Case study

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

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)[1]. 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[2,3]. 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[4]. 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 thorugh 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[4,5,6,8].

Flow cytometry (FCM) plays a central role in the immunophenotyping of hematologic malignant neoplasms[7]. In spite of its outstanding advantages, the use of flow cytometry for the diagnosis of non-hematologic malignancies is rare[4]. Studies show that the multiparameter flow cytometric analysis is sensitive and rapid and allows for the immunophenotypic evaluation of a large number of cells[9,10]. 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 µL 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^3 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).

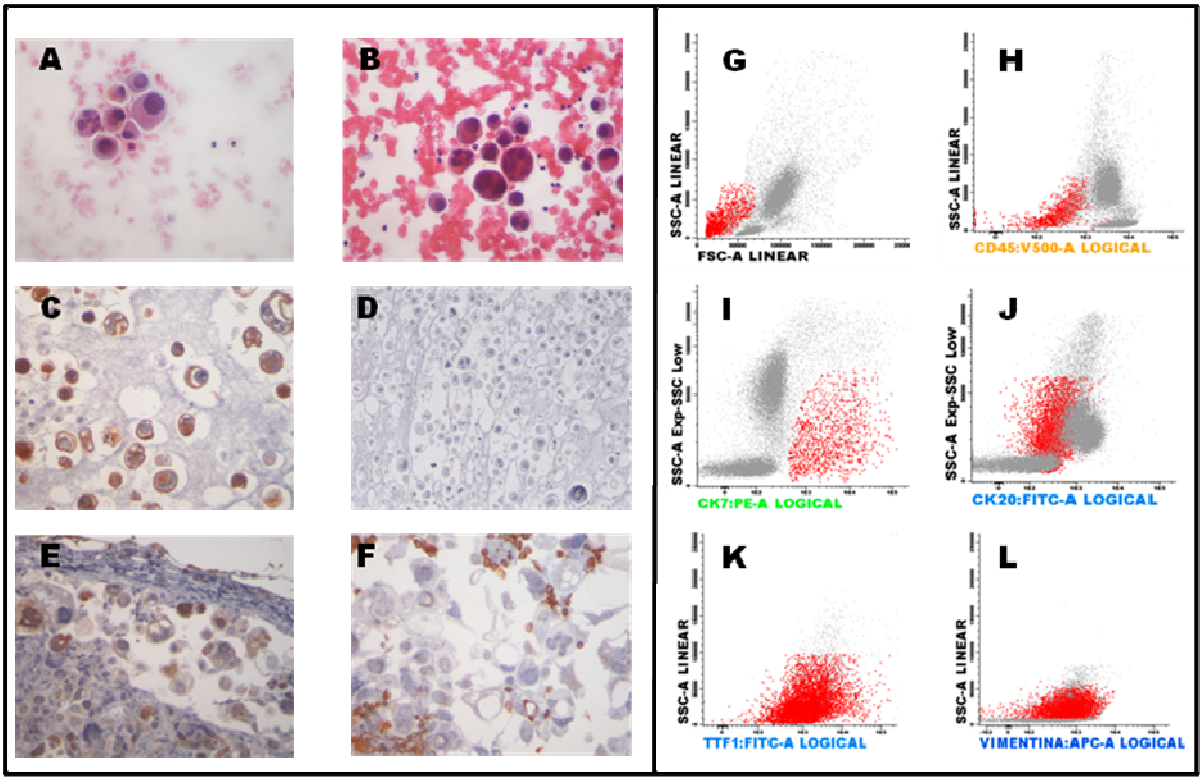

Figure 1: Morphological and immunocytochemical evaluation and flow cytometric evaluation of pleural effusion. Adenocarcinoma of lung showing **(A)** and **(B)** tumor cell morphology analyzed by hematoxylin and eosin (X 400); **(C)** positive immunostaining for CK7, **(D)** negative immunostaining for 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

Flow cytometry is an effective and quick method for detecting neoplastic cells in pleural fluids. This methodology provided contributions to other sectors of laboratory exams, which are now able to characterize and differentiate populations of phenotypically abnormal cells from normal ones, even when they are in low percentage in the sample[4]. However, further studies should be done using flow cytometry to detect malignant cells originating from solid tumors. In the case report described, flow cytometry was an effective and quick method for detecting neoplastic cells in pleural fluids.

CONCLUSION

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.

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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