compared to other states.[2] The study was carried out to ascertain the aetiology of exudative pleural effusion and to differentiate between tuberculosis and malignancy in cases clinically suspicious of malignancy. The study also compares sample adequacy and different histopathological diagnosis encountered in various studies published by other authors.

MATERIALS AND METHODS

This retrospective study was carried out on pleuroscopic biopsy samples obtained from 1st January 2010 to 31st December 2011. Pleural biopsy was performed on patients with undiagnosed exudative pleural effusion with a non-diagnostic cytology and a clinical suspicion of tuberculosis or malignancy. Patients with

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lack of pleural space due to severe and dense pleural adhesions, severe cardiac or respiratory insufficiency and coagulation disorders were excluded. Each patient was explained about the procedure of pleural biopsy and written informed consent was taken before carrying out the procedure.

Pleuroscopic biopsies of parietal pleura were obtained by respiratory physicians in the endoscopic suite with patients under conscious sedation and local anesthesia using flexi-rigid fiber-optic pleuroscope. The procedure was performed with minimal patient discomfort and no serious complications were encountered.

Tissues obtained were placed in 10% formalin and were then processed into Haematoxylin and Eosin (H&E) stain slides. Ziehl-Nielsen (ZN) stain was performed in all suspected tuberculosis cases. Transcription Factor 1 (TTF1) stain and other immunohistochemistry (IHC) markers were carried out in all cases suspicious of malignancy.

RESULTS

Two hundred and nineteen (219) cases were biopsied in the study period. Based on histological evaluation of the H&E stained slides, adequate biopsy material for interpretation was obtained in 210 cases (95.9%) of the 219 cases.

Pathological diagnosis

Histological examination revealed 79 (37.6%) cases of tuberculosis. 64 (30.5%) biopsies revealed malignancy (primary and secondary). 62 (29.5%) were non-specific inflammation and 5 (2.4%) were empyema.

Tuberculosis

Of the 79 tuberculosis cases, 45 were males and 34 were females. The male:female ratio was 1.32:1. The youngest patient was 12-years-old and oldest patient was 86-years-old, with a mean age of 49 years. The maximum age incidence of tuberculosis was in the 3rd, 4th & 5th decade of life.

Typical caseating granulomas were detected in 69 cases and non-caseating granulomas seen in 10 of 79 cases diagnosed as tuberculosis on histopathological examination. ZN stain for AFB was positive in only 2 of the 79 cases. Sensitivity for detection of AFB by ZN stain was found to be very low (2.5%). In 10 patients with non caseating granulomas, additional PAS/GMS stains were done to rule out fungal infections.

Malignancy

Of the 64 cases diagnosed as malignancy, 35 were males and 29 were females. The male:female ratio was 1.2:1. The youngest patient was 30-years-old and the oldest patient was 87-years-old. The mean age was 63 years. The maximum age incidence of malignancy was in the 5th, 6th &7th decade of life. Malignancy was found to be more common in older age-groups as compared to tuberculosis cases (Figure 1).

Table 1 shows histopathological type of malignancy encountered in these 64 cases. 63 (98%) were metastasis to the pleura and one was a primary malignancy of the pleura. Lung adenocarcinoma (51 cases) was the most common malignancy metastasized to pleura. The biopsies typically revealed malignant tumour cells forming tubules, papillae or sheets surrounded by desmoplastic stroma. Tumour cells were pleomorphic having enlarged hyperchromatic, prominent nucleoli and mitosis. All expressed TTF-1 on immunohistochemistry (IHC) (Figure 2).

Metastatic squamous cell carcinoma (6 cases) comprised of malignant cells displaying squamous differentiation in the form of keratinized cytoplasm, pearl formation and intercellular bridges. IHC revealed the tumour cells to express cytokeratin 5/6 (Figure 3).

Metastatic small cell carcinoma (3 cases) comprised of tumour cells arranged in nests, trabeculae or diffuse sheet pattern. Tumour cells were small round or oval with round or oval nuclei, scanty cytoplasm, indistinct cell borders, nuclear molding and inconspicuous nucleoli. Areas of necrosis and frequent mitosis were seen. The tumour cells expressed TTF1, chromogranin and/or synaptophysin (Figure 4).

Metastatic carcinoma from breast (1 case) showed malignant tumour cells forming sheets and tubules surrounded by desmoplastic stroma. The tumour cells were TTF1 negative but expressed estrogen receptor protein (Figure 5).

The one case of metastasis from a colonic adenocarcinoma showed malignant tumour cells forming glands, surrounded by desmoplastic stroma. The tumour cells were TTF1 negative but expressed cytokeratin 20.

Metastatic fibrous histiocytoma (1 case) showed malignant spindle-shaped tumour cells arranged in storiform pattern. Tumour cells were large, pleomorphic with enlarged hyperchromatic nuclei and prominent nucleoli. Bizarre tumour giant cells and abundant mitotic figures including atypical forms were seen. The tumour cells expressed vimentin and CD68.

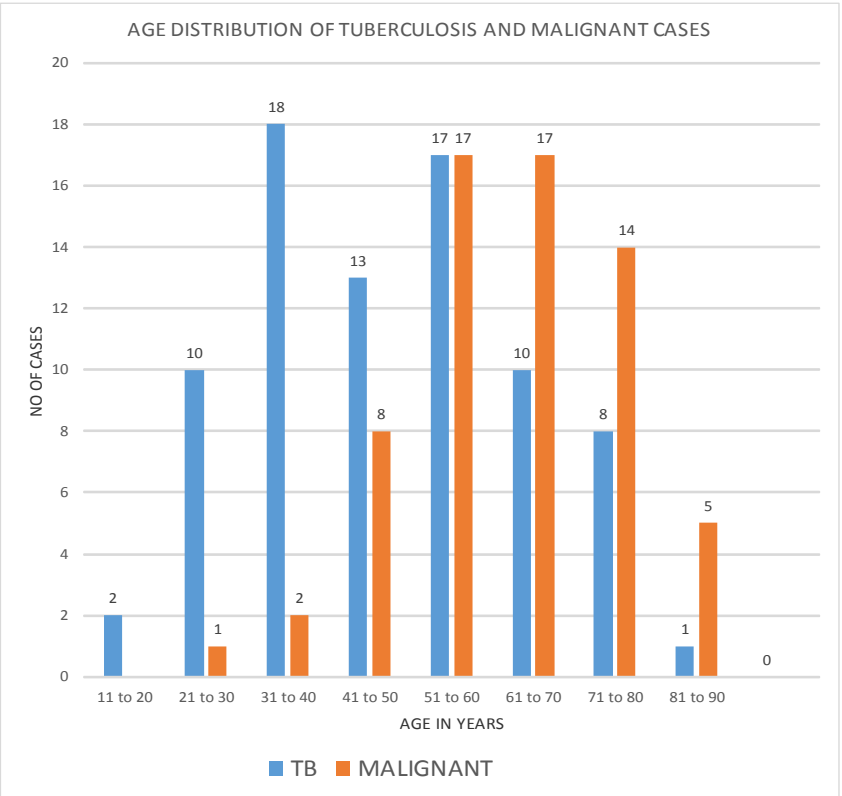

FIG. 1: Age distribution of tuberculosis and malignancy cases

The case of malignant mesothelioma showed a biphasic pattern with both epithelioid and sarcomatous components comprising of malignant round and spindle-shaped cells. The epithelial component exhibited round cells arranged in tubulopapillary arrangement and sarcomatous component showed spindle cells arranged in short fascicles. The tumour cells expressed Pan Keratin AE1/AE3, vimentin, CK5/6, calretinin and were negative for TTF1.

TABLE 1: Histological types of malignancy encountered

Type of malignancy	No. of cases	Immunohistochemical profile
Metastatic adenocarcinoma from lung	51	TTF1 (+)
Metastatic squamous cell carcinoma from lung	6	CK 5/+6 (+)
Metastatic small cell carcinoma from lung	3	TTF1(+), synaptophysin(+) & or chromogranin (+)
Metastatic carcinoma from breast and GIT (sites other than lung)	2	Breast : TTF1 (-) & ER (+) GIT: TTF1(-), CK20(+)
Metastatic malignant fibrous histiocytoma (MFH) from lung	1	Vimentin (+), CD68 (+)
Malignant mesothelioma (primary from pleura)	1	Pan keratin AE1/AE3(+), vimentin(+), CK5/6(+), calretinin (+), TTF1(-)
Total	64	

Key: thyroid transcription factor 1(TTF1), cytokeratin 5/6 (CK5/6), oestrogen receptor (ER), gastrointestinal tract (GIT), cytokeratin 20 (CK 20).

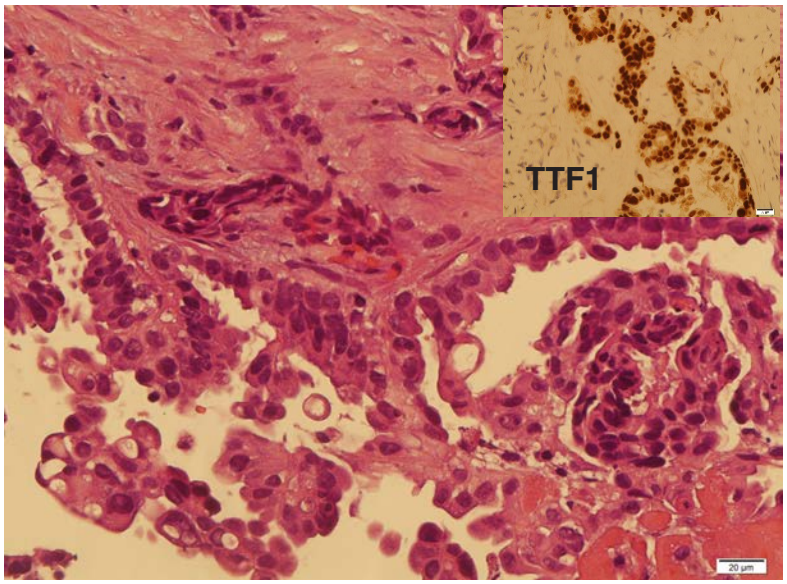

FIG. 2: Metastatic adenocarcinoma from lung. Malignant cells form glands infiltrating into the fibrous stroma. H&E stain X400. Inset shows positive nuclear staining in tumour cells for Thyroid Transcriptor Factor 1 (TTF1). IHC stain

We were able to achieve a definitive diagnosis for TB, malignancy and empyema in 70.5% of on cases on pleuroscopic biopsy. In the remaining 29.5% of cases, the findings were non-specific. This group of non-specific pleuritis needed additional pathological tests to know the exact aetiology of pleural effusion.

DISCUSSION

In our study, 95.9% samples were adequate. Only 4.1% of samples obtained insufficient or non-representative material and required repeat sampling. In various published series adequate material was obtained in 93-100% of cases and

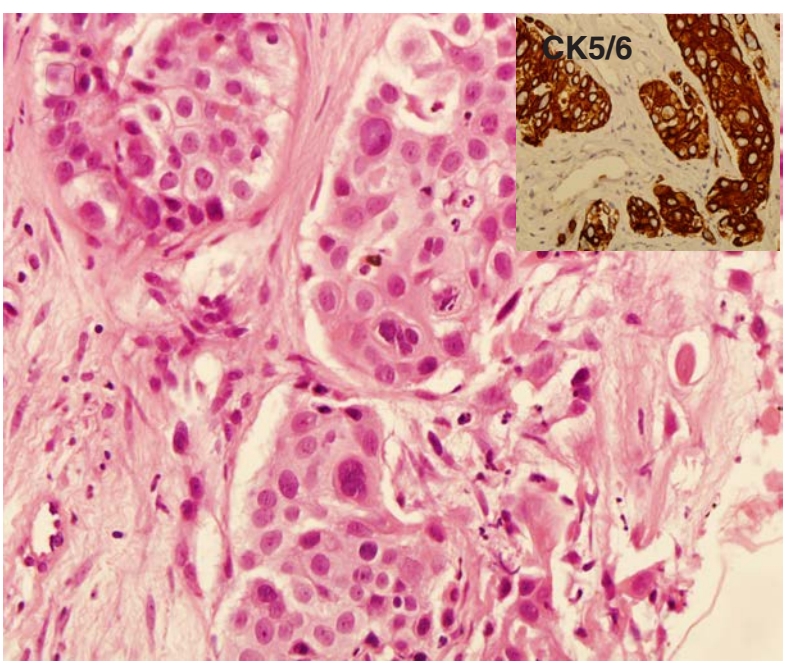

FIG. 3: Metastatic squamous cell carcinoma from lung: Malignant large round cells in sheets with keratinized cytoplasm surrounded by desmoplastic stroma. H& E Stain X400. Inset shows positive cytoplasmic staining in tumour cells for CK5/6. IHC Stain

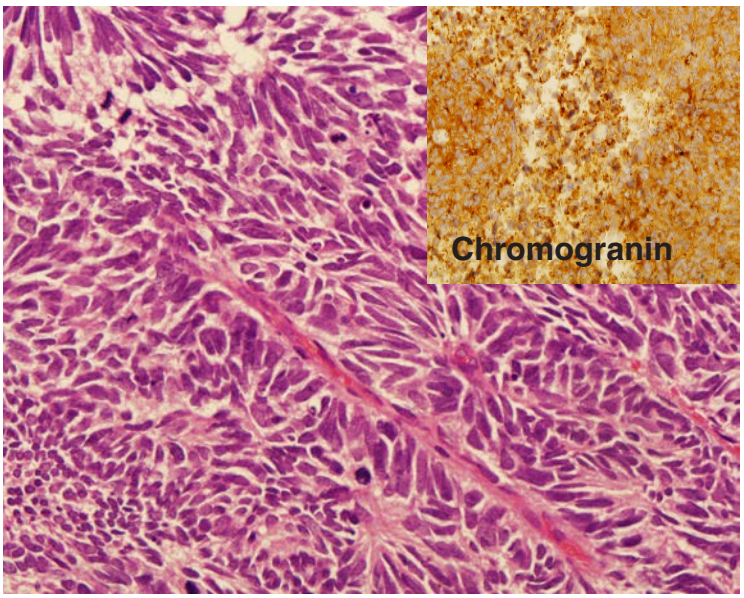

FIG. 4: Metastatic small cell carcinoma from lung. Malignant small round cells, with enlarged hyperchromatic nuclei, inconspicuous nucleoli, having scanty cytoplasm and arranged in diffuse pattern. H&E stain X400. Inset shows positive cytoplasmic staining for chromogranin in tumour cells. IHC stain

our findings were concordant with the findings of other workers (Table 2).

Table 3 shows histopathological diagnosis encountered in various published series. In the present study, tuberculosis was the most common diagnosis followed by malignancy -and non-specific pleuritis. Tuberculosis was more common in a younger age-group (3rd, 4th and 5th decade) than malignancy. Tuberculosis comprised of 37.6% of diagnoses. In various published reports, tuberculosis was diagnosed in 22.8% to 70.6% of biopsies (Table 3). The ZN stain for AFB was positive in only 2 of our 79 cases of tuberculosis. Tan *et al*[3] reported 3 out of 48 cases, Sakuraba *et al*[4] reported 4 out of 32 cases and Mootha *et al*[9] reported 1 in 35 cases positive for AFB on ZN stain. In our experience and those of other workers, TB bacilli

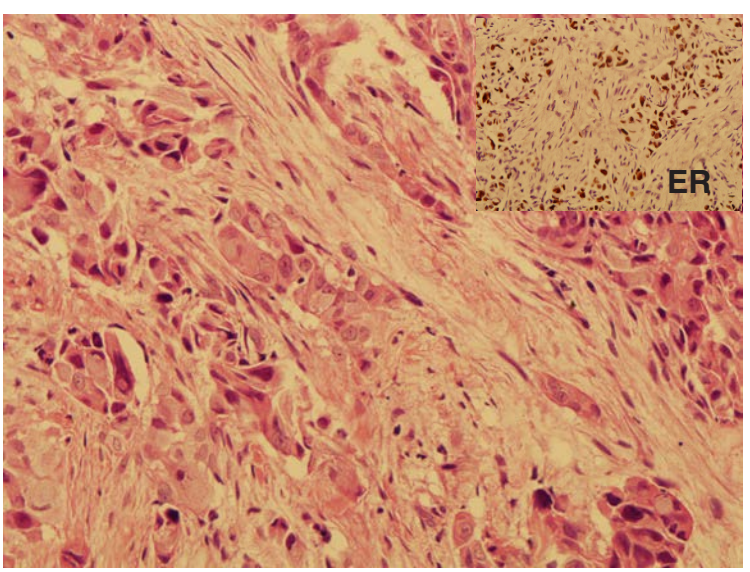

FIG. 5: Metastatic carcinoma from breast. Malignant tumour cells in short cords and forming tubules surrounded by desmoplastic stroma. H & E stain X400. Inset shows positive nuclear staining of tumour cells for ER. IHC stain

Table 2: Percentage of adequate biopsies obtained by various published series

Author	No. of samples	No. of adequate samples	% of adequate biopsies
Tan KK *et al*[3] (1968)*	72	68	95.95%
Sakuraba M *et al*[4] (2006)	32	30	93.8%
Tong ZH *et al*[5] (2007)	60	57	95%
Wang Z *et al*[6] (2008)	27	25	93%
Huang GH *et al*[7] (2011)	47	44	93.6%
Gao BA *et al*[8] (2014)**	215	215	100%**
Present series	219	210	95.9%

*Biopsy material obtained by Harefield needle.[3].** Gao BA *et al*[8] reported unknown aetiology in 25 of 215 cases which we presume was based on adequate material.

are not easily identified from pleural biopsy specimens. We found the sensitivity of the ZN stain for detection of AFB was found to be very low (2.5%). Other published reports show a sensitivity ranging from 2.85% to 12.5%.

Typical caseating granulomas were seen in 69 cases and non-caseating granulomas were seen in 10 cases of our 79 TB cases. In the 10 patients with non-caseating granulomas, additional PAS / GMS stains were done to rule out fungal infections. The physicians excluded sarcoidosis, fungal infections and other granulomatous diseases before starting anti-tuberculosis treatment and patients were followed up for response to therapy. If additional pleural biopsy sample is obtained for polymerase chain reaction (PCR) and TB culture, it can further help to confirm a diagnosis of TB in all these TB cases reported by histopathological examination.[4]

The malignancy group comprised of 30.5% of cases. In various published data, malignancy ranged from 22-60% (Table 3). Immunohistochemical markers were used to identify the type of malignancy. Metastasis was the most common malignancy encountered in the pleura, with the majority being lung adenocarcinoma. Malignant mesothelioma was very rare in the present study.

Non-specific pleuritis comprised of 29.5% cases, comprising of mostly para pneumonic effusions. Infections, collagen vascular diseases, pulmonary infarct, drug reactions etc. needed to be ruled out in these 29.5% of cases. A proper clinicopathological correlation with detail clinical history, signs and symptoms, X-ray and

TABLE 3: Histopathological diagnosis obtained in various published series

Author	No. of cases	Tuberculosis No. (%)	Malignancy* No. (%)	Other diagnosis** No. (%)
Gao BA *et al*[8] (2014)	215	91 (42.3%)	97 (45.1%)	27 (12.6%)
Huang GH *et al*[7] (2011)	47	17 (36.2%)	21 (44.7%)	09 (19.1%)
Mootha VK *et al*[9] (2011)	35	08 (22.9%)	17 (48.6%)	10 (28.6%)
Wang Z *et al*[6] (2008)	25	06 (24.0%)	15 (60.0%)	04 (16.0%)
Tong ZH *et al*[5] (2007)	60	16 (27.0%)	32 (53.0%)	12 (20.0%)
Tan KK *et al*[3] (1968)	68	48 (70.6)	15 (22.0%)	03 (04.4%)
Present Series	210	79 (37.6%)	64 (30.5%)	67 (31.9%)

*Malignancies included primary/metastatic/lymphoma diagnosis. **Other diagnosis included empyema/ non-specific pleuritis/ idiopathic or unknown and schistosomiasis etc.

CT chest diagnosis, blood counts, Adenosine deaminase activity (ADA) test, serological and culture investigations for bacterial, viral and fungal infections, antinuclear antibody (ANA) and double stranded DNA (ds DNA) tests for collagen vascular disorders can yield definitive diagnoses in this group. This will further improve diagnosis and reduce the number of cases with non-specific pleuritis diagnosis.

Empyema comprised of only 2.4% of cases. Various published reports included empyema in other diagnosis category and number of cases encountered was too few for study comparison.

In the present study definitive diagnosis for TB, malignancy and empyema was obtained in 70.5% of cases. Gao *et al*[8] obtained definitive diagnosis in 88.4% of cases. However they combined histopathology and microbiology for the final diagnosis in tuberculosis patients and 3 patients with atypical malignant cells were considered as malignant while calculating the diagnostic rate for pleural biopsy examination. Sakuraba *et al*[4] reported 93.6% sensitivity in TB and 92.3% sensitivity in carcinoma for thoracoscopic pleural biopsy. They also combined pleural biopsy examination and other pathological examination while working out diagnostic sensitivity and specificity of pleural biopsy examination. Agarwal *et al*[10] reported 91% sensitivity and 100% specificity in a meta-analysis of 755 patients with undiagnosed exudative pleural effusion from 17 published studies.

Conclusion: Flex-rigid pleuroscopic biopsies provided a definitive diagnosis in 70.5% cases of exudative pleural effusion of unknown origin where other less invasive procedures like pleural fluid cytology and sputum examination were inconclusive. Pleuroscopic biopsy of parietal pleura was found to be of value in differentiating between tuberculosis and malignancy involving the pleura especially in cases where the clinical findings were suspicious for malignancy. If additional a pleural biopsy sample is obtained for PCR and TB culture, it may confirm the diagnosis of TB in all cases reported on histopathological examination as tuberculosis.

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REFERENCES

1. Lee P, Colt HG. State of the art: pleuroscopy. J Thorac Oncol. 2007; 2(7): 663-70.
2. Dony JF, Ahmad J, Khen Thiong Y. Epidemiology of tuberculosis and leprosy, Sabah, Malaysia. Tuberculosis (Edinb). 2004; 84(1-2): 8-18.
3. Tan KK, Hin CC. Histological features in diagnostic pleural biopsy. Singapore Med J. 1968; 9(2): 81-5.
4. Sakuraba M, Masuda K, Hebisawa A, Sagara Y, Komatsu H. Thoracoscopic pleural biopsy for tuberculous pleurisy under local anaesthesia. Ann Thorac Cardiovasc Surg. 2006; 12(4): 245-8.
5. Tong ZH, Wang Z, Xu LL, Wang XJ, Li HJ, Wang C. [The application of flexirigid thoracoscopy in the diagnosis of pleural effusions with unknown aetiology] (Article in Chinese). Zhonghua Jie He He Hu Xi Za Zhi. 2007; 30(7): 533-7.
6. Wang Z, Tong ZH, Li HJ, *et al*. Semi-rigid thoracoscopy for undiagnosed exudative pleural effusions: a comparative study. Chin Med J (Engl). 2008; 121(15): 1384-9.
7. Huang GH, Cheng YX, Su J, *et al*. [Application of flexirigid thoracoscopy in the diagnosis of pleural disease with unknown aetiology] (Article in Chinese). Nang Fang Yi Ke Da Xue Xue Bao. 2011; 31(4): 669-73.
8. Gao BA, Zhou G, Guan L, Zhang LY, Xiang GM. Effectiveness and safety of diagnostic flexi-rigid thoracoscopy in differentiating exudative pleural effusion of unknown aetiology: a retrospective study of 215 patients. J Thorac Dis. 2014; 6(5): 438-43.
9. Mootha VK, Agarwal R, Singh N, Aggarwal AN, Gupta D, Jindal SK. Medical thoracoscopy for undiagnosed pleural effusions: experience from a tertiary care hospital in north India. Indian J Chest Dis Allied Sci. 2011; 53(1): 21-4.
10. Agarwal R, Aggarwal AN, Gupta D. Diagnostic accuracy and safety of semirigid thoracoscopy in exudative pleural effusions: a meta-analysis. Chest. 2013; 144(6): 1857-67.