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ORIGINAL ARTICLE

Disease Site

Blood/Bone Marrow

2205 **Rapid Engraftment after Allogeneic Transplantation of Density-Enriched Peripheral Blood CD34+ Cells in Patients with Advanced Hematologic Malignancies**

Thai M. Cao, Claus Kusnierz-Glaz, Frank Valone, Keith E. Stockerl-Goldstein, Wendy W. Hu, Laura Johnston, Karl G. Blume, Samuel Strober, and Robert S. Negrin

Allogeneic hematopoietic cell transplantation utilizing a peripheral blood allograft manipulated via low-density enrichment with a Percoll class gradient was feasible and safe, resulting in rapid engraftment and no occurrence of graft failure in examinable patients. However, the anticipated benefit of enrichment for a CD4⁺/CD8⁺ double-negative cell population, which in preclinical studies suppressed graft versus host disease (GVHD), was observed in only a few patients, and there was no reduction in the expected incidence of GVHD.

2214 **Anemia As an Independent Prognostic Factor for Survival in Patients with Cancer: A Systematic, Quantitative Review**

J. Jaime Caro, Maribel Salas, Alexandra Ward, and Glenwood Goss

Anemia is a predictor of survival for patients with cancer independent of the type of malignancy involved, and it must be considered in planning the treatment of these patients.

2222 **Methylation of *p15* and *p16* Genes in Adult Acute Leukemia: Lack of Prognostic Significance**

Chor S. Chim, Chris Y.Y. Tam, Raymond Liang, and Yok L. Kwong

Gene methylation of *p15* and *p16* was found to lack prognostic significance in adult acute leukemias.

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Dynamic Magnetic Resonance Imaging of Regional Contrast Access as an Additional Prognostic Factor in Pediatric Osteosarcoma

Wilburn E. Reddick, Sihong Wang, Xiaoping Xiong, John O. Glass, Shengjie Wu, Sue C. Kaste, Charles B. Pratt, William H. Meyer, and Barry D. Fletcher

Dynamic magnetic resonance imaging estimates of regional contrast access in patients with resectable primary nonmetastatic osteosarcoma after preoperative chemotherapy, when combined with tumor size, holds promise for the identification of patients at risk of recurrence. The availability of such imaging-based response predictors could facilitate the development of risk-adapted treatment approaches.

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Matthew R. Smith, Francis J. McGovern, Mary Anne Fallon, David Schoenfeld, Philip W. Kantoff, and Joel S. Finkelstein

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Catherine Mayers, Tony Panzarella, and Ian F. Tannock

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Patricia G. Moorman, Ali Hamza, Jeffrey R. Marks, and John A. Olson, Jr.

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Tohru Ohtake, Izo Kimijima, Toshihiko Fukushima, Mitsuhiko Yasuda, Kohji Sekikawa, Seiichi Takenoshita, and Rikiya Abe

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2273 **Form or Function? Part 1. Subjective Cosmetic and Functional Correlates of Quality of Life in Women Treated with Breast-Conserving Surgical Procedures and Radiotherapy**

Annette L. Stanton, Leela Krishnan, and Charlotte A. Collins

In 185 women who were diagnosed with Stage 0–II breast carcinoma from 3 months to 18 years previously and who underwent breast-conserving surgical treatment and radiotherapy, self-reported functional status (e.g., arm stiffness), and particularly breast specific pain, was related more consistently to quality-of-life indicators than was self-reported cosmetic status.

See accompanying article on pages 2282–7, this issue.

2282 **Form or Function? Part 2. Objective Cosmetic and Functional Correlates of Quality of Life in Women Treated with Breast-Conserving Surgical Procedures and Radiotherapy**

Leela Krishnan, Annette L. Stanton, Charlotte A. Collins, Vici E. Liston, and William R. Jewell

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Yoon T. Jeon, So Y. Yoon, Sang W. Shin, Byung S. Kim, Young J. Mok, Chong S. Kim, Jin H. Hyun, Jun S. Kim, and Yeul H. Kim

Epirubicin, cisplatin, uracil and tegafur, and leucovorin combination chemotherapy is highly active in the treatment of advanced gastric carcinoma.

2294 **Concurrent Hypermethylation of Multiple Tumor-Related Genes in Gastric Carcinoma and Adjacent Normal Tissues**

Wai K. Leung, Jun Yu, Enders K. W. Ng, Ka Fai To, Po K. Ma, Tin Lap Lee, Minnie Y.Y. Go, S. C. Sydney Chung, and Joseph J. Y. Sung

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Michael J. Barry, Peter C. Albertsen, Malcolm A. Bagshaw, Michael L. Blute, Richard Cox, Richard G. Middleton, Donald F. Gleason, Horst Zincke, Erik J. Bergstralh, and Steven J. Jacobsen

This article defines the outcomes of treatment for patients with nonmetastatic prostate carcinoma who were diagnosed in the era before the advent of prostate specific antigen screening and were treated with watchful waiting, radiotherapy, or radical prostatectomy. However, comparisons of outcomes for patients in different treatment groups are treacherous given the bias due to patient selection.

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Artur L. R. Bezerra, Geraldo H. Santiago, Karina C. B. Ribeiro, Maria R. D. O. Latorre, and Luisa L. Villa

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2322 Prediction of Prostate Carcinoma Stage by Quantitative Biopsy Pathology

Robert W. Veltri, M. Craig Miller, Alan W. Partin, Edward C. Poole, and Gerard J. O'Dowd

With the aid of advanced computational tools, quantitative pathology information extracted from biopsy specimens has been shown to enhance the prediction of organ-confined prostate carcinoma. Ordinal logistic regression and artificial neural network methodologies were used to train patient-specific statistical models to predict disease stage within a well-characterized set of patients. When challenged with an independent testing set, these trained computational models were able to predict with accuracy an organ-confined prostate carcinoma.

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Neoadjuvant chemotherapy in ovarian carcinoma help to select patients for feasible and less aggressive interval debulking.

Head and Neck Disease
2335 Completely Resected Anaplastic Thyroid Carcinoma Combined with Adjuvant Chemotherapy and Irradiation Is Associated with Prolonged Survival

Philip I. Haigh, Philip H. G. Ituarte, Hurng Sheng Wu, Patrick A. Treseler, Marc D. Posner, Jeanne M. Quivey, Quan Yang Duh, and Orlo H. Clark

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Gwi Eon Kim, Woo-Ick Yang, Sang-wook Lee, Yong Bae Kim, Chang Ok Suh, Joo-Heon Yoon, Young Taik Oh, Hyun Cheol Chung, and Byung Soo Kim

By dividing patients with angiocentric lymphoma of the head and neck into two groups according to whether their tumors were positive or negative for the cytotoxic granule-associated protein, granzyme B, and comparing their clinicopathologic and immunohistochemical findings, patterns of disease failure, and survival data, it was found that granzyme B may be useful as an additional marker for identifying patients with natural killer/T-cell lymphoma and as a prognostic indicator for risk assessment in patients with angiocentric lymphoma of the head and neck.

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Advanced Head and Neck Carcinoma in Women: Treatment Outcomes May Not Improve with Accelerated Hyperfractionated Radiotherapy

Felix Leborgne, José H. Leborgne, Jack F. Fowler, Eduardo Zubizarreta, and Julieta Mezzera

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Arja M. Lilja, Raija I. Portin, Päivi I. Hämäläinen, and Eeva K. Salminen

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Tony S. K. Mok, Sarath Kanekal, Xiao Rong Lin, Thomas W. T. Leung, Anthony T. C. Chan, Winnie Yeo, Simon Yu, Karen Chak, Rich Leavitt, and Philip Johnson

The authors performed a pharmacokinetic study of direct intralesional injection of cisplatin/epinephrine/bovine collagen gel into hepatocellular carcinoma. The results showed that this novel technology is capable of delaying cisplatin release from the tumor into systemic circulation.

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Association between Insulin-Like Growth Factor-2 and Metastases after Transcatheter Arterial Chemoembolization in Patients with Hepatocellular Carcinoma: A Prospective Study

Byung-Cheol Song, Young-Hwa Chung, Jeong A. Kim, Han Chu Lee, Hyun-Ki Yoon, Kyu-Bo Sung, Soo Hyun Yang, Kwon Yoo, Yung Sang Lee, and Dong Jin Suh

Increased levels of insulin-like growth factor-2 (IGF-2) after transcatheter arterial chemoembolization (TACE), a common occurrence in patients with large-sized tumors and high serum α -fetoprotein levels, are reported to be associated with the development of metastatic hepatocellular carcinoma after TACE.

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Lung Disease

2394 Patterns of Relapse of N2 Non-small-Cell Lung Carcinoma Patients Treated with Preoperative Chemotherapy: Should Prophylactic Cranial Irradiation Be Reconsidered?

F. Andre, D. Grunenwald, J. L. Pujol, P. Girard, A. Dujon, L. Brouchet, P. Y. Brichon, V. Westeel, and T. Le Chevalier

Preoperative chemotherapy was shown to decrease the risk of visceral metastasis in N2 non-small-cell lung carcinoma patients but was associated with a high rate of brain metastasis. It was concluded that prophylactic cranial irradiation should be reinvestigated in future clinical trials.

Skin and Soft Tissue

2401 The Sentinel Lymph Node Status Is an Important Factor for Predicting Clinical Outcome in Patients with Stage I or II Cutaneous Melanoma

Markwin G. Statius Muller, Paul A. M. van Leeuwen, Elly S. M. de Lange-de Klerk, Paul J. van Diest, Rik Pijpers, Charlotte C. Ferwerda, Ronald J. C. L. M. Vuylsteke, and Sybren Meijer

The sentinel lymph node (SLN) status has a strong additional value in predicting a minimum disease free period of 3 years after the SLN procedure. Patients with a positive SLN have a poorer prognosis than those with a negative SLN.

2409 Efficacy of Ultrasound B-Scan Compared with Physical Examination in Follow-Up of Melanoma Patients

Christiane Voit, Thomas Mayer, Martina Kron, Alfred Schoengen, Wolfram Sterry, Lutz Weber, and Thomas M. Proebstle

Regional tumor recurrences of melanoma were detected earlier with ultrasound B-scan than with physical examination. Better overall survival was noted in patients who had smaller metastases and a lower number of metastases.

Discipline

Translational Research

2417 Tumor-Infiltrating Lymphocytes Are a Marker for Microsatellite Instability in Colorectal Carcinoma

Thomas C. Smyrk, Patrice Watson, Karen Kaul, and Henry T. Lynch

Colorectal carcinomas with microsatellite instability have distinct molecular etiology and clinicopathologic features. Tumor-infiltrating lymphocytes may provide an economical indicator of colorectal carcinomas to test for microsatellite instability.

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Medical Oncology

2423 The Role of Surgery and Postoperative Chemoradiation Therapy in Patients with Lymph Node Positive Esophageal Carcinoma

Eric L. R. Bédard, Richard I. Inculet, Richard A. Malthaner, Edward Brecevic, Mark Vincent, and Rashid Dar

Patients who have undergone resection for lymph node positive esophageal carcinoma are at high risk of disease recurrence and early death. The results of this study indicate that postoperative chemoradiation therapy appears to prolong survival in this patient population.

2431 A Phase I Trial of Pulse Calcitriol in Patients with Refractory Malignancies: Pulse Dosing Permits Substantial Dose Escalation

Tomasz M. Beer, Myrna Munar, and W. David Henner

Substantial dose escalation with minimal toxicity was permitted by weekly dosing with oral calcitriol. Peak serum calcitriol levels in the range required for antiproliferative effects in vitro were achievable with weekly dosing.

2440 Bulky Disease Is an Adverse Prognostic Factor in Patients Treated with Chemotherapy Comprised of Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone with or without Radiotherapy for Aggressive Lymphoma

Richard B. Wilder, Maria A. Rodriguez, Chul S. Ha, Barbara Pro, Mark A. Hess, Fernando Cabanillas, and James D. Cox

More than one extranodal site of disease, bulky disease, and an at least 1.1-fold elevation in the serum lactate dehydrogenase (LDH) level were found to constitute independent, adverse prognostic factors for progression-free survival in patients with aggressive lymphoma treated with a chemotherapy regimen comprised of cyclophosphamide, doxorubicin, vincristine, and prednisone with or without consolidated radiotherapy. Age > 60 years, bulky disease, and an at least 1.1-fold elevation in the serum LDH level also were found to be adverse prognostic factors for overall survival. Involved-field radiotherapy should be considered in patients with bulky lymphomas.

Surgical Oncology

2447 Effect of Myocutaneous Inflammatory Changes Caused by Intra-Arterial Chemotherapy on the Outcome of Patients who Undergo Limb-Saving Surgery

Hiroyuki Tsuchiya, Toshio Morinaga, Junichi Taki, Hisashi Sumiya, Osamu Matsui, and Katsuro Tomita

Myocutaneous inflammatory reactions caused by intra-arterial chemotherapy should be prevented, if possible, to obtain good limb function and chemotherapeutic effects in patients who undergo this type of chemotherapy. Resection of necrotic tissue is mandatory to relieve pain.

(Continued)

Pediatric Oncology**2454** What Constitutes Optimal Therapy for Patients with Rhabdomyosarcoma of the Female Genital Tract?

Carola A. S. Arndt, Sarah S. Donaldson, James R. Anderson, Richard J. Andrassy, Fran Laurie, Michael P. Link, R. Beverly Raney, Harold M. Maurer, and William M. Crist

Females with localized rhabdomyosarcoma (RMS) of the genital tract are reported to have a 5-year survival of 87%. RMS at this site should be treated with combination chemotherapy, a conservative surgical approach, and the use of radiotherapy in selected patients. The best outcome is observed in patients ages 1–9 years, in whom the 5-year survival is reported to be 98% with multimodal therapy.

Epidemiology**2469** Survival Patterns of Cancer Patients

R. H. Riffenburgh and P. A. S. Johnstone

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2476 Lung Carcinoma in African Americans: A Review of the Current Literature

John H. Stewart IV

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Tumor-Infiltrating Lymphocytes Are a Marker for Microsatellite Instability in Colorectal Carcinoma

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BACKGROUND. Cells with deficient DNA mismatch repair develop microsatellite instability. Extensive microsatellite instability (MSI-high) is characteristic of colorectal carcinomas in hereditary nonpolyposis colorectal carcinoma (HNPCC) and in 10–15% of sporadic colorectal carcinomas. Microsatellite instability-high colorectal carcinomas differ from others in important clinical and pathologic features. However, MSI typing is expensive and not widely available. Microsatellite instability type may be predicted by tumor-infiltrating lymphocytes (TILs), which can be evaluated with ordinary light microscopy.

METHODS. The authors evaluated TILs as a pathology screen for MSI-high status in 138 colorectal carcinomas that had been evaluated for MSI in a variety of studies. This case series was systematically enriched with HNPCC and other MSI-high cases to allow accurate sensitivity and specificity estimation. Tumor-infiltrating lymphocytes were quantitated as TILs per 10 high-power microscopic fields by an observer blinded to MSI status.

RESULTS. Of the 138 carcinomas studied, 67 (48.6%) were MSI-high, 22 (15.9%) were MSI-low, and 49 (35.5%) were MSI-stable. All 25 HNPCC colorectal carcinomas were MSI-high. Tumor-infiltrating lymphocytes counts ranged from 0 to 300, with a markedly skewed distribution (median, 11; mean, 36). Sensitivity and specificity for selected cut points of TIL count were computed. Using a TIL count of 5 as a cut point yields a sensitivity of 93% and specificity of 62%. In a population in which 12% were MSI-high, consideration of TIL could reduce the number of colorectal carcinomas referred for MSI testing by greater than one-half, and still 93% of the MSI-high carcinomas would be identified.

CONCLUSIONS. The presence of MSI defines a subset of colorectal carcinomas with special molecular etiology and characteristic clinical, pathologic features, inclusive of increased survival. The authors conclude that quantification of TILs may provide a simple, single criterion for choosing which colorectal carcinomas are candidates for MSI testing. *Cancer* 2001;91:2417–22. © 2001 American Cancer Society.

KEYWORDS: tumor-infiltrating lymphocytes (TILs), hereditary nonpolyposis colorectal carcinoma (HNPCC), pathology, microsatellite instability, colorectal carcinoma.

Carcinogenesis is a multistep process involving the accumulation of multiple mutations. Colorectal carcinoma can be divided into two broad categories based on the mechanisms by which those mutations are acquired. One category, characterized by chromosomal instability and usually featuring multiple allelic deletions, accounts for roughly 85% of colorectal carcinomas. Colorectal carcinomas in the second category have defective DNA mismatch repair mechanisms, which allow mutations to be accumulated at many times the normal rate. The mutations are DNA base pair additions or deletions rather than coarser chromosomal derangements, and tumors arising in this setting typically have diploid DNA content. One manifestation

of the inability to repair DNA mismatches is cell-to-cell variability in the length of DNA microsatellites (mono- and dinucleotide repeat sequences); the phenomenon is known as microsatellite instability, and tumors with extensive microsatellite instability are designated microsatellite instability (MSI)-high. Patients with the syndrome of hereditary nonpolyposis colorectal carcinoma (HNPCC), who carry germ-line mutations of DNA mismatch repair genes, almost always develop MSI-high carcinomas. Approximately 15% of sporadic colorectal carcinomas are also MSI-high.

Colorectal carcinoma in HNPCC has a characteristic clinicopathologic profile, and that profile is shared with sporadic MSI-high carcinoma. Such tumors typically arise proximal to the splenic flexure. Mucinous carcinoma, signet cell carcinoma, and undifferentiated medullary carcinoma all are more common in this setting, and there is often morphologic evidence of host lymphoid response to tumor. Relatively improved prognosis, independent of stage, has been documented in HNPCC,¹⁻⁴ and a similar benefit may accrue in sporadic MSI-high colorectal carcinoma,⁵⁻⁷ although some studies dispute this point.^{8,9} Finally, theoretic and experimental considerations suggest that MSI-high carcinomas have unique responses to certain classes of chemotherapeutic agents.^{10,11}

There are several reasons, then, why it could be worthwhile to identify a particular colon carcinoma as MSI-high: 1) the chance to uncover a hereditary colorectal carcinoma-prone syndrome; 2) the ability to predict prognosis; and 3) the potential to influence therapy. The expense of performing MSI testing on all colon carcinomas makes it difficult to justify mass screening. We suggest that routine histologic examination can predict MSI-high status. If this is true, then pathologists can provide an essentially free screen for finding cases that warrant molecular assessment of MSI. The pioneering work of Jass et al. indicates that clinicopathologic features can identify MSI-high colorectal carcinoma with 82% sensitivity and 96% specificity.¹² In constructing his diagnostic algorithms, Jass et al. made use of a phenomenon first noted to our knowledge by Krishna et al.,¹³ i.e., that sporadic MSI-high colon carcinomas tend to have high numbers of tumor-infiltrating lymphocytes (TILs).

In this study, we have evaluated the value of TILs as a screen for MSI-high colon carcinomas. To accurately estimate sensitivity and specificity, we assembled a collection of cases that was enriched with MSI-high tumors by including a series from HNPCC patients and by selectively oversampling MSI-high tumors from another case series.

MATERIALS AND METHODS

One hundred thirty-eight colorectal carcinomas that had been evaluated for MSI status in other studies were included. Twenty-five of these carcinomas came from patients with HNPCC. Thirty-three carcinomas, family history unknown, collected as comparison cases in a previous study of prognosis in HNPCC colon carcinoma also were included. Finally, 80 carcinomas, family history unknown, were selected through a process that aimed to include equal numbers of MSI-high, MSI-low, and microsatellite-stable (MSS) carcinomas.

Histologic review was performed by a pathologist (T.C.S.) not aware of familial status or results of MSI testing. All test tissues had been processed in a routine fashion at the time of surgery; i.e., tissue was fixed in formalin for 12-24 hours and embedded in paraffin. Sections were cut 4 μ m thick and stained with hematoxylin and eosin. Tumor-infiltrating lymphocytes were quantitated as TILs per 10 high-power microscopic fields (field diameter 0.5 mm). The 10 fields were selected randomly from superficial and deep components of the tumor (not including intramucosal carcinoma), guided only by an effort to maximize the number of tumor cells per field. There was no adjustment made for cell-poor tumors such as mucinous tumors with only strips of neoplastic epithelium. Figure 1 shows a colon carcinoma with many TILs, visible as black dots with slight perinuclear haloes. For comparison, Figure 2 shows a tumor with few TILs.

Extraction of DNA from Specimens

For microsatellite instability analysis, normal and tumor DNA were extracted from formalin fixed paraffin embedded tumor and corresponding normal sample. DNA was extracted using standard procedures with proteinase K digestion, phenol/chloroform extraction followed by ethanol precipitation. Purified DNA then was quantified spectrophotometrically before polymerase chain reaction (PCR) amplification.

Marker Selection

A standard panel of five microsatellite markers (BAT26, BAT40, D2S123, D5S346, and D17S250) were studied.¹⁴ This panel is composed of two mononucleotide and three dinucleotide repeats. The sequences of the microsatellite PCR primers were described.¹⁵ One member of each primer pair was labeled with carbocyanine (Cy 5.5) fluorescence dye to permit detection by using an automated fluorescent DNA sequencing apparatus.

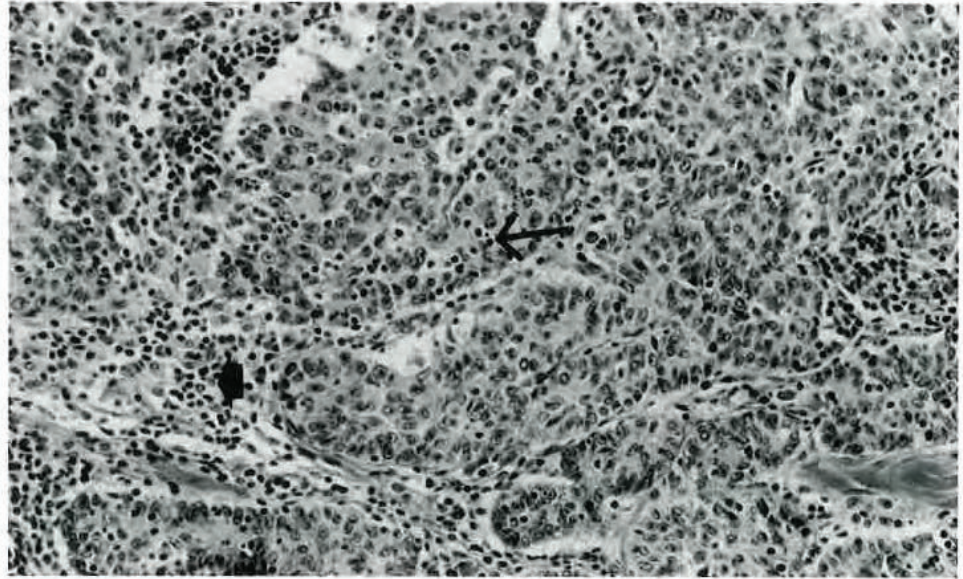


FIGURE 1. A colon carcinoma with many tumor-infiltrating leukocytes (TILs), visible as black dots with perinuclear halo (arrow). The stromal lymphocytes (arrowhead) are not counted as TILs.

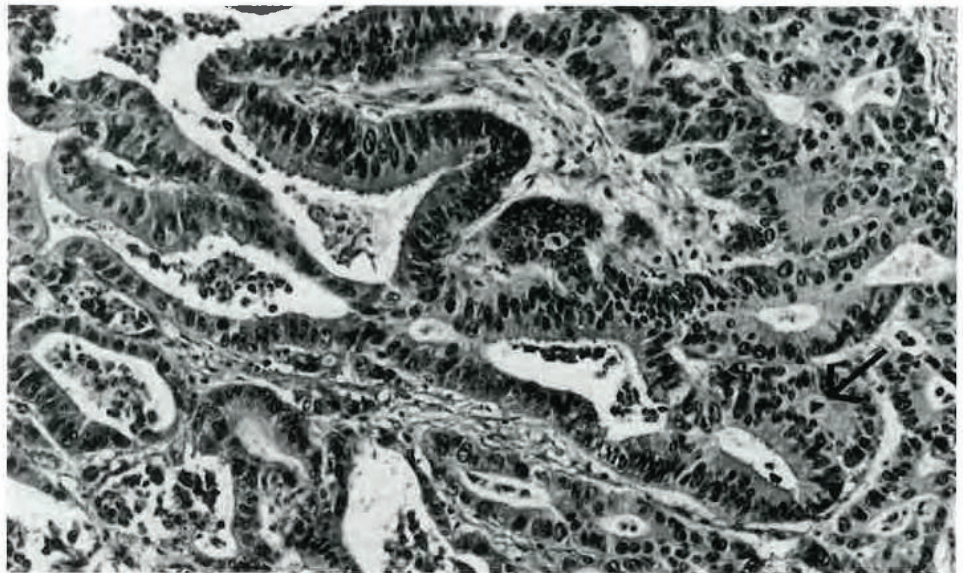


FIGURE 2. A colon carcinoma with few tumor-infiltrating leukocytes (TILs). Mitotic figures (arrow) are a potential pitfall and should not be mistaken for TILs.

Polymerase Chain Reaction

Polymerase chain reaction was performed in a final volume of 20 μ L containing 100 ng DNA, 1 $\times$ PCR buffer (Perkin-Elmer, Norwalk, CA), 300 μ M each dNTP, 3.0 mM Mg²⁺, 5% dimethyl sulfoxide (Sigma, St. Louis, MO), 5 pmol each primer and 1 U of Taq DNA polymerase (Perkin-Elmer). The DNA was amplified using a hot-start approach in a GeneAmp PCR System 2400 (Perkin-Elmer). Polymerase chain reaction initially denatured at 94 °C for 5 minutes followed by 35 cycles of denaturing at 94 °C for 30 seconds, annealing at 58 °C for 30 seconds for BAT26 and BAT40, 60 °C for 30 seconds for D2S123, 55 °C for 30 seconds for D5S346, and 52 °C for 30 seconds for D17S250, with extension at 72 °C for 1 minute. The final extension at 72 °C for 5 minutes was followed by

cooling to 4 °C. After amplification, 2 μ L from each reaction was added to 2 μ L of the size standards/stop mix solution (Visible Genetics, Inc., Toronto, Ontario, Canada). The reaction mixture was denatured by heating to 95 °C for 2 minutes and then rapidly chilled on ice immediately before gel loading.

Analysis was performed on a Micro Gene Blaster automated Fluorescent DNA Sequencer (Visible Genetics, Inc.) using GeneObject fragment analysis software. Samples showing novel bands in the tumor DNA relative to the normal DNA, present in two or more of the five markers analyzed, were designated as MSI-high, in accordance with National Institutes of Health Consensus Conference Guidelines.¹⁴ Samples showing a size shift at a single microsatellite locus were designated as MSI-low.

Statistical Analysis

The significance of the difference between MSI-high and MSI-low/stable (pooled) was evaluated using the Wilcoxon rank-sum test. To evaluate the validity of our assumption that the MSI-low cases are similar to the MSS cases in TIL count, we used one-way analysis of variance of TIL values classified by MSI (high, low, stable) with Tukey studentized range test comparing all possible pairs of groups. Sensitivity and specificity of TIL count as a predictor of MSI-high status was calculated using selected cut points of TIL count, with 95% confidence intervals. Statistical analysis was performed using SAS release 6.12 (SAS Institute, Cary NC); confidence intervals were calculated using exact methods following Fleiss (p. 14).¹⁶

RESULTS

Of the 138 carcinomas evaluated, 67 (48.6%) were MSI-high, 22 (15.9%) were MSI-low, and 49 (35.5%) were MSS. All 25 HNPCC tumors tested had microsatellite instability (MSI-high). Tumor-infiltrating lymphocyte count ranged from 0 to 300, with a markedly skewed distribution (median, 11; mean, 36; standard deviation, 56). In tumors with the highest TIL counts (> 200 per 10 fields), lymphocyte distribution was uniform: every field examined had 10 or more TILs. Tumors with 30–200 TILs per 10 fields showed some variability from field to field, but TILs were always present in at least half of the fields examined.

As shown in Table 1, TIL counts in MSI-high cases differed significantly from the pooled set of MSI-low and MSS ($P < 0.0001$). The MSI-low cases, with a mean of 13.9, did not differ significantly from the MSS cases, with a mean of 8.7.

A receiver-operator characteristic curve, showing sensitivity and specificity for selected cut points of TIL count is given in Figure 3.

DISCUSSION

We studied a series of colorectal carcinoma cases with known MSI status to estimate the sensitivity and specificity of TIL count as an indicator of MSI. As shown in Figure 3, the best combination of sensitivity and specificity is obtained using a TIL count of 7 as a cut point (sensitivity, 88%; specificity, 75%). Different cut points can be chosen depending on whether the clinical situation calls for a sensitive or specific test. If TIL count is to be used as a screening test, then a high-sensitivity, low-specificity point of the curve would be preferred. For example, using a TIL count of 5 as a cut point yields a sensitivity of 93% and specificity of 62%.

Our series were enriched for MSI-high cases. In practice, test performance depends in part on the

TABLE 1
TIL/10 HPF in MSI-High vs. MSI-Low/MSS CRC

Group	Median	Mean	SD
MSI-high	42	63.3	67.6
MSI-low/MSS	2	10.3	21.7

TIL: tumor-infiltrating leukocyte; HPF: high power field; MSI: microsatellite instability; MSS: MSI-stable; CRC: colorectal carcinoma; SD: standard deviation.

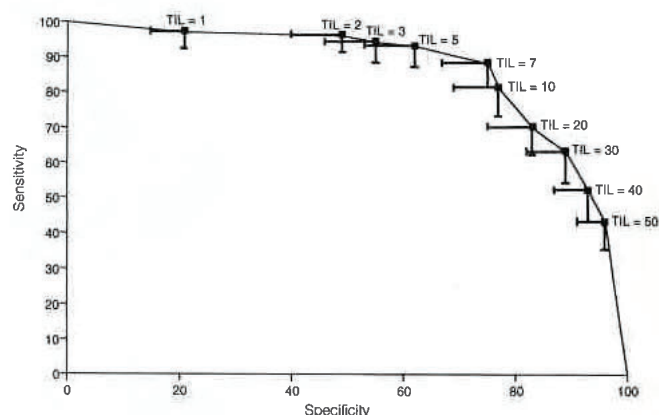


FIGURE 3. Predicting microsatellite instability (MSI)-high from TIL count: sensitivity and specificity by TIL cut point. Estimates are based on 67 MSI-high and 71 MSS/MSI-low colorectal carcinoma cases. Lower limits of the 95% confidence interval for both sensitivity and specificity are indicated by error bars. TIL: tumor-infiltrating lymphocyte.

prevalence of the disease being tested for. In a recent study of 535 consecutive patients with colorectal carcinoma, 12% had MSI-high carcinomas.¹⁷ Using this figure, and a TIL cut point of 5, we would expect that 55.4% of an unselected series would have a “negative” test (TIL count < 5); of these cases, 1.5% would be false-negatives (i.e., MSI-high cases). Thus, the predictive value of a negative test is 98.5%. We would expect 44.6% of the series to have a “positive” TIL count, and of these 75% would be false-positives (i.e., MSI-low or MSS cases), making the predictive value of a positive test 25%. By using TIL count as a screen, the numbers of cases referred for MSI testing can be reduced by greater than half, while still identifying 93% of the MSI-high carcinomas. Immunohistochemistry for the protein products of DNA mismatch repair genes *MLH1*, *MSH2*, and *MSH6* has been shown to be an effective way to document mutation or inactivation of those genes.¹⁸ One might envision a three-tiered system, with histology (TIL) screen identifying cases for immunohistochemical staining, followed by molecular testing to confirm MSI-high status.

Finding MSI-high colorectal carcinomas is motivated by three clinical goals: diagnosing HNPCC, predicting prognosis, and influencing therapy. Note that

no step discussed thus far (TIL count; immunohistochemistry for *MLH1*, *MSH2*, and *MSH6*; or molecular testing for microsatellite instability) has been shown to distinguish sporadic MSI-high colorectal carcinoma versus HNPCC. Our study was not designed to answer questions about differences between sporadic and HNPCC carcinomas. A two-stage process with the objective of identifying HNPCC patients (first MSI typing then mutation testing only MSI-high cases) has been evaluated,¹⁷ and was found to be effective. In this setting, an additional screening step using clinical features that distinguish HNPCC from non-HNPCC colorectal carcinoma cases can resolve the requirement for MSI typing. Salovaara et al.¹⁷ suggest that for detection of HNPCC, MSI testing can be limited to those patients with colorectal carcinoma diagnosed when the patient was younger than 50 years of age, or with multiple primary carcinomas of the colorectum or endometrium, or with a first-degree relative affected by colorectal or endometrial carcinoma. Using these screening parameters, only 22% of 535 cancer patients required MSI testing, but 17 of 18 HNPCC patients in the series were identified. These selection criteria are not sensitive predictors of MSI-high status (nor were they designed to be); 66 of the 535 patients had MSI-high colorectal carcinomas, but the selection criteria identified only 34 of them.

If the goal is to identify all MSI-high carcinomas regardless of HNPCC status, then TIL count alone, a simple parameter easily quantitated by pathologists using routine stains and equipment, is a valuable first step in the process. Building on the work of Jass et al.,¹² which identifies other clinicopathologic discriminators such as tumor site and mucin content, it should be possible to further improve sensitivity and specificity without adding cost.

Are TILs more than a marker of MSI-high/improved prognosis carcinomas? Might they have a direct effect on prognosis? In support of this idea, Dolcetti et al.¹⁹ have shown that MSI-high colorectal carcinomas harbor increased numbers of activated cytotoxic T lymphocytes, and have a higher percentage of apoptotic tumor cells, than MSS carcinomas. Activated cytotoxic lymphocytes have been linked to prognosis in extracolonic malignancies as well, including medullary carcinoma of the breast²⁰ and malignant melanoma.²¹

The pathophysiology of TIL accumulation in MSI-high colorectal carcinoma has not been elucidated. An early proposal was that MSI-high tumors make so many abnormal proteins that they are particularly likely to incite a host immune response. No experimental evidence supporting this theory has been developed. Fas ligand may play a role; tumor cells that

express Fas ligand can kill lymphoid cells by Fas-mediated apoptosis in vitro, the so-called "Fas counterattack".²² It may be that MSI-high colorectal carcinomas are less able to express functional Fas ligand. There is some evidence that Fas ligand expression is related to the ability of colorectal carcinoma to metastasize,²³ so it would seem that failure to express Fas ligand might result in improved prognosis as well as increased numbers of TILs. These and other theories remain to be investigated.

The presence of microsatellite instability defines a subset of colorectal carcinoma with special molecular etiology and characteristic clinicopathologic features. Because it is a small subset (approximately 15% of all colorectal carcinoma), it may not be feasible to test all colorectal carcinoma for MSI. A variety of clinicopathologic features can be used to enrich the test population, including those suggested by Jass et al.¹² (right-sided location, excess mucin production, undifferentiated morphology, TILs) and those suggested by Salovaara et al.¹⁷ (diagnosis at younger than 50 years of age, multiple colorectal carcinomas, colorectal or endometrial carcinoma in a first-degree relative). We suggest that quantitation of TILs provides a simple, single criterion for choosing which colorectal carcinoma to test for MSI.

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