Prognostic Factors in Colorectal Cancer

College of American Pathologists Consensus Statement 1999

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● ***Background.*—Under the auspices of the College of American Pathologists, the current state of knowledge regarding pathologic prognostic factors (factors linked to outcome) and predictive factors (factors predicting response to therapy) in colorectal carcinoma was evaluated. A multidisciplinary group of clinical (including the disciplines of medical oncology, surgical oncology, and radiation oncology), pathologic, and statistical experts in colorectal cancer reviewed all relevant medical literature and stratified the reported prognostic factors into categories that reflected the strength of the published evidence demonstrating their prognostic value. Accordingly, the following categories of prognostic factors were defined. Category I includes factors definitively proven to be of prognostic import based on evidence from multiple statistically robust published trials and generally used in patient management. Category IIA includes factors extensively studied biologically and/or clinically and repeatedly shown to have prognostic value for outcome and/or predictive value for therapy that is of sufficient import to be included in the pathology report but that remains to be validated in statistically robust studies. Category IIB includes factors shown to be promising in multiple studies but lacking sufficient data for inclusion in category I or IIA. Category III includes factors not yet sufficiently studied to determine their prognostic value. Category IV includes factors well studied and shown to have no prognostic significance.**

***Materials and Methods.*—The medical literature was critically reviewed, and the analysis revealed specific points of variability in approach that prevented direct comparisons among published studies and compromised the quality of the collective data. Categories of variability recognized included the following: (1) methods of analysis, (2) interpretation of findings, (3) reporting of data, and (4) statistical evaluation. Additional points of variability within these categories were defined from the collective experience of the group. Reasons for the assignment of an individual prognostic factor to category I, II, III, or IV (categories defined by the level of scientific validation) were outlined with reference to the specific types of variability associated with the supportive data. For each factor and category of variability related to that factor, detailed recommendations for improvement were made. The recommendations were based on the following aims: (1) to increase the uniformity and completeness of pathologic evaluation of tumor specimens, (2) to enhance the quality of the data needed for definitive evaluation of the prognostic value of individual prognostic factors, and (3) ultimately, to improve patient care.**

***Results and Conclusions.*—Factors that were determined to merit inclusion in category I were as follows: the local extent of tumor assessed pathologically (the pT category of the TNM staging system of the American Joint Committee on Cancer and the Union Internationale Contre le Cancer [AJCC/UICC]); regional lymph node metastasis (the pN category of the TNM staging system); blood or lymphatic vessel invasion; residual tumor following surgery with curative intent (the R classification of the AJCC/UICC staging system), especially as it relates to positive surgical margins; and preoperative elevation of carcinoembryonic antigen elevation (a factor established by laboratory medicine methods rather than anatomic pathology). Factors in category IIA included the following: tumor grade, radial margin status (for resection specimens with nonperitonealized surfaces), and residual tumor in the resection specimen following neoadjuvant therapy (the ypTNM category of the TNM staging system of the AJCC/UICC). Factors in category IIB included the following: histologic type, histologic features associated with microsatellite instability (MSI) (ie, host lymphoid response to tumor and medullary or mucinous histologic type), high degree of MSI (MSI-H), loss of heterozygosity at 18q (*DCC* gene allelic loss), and tumor border configuration (infiltrating vs pushing border). Factors grouped in category III included the following: DNA content, all other molecular**

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markers except loss of heterozygosity 18q/DCC and MSI-H, perineural invasion, microvessel density, tumor cell–associated proteins or carbohydrates, peritumoral fibrosis, peritumoral inflammatory response, focal neuroendocrine differentiation, nuclear organizing regions, and proliferation indices. Category IV factors included tumor size and gross tumor configuration. This report records findings and recommendations of the consensus conference group, organized according to structural guidelines defined herein.
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CATEGORY I FACTORS

Local Extent of Tumor Assessed Pathologically (pT Category)

Overview.—Despite universal recognition of the prognostic importance of the local extent of disease as determined by pathologic assessment,[1] variations in approach to the acquisition, interpretation, reporting, and analysis of this vital information still exist on a fundamental level. Lack of uniformity in methodologic approach and variations in the interpretation and reporting of pathologic findings are currently the most problematic issues associated with this factor.[1–6]

Method Issues

- Specimen processing variation
  - Processing fresh versus fixed tissue from specimen
  - Fixing specimen closed or opened
  - Opening of specimen along long axis versus across short axis
  - Fixation time before sampling for microscopic evaluation
  - Pinning techniques versus no pinning
  - Inking versus no inking (if inked, which surfaces marked)
  - Type of fixative used
  - Number of blocks submitted
- Variability in handling of cases with nonperitonealized surfaces (radial) margins (discussed separately in category IIA variables)

Recommendation.—For standardization purposes, inking of radial margins should be carried out. Tissue should be fixed in 10% buffered formalin before processing. The duration of fixation, open versus closed fixation, and pinned versus unpinned should be at the pathologist's discretion. Overfixation should be avoided, however (see p 985). At least 3 blocks of tumor should be submitted (5 blocks may be submitted to optimize identification of extramural venous invasion; see below), or the entire tumor should be submitted if it is less than 3 blocks, taken perpendicular to the bowel wall and cut transversely to demonstrate deepest extent of tumor and tumor border configuration.

Interpretation Issues

- pTis: variability in use of the term *carcinoma in situ*. Used descriptively (and traditionally), the term connotes malignant epithelial cells that do not penetrate their basement membrane and do not invade the underlying stroma. Used as a staging term in colorectal cancer, the term also includes malignant cells that invade the lamina propria up to and including the muscularis mucosae.
- pT4: confusion regarding the definition of serosal perforation and the microscopic features by which it is recognized. Breach of the serosal surface (serosal involvement by tumor) may have variable histopathologic manifestations, many of which are not interpreted by pathologists as serosal perforation, leading to an underestimation of pT4b disease.
- pT3 with positive radial margin versus pT4b: confusion about evaluation of peritonealized versus nonperitonealized surfaces of the specimen.

Recommendation.—For pTis (carcinoma in situ): specify as either (1) high-grade dysplasia/intraepithelial carcinoma (pTie) or (2) intramucosal carcinoma (pTim). Clarification of the definition for pT3 is required to indicate that the serosal surface is to be uninvolved by tumor.[5] Clarification of category pT4b (serosal perforation) is needed, and its definition should include disruption of the serosal (mesothelial) cells on the bowel surface. This disruption may include the following: (1) mesothelial inflammatory and/or hyperplastic reactions with tumor close to but not at the serosal surface; (2) tumor present at the serosal surface with inflammatory reaction, mesothelial hyperplasia, and/or erosion or ulceration; and (3) free tumor cells on the serosal surface (in the peritoneum) with underlying ulceration of the visceral peritoneum.[5]

Reporting Issues

- Variability in staging system and terms used (eg, Dukes' or Astler-Coller staging systems used instead of TNM system)
- Variable reporting of pT4 tumors as pT4a versus pT4b

Recommendation.—Use of terms and definitions of the T categories set forth by the American Joint Committee on Cancer and the Union Internationale Contre le Cancer (AJCC/UICC) in the 1997 AJCC/UICC *Cancer Staging Manual*[6] and the 1993 *TNM Supplement*.[7]

Statistical Issues

- Considerable variation in staging system used and in the use of pathologic stage data in prognostic marker studies

Recommendation.—Report pT, pN, and pM categories in all cases. Each surgical margin (proximal, distal, and radial) should be reported separately.

Other

- Obtaining fresh tissue or additional fixed tissue for research while maintaining integrity of surgical pathology evaluation

Recommendation.—There are currently insufficient data to make recommendations, but archiving of additional tumor for molecular studies may be advisable.

Regional Lymph Node Metastasis Assessed Pathologically (pN Category)

Overview.—Metastasis to regional lymph nodes as determined by pathologic assessment is, among the factors that most strongly predict outcome following surgical resection, second only to distant metastatic disease in importance. Nevertheless, significant methodologic variation still exists in routine pathology practice with regard to

both lymph node harvesting and processing of lymph nodes for microscopic examination. Lack of uniformity in approach is currently the most problematic issue associated with this factor. Newer (nontraditional) methods of lymph node examination for micrometastatic disease and the biologic significance of metastasis identified by these methods currently lack validation.[8–20]

Method Issues

- Variations in surgical technique contributing to variation in number of nodes contained in resection specimens
- Variations in handling of specimen (using conventional techniques)
  - Diligence of search for nodes
  - Use of clearing or other solutions to increase macroscopic visualization of nodes
  - Threshold for acceptable number of nodes
  - Submission of whole versus half of each node found for microscopic examination
  - Acquisition of tissue levels (and in number of levels, if acquired) for microscopic examination
  - Separation of lymph nodes by anatomic site in large specimens (ie, regional vs nonregional as pertains to the anatomic site of the tumor)
- Use of special techniques as adjuncts to or replacement for light microscopy (nonhistologic or nonconventional techniques)
  - Immunohistochemical staining: cytokeratin, carcinoembryonic antigen (CEA), epithelial membrane antigen
  - Polymerase chain reaction amplification of tumor RNA and DNA—considerable variation in method and control comparisons in investigational studies

Recommendation.—All identified lymph nodes should be sectioned. It has been shown that 12 to 15 negative lymph nodes predict for regional node negativity.[9, 19] If fewer than 12 nodes are found, additional visual enhancement techniques should be considered. All grossly negative or equivocal lymph nodes are to be submitted entirely. For grossly positive lymph nodes, a representative sample should be submitted for microscopic confirmation. Data are insufficient to recommend routine use of tissue levels or ancillary special techniques.

Interpretation Issues

- Variation in lower limit of acceptable nodal harvest
- Failure to interpret tumor directly invading node as metastatic disease
- Failure to recognize nonregional lymph node metastasis as pM1 disease
- Variable interpretation of micrometastasis by light microscopy
- Variable interpretation of minute foci of tumor (including single cells) or tumor detected by nonhistologic or nonconventional methods as biologically significant

Recommendation.—Use guidelines for definitions of nodal metastasis given in the 1997 TNM *Cancer Staging Manual*.[6] Any histologically confirmed focus of tumor that measures 2 mm or less in greatest dimension is to be regarded as a micrometastasis and classified as N1. Tumor detected by nonhistologic methods is classified as pN0.[20]

Reporting Issues

- Variability in reporting of regional lymph node status (pN missing from many reports)
- Variability in assignment of a pN category by the pathologist

Recommendation.—Regional lymph node status (both numbers of nodes examined and number of nodes positive) always should be reported and always assigned a pN category by the pathologist.

Statistical Issues

- Various methods for analyzing nodal data: categorical, continuous, percentage positive

Recommendation.—There are currently insufficient data to make recommendations.

Blood or Lymphatic Vessel Invasion

Overview.—The prognostic importance of involvement of small (thin-walled, presumably lymphatic) vessels in the submucosa has been well documented with respect to polypectomies of malignant polyps and shown to be associated with an increased risk of regional lymph node metastasis. The prognostic importance of involvement of extramural veins by tumor and its association with increased risk of liver metastasis has also been demonstrated. Despite recognition of the importance of blood or lymphatic vessel involvement by tumor, considerable heterogeneity exists in the methodologic approach to, assessment of, and reporting of this feature.[1,3,8,21–57]

Method Issues

1. Malignant polyps or local excisions (pT1 tumors)
   - Variable number of tissue levels examined
   - Variable use of special stains or immunohistochemical staining to visualize vessels
2. pT2, pT3, and pT4 tumors
   - Variable sectioning of specimens and number of samples submitted
     - Data demonstrating increased likelihood of finding venous invasion with submission of additional sections suggest that 5 blocks of tumor may be optimal[8]
     - Variable estimates of cost-effectiveness of more extensive examination
     - Practical feasibility of more extensive examination
   - Variable number of tissue levels examined
   - Variability in use of special stains or immunohistochemical staining to visualize vessels

Recommendation.—At least 3 blocks of tumor (optimally 5 or more blocks) should be submitted. A single hematoxylin-eosin–stained section from each block should be examined for blood or lymphatic vessel invasion; data are insufficient to recommend that additional tissue levels be examined. No special stains or immunohistochemical stains are recommended.

Interpretation Issues

- Differentiation of postcapillary venules from lymphatic vessels (both thin-walled, small-caliber vessels) often not possible, but these vessels variably definitively diagnosed
- Malignant polyps: interobserver variability in diagnosis

of small vessel invasion (impact of retraction or cautery artifact on interpretation)
- The importance of *suspicion* of small vessel invasion in a malignant polyp variably recognized (outcome resembles that of diagnostic small vessel invasion)
- Mural penetration of tumor variably interpreted as large vessel invasion

Recommendation.—Identification of tumor within an endothelial-lined channel or surrounded by an elastic lamina is required for diagnosis of vessel invasion. Small vessels not definitively interpreted as lymphatics or venules should be identified as angiolymphatic vessels.

Reporting Issues

- Variability in reporting of small vessel invasion
- Variability in reporting of large vessel invasion
- Variability in reporting anatomic location of small vessel invasion (eg, submucosal, mural, extramural)
- Variability in reporting anatomic location of large vessel invasion (eg, submucosal, mural, extramural)

Recommendation.—For all tumors, including malignant polyps and rectal tumors removed by transanal disk excision, venous and angiolymphatic invasion should always be reported as present or absent and its anatomic location specified as intramural or extramural.

Statistical Issues

- Considerable variation in types of vascular invasion data in prognostic marker studies (eg, lymphatic only, venous only, both lumped together, separation by anatomic location, or any vessel invasion without specification of type or site)—unclear whether vessel type, vessel location, or both are prognostically significant

Recommendation.—In prognostic marker studies, large vessel and small vessel invasion should be designated separately. Anatomic site within the bowel wall should be considered a separate variable.

Residual Tumor Classification (R Classification)

Overview.—The residual tumor (R) classification has been shown to have prognostic significance. The following discussion is included for anatomic pathologists for educational purposes and for its relationship, in some circumstances, to a positive surgical margin. The presumption underlying the finding of tumor at a surgical resection margin is that tumor remains in the patient at the surgical interface, and based on this premise, classification of a positive margin as residual tumor (R) is appropriate.[1,6]

Method Issues

- Lack of understanding of appropriate usage of R classification for tumor remaining in patient following therapy of any type
- Variable inappropriate use of the R category to refer to residual tumor in the resection specimen after neoadjuvant therapy
- Variable use of R category to refer to residual tumor in the patient after incomplete resection (eg, a positive radial margin)
- Variable inappropriate use of ypTNM to modify the R category
- Lack of guidelines for the appropriate use of ypTNM

Recommendation.—Tumor at a surgical resection margin should be considered the counterpart of residual tumor in the patient and classified according to the R classification as defined in the 1997 AJCC/UICC *Cancer Staging Manual*.[6] Further definition of the residual tumor category is needed to distinguish residual tumor in the patient following treatment versus residual tumor in a resection specimen following neoadjuvant treatment (see ''Tumor Classification After Neoadjuvant Therapy (ypTNM)'' in the ''Category IIA'' section).

Interpretation Issues

- Positive margins (including radial) may or may not be interpreted as evidence of residual disease in the patient—role of pathologist versus surgeon in defining residual disease in incomplete resections

Recommendation.—Positive margins should be interpreted as the counterpart of residual tumor in the patient unless proven otherwise.

Reporting Issues

- Reporting of surgical margin status alone versus surgical margin status plus corresponding R classification

Recommendation.—Surgical margin status should always be reported. If positive, the appropriate R category (R1 indicates microscopic residual disease; R2 indicates macroscopic residual disease) should be assigned.

Statistical Issues

- Few data on the relevance of the R category as it relates to radial margins due primarily to the lack of recognition, reporting, and studies on prognostic significance of radial margins, except in rectal cancer

Recommendation.—All studies on prognostic importance of residual disease as it relates to margin status (including the radial margin) should also include the R classification.

Preoperative CEA Elevation

Overview.—Preoperative CEA has been shown to have prognostic significance. The following discussion is included for anatomic pathologists for educational purposes only. Anatomic pathologists rarely know if testing is performed or, if performed, what the results are.[58–66]

Method Issues

- Variation in laboratory measurement methods
- Variation in preoperative testing for CEA according to treating physician

Recommendation.—Standard laboratory testing of a preoperative serum sample should be performed for all patients.

Interpretation Issues

- Variation in level of elevation that is regarded as significant

Recommendation.—Significant level of elevation is greater than 5 ng/mL. (This should not be taken to preclude the individual laboratory's right to determine local normal ranges for this analyte.)

Reporting Issues

- Ordering physician may or may not report results as part of tumor staging

Recommendation.—Preoperative CEA levels, if known, should be reported as a clinical or (clinical) pathologic parameter as follows: CX, CEA level not assessed; C0, CEA level not elevated (<5 ng/mL); or C1, CEA level elevated (≥5 ng/mL).

Statistical Issues

- Use of different cutoffs for elevation (eg, anything greater than hospital norm, 5 ng/mL, 7.5 ng/mL, 10 ng/mL) in analyses demonstrating the significance of CEA
- Variable adjustment for preoperative treatment

Recommendation.—In prognostic marker studies, preoperative CEA levels should be reported as elevated if 5 ng/mL or greater and handled as a separate element in multivariate analyses.

CATEGORY IIA FACTORS

Histologic Grade

Method Issues

- Multiple grading systems suggested during the past several decades but none widely accepted
- Variation in number of strata in different grading systems
- Assessment of grade largely subjective overall
- Semiquantitative grading suggested by the College of American Pathologists despite lack of data to justify use
- Prognostic significance of grade demonstrated in most studies by collapsing 4 into 2 grades as follows: well and moderately (grades 1 and 2) defined as low grade, and poorly and undifferentiated (grades 3 and 4) defined as high grade

Recommendation.—A 2-tiered grading system (high grade and low grade) would both reduce interobserver variation and retain or improve prognostic significance.[1–3,8,9,62,65,68–80]

Interpretation Issues

- Determination of grade largely a subjective exercise with few or no defined criteria
- Substantial interobserver variability demonstrated[8]
- Fundamental basis of grade controversial:
  - Overall impression
  - Worst area
  - Amount of gland formation alone
  - Combination of gland formation and other structural or cytologic features (eg, nuclear grade)
- Relationship between grade, DNA replication error, and high degree of microsatellite instability (MSI-H) status may be the most important issue in high-grade tumors
  - For medullary tumors, problem may be obviated if these are defined by the World Health Organization (WHO) as a separate histologic type (WHO classification now under revision) and not assigned a grade by convention
  - For mucinous tumors, grade assignment may be inappropriate

Recommendations.—Gland formation should be the only feature used to assign grade (<50% gland formation defines high grade).

Reporting Issues

- Variation in wording used in surgical pathology reports
- Variable use of numerical versus descriptive grade
- Variable reporting of single grade for entire tumor versus range of grades within a single tumor
- Number of categories from 2 to 4 often goes unstated
- Variable or inappropriate assignment of grade to histologic types that should not be graded or are always assigned a specific grade by convention

Recommendation.—Report grade in a 2-tiered descriptive system as either high grade or low grade.

Statistical Issues

- Variable practice of grouping of grades to reduce the number of categories

Recommendation.—Use 2 categories to include well- and moderately differentiated tumors in a low-grade category and poorly and undifferentiated tumors in a high-grade category for all statistical analyses.

Radial Margin (Specimens With Nonperitonealized Surfaces)

Method Issues[81–84]

- Lack of understanding of definition, importance, and need for separate analyses of radial margins by both pathologists and surgeons
- Open or closed fixation at the discretion of the pathologist, but closed fixation and gross serial sectioning through the bowel used in most studies demonstrating the importance of radial margins
- Variable use of ink to mark radial margin

Recommendation.—Radial margin status (positive or negative) and, if negative, surgical clearance (the distance between the tumor and the radial margin at its closest approach) should be a standard part of assessment for all specimens with nonperitonealized surfaces. Grossly identified radial margins should be inked. Open or closed fixation is at the discretion of the pathologist.

Interpretation Issues

- Peritonealized surface misinterpreted as a radial margin when mesothelial cells are missing
- Variability in the consideration of specimen-specific anatomical issues, with all external surfaces variably treated as peritonealized surfaces and involvement variably misinterpreted as pT4b

Recommendation.—As recommended by the Report from the National Cancer Institute Colorectal Cancer Surgery Guidelines Conference (April 1–2, 1999), the surgeon should be aware of the radial margins at the time of operation. Labeling the specimen and marking areas of concern so the specimen can be properly oriented and areas of specific concern can be correctly identified by the pathologist should be considered in every case and performed when appropriate. For the pathologist, careful gross assessment to identify the location of the peritoneal reflection should be performed in the fresh state, if possible, on specimens with both peritonealized and nonperitonealized surfaces. The nonperitonealized surface should be marked with ink and reported separately. For

rectal specimens that lack a peritonealized surface, the entire external surface of the specimen is a radial margin.

Reporting Issues

- Variable lack of reporting of positive radial margins by pathologists
- If positive radial margin reported, variable reporting of anatomic location of involvement
- If radial margin assessed and reported to be negative, variable reporting of the surgical clearance (the distance between the tumor and the radial margin at its closest approach)

Recommendation.—Radial margin status (positive or negative) and surgical clearance in all cases with negative radial margins should be a standard part of reporting for all specimens with nonperitonealized surfaces. Whenever orientation of the specimen is possible, the anatomic location of the positive radial margin should be reported. Positive radial margins should be classified by the R classification, which connotes residual disease in the patient (see above).

Statistical Issues

- Current limitation of studies on the importance of radial margins are limited to rectal cancers; no data at all on radial margins in resection specimens with partially peritonealized surfaces
- Among current studies, numbers of patients relatively small; no statistically robust studies with multivariate analysis

Recommendation.—All studies on prognostic factors should include assessment of radial margin and surgical clearance (see below).

Tumor Classification After Neoadjuvant Therapy (ypTNM)

Method Issues[1,6,7]

- Lack of understanding of restriction of use of cTNM or pTNM to previously untreated tumors
- Lack of understanding that the prognostic significance of a ypTNM stage grouping cannot be equated with the prognostic significance of a p/cTNM stage grouping (based on previously untreated tumor)
- Variable use of the ypTNM category to refer to residual tumor in resection specimen after neoadjuvant therapy

Recommendation.—Tumor remaining in a resection specimen following neoadjuvant therapy should always be classified by ypTNM to distinguish it from untreated tumor.

Interpretation Issues

- Viable versus necrotic tumor in determining residual disease
- Acellular mucin pools or other probable ''footprints'' of tumor interpreted as evidence of residual tumor

Recommendations.—Only histologically viable tumor should be interpreted as residual disease and classified by ypTNM.

Reporting Issues

- Variable reporting of uncertainty about viability of residual tumor
- ypTNM variably assigned and reported

Recommendation.—Histologically viable tumor remaining in a resection specimen following neoadjuvant therapy should always be classified by ypTNM to distinguish it from untreated tumor are reported using this classification.

Statistical Issues

- Limited number and size of studies on the importance of residual disease in the resection specimen after neoadjuvant therapy
- Importance of level of precision in assessment of residual disease (ie, assessment by ypTNM) unknown

Recommendation.—All studies on the prognostic importance of residual disease in resection specimens following neoadjuvant therapy should include ypTNM.

CATEGORY IIB FACTORS

Histologic Type

Method Issues[3,38,39,46,47,51,70,72,78,85–96]

- Variable use of routine histology alone versus use of special studies (eg, assessment of neuroendocrine differentiation in small cell carcinomas by immunostaining)

Recommendation.—Perform assessment of tumor type by routine histologic method alone. Special studies may be used at the discretion of the pathologist.

Interpretation Issues

- Mixed patterns variably interpreted (eg, signet ring cells in a mucinous carcinoma)

Recommendations.—Mixed patterns should be classified by predominant type.

Reporting Issues

- WHO classification variably used
- Medullary carcinoma variably reported as a distinct entity

Recommendation.—Report histologic type according to the WHO classification. Medullary carcinoma should be reported separately from undifferentiated carcinoma (see below).

Statistical Issues

- Histologic type not statistically significant in most studies of prognostic variables except for tumor types that are, by definition, high grade (poorly or undifferentiated), specifically, signet-ring cell carcinoma and small cell carcinoma
- Limited number of studies on the prognostic significance of histologic type after stratification by MSI status

Recommendation.—Tumor type should be correlated with outcome after adjustment for MSI status in statistically robust studies with multivariate analysis in order to definitively determine its prognostic significance.

Histologic Features Associated With MSI-H: Host Lymphoid Response to Tumor and Medullary or Mucinous Histologic Type

Host Lymphoid Response to Tumor

Method Issues[38,89,97–106]

- Variable use of histologic appearance alone versus use of immunohistochemical staining for lymphocytes

- Variation in sampling location and amount—unclear affect on assessment of this parameter

Recommendation.—Perform assessment by routine histology only. Both the perimeter and center of tumor should be examined in this assessment.

Interpretation Issues

- Variable interpretation of pattern of lymphoid response
  - Intratumoral lymphocytes
  - Peritumoral lymphoid reaction
  - Transmural Crohn's-like lymphoid reaction
- Intensity of lymphoid response variably interpreted (eg, all or none versus graded intensity)
- One or more than one pattern variably recognized
- Variable differentiation between an inflammation versus immune response
- Intratumoral lymphocytes variably interpreted as a separate feature versus integral feature of a medullary carcinoma
- Variable recognition of the relationship between replication error status and intratumoral lymphocytes

Recommendations.—Intratumoral lymphocytic infiltrates should be distinguished from peritumoral inflammatory infiltrates. The former are closely associated with MSI-H and medullary architecture (see below). Only moderate- and high-density intratumoral infiltrates (4 or more per high-power field) should be considered significant.

Reporting Issues

- Variable reporting of host lymphoid response
- Variation in description of type and grading of host lymphoid response

Recommendation.—Separate reporting of host lymphoid response is optional. If reported, distinction should be made between peritumoral and intratumoral lymphoid infiltrates.

Statistical Issues

- Statistically robust studies are needed to confirm the relationship between host lymphoid response and prognosis and among intratumoral lymphocytes, MSI-H, and prognosis.

Recommendation.—Lymphoid response as outlined above (especially intratumoral lymphocytes) should be included in multivariate analyses to correlate with outcome and MSI-H status in statistically robust studies.

Histologic Type: Medullary Carcinoma, Mucinous Carcinoma[98,107–113]

Method Issues

- Variable use of routine histology alone versus use of special studies

Recommendation.—Perform assessment by routine histologic method alone. Special studies should be performed at the discretion of the pathologist.

Interpretation Issues

- Variable interpretation of mixed patterns common in MSI-H tumors (eg, signet ring cells in a mucinous carcinoma)
- Medullary type not included in the WHO classification prior to 2000
- Medullary types commonly confused with neuroendocrine carcinomas

Recommendations.—Medullary carcinoma should be distinguished and separately classified. Mixed patterns should be classified by predominant type.

Reporting Issues

- WHO classification variably used

Recommendation.—Report histologic type according to WHO classification as revised in 2000. Medullary carcinoma should be reported separately as a specific type according to the newly revised classification.

Statistical Issues

- Histologic type not proven statistically significant independent of tumor grade
- Relationship among tumor type, MSI-H, and outcome lacking

Recommendation.—Correlate tumor type with outcome and MSI status in statistically robust studies with multivariate analysis.

High Degree of MSI

Method Issues[114–118]

1. Molecular techniques
   - Tissue source may be variably contaminated with nonneoplastic cells
   - Variable probes may be used
   - Variable duration of fixation and type of fixative used (eg, containing heavy metals)
   - Variability in quality control
   - Variable use of requisite paired tumor and normal specimens for comparison
   - Innate variability in technique using polymerase chain reaction assays
2. Immunostaining techniques
   - Various antibodies (different clones) used
   - Tissue source fresh or fixed
   - Variable use of antigen retrieval methods leading to variable staining quality with individual antibodies to the major DNA repair enzymes (hMLH1 and hMSH2) (eg, hMLH1 is articularly dependent on appropriate antigen retrieval techniques)

Recommendations.—For molecular methods, good general polymerase chain reaction quality control is required. Overfixation (>72 hours) should be avoided. Manual microdissection is usually required to obtain ≥70% tumor DNA. Consensus probe panels developed by the National Cancer Institute for MSI are recommended. Paired tumor and normal samples must be used. For immunostaining methods, immunostaining procedures for hMLH1 and hMSH2 should include antigen retrieval based on steam heating with EDTA or citrate buffer.

Interpretation Issues

1. Molecular techniques
   - Variable recognition of cutoff of 30% instability for diagnosis of MSI-H
   - Misinterpretation of dilution by normal cells as a negative assay
2. Immunostaining techniques
   - Low sensitivity of staining and/or high background leading to misinterpretation

- Focal chromogen deposition occurring within truly negative nuclei with antigen retrieval techniques misinterpreted as positivity

Recommendations.—For molecular methods, interpretation of MSI-H should be strictly based on 30% or more of microsatellites assayed showing instability. Paired tumor and normal samples must be used. Dilution of tumor sample by normal cells (>30%) should be avoided. For immunostaining methods, internal and external staining controls should be used. Only diffuse nuclear staining should be interpreted as positive.

Reporting Issues

1. Molecular techniques
   - Variable use of multiple synonyms and related terms: microsatellite stable, low-level instability, MSI-H, replication error positive, mutator phenotype, ubiquitous somatic mutation
2. Immunostaining techniques
   - Uniform reporting format lacking

Recommendations.—For molecular methods, report should include specific probes used. The terms proposed by the National Cancer Institute Workshop on Microsatellite Instability (microsatellite stable, low-level instability, MSI-H) should be used.[118] The MSI-H status should be defined as more than 30% of markers analyzed demonstrating instability. For immunostaining methods, hMLH1 and hMSH2 expression should be reported as intact or absent.

Statistical Issues

1. Molecular techniques
   - Incomplete data on MSI-H as a therapeutic predictive factor
   - The importance of low-level instability uncertain
2. Immunostaining techniques
   - Familial MSI-H cases may have intact expression of both hMLH1 and hMSH2 due to either (1) missense mutation or (2) mutation in another of the DNA mismatch repair genes

Recommendations.—For molecular methods, prospective therapeutic trials are required to test predictive value. For immunostaining methods, when being used for heredity nonpolyposis colorectal cancer proband identification, MSI molecular testing should also be included in the testing algorithm.

Loss of Heterozygosity at 18q and Allelic Loss of Deleted in Colon Cancer Gene

Method Issues[119–128]

1. Molecular techniques
   - Variable contamination of tissue source with nonneoplastic cells
   - Variable probes used (eg, different clones)
   - Tissue source fresh or fixed
2. Immunostaining techniques
   - Various different monoclonal and polyclonal antibodies used
   - Variations in tissue antigenicity

Recommendations.—For molecular methods, good general polymerase chain reaction quality control is required. Overfixation (more than 72 hours) should be avoided. Manual microdissection is usually required to obtain ≥70% tumor DNA. Paired tumor and normal samples must be used. For immunostaining methods, internal and external positive and negative staining controls should be closely monitored. Antigen retrieval techniques are recommended by most authors.

Interpretation Issues

1. Molecular techniques
   - Variable interpretation of differential band intensity for diagnosis of allele loss if less than twofold.
   - Misinterpretation of noninformative assays as loss of heterozygosity (LOH) or as normal
   - Potential misinterpretation of dilution by normal cells as a negative assay
2. Immunostaining techniques
   - Weak positive staining variably interpreted as loss of expression

Recommendations.—For molecular methods, interpretation of allelic loss should be based on a twofold difference in band intensity. Paired tumor and normal samples must be used. Dilution of tumor sample by normal cells (>30%) should be avoided. For immunostaining methods, internal and external staining controls should be carefully monitored. Only completely negative nuclei should be interpreted as showing loss of deleted in colon cancer (DCC) gene expression. Grading of intensity of nuclear staining is inappropriate.

Reporting Issues

1. Molecular techniques
   - Multiple terms used as if synonymous: LOH, allelic loss, allelic imbalance, DCC (eg, not the only gene on 18q)
2. Immunostaining techniques
   - Uniform reporting format not yet established

Recommendations.—For molecular methods, report should include specific probes used and quantitative threshold for LOH (allelic imbalance). For immunostaining methods, DCC expression should be reported as intact or absent.

Statistical Issues

1. Molecular techniques
   - Variations in strength and utility of DCC as a prognostic marker vary among studies
2. Immunostaining techniques
   - Variation in relationship between loss of DCC expression and 18q LOH
   - Incomplete data on DCC loss as a therapeutic predictive factor

Recommendation.—For molecular methods, statistically robust prospective studies are needed to confirm prognostic value. For immunostaining methods, statistically robust studies are needed to establish the relationship among loss of DCC expression, LOH with various 18q probes, and patient outcome.

Tumor Border Configuration

Method Issues[38,51,85,89,96–98,129–132]

- Variation in criteria for assessment according to author
- Gross versus microscopic versus combination approaches to assessment
- Assessment variably subjective

Recommendation.—The 2-tiered evaluation system (pushing border vs infiltrating border) that has been defined by Jass et al and tested for interobserver variability should be used.

Interpretation Issues

- Substantial interobserver variability unless pathologists educated to definition
- Variation in opinions as to what features should be included in the definition

Recommendation.—Definitions of features of pushing versus infiltrating border published by Jass et al should be followed for interpretation and to reduce interobserver variability.

Reporting Issues

- Tumor border configuration rarely reported
- Recognition of significance of tumor border configuration not widely recognized

Recommendation.—If reported, report tumor border configuration described as pushing or infiltrating.

Statistical Issues

- Need for statistically robust studies with multivariate analysis

Recommendation.—Evaluation of tumor border configuration as a 2-tiered variable should be carried out in large studies on prognostic factors using multivariate analysis.

CATEGORY III FACTORS

DNA Content

Method Issues[73,133–143]

1. Flow cytometry
   - Methodology not standardized
   - Difficult to quality control
   - Variation with fresh versus archived tissue
   - Variation in quality of histograms with preparatory techniques
   - Variation with ratio of stromal to neoplastic cells
   - Channel setting (for linearity) variably machine dependent
2. Image analysis
   - Methods not standardized
   - Methods not widely available

Recommendation.—Data are insufficient to recommend specific methods. Comparative evaluation of methods for DNA content is needed.

Interpretation Issues

- Variation in determination of aneuploidy
  - Variation in setting of cutoffs
  - Variation in number of repeat analyses (discretion of investigator)
- Variation in basic definitions and terms (eg, diploid, diploid low, diploid high, nondiploid, aneuploid, tetraploid)

Recommendation.—Data are insufficient to recommend specific interpretation guidelines.

Reporting Issues

- Variation in terms (eg, diploid, diploid low, diploid high, nondiploid, aneuploid, tetraploid)

Recommendation.—Although standardized terms are needed, data are insufficient to recommend specific terminology. Definition should be reported for specific terms used.

Statistical Issues

- Univariate versus multivariate analyses
- Lumping of DNA ploidy and cell proliferation analysis into a single variable
- Adjustment for treatment variations

Recommendation.—DNA content should be evaluated using consistent and reproducible methods in large studies using multivariate analysis.

Other Molecular Markers

Overview.—A wide variety of molecular markers has been defined in colorectal cancer, but aside from LOH 18q/DCC loss and MSI-H (see category IIB above), the prognostic significance of these factors remains unproven.[1,119,144–183] A critical analysis of the variables related to each of these molecular markers is beyond the scope of this review, but the general limitations of the existing data defining the prognostic significance of these markers is outlined below. The categories of molecular markers linked with colorectal cancer include the following:

- Tumor suppressor genes (*LOH 1p/p53*, *LOH 8p*, *LOH 1p*, *LOH 5q*)
- Oncogenes (K-*ras*, c-*myc*)
- Apoptosis and cell suicide-related genes (*bcl*-2; *BAX*)
- DNA synthesis-related genes (thymidylate synthase; thymidine phosphatase)
- Transforming growth factors (TGF) and epidermal growth factor receptor (EGF-R) genes (*TGF-α*, *TGF-β*, c-*erb*-b/*her2/neu*, *EGF-R*)
- Cyclin-dependent kinase inhibitor genes (*p27*, *p21*)
- Angiogenesis-related genes (vascular endothelial growth factor)
- Adhesion molecule and glycoprotein genes (CD44, E-cadherin, sialo-Tn antigen)
- Matrix metalloproteases and inhibitors (urokinase-type plasminogen activator)
- Metastasis suppressor genes (*nm23-H1*)

Method Issues

- Variation of method according to investigator and factor
- Various methods applied to investigation of a single genetic factor producing different results (various types of aberrant genetic events may ultimately produce the same effect in the cell)
- No standard guidelines for clinical testing

Recommendation.—Data are not sufficient for specific recommendations.

Interpretation Issues

- Investigator-dependent interpretation
- Method-dependent interpretation

Recommendation.—Data are not sufficient for specific recommendations.

Reporting Issues

- Investigator-dependent reporting and terminology

Recommendation.—Data are not sufficient for specific recommendations.

Statistical Issues

- Large number of single studies on single factors
- Small number of studies on a large number of individual molecular factors
- Conflicting results from various studies of same factor (eg, p53)
- Almost no statistically robust studies on most factors
- Almost no multivariate analyses of most factors

Recommendation.—Individual factors should be evaluated as single variables in large studies on prognostic factors using multivariate analysis.

Perineural Invasion

Method Issues[1,2,44,87,184,185]

- Variable use of routine histology alone versus immunostaining to highlight nerves

Recommendation.—Use routine histology alone.

Interpretation Issues

- None

Recommendation.—None.

Reporting Issues

- Variably reported

Recommendation.—Report perineural invasion as present or absent in all cases.

Statistical Issues

- Univariate versus multivariate analyses
- Study size variation

Recommendation.—Perineural invasion should be evaluated as an individual variable in large studies on prognostic factors using multivariate analysis.

Microvessel Density

Method Issues[42,185–191]

- Variation in dilution of factor VIII (Dako Corporation, Carpinteria, Calif) used for immunostaining of endothelium
  - 1:250
  - 1:2400
- Variation in definition of a high-power field
  - ×40 field
  - ×20 field
- Variation in number of fields

Recommendation.—Standard guidelines for staining and evaluation should be established.

Interpretation Issues

- Granulation tissue due to ulceration versus tumor-induced neovascularization variably interpreted
- Variation in which vessels are counted
- Variation in interpretation of a vessel (eg, stained cells in clusters without lumens variably assessed as vessels)

Recommendation.—Interpretation guidelines published by Weidner should be followed.

Reporting Issues

- Variably reported as a density measurement: mean number of microvessels per high-power field
- Variably reported as a total number (microscopic area examined fixed) (eg, <25 or ≥ 25)

Recommendation.—Tumor angiogenesis should be reported as a maximum density measurement.

Statistical Issues

- Univariate versus multivariate analyses
- Small numbers of cases

Recommendation.—Microvessel density should be evaluated in large studies on prognostic factors using multivariate analysis.

Cell Proteins and Carbohydrates

Overview.—Among the numerous cell proteins and carbohydrate markers that have been reported in colorectal cancer, none have been extensively studied in clinical trials.[1] This class of tumor markers includes all the following substances:

- Class I HLA molecules
- Class II HLA molecules
- CA 19–9
- CA 72–4
- Sialyl Le^x
- Sialosyl-Tn
- Urokinase-type plasminogen activator
- Plasminogen activator inhibitor 2
- Glycoprotein 72
- P-glycoprotein (multidrug resistance gene product)
- MUC-1 mucin
- E-cadherin
- α-Catenin
- Integrins
- Type IV collagen
- Gelatinase B (metalloproteinase-9)
- Laminin
- Tenascin
- Autocrine mobility factor receptor (gp78)
- Phospholipase C
- Secretory component of immunoglobulin A
- Metallothionein
- EGF-R
- Gastrin receptor
- Somatostatin receptors
- Sucrase-isomaltase
- Cathepsin B, L, and D (cysteine/aspartyl proteases)
- Ferritin
- CD44
- Vitamin D receptor protein
- Cytokeratin 20

Method Issues

- Variation of method according to investigator and factor
- No standard guidelines

Recommendation.—Data are not sufficient for specific recommendations.

Interpretation Issues

- Investigator-dependent interpretation of results

Recommendation.—Data are not sufficient for specific recommendations.

Reporting Issues

- Investigator-dependent reporting of results and use of terminology

Recommendation.—Data are not sufficient for specific recommendations.

Statistical Issues

- Large number of single studies on single factors
- Small number of studies on a large number of individual cell elements
- Almost no statistically robust studies
- Almost no multivariate analyses

Recommendation.—Individual factors should be evaluated as single variables in large studies on prognostic factors using multivariate analysis.

Peritumoral Fibrosis (Desmoplasia)

Method Issues[5,97–99]

- Histopathologic examination alone versus special stains
- Sampling variation

Recommendation.—Assessment of tumor-associated stromal response should be performed by routine histopathologic examination of the tumor periphery (no special stains recommended).

Interpretation Issues

- Interobserver variation and intraobserver variation[99]
- Variation in judgment threshold for how much fibrosis constitutes desmoplasia
- Peritumoral fibrosis sometimes graded: little, moderate, extensive
- Variably considered part of tumor border configuration instead of a separate variable

Recommendation.—Explicit guidelines for analysis and interpretation of peritumoral fibrosis should be established.

Reporting Issues

- Usually not evaluated
- Usually not reported

Recommendation.—Guidelines for reporting of peritumoral fibrosis should be established.

Statistical Issues

- Significance of desmoplasia independent of tumor border configuration unclear
- Small studies insufficient to determine significance

Recommendation.—Peritumoral fibrosis should be evaluated by uniform method as an individual variable in large studies on prognostic factors using multivariate analysis.

Purulent Peritumoral Inflammatory Reaction

Method Issues[71,98,107,192–194]

- Variable sampling with avoidance of necrotic areas likely to be inflamed

Recommendation.—Data are insufficient to recommend specific methodologic guidelines.

Interpretation Issues

- Variation in interpretation of immune cells as inflammatory cells
- Variation in interpretation of abscesses as a primary versus secondary phenomenon in relationship to tumor perforation (itself an adverse prognostic factor)

Recommendation.—Inflammatory reactions should be evaluated as a feature separate and distinct from host lymphoid responses (see ''Host Lymphoid Response to Tumor'').

Reporting Issues

- Variably reported at all
- Variable reporting of inflammatory reaction in association with tumor perforation

Recommendation.—Tumor perforation should be reported in all cases. Inflammatory reaction should be reported as purulent in type to distinguish it from a host lymphoid (immune) response.

Statistical Issues

- Few studies with little data
- Variation in recognition and analysis as a unique factor distinguished from lymphoid response

Recommendation.—Peritumoral inflammation should be analyzed as a unique variable in large studies using multivariate analysis.

Foci of Neuroendocrine Differentiation Within Any Histologic Type

Method Variation Issues[195–197]

- Light microscopy—variable identification of neuroendocrine cells in routine hematoxylin-eosin–stained sections
- Histochemical stains—variable use of argentaffin and argyrophil reactions
- Immunohistochemical methods—variable use
  - Chromogranin
  - Neuron-specific enolase
  - Synaptophysin
  - Leu-7
  - Specific peptides

Recommendation.—Assessment should be performed by hematoxylin-eosin staining alone; data are insufficient to recommend special stains or immunohistochemical stains.

Interpretation Variation Issues

- Definition of significant neuroendocrine differentiation (eg, any positive cells detected by immunohistochemical staining or some specific number of cells)

Recommendation.—Data are insufficient to recommend specific interpretation guidelines.

Reporting Issues

- Dependent on interpretation and relationship to histologic type

Recommendation.—Documentation of neuroendocrine differentiation may be reported as a confirmation of small cell histologic type or rare composite or amphicrine tumors.

Statistical Issues

- Cut point determination varies

Recommendation.—Data are insufficient to recommend specific statistical guidelines.

Nucleolar Organizing Regions

Method Issues[71,192–194,197–199]

- Variation in thickness of sections (2–5 μm) used in different studies
- Variation in staining techniques
- Nucleolar organizing region analysis by automated image analyses versus counting under oil immersion
- Variation in number of nuclei counted

Recommendation.—Data are not sufficient to recommend specific method.

Interpretation Issues

- Variation in number of nucleolar organizing regions with plane of section (focusing up and down)
- Counting alone versus separation into patterns (eg, clusters vs individual) versus area of nucleolar organizing regions per nucleus
- Interobserver and intraobserver variability[193]

Recommendation.—Data are not sufficient to recommend specific method of interpretation.

Reporting Issues

- Median numbers versus ranges

Recommendation.—Data are not sufficient to recommend specific method of reporting.

Statistical Issues

- Small patient numbers
- Univariate versus multivariate analyses

Recommendation.—Data are insufficient to recommend specific statistical guidelines.

Proliferation Indices

Method Issues[200–216]

- Immunohistochemistry (Ki-67, proliferating cell nuclear antigen) method variation
- Flow cytometric method variation
- Mitotic counts rarely used for carcinomas—variation in approach
- Variation in number of counts performed using any method

Recommendation.—Data are insufficient to recommend specific method.

Interpretation Issues

- Interobserver variability in interpretation of a mitotic figure
- Immunostaining: variable interpretation of strong versus weak staining
- Overall interpretation variation—average or region of most intense activity only
- Specificity: Ki-67 variably expressed in noncycling cells

Recommendation.—Data are insufficient to recommend specific interpretation guidelines.

Reporting Issues

- Morphologic methods: variable expression of rate as number of cycling cells per high-power field or per fixed number of cells
- Proliferation index rarely reported at all

Recommendation.—Data are insufficient to recommend specific reporting guidelines.

Statistical Issues

- Univariate versus multivariate analyses
- Conflicting data

Recommendation.—Data are insufficient to recommend specific statistical guidelines.

CATEGORY IV FACTORS

Tumor Size

Method Issues[2,3,42,68,76,78,216,217]

- Variable number of dimensions recorded

Recommendation.—One dimension (largest diameter) is sufficient.

Interpretation Issues

- None

Recommendation.—None.

Reporting Issues

- Variably recorded as part of the gross description

Recommendation.—Tumor size should be report as part of permanent record of tumor description. Although the size of the tumor is of no prognostic significance, it may be important for quality control of tumor size determined by nonpathologic means (eg, imaging modalities).

Statistical Issues

- None.

Recommendation.—Stay in category IV.

Gross Tumor Configuration

Method Issues[3,67,74,86–88]

- None

Recommendation.—None.

Interpretation Issues

- Variable interpretation of complex configurations
- Variable numbers of individual configurations considered in evaluation

Recommendation.—Data are insufficient to recommend specific interpretation guidelines.

Reporting Issues

- Variable reporting of configuration
- When reported, variable interpretation (see above) produces variable reporting

Recommendation.—Data are insufficient to recommend specific reporting guidelines. Reporting is optional as a point of description and documentation.

Statistical Issues

- Multivariate versus univariate analyses

- Variable study size

Recommendation.—Stay in category IV.

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