C282Y mutated protein (i.e., differential allele expression, HFE protein accumulation in the endoplasmic reticulum, or degradation of the protein). Finally, we agree with the need for further studies to determine whether RNA stability rather than RNA transcription is involved in this regulation.

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Microsatellite Instability as a Molecular Marker for Very Good Survival in Colorectal Cancer Patients Receiving Adjuvant Chemotherapy

Dear Sir:

Hemminki et al. present some interesting prospective data showing that microsatellite instability (MSI) is associated with excellent survival in patients with Dukes C colorectal carcinoma (CRC) receiving 5-fluorouracil (5-FU)-based chemotherapy. Although not discussed in their study, it would be interesting to know if the survival of MSI patients in the nonchemotherapy group was also better than that of MSI patients. Earlier this year our group published virtually identical survival curves for CRC patients receiving chemotherapy; however, we did not observe prognostic value for MSI in patients not treated with chemotherapy (Figure 5 in Elsaleh et al.). Our results suggest that MSI could be an important molecular predictive marker allowing more refined selection of CRC patients to receive chemotherapy. The MSI phenotype is strongly associated with normal p53 gene status, a known marker of good response to chemotherapy both in vitro and in vivo. MSI is also associated with the methylator phenotype, characterized by frequent hypermethylation of CpG sites within promoter regions and often resulting in transcriptional silencing. As suggested by our group, the methylator phenotype may be an even stronger predictive factor than MSI status. Methylation of both the p16 and hMLH1 genes occurs more frequently in CRC from female than male patients; it is tempting to speculate that these observations are related to our previous finding that female CRC patients derive more survival benefit from chemotherapy than males.

It will be interesting to determine in future studies whether MSI tumors that are methylated for hMLH1 are phenotypically different to MSI tumors that carry a germline or somatic mutation of one of the DNA repair genes.

A recent report shows that methylation of the DNA repair gene MGMT is associated with good response of gliomas to alkylating agents, suggesting this epigenetic alteration may be a predictive factor for various cancer types and treatments. Regardless of the mechanism by which MSI and DNA methylation are associated with response to therapies, it is clear that phenotypes defined by genetic and epigenetic alterations can help to identify cancer patients who stand to gain benefit from the use of adjuvant therapies.

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Reply. We found that patients with MSI stage C colorectal cancer (CRC) had better disease-free survival than patients with MSI CRC when both groups were treated with 5-FU-based chemotherapy. Patients with MSI CRC had as high as 90% 3-year disease-free survival rate compared with only 43% among those with MSI CRC, and the 3-year overall survival also tended to be more favorable in MSI CRC (90% vs. 62%). Elsaleh et al. ask whether patients with MSI CRC have a survival advantage over those with MSI CRC also in the subset of our series that was not treated with 5-FU-based adjuvant therapy.

Many studies have found that the outcome of MSI stage C CRC is superior to that of MSI CRC, as reviewed in our study. Because most of these series are retrospective and the benefit of adjuvant chemotherapy has been debated until recently, it is likely that many of these patients have probably not been treated with adjuvant 5-FU. For example, Wright et al. studied 255 patients with stage C CRC, none of whom received adjuvant chemotherapy, and found improved prognosis for the MSI group. Based on these results, MSI CRC has been generally considered biologically less aggressive than MSI CRC and to have more favorable outcome. The purpose of our study was to investigate whether 5-FU-based chemotherapy could be safely given to patients with MSI CRC, and whether it influences prognosis of these patients. Therefore, we limited our analysis to the MSI group, and we have not yet detailed the treatment data available for the MSI group.

In accord with previous studies, Elsaleh et al. found a better outcome for MSI cancers over MSI cancers. Importantly, in their series adjuvant chemotherapy appeared to benefit only patients with MSI CRC, suggesting that the favorable outcome related to MSI CRC may in part be a treatment effect. Although our results provide some support to this hypothesis, we do not recommend limiting
adjuvant chemotherapy to MSI* CRC based on the presently available evidence only. In most studies, 12%–15% of stage C CRCs are MSI*,1 and the proportion of MSI* CRC was 9% in the series of Elsaleh et al. Because prospective randomized trials have found a survival difference of 25%–30% in favor of patients treated with adjuvant chemotherapy in Dukes C colon cancer,4 the proportion of MSI* CRC appears to be too small to account for the chemotherapy effect. Unequal distribution of prognostic factors between the chemotherapy and nonchemotherapy groups in nonrandomized series may also explain some of the observed lack of effect of adjuvant chemotherapy in the MSI* group.

Further studies assessing the relative benefit of adjuvant chemotherapy are particularly interesting in the MSI* subgroup of CRC. Ideally, this question could be answered using paraffin-embedded tissue of patients who have participated in a randomized controlled trial in which a surgery-only arm is available. Regarding MSI* CRC, both our results and those of Elsaleh et al. suggest that adjuvant 5-FU–based chemotherapy benefits these patients.

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**Intestinal Motility and Bacterial Overgrowth in Patients With Gallstones**

**Dear Sir:**

We read with a great interest the article of Thomas et al.1 who reported that patients with cholesterol-rich gallbladder stones have slow colonic transit times, increased numbers of gram-positive anaerobes, and greater 7α-dehydroxylation activity and that all 3 favor enhanced deoxycholic acid (DCA) formation and its passive absorption from the large intestine.

These observations are very interesting because both intestinal hypomotility and bacterial overgrowth may also facilitate enhanced unconjugated bilirubin (UCB) formation, its passive absorption, and increased levels of bilirubin conjugates in bile. Therefore, we believe that increased DCA formation is important in the pathogenesis of cholesterol and pigment gallstones, and it raises the possibility that the origins of cholesterol gallstones and pigment gallstone disease are linked through this secondary bile acid. There is published evidence to support this hypothesis.

First, bilirubin pigments are part of the composition of both cholesterol and pigment gallstones. Second, cholesterol gallstone characteristic contains a central pigmented nodule with either radial or lamellar pigmented bands alternating with nonpigmented areas. Third, analysis of the matrix associated with the pigment area in the cholesterol stones indicates that 85% is UCB, 15% bilirubin monoglucuronides, and traces of deconjugated 2-alanine as evident by calcium salts.

There is also some evidence that argues in favor to the role of the intestinal hypomotility in an increasing absorption of UCB; this in turn may contribute in the pathogenesis of hyperbilirubinemia and pigment gallstones. Kotal et al.1 observed in rats that fasting decreases intestinal motility and fecal elimination of bile pigments; this alteration in turn enhanced enterohepatic circulation of UCB and resulted in an increased reflux to plasma because of the low first-pass clearance of UCB by the liver. Furthermore, the ratio of biliary bilirubin to bile acids doubled during fasting. If this also happens in humans, such changes could contribute to bile pigment sludge and stone formation observed during fasting, for example, prolonged parenteral nutrition.

In recent studies we found that ileal resection in rats leads to a doubling of conjugated bilirubin secretion rates in bile, compared with proximal intestinal resection or sham-operated controls.4 We hypothesized that this is a result of the enterohepatic cycling of UCB induced by small intestinal bile salt loss. Some possible considerations that support this hypothesis are that UCB is known to be absorbed by passive diffusion from the intestine, whereas its conjugated form is not, and at the intestinal level, both UCB solubility and absorption are bile salt dependent. Interestingly, this observation has been confirmed in patients with inflammatory (Crohn’s) bowel disease.5

One would think that in patients with anaerobic bacterial overgrowth in the small intestine, bilirubin deconjugation and enterohepatic cycling would also occur. There are some clinical conditions associated with bacterial overgrowth such as hypochlorhydria, or achlorhydria. Affector loop of Billroth II, duodenaljejunal diverticulosis, surgical blind loop (end-to-side anastomosis), obstruction (stricture, adhesion), inflammation, neoplasm, scleroderma, diabetic autonomic neuropathy, resection of diseased ileocecal valve, chronic pancreatitis, immunodeficiency syndromes, and cirrhosis may potentially induce bacterial overgrowth.

In addition, Berr et al.6 have proposed that DCA excess in patients with cholesterol-rich gallbladder stones could be caused by one or more of the following mechanisms: (1) decreased absorption of cholic acid in the ileum attributable to impairment of ileal bile acid transport or binding of cholic acid to luminal contents, (2) enhanced colonization of the large and possibly the small intestine with anaerobic bacteria capable of CA-7α-dehydroxylation, and (3) increased absorption of DCA from the colon possibly caused by some alteration in the physical state of DCA in the colon or by slow colonic transit. Finally, according to our hypothesis, deconjugation of DCA concomitantly with bilirubin conjugates probably facilitates the UC bile absorption, and thereby enterohepatic cycling of bilirubin could occur. This question could be answered whether Thomas et al.1 have measured the molecular bilirubin species in bile of patients with cholesterol-rich gallbladder stones.

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