Flow cytometry in the diagnosis of peritoneal carcinomatosis

CRISTIANE TOVO BOTH[1], MD, PhD
ANGELO ALVES DE MATTOS[1], MD, PhD
JORGE NEUMANN[2], MD
MARCIANO D. REIS[3], MD

From the Post-Graduation Course in Hepatology, Santa Casa Hospital[1] and Lab. Transplantation Immunology[2], Porto Alegre, Brazil, and Department of Clinical Pathology, Sunnybrook and Women's College Health Sciences Centre, and Department of Laboratory Medicine and Pathobiology[3], University of Toronto, Toronto, Ontario, Canada

Address:
Rua Aurélio Bitencourt 115/201
CEP 90.430-080, Porto Alegre, RS, Brazil
00 21 55 51 33353349
00 21 55 51 32284346
both@zaz.com.br

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ABSTRACT

Objectives: 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, determinded 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 cytopathological 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 cytopathological 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 cytopathological 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 cytopathological 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 cytopathological examination. However, DNA index was able to detect 50.0% of peritoneal carcinomatosis whose conventional cytopathological 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 alities 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 cytopathological 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 establised by histopathological examination in 11 patiens and by echographic-clinical-laboratorial data in 23 patients.

In 6 cases in wich hepatic tumor was detected, peritoneal involvement was excluded by paparoscopic examination. In the other remainig cases with NCA ascites, peritoneal neoplasic 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 cytopathological investigation 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 Papanicolaou 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.

The material was then put on ice bath while the freezing solution was prepared: 1.5 ml of Hanks solution + 1.0 ml of dimethylsulfoxide (DMSO) + 2.5 ml of FCS. The cells were frozen at -80^{0}C for 24 h and then transferred to liquid N_2.

The DNA analysis by FCM was performed in the Department of Clinical Pathology, Sunnybrook and Women's College Health Sciences Centre, using a Coulter EPICS XCLâ instrument. For this analysis, the samples were quickly thawed, washed with Hanks solution, and the resultant cell pellet was resuspended in 200 ml of Hanks solution, filtered twice, and the cells were counted again. A tube containing 1 x 10^6 chicken lymphocytes and a tube containing 5 ml chicken erythrocytes were prepared for equipment calibration. An Eppendorf type tube with 1 x 10^6 cells from the patient and another with cells from the patient and control lymphocytes in a 4:1 mix ratio were prepared. The Coulter DNA-prepâ reagents were used as indicated by the manufacturer. After data acquisition, analysis was done employing the Multicycleâ program the accuracy and the method resolution were guided by the use of the variation coefficient (CV) of less than 3,0 %.

The tumor cells ploidy was determined by the average DNA quantity of the cells of a neoplastic population that are at the G0/G1 phase compared to a normal quantity of a similarly processed control sample. This rate can be determined by the following equation (18,24,25,26):

$$\text{DNA index (DNAI)} = \frac{\text{DNA quantity of the testing G0/G1 cells peak}}{\text{DNA quantity of the standard G0/G1 cells peak}}$$

The DNA histograms were classified as aneuploid, diploid, hypodiploid, tetraploids or hypertetraploids based on the DNA quantity related to the normal control. DNA aneuploidy was defined as the presence of two different peaks of the G0/G1 phase in the histogram. In this study a safety limit of 10 % was used (27). So, a histogram was considered diploid when the DNAI was equal to 1.0 (oscillating from 0.9 to 1.1), hypodiploid when less than 0.9, tetraploid when 2.0 (varying from 1.8 to 2.2) or hypertetraploid when higher than 2.2.

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 categoric variables, we used the Pearson X^2 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 lymphangioleiomiomatosis.

The mean SAAG was 0.77 ± 0.36 g/dl in the CA group and 1.81 ± 0.64 g/dl in the NCA gropu ($p<0.05$), and in all the CA patiens, 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 09/21 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.

There was no statistically significant difference between accuracy and usefulness of DNAI and CP when used individually or in association.

DISCUSSION

FCM is a method that can evaluate a great number of cells in a short period of time and it could be complementary to the cytopathological examination (28,29,30,31). One of its most accepted applications is the measurement of DNA content which allows the identification of cell populations with abnormal quantities of DNA (aneuploidy) and provides information on the cellular proliferative activity by analyzing the distribution of cells in their different phases of the cell cycle (26, 28, 31, 32, 33).

Recently, the analysis of the DNA aneuploidy through FCM has been used in an attempt to increase the accuracy of the analysis of effusions by cytology (16, 17, 20, 21, 22, 34, 35, 36, 37, 38, 39) and it is able to identify abnormal cells populations not recognized by conventional cytopathological examination (4, 16, 21, 22, 34, 35, 36, 37, 38). However, the greatest limitation to the widespread application of this method is the lack of concordance amongst the published studies (6).

The cytopathologic examination of the sediment of effusion has for many years been the gold standard for detecting the presence of neoplasms (20,40). It generally presents a low sensitivity and a high specificity (5, 7, 8, 9, 41, 42, 43). The increased sensitivity observed in some studies is obtained with a decrease in specificity, which is far from ideal (15, 23, 44, 45, 46). In the present study, conventional CP examination produced a sensitivity of 42% and a specificity of 100%, in accordance with many other studies (5, 7, 8, 9, 41, 42, 43).

We observed that despite a high average DNAI, this did not result in significant differences between the CA and NCA groups, regardless of wheter 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 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

Table 1 – DNAI in patients with and without CA

DNAI	CA n (%)	NCA n (%)
Altered (<0.9 or >1.1)	12 (57.1)	3 (6.5)
Normal (0.9 – 1.1)	09 (42.9)	43 (93.5)
Total	21 (100.0)	46 (100.0)

Fisher test: p = 0.00001 (p<0.05)

Table 2 – DNAI associated to CP examination in the cases of CA

	CA n (%)	NCA n (%)
Altered DNAI and /or CP	15 (71.4)	03 (6.5)
Normal DNAI and CP	06 (28.6)	43 (93.5)
Total	21 (100.0)	46 (100.0)

X^2 Pearson = 30.91; p = 0.000004 (p< 0.05)

Table 3 – Comparison between methods (DNAI and CP) when used individually or in association

	DNAI	CP	DNAI +CP	p
sensitivity (%)	57.1	42.9	71.4	0.174
specificity (%)	93.5	100.0	93.5	0.246
+ PV (%)	80.0	100.0	83.0	0.460
– PV (%)	82.7	79.3	87.8	0.509

PV = predictive value

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 Ghilain 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, 38, 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 efusions 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.

REFERENCES

1. BARLOGIE B, DREWINKO B, SCHUMANN J, GOHDE W, DOSIK G, LATREILLE F, et al. Cellular DNA content as a marker of neoplasia in man. Am J Med 1980;69:195-203.
2. DEWALD G, DINES DE, WEILAND LH, GORDON H. Usefulness of chromossome examination in the diagnosis of malignant pleural effusions. N Engl J Med 1976;295:1494-1500.
3. GIVAN A L. Flow cytometry: first principles. New York: Wiley-Liss, 1992:103-133: Cells from within: DNA and molecular biology.
4. HEDLEY DW, SHANKEY TV, WHEELESS LL. DNA cytometry consensus conference. Cytometry 1993; 14:471.
5. JONES MA, HITCHCOX S, D'ASCANIO P, PAPILLO CT, TARRAZA HM. Flow cytometric DNA analysis versus cytology in the evaluation of peritoneal fluids. Gynecol Oncol 1991; 43:226-232.
6. SHANKEY TV, RABINOVITCH PS, BAGWELL B, BAUER KD, DUQUE RE, HEDLEY DW, et al. Guidelines for implementation of clinical DNA cytometry. Cytometry 1993; 14:472-477.
7. MATTOS AA. Estudo do líquido de ascite, contribuição da biópsia peritoneal às cegas. Porto Alegre: Faculdade de Medicina da Universidade Federal do Rio Grande do Sul, 1981. 104 p. (Dissertação, Mestrado em Gastroenterologia).
8. _____. Diagnóstico da ascite. propostas propedêuticas e contribuição do exame citopatológico do peritôneo. São Paulo: Escola Paulista de Medicina, 1985. 121 p. (Tese, Doutorado em Gastroenterologia).
9. MATTOS AA, LIMA JP. Estudo do líquido de ascite. Rev AMRIGS 1983; 27:294-301.
10. RUNYON BA. Ascites and spontaneous bacterial peritonitis. In: Sleisenger MH, Fordtran JS Gastrointestinal Disease. 6. ed. Philadelphia: WBSC, 1998:1310-1333.
11. BERGE T, HELLSTEN S. Cytological diagnosis of cancer cell in pleural and ascitic fluid; comparision of results obtained by pathologist and cytologist. ActaCcyto 1966;10:138-140.
12. DECKER A, BUPP PA. An investigation into the usefulness of cell block versus smears. Am J Clin Pathol 1978; 70:855-860.
13. JOHNSON WD. The cytological diagnosis of cancer in serum effusions. Acta Cyto 1966;10:161-172.
14. KOSS LG. Diagnostic cytology and its histological bases. 3. ed. Philadelphia: J.B. Lippincott, 1979:911-970: Efusion in the presence of cancer.
15. LUSE AS, REAGAN JW. A histocytological study of effusions. effusions associated with malignant tumors. Cancer 1954;7:1167-1181.
16. EVANS DA, Thornthwaite JT, Ng ABP. DNA flow cytometry of pleural effusions. comparision with pathology for the diagnosis of malignancy. Anal Quant Cytol Histol 1983;5:19-27.
17. RIJKEN A, DEKKER A, TAYLOR S, HOFFMAN P, BLANK M, KRAUSE JR. Diagnostic value of DNA analysis in effusions by flow cytometry and image analysis. Am J Clin Pathol 1991;95:6-12.
18. ROSS JS. DNA ploidy and cell cycle analysis in pathology. New York: Igaku Shoin Medical Publication, 1996.
19. SAHNI K, TRIBUKAIT B, EINHARN N. Flow cytometric measurement of ploidy and proliferation in effusions of

ovarian carcinoma and their possible prognostic significance. Gynecol Oncol 1989;35:240-245.
20. SCHNELLER J, EPPICH E, GREENEBAUM E. Flow cytometry and feulgen cytophotometry in evaluation of effusions. Cancer 1987;59:1307-1313.
21. UNGER K, RABER M, BEDROSAIN CWM, STEIN DA, BARLOGIE B. Analysis of pleural effusions using automated flow cytometry. Cancer 1983; 52:873-877.
22. WEISSMAN GS, MCKINLEY MJ, BUDMANN DR, CACCESE WJ, SCHULMAN P, GRUENEBERG D, et al. Flow cytometry-a new technique in the diagnosis of malignant ascites. J Clin Gastroenterol 1987; 9:599-602.
23. ZARBO RJ. Flow cytometric DNA analysis of effusion (editorial). Am J Clin Pathol 1991; 95:2-4.
24. PROLLA G, PREGER R, FLECK J. A cinética celular. In: Fleck J. Câncer: integração clínico-biológica. Rio de Janeiro: Medsi, 1992:123-142.
25. RILEY RS, MAHIN EJ, ROSS W. Clinical applications of flow cytometry. New York: Igaku Shoin Medical Publication, 199:3251-322: DNA ploidy and cell cycle analysis.
26. SHAPIRO HM. Practical flow cytometry. 3. ed. New York: Wiley-Liss, 1995:367-428: Using flow cytometers.
27. JOSEPH MG, BANERJEE D, HARRIS P, GIBSON S, MCFADDEN RG. Multiparameter flow cytometric DNA analysis of effusions: a prospective study of 36 cases compared with routine cytology and immunohistochemistry. Mod Pathol 1995;8:686-693.
28. CIBAS ES. Applications of flow cytometric DNA analysis to diagnostic cytology. Diagn Cytopathol 1995; 13:166-171.
29. FUHR JE, KATTINE AA, SULLIVAN TA, NELSON HS. Flow cytrometric analysis of pulmonary fluids and cells for the detection of malignancies. Am J Pathol 1992;141:211-215.
30. GREEN LK, GRIFFIN J. Increased natural killer cells in fluids. a new, sensitive means of detecting carcinoma. Acta Cytol 1996; 40:1240-1245.
31. O'LEARY TJ. Flow cytometry in diagnostic cytology. Diagn Cytopathol 1998; 18:41-46.
32. ORFAO A, CIUDAD J, GONZALEZ M, LOPEZ M, DEL MAR ABAD M, PAZ BOUZA JI, et al. Flow cytometry in the diagnosis of cancer. Scand J Clin Lab Invest 1995; 221:145-152.
33. SHAPIRO HM. Practical flow cytometry. 3. ed. New York: Wiley-Liss, 1995:1-32: Overture.
34. CEYHAN BB, DEMIRALP E, CELIKEL T. Analysis of pleural effusions using flow cytometry. Respiration 1996;63:17-24.
35. HEDLEY DW PHILIPS J, RUGG CA. Measurement of cellular DNA content as an adjunct to diagnostic cytology in malignant effusions. Eur J Cancer Clin Oncol 1984;20:749-752.
36. HOSTMARK J, VIGANDER T, SKAARLAND E. Characterization of pleural effusions by flow-cytometric DNA analysis. Eur J Respir Dis 1985;66:315-319.
37. KAPUSTA L, ZBIERANOWSKI I, DEMIANIUK C. The role of DNA flow and image cytometry in the evaluation of body cavity fluids. Anal Quant Cytol Histol 1991;13:187-192.
38. KATZ RL, JOHNSON TS, WILLIAMSON KD. Comparision of cytologic and acridine-orange flow-cytometric detection of malignant cells in human body cavity fluids. Anal Quant Cytol Histol 1985;7:227-235.
39. SINTON EB, CARVER RK, MORGAN DL. Prospective study of concurrent ploidy analysis and routine cytopathology in body cavity fluids. Arch Pathol Lab Med 1990;114:188-194.
40. TAVEL ME. Ascites: etiologic considerations with emphasis on the value of several laboratory findings in diagnosis. Am J Med Sci 1959;237:727-733.
41. GHILAIN JM, HENRION J, SCHAPIRA M, MAJOIS F, BEAUDIN M, HELER FR. Le liquid d'ascite: intérêt de divers tests biologiques dans le diagnostic différential entre ascite cirrhotique et néoplasique. Acta Gastroenterol Belg 1990;53:168-179.
42. MATTOS AA. Ascite. Porto Alegre: Byk, 1994.
43. McGUCKIN WF, SOULE EH, CAIN JC. The value of glycoprotein determination and cytology in the study of ascitic fluid. Gastroenterology 1959;37:332-338.
44. CEELEN GH. The cytologic diagnosis of ascitic fluid. symposium on diagnostic accuracy of cytologic technics. Acta Cytol 1964;8:165-185.
45. LUSE AS. A hystocytologic and electron microscopic study of effusions associated with malignant disease. Ann N Y Acad Sc 1956;63:1331-1347.
46. VON HAAM E. A comparative study of the accuracy of cancer cell detection by cytological methods. Acta Cytol 1962; 6:508-518.
47. BANKS ER, JENNINGS CD, JACOBS S, DAVEY DD. Comparative assessment of DNA analysis in effusions by image analysis and flow cytometry. Diagn Cytopathol 1994;10:62-67.
48. BARDALES RH, STANLEY MW, SCHAEFER RF, LIBLIT RA, OWENS RB, SURHLAND MF. Secondary pericardial malignancies. a critical appraisal of the role of cytology, pericardial biopsy and DNA ploidy analysis. Am J Clin Pathol 1996; 106:29-34.
49. FRIERSON HF. Ploidy and proliferative fraction analysis of cytologic specimens. In: Keren DF, Hanson CA, Hurtubise PE. Flow cytometry and clinical diagnosis. Chicago: American Society of Clinical Pathologists, 1994:596-613.
50. SIKORA J, DVORACKI G, ZEROMNSKI J. DNA ploidy, S-phase and Ki-67 antigen expression in the evaluation of cell content of pleural effusions. Lung 1996;174:303-313.
51. KEHOE S, WARD K, LUESLEY D, CHAN KK. The application of flow cytometric DNA analysis in detecting the presence of malignant cells in ovarian carcinoma peritoneal fluids. Br J Obstet Gynaecol 1995; 102:656-659.
52. LEE JS, LEE MC, PARK CS, JUHNG SW. Diagnostic value of p53 protein and flow cytometric DNA analysis in the study of serous effusions. Acta Cytol 1997; 41:1719-1725.
53. CLARK GM, MATHIEU MC, OWENS MA, DRESSLER LG, EUDEY L, TORMEY DC, et al. Prognostic significance of S-phase fraction in good-risk, node-negative breast cancer patients. J Clin Oncol 1992; 10:428-432.
54. JOENSUU H, KALLIONIEMI OP. Different oppinions on classification of DNA histograms produced from paraffin-embedded tissue. Cytometry 1989;10:711-717.
55. SHAPIRO H.M. Practical flow cytometry. 3. ed. New York: Wiley-Liss, 1995:43-74: Hystory.