Character of the Invasive Margin in Colorectal Cancer

Does It Improve Prognostic Information of Dukes Staging?

Fabio Cianchi, M.D.,* Luca Messerini, M.D.,† Annarita Palomba, M.D.,†
Vieri Boddi, B.S.,‡ Giuliano Perigli, M.D.,* Filippo Pucciani, M.D.,*
Paolo Bechi, M.D.,* Camillo Cortesini, M.D.*

*From the *Clinica Chirurgica Generale, the †Istituto di Anatomia ed Istologia Patologica, and the ‡Istituto di
Patologia Generale, Universita' di Firenze, Florence, Italy*

PURPOSE: The clinical significance and prognostic value of
the histopathologic parameters used in both the Dukes and
Jass classifications were evaluated to select those with an
independent effect on survival after radical surgery for colo-
rectal cancer. METHODS: The depth of local spread (limited
to the bowel wall or extended beyond it), the number of
metastatic lymph nodes (none, 1-4, more than 4), the
character of the invasive margin (pushing or infiltrating),
and the presence or absence of conspicuous peritumoral
lymphocytic infiltration were assessed in 235 patients who
had undergone radical resection for colorectal cancer. The
influence of these variables on survival was studied by
univariate and multivariate analysis. RESULTS: No signifi-
cant difference in survival was found between patients with
conspicuous peritumoral infiltrate and those without it;
moreover, multivariate analysis failed to show any indepen-
dent prognostic value for either lymphocytic infiltration or
depth of local invasion. However, the character of the
invasive margin and the number of metastatic lymph nodes
were identified as the only variables with any independent
importance on survival. Based on these data, a new prog-
nostic model may be proposed; it uses the character of the
infiltrative margin as a discriminating factor among patients
within the lymph node-negative (Dukes A and B stages) and
lymph node-positive (Dukes C1 and C2 subsets) groups. A
good prognosis for Dukes A, B, and C1 patients was asso-
ciated with pushing tumors; C1 and C2 patients with infil-
trating tumors had a poor prognosis. On the whole, the new
prognostic model has allowed for the placement of 59.6
percent of our patients into groups that provide a confident
prognosis. The clinical outcome of Dukes A and B patients
with infiltrating tumors is still uncertain. CONCLUSIONS:
The character of the invasive margin is an important prog-
nostic factor in colorectal cancer. The association of this
parameter with the traditional Dukes classification may pro-
vide additional useful prognostic information and aid in the
selection of those patients who could most benefit from
adjuvant therapy. [Key words: Colorectal cancer; Dukes
staging; Jass system; Prognosis]

Cianchi F, Messerini L, Palomba A, Boddi V, Perigli G,
Pucciani F, Bechi P, Cortesini C. Character of the invasive
margin in colorectal cancer: does it improve prognostic
information of Dukes staging? Dis Colon Rectum 1997;40:
1170-1176.

Address reprint requests to Dr. Cianchi: Istituto Clinica Chirurgica
Generale e D.C., Viale Morgagni, 85 50134 Firenze, Italy.

The Dukes classification, including recent modifi-
cations,[1,2] is still widely used in the prognostic
evaluation of patients operated on for colorectal can-
cer, because of its simplicity and good relationship
with survival. Nevertheless, the most important weak-
ness of this classification is the small number of pa-
tients who can be included in well-predicted prog-
nostic groups. Jass and coworkers[3] developed a new
classification for rectal cancer (also commonly used
for colonic cancer) that allows for identification of a
larger number of patients within groups and that pro-
vides a confident prediction of clinical outcome.
However, many doubts have recently been raised
concerning the reproducibility of one of the Jass
pathologic components, the degree of peritumoral
lymphocytic infiltration as expressed by the distinc-
tion between a "conspicuous" and a "less conspicu-
ous" infiltrate. Because this distinction primarily de-
pends on the pathologist's interpretation, this
parameter remains potentially subjective.

The aim of this study was to evaluate the clinical
significance and prognostic value of the histopatho-
logic parameters used in both the Dukes and Jass
classifications. Furthermore, those parameters with an
independent effect on survival were clearly identified
so the largest number of patients possible could be
placed in well-defined groups with either good or
poor prognoses.

MATERIALS AND METHODS

Between 1987 and 1993, 306 patients seen at our
Operative Unit were histologically diagnosed as hav-
ing colorectal adenocarcinoma. Two hundred thirty-
five subjects, 145 males (61.7 percent), with a median
age of 66 (range, 36–88) years, underwent radical
surgical resection by one surgeon (CC). A retrospec-
tive study was performed on the clinical outcome of

these patients. One hundred thirty-one patients (55.7 percent) had neoplasms of the colon; 104 (44.3 percent) had neoplasms of the rectum. Operations were defined as radical when there was no evidence of distant metastases or incomplete clearance of tumor. No adjuvant therapeutic protocols were performed. Average duration of follow-up for surviving patients was 53.4 (range, 25–108) months. Patients who had died within 30 days of surgery were excluded from the study.

For each colorectal tumor specimen, the following histopathologic variables were assessed by the same pathologist (LM): depth of direct spread (limited to the bowel wall or extended beyond it); number of positive lymph nodes (none, 1–4, more than 4); character of the invasive margin (pushing or infiltrating), and the presence or absence of conspicuous peritumoral lymphocytic infiltration. The last two parameters were assessed according to criteria as defined by Jass et al.[4] In particular, the character of the invasive margin was classified as pushing when advancement of the tumor was clearly evident and the tumor had pushed the surrounding tissues, thus creating a well-delineated border. It was defined as infiltrating when the cancer cell spread in the surrounding tissues had no distinct border.

The relationship of these variables with survival was analyzed using the Kaplan-Meier survival method.[5] Cox model analysis,[6] in a stepwise regression, was used to identify those factors that influenced survival independently.

All patients were staged according to Dukes classification as modified by the Gastrointestinal Tumor Study Group[7] as follows: Stage A, tumors confined to the bowel wall; Stage B, tumors extending beyond the wall and lymph nodes negative; Stage C1, invasion of one to four lymph nodes by the tumor, irrespective of extent of local spread; Stage C2, more than four lymph nodes involved by tumor, irrespective of extent of local spread. This classification took into consideration the prognostic importance of the subdivision of Dukes Class C, which is based on the number of positive lymph nodes.[3, 8, 9] We also classified our patients according to the prognostic scoring system as proposed by Jass et al.,[3] which is based on the results of four histopathologic variables that we had examined. Each of the four variables was given an arbitrary score, which could add up to a maximum of five. The total score was then assigned to a prognostic group (Table 1). The relationship between the Dukes and Jass groups and survival was estimated using the

Table 1.
Jass Scoring Prognostic System

Variable	Score
Depth of direct spread	
Limited to the bowel wall	0
Beyond the bowel wall	1
No. of positive lymph nodes	
None	0
1–4	1
>4	2
Character of invasive margin	
Pushing	0
Infiltrating	1
Conspicuous peritumoral lymphocytic infiltration	
Present	0
Absent	1

Total score	Group
0–1	I
2	II
3	III
4–5	IV

Kaplan-Meier method. All survival curves were compared using the Cox-Mantel text.

RESULTS

Clinical and pathologic data relating to the 235 patients are summarized in Table 2. When all 235 patients were considered, the five-year survival rate was 63.1 percent. Forty-four patients (18.7 percent) had Stage A, 116 (49.4 percent) had Stage B, 58 (24.7 percent) had Stage C1, and 17 (7.2 percent) had Stage C2. The five-year survival rates were 87.4 percent for patients with Stage A, 70 percent with Stage B, 48 percent with Stage C1, and 0 percent with Stage C2 ($P < 0.0001$; Fig. 1). Using the Jass classification,[3] 51 patients (21.7 percent) fell into Group I, 55 (23.5 percent) into Group II, 80 (34 percent) into Group III, and 49 (20.8 percent) into Group IV. Five-year survival rates were 91.6, 71.7, 67.8, and 22.1 percent ($P < 0.0001$) for each group, respectively (Fig. 2).

In evaluating survival with respect to the type of invasive margin, 76 patients (32.4 percent) with pushing tumors had an 89 percent five-year survival rate, whereas 159 patients (67.6 percent) with infiltrating tumors had a 50.4 percent five-year survival rate ($P < 0.0001$; Fig. 3). The five-year survival rate of the 160 patients (68.1 percent) with no metastatic lymph nodes was 75.3 percent, whereas the survival rates of patients with one to four metastatic lymph nodes and those with more than four were the same as those

Table 2.
Clinical and Pathologic Features in 235 Patients with Colorectal Cancer

	No.	%
Gender		
Male	145	61.7
Female	90	38.3
Tumor site		
Colon	131	55.7
Rectum	104	44.3
Direct spread		
Within the wall	55	23.4
Beyond the wall	180	76.6
Positive lymph nodes		
None	160	68.1
1–4	58	24.7
>4	17	7.2
Lymphocytic infiltration		
Present	45	19.2
Absent	190	80.8
Type of invasive margin		
Pushing	76	32.4
Infiltrating	159	67.6
Dukes stage		
A	44	18.7
B	116	49.4
C1	58	24.7
C2	17	7.2
Jass stage		
I	51	21.7
II	55	23.5
III	80	34.0
IV	49	20.8

Figure 2. Survival of 235 patients with colorectal cancer according to Jass classification.
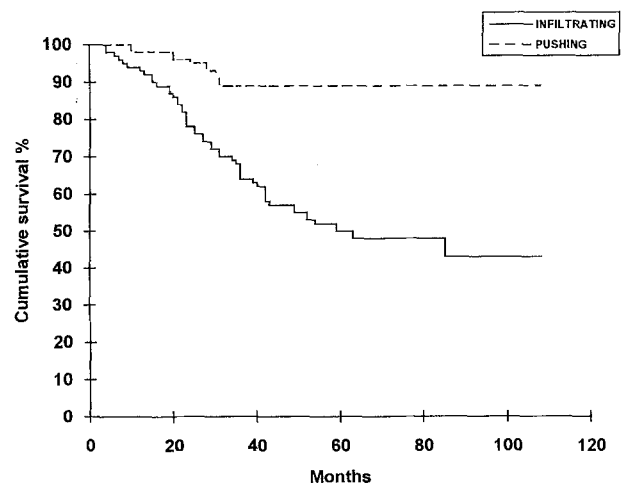
Figure 3. Survival of 235 patients with colorectal cancer according to type of invasive margin.
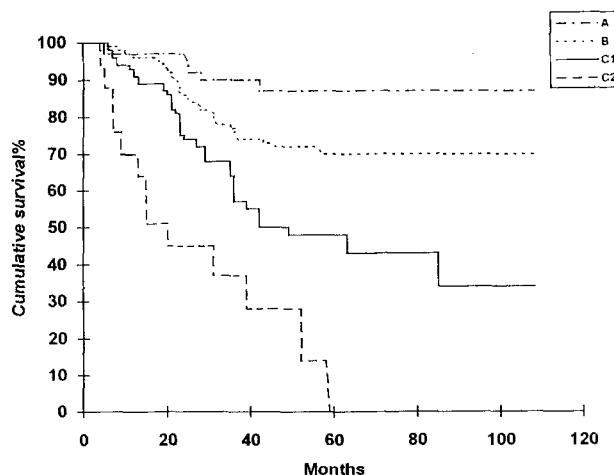

Figure 1. Survival of 235 patients with colorectal cancer according to Dukes stage.

who had been classified as C1 and C2, respectively ($P < 0.0001$). With regard to direct spread, the five-year survival rate of the 55 patients (23.4 percent) with tumors confined to the bowel wall was 83.2
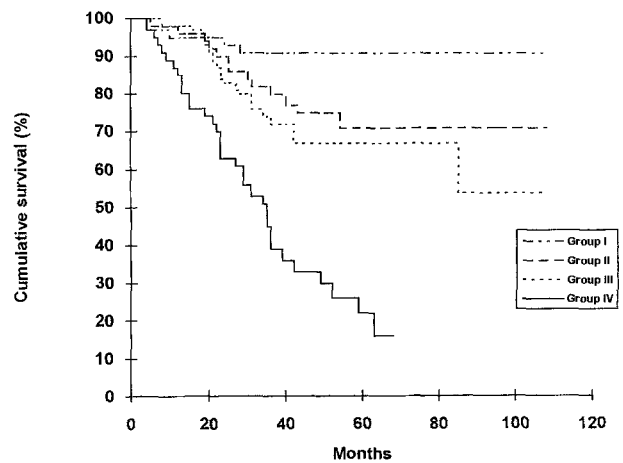
percent, whereas the five-year survival rate of the 180 patients (76.6 percent) with tumors that had extended beyond the wall was 56.2 percent ($P < 0.001$). When the absence or presence of conspicuous peritumoral lymphocytic infiltration was considered, the difference in survival was not statistically significant. In fact, the 45 patients (19.2 percent) with conspicuous infiltration had a 74.3 percent five-year survival rate, whereas the 190 patients (80.8 percent) without it had a 61.4 percent five-year survival rate ($P = 0.15$).

By using Cox regression analysis, two of the four variables analyzed were selected as having an independent influence on survival—the character of the invasive margin and the number of lymph nodes invaded by the tumor. The depth of local spread and lymphocytic infiltration had no additional influence on survival when the selected variables were taken into consideration (Table 3).

Based on these data, the type of invasive margin as a discriminating variable among lymph node-negative (Dukes A and B stages) and lymph node-positive (Dukes C1 and C2 stages) patients was hypothesized. In the former group, 65 patients (27.7 percent) had tumors with a pushing margin, and 95 patients (40.4 percent) had tumors with an infiltrating one. Five-year survival rates were 88.5 and 66.1 percent for each subset, respectively ($P < 0.01$; Fig. 4). Among those patients with one to four positive lymph nodes, 11 patients (4.7 percent) had pushing tumors and 47 (20.0 percent) had infiltrating ones. Five-year survival rates were 100 and 28.7 percent for the two subsets, respectively ($P < 0.001$; Fig. 5). The 17 patients (7.2 percent) with more than four positive lymph nodes had tumors that showed an infiltrating margin; their five-year survival rate was 0 percent.

DISCUSSION

Although survival after radical surgery for colorectal carcinoma is quite satisfactory in comparison with other types of cancer of the digestive tract, improvement in survival rates by means of adjuvant therapeutic protocols is an on-going task. Rational use of radiotherapy and chemotherapy requires careful patient selection. In fact, only those patients who would die, even after having radical resection of the tumor, might receive the most benefit from such types of therapy. Since 1932, the Dukes classification has certainly been the most widely used prognostic tool in evaluation of these patients. This has been mainly the result of its simplicity in clinical use and its good relationship to survival. Among the modifications successively proposed to improve the prognostic value
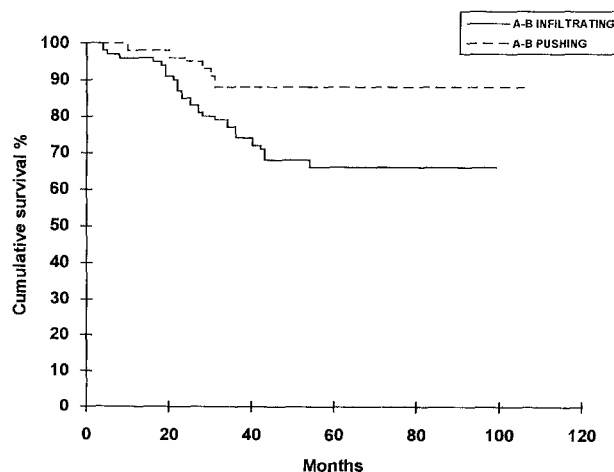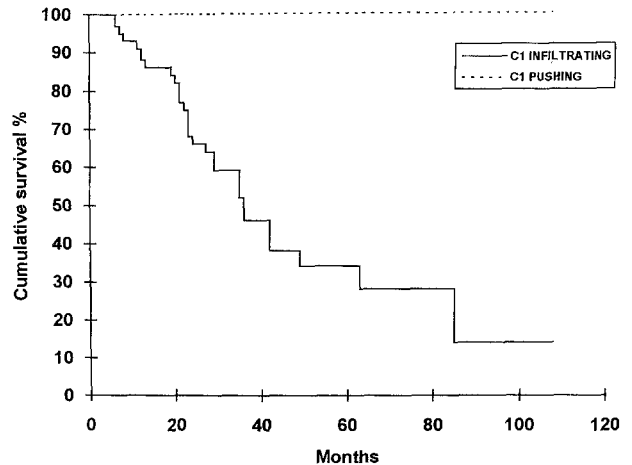

Figure 5. Survival of Dukes C1 patients according to type of invasive margin.

of the Dukes system, only the subdivision of Dukes C lesions into "C1" and "C2" subsets on the basis of the level of the positive lymph nodes[2, 10] or their number[7–9] has contributed any new significant prognostic data.

Our results are consistent with previous reports,[8, 11–13] which confirm the strong relationship between Dukes staging and survival. Nevertheless, the most important drawback in the Dukes system is the identification of only 15 to 20 percent of patients in Stage A with a good prognosis and less than 10 percent of those in Stage C2 with a poor prognosis. In fact, our series showed an 18.7 and 7.2 percent rate, respectively. To overcome this limitation, Jass and coworkers[3] introduced a new classification based on four pathologic variables: local spread, number of lymph nodes invaded by tumor, character of the invasive margin, and peritumoral lymphocytic infiltration. These had been selected by means of Cox regression analysis as having independent prognostic value and given weighted scores to identify four prognostic groups. In our series, the Jass groups were significantly related to the survival rate, even if the difference in survival between Group II and Group III, which accounted for the majority of our patients, was not significant. These data are consistent with those in a previous study by Secco et al.[14] Group I, which was characterized by an excellent prognosis, and Group IV, which was characterized by a poor prognosis, accounted for 21.7 and 20.8 percent of our patients, respectively. Therefore, the Jass classification may be considered superior to Dukes staging based on the fact that twice as many patients can be placed into definite groups with a confident progno-

Figure 4. Survival of Dukes A and B patients according to type of invasive margin.

Table 3.
Multivariate Analysis (Cox Regression Model) of the Four Parameters Used in the Dukes and Jass Classifications

Variable	Categories	HR	95% CB	P Value
No. of positive lymph nodes	0	1.000		
	1–4	2.066	1.220–3.498	
	>4	3.713	1.780–7.745	<0.001
Invasive margin	Pushing	1.000		
	Infiltrating	4.077	1.790–9.284	0.001
Lymphocytic infiltration	Present	1.000		
	Absent	0.717	0.365–1.409	0.334
Direct spread	Within the wall	1.000		
	Beyond the wall	1.515	0.698–3.290	0.293

HR = hazard ratio; CB = confidence bounds.

sis.[3, 4, 15] However, if a prognostic system is to be of clinical relevance, it must also be reproducible. Unfortunately, the assessment of the degree of peritumoral lymphocytic infiltration has revealed poor intraobserver and interobserver consistency in many of the compiled series.[9, 16] These results may be explained by the variability in the lymphocytic infiltrate distribution in the different areas of the tumor[16, 17] and the subjective nature of this parameter, given that it depends on the transforming of a visual interpretation into categories by the pathologist. In our experience, no significant difference in survival was found between patients with conspicuous infiltrate and those without it. These data were confirmed using Cox regression analysis. In fact, no independent prognostic value of this parameter was found in tumors of the colon and the rectum, which had been analyzed together. Similar findings have been found in colonic cancers by Shepherd *et al.*[18] and in colorectal lesions by Ponz de Leon *et al.*[19]

Previous studies[9, 16, 17] demonstrated that the other subjective variable of the Jass system, *i.e.*, the type of invasive margin, can be reliably assessed with high levels of intraobserver and interobserver agreement. Moreover, assessment is not significantly affected by the site of sampling of the invasive tumor margin.[16] In agreement with previous reports,[3, 4, 18, 19] the present study shows that this parameter is a good prognostic factor and was selected for its independent value on survival by multivariate analysis.

The other variable, which was found to be of independent prognostic significance, is the number of metastatic lymph nodes. On the other hand, the depth of local invasion did not provide any further prognostic information in our series.

These findings suggested that a new prognostic model might be possible using the character of the invasive margin as a discriminating factor within

lymph node-negative and lymph node-positive patients. Among the first group, which included those subjects in Dukes A and B stages, our patients with pushing tumors had a 88.5 percent five-year survival rate. Thus, they should have been considered as having as good a prognosis as that of patients with Stage A or those who had been placed in Jass Group I. Instead, those patients with infiltrating tumors were still classified as having an indefinite clinical outcome, given that their five-year survival rate was 66.1 percent. With regard to those patients with positive lymph nodes and, in particular, those with one to four positive lymph nodes or Stage C1, the 100 percent five-year survival rate associated with pushing tumors was probably overrated given that the number of patients was small. However, it does represent both an unexpected and significant result that has not been reported previously. In fact, only Fisher and coworkers[9] noted that patients with C1 lesions, who had Jass scores of I and II, exhibited survival patterns similar to those patients lacking nodal metastases or Stage B patients. However, further confirmation with larger patient populations may be necessary. On the other hand, patients with C1-infiltrating tumors and patients with more than four lymph nodes involved, that is, Stage C2 (all with infiltrating tumors), were consistently associated with a poor prognosis (28.7 and 0 percent at 5 years, respectively).

When subsets of our patients with good and poor prognoses were considered together, the new prognostic model, which can be easily used in clinical practice by the addition of the type of invasive margin to the Dukes classification, allowed for placement of 59.6 percent of the patients into groups that provided more confident prognoses. The percentage of patients was higher than when the Dukes and Jass classifications (25.9 and 42.5 percent, respectively) were used. Unfortunately, patients classified as Dukes A

and B with infiltrating tumors still represent a prognostic group with unsatisfactory individual patient prediction. Further prognostic discrimination is necessary, and new biologic parameters, such as DNA ploidy[20] and cell kinetics,[21] will no doubt help in this direction.

CONCLUSION

The character of the invasive margin in colorectal cancer is a valid factor in predicting survival. Moreover, the use of the combination of this parameter with the traditional Dukes classification may add useful prognostic information and may be of value in selecting patients who could receive the most benefit from adjuvant therapy.

REFERENCES

1. Dukes CE. The classification of cancer of the rectum. J Pathol Bacteriol 1932;35:323–32.
2. Gabriel WB, Dukes C, Bussey HJ. Lymphatic spread in cancer of the rectum. Br J Surg 1935;23:395–413.
3. Jass JR, Love S, Northover JM. A new prognostic classification for rectal cancer. Lancet 1987;1:1303–6.
4. Jass JR, Atkin WS, Cusick J, et al. The grading of rectal cancer: historical perspectives and a multivariate analysis of 447 cases. Histopathology 1986;10:437–59.
5. Kaplan E, Meier P. Non parametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457–81.
6. Cox DR. Regression model and life tables. J R Stat Soc (B) 1972;34:187–220.
7. Gastrointestinal Tumor Study Group. Adjuvant therapy of colon cancer: results of a prospectively randomized trial. N Engl J Med 1984;310:737–43.
8. Wolmark N, Fisher B, Wieand HS. The prognostic value of the modifications of the Dukes' C class of colorectal cancer: an analysis of the NSABP clinical trials. Ann Surg 1986;203:115–22.
9. Fisher ER, Robinsky B, Sass R, Fisher B, and other NSABP collaborators. Relative prognostic value of the Dukes and the Jass systems in rectal cancer: findings from the National Surgical Adjuvant Breast and Bowel Projects (Protocol R-01). Dis Colon Rectum 1989;32:944–9.
10. Dukes CE, Bussey HJ. The spread of rectal cancer and its effect on prognosis. Br J Cancer 1958;12:309–20.
11. Carlon CA, Fabris G, Arslan-Pagnini C, Pluchinotta AM, Chinelli F, Carniato S. Prognostic correlations of operable carcinoma of the rectum. Dis Colon Rectum 1985;28:47–50.
12. Newland RC, Dent OF, Lyttle MN, Chapuis PH, Bokey EL. Pathologic determinants of survival associated with colorectal cancer with lymph node metastases: a mul-tivariate analysis of 579 patients. Cancer 1994;73:2076–82.
13. Deans GT, Parks TG, Rowlands BJ, Spence RA. Prognostic factors in colorectal cancer. Br J Surg 1992;79:608–13.
14. Secco GB, Fardelli R, Campora E, et al. Prognostic value of the Jass histopathologic classification in left colon and rectal cancer: a multivariate analysis. Digestion 1990;47:71–80.
15. Jass JR, Morson BC. Reporting colorectal cancer. J Clin Pathol 1987;40:1016–23.
16. Dundas SA, Laing RW, O' Cathain A, et al. Feasibility of new prognostic classification for rectal cancer. J Clin Pathol 1988;41:1273–6.
17. Lanza G Jr, Borghi L, Ballotta MR, et al. Valutazione di parametri prognostici nel carcinoma del colon retto. 1. Variabili istopatologiche. Pathologica 1992;84:131–53.
18. Shepherd NA, Saraga E-P, Love SB, Jass JR. Prognostic factors in colonic cancer. Histopathology 1989;14:613–30.
19. Ponz de Leon M, Sant M, Micheli A, et al. Clinical and pathologic prognostic indicators in colorectal cancer: a population-based study. Cancer 1992;69:626–35.
20. Chapman MA, Hardcastle JD, Armitage NC. Five-year prospective study of DNA tumor ploidy and colorectal cancer survival. Cancer 1995;76:383–7.
21. Pietra N, Sarli L, Sansebastiano G, Jotti GS, Peracchia A. Prognostic value of ploidy, cell proliferation kinetics, and conventional clinicopathologic criteria in patients with colorectal carcinoma: a prospective study. Dis Colon Rectum 1996;39:494–503.

Invited Editorial

By linking the histologic nature of the tumor margin to a modification of Dukes staging, Dr. Cianchi and associates have been able to identify a greater percentage of patients with either a good or poor prognosis than is possible using either a modification of Dukes staging or the Jass prognostic classification. Interestingly, in their analysis of the "Jass" variables (direct spread and peritumoral lymphocytic infiltration), the authors failed to demonstrate that either had a significant independent prognostic effect. This may be explained by a difference in the method of data collection and/or by the fact that the series included both colonic and rectal cancers. The Jass system was developed specifically for rectal cancer. Subsequent work has shown that lymphocytic infiltration has no independent prognostic significance in colonic cancer. The authors suggest that difficulty in assessing this subjective variable may have been a factor in explaining the difference in their findings.

When only pathology variables are considered, di-

rect spread is widely accepted as having an important independent prognostic effect. The reason for the failure to demonstrate such an effect in the present series is not readily apparent. For the above reasons, it would seem prudent to suggest that further studies be undertaken on a larger series of patients before making recommendations on the use of the proposed system.

In the writer's view, formulation of a new prognostic classification should ideally evolve from the results of a multivariate analysis on a comprehensive range of prognostic variables performed for each tumor stage. Tumor site should be included in the model, and if shown to have a significant independent effect, analysis should be done separately for the colon and rectum.

Ronald C. Newland, M.D.
Sydney, Australia

The Authors Reply

Both the Dukes and Jass classifications were originally developed for rectal cancer,[1,2] but they are currently used by pathologists and surgeons even for prognostic evaluation of colonic cancer. Both of these systems showed a clear correlation with survival in our series.

With regard to direct spread of tumor (classified as limited to the bowel wall or extending beyond it), we found that it significantly influenced survival at univariate analysis. Nevertheless, when all histopathologic parameters used in the two systems had been evaluated at multivariate analysis, we found that only the type of invasive margin and the number of metastatic lymph nodes had any independent prognostic significance. The lack of prognostic value for direct spread at multivariate analysis may be explained by the valid effect of the type of invasive margin in predicting death as the result of either distant metastases or local recurrence. The direct spread of the tumor is likely to be related to only local recurrence, and thus, it shows a less meaningful prognostic significance.

Even if peritumoral lymphocytic infiltration may have potential prognostic value for only rectal tumors,

as Dr. Newland suggests, in our opinion, this variable cannot be reliably used by pathologists for prognostic evaluation regarding either colonic or rectal tumors. In fact, assessment of this parameter is not objectively reproducible, and the degree of its observer consistency, as reported in some studies, has been little better than that expected by pure chance.[3,4]

We agree with Dr. Newland when he suggested that further studies be undertaken on a larger series of patients. This may be necessary to confirm our finding and obtain more detailed data by considering colonic and rectal cancers separately.

In any case, we sought and finally proposed a prognostic model that can be reliably adopted for both colonic and rectal cancer by adding the type of invasive margin to Dukes staging. Thus, the clinical use of this prognostic model is very easy. Moreover, the most important finding from a clinical point of view is that this model can provide more prognostic information than either the Dukes or Jass classification. In fact, we have constructed a model that can identify a greater percentage of patients with either a good or poor prognosis than the above-mentioned classifications.

REFERENCES

1. Dukes CE. The classification of cancer of the rectum. J Pathol Bacteriol 1932;35:323–32.
2. Jass JR, Love S, Northover JM. A new prognostic classification for rectal cancer. Lancet 1987;1:1303–6.
3. Deans GT, Parks TG, Rowlands BJ, Spence RA. Prognostic factors in colorectal cancer. Br J Surg 1992;79:608–13.
4. Dundas SA, Laing RW, O'Cathain A, *et al.* Feasibility of new prognostic classification for rectal cancer. J Clin Pathol 1988;41:1273–6.

Fabio Cianchi, M.D.
Luca Messerini, M.D.
Annarita Palomba, M.D.
Vieri Boddi, B.S.
Giuliano Perigli, M.D.
Filippo Pucciani, M.D.
Paolo Bechi, M.D.
Camillo Cortesini, M.D.
Florence, Italy