Flow cytometry in the diagnosis of peritoneal carcinomatosis

ABSTRACT

Objective: Peritoneal carcinomatosis is the second major cause of ascites. Due to its frequency and poor prognosis, it is important to establish an accurate diagnosis. The aim of this study was to analyse the use of a DNA index, determined by flow cytometry, in the differential diagnosis of ascites, and to compare it to the cytopathological examination.

Methods: A prospective analysis was carried out on 67 patients with ascites of various etiologies. There were 39 women and 28 men, with a mean age of 53 ± 14 (5 to 82 years). Peritoneal carcinomatosis was detected in 21 patients, whereas in 46 the ascites was of non-carcinomatosis origin.

Results: The sensitivity of the cytological examination for the diagnosis of peritoneal carcinomatosis was 42.9%, and the specificity was 100.0%. The mean DNA index determined by flow cytometry was similar for peritoneal carcinomatosis and non carcinomatosis patients, being 1.28 x 1.01, respectively, in the preparations without control lymphocytes, and 1.28 x 1.04, respectively, when control lymphocytes were added. The sensitivity of DNA index cytometry was 57.1% and specificity 93.5%. The combined use of DNA index and cytological examination did not show advantage over the use of any of the tests individually, although DNA index was able to detect half of the cases of peritoneal carcinomatosis in which cytological examination was negative. Although the sensitivity was higher when the parameters were associated, DNA index did not offer statistically significant advantage over the use of cytological alone, which in turn had higher specificity.

Conclusion: DNA index presented lower sensitivity for the diagnosis of peritoneal carcinomatosis when used alone, showing no advantage over conventional cytological examination. However, DNA index was able to detect 50.0% of peritoneal carcinomatosis whose conventional cytological examination were negative, and could be valuable in these situations.

KEY WORDS: Flow Cytometry; Ascites; Peritoneal Carcinomatosis.

INTRODUCTION

In the last 30 years, it has been shown that many types of tumor cells have abilities resulting in altered cellular DNA content (1,2,3,4,5,6). With the introduction of flow cytometry (FCM), the investigation of those abnormalities became more accurate and objective (1).

As peritoneal carcinomatosis (CA) is the second most frequent cause of ascites, its accurate diagnosis is of fundamental importance (7,8,9,10). Considering the difficulties associated with diagnostic assessment of neoplastic involvement of the peritoneal membrane (11,12,13,14,15) and the historically discrepant results of FCM (5,16,17,18,19,20,21,22,23), the present study analyzed prospectively the value of FCM compared with conventional cytological examination (CP) of peritoneal fluid in establishing the diagnosis of peritoneal carcinomatosis.

MATERIALS AND METHODS

Ascitic fluid from 67 patients seen at Santa Casa Hospital, in Porto Alegre, Brazil, were prospectively analyzed during a one and a half year period.

The only criterion for inclusion in this study was the presence of ascites of any etiology. The study protocol was previously submitted to and approved by the Hospital Ethics Committee. The purpose and risks of the procedure were explained to all the patients and an informed consent was obtained.

There were 39 female and 28 male patients. The average age was 53 ± 14 years (5 to 82 years old). Fifty five patients were caucasian and 12 were black.

The diagnosis of CA was made in 21 patients, and in the remaining 46, the ascites was of other etiologies. The diagnosis for CA was established through a peritoneal biopsy obtained either through laparoscopy (12 patients) or laparotomy (8 patients). In one case the diagnosis was obtained after CT guided biopsy of an abdominal mass. Seventeen patients had adenocarcinoma and in 4 the ascites was secondary to lymphoma. Although etiologically not related to carcinomas, we included these cases of lymphoma as they may represent similar diagnostic challenge and may harbor aneuploid cells.

All patients with CA were submitted to ultrasonography, and none presented signs of portal hypertension.
In the group with non-carcinomatous (NCA) ascites, the diagnosis of cirrhosis was established by histopathological examination in 11 patients and by echographic-clinical-laboratorial data in 23 patients.

In 6 cases in which hepatic tumor was detected, peritoneal involvement was excluded by laparoscopic examination. In the other remaining cases with NCA ascites, peritoneal neoplastic involvement was excluded by laparoscopy or laparotomy.

For inclusion in the study, paracentesis was performed according to well-established techniques (8,10). The serum-ascites-albumin gradient (SAAG) was chieved and cytological examination followed standardized methods (24). An aliquot of 20 ml of ascites was collected and centrifuged, and the sediment was obtained to process the slides. Two slides were fixed in 95% alcohol and stained by the Panicaloau technique, and one slide was air dried and stained with Giemsa stain. Results were expressed as “positive” or “negative”. “Suspicious” or “doubtful” results were considered negative. The material was submitted blindly to a cytopathologist.

Besides the sample volume necessary for the determinations described, a minimum aliquot of 20 ml of peritoneal fluid was centrifuged for 10 minutes at 3 000 rpm (600 g). The resultant cell pellet was resuspended in Hanks solution and centrifuged once again. After resuspension, an aliquot of cells were counted in a Neubauer chamber, the cell pellet was finally resuspended in 0.5 ml of a previously prepared solution, composed of 1.0 ml of Fetal Calf Serum (FCS in final concentration of 20%) diluted in 4 ml of Hanks solution, filtered twice, and the sediment was obtained to process the slides. Two slides were fixed in 95% alcohol and stained by the Panicaloau technique, and one slide was air dried and stained with Giemsa stain. Results were expressed as “positive” or “negative”. “Suspicious” or “doubtful” results were considered negative. The material was submitted blindly to a cytopathologist.

For statistical analysis, the Student t test was used for comparison of the continuous variables concerning the presence of CA. In order to verify the association among the categorical variables, we used the Pearson χ² test or the Fisher test, according to the size and/or the sample distribution. The statistic programs used were SPSS for Windows (SPSS, Chicago, IL) and the EPIINFO version 6.03 (Centers for Disease Control and Prevention. Atlanta, GA). The highest significance level adopted was 0.05.

RESULTS

In the CA group, 17 patients had adenocarcinoma and in four the ascites was secondary to lymphoma.

In the NCA group 34 had cirrhosis caused by hepatitis C virus in 14 cases, by hepatitis B virus in five cases and by a coinfection of hepatitis B and C viruses in one patient. Cirrhosis was caused by the use of alcohol in eight patients, and in five additional cases there was a combination of the HCV and alcoholism. One patient had primary biliary cirrhosis. There was six cases with hepatic tumor with no peritoneal involvement, and the remaining cases comprised two peritoneal tuberculosis, one with heart failure and one with lymphangioleiomyomatosis.

The mean SAAG was 0.77 ± 0.36 g/dl in the CA group and 1.81 ± 0.64 g/dl in the NCA group (p<0.05), and in all the CA patients, the SAAG was under 1.1 g/dl.

The sensitivity of conventional CP examination for the diagnosis of CA was 42.9% (95% CI: 22.6 to 65.6%), detecting 9/21 of CA cases, and the specificity was 100.0% (95% CI: 90.4 to 100.0%). The average DNAI was not statistically different in patients with and without CA, being 1.28 ± 0.71 and 1,01 ± 0.05, respectively, in the preparation without control lympho-
cytes, and 1.28 ± 0.91 and 1.04 ± 0.19, respectively, in the preparations with control lymphocytes.

The number of patients with normal and altered DNAI in patients with and without CA is presented in Table 1.

The DNAI sensitivity for the diagnosis of CA was 57.01% (95% CI: 34.4 to 77.4%) and the specificity was 93.5% (95% CI: 81.1 to 98.3%). Table 2 depicts the distribution of cases with a diagnosis of CA when the two methods are used in association (DNAI and CP).

The use of DNAI enabled the detection of 06/12 patients with CA where CP examination was negative. The sensitivity of the two associated methods for diagnosing CA was 71.4% (95% CI: 47.7 to 87.8%) and the specificity was 93.5% (95% CI: 81.1 to 98.3%). The comparison of the methods when used individually or in association is shown in Table 3.

We observed that despite a high average DNAI, this did not result in significant differences between the CA and NCA groups, regardless of whether control lymphocytes were used or not. Some authors have described a much higher DNAI in patients with neoplasias compared to normal controls (5, 34). Nevertheless, the majority of published studies does not perform that comparison (21, 27, 50, 51). On the other hand, some authors (17, 19, 41, 47, 48) do not explore the DNAI average and just refer to the FCM results in terms of aneuploid or diploid.

In our study, when the number of patients with altered DNAI was analysed, a statistically significant association between this data and the presence of CA was observed. Analysis of the role of DNAI in the differential diagnosis of ascites yielded a sensitivity of 57.1% (12/21 patients). Among the non-neoplastic ascites, aneuploid histograms were seen in three cases (6.5% false positivity), which showed a specificity of 93.5% (Table 1).

Frieson (49), Cibas (28) and Joseph et al. (27) suggest that the false-negative results that can be produced by FCM are probably a consequence of samples containing only a small quantity of aneuploid neoplastic cells, as 3 to 5% from the examined cells must be aneuploid for their detection as abnormal cells in the G0/G1 phase peak. Perhaps it is important to analyze a...
higher number of events with the goal of increasing the sensitivity.

Some authors refer to FCM sensitivity ranging from 60 to 68%, with specificity ranging between 91.0 and 100.0 % (17, 19, 37, 34, 50). However, others groups, encountered more modest levels of aneuploidy ranging between 43.0 and 48.5 % (20, 47, 51, 52). They suggest that this test can help in the differential diagnosis of effusions and that the low sensitivity is a limiting factor in the usefulness of FCM. The most disappointing results were obtained by Gihlain et al. (41) and by Jones et al. (26), with sensitivity of 23.5 % and 8 % respectively, in peritoneal effusions. Some authors observed higher sensitivity, of about 80%, used the CP examination as the gold standard, thus biasing the final analysis (29, 48).

The results of our report, indicating FCM sensitivity of 57.1 % and specificity 93.5 % are similar to those obtained by other groups (5, 17, 19, 20, 27, 33, 34, 41, 47, 48, 50, 52, 53). As it was observed in our series, some authors report false positive results by FCM ranging from 0.5 to 4.0 % (5, 23, 49). The explanation for this variation include technical issues in samples preparation, more flexible criteria for the diagnosis of aneuploid DNA, and errors in histogram interpretation, as on occasions there is some degree of subjectivity in the interpretation (25, 54).

When comparing DNAI with conventional CP examination in patients with CA, we observed that DNAI was capable of detecting six cases (50 %) of patients with CA whose CP examination had been negative. Thus, as seen in Table 2, combining DNAI with CP examination increases the diagnostic sensitivity in peritoneal carcinomatosis. This evidence reinforces the opinion of some authors who suggest that FCM is important only in the patient population with negative CP examination of their peritoneal fluid (5, 17, 34, 48, 50, 53). However, when the two methods were compared separately or together, we did not observe any statistically significant difference concerning their contribution to diagnostic accuracy in CA. (Table 3). We emphasize the fact that no studies found in the literature analysed this association.

There is a possibility that multiparametric FCM studies increase the diagnostic accuracy in CA. It is a technique that permits the determination of DNA content and of several specific markers of the cell cycle through the use of specific monoclonal antibodies (27, 28, 32, 50). As such, the use of FCM with the determination of proteins related to specific points of the cell cycle, oncoproteins, hormones and growth factors receptors can provide relevant information in specific cancer subtypes (30, 32, 55), and one can envisage that they will be used routinely in the near future.

We conclude from our study that despite the fact that FCM was able to detect cases of malignant effusions which were not diagnosed by CP examination, the low sensitivity shown prevents its widespread use for the screening of effusions and should be used only in the cases where, in addition to a strong clinical evidence of peritoneal involvement by neoplasia, the CP examination of the ascitic fluid is negative.

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