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ORIGINAL ARTICLE

Clinicopathological predictors of benefit from adjuvant chemotherapy for stage C colorectal cancer: Microsatellite unstable cases benefit

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Abstract

Aim:　In colorectal cancer (CRC), adjuvant therapy is offered on the basis of stage and attempts to identify factors to better target treatment have not been successful. Recent work suggested that mismatch repair deficient CRCs may not benefit from 5FU adjuvant chemotherapy but studies remain conflicting. We aimed to determine if gender, tumor site, tumor pathological characteristics and microsatellite instability (MSI) predict survival benefit from adjuvant chemotherapy in stage C CRC.

Methods:　Data were collated on ACPS (Australian Clinico-pathological Staging System) stage C CRC cases that underwent curative resection over a 23-year period. Pathology was reevaluated, DNA was extracted from the formalin-fixed paraffin specimen, and MSI status was established by BAT26 instability. Multivariate analysis was performed using Cox proportional hazard model and effects modification interaction testing.

Results:　In total 814 unselected cases were included, of whom 37% received chemotherapy. Seventy-seven cases exhibited MSI. Overall, adjuvant chemotherapy produced a cancer-specific survival benefit (HR 0.52, 95% CI 0.39–0.70; $P < 0.0001$). On interaction testing, none of the examined parameters significantly influenced the magnitude of that survival benefit. Chemotherapy was beneficial in both the MSI (HR 0.08, 95% CI 0.02–0.27; $P = < 0.0001$) and the microsatellite stable cohort (HR 0.62, 95% CI 0.47–0.81; $P = 0.001$).

Conclusion:　These results suggest that survival benefit from 5FU adjuvant chemotherapy for stage C CRC does not vary according to gender, site of tumor, pathological characteristics or MSI status. This study suggests that it would be unwise to exclude patients from being offered adjuvant chemotherapy on the basis of MSI.

Key words: adjuvant, chemotherapy, colorectal cancer, microsatellite instability, predictive biomarker.

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INTRODUCTION

Much recent emphasis in cancer treatment has been toward individualizing cancers and better targeting treatment. In colorectal cancer (CRC), the decision for adjuvant chemotherapy is still based largely on tumor stage. Attempts to stratify risk within heterogeneous stage groups have not produced clinically useful results and identification of factors to target treatment has proven elusive.

It has been suggested that female gender and patients with proximal colonic tumors may receive greater benefit from adjuvant chemotherapy.[1] However, this advantage is not marked. It is also often assumed that poor prognostic stage C tumors will have a greater benefit from adjuvant chemotherapy but evidence for this is lacking.

In an attempt to understand which patients may derive greater benefit from adjuvant chemotherapy, recent work has focused on tumors with microsatellite instability (MSI), being indicative of tumors with deficiency of a mismatch repair (MMR) protein. Initial *in vitro* work predicted resistance in MSI tumors.[2–4] In contrast, early clinical studies suggested a trend to improved survival for MSI cases given 5-fluorouracil (5FU) adjuvant chemotherapy,[1,5–7] including the suggestion that they would be the only ones to benefit.[1] Other studies have shown no difference in the survival benefit between MSI high and microsatellite stable (MSS) tumors,[8,9] while others suggest the MSI cases are not benefitting.[10–16] Furthermore, some studies have suggested there may be a negative impact on survival in MSI tumors.[12,14]

The aim of this study was to determine if gender, tumor site, tumor pathological characteristics and MSI status could predict survival benefit from 5FU-based adjuvant chemotherapy given post curative resection for node-positive CRC.

METHOD

This study is a retrospective cohort study, comparing survival in patients who received chemotherapy with those who did not, looking for a compounding effect from clinical and pathological parameters and MSI status. Patients were eligible if they underwent a curative resection for an ACPS (Australian Clinico-pathological Staging System) stage C[17] colon or rectal adenocarcinoma (equivalent to AJCC stage C). All identifiable cases from three hospitals over a 23-year period were included (1980–2003). Cases from years prior to the use of standard chemotherapy in our institutions were included to minimize the selection bias in the non-chemotherapy cohort. Cases were excluded if death occurred perioperatively or if non-CRC cancers were present. Cases of multiple CRC were included only if an index stage C cancer could be clearly established; synchronous cancers were included if the second cancer was clearly an earlier stage and metachronous cancers were included if the previous cancer occurred more than 5 years beforehand. Only patients who received single

agent 5FU-based regimens were included. This included infusional and bolus 5FU (±folinic acid). Patients who received combination therapy (i.e. with oxaliplatin) were excluded. Cases were classified by intention to treat – if the patient at least commenced treatment, the case was included in the chemotherapy cohort. Administration of radiotherapy was recorded. Appropriate ethics approval was obtained from the hospitals' human research ethics committees. Cases were identified from hospital and state registries and pathology databases. Demographic and operative data were collated and case notes were reviewed if required. Death data were sourced from the South Australian State Cancer Registry, which is cross-checked regularly with national databases.[18]

Tumor site was determined from operative report and proximal tumors were defined as including the splenic flexure. Obstruction and perforation were assessed from the pathological specimen. Pathology reports and archived slides for all cases were retrieved. A single specialist colorectal pathologist (ARR) reevaluated all pathological slides. If slides were inadequate or lost, further sections were taken from archived tissue blocks. Negative margins were confirmed.

MSI analysis

MSI was established by identification of instability in the mononucleotide sequences BAT 40 and BAT 26, the latter being highly specific for MSI high.[19] Tumor and normal DNA samples were taken from formalin-fixed paraffin embedded archived specimens. Tissue was dewaxed and DNA was extracted using Tris/EDTA DNA buffer and proteinase K, 6 M NaCl for salt extraction, and ethanol. One of the microsatellite primer pairs was fluorescently 5′-labeled during synthesis. DNA (2.5–10 ng) was amplified in a standard 50 μL reaction mix: 200 μM of each dNTP, 2 mM of $MgCl$, 0.2 μM of forward fluorescent and reverse non-labeled primers, 0.5 U of AmpliTaq Gold DNA (Perkin-Elmer/Cetus, Waltham, Massachusetts, USA). Cycling conditions involved an initial denaturation at 95°C, 30 s at 55°C and 90 s at 68°C with a final extension of 10 min at 68°C. PCR products of paired normal and tumor tissue were electrophoresed using an ABI Prism 377 or 3700 DNA sequencer (Perkin-Elmer) and analyzed using Gene scan and Genotyper (Applied Biosystems, Carlsbad, California, USA). The complete National Cancer Institute (NCI) panel[20] was run on equivocal cases including when there was discordance between the BAT

26 and BAT 40 result, MSI defined by instability in two or more markers.

MSI status was categorized as positive or negative. "Positive" in this study is equivalent to MSI high (given the specificity of the markers used). Patients with an equivocal NCI panel result were classified as negative.

Statistical analysis

The primary study endpoint was cancer-specific survival. Noncancer deaths were censored. Univariate analysis was performed using Kaplan–Meier survival curves and compared by log rank test. A Cox regression proportional hazard model was used for multivariate analysis with backward elimination by likelihood ratio. Significance was set at 0.05 and confidence intervals at 95%. Interaction testing was incorporated into the adjusted analysis model to test for effects modification. Chi-square testing was used to compare groups. Analysis was performed using PASW Statistics for Windows (version 18, Copyright 2009, SPSS Inc, Chicago, IL, USA) and Intercooled Stata for Windows (version 8.2, Copyright 2004, StataCorp LP, College Station, Texas, USA).The association between MSI and pathological factors was tested by logistic regression. *A priori* power calculations determined that 372 patients were required per group to achieve 80% power to show a 10% survival difference between groups (chemotherapy *vs* no chemotherapy).

RESULTS

Over the 23-year study period, 993 ACPS stage C cases were identified that had undergone a curative resection. Of these, 179 cases were excluded, 128 because pathology was unavailable, 42 died in hospital perioperatively, 7 had multiple CRCs that met the exclusion criteria, and in 2 cases there was inadequate clinical information. This left 814 cases for study. Cases were near consecutive apart from the first 5-year period when not all cancers were registered in the available databases. Twelve specimens could not be assessed for MSI due to lack of tissue and were not included in the MSI analysis. Three cases with synchronous CRCs were not included in the site analysis. Median follow-up was 36.3 months overall (range 0.6–290 months) and 72 months (6 years) for living patients. Median age was 71 years (range 30–96 years). Gender distribution was equal (male 49.6%, female 50.4% ns). Sixty-one percent of tumors were located distal to the splenic flexure. The pathological data frequency and prognostic influence are shown in Table 1.

Of the 802 cases where MSI status was successfully established, 9.6% (77) were positive. Of these, 27 (35%) received chemotherapy. MSI cases were more likely to be women (70% compared with 48% of MSS cancers, $P < 0.0001$) and the tumors were more likely to be proximal (83% compared with 40%, $P < 0.0001$) (Table 2), although on adjusted analysis only proximal site remained significant (HR of MSI in a distal cancer was 0.16, 95% CI 0.08–0.33; $P < 0.0001$). They were more likely to be poorly differentiated (83% *vs* 35%, $P < 0.0001$) and there was a strong association with tumor infiltrating lymphocytes found in 30% of MSI cases, but only 1.5% of MSS cases ($P < 0.0001$).

Gender or tumor site did not affect outcome on univariate or multivariate analysis. Perforation but not obstruction had an adverse effect on survival (HR 2.27, 95% CI 1.46–3.53; $P < 0.0001$). The pathological parameters that were associated with a significantly poorer survival were higher nodal stage, poor differentiation, mucinous component to the tumor, extramural vascular invasion and perineural invasion (Table 1). T stage, tumor size, nature of advancing edge (infiltrative or pushing), type of stroma and budding did not independently influence survival. MSI status did not significantly affect survival across the whole group but was associated with a better prognosis in the untreated cohort (HR 1.98, 95% CI 1.23–3.20; $P = 0.005$).

Chemotherapy was commenced in 37% of patients and radiotherapy in 12%. Cases prior to 1993 (the year of implementation of standard adjuvant chemotherapy in our institutions) comprised half of the non-chemotherapy cohort. The chemotherapy cohort was younger (65.4 years *vs* 75.1 years, $P < 0.0001$), had a slight male predominance (56.0% *vs* 45.8%, $P = 0.005$) and had a higher proportion of poor prognostic indicators (Table 3). Overall, 469 (57.6%) patients died during the study period. Of these, 76% were CRC-related deaths.

Chemotherapy significantly improved outcome. Adjusting for all other variables, the hazard ratio of dying of cancer if chemotherapy was given was 0.52 (95% CI 0.39–0.70; $P < 0.0001$). Five-year cancer-specific survival was 58% compared with 40% in the non-chemotherapy cohort ($P < 0.0001$). Gender, site and combination subgroups using these parameters all showed significant benefit from adjuvant treatment (men/proximal given chemotherapy HR cancer death 0.47 [95% CI 0.25–0.87, $P = 0.02$], men/distal 0.61 [0.69–0.97, $P = 0.04$], women/proximal 0.43 [0.24–0.78, $P = 0.005$], women/distal 0.40 (0.24–0.68, $P = 0.001$). There was a trend to less effect in men

Table 1   Frequency of pathology parameters and hazard ratio of cancer death for factors associated with poorer prognosis

Overall	Subcategory	n	%	HR	95% CI	P
T stage	1/2	65	8.0%	ns		
	3/4	743	92.0%			
	NA	3				
N stage	1	577	71.1%			
	2	234	28.9%	2.11	1.67–2.66	<0.0001
Differentiation	Well/Moderate	667	82.2%			
	Poor	143	17.6%	1.40	1.05–1.87	0.02
	NA	1	0.1%			
Mucinous component	Present	314	39.3%	1.29	1.02–1.63	0.03
Vascular invasion	Mural	558	68.6%			
	Extramural	453	55.8%	1.78	1.37–2.31	<0.0001
Perineural invasion	Present	142	17.5%	1.45	1.10–1.91	0.01
Advancing edge	Infiltrative	256	31.6%	ns		
	Pushing	550	67.8%			
	NA	5	0.6%			
Budding	Present	314	38.7%	ns		
	Absent	492	60.7%			
	NA	5	0.6%			
Stroma	Fibroid	536	66.1%	ns		
	Keloid	262	32.3%			
	Myxoid	8	1.0%			
	NA	5				
Lymphocytes[†]	Peritumoral	685	84.5%	ns		
	Crohn's-like	175	21.6%			
	TILs	35	4.3%			
	None	96	11.8%			
Obstruction		129	15.8%	ns		
Perforation		30	3.7%	2.27	1.46–3.53	<0.0001

811 cases as 3 cases had synchronous cancers so were not included in the adjusted analysis which included tumor site. [†]More than one type may be present. NA, not assessable due to radiotherapy effect; TIL, tumor infiltrating lymphocyte.

especially for distal tumors, but on interaction testing gender or tumor site did not influence the magnitude of the survival benefit from adjuvant chemotherapy. On interaction testing, no individual pathological factor was significantly associated with a greater benefit from chemotherapy.

When chemotherapy effect was tested in the MSI and MSS cohorts, both derived a significant benefit from treatment (Fig. 1). Five-year cancer-specific survival results show the significant benefit in MSI cases (76% *vs* 43%, $P = 0.009$) and MSS cases (59% *vs* 52%, $P = 0.005$). Adjusted analysis is shown in Table 4. Chemotherapy improved survival in both the MSI cases (HR 0.08, 95% CI 0.02–0.27; $P = < 0.0001$) and the MSS cases (HR 0.62, 95% CI 0.47–0.81; $P = 0.001$). While the hazard ratio of dying in the MSI group was markedly decreased by chemotherapy, seemingly much more than the MSS group, effect modification interaction testing did not reach significance (0.08). Thus, the mag-

nitude of the survival benefit from chemotherapy was not significantly influenced by MSI status.

DISCUSSION

It is well recognized that there is survival advantage from adjuvant chemotherapy in node-positive CRC, but it is equally recognized that a significant number of patients are being overtreated because they are either cured by surgery alone or have disease that is not curable with chemotherapy.[21–23] Accordingly, there is great interest in trying to predict which patients derive benefit from adjuvant chemotherapy following apparently curative resection of CRC.

This study is one of the larger to examine gender, tumor site and pathological factors that might differentially influence response to adjuvant chemotherapy. Our results show that neither gender nor tumor site (or in combination) influenced the survival benefit from

Table 2 MSI/MSS cohort comparison

		MSI (77)		MSS (725)		
		n	%	n	%	P
Median age		72.8		70.7		0.02
Gender	Male	23	29.9%	375	51.7%	<0.0001
	Female	54	70.1%	350	48.3%	
Proximal		64	83.1%	250	34.6%	<0.0001
Median size			53 mm		40 mm	<0.0001
T stage	1/2	0		65	8.0%	
	3/4	77	100%	654	90.2%	
	NA			6		
N stage	1	55	71.4%	516	71.2%	
	2	22	28.6%	209	28.8%	
Differentiation	Moderate	34	44.2%	623	86.4%	<0.0001
	Poor	43	55.8%	98	13.6%	
	NA			4		
Mucinous component	Present	53	68.8%	257	35.4%	<0.0001
Vascular invasion	Mural	46	59.7%	506	70.3%	0.06
	Extramural	42	54.5%	403	56.0%	0.81
Perineural invasion	Present	7	9.1%	134	18.7%	0.04
Infiltrating advancing edge		12	15.6%	241	33.6%	0.001
Budding		13	16.9%	298	41.5%	<0.0001
Stroma	Fibroid	55	71.4%	472	65.8%	0.51
	Keloid	21	27.3%	238	33.2%	
	Myxoid	1	1.3%	7	1.0%	
	NA			8		
Lymphocytes[†]	Peritumoral	69	89.6%	606	84.5%	0.23
	Crohn's	32	41.6%	141	19.6%	<0.0001
	TILs	24	31.2%	11	1.5%	<0.0001
Obstructed		13	17.1%	112	15.6%	0.7
Perforated		2	2.7%	28	3.9%	0.59

802 cases as MSI was unable to be established in 12 cases. [†]More than one type may be present. NA, not assessable due to radiotherapy effect; TIL, tumor infiltrating lymphocyte.

adjuvant chemotherapy. Subgroup analyses in the early chemotherapy trials failed to show significant gender variation in chemotherapy responsiveness.[21–23] This was supported by Gill et al. in their reanalysis of seven adjuvant chemotherapy randomized controlled trials (RCTs).[24] In contrast, in their study of 656 stage C cases, Elsaleh et al. showed that proximal cancers and women (and in combination) had the greatest benefit from adjuvant chemotherapy while men with distal lesion did not derive benefit.[1] We showed a similar trend; however, men with distal lesions did still have a significant benefit from chemotherapy.

While it is well recognized that the histological features of CRCs have a prognostic effect, none of the factors we investigated were shown to predict the effect of chemotherapy. This is supported by Gill et al. who found that neither T stage, N stage, nor grade influenced the effect of chemotherapy in stage B and C colon cancers.[24] This suggests that none of the histological factors we investigated could be used to target treatment.

We found that both MSI and MSS cancer groups derived a survival benefit from 5FU-based chemotherapy, and that the effect was not significantly greater in one or the other. The views in the literature on predictive value of MSI status on chemotherapy effect have been quite disparate. Some of the earlier studies suggested that there was greater chemotherapy benefit in MSI-high cases when these cases were shown to do well with treatment.[5,7,10] However, this may have only highlighted the better prognosis often observed in MSI cases.[6,25,26] In 2001, Elsaleh et al. showed that MSI-positive cases (8.5% of 656 stage C CRC cases) had a marked improvement in 5-year survival with 5FU chemotherapy (37% without treatment vs 90% with

Table 3 Chemotherapy cohort matching

Subgroup	Category	Chemo (307)	%	No chemo (507)	%	P-value
Median age		65.4 years		75.1 years		<0.0001
Gender	Male	172	56.0%	232	45.8%	0.005
	Female	135	44.0%	275	54.2%	
Site†	Proximal	114	37.3%	204	40.4%	0.37
	Distal	192	62.7%	301	59.6%	
T stage	T1/2	25	8.2%	40	8.0%	0.90
	T3/4	280	91.8%	463	92.0%	
	NA	2		4		
N stage	N1	204	66.4%	376	74.2%	0.02
	N2	103	33.6%	131	25.8%	
Differentiation	Moderate	258	84.3%	409	81.2%	0.25
	Poor	48	15.7%	95	18.8%	
	NA	1		3		
Mucinous component	Present	119	39.4%	195	39.3%	0.98
Vascular invasion	Mural	223	73.4%	335	66.5%	0.04
	Extramural	184	60.3%	269	53.4%	0.05
Perineural	Present	59	19.5%	83	16.6%	0.30
Advancing edge	Infiltrating	105	34.7%	151	30.0%	0.17
Budding	Present	118	38.9%	196	39.0%	0.99
Stroma	Fibroid	194	64.0%	342	68.0%	0.14
	Keloid	108	35.6%	154	30.6%	
	Myxoid	1	0.3%	7	1.4%	
	NA	4		4		
Lymphocytes	Peritumoral	283	93.7%	402	79.9%	0.0001
	Crohn's	69	22.8%	106	21.0%	0.53
	TILs	12	3.9%	23	4.6%	0.68
Obstructed		43	14.1%	86	17.2%	0.26
Perforated		11	3.6%	19	3.8%	0.89
MSI positive		27	9.0%	50	10.0%	0.66

†Three cases of synchronous tumors not included in site analysis. NA, not assessable due to radiotherapy effect; TIL, tumor infiltrating lymphocyte.

chemotherapy) whereas MSS cases did not benefit at all (32% *vs* 35%) . While this part of the analysis was unadjusted and groups were not matched, the findings were striking.

However in 2003, Ribic *et al.* published results that were contradictory.[12] Five hundred and seventy stage 2 and 3 colon cancer cases were included from five past randomized 5FU adjuvant chemotherapy trials. The 5-year overall survival for MSI cases without treatment was 75.5% while with treatment was only 70.7%, although this did not reach significance (*P* = 0.07). In contrast, the MSS cases showed improved survival from 68.4 to 88% with chemotherapy (*P* = 0.02). Interestingly on adjusted analysis, no significant chemotherapy benefit was observed across the whole patient group but a trend to better outcome in the stage C group with treatment (HR 0.69, CI 0.47–1.01). There was a non-significant trend to a worse outcome in MSI cases given

chemotherapy (HR of dying 2.14, 95% CI 0.83–5.49 ns) and interaction testing showed that MSI status influenced the effect of adjuvant chemotherapy (*P* = 0.01). They suggested chemotherapy may be detrimental in MSI cases.

This study group was expanded in 2010 by Sargent *et al.* to include a further 457 cases again from past randomized trials and mismatch repair protein (MMR) status was established by either MSI testing or lack of MMR protein on immunohistochemistry.[14] Multivariate analysis of the pooled data (1027 cases, adjusted for stage, age and sex) showed that only the stage 3 MMR proficient cases had a survival advantage from chemotherapy. In the MMR deficient group, there was no survival benefit from chemotherapy. In the stage 2 MMR deficient cases, the overall survival was worsened by chemotherapy (HR 2.95, 95% CI 1.02–8.54; *P* = 0.04). No detrimental effect was observed in the stage 3 cases.
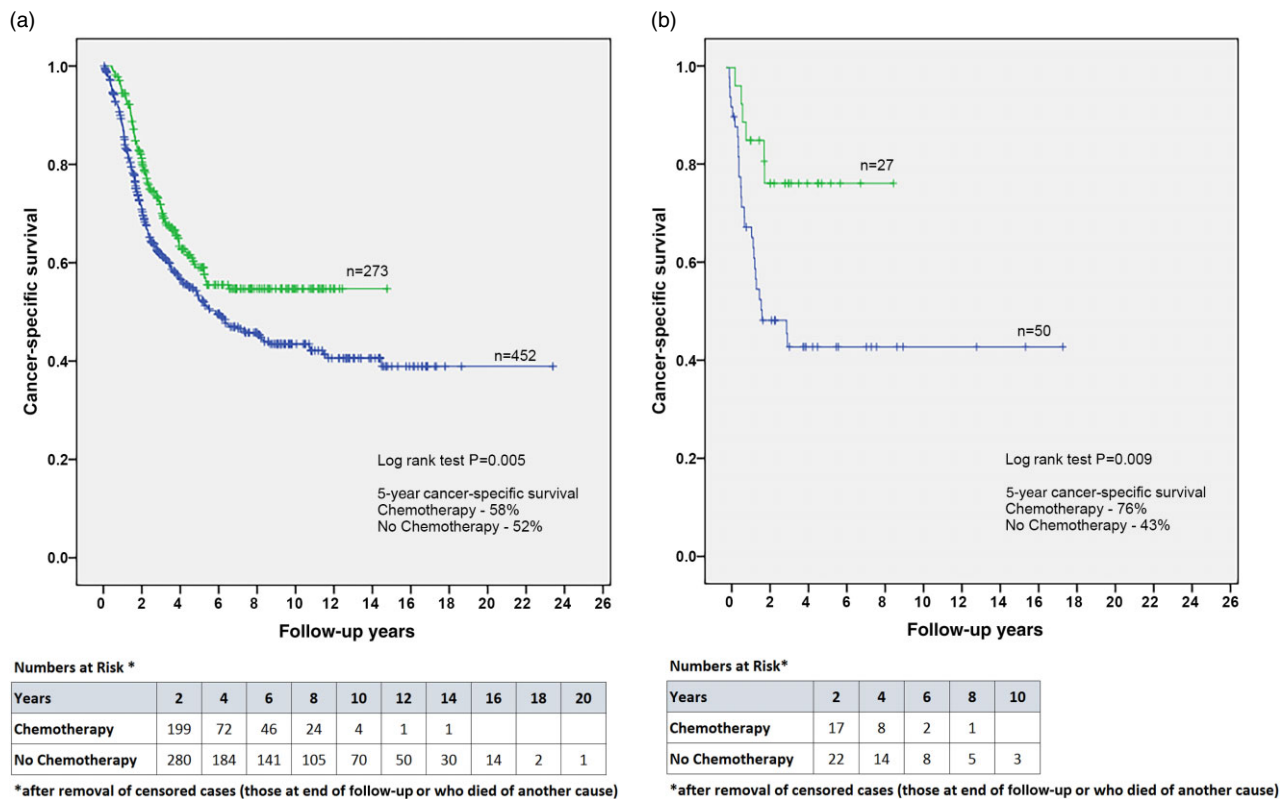
Figure 1 (a) Chemotherapy effect in MSS cases. (b) Chemotherapy effect in MSI-positive cases. Chemotherapy: ⌐, no; ⌐, yes; +, no – censored; +, yes – censored.

Table 4 Multivariate analysis of adjuvant chemotherapy effect according to MSI

		HR of dying	95% CI	P
Chemotherapy effect	All patients	0.52	0.39–0.70	<0.0001
	For MSS cases	0.62	0.47–0.81	0.001
	For MSI cases	0.08	0.02–0.27	<0.0001

Multivariate analysis adjusted for age, gender, site (proximal/distal) and pathological factors as per Table 1. Cancer-specific survival.

This finding, that there was no benefit from chemotherapy in MSI cases, was supported by two subsequent studies[13,15] and many clinicians reconsidered their practice and became disinclined to give 5FU adjuvant chemotherapy to MSI cases. However, there have been more recent studies that challenge the appropriateness of this approach and show that MSI status does not influence adjuvant chemotherapy benefit. Reanalyses of patients from both the National Surgical Adjuvant Breast and Bowel Project (NSABP) and QUASAR trial found that MSI status did not affect chemotherapy responsiveness.[8,9] Interestingly, in their analysis of 2141 cases from various past adjuvant chemotherapy RCTs (inclusive of trial patients from the Ribic and Sargent studies), Sinicrope et al. found that overall stage 3 MMR deficient cases did benefit from 5FU-based adjuvant therapy, although it was limited to the cases they identified as likely germline rather than sporadic (loss of MSH2 protein or less than 55 years at time of treatment with loss of MLH1 protein and/or MSI high).[16]

The current study has limitations. We did not use randomized data but in an attempt to overcome this we comprehensively adjusted for prognostic influences. We drew as many cases as possible from the time before the standard use of adjuvant chemotherapy to minimize selection bias. There were slightly more cancers with

poor prognostic factors in the chemotherapy group, but this would only have diminished the improved survival we observed with chemotherapy. Despite having reasonable numbers, the current study was underpowered to show subtle differences. This was partly due to the low percentage of MSI-positive cases. Our frequency of 9.6% is not dissimilar to other studies of stage C disease where MSI was found in around 10% of cases,[1,25] less than the 15% cited overall for CRC.[27–30] We analyzed BAT 26 and 40 to determine MSI status, performing the entire NCI panel[20] only on equivocal cases, but this should not have underestimated the frequency given the sensitivity of BAT26 for MSI high is close to 99%.[19,31,32]

The included data are robust. All pathology was reexamined to ensure the accuracy and consistency of histological factors and allowed for inclusion of more recently recognized histological factors. Data for this study were gathered from several prospective databases, including the South Australian Cancer Registry that is dedicated to the accurate and thorough collation of cancer data.[18] Inaccuracies relating to retrospective review of data were minimized by extensive cross-checking. Certain factors were not included if accuracy could not be assured, such as patient comorbidities. While this factor may have influenced whether chemotherapy was given, we attempted to minimize its influence by studying cancer-specific survival.

There is not an obvious reason why our findings should vary from the Ribic and Sargent studies. We included rectums and only stage C cases but the trends they observed persisted on stage subgrouping. We did not use randomized data but adjusted more extensively for covariates that may have influenced outcome. The patient groups were similar except for age. The median age in our study was older than those in the Ribic study (71 *vs* 59.8 years) but our findings did not vary even on exclusion of older patients (data not included) and we used cancer-specific survival rather than their overall survival. The different methods of establishing MMR deficiency should not have influenced results given the high concordance between detecting MMR deficiency by lack of the protein on immunohistochemistry and establishment of MSI high on molecular testing.[19]

In conclusion, this study showed that patient gender, tumor site, pathological tumor characteristic and MSI status did not influence the benefit observed from 5FU adjuvant chemotherapy and, at least in this patient group, none of these factors could have been used to target treatment. While the use of adjuvant chemotherapy in microsatellite unstable cancer cases has been questioned, there remains significant controversy and

there is now sufficient conflicting evidence to question the emerging dogma that MSI cases do not benefit from 5FU chemotherapy. The contrary studies show a lack of benefit at worst, not a significant detrimental effect in the stage C cases. In our group, if chemotherapy had not been offered on the basis of MSI, there would have been a significantly worse outcome, given 57% of MSI cases who did not get chemotherapy died from their cancer within 5 years compared with only 24% of those who received treatment. It is likely that further studies of MSI alone as a predictive biomarker will be unhelpful and continue to produce contradictory results, but it may be that study of related biomarkers such as Braf will be useful. Given the findings of the current study and the remaining controversy, it would be unwise not to offer MSI-high cases adjuvant chemotherapy.

ACKNOWLEDGMENT

REFERENCES

1 Elsaleh H, Joseph D, Grieu F, Zeps N, Spry N, Iacopetta B. Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. *Lancet* 2000; **355**: 1745–50.

2 Carethers JM, Chauhan DP, Fink D *et al.* Mismatch repair proficiency and in vitro response to 5-fluorouracil. *Gastroenterology* 1999; **117**: 123–31.

3 Arnold CN, Goel A, Boland CR. Role of hMLH1 promoter hypermethylation in drug resistance to 5-fluorouracil in colorectal cancer cell lines. *Int J Cancer* 2003; **106**: 66–73.

4 Meyers M, Wagner MW, Hwang HS, Kinsella TJ, Boothman DA. Role of the hMLH1 DNA mismatch repair protein in fluoropyrimidine-mediated cell death and cell cycle responses. *Cancer Res* 2001; **61**: 5193–201.

5 Lukish JR, Muro K, DeNobile J *et al.* Prognostic significance of DNA replication errors in young patients with colorectal cancer. *Ann Surg* 1998; **227**: 51–6.

6 Halling KC, French AJ, McDonnell SK *et al.* Microsatellite instability and 8p allelic imbalance in stage B2 and C colorectal cancers. *J Natl Cancer Inst* 1999; **91**: 1295–303.

7 Hemminki A, Mecklin JP, Jarvinen H, Aaltonen LA, Joensuu H. Microsatellite instability is a favorable prognostic indicator in patients with colorectal cancer receiving chemotherapy. *Gastroenterology* 2000; **119**: 921–8.

8 Hutchins G, Southward K, Handley K *et al.* Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. *J Clin Oncol* 2011; **29**: 1261–70.

9 Kim GP, Colangelo LH, Wieand HS *et al.* Prognostic and predictive roles of high-degree microsatellite instability in

colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project Collaborative Study. *J Clin Oncol* 2007; **25**: 767–72.

10 Watanabe T, Wu TT, Catalano PJ *et al*. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. *N Engl J Med* 2001; **344**: 1196–206.

11 Barratt PL, Seymour MT, Stenning SP *et al*. DNA markers predicting benefit from adjuvant fluorouracil in patients with colon cancer: a molecular study. *Mech Dis* 2002; **360**: 1381–7.

12 Ribic CM, Sargent DJ, Moore MJ *et al*. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 2003; **349**: 247–57.

13 Carethers JM, Smith EJ, Behling CA *et al*. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. *Gastroenterology* 2004; **126**: 394–401.

14 Sargent DJ, Marsoni S, Monges G *et al*. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Clin Oncol* 2010; **28**: 3219–26.

15 Jover R, Zapater P, Castells A *et al*. Mismatch repair status in the prediction of benefit from adjuvant fluorouracil chemotherapy in colorectal cancer. *Gut* 2006; **55**: 848–55.

16 Sinicrope FA, Foster NR, Thibodeau SN *et al*. DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. *J Natl Cancer Inst* 2011; **103**: 863–75.

17 Davis NC, Newland RC. The reporting of colorectal cancer: the Australian Clinico-pathological Staging (ACPS) System. *Med J Aust* 1983; **1**: 282.

18 South Australian Cancer Registry. Available from URL: http://data.sa.gov.au/data/dataset/sa-cancer-registry.

19 Dietmaier W, Wallinger S, Bocker T, Kullmann F, Fishel R, Ruschoff J. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. *Cancer Res* 1997; **57**: 4749–56.

20 Boland CR, Thibodeau SN, Hamilton SR *et al*. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998; **58**: 5248–57.

21 Laurie JA, Moertel CG, Fleming TR *et al*. Surgical adjuvant therapy of large-bowel carcinoma: an evaluation of levamisole and the combination of levamisole and fluorouracil. The North Central Cancer Treatment Group and the Mayo Clinic. *J Clin Oncol* 1989; **7**: 1447–56.

22 Moertel CG, Fleming TR, Macdonald JS *et al*. Fluorouracil plus levamisole as effective adjuvant therapy after resection of stage III colon carcinoma: a final report. *Ann Intern Med* 1995; **122**: 321–6.

23 Marsoni S. Fluorouracil and folinic acid in colon cancer. IMPACT Investigators. *Lancet* 1995; **345**: 1582–3.

24 Gill S, Loprinzi CL, Sargent DJ *et al*. Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III colon cancer: who benefits and by how much? *J Clin Oncol* 2004; **22**: 1797–806.

25 Wright CM, Dent OF, Barker M *et al*. Prognostic significance of extensive microsatellite instability in sporadic clinicopathological stage C colorectal cancer. *Br J Surg* 2000; **87**: 1197–202.

26 Lim SB, Jeong SY, Lee MR *et al*. Prognostic significance of microsatellite instability in sporadic colorectal cancer. *Int J Colorectal Dis* 2004; **19**: 533–7.

27 Konishi M, Kikuchi-Yanoshita R, Tanaka K *et al*. Molecular nature of colon tumors in hereditary nonpolyposis colon cancer, familial polyposis, and sporadic colon cancer. *Gastroenterology* 1996; **111**: 307–17.

28 Aaltonen LA, Salovaara R, Kristo P *et al*. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *N Engl J Med* 1998; **338**: 1481–7.

29 Malkhosyan SR, Yamamoto H, Piao Z, Perucho M. Late onset and high incidence of colon cancer of the mutator phenotype with hypermethylated hMLH1 gene in women. *Gastroenterology* 2000; **119**: 598.

30 Ward R, Meagher A, Tomlinson I *et al*. Microsatellite instability and the clinicopathological features of sporadic colorectal cancer. *Gut* 2001; **48**: 821–9.

31 Hoang J, Cottu PH, Thuille B, Salmon RJ, Thomas G, Hamelin R. BAT-26, an indicator of the replication error phenotype in colorectal cancers and cell lines. *Cancer Res* 1997; **57**: 300–3.

32 Loukola A, Eklin K, Laiho P *et al*. Microsatellite marker analysis in screening for hereditary nonpolyposis colorectal cancer. *Cancer Res* 2001; **61**: 4545–9.