Inclusion of Vasculature-Related Variables in the Dukes Staging System of Colon Cancer

Michael I. Koukourakis,1 Alexandra Giatromanolaki,1 Efthimios Sivridis,1 Kevin C. Gatter,2 and Adrian L. Harris3 for Tumour and Angiogenesis Research Group

Abstract

Purpose: The Dukes stage is used to stratify colorectal cancer patients into groups of different prognosis and need of adjuvant radiotherapy and chemotherapy. However, ~80% of patients with Dukes stage C colorectal cancer receive cytotoxic therapy without any expected benefit, for such patients would either not relapse without adjuvant therapy or they would inevitably do so because of tumor resistance to the available regimens. On the other hand, as 20% of Dukes stage B patients would relapse after surgery, adjuvant therapy could improve their survival. Improvement of the Dukes stage predictive accuracy is necessary to better assign patients for adjuvant therapies, especially nowadays when antiangiogenic agents are being incorporated in the clinical practice.

Patients and Methods: In this study, we examined the prognostic role of Dukes staging system in parallel with three vasculature-related variables (vascular invasion, tumor angiogenic activity, and vascular survival ability) in a series of 130 stage B/C patients with colorectal cancer treated with surgery alone (without adjuvant radiotherapy or chemotherapy).

Results: Inclusion of vasculature-related variables in the Dukes staging system significantly improved the prognostic categorization of patients, identifying subgroups of B-stage and C-stage patients with an up to 40% and 60% 5-year survival difference, respectively.

Conclusions: Preliminary results show that the prognostic value of Dukes staging system is significantly improved after taking into account vasculature-related variables, which may be useful in stratifying patients for adjuvant therapies, highlighting also subgroups that may benefit the most from antiangiogenic agents.

Colorectal cancer is a common malignant disease affecting 40 new patients per 100,000 population yearly (1). The Dukes stage (2) and several modified staging systems (Unio Internationale Contra Cancrum-American Joint Committee on Cancer and tumor-node-metastasis system; refs. 3–6) are used to stratify patients into groups of different prognosis and need of adjuvant radiotherapy and chemotherapy. Although the number of nodes (<4 versus ≥4) or the extent of the transmural extension, as scored in the American Joint Committee on Cancer/Unio Internationale Contra Cancrum system (6), influence drastically the prognosis of patients, lymph node positivity (C stage) is the principal feature, if not the only feature, indicating indisputably the necessity for adjuvant therapy (7). Patients of this stage experience a small, but significant, benefit from adjuvant chemotherapy and radiotherapy in the case of rectal cancer (8–10). The usefulness of chemotherapy in stage B disease (no lymph node metastasis) is controversial although there may be still a subset of patients, unrecognizable by revised Dukes staging systems, who would benefit from current chemotherapy protocols (11, 12).

In any case, not less than 80% of patients with Dukes stage C colorectal cancer treated with postoperative chemotherapy and/or radiotherapy receive cytotoxic therapy without any expected benefit; such patients would either not relapse without adjuvant therapy or they would inevitably do so because of tumor resistance to the available regimens. On the other hand, as 20% of Dukes stage B patients would relapse after surgery, adjuvant therapy could improve their survival (13).

The recognition of reliable prognostic factors that would distinguish colorectal cancer patients in subsets of high and low risk for tumor relapse is of great importance. This may allow treatment of those at highest risk within stage B tumors while sparing overtreatment, toxicity, and expense in stage C patients with low risk of relapse.

Vascular invasion (i.e., the presence of cancer cells in peritumoral or blood vessels) has been recognized as a strong prognostic factor in colorectal cancer (14, 15). Presumably, detection of cancer cells in peritumoral or intratumoral...
vessels provides a sign that the metastatic process has commenced and, therefore, is generally regarded as an indicator of poor prognosis. This is the first ever vasculature-related variable recognized and, as it forms a feature in routine histopathologic reports, it should be taken into account for the prognostic categorization of patients. Furthermore, at the discretion of physicians, patients with Dukes stage B colorectal cancer and vascular invasion can refer to chemotherapy or radiotherapy although none of the known histopathologic factors can reliably stress the necessity of adjuvant treatment (16).

During the past decade, many clinicopathologic studies indicated the importance of tumor angiogenic activity (i.e., the endothelial cell proliferation activity at the edge of the tumor) in defining the aggressiveness of tumor behavior (local invasion and distant metastasis; ref. 17). Despite the strong evidence, only very recently this vasculature-related variable became a certainty, following the clinical proof of therapeutic efficacy of the antiangiogenic agents (18). The Food and Drug Administration approval of the bevacizumab (an anti-vascular endothelial growth factor monoclonal antibody) as a first-line agent for metastatic colorectal cancer established “angiogenesis” as an important pathway that, apart from its prognostic value, can be used for targeted therapies. Increased vascular density, being a prognostic marker independent from stage (in most published studies), could be useful in deciding referral of even Dukes stage B patients for chemotherapy and eventually for antiangiogenic agent combination therapy.

Vascular survival ability (i.e., the ability of tumors to maintain the newly formed vasculature in the inner tumor areas) has been recently recognized as another vasculature-related prognostic variable, independent from tumor angiogenic activity (19). In an earlier study, we showed that colorectal carcinomas with a relatively low angiogenic activity can be as lethal as those of high angiogenic proliferation (as assessed by vessel density) provided that an adequate vasculature in their inner tumor areas is maintained (19, 20). This is probably because an increased vasculature surface offers increased chances for intravascular invasion to cancer cells. It could be also postulated that cancer cell clones producing survival growth factors able to sustain the neo-vascularization triggered, once the target organ is reached, bear a higher adhesive and invasive ability than simple angiogenic clones.

In this study, we examined the combined role of the three aforementioned vasculature-related variables (vascular invasion, tumor angiogenic activity, and vascular survival ability) in the prognosis of Dukes stage B and C colorectal carcinomas treated with surgery alone. In this way, the prognostic significance of vasculature-related variables could be assessed without any interference from the effects of adjuvant chemotherapy or radiotherapy. A modification of Dukes staging system is proposed after taking into account these features, which may be useful in stratifying patients for adjuvant therapies with or without antiangiogenic agents.

### Materials and Methods

Formalin-fixed, paraffin-embedded tissues from 130 consecutive patients with colorectal adenocarcinoma treated with surgery alone were retrieved from the files of the Nuffield Department of Pathology, John Radcliffe Hospital (Oxford, United Kingdom). Histologic staging was based on macroscopic examination of the surgical specimen and microscopic evaluation of H&E sections of the primary tumor and the excised lymph nodes. Sixty-three cases were staged as Dukes B and 67 as Dukes C. Fifty of 130 cases had tumors with rectal location. Nodal status was based on the evaluation of 3 nodes in 11 patients, of 4 to 6 nodes in 32 patients, and of 7 to 27 nodes in 95 patients. Although a higher number of nodes should have been identified and assessed in some patients, the magnitude of an erroneous staging and introduction of bias in the analysis is minimal. Five of 11 and 19 of 32 patients with three and four to six nodes, respectively, were found with involved nodes. The distribution of the remaining cases was balanced in the vasculature-related variable categories. Fifty-seven of the cases were female and 73 were male. The median age was 67 years (range, 41-88 years).

**Assessment of vascular invasion.** The presence of cancer cells within vascular channels of the primary tumor (inner tumor areas and tumor periphery) and in lymph node deposits was detected in H&E sections.

**CD31 immunohistochemistry.** For the assessment of tumor angiogenic activity and vascular survival ability, 3-μm-thick tissue sections were obtained from the invading part of the primary tumor and stained with the anti-CD31 pan-endothelial cell marker. Briefly, the J70 monoclonal antibody (DAKO, Glostrup, Denmark) recognizing CD31 (platelet/endothelial cell adhesion molecule-1; ref. 21) and the alkaline phosphatase/anti–alkaline phosphatase procedure were used. Sections were dewaxed, rehydrated, and predigested with protease type XXIV for 20 minutes at 37°C. JC70 (1:20) was applied at room temperature for 30 minutes and washed in TBS. Rabbit anti-mouse antibody (1:50) was applied for 30 minutes, followed by application of alkaline phosphatase-anti–alkaline phosphatase complex (1:1) for 30 minutes. After washing in TBS, the last two steps were repeated for 10 minutes each. The color was developed by 20 minutes incubation with New Fuchsin solution.

**Assessment of the tumor angiogenic activity.** Sections from primary tumors were scanned at low power (40× and 100×), areas of the highest vascularization at the invading tumor front (adjacent to the normal colon) were chosen, and vessel counting was done on three 200× fields of the highest density. Vessels adjacent to necrotic areas were excluded from the appraisal. The final vascular density was the mean of the vessel counts obtained in these fields. The median value of the vascular density recorded in tumors was used as a cutoff point to define two groups of tumors with high and low tumor angiogenic activity.

**Assessment of the vascular survival ability.** The method for the assessment of vascular survival ability has been previously reported (19, 20). Three areas of tumor adjacent to normal colon bearing the highest vascularization were identified per case as above. Vessel counting followed in three consecutive 200× fields starting from the tumor tissue adjacent to the normal colon (t1 field, for tumor periphery) and moving twice the optical field towards the tumoral center (t2 and t3 fields, for intermediate and inner tumor area, respectively). These three fields are estimated to cover a course of 6 mm (~2 mm each) from the periphery to the center. The mean vessel density in peripheral, intermediate, and inner tumor areas was the mean value obtained from the t1, t2, and t3 assessed areas, respectively (VDt1, VDt2, and VDt3, respectively). Cases were divided into two groups according to their ability to maintain the vascular density in inner tumor areas. Cases with a VDt1 <VDt2 + t3 (mean vascular density in t2 + t3 areas higher than the 50% of the vascular density in the t1 area) were considered to have a high vascular survival ability.

**Statistical analysis.** Statistical analysis and graphs were done using the GraphPad Prism 4.0 and the Instat 3.1 statistical packages (GraphPad, San Diego, CA; http://www.graphpad.com). The χ2, t test, or Fisher’s exact test was used for testing relationships between categorical tumor variables as appropriate. Nonparametric correlation analysis was used to assess interobserver variability of the score obtained for the vasculature-related variables. Survival curves were plotted using the Kaplan-Meier method and statistical differences
between life tables were determined by the log-rank test. A Cox proportional hazard model was used to assess the effect of tumor variables on local relapse, distant metastases, and death events. $P < 0.05$ was considered statistically significant.

**Results**

**Association among variables.** In this study, vascular invasion by cancer cells was noted in 40 of 130 cases (30.7%). The vascular density at the invading tumor front (tumor angiogenic activity) ranged from 10 to 117 with a median of 45 vessels. At the front, 63 cases (48.4%) showed a vascular density higher than 45 and grouped into the high tumor angiogenic activity category (Fig. 1B) whereas 65 of 130 cases (50%) fulfilled the criteria for high vascular survival ability. Interobserver variability was minimal for all three vasculature-related variables ($r > 0.92, P < 0.0001$). Immunohistochemical images from representative cases with high and low vascular survival ability are shown in Fig. 1.

Table 1 shows the association between the above vasculature-related variables and the Dukes stage. There was a significant association of vascular invasion with Dukes stage C ($P = 0.0001$) but none of the remaining variables was related to another variable or with Dukes stage.

**Association with survival.** To assess whether tumor location (rectal versus colonic) had an effect on the survival of patients, analysis was done showing that there was overlapping survival figures between 50 patients with rectal location and 80 with colonic location ($P = 0.95$, hazard ratio = 1.01). Further analysis within stage B (rectal versus colonic location: 25 versus 38 patients, respectively) and stage C (rectal versus colonic location: 25 versus 42 patients, respectively) showed similar results (Table 2).

Survival curves were plotted for Dukes stage (C versus B) and the three vasculature-related variables (vascular invasion: yes versus no; vascular survival ability and tumor angiogenic activity: high versus low) according to the Kaplan-Meier method. Table 2 shows the $P$ values and hazard ratios obtained for each variable (overall and for Dukes B and C stages separately). Dukes stage and all vasculature-related variables were significantly related to poor postoperative outcome. Separate analysis within stage B and C showed that only vascular survival ability was significantly associated with prognosis in stage B.

The Kaplan-Meier survival curves using double stratification for Dukes stage and the vasculature-related variables are shown in Fig. 2.

**Multivariate analysis of death events.** Table 3 shows the multivariate analysis for vasculature-related variables and Dukes stages B, C, and B/C combined. All four variables had an independent prognostic role when all patients were considered together. In stage B, however, only vascular survival ability had an independent prognostic relevance. Introduction of the location (rectum versus colon) in the multivariate model did not affect the above significant results (data not shown).

The same multivariate model was applied separately in patients with rectal and colonic location (data not shown). Presumably, due to the low number of cases, vascular survival ability was the only variable with an independent prognostic relevance in rectal cancer ($P = 0.002$, $t$ ratio 3.25). In colonic cases, vascular survival ability and vascular invasion had an independent prognostic meaning ($P = 0.002$, $t$ ratio 3.16; $P < 0.0001$, $t$ ratio 4.11, respectively). Further analysis within B and C stages was not done due to the low number of cases.

**Combined vasculature-related variable analysis within Dukes stage.** To investigate how the three vasculature-related variables combined affect prognosis within Dukes staging system, Kaplan-Meier survival curves were plotted separately for stage B and C after scoring patients from 0 to 3 according to the number of unfavorable vasculature-related variables their tumors bore (Fig. 3).

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**Fig. 1.** Shows two cases colorectal adenocarcinoma with low vascular survival ability (A, invading front; B, inner area) and with high vascular survival ability (C, invading front; D, inner area).
Vasculature-related variables dissected three groups within Dukes stage B: B0 (absence of any unfavorable vasculature-related variable), B1 (presence of one unfavorable vasculature-related variable), and B2/3 (presence of two or three unfavorable vasculature-related variables). B3 is also mentioned although none of the stage B patients was classified as B3. Similarly, three major groups were identified within stage C: C0 (up to one unfavorable vasculature-related variable), C1 (one unfavorable vasculature-related variable), and C2/3 (two or three unfavorable vasculature-related variables).

A four-variable prognostic model. Patients were subsequently scored according to the number of unfavorable prognostic variables (C-stage, positive vascular invasion, high tumor angiogenic activity, and high vascular survival ability). Thus, the score ranged from 0 to 4. Kaplan-Meier survival analysis for these five subgroups is shown in Fig. 4. Patients with absence of any of the four vasculature-related prognostic variables (16 of 130) showed an excellent (100%) 5-year survival. Patients with one (44 of 130) or two (34 of 130) unfavorable variables had a 5-year survival rate of 65% to 78%. Patients with three unfavorable variables had 29% 5-year survival rate whereas all 10 patients with four unfavorable vasculature-related variables died within 2 years from surgery.

Discussion

During the past decades, chemotherapy and pelvic radiotherapy established their place in the adjuvant postoperative treatment of colorectal cancer. The overall accepted guidelines can be summarized as follows: (a) all patients with stage C colorectal cancer receive chemotherapy, combined with pelvic radiotherapy in rectal cancer; (b) chemotherapy for stage C tumors should include 5-fluorouracil and leucovorin (or alternatively capcitabine) whereas the addition of oxaliplatin or irinotecan offers a very small benefit which does not, as yet, justify their inclusion in the standard regimen; (c) chemotherapy for stage B tumors is optional whereas pelvic radiotherapy is recommended in rectal cancer with extramural invasion; and (d) the use of antiangiogenic agents is, at present, restricted in the treatment of advanced metastatic disease where the addition of bevacizumab to standard chemotherapy provided a 10% increase in response rate and up to 5-month improvement in median survival rate (18).

Using the aforementioned guidelines, it is disappointing to realize that, from the clinical studies done, the 5-year survival benefit in stage-C cancer patients improved by no more than 15% to 20% (10, 22). Approximately 45% of stage C tumors will not relapse even without adjuvant therapy whereas ~35% will relapse despite the administration of chemotherapy. This, in simple terms, means that some 80% of stage C cases do not benefit from adjuvant therapy. In an effort to improve the results, the following questions were raised: (a) Is there a marker to identify the subgroup of stage C patients who need no chemotherapy? (b) Is there a subgroup of stage C cases who require aggressive adjuvant chemotherapy, probably combined with antiangiogenic agents? On the other hand, classification of a tumor as Dukes stage B does not really help the opinion on the recommended adjuvant treatment.

Vascular invasion by tumor has been recognized as an unfavorable prognostic variable in colorectal cancer for almost

| Table 1. The association among vasculature-related variables and Dukes stage |
|---------------------------------|-----------------|---------------------|-----------------|
|                                | Dukes stage     | Vascular invasion  | Tumor angiogenic activity |
|                                | B               | C                  | No   | Yes | P    | Low | High | P    |
| Vascular invasion              |                 |                    |      |     |      |      |      |      |
| No                              | 54              | 36                 | —    |     | 0.0001 |      |      |      |
| Yes                             | 9               | 31                 | —    |     | —    | —    | —    | —    |
| Tumor angiogenic activity       |                 |                    |      |     |      |      |      |      |
| Low                             | 33              | 34                 | 0.86 |     | 49   | 18   | 0.34 | —    |
| High                            | 30              | 33                 |      |     | 41   | 22   | —    | —    |
| Vascular survival ability       |                 |                    |      |     |      |      |      |      |
| Low                             | 35              | 30                 | 0.29 |     | 45   | 20   | 1    | 36   | 29   | 0.48 |
| High                            | 28              | 37                 |      |     | 45   | 20   |      |      |      |      |

Table 2. Statistics from Kaplan Meier survival analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>B and C stage (Hazard ratio)</th>
<th>P</th>
<th>B stage (Hazard ratio)</th>
<th>P</th>
<th>C stage (Hazard ratio)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dukes stage (C vs B)</td>
<td>3.49</td>
<td>0.0001</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Vascular invasion (yes vs no)</td>
<td>4.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>1.58</td>
<td>0.54</td>
<td>3.72</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>Tumor angiogenic activity (high vs low)</td>
<td>2.11</td>
<td>0.01</td>
<td>1.56</td>
<td>0.46</td>
<td>2.38</td>
<td>0.01</td>
</tr>
<tr>
<td>Vascular survival ability (high vs low)</td>
<td>3.68</td>
<td>&lt;0.0001</td>
<td>5.40</td>
<td>0.01</td>
<td>2.91</td>
<td>0.005</td>
</tr>
<tr>
<td>Location (colon vs rectum)</td>
<td>1.01</td>
<td>0.95</td>
<td>1.00</td>
<td>0.99</td>
<td>0.95</td>
<td>0.90</td>
</tr>
</tbody>
</table>
Thus, in 1988 Minsky et al. (14) reported the postoperative outcome of 294 colorectal carcinomas treated with surgery. Vascular invasion defined a group of stage C carcinomas with poor prognosis and the authors suggested that tumor bed irradiation might have a contributory role in the overall management. In the same year, Krasna et al. (23) reported that vascular invasion was associated with significantly worse prognosis even among patients in the same Dukes stage. An analysis of 154 colorectal carcinoma patients published from the University of Leiden in 1991 indicated that vascular invasion is as good as a prognostic factor as Dukes stage (24). In a synchronous study, involving 344 patients, Inada et al. (25) showed that vascular invasion is intimately related to the development of liver metastasis and, subsequently, Ouchi et al. (26) confirmed these results. The importance of identifying additional prognostic variables within stage B colorectal cancer was addressed in a more recent study from the University of Dublin, Ireland (27). In an analysis of 117 Dukes stage B patients, the authors associated vascular invasion with poor long-term outcome in the subgroup of patients with rectal, but not colonic, location. In a study by Zarbo et al. (28), in 309 colorectal carcinomas, vascular invasion emerged as an independent prognostic indicator of survival. Another large study of 211 patients was reported in 1998 from the Memorial Sloan-Kettering Cancer Center, New York (15). Again, vascular invasion was found to be a strong and independent variable defining prognosis. In another study by Galindo et al. (29) in 126 colorectal adenocarcinomas, vascular invasion and Dukes stage were significant independent prognostic variables. Similar results were reported in three more recent studies (30–32).

The tumor vascular density, assessed after immunohistochemical staining with pan-endothelial cell markers, has been recognized as an important prognostic variable over the past 10 years. Early studies by Saclarides et al. (33), Frank et al. (34), and Engel et al. (35) provided evidence that increased vascularity promotes tumor dissemination and adversely affects survival in rectal and colon cancer. In 1995, Bossi et al. (36) reported that angiogenesis is an early critical step in the development of colorectal cancer but failed to show any association between high vascular density and survival. Subsequent studies, however, provided evidence that vascular density is an important prognostic variable in colorectal cancer. A study by Lindmark et al. (37) in 212 cases showed that high vascular density was a strong prognostic factor independent of Dukes stage in colorectal cancer. Large studies from Japanese institutes also confirmed the close association of high vascular density with metastatic behavior and prognosis in colorectal cancer patients (38–40). In a study by Vermeulen et al. (41) in 145 cases, high angiogenic activity was linked with hematogenous metastasis and poor prognosis in both univariate and multivariate analyses. The authors suggested that Dukes stage B patients could be subclassified using vascular density and p53 protein expression. A study of 111 colorectal carcinomas patients from the University of Manchester, United Kingdom reported a paradoxical association of vascular density with good prognosis, indicating that in assessing vascularity (42), areas of ulceration or inflammation should be avoided for these give erroneously high scores. In one of our studies (43), 107 specimens from stage B and C colorectal carcinomas were analyzed. High vascular density was the only variable defining a subgroup of stage C carcinomas with poorer prognosis. Sternfeld et al. (44) also found that vascular density is an independent prognostic variable in a series of 146 operable colorectal carcinomas. A large number of more recent studies also confirm the important prognostic role of vascular density in the postoperative outcome of colorectal cancer (45–49).

**Table 3.** Multivariate analysis for vasculature-related variable and Dukes stage B and C, and B and C stages combined

<table>
<thead>
<tr>
<th>Variable</th>
<th>B and C stage</th>
<th>B stage</th>
<th>C stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t$ ratio</td>
<td>$P$</td>
<td>$t$ ratio</td>
</tr>
<tr>
<td>Dukes stage</td>
<td>2.14</td>
<td>0.03</td>
<td>—</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>3.59</td>
<td>0.0005</td>
<td>0.99</td>
</tr>
<tr>
<td>Tumor angiogenic activity</td>
<td>2.21</td>
<td>0.02</td>
<td>1.12</td>
</tr>
<tr>
<td>Vascular survival ability</td>
<td>4.41</td>
<td>&lt;0.0001</td>
<td>2.96</td>
</tr>
</tbody>
</table>
Studies from our group focused on a third vasculature-related variable, vascular survival ability (19). The angiogenic activity of tumors is apparently exerted at the invading tumor edge (i.e., in the interface between the growing tumor and the surrounding normal tissues). This layer serves for the growth and invasion of cancer cells and it is incorporated into the main tumor mass once a new tumor front layer is formed, compatible with a "relay-race" model of growth. The newly created vasculature will thus face the unfavorable conditions of the inner tumor areas (hypoxia and acidity) and may be subjected to apoptosis, as was indeed observed in an earlier study of ours (50). However, the ability of tumors to sustain the newly formed vasculature within the main tumor mass varies among tumors and, as it can be scored by a method described previously, it may provide important prognostic information (51–54). In a large colorectal cancer study, we showed that vascular survival ability is a stronger prognostic variable than vascular density, predicting postoperative outcome independently of Dukes stage (20).

The assessment of vasculature-related variables assumes a particular importance nowadays when antiangiogenic agents are massively tested in the clinical practice. An anti–vascular endothelial growth factor monoclonal antibody has been already approved for the treatment of metastatic colorectal cancer (18) whereas several other antibodies and pharmaceutical compounds are expected to be of clinical relevance (55). The incorporation of vasculature-related variables in the clinical staging of colorectal cancer provides important clinical information and splits the traditional stages B and C of colorectal adenocarcinomas into subgroups of patients with wide prognostic differences. The problem on what adjuvant therapy should be used in the treatment of B-stage patients is at least in part resolved whereas subgroups of stage C-stage patients who need aggressive multidrug adjuvant chemotherapy are identified. Furthermore, patients are characterized according to their angiogenic profile, a feature which is useful in identifying cases and C1 (19 of 67, 28% of stage C cases) that share a far better prognosis compared with stage C2/C3 (36 of 67, 54% of stage C cases). The latter prognostic group should receive the most aggressive chemotherapy available, including antiangiogenic agents. Randomized trials in stage C2/C3 patients are expected to show the benefits from such an aggressive treatment. Chemotherapy for stage C0/C1 becomes optional or should include less aggressive schemes (5-fluorouracil/leucovorin or capecitabine alone). Nevertheless, combination with antiangiogenic agents could prove to be of importance in stage C1 with high tumor angiogenic activity or vascular survival ability.

In the traditional Dukes classification system, the use of chemotherapy is optional for stage B patients. The modified system recognizes a B2/3 category (20 of 63, 31% of Dukes stage B cases) that has a definitely poor prognosis. For these cases, the administration of chemotherapy is well justified although it less clear and, therefore, deserves clinical testing whether or not the combination with antiangiogenic agents will be of additional benefit. On the other hand, the group B0 (16 of 63, 25% of stage B cases) should be excluded from any chemotherapeutic protocol because of excellent survival. In this way, 25% of stage B cases will be spared from overtreatment and the accompanied unnecessary cost of chemotherapy whereas some 30% of stage B patients will experience the benefit of chemotherapy, which would have been lost if the policy of the Institute was not to offer chemotherapy in patients with Dukes stage B disease.

The necessity to identify subsets of patients with different prognosis within Dukes B and C stages is therefore obvious. By incorporating the vasculature-related variables in Dukes staging, the prognostic accuracy of the system is improved and more objective criteria for adjuvant therapy are introduced.

Thus, for Dukes stage C, the proposed modified system (Table 4) recognizes the subgroups C0 (12 of 67, 18% of stage C cases) and C1 (19 of 67, 28% of stage C cases) that share a far better prognosis compared with stage C2/C3 (36 of 67, 54% of stage C cases). The latter prognostic group should receive the most aggressive chemotherapy available, including antiangiogenic agents. Randomized trials in stage C2/C3 patients are expected to show the benefits from such an aggressive treatment. Chemotherapy for stage C0/C1 becomes optional or should include less aggressive schemes (5-fluorouracil/leucovorin or capecitabine alone). Nevertheless, combination with antiangiogenic agents could prove to be of importance in stage C1 with high tumor angiogenic activity or vascular survival ability.

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The incorporation of vasculature-related variables in staging colorectal cancer provides important clinical information and splits the traditional stages B and C of colorectal adenocarcinomas into subgroups of patients with wide prognostic differences. The problem on what adjuvant therapy should be used in the treatment of B-stage patients is at least in part resolved whereas subgroups of stage C-stage patients who need aggressive multidrug adjuvant chemotherapy are identified. Furthermore, patients are characterized according to their angiogenic profile, a feature which is useful in identifying
Table 4. A modified Dukes staging system, proposed for further evaluation, based on three vasculature-related variables

<table>
<thead>
<tr>
<th>Stage</th>
<th>Postoperative prognosis (5-y survival, %)</th>
<th>Adjuvant treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>B0: No lymph node involvement; absence of unfavorable vasculature-related variables</td>
<td>100</td>
<td>None</td>
</tr>
<tr>
<td>B1: No lymph node involvement; presence of one unfavorable vasculature-related variable</td>
<td>80</td>
<td>Optional*</td>
</tr>
<tr>
<td>B2/B3: No lymph node involvement; presence of two or three unfavorable vasculature-related variables</td>
<td>60</td>
<td>Chemotherapy + radiotherapy† (antiangiogenic agents may prove of significant value)</td>
</tr>
<tr>
<td>C0: Lymph node involvement but absence of vascular invasion, high tumor angiogenic activity, or vascular survival ability</td>
<td>80</td>
<td>Optional*</td>
</tr>
<tr>
<td>C1: Lymph node involvement with the presence of one unfavorable vasculature-related variable</td>
<td>80</td>
<td>Chemotherapy + radiotherapy† (antiangiogenic agents may prove of significant value in cases with high tumor angiogenic activity or high vascular survival ability)</td>
</tr>
<tr>
<td>C2/3: Lymph node involvement with the presence of two or three unfavorable vasculature-related variables</td>
<td>0-25</td>
<td>Aggressive chemotherapy † † + radiotherapy† (antiangiogenic agents expected to show a marked benefit)</td>
</tr>
</tbody>
</table>

NOTE: The expected postoperative survival rates (without adjuvant therapy) together with the suggested adjuvant therapy are also indicated.
*Chemotherapy or radiotherapy can be either omitted or simple 5-fluorouracil/leucovorin (or capecitabine) chemotherapy may be administered.
†Radiotherapy indicated for rectal location.
††Combined chemotherapy of 5-fluorouracil/leucovorin (or capecitabine) with irinotecan and/or oxaliplatin.

patients for inclusion in randomized trials with antiangiogenic regimens. The preliminary results of the study encourage the conduct of large retrospective studies to evaluate whether or not vasculature-related variables identify groups with large survival differences even within the B and C subgroups of the American Joint Committee on Cancer/Unione Internationale Contro Cancrum system. The most interesting aspect of the proposed staging modification is, however, its eventual application as a tool to prospectively stratify patients recruited in trials with antiangiogenic therapy.

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