Clinical value of cytology in pleural effusions versus histology by medical thoracoscopy

Utilità della citologia nel versamento pleurico: confronto con l'istologia ottenuta con toracoscopia medica

Pier Aldo Canessa *(foto)*
Carmen Manta
Massimiliano Sivori
Donatella Intersimone*
Franco Fedeli*

SC Pneumologia, Ospedale San Bartolomeo, Sarzana (SP);
** Anatomia Patologica, Ospedale Sant'Andrea, La Spezia*

Summary

Background. Pleural effusions are frequently the presenting symptom of neoplastic disease. The cytological examination of pleural effusion is recognized as being the most commonly used investigation in the diagnosis of malignancy.
Objectives. The aim of this study was to assess the sensitivity and specificity of cytology in pleural exudate effusions compared to the histology obtained by medical thoracoscopy as the diagnostic gold standard examination.
Methods. We assessed 256 consecutive thoracoscopies performed between 2006 and 2010 in the Pneumology ward of the Sarzana Hospital (Italy). Pleural disease was diagnosed based on histological criteria.
Results. We had 80 mesotheliomas, 52 pleural metastasis and 124 non malignant pleural diseases. Cytologic examination permitted the diagnosis of 35 mesotheliomas (28 epithelioid, 2 sarcomatoid, 2 desmoplastic, 3 biphasic), 37 pleural metastasis (21 lung, 8 breast, 1 lymphoma, 3 ovary, 1 stomach, 1 liver, 2 uterus). Cytology remained negative in 45 mesotheliomas (28 epithelioid, 11 sarcomatoid, 2 desmoplastic, 4 biphasic) and in 15 pleural metastasis (6 lung, 4 breast, 3 lymphoma, 1 sarcoma, 1 thyme). The sensitivity of cytology was 53.8% and the specificity was 97.6%; the sensitivity of pleural metastasis was greater than the sensitivity of malignant mesothelioma (71.2% *vs* 43.7%).
Conclusions. We conclude that sensitivity of first cytology samples in pleural effusion remains about 50% in the immunocytochemical era and that it is too low to avoid a diagnostic thoracoscopy. A negative cytologic examination of pleural effusion does not exclude a diagnostic thoracoscopy; a positive cytology for metastasis could exclude diagnostic thoracoscopy, even though thoracoscopy might be performed for talc poudrage. A positive cytology for mesothelioma requires confirmation by histology obtained by thoracoscopy.

Key words
Cytological examination • Pleural effusion • Mesothelioma • Medical thoracoscopy

Parole chiave
Citologia • Versamento pleurico • Mesotelioma • Toracoscopia medica

Riassunto

Premesse. *I versamenti pleurici sono spesso sintomi di una malattia neoplastica. L'esame citologico del versamento pleurico è considerato la metodica più utilizzata nella diagnosi delle malignità.*
Obiettivi. *Lo scopo di questo studio è quello di valutare la sensibilità e la specificità della citologia nel versamento pleurico di tipo essudatizio paragonato all'istologia ottenuta da toracoscopia medica, ritenuta esame gold standard.*
Materiali e metodi. *Abbiamo valutato la citologia su liquido pleurico (il primo campione) su 256 toracoscopie consecutive eseguite dal 2006 al 2010 presso il reparto di Pneumologia dell'Ospedale di Sarzana (La Spezia).*
Risultati. *I referti istologici hanno permesso le seguenti diagnosi: 80 mesoteliomi, 52 metastasi pleuriche e 124 patologie non maligne. La citologia è risultata positiva in 35 mesoteliomi (28 epitelioidi, 2 sarcomatoidi, 2 desmoplastici e 3 bifasici), in 37 metastasi e in 3 casi in cui la diagnosi istologica era non maligna. La sensibilità della citologia è risultata del 53,8% e la specificità del 97,6%; la sensibilità nelle metastasi è più elevata rispetto ai mesoteliomi (71,2% vs. 43,7%).*
Conclusioni. *La sensibilità del primo esame citologico su liquido pleurico rimane intorno al 50% anche nell'era della immunoistochimica ed è troppo bassa per escludere la toracoscopia. Un esame citologico negativo del liquido pleurico non esclude la toracoscopia diagnostica; un esame citologico positivo per metastasi può escludere la toracoscopia, anche se la toracoscopia potrebbe essere eseguita per effettuare il talcaggio. Un esame citologico positivo per mesotelioma pleurico richiede conferma istologica attraverso il prelievo di campioni bioptici ottenuti durante la toracoscopia.*

Ricevuto il 27-8-2014.
Accettato il 1-12-2014.

Pier Aldo Canessa
SC Pneumologia, Ospedale San Bartolomeo
via Cisa Sud
19038 Sarzana (SP)
pieraldo.canessa@asl5.liguria.it

Introduction

Pleural effusions are a common finding in many pathological conditions including infections, organ failure and malignancies. Epidemiological data estimate there to be 50,000 new cases of malignant pleural effusion per year in the UK: this would translate to one new case per 1,000 population per year [1]. Pleural effusion diagnosis is essential for management and therapy, particularly in malignancy.

To date, a number of tumor markers have been evaluated for their ability to improve the diagnosis of pleural effusions; nevertheless, none of them have proved to be optimal in identifying the appropriate patients that could benefit from their use [2 3].

The cytological examination of pleural effusion is the most commonly used investigation in the diagnosis of malignancy.

The cytological examination of pleural effusion is recognized as being the most commonly used investigation in the diagnosis of malignancy even though malignant effusions can be diagnosed by a single pleural fluid cytology specimen in 60% of cases for carcinomatous effusions [1] and in 51% of cases for mesothelioma [4].

This yield increases only slightly if repeated cytology specimens are analyzed [5].

The aim of this study based on the third phase of the architecture of diagnostic research [6] was to assess the sensitivity and specificity of cytology in pleural exudative effusions compared to the histology obtained by medical thoracoscopy as the diagnostic gold standard examination. We are not aware of any studies with this architecture.

Materials and methods

We assessed 256 consecutive thoracoscopies performed between January 1, 2006 and December 31, 2010 for pleural effusion in the Pneumology Ward of the Sarzana Hospital (Italy). All patients underwent diagnostic pleural drainage through an 8 fr catheter and almost 150 mL [7] of aspirated pleural fluid was sent to the Pathology Laboratory. Medical thoracoscopy was performed in a fully equipped operating theatre under conscious sedation (i.v. propofol) and before to have and know cytologic response. In each patient, a minimum of ten parietal pleural biopsies were taken [3]. Histological specimens, obtained by medical thoracoscopy, were assessed by standard protocols used in the Division of Histopathology and Cytopathology (La Spezia, Italy) [8].

The cytologic specimens were stained using the H&E and Papanicolau methods, after fixation with 95% ethanol. A sample of fluid was cytocentrifuged and then stained with H&E. If the fluid clotted, a cell block was prepared after being fixed and sectioned as a histological sample.

In further detail, one slide of those stained with H&E and Papanicolau, was de-stained and consecutive sections (4-6 *mm* thick) from the cell block were mounted onto positively charged slides (superfrost plus; Menzel, Braunschweig, Germany), de-paraffinised with xylene and re-hydrated through a descending graded series of ethanol.

Immunocytochemical analyses of effusion samples with positive cytology were performed using various primary antibodies, including mesothelium-associated markers, adenocarcinoma-associated markers, and markers that distinguish neoplastic cells from reactive mesothelial cells [9]. The primary antibody-HRP labelled antibody complex was visualized using DAB (ultraView Universal DAB, a multimer-technology based detection system intended for the specific and sensitive detection of mouse and rabbit primary antibodies). This detection system was optimized for use on the NexES IHC and BenchMark Series automated slide stainers.

The presence or absence of malignant cells in the cytologic material was reported as follows:

1) specimens were called cytologically negative if there was no increase in the number of mesothelial cells and no cell atypia;
2) specimens were diagnosed as positive when they showed marked hypercellularity and significant mesothelial cell atypia with enlargement of cells, nuclei and nucleoli.

The positive specimens were re-evaluated by two observers (FF or DI).

The study was performed after obtaining informed consent from the patients and approval of the study protocol from the ethics committee of the ASL5 of La Spezia, Italy.

Results

We had 80 mesotheliomas (56 epithelioid, 13 sarcomatoid, 4 desmoplastic, 7 biphasic), 52 pleural metastasis (27 lung, 12 breast, 4 lymphoma, 3 ovary, 1 stomach, 1 liver, 2 uterus, 1 sarcoma, 1 thymus) and 124 non-malignant pleural diseases (6 mesothelial hyperplasias, 6 tuberculosis, 2 eosinophilic pleurisies, 4 empyemas, 106 chronic pleurisies).

The cytologic examination permitted the diagnostic suspicion of 35 mesotheliomas (final diagnosis: 28 epithelioid, 2 sarcomatoid, 2 desmoplastic, 3 biphasic), 37 pleural metastases (21 lung, 8 breast, 1 lymphoma, 3 ovary, 1 stomach, 1 liver, 2 uterus). The cytology remained negative in 45 mesotheliomas (28 epithelioid, 11 sarcomatoid, 2 desmoplastic, 4 biphasic) and in 15 pleural metastases (6 lung, 4 breast, 3 lymphoma, 1 sarcoma, 1 thymus).

Table I shows the comparison of the cytologic examination results with the histologic results.

The sensitivity of cytology was 53.8% and the specificity was 97.6%; the sensitivity of pleural metastasis is

Table I. Cytologic examination results compared to histologic results.

Histology	Total	Positive cytology (%)
• Mesothelioma	80	35 (43.7)
• Metastasis	52	37 (71.2)
• Non-malignant diseases	124	3 (2.4)
• TOTAL	256	75 (29.2)

greater than the sensitivity of malignant mesothelioma (71.2% *vs* 43.7%).

When the mesotheliomas group is divided into histologic subgroups, the sensitivity of cytology for sarcomatoid mesotheliomas is very low (15.4%), whereas it is 50% for epithelioid mesotheliomas, 50% for desmoplastics and 42.9% for biphasics.

We subjected the three patients who received a positive cytology but a negative histology to a follow-up. One asbestosic patient had a positive cytology for epithelioid mesothelioma and a histologic pattern of atypical hyperplasia with no signs of submucosa infiltration of the parietal pleura: after six months the patient did not present disease evolution. Another patient was suspected of having breast cancer, but upon clinical check-up and follow-up after six months, she was considered a likely false-positive case. The third subject had a carcinoma cytologic diagnosis, but a negative thoracoscopic and histologic pattern: he did not return for follow-up.

Discussion

The necessity to understand the value of cytology of pleural effusion with respect to the gold standard exam, comes from our daily clinical practice. In fact, the area surrounding our hospital has a high asbestos contamination, which is responsible for most of the pleural pathology (mesotheliomas and chronic pleurisies) that is largely present in our clinical practice [10].

Pleural effusion diagnosis is essential for management and therapy, particularly in malignancy.

In literature, the sensitivity of cytology varies from 40% to 87% [9]. In those studies, the sensitivity of cytology was calculated on pleural fluid in patients with a known diagnosis so the specificity was always 100%. In our study, on the other hand, the specificity does not reach 100%: the explanation for this may be that the origin of the pleural fluid we studied was not known, hence resulting in three false-positive cases.

The plan of this study was based on the third phase of architecture of diagnostic research, according to Sackett and Haynes [6], that compares the test (cytology) to the gold standard test (histology by thoracoscopy). There have not yet been any studies published with this plan on this subject.

Our values of sensitivity are similar to those found in previous studies, even though we only used the first sample of pleural fluid to execute the cytologic examination.

Our values of sensitivity are similar to those in literature, even though only the first sample of pleural fluid was evaluated.

In actual fact, a second sample would have contributed, albeit slightly, in making a diagnosis. It would have increased sensitivity by another 27-28% [5]. Therefore in this study, that would translate into having a sensitivity of about 66% so that out of a total of 61 patients with a negative cytology, 16 of them would have resulted positive.

This study supports the association between histologic subtypes of mesothelioma and the sensitivity of cytology.

Furthermore, this study supports the association between histologic subtypes of mesothelioma and the sensitivity of cytology [4]: we confirm a very low cytology sensitivity for sarcomatoid mesotheliomas (15.4%) [4].

The result of this study induces us to consider the clinical value of the cytologic examination in all cases of unknown pleural effusion as less valuable in our area where a very high incidence of mesotheliomas is present and a medical thoracoscopy can be performed immediately also to performed talc puodrage for a massive pleural effusion. Furthermore in our study we had a high incidence of benign chronic pleurisies caused by the past asbestos contamination of La Spezia that are not present in others studies, but we had also 6 mesothelial hyperplasia, 6 tuberculosis, 2 eosinophil pleurisies, 4 empyemas that only thoracoscopy could diagnose. Pleural effusion by asbestos chronic pleurisy could precede mesothelioma and could give a false negative thoracoscopy exam that in our data is 10.5% after 18 months follow up [11]. In this study, we did not do a systematic follow up to benign patients.

Sensitivity of first cytology samples in pleural effusion remains about 50% in the immunocytochemical era and it is too low to be useful in clinical practice.

We conclude that sensitivity of first cytology samples in pleural effusion remains about 50% in the immunocytochemical era and that it is too low to be useful in clinical practice when we have a suspicion of mesothelioma or in case of massive pleural effusion and need of talc poudrage.

A negative cytologic examination of pleural effusion does not exclude a malignant disease and often does not diagnose a non-malignant disease; a positive cytology for metastasis could exclude diagnostic thoracoscopy, even though thoracoscopy might be

performed for genetic mutation research and talc poudrage. A positive cytology for mesothelioma requires confirmation by histology obtained by thoracoscopy [12]. When a medical thoracoscopy must be performed immediately, pleural cytology may be not appropriate.

Acknowledgements

The authors thank A. Camaiora, V. Balestracci and R. Tome for collecting the PE samples.

This work was funded by a grant from Fondazione Carispe.

References

1 Rahman NM, Ali NJ, Brown G, et al. *Local anaesthetic thoracoscopy: British Thoracic Society pleural disease guideline 2010*. Thorax 2010;65:ii54-60.

2 Filiberti R, Parodi S, Libener R, et al. *Diagnostic value of mesothelin in pleural fluids: comparison with CYFRA 21-1 and CEA*. Med Oncol 2013;30:543-52.

3 Canessa PA, Ferro P, Manta C, et al. *Clinical value of mesothelin in pleural effusions versus histology by medical thoracoscopy: brief report*. Med Oncol 2013;30:649.

4 Rakha EA, Patil S, Abdulla K, et al. *The sensitivity of cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma.* Diagn Cytopathol 2010;38:874-9.

5 Bielsa S, Panades MJ, Egido R, et al. *Rentabilidad del estudio citologico del liquido pleural en el derrame maligno*. An Med Interna (Madrid) 2008;25:173-7.

6 Sackett DL, Haynes RB. *Evidence base of clinical diagnosis. The architecture of diagnostic research.* BMJ 2002;324:539-41.

7 Swiderek J, Morcos S, Donthireddy V, et al. *Prospective study to determine the volume of pleural fluid required to diagnose malignancy*. Chest 2010;137:68-73.

8 Canessa PA, Franceschini MC, Ferro P, et al. *Evaluation of solubile mesothelin-related peptide as a diagnostic marker of malignant pleural mesothelioma effusions: its contribution to cytology*. Cancer Invest 2013;31:48-55.

9 Ikeda K, Tate G, Suzuki M, et al. *Comparison of immunocytochemical sensitivity between formalin-fixed and alcohol-fixed specimens reveals the diagnostic value of alcohol-fixed cytocentrifuged preparations in malignant effusion cytology*. Am J Clin Pathol 2011;136:934-42.

10 Gennaro V, Ugolini D, Viarengo P, et al. *Incidence of pleural mesothelioma in Liguria Region, Italy (1996-2002)*. Eur J Cancer 2005;41:2709-14.

11 Canessa PA, Ferro P, Franceschini MC, et al. *Clinical relevance of positive pleural effusion soluble mesothelin-related peptide in non-malignant pleuritis*. Chest 2013;144:517A.

12 Scherpereel A, Astoul P, Baas P, et al. *Guidelines of the European Respiratory Society and the European Society of Thoracic Surgeons for the management of malignant pleural mesothelioma*. Eur Respir J 2010;35:479-95.

Gli Autori dichiarano di non avere alcun conflitto di interesse con l'argomento trattato nell'articolo.