



High fluorescence cell count in pleural fluids for malignant effusion screening



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ABSTRACT

Malignant pleural effusion (MPE) is mainly secondary to pleural metastasis. Its prevalence is 15 to 35% of all the pleural effusions, and the median of survival oscillates between 4 and 6 months, reason why it is very important to know how to diagnose it. The Sysmex XN-350® is an automated hematological analyzer that allows white blood cell count and differentiation, as well as high fluorescence cells (HFC) which includes macrophages, mesothelial and neoplastic cells.

For MPE screening, the best combinations obtained were HF-BF# $\geq 17/\mu\text{L}$ and HF-BF# $> 10/\mu\text{L}$, both in the absence of heart failure and/or low respiratory infection.

The results of this study show that the automated analysis of the pleural fluid with the Sysmex XN-350® analyzer is effective for the screening of the MPE.

To the Editor:

Malignant pleural effusion (MPE) is mainly secondary to pleural metastasis. Its prevalence is 15 to 35% of all the pleural effusions, and the median of survival oscillates between 4 and 6 months, reason why it is very important to know how to diagnose it [1]. Cellular evaluation includes the determination of the number of cells and their differentiation, but the cell identification by microscope review has limitations like being time consuming, which is especially important in an emergency laboratory.

The Sysmex XN-350® is an automated hematological analyzer that allows white blood cells count as well as fluorescence flow cytometry for their differentiation. It uses different dyes to stain the cellular content, which then is exposed to a laser beam that passes through each cell, emitting a scattering of light. For the cellular identification, different parameters are combined such as the frontal scatter, which measures the size; lateral scatter, intracellular complexity; and the fluorescence that measures the nucleic content [3,4]. Combining these parameters and using the “body fluid mode” it is possible to identify neutrophils, eosinophils, monocytes and lymphocytes, excluding from

this differential count the high fluorescence cells (HFC) that includes macrophages, mesothelial and neoplastic cells [5]. In this way, HFC groups cells that, due to their size, complexity and nucleic content, can be differentiated from blood cells.

We have previously published a study for the screening of peritoneal carcinomatosis by the use of high fluorescence cells [6], but since these two types of effusion present different incidences of neoplastic infiltration (pleural effusion 15 to 35%, ascites 7 to 10%) [1,2] we have preferred to develop two different algorithms.

Our objective was to evaluate the utility of counting the high fluorescence cells in pleural liquids for the screening of MPE.

We analyzed consecutively all the pleural liquids obtained between January 2018 and March 2019 from diagnostic thoracentesis. This study was approved by the ethics committee of Donostia University Hospital. Samples were collected in 15 mL conical polypropylene tubes (Falcon), and were processed in the clinical laboratory within 1 h of collection using a Sysmex XN-350® analyzer in “body fluid (BF) mode” [6]. Before analysis, the following steps were performed: (1) samples were examined for blood clots, and (2) each tube was gently rolled between the palms of the hand for 1 min, turning it upside down 10

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Table 1
The best combinations of cut-off points for the screening of malignant pleural effusions.

	AUC	S	E	PPV	NPV	LR +	Smears that should be performed
HF-BF# \geq 17/ μ L	0.718	94 (89–97%)	50 (44–55%)	46 (43–48%)	95 (91–97%)	1.9 (1.7–2.1)	64% (324/505)
HF-BF# > 10/ μ L	0.70	98 (94–100%)	42 (37–47%)	43 (41–45%)	98 (94–99%)	1.7 (1.5–1.8)	70% (356/505)
HF-BF# \geq 17/ μ L No ICC, no infection	0.849	91 (85–95%)	79 (74–83%)	66 (61–71%)	95 (92–97%)	4.3 (3.5–5)	43% (216/505)
HF-BF# > 10/ μ L No ICC, no infection	0.842	94 (89–97%)	74 (69–79%)	62 (58–66%)	97 (94–98%)	3.7 (3–4.4)	47% (237/505)
HF-BF# \geq 17/ μ L No ICC, no infection Positive OM	0.918	87 (81–92%)	97 (94–98%)	92 (91–98%)	92 (87–95%)	25 (14–44)	
HF-BF# > 10/ μ L No ICC, no infection Positive OM	0.925	88 (82–93%)	96 (94–98%)	92 (87–95%)	94 (92–97%)	26 (15–45)	

AUC, area under the curve; **S**, sensitivity; **E**, specificity; **PPV**, positive predictive value; **NPV**, negative predictive value; **LR +**, positive likelihood ratio. **TC-BF#**, total count of nucleated cells/ μ L; **HF-BF#**, number of high fluorescence cells/ μ L. **No ICC, no infection**, exclusion of patients with heart failure and/or low respiratory infection at the time of analysis; **Positive OM**, presence of atypical cells in the smear performed in the Biochemistry laboratory by cyto centrifugation and staining with May Grünwald-Giemsa; **Smears that should be performed**, percentage of smears that would be performed if the described algorithm would be used.

times.

The variables studied were the total number of nucleated cells/ μ L (TC-BF#), the total number of high fluorescence cells/ μ L (HF-BF#), the number of high fluorescence cells per 100 leucocytes (HF-BF%), the total number of neutrophils (Neut #), the number of neutrophils per 100 leucocytes (Neut%) and the total number of red blood cells (RB #).

We reviewed the medical records of the patients from whom pleural fluid samples had been collected. Patients were considered to have a MPE if they met at least one of the following criteria: (1) neoplastic infiltration associated with pleural effusion diagnosed by computed tomography (CT) and/or (2) positive cytology for malignant cells performed in the department of pathology (PA); in both cases, the presence of radiological pleural effusion and clinical course compatible with neoplastic disease was essential. Those fluids in which neither CT nor AP had been performed were discarded. Areas under the curves (AUCs) were calculated using MedCalc version 12.7.0.

A total of 523 pleural fluids were collected, 18 of which were excluded due to the presence of visible clots. MPE was identified in 31% (156/505). The AUCs for the MPE screening were: 0.78 for HF-BF% (95% CI, 0.75–0.82, $p < .0001$); 0.76 for HF-BF# (95% CI, 0.72–0.80, $p < .0001$); 0.69 for Neut% (95% CI, 0.65–0.73, $p < .0001$); 0.59 for Neut# (95% CI, 0.55–0.64; $p = .0003$); 0.51 for TC-BF# (95% CI, 0.45–0.56, $p = .90$) and 0.52 for RB# (95% CI, 0.47–0.56, $p = .55$).

RB# and TC-BF# were discarded because they were not statistically significant, while HF-BF#, HF-BF%, Neut# and Neut% were analyzed separately and together at different cut-off points, also adding diagnostic suspicion at the time of thoracentesis. For MPE screening, the best combinations obtained were HF-BF# \geq 17/ μ L and HF-BF# > 10/ μ L, both in the absence of heart failure and/or low respiratory infection (see Table 1).

To improve the results, the analysis by optical microscopy (OM) performed in the biochemistry laboratory was added. Smears were prepared by cyto-centrifugation and subsequently stained. This was performed using a Bunsen Citocentrifuga® cyto centrifuge with a constant centrifugation speed and duration (30 rcf for 5 min) and a sample volume that varied according to cell density (100 μ L for densities of 0–1000/ μ L and 50 μ L for densities \geq 1000/ μ L). Cell staining was carried out with the Sysmex SP-10 slide maker and stained using the May Grünwald-Giemsa staining method. Two cytologists, who were blinded to the clinical data related to each smear, conducted the OM analysis and prepared reports classifying samples as positive or negative for atypical cells.

The results of this study show that the automated analysis of the pleural fluid with the Sysmex XN-350® analyzer is effective for the screening of the MPE. The cut-off point of HF-BF# \geq 17/ μ L was originally published by Labarere et al. [5]. Subsequently, this value was

validated for the screening of peritoneal carcinomatosis [6]. This study maintains the cut-off point of HF-BF# \geq 17/ μ L; but unlike the previous one this focuses on the study of MPE and shows that associating other variables does not clearly improve their diagnostic performance, except those that depend on the clinical data.

The microscopic study from PA continues to be the gold standard for the diagnosis of MPE. Its diagnostic rate varies with the series, which have reported a mean sensitivity of about 60% (40–87%) [7]. A recent study has shown that such sensitivity has not improved despite advances in immunohistochemical methods [8]. On the other hand, the specificity of this technique is higher than 92%. In our study, only 51% (80/156) of the MPE cases were classified as positive by cytopathology, in the rest (76/156) the diagnosis of MPE was based on the presence of pleural effusion with a positive CT report and a clinical picture compatible with neoplastic disease, presenting a median HF-BF# of 45/ μ L.

Computed tomography is an important investigation in patients of MPE. The CT features highly suggestive of malignancy are nodular pleural thickening, pleural irregularity, mediastinal pleural thickening, circumferential pleural thickening, and pleural thickness > 10 mm. The reported sensitivity is 36–68% with specificity of 78–100% [9,10].

The strengths of this study include the large number of samples analyzed, the application of clinical, radiological and cytological criteria for the diagnosis of malignant pleural effusion, and having been conducted in the context of routine clinical practice.

The implementation of high fluorescence cells in algorithms for the screening and follow-up of neoplastic effusions would mean earlier diagnosis of MPE, and would allow stricter follow-up in patients with negative PA cytology.

In summary, the screening of neoplastic infiltration in pleural and ascitic fluids is possible using the same cut-off point for high fluorescence cells, which facilitates the implantation of a screening algorithm for the neoplastic serous fluids arriving at the laboratory.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cca.2019.09.008>.

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