

**Detection of Metastatic Lung Cancer by Immunocytochemistry and Flow Cytometry in a  
Sample of Pleural Fluid**

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**ABSTRACT**

Malignant pleural effusions (MPE) are common complications in cancer patients and indicate the spread of the primary disease (metastasis). For over a century, malignant effusion has been diagnosed through the observation of changes in the cavity effusion cells, such as the gold-standard cytomorphological analysis. Studies show that the multiparameter flow cytometric analysis is sensitive and rapid and allows for the immunophenotypic evaluation of a large number of cells. This report describes the case of a 64-year-old man diagnosed with lung cancer who had never had any kind of treatment for this disease. The pleural sample was analyzed by morphological (quantitative and differential cytology) and immunocytochemical analyses. As a new tool for diagnosis of malignant pleural effusion, the sample was also analyzed by flow cytometry. In the case report described, flow cytometry was an effective and quick method for detecting neoplastic cells in pleural fluids.

**Keywords:** Malignant pleural effusions, new tool for diagnosis, flow cytometry.

## INTRODUCTION

Malignant pleural effusions (MPE) are common complications in cancer patients and indicate the spread of the primary disease (metastasis)<sup>1</sup>. Lymphomas and tumors of lung, breast, and ovary constitute more than 75% of the primary neoplasms in MPE cases. The presence of MPE signifies an advanced stage of the disease. In these cases, death will likely result within a few months of the pleural fluid being first detected<sup>2,3</sup>. For over a century, malignant effusion has been diagnosed through the observation of changes in the cavity effusion cells, such as the gold-standard cytomorphological analysis<sup>4</sup>. However, this methodology is laborious and, consequently, causes delays in the conclusion of the diagnosis. Another problem is related to the similarity among hyperplastic mesothelial cells, neoplastic mesothelial cells, and metastatic adenocarcinoma, which are difficult to differentiate through morphology. Thus, complementary methods are needed in order to identify tumor cells within body cavity fluids, such as immunocytochemistry, cytogenetics, and molecular biology, to conclude the diagnosis<sup>4,5,6,8</sup>.

Flow cytometry (FCM) plays a central role in the immunophenotyping of hematologic malignant neoplasms<sup>7</sup>. In spite of its outstanding advantages, the use of flow cytometry for the diagnosis of non-hematologic malignancies is rare<sup>4</sup>. Studies show that the multiparameter flow cytometric analysis is sensitive and rapid and allows for the immunophenotypic evaluation of a large number of cells<sup>9,10</sup>. In face of the above, we report a case of lung cancer whose diagnosis of malignant pleural effusion was confirmed by morphological, immunohistochemistry, and flow cytometry analyses.

## PRESENTATION OF CASE

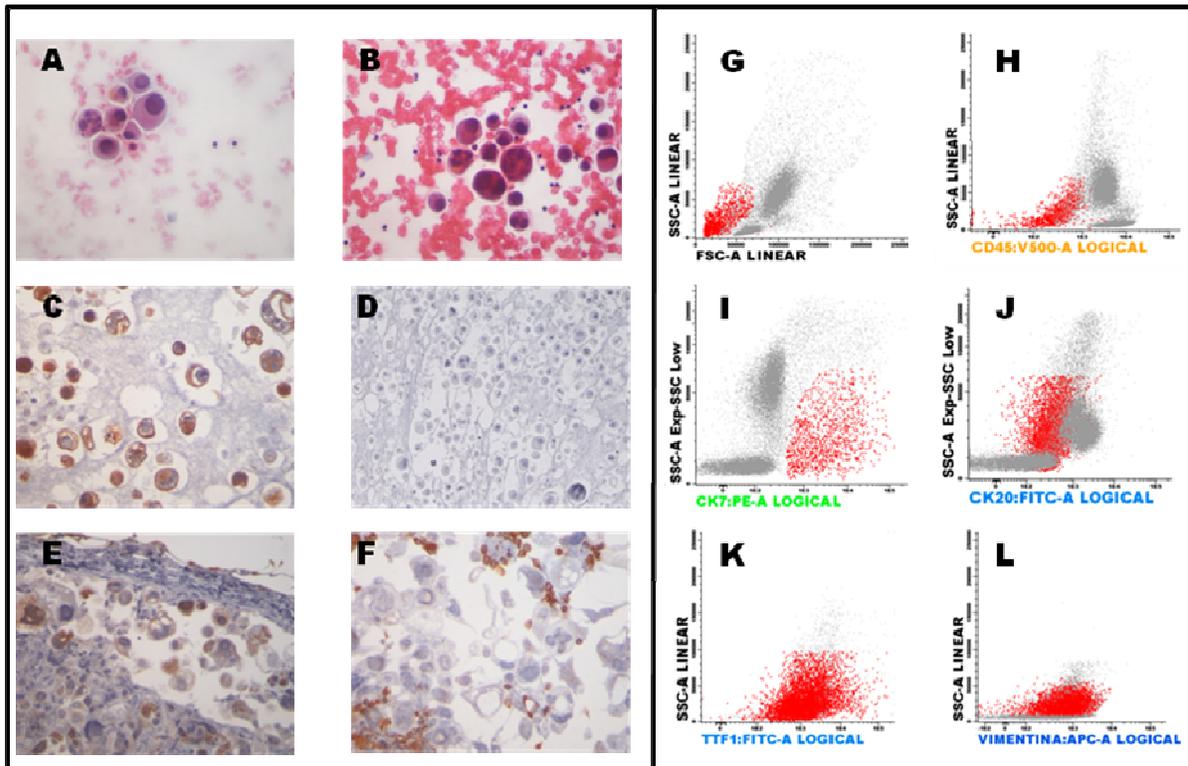
This report describes the case of a 64-year-old man diagnosed with lung cancer who had never had any kind of treatment for this disease. After suspicions that the lung cancer had advanced, the X-ray exam confirmed pleural effusion and the patient underwent thoracentesis. The pleural sample was analyzed by morphological (quantitative and differential cytology) and immunocytochemical analyses (Table 1; Figure 1).

**Table 1** - Results of pleural fluid immunocytochemistry.

Markers	Expression
CK7	+
CK20	+
TTF-1	+
CK POOL	+
NepsinA	-
Vimentin	-

As a new tool for diagnosis of malignant pleural effusion, the sample was also analyzed by flow cytometry (Figure 1). To ensure cell viability of the sample, all analyses were made immediately after thoracentesis. For total cell count, a fresh pleural fluid sample, non-centrifuged and properly homogenized, was used. The differential count used the pellet obtained by low-speed centrifugation (200  $\mu$ L of the sample were centrifuged in a Cytospin centrifuge (CYTOPROTM - Wescor) and stained by the May-Grumwald-Giemsa method by a SYSMEX SP-1000i device). The phenotypic evaluation by immunocytochemistry was performed using the streptavidin-biotin tagging method, the immunophenotyping was performed by eight-color multiparameter flow cytometry (FacsCanto II - Becton Dickinson (BD), San Jose, CA, USA), and the analysis was carried out using the software Infinicyt version 1.7 (Cytognos, Salamanca, Spain). The global

leucocyte count detected 700 cells/mm<sup>3</sup> and the morphology evaluation observed 18% neutrophils, 18% mononuclear, and 64% mesothelial cells. The analysis by flow cytometry used 7AAD to evaluate cell viability and CD45 to exclude debris and hematologic cells. Thus, the immunophenotype by flow cytometry observed 45% CD45 (+) hematologic cells, consisting of 2.38% monocytes, 0.96% natural killer T cells, 28.79% T cells, 10.31% B cells, 18.03% neutrophils, and 39.53% other kinds of hematological cells that were not marked with appropriate markers. The immunophenotype of CD45-negative cells had CK7 (+), Vimentin (-), and TTF-1 (+), which suggests that 18% of the cells analyzed by flow cytometry were tumor cells (Figure 1). This result was the same observed in the immunocytochemistry evaluation (Figure 1).



**Figure 1:** Morphological and immunocytochemical evaluation and flow cytometric evaluation of pleural effusion. Adenocarcinoma of lung showing (A) and (B) tumor cell morphology analyzed by hematoxylin and eosin (X 400); (C) positive immunostaining for CK7, (D) negative immunostaining for CK20; (E) positive immunostaining for TTF-1; and (F) negative immunostaining for Vimentin immunocytochemistry (X 400); (G-L) Show representative dot plots of immunophenotyping by flow cytometry. Tumor cells were stained in red and hematology cells, in gray. (G) Shows forward and side light scatter properties of tumor cells; (H): negative expression of CD45; (I) positive expression of CK7; (J) negative expression of CK20; (K) positive expression of TTF-1; and (L) negative expression of Vimentin.

In addition, 37% of CD45 (-) cells had CK7 (-), CK20 (-), and TTF-1 (-), and Vimentin (-) and were considered as other cell types such as mesothelial cells. As lymphomas are very often the cause of malignant pleural effusions, the cells were marked with CD8/LAMBDA, CD56/KAPPA, CD5, CD19, CD3, CD38, and CD20/CD4 and the results confirmed that the present case was not a lymphoma.

## **DISCUSSION**

The malignant pleural effusion (MPE) is a frequent complication in patients with advanced tumors. According to reports, in the vast majority of cases, a MPE signifies incurable disease associated with high morbidity and mortality<sup>11,12</sup>.

Cytologic or histologic examination of fluids from serous cavities for confirmation of malignant cells is an essential component in the management of adult with cancer and it is currently important in establishing a diagnosis and treatment protocols<sup>12, 13</sup>. Various studies have shown a sensitivity of 57.3% and specificity of 89% by conventional cytology for the detection of malignant cells in effusion samples. Studies have shown that positive and negative predictive values for detection of malignancy by cytomorphology are 89.3% and 69.4% respectively<sup>8,14</sup>. Although cytology is the gold standard method for the diagnosis of pleural effusion, it presents limitations when the number of cells is low or it is difficult to discriminate cancer cells from reactive mesothelial or inflammatory cells<sup>15</sup>. The most common difficulty encountered by cytopathologists worldwide is the inability to separate without dispute the exfoliated atypical benign mesothelial cells from metastatic cells of adenocarcinoma in effusion<sup>8</sup>. Thus, other tools to conclusion of diagnosis are necessary such as immunocytochemical or immunohistochemical<sup>14</sup>. These techniques use various antibodies to differentiate between epithelial and mesothelial cells or to identify other types of tumors<sup>16</sup>. Immunocytochemical is essential when the morphology is difficult to distinguish tumor from reactive cells. However, in some cases these methodologies are

insufficient to do the diagnostic, especially when there is sample hypocellular, and in the cases that thoracentesis procedure is difficult<sup>17</sup>. Thus, numerous studies suggest that flow cytometry presents higher accuracy than cytomorphology in the analysis of serous effusions, which improve significantly the diagnostic by adding immunophenotype<sup>4,8</sup>.

Therefore, in this case it was used the flow cytometry as a new laboratory tool together with cytomorphology and immunocytochemistry. As it can be seen in Figure 1 (Panel G to L), flow cytometry was effective in detecting neoplastic cells and the phenotype was the same of those observed by immunocytochemistry (Panel A to F). However, further studies should be done using flow cytometry to detect malignant cells originating from solid tumors. In the future, this methodology can contribute with the other exams to the conclusion of cancer diagnosis, particularly because with this analysis it is possible to use sample with hypocellular and the methodology is less laborious than immunocytochemistry. This analysis delivers the conclusion of the diagnosis fast, which is important to decide the treatment.

## **CONCLUSION**

In the case report described, flow cytometry was an effective and quick method for detecting neoplastic cells in pleural fluids. It is believed that the development of new diagnosis tools such as flow cytometry can be used to bring great advances in the detection of malignant cells, thus contributing not only in the diagnosis of malignant effusions, but also in the early detection of solid tumors.

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**COMPETING INTERESTS**

The authors declare no conflicts of interest

**AUTHORS' CONTRIBUTIONS,**

Aline Costa and Teresa Maria da Silva Figueiredo performed morphology and immunocytochemistry experiments and analyzed data.

Manoela Lira Reis, Rafaela Pilon, Alisson Bigolin, Renata Cristina Messores Rudolf de Oliveira and Ana Carolina Rabello de Moraes performed flow cytometry experiments and analyzed data.

Manoela Lira Reis and Maria Cláudia Santos da Silva designed the research study, analyzed the data, and wrote the paper.

All authors read and approved the final manuscript.

**ETHICS**

This study was approved by the human research ethics committee of the Federal University of Santa Catarina, Brazil – approval number CAAE 18715613.0.0000.0121 (Supplementary File)

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