FLOW CYTOMETRY IN COLON CANCER: DOES FLOW CYTOMETRIC CELL CYCLE ANALYSIS HELP PREDICT FOR SHORT-TERM RECURRENCE IN PATIENTS WITH COLORECTAL CARCINOMA?

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The purpose of this prospective study was to determine whether tumor ploidy or S-phase fraction measurements were of prognostic value in predicting short-term recurrence for colorectal carcinoma. A total of 52 patients underwent curative resection of colorectal carcinoma. Fresh suspensions of tumor cells were used for flow cytometric analysis. Patients underwent follow-up for possible recurrence, which then was related to ploidy status, S-phase fraction measurement, adjuvant therapy received, and Dukes stage. Disease-free probability was determined using Kaplan-Meier actuarial curves for various subgroups of the study populations. Results revealed that elevated S-phase fraction (>16%) did predict for a higher probability of recurrence in those patients receiving no postoperative adjuvant treatment. Among patients receiving postoperative adjuvant therapy, however, a higher S-phase fraction predicted for a lower probability of recurrence. These data suggest that the S-phase fraction does predict for disease-free probability and also predicts response to adjuvant therapy in patients with colorectal carcinoma. (*J Natl Med Assoc.* 1995;87:803-806.)

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Large-bowel carcinomas represent a rather morphologically uniform group of tumors that show great clinical variability and unpredictability.[1] Traditionally, Dukes stage, which includes lymph node metastases, has been used to identify subgroups of patients with different prognoses. Other prognostic variables such as size, location, nuclear grade, angioinvasion, and carcinoembryonic antigen levels, also have been used to predict the likelihood of recurrence.

Recently, a number of new variables have been tested for their potential prognostic value.[2,3] The determination of DNA ploidy status by flow cytometry has emerged as a potential prognostic aid in a variety of human malignancies. However, previous retrospective studies in which DNA content was measured in specimens retrieved from paraffin-embedded tissue blocks have not been conclusive with regard to recurrence or survival.[4-6] The study described here was performed on fresh suspensions of tumor cells to evaluate the prognostic significance of tumor ploidy and S-phase fraction measurements in predicting short-term recurrence for colorectal carcinoma.

MATERIALS AND METHODS

Patients

The initial study population included 56 patients who underwent primary colon or rectal carcinoma resection at St Joseph Mercy Hospital between 1987 and 1988. All patients underwent curative resections, and fresh tissue specimens were evaluated using flow cytometry. Four

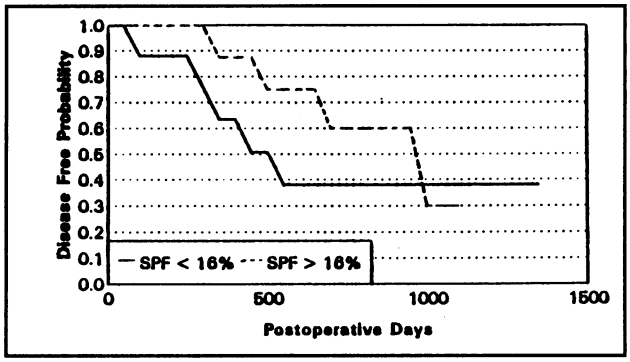

Figure 1. Tumor ploidy for all colorectal cancer.

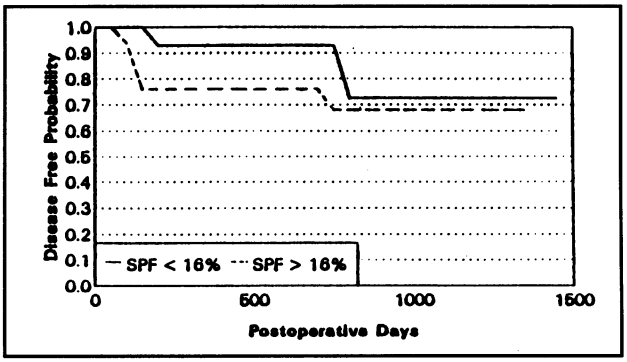

Figure 2. S-phase fraction for untreated colorectal cancer.

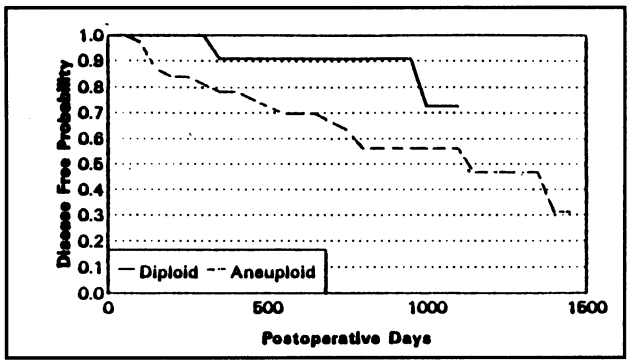

Figure 3. S-phase fraction for treated colorectal cancer.

patients were eliminated from the study due to a less than 30-day survival. The remaining 52 patients formed the basis of the study and underwent prospective follow-up for evaluation of short-term recurrence and survival.

DNA Analysis

Samples of non-necrotic tissue were obtained from several grossly abnormal areas of the tumor to include a final wet mass of approximately 0.5 g to 1.0 g. Approximately 0.1 g of grossly normal adjacent colonic mucosa was stripped free for use as an internal diploid DNA standard. Cells were harvested by mechanical disaggregation or fine-needle aspiration. Two separate aliquots of approximately 6×10^6 tumor cells were prepared for each tumor, and to one of these, approximately 20×10^3 cells representing a mixture of chicken and trout erythrocyte nuclei were added as additional internal DNA calibrators.

Both samples were processed immediately and stained according to the method of Vindelov et al.[7] The samples were analyzed on an FACScan flow cytometer equipped with an electronic doublet discriminator (Becton Dickinson Immunocytometry Systems, Mountain View, California). Histograms were collected on approximately 20,000 nuclei from each aliquot. The list mode data files then were ported to a 33-MHz 80386 desktop computer and analyzed using ModFit DNA histogram modeling software (Verity Software House, Topsham, Maine).

Tumor ploidy was determined using the aliquot containing the chicken and trout cells. The aliquot containing only colonic cells was used to measure S-phase fraction. A conservative rectangular model, which included background debris subtraction, was used to estimate S-phase fraction.

Statistical Analysis

Data were analyzed using Kaplan-Meier actuarial curves that were constructed for various subgroups of the study population. The Wilcoxon sign rank test of significance was used to evaluate the differences between the Kaplan-Meier curves. Fisher's exact test also was used to evaluate 2×2 contingency tables for patients with at least 2 years of follow-up.

RESULTS

All of the resected tumors from the 52 patients were adenocarcinomas. Patients ranged in age from 43 to 90 years. Ploidy determinations revealed 40 patients with aneuploid lesions and 12 patients with diploid lesions.

There were 33 males and 19 females (male:female ratio of 1.7:1). The majority of the tumors were right-sided, with 25 tumors occurring in the cecum and ascending colon, 1 lesion in the transverse colon, 6 tumors in the left colon, and 15 tumors in the sigmoid colon, and 5 lesions in the rectum. There were 8 (15%) patients with Dukes A lesions, 25 (48%) patients with Dukes B lesions, and 19 (36%) patients with Dukes C lesions.

Most of the patients did not receive adjuvant therapy postoperatively (65%); however, of the 18 patients who did, 6 (33%) were female and 12 (67%) were male. Postoperative adjuvant therapy consisted of chemotherapy

(three patients), radiation therapy (two patients), and combined chemotherapy and radiation therapy (three patients).

Among a subgroup of patients with a minimum of 2 years of clinical follow-up (n=42), ploidy alone was not a significant predictor of short-term recurrence ($P>.05$). Among the entire study population, however, ploidy did significantly predict for probability of short-term recurrence ($P<.0001$) (Figure 1).

The median S-phase fraction for the entire study population was 16%. In patients receiving no postoperative adjuvant treatment, elevated S-phase fraction (>16%) predicted for higher probability of recurrence ($P=.0156$) (Figure 2). Among patients receiving postoperative adjuvant treatment, a higher S-phase fraction (>16%) predicted for a lower probability of recurrence (Figure 3).

DISCUSSION

Previous flow cytometric DNA studies of colorectal carcinoma have given variable degrees of correlation.[8-11] The majority of these studies were retrospective, and DNA content was measured in specimens retrieved from paraffin-embedded tissue blocks. Wolley et al[12] gave the first prospective report on the prognostic value of flow cytometric DNA determinations in colorectal carcinoma and suggested that the DNA content of the tumor is important for the prognosis. It was unclear, however, if tumor ploidy was a manifestation of advanced disease or other known prognostic variables.

High S-phase values indicate that a large proportion of cells are proliferating, resulting in rapid tumor growth. The finding that aneuploidy in colorectal carcinoma is related to S-phase values may indicate that the S-phase value rather than the ploidy level is the factor governing prognosis.[9] Therefore, DNA content of a tumor could be a secondary reflection of tumor growth rate.

Cytometric means of measuring S-phase values are technically difficult when compared with the relatively simple measurement of tumor ploidy. For this reason, there is often great interlaboratory variability in determining S-phase fraction. Normal ranges for each laboratory performing this test must be established based on that particular laboratory's methods and patient population. The S-phase value, however, has clearly demonstrated prognostic value in tumors of the breast and lymphatic system. It also has been shown to be useful in predicting response to therapy in head and neck malignancies. Although the independence of ploidy as a significant prognostic indicator was not realized in a subgroup of our study population with 2 years of follow-up, it is clear that DNA content and S-phase fraction determinations reveal important information about the biologic behavior of colorectal carcinomas.

The measurement of DNA ploidy has been shown to be an independent prognostic variable for predicting both the likelihood of recurrent disease and tumor-related death when the data are adjusted for other clinical and pathological characteristics, including gender, serum carcinoembryonic antigen, size, grade, and stage of tumor.[8] It also must be noted that there is a lack of consensus with regard to the prognostic value of DNA ploidy status. Rognum et al[13] and Melamed et al[14] found no significant differences in survival between patients with DNA diploid and DNA aneuploid tumors. Goh et al[15] reported that the prognostic significance of DNA ploidy was of little value when included in a regression analysis model. These conflicting results may be due to the relatively small number of patients studied or differing laboratory techniques. Also, studies using paraffin-embedded material may give a less than optimal interpretation of collected data.

CONCLUSION

These data suggest that the S-phase fraction does, in fact, predict for short-term disease-free probability in patients with colorectal carcinoma. The S-phase fraction also may identify those patients who would most likely benefit from postoperative adjuvant therapy. Technical refinements in S-phase fraction measurement may improve the predictive potential of this unique variable further. However, the results of this study are based on a small group of patients, and larger series are needed to determine the relative significance of ploidy and S-phase fraction to other variables.

Literature Cited

1. Wiggers T, Arends JW, Schatte B, Volovies L, Bosman F. A multivariate analysis of pathologic prognostic indicators in large bowel cancer. *Cancer*. 1988;61:386-395.

2. Scott NA, Rainwater LM, Wieand HS. The relative prognostic value of flow cytometric DNA analysis and conventional clinicopathologic criteria in patients with operable rectal carcinoma. *Dis Colon Rectum*. 1987:513-520.

3. Bauer KD, Lincoln ST, Vera-Roman JM, Wallemark CB, Chmiel JS, Madurski ML, et al. Prognostic implications of proliferative activity and DNA aneuploidy in colonic adenocarcinomas. *Lab Invest*. 1987;57:329.

4. Halvorsen TB, Johannesen E. DNA ploidy, tumor site and prognosis in colorectal cancer. A flow cytometric study of paraffin-embedded tissue. *Scand J Gastroenterol*. 1990;25:141-149.

5. Robey-Cafferty S, El-Naggar AK, Grignon DJ. Histologic parameters and DNA ploidy as predictors of survival in stage B adenocarcinoma of the colon and rectum. *Mod Pathol*. 1990;3:261-265.

6. Scivetti P, Danova M, Riccardi A. Prognostic significance of DNA content in large bowel carcinoma: a retrospective flow cytometric study. *Cancer Lett*. 1989;49:213-219.

7. Vindelov LL, Christensen IJ, Nissen NI. A detergent-trypsin

method for the preparation of nuclei for flow cytometric DNA analysis. *Cytometry*. 1983;3:323-327.

8. Kokal W, Sheibani K, Terz J, Harada JR. Tumor DNA content in prognosis of colorectal carcinoma. *JAMA*. 1986;255:3123-3127.

9. Emdin SO, Stenling R, Roos G. Prognostic value of DNA content in colorectal carcinoma. *Cancer*. 1987;60:1282-1287.

10. Quirke P, Dixon MF, Clayden AD. Prognostic significance of DNA aneuploidy and cell proliferation in rectal adenocarcinomas. *J Pathol*. 1987;151:285-291.

11. Jones DJ, More M, Schofield PF. Prognostic significance of DNA ploidy in colorectal cancer: a prospective flow cytometric study. *Br J Surg*. 1988;75:28-33.

12. Wolley RC, Schreiber K, Koss LG, Karas M. DNA distribution in human colon carcinomas and its relationship to clinical behavior. *J Natl Cancer Inst*. 1982;69:15-22.

13. Rognum TO, Thorud E, Lund E. Clinical behavior in large bowel carcinoma patients with different DNA ploidy pattern. Presented at the 14th International Cancer Congress; 1986; Budapest. Abstract 2439.

14. Melamed MR, Enker WE, Banner P, Janov AJ, Kessler G, Daryznkiewicz A. Flow cytometry of colorectal cancer with three year follow-up. *Dis Colon Rectum*. 1986;29:184-186.

15. Goh HS, Jass JR, Atkin WS, Cuzick J, Northover JMA. Value of flow cytometric determination of ploidy as a guide to prognosis in operable rectal cancer: a multivariate analysis. *Int J Colorectal Dis*. 1987;2:17-21.