Journal of Molecular Biomarkers & Diagnosis

Roca, et al., J Mol Biomark Diagn 2017, S2:028
DOI: 10.4172/2155-9929.S2-028

Case Report Open Access

False Negative Pleural Cytology in Patient with Malignant Pleurisies: Is Pleural EpCAM-Positive Microparticles a Complementary Tool for Diagnosis?

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Rec Date: November 27, 2016, **Acc Date:** January 18, 2017, **Pub Date:** January 20, 2017

Abstract

The diagnosis of neoplastic pleurisies remains difficult because of frequent false negative cytological analysis. Thoracoscopy remains the gold standard to distinguish benign from malignant pleural effusion, but it is invasive and not suitable for all patients.

Microparticles (MPs) are promising potential biomarkers and could represent a new approach to identify patients with malignant pleural effusions.

We have recently reported the presence of EpCAM-positive-MPs (EpCAM+MPs) in malignant pleurisies that could be routinely used as a complementary tool with cytology for the diagnosis of pleural malignancy.

Here we describe three cases of pleural cancer patients negative for cancer cells but positive for EpCAM+MPs in the pleural fluid.

This study confirmed the possibility to apply in clinical practice recent data about EpCAM+MPs, detected in pleural fluid, as a non-invasive pleural biomarker useful to increase sensitivity of cytology. In particular, we suggest in patients with a strong suspicion of pleural cancer to search pleural EpCAM+-MPs as a complementary diagnostic tool in case of negative cytological analysis of pleural fluid.

Keywords: Malignant pleural effusion; Thoracoscopy; Microparticles; EpCAM

Introduction

Non-invasive biomarkers to differentiate benign from malignant pleural effusion are needed [1,2]. If thoracentesis is usually the first step to achieve cytological analysis in patients with pleural effusion thoracoscopy remains the gold standard to obtain tissue for final diagnosis [3].

However, cytology can produce false negative results and thoracoscopy is an invasive approach not suitable for all patients.

Microparticles (MPs) are extracellular vesicles released by all cell types and are considered promising potential biomarkers for diagnosis, prognosis, and disease monitoring.

These extracellular vesicles with a size of 0.1-1 micron, originate from blebbing of cell membranes after cell activation or apoptosis. They generally express the anionic phospholipid phosphatidylserine, and membrane antigens derive from their parental cells [4]. Microparticles are also released by tumor cells [5-9]. However, very few data have reported the presence of these MPs in pleural fluid [10-12].

We have recently reported the presence of EpCAM-positive microparticles in pleural fluid that could represent a new approach to non-invasively identify patients with malignant pleural effusions [13].

Here we describe three cases of cancer patients negative for cancer cells but positive for EpCAM-MPs in the pleural fluid after thoracentesis, illustrating a potential interest of this analysis to improve the diagnosis of patients suffering from malignant pleurisy.

Case Reports

Case 1

A 66-year-old man presented with dyspnea and a pleural effusion on the left side at chest radiograph. His past medical history was significant for head and neck cancer.

Physical examination revealed a good performance status (PS 1, ECOG classification), no weight loss, and, at the lower part of the left hemithorax, decreased breath sounds, and dullness to percussion. A CT scan showed a left-sided pleural effusion, and no pleural or parenchymal abnormalities were noted. The patient underwent thoracentesis and the cytological analysis did not detect the presence of cancer cells.

Given the high suspicion for relapse of cancer disease, thoracoscopy was performed It revealed diffuse pleural nodules suggesting metastases and about 2300 ml of hematic pleural fluid were drained (Figure 1a). We searched the presence of EpCAM positive MPs in pleural fluids using highly sensitive flow cytometry. As illustrated in Figure 2a, compared to control, a huge number of MPs positive for EpCAM was detectable in the pleural fluid of this patient (Figure 2 and Case 1).

Although the cytology was negative, the histological report of pleural biopsy was consistent with the diagnosis of metastasis from head and neck cancer.

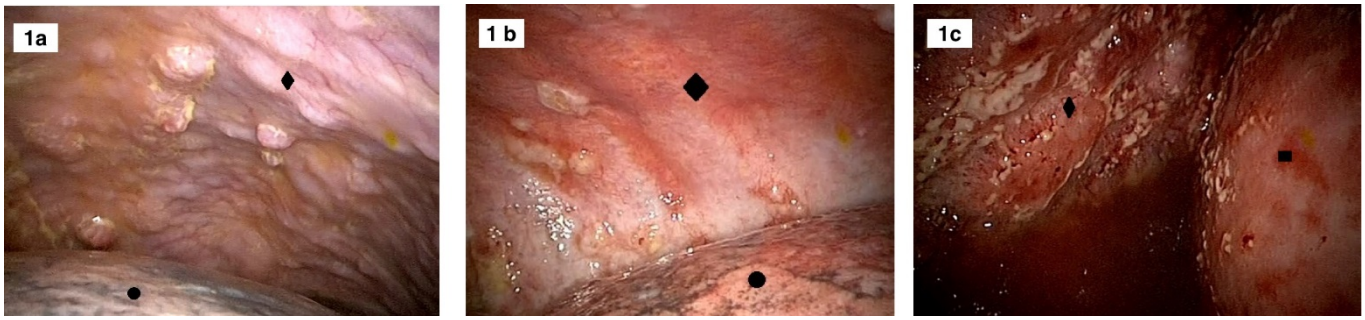

Figure 1: Thoracoscopic features of the three clinical cases.

(Lung: , Parietal Pleura: , Diaphragm:)

a: Case 1- Metastatic pleural cancer with huge nodules and neoplastic lymphangitis.

b: Case 2- Pleural metastasis of prostatic adenocarcinoma.

c: Case 3- Costo-diaphragmatic gutter in patient with breast cancer, multiple nodules are disseminated on the parietal pleural () and on the diaphragm ().

Case 2

A 67-year-old patient visited the outpatient clinic for a right pleural effusion documented at chest radiography. He had never smoked. Physical examination revealed a good performance status (PS-0, ECOG classification), and it was unremarkable. The thoracentesis performed to improve its symptoms showed after biological analysis an exudate without malignant cells. A chest CT scan confirmed right pleurisy without pulmonary or pleural lesions. The patient underwent thoracoscopy for diagnostic and medical purposes. Pleural biopsies were taken and a pleurodesis using dedicated talc was carried out due to the strong macroscopic suspicion of malignant disease (Figure 1b). Compared to control, EpCAM positive MPs were also detected in pleural liquid by cytometry (Figure 2 and Case 2).

The histological diagnosis of pleural biopsy revealed metastasis from adenocarcinoma of probable prostatic origin. A total body CT scan showed a prostate cancer with bone metastasis.

Representative flow cytometry graphs of EpCAM labeling on MPs from pleural fluid. The control experiments with appropriate isotype antibodies are displayed below.

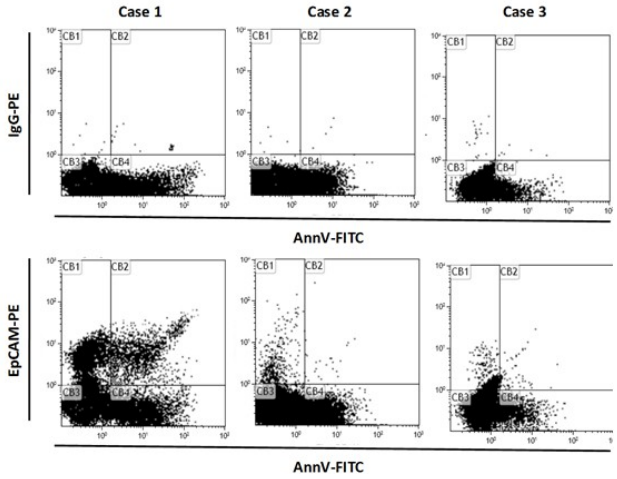

Figure 2: EpCaM-positive microparticles in pleural effusion.

Case 3

A 37-year-old woman with a significant smoking history presented with dyspnea, fatigue, and weight loss. The physical examination revealed dullness to percussion with decreased breath sounds at the lower part of the left hemithorax.

A left-sided pleural effusion was identified on chest X-ray and confirmed by the chest CT without lung or mediastinal abnormalities. Thoracentesis yielded pleural fluid consistent with an inflammatory pleurisy without malignant cells.

Compared to control, EpCAM positive MPs were detected in pleural liquid by cytometry (Figure 2 and Case 3), at a level comparable to patient 2.

By thoracoscopy 500 ml of citrine pleural liquid were removed and pleural samples were taken (Figure 1c).

Pleural biopsies during the procedure using an optical biopsy forceps confirmed the diagnosis of pleural metastasis from breast cancer. Mammography identifies a nodular lesion at the level of the left-upper-outer lobe strongly suggestive of breast cancer.

Discussion

Cytological analysis has variable diagnostic yields depending on multiple factors and situations. However, according to the recommendations, thoracentesis for sampling the fluid still remains the first step leading to cytological analysis for the diagnosis of pleural cancer. Indeed, in pleurisy, it is largely used to distinguish benign from malignant pleural effusion. Nevertheless, this technique presents some limitations. In fact, the cell poorness, or the difficulties to distinguish tumor cells from reactive or inflammatory mesothelial cells can cause false negative results [14].

At contrary, thoracoscopy allowing pleural biopsies usually achieves the diagnosis between benign and malignant effusion. For this reason, it is the gold standard technique in case of pleural abnormalities (nodules, thickenings...) and pleural effusion after imaging procedures. However, thoracoscopy has some limitations and cannot be applied for all patient. In fact, advanced age, poor performance status, and comorbidities can limit this invasive procedure.

The presence of specific microparticles in the pleural fluid could be considered as promising non-invasive biomarkers beside the cytological analysis of the fluid and can increase the diagnostic yield.

Microparticles can easily be detected from different body fluids, including peripheral blood, urine, cerebrospinal fluid, saliva, or synovial and vitreous fluids [15].

Moreover, MPs contain many antigens also present in the cell of origin [16,17]. For this reason, they could have considered useful as biomarkers for the screening and the diagnosis of a cancer at an early stage [18-23].

In a recent study, we documented the presence of high amounts of normal cells-derived and tumor-derived MPs in pleural fluids [24]. In this study, we found EpCAM+ MPs deriving from cancer cells, which identified patients suffering from carcinoma although the pleural cells were no longer detectable. Therefore, EpCAM+MPs could be considered as promising biomarker complementary to cytology to distinguish benign and malignant pleurisy (Figure 2).

For the first time, we reported here three clinical cases of cancer patients negative for cancer cells at pleural cytology but positive for EpCAM-MPs, illustrating the potential of MPs detection to improve the diagnosis of patients suffering from malignant pleurisy.

These clinical cases show the possibility to apply a non-invasive method, based on the detection of pleural EpCAM-positive MPs, which is complementary to pleural cytological analysis.

Therefore, even if the cytological analysis is negative, the detection of EpCAM-positive MPs could be added to cytology to better discriminate benign from malignant pleural effusions. The positivity of MPs in patient with negative cytology could be explained by the diffusion properties of the vesicles in the pleural fluid. In addition, the fact those MPs, deriving from apoptotic cancer cells, remain present even if parental cells are no longer detectable because of the process of apoptosis. Moreover, in contrast to cytology it is important to remember that flow cytometry is an operator-independent technique allowing counting MPs.

The MPs also contain protein nucleic acids and therefore carry a genetic signature and could be implicated in carcinogenesis. In particular, the MPs have a role in the progression of the disease, in cellular function and in genetic regulation. Further molecular biology techniques applied on MPs of neoplastic pleurisies could be interesting to study their genetic signature and their carcinogenic potential. Therefore, this present report opens new perspectives about the utility of MPs from pleural fluid. Moreover, it offers the prospect of designing new therapeutic approaches.

In particular, since anti-EpCAM target therapy are available [25], it could be of interest to identify patients with EpCAM-positive MPs to define more personalized therapeutic approaches [26].

Conclusion

We have recently reported that EpCAM+MPs detected in the pleural fluid are correlated with the presence of neoplasia and in particular, with adenocarcinoma type. These reported cases illustrate how this marker can complete the usual cytological analysis of the pleural fluid.

Consequently, pleural EpCAM+MPs combined with pleural cytological analysis could be relevant for poor performance status patient for whom more invasive procedure is not available in case of suspicion of pleural cancer.

Moreover, since cancerous MPs have also several activities and genetic signatures related to malignant responses, EpCAM+ MPs could be involved in carcinogenesis of the pleural microenvironment. Finally, EpCAM+MP detectable in pleural fluid could act as a target for specific treatment as already reported in others cancers [27,28].

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This article was originally published in a special issue, entitled: **"Cancer Biomarkers"**, Edited by Kenneth Maiese