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Microsatellite instability as a marker of prognosis and response to therapy: A meta-analysis of colorectal cancer survival data

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ABSTRACT

Background and methods: We have reviewed and pooled data from published studies to evaluate the relationship between microsatellite instability (MSI) and colorectal cancer (CRC) prognosis. Thirty-one eligible studies reporting survival in 12782 patients characterised for MSI were pooled using a fixed- or random-effects model.

Results: The summary odds ratio (OR) estimate for overall survival (OS) associated with MSI was 0.6 (95%CI 0.53–0.69, $p < 0.0001$), with no evidence of heterogeneity. The effect was similar for disease-free survival (DFS) (OR = 0.58, 95%CI 0.47–0.72, $p < 0.0001$). In a subset of patients treated with 5-fluorouracil (5-FU)-based chemotherapy a significant improved prognosis was found for microsatellite stable (MSS) tumours (OR = 0.52, 95%CI 0.4–0.6, $p < 0.0001$) with no heterogeneity ($p = 0.53$; $I^2 = 0\%$). By contrast a large heterogeneity characterised the data relative to 396 patients with MSI tumours (OR = 0.69, 95%CI 0.3–1.5, $p = 0.1$; heterogeneity: $p = 0.03$; $I^2 = 58\%$).

Conclusions: This study confirmed the association between MSI and favourable prognosis as determined by both OS and DFS of CRC patients. A significant beneficial effect of 5-FU therapy was found for MSS tumours whilst no clear conclusion was reached for MSI tumours due to the high inter-study heterogeneity. We propose that this inconclusive result is due to the use of a single marker, such as MSI, that cannot account alone for the complexity of the mechanisms underlying 5-FU cytotoxicity. Future studies to predict response to 5-FU chemotherapy should include additional genome stability markers.

1. Introduction

Colorectal cancer (CRC) is one of the most common malignancies in western countries, representing the third most common cancer in both women and men. Despite advances in early detection and improvements in dosing and scheduling of therapies in both adjuvant and advanced settings, CRC is still responsible for a substantial mortality and represents the second leading cause of cancer-related death in the United States, with a relative 5-year survival rate of about 67%.[1]

CRC shows a significant heterogeneity even within the same pathologic stage in both prognosis and response to therapy.[2] The clinical heterogeneity may be a sign of underlying molecular heterogeneity in the pathogenesis of CRC. Current

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knowledge on molecular mechanisms involved in colorectal carcinogenesis indicates that CRC is a multi-pathway disease and two major genomic instability pathways are involved in its pathogenesis: microsatellite instability (MSI) and chromosome instability (CIN). Approximately 15% of sporadic CRC are characterised by MSI, showing insertions and deletions at DNA microsatellite sequences, whereas the remaining 85% of CRC develop through the CIN pathway and are characterised by gross chromosomal alterations, either qualitative or quantitative. Although the MSI and CIN phenotypes can be distinguished from one another, there is evidence suggesting some degree of overlap.[3] In CRC MSI is mainly caused by mutations in the DNA mismatch repair (MMR) genes *hMLH1* and *hMSH2* and, less frequently, *hMSH6, hPMS1*and *hPMS2*. Genetic and epigenetic inactivation of MMR genes leads to mutations in cancer-related genes and to cancer development and progression.[4]

MSI is typically assessed by analysing five microsatellite markers: three dinucleotide (D2S123, D5S346, D17S250) and two mononucleotide (BAT25 and BAT 26) repeats referred to as the National Cancer Institute (NCI) consensus panel.[5] Three levels of MSI can be identified: high-level MSI (MSI-H), generally defined as MSI in more than 30% of the standard markers; low-level MSI (MSI-L), when changes are exhibited in less than 30% of the markers and microsatellite stable (MSS) in the absence of microsatellite alterations. Mononucleotide repeat markers have been shown to be highly sensitive in detecting MSI-H tumours, indeed the use of BAT 26 alone has been found highly correlated with the NCI panel and has been shown to identify nearly all MSI-H CRC cancers.[6] MMR deficiency leading to MSI can be also detected by lack of expression of one or more of the MMR proteins by immunohistochemistry (IHC). Recently a scoring system (MsPath), based on easily assessable clinicopathological features, was developed to predict MSI-H status in CRC and proposed to triage tumours for MSI or IHC testing.[7]

MSI-H identifies a well-defined subset of CRC that tends to show diploid state, to be more proximal, poorly differentiated, mucinous and to have marked lymphocyte infiltration.[8] Thus, MSI genotyping may allow the identification of discrete molecular CRC subtypes and MSI seems to represent one of the most promising molecular markers with both prognostic and predictive value for chemosensitivity.[9] Indeed, a pooled analysis of survival data support the notion that patients with tumours showing MSI-H phenotype have a better prognosis than those with MSS tumours.[10] MSI testing could also result in more patients being assigned to proper treatment based on their disease profile. An intact MMR system is a determinant of sensitivity to a variety of chemotherapeutic agents, it is therefore reasonable to consider MSI-H tumours as a separate entity when determining response to chemotherapy. Indeed non-responsiveness to 5-fluoruracil (5-FU) chemotherapy was shown in two clinical trials although its significance was limited by the small number of treated MSI-H patients.[10] Other studies including prospective studies have addressed this issue.[11] To gain better insight into the clinical value of MSI status we have undertaken a systematic review of published studies and used meta-analysis techniques to derive a more precise and updated estimate of the prognostic and predictive significance of this CRC phenotype.

2. Materials and methods

2.1. Eligibility criteria

Studies were eligible for pooling if survival was assessed in CRC patients stratified by MSI status. The primary outcomes of interest were overall survival (OS), disease-specific survival (DSS) and disease-free survival (DFS). Only studies providing information on survival were included. Studies assessing MSI by analysing DNA molecular markers and/or IHC were included. Studies only quoting the p value of the logrank statistics were not eligible. Care was taken to account for overlapping and duplicated datasets.

2.2. Identification of studies

Studies were identified using the PubMed electronic database (www.ncbi.nlm.nih.gov/pubmed) from 01/01/1999 until 30/03/2009. The search strategy included the keywords “colorectal cancer” or “colon cancer” combined with either “outcome” or “prognosis” combined with any of “microsatellite instability”, “replication error” and “mismatch repair”, and combined with any of “hazard ratio”, “relative risk” and “logrank test”. All studies matching eligibility criteria were retrieved and the bibliographies checked for other relevant publications. Review articles and bibliographies of other relevant studies identified were hand-searched to identify additional studies. Only published studies in peer-reviewed journals were included. Unpublished data were not sought and primary study authors were not contacted.

2.3. Statistical analysis

The association between MSI status and the outcomes of interest was derived as a weighted average of study-specific estimates of the odds ratio (OR) using the Mantel–Haenszel method, which determines the weight of each study by using the inverse variance approach.[12] The log OR (MSI/MSS) and the corresponding 95% confidence intervals (CI) were used as data points for the meta-analysis. Survival data were retrieved as dichotomous outcomes (dead or not dead, relapse or not relapse) according to MSI status during the follow-up indicated in the primary study (mean = 4.75 years; range 3–6.2 years). As the time-to-event used for data extraction within each study was the same for MSI-H and MSS patients, the OR statistics was virtually independent from the length of the follow-up. The Mantel–Haenszel approach is specific for dichotomous data, providing a pooled OR across the strata of fourfold tables, where each stratum represents a single study included in the meta-analysis and assuming a fixed- or random-effects model.

Studies were searched for, identified and eligibility confirmed independently by two authors. Authors extracted the data and assessed the quality of studies independently of each other, and disagreements were resolved by discussion. Characteristics of the studies were extracted from published articles and summarised in a consistent manner to aid comparison. Studies matching the eligibility criteria in which no dichotomous survival data were available were included for systematic review but excluded from the meta-analysis

according to MSI status. This applied to six studies reporting OS relative risk (RR),[13–15] OS hazard ratio (HR),[16,17] the HR of DSS[18] and the HR of 5-FU-based beneficial survival.[17]

Heterogeneity was formally tested by Cochran's Q statistics[19] to discriminate the appropriate model to be used. In the absence of heterogeneity ($p > 0.05$) the fixed-effects model was used, whilst in the presence of heterogeneity ($p < 0.05$) the random-effects model was considered.[20] The percentage of variability across studies due to heterogeneity rather than chance was quantified using the I^2 statistic.[21] Studies were plotted in order of publication from the most recent. Horizontal lines represent 95%CI, and their width is inversely proportional to the weight of the study. Therefore estimates from small studies which have less precision will scatter widely. Each circle in the forest plot represents the OR point estimate. The overall summary estimate under fixed- or random-effects with its CI is also shown. The vertical line is at the null value (OR = 1.0). Statistical computations were undertaken using MedCalc for Windows, Version 10.0.2 (MedCalc Software, Mariakerke, Belgium).

3. Results

3.1. Eligible studies

Fifty-five potentially eligible studies were identified and retrieved. Seven studies[22–28] were excluded as survival data were not stratified by MSI status, three[29–31] as survival data cannot be extrapolated separately for MSI and MSS patients, two[32,33] as they only quoted the median survival time, five[34–38] as they reported only the *p*-value of the logrank statistics. Datasets were duplicated in seven studies[6,39–44] and in order to reduce bias the most recently published papers were used. There were therefore thirty-one studies[11,13–18,45–68] assessing survival by MSI status eligible for systematic review and meta-analysis.

3.2. Study characteristics

Characteristics of the eligible studies are summarised in Table 1. Eligible studies analysed a total of 12782 patients for different survival outcomes according to MSI status. The mean number of patients was 412 per study (range 43–1263). Twenty-one studies referred to patients with colorectal cancers, whilst ten studies[17,49,51,53,54,57,59,62,64,66] restricted analysis to patients with colonic cancers. In seven studies[14,17,49,51,53,59,62] authors explicitly stated that patients were previously enrolled in randomised clinical trials. In the remaining studies study type was not stated. Nine studies (3049 patients)[15,17,45–51] overlapped with those analysed by Popat et al.[10] Twenty-three studies considered in the previous systematic review[10] were either antecedent to 1999 or reported only multivariate and *p*-value data.

In our retrieved papers different tumour staging systems were used and in order to make them comparable Duke's and Astler-Coller stages were transformed into the TNM staging system.[69] Most studies (11278 patients) examined various tumour stages, whilst seven studies[15,45,46,48–50,53,57] were based on single-stage disease, one of which examined only stage IV patients.[50] The median length of the follow-up on subjects in the selected studies was five years.

The relationship between MSI status and the effectiveness of standard 5-FU-based chemotherapy was analysed in a subset of retrieved studies which stratified patients by 5-FU therapy and reported survival data separately for MSI and MSS patients. Data from seven studies[11,14,48,51,52,57,62] (2863 patients, 396 MSI and 2467 MSS) responding to the above criteria were pooled for meta-analysis. Due to the low number of studies responding to these criteria, in three cases[14,48,52] data were manually obtained from Kaplan–Meier survival curves. All studies considered stages II–III colon[51,57,62] and colorectal[11,14,48,52] cancer patients. Patients received standard 5-FU adjuvant chemotherapy in combination with levamisole or leucovorin in six studies, whilst in one study[14] intraportal infusion of 5-FU was administered in combination with mitomycin. In three studies[14,51,62] the authors retrospectively analysed the 5 years survival benefit of patients treated with 5-FU enrolled in prospective randomised clinical trials. Approximately half of the total patients enrolled in each of the seven studies received chemotherapy (mean 48%; range 32–68%).

3.3. Microsatellite instability allocation

A variety of dinucleotide and mononucleotide microsatellite markers were used for MSI status assignment within our systematic review (Table 1). The median number of genotyped markers was five (range 1[48,65]–12[54]). Two studies[17,59] analysed only dinucleotide markers, whilst six studies[11,48,63,65,67,68] considered only mononucleotide markers. Five studies[11,55,58,59,67] classified MSI status by both DNA microsatellite markers and by IHC analysis. Only one study[16] assigned MSI status exclusively by IHC. The median threshold of microsatellite markers showing instability required to assign MSI-H was 36% (range 14%[45] to 100%).[48,65] The proportion of MSI-H in sporadic tumours of all stages has been found to be 16.3%.[70] In accordance with this result the mean percentage of MSI-H cancers in our dataset was 15.4%. Most studies divided patients into MSI-H and MSS. In such cases MSS group included both MSI-L and real MSS patients. The existence of MSI-L has not been established definitely as a distinct phenotype,[71] thus in one study[61] survival data separately available for MSI-L and MSS have been combined. In one study[47] the survival data of MSI-L cancer patients were omitted.

3.4. Overall and disease-specific survival analysis

Twenty studies (9243 patients, 1278 MSI-H) provided OS data suitable for pooling. The OR and 95%CI for each study and the summary OR are shown in Fig. 1A. The overall summary estimate was 0.60 (95%CI: 0.53–0.69, $p < 0.0001$), with no evidence of heterogeneity (Q = 26.1, $p = 0.13$; $I^2 = 27.3\%$). A subset of ten studies (4014 patients) reporting the OS for stages II–III cancer patients were pooled separately and the forest plot is shown in Fig. 1B. This pooling considered 645 MSI-H cases and the summary OR estimate was 0.65 (95%CI 0.53–0.79, $p < 0.0001$), suggesting that MSI-H cancers have a better prognosis irrespective of the tumour stage. The HRs of the two studies not included in our pooling for OS were 0.6 (95%CI 0.39–0.91)[16] and 0.84 (95%CI 0.58–1.23),[17] whilst the three RRs

Table 1 – Summary of studies of MSI status and colorectal cancer survival.

Study	Study details			Assessment of MSI					Survival data		
	No. Patients	Tumour	TNM stage *Duke's **Astler–Coller	No. Markers	Mono-markers	Positive marker threshold to assign MSI-H	IHC testing (MLH1, MSH2)	MSI-H (%)	OS	DSS	DFS
Soreide (2009)	186	Colorectal	I, II, III	5	5	⩾2		20		▲	▲
Jover (2009)	754	Colorectal	I, II, III, IV	5	5	⩾30%	Y	10	▲		▲
Jensen (2009)	311	Colorectal	II, III, IV	5	5	⩾1	Y[c]	14	▲		▲
Ogino (2009)	649	Colon	I, II, III, IV	10	3	⩾30%		19	▲	▲	
Banerjea (2009)	91	Colorectal	I, II, III	1	1	1		18			▲
Lee (2008)	134	Colorectal	I, II, III, IV	5	2	⩾2		14	▲		
Barault (2008)	554	Colon	I, II, III, IV	5	2	⩾40%		14	▲		
Malesci (2007)	893	Colorectal	I, II, III, IV	2	2	⩾1		10	▲	▲	
Kim (2007)[a]	542	Colon	B, C*	5	2	⩾30%		18	▲		▲
Maestro (2007)	350	Colorectal	A,B,C,D*	5	2	⩾2		7	▲		
Lamberti (2007)	416	Colorectal	I, II, III, IV	5	2	⩾2		13	▲		▲
Sinicrope (2006)[a]	528	Colon	B2 ,C**	11	0	⩾30%	Y	18	▲		▲
Lanza (2006)	363	Colorectal	II, III	6	2	⩾30%	Y	21		▲	
Dietmaier (2006)	170	Colon	III	5	2	>40%		14	▲		
Chang (2006)	213	Colorectal	I, II, III, IV	5	2	⩾2		9	▲		▲
Ward (2005)	528	Colorectal	II, III	5	3	⩾2	Y	14	▲		
Storojeva (2005)[a]	160	Colorectal	B, C*	9	3	⩾40% or 2 mono-		13	▲		
Samowitz (2005)	903	Colon	I, II, III, IV	12	2	⩾30% or 1 mono-		9	▲	▲	
Benatti (2005)	1263	Colorectal	I, II, III, IV	6	3	⩾2		20		▲	
Westra (2005)[a]	273	Colon	III	9	4	>3		16			▲
Kakar (2004)	248	Colorectal	A,B,C,D*	No genetic testing			Y[b]	29	▲		
Carethers (2004)	204	Colorectal	II, III	5	2	⩾2		18	▲		
Ribic (2003)[a]	570	Colon	II, III	11	2	⩾30%		17	▲		▲
Brueckl (2003)	43	Colorectal	IV	10	3	⩾2		16	▲		
Barratt (2002)[a]	368	Colon	B, C*	4	0	⩾2		24	▲		
Watanabe (2001)[a]	229	Colon	III	10	2	⩾30%		32	▲		
Elsaleh (2001)	721	Colorectal	III	1	1	1		9	▲		
Wright (2000)	238	Colorectal	III	7	3	⩾40%		9	▲		
Gryfe (2000)	587	Colorectal	I, II, III, IV	10	4	⩾2		17	▲		
Gafà (2000)	215	Colorectal	I, II, III, IV	6	2	⩾2		20		▲	
Liang (1999)	78	Colorectal	B2*	7	1	1		32	▲		

a Randomised clinical trials.
b MLH1, MSH2 and/or MSH6.
c MLH1, MSH2, MSH6 and/or PMS2.
* Duke's.
** Astler–Coller.

were 0.23 (95%CI 0.06–0.95),[13] 1.13 (95%CI 0.43–2.94)[14] and 0.5 (95%CI 0.25–0.99).[15] All but one study[14] confirmed a better OS outcome for MSI-H patients.

Six studies[46,54,58,63,66,68] (3209 patients, 449 MSI-H) were suitable for pooling data on DSS and the plot is shown in Fig. 2. The overall summary estimate of the OR was 0.33 (95%CI 0.25–0.44, $p < 0.0001$), with some evidence of heterogeneity (Q = 9.98, $p = 0.08$) and a I^2 value of 49.9%. One study[18] reported only DSS univariate HR of 0.51 (95%CI 0.39–0.67) confirming a better outcome for MSI-H patients.

3.5. *Effect of MSI on disease-free survival*

The forest plot in Fig. 3 shows the OR and 95%CI of ten studies[11,51,53,56,59,60,62,65,67,68] pooled for DFS (3884 patients, 575 MSI-H). The summary OR was 0.58 (95%CI 0.47–0.72, $p < 0.0001$), with no evidence of heterogeneity (Q = 11.5, $p = 0.25$; $I^2 = 21.5\%$). The DFS result suggests that the better OS of MSI-H patients is associated with non-relapsing cancers.

3.6. *MSI status and effectiveness of therapy*

Clinical evidence is conflicting on the benefit of 5-FU chemotherapy on survival of MSI-H cancer patients.[72] Seven studies[11,14,48,51,52,57,62] reported OS data stratified by 5-FU chemotherapy separately for MSI-H and MSS in stages II–III CRC patients. We pooled the data of 2863 cancer patients, of whom 396 are MSI-H tumours. The forest plots are shown in Fig. 4A (MSS) and Fig. 4B (MSI-H). The MSS 5-FU-treated patients showed a significant better survival as compared to

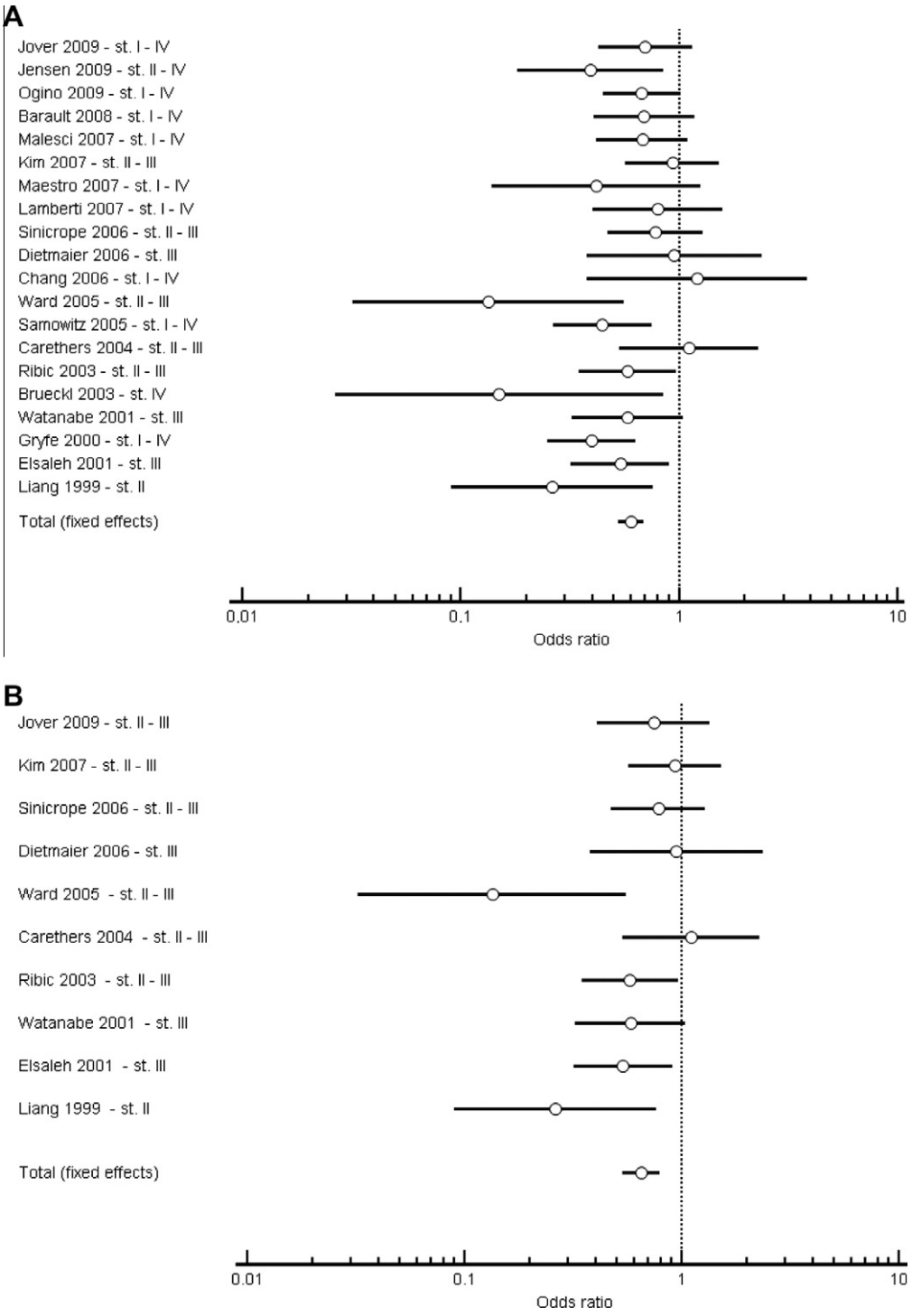

Fig. 1 – Forest plots of odds ratios (ORs) for overall survival (OS) from eligible studies of colorectal cancer associated with microsatellite instability. (A) patients with all tumour stages; (B) patients with stages II–III. Studies are plotted in order of publication. Horizontal lines represent 95%CI. Each circle represents the OR point estimate and the lower circle is the overall summary estimate. The vertical line is at the null value (OR = 1.0).

untreated MSS patients (summary OR 0.52, 95%CI 0.4–0.6, $p < 0.0001$), with no heterogeneity ($Q = 5.12$, $p = 0.53$; $I^2 = 0\%$). For 5-FU-treated MSI-H patients the summary OR was 0.69 (95%CI 0.3–1.5, $p = 0.1$) with evidence of heterogeneity ($Q = 14.1$, $p = 0.03$; $I^2 = 57.5\%$).

The pooling of the three studies[14,51,62] reporting data from 5-FU–randomised trials confirmed the benefit of 5-FU treatment for MSS patients (OR = 0.62, 95%CI 0.47–0.82; $p = 0.003$), with no heterogeneity. Pooling the data of 214 MSI-H patients from the same three[14,51,62] studies resulted in summary OR of

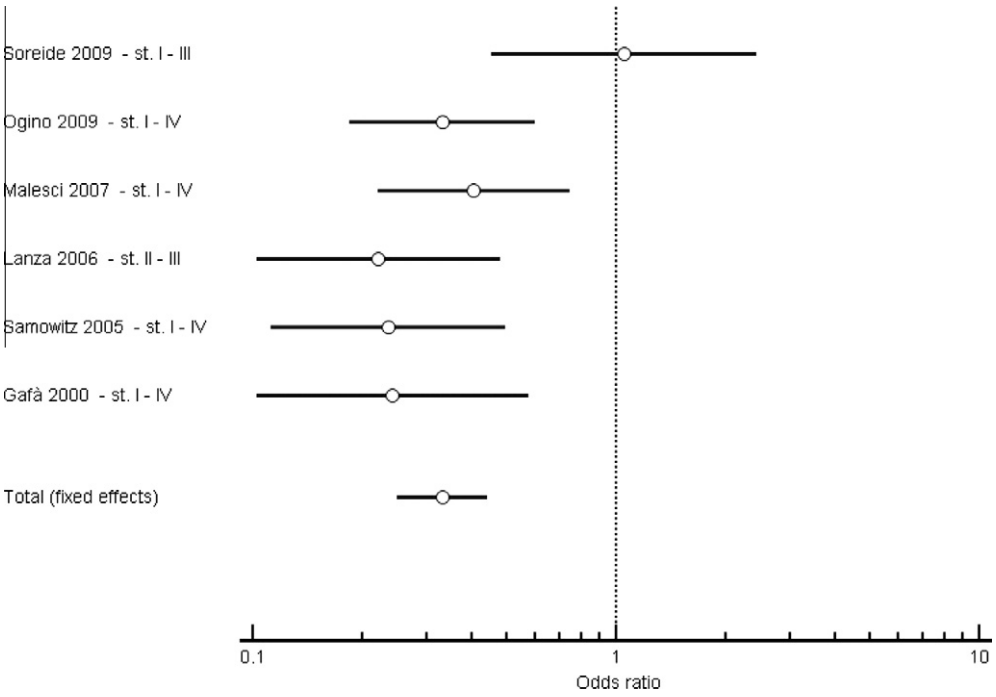

Fig. 2 – Forest plot of odds ratios (ORs) for disease-specific survival (DSS) from eligible studies of colorectal cancer associated with microsatellite instability.

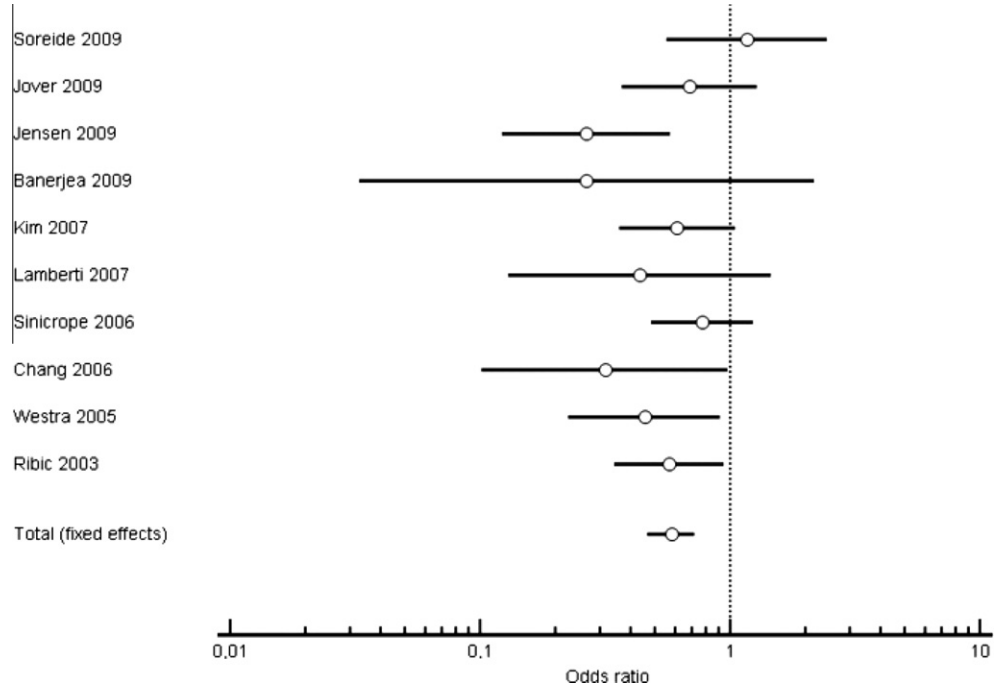

Fig. 3 – Forest plot of odds ratios (ORs) for disease-free survival (DFS) from eligible studies of colorectal cancer associated with microsatellite instability.

1.03 (95%CI 0.25–4.3), with evidence of heterogeneity ($p = 0.03$). This result combined with the presence of heterogeneity hampers any clear conclusion on the benefit from 5-FU-based chemotherapy for MSI-H patients. A study[17] included in our systematic review reported univariate HR from a randomised trial on MSS and MSI-H colon cancer patients treated with intraportal infusion of 5-FU. The HR for MSS patients was 0.73 (95%CI 0.51–1.05) and the HR for MSI-H patients was 0.94 (95%CI 0.48–1.84) confirming that the beneficial effect of 5-FU therapy is not obvious for MSI-H CRC.

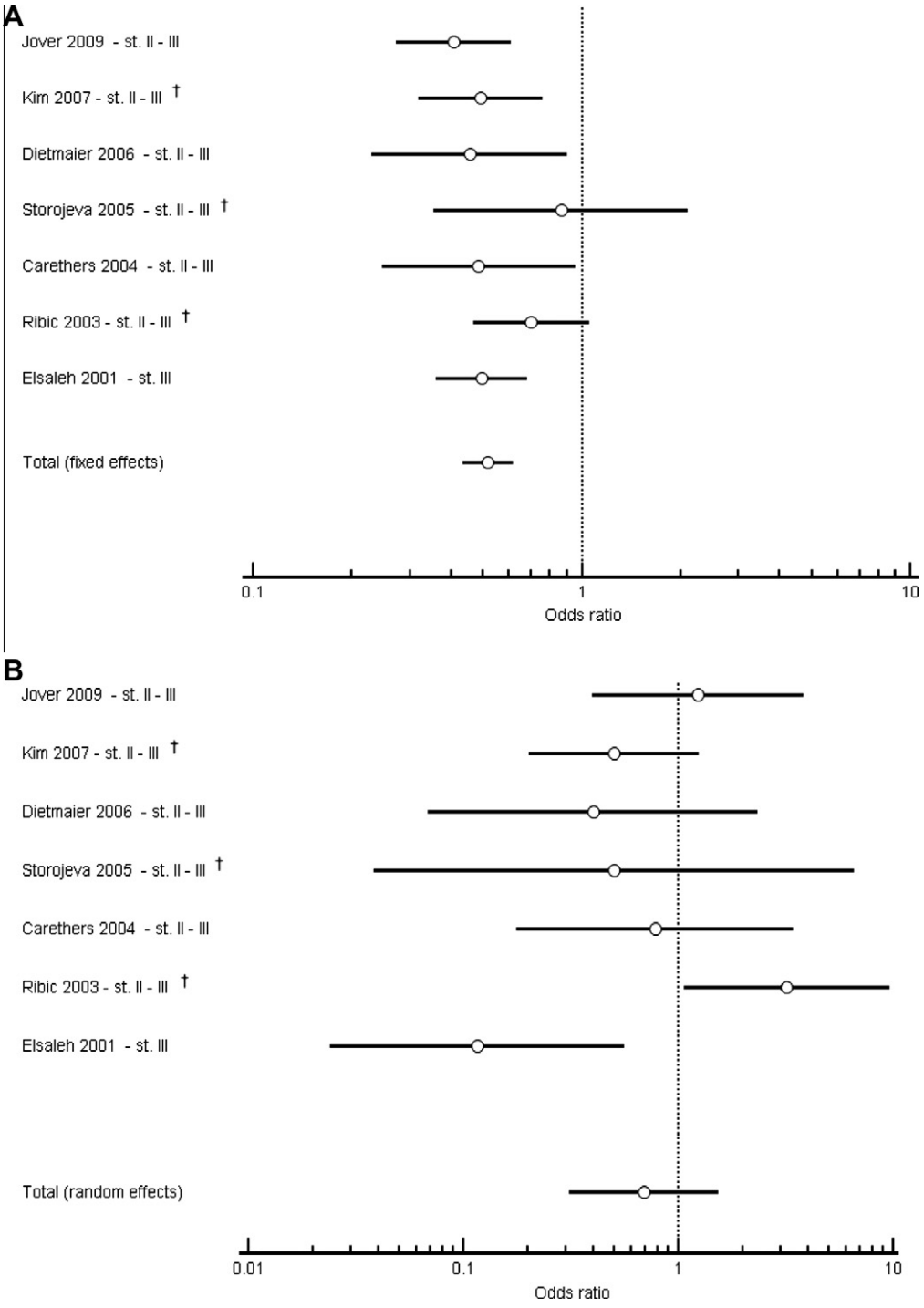

Fig. 4 – Forest plots of odds ratios (ORs) for OS from eligible studies of colorectal cancer associated with 5-FU treatment. (A) MSS tumours; (B) MSI-H tumours. † Randomised clinical trials.

3.7. *Publication bias and heterogeneity*

No evidence of publication bias was found towards studies reporting a better OS associated with MSI status as shown in the funnel plot of Fig. 5A, and this was confirmed by the formal evaluation using Egger's test ($p = 0.68$). No statistical heterogeneity for OS-pooled studies was present. Small qualitative heterogeneity was due to one study[50] examining only stage IV CRC patients, but the exclusion of this study from the pooling did not change the summary statistics. The funnel plot of DFS-pooled studies showed no evidence of publication bias (Fig. 5B) with an Egger's test p value of 0.32. The publication bias assessment of DSS-pooled studies was not possible due to the small number of studies. However, the very similar results for OS, DFS and DSS in some overlapping sets of patients suggest that the finding of a better prognosis in MSI-H CRC patients is qualitatively and quantitatively correct. When all the analyses were performed using a random-effects model

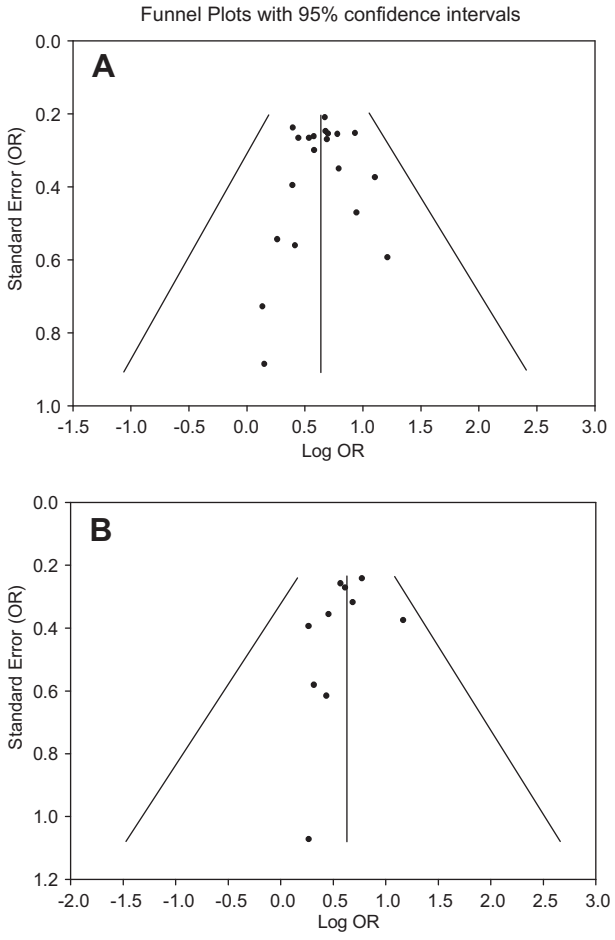

Fig. 5 – Detecting publication bias by a funnel plot. The funnel plots display the OR associated with microsatellite instability in each study included in the OS (panel A) and DFS (panel B) meta-analyses. Each OR is reported on a log scale against its standard error. The vertical line indicates the pooled estimate of the overall OR, with the sloping lines representing the expected 95%CI for a given SE.

assuming missed heterogeneity no change in the direction or significance of our summary statistics was found.

Another potential source of heterogeneity is the variable length of follow-up amongst studies. In order to overcome this potential bias survival data in each study were extracted with the same observational period for MSS and MSI-H patients. In this way the OR estimates are identical to the HR from the time-dependent hazard rates calculated according to Samson et al.[73]

4. Discussion

We have shown that the published data support the view that MSI-H is associated with a better prognosis in CRC, and it appears that this molecular marker can stratify CRC patients further after standard pathological staging. There is a great variability in clinical outcome of CRC patients with the same staging, underlining the need for robust prognostic and predictive molecular markers. Patients with stages I–IV MSI-H CRC appear to have a better survival and we determined that MSI-H in stages II and III did not show any further better prognostic value. The better outcome is found in terms of OS, DSS and DFS. Our findings include 1972 MSI-H patients and the number of studies in the survival analyses which did not conform to the majority was small adding confidence to our survival results.

In line with this report, in a previous systematic review on the prognostic value of MSI status, it was found that MSI-H is associated with a better outcome in stages I–IV disease although the DSS and DFS were not estimated.[10] Therefore the determination of MSI-H might guide surgical procedure and clinical approach. Survival results for MSI-H are consistent independently from the number/type of genetic markers and from the application of genetic or IHC criteria to assign MSI status. The gold standard for MSI testing is tumour genotyping but since this procedure is time, cost and labour intensive, alternative method such as the cost effective IHC which has been shown to be highly concordant with MSI genotyping should be considered by the clinicians.[74]

The ability to identify a subset of colorectal cancer patients who benefit from the standard chemotherapy based on 5-FU could be of great clinical advantage as it is estimated that response rates with 5-FU-based therapy are about 25%.[75] Almost all adjuvant chemotherapy for node positive CRC involves the agent 5-FU, typically in combination with levamisole or leucovorin, still after 40 years from its introduction 5-FU is the dominant first line chemotherapeutic agent in CRC.[76] Currently Duke's staging and histological grade are the criteria used to guide decisions on adjuvant therapy. The translation of molecular markers into the clinical decision making is of great relevance for both personal and societal cost benefit balance.

In the recent years several efforts have been made to clarify the mechanisms underlying the 5-FU cytotoxicity and this knowledge should impact on the design of therapeutic strategy. Intracellular metabolites of 5-FU can exert cytotoxic effects via inhibition of thymidylate synthetase, or through incorporation into RNA and DNA. On the basis of current knowledge the DNA-directed cellular effects of 5-FU should reflect the excision of either 5-FU or uracil mainly by uracil DNA glycosylases (UDG) within the base excision repair (BER) pathway[77] and the excision of mismatched nucleotides mainly by MMR.[78] In agreement with these mechanisms, resistance to 5-FU in *in vitro* studies has been observed by inactivating either BER or MMR, with a strong influence of the cell model and treatment schedule used. In the clinical studies where the 5-FU response was evaluated MSI was characterised as a marker of MMR capacity but no one examined simultaneously BER capacity that in the absence of MMR would become the driving factor for 5-FU cytotoxicity. In a previous study[79] we have shown that gastric tumours with MSI are characterised not only by defective MMR but also by downregulation of one of the 5-FU-repair UDG (i.e. SMUG1) as well as by altered expression of other DNA repair genes. Variability in the functionality of DNA repair pathways other than MMR might account for the high variability in the response to 5-FU of MSI-H CRC patients. A recent randomised clinical trial has found a significant improvement in DFS of CRC patients treated with 5-FU in combination with leucovorin and oxaliplatin (FOLFOX).[80] Based on these findings, the FOLFOX regimen has became the new standard adjuvant treatment for patients with stage III colon cancer. There are some indications that MSI-H patients receiving adjuvant FOLFOX therapy

have the same response of MSS patients.[81] This is expected since MMR proteins do not recognise oxaliplatin-related adducts.[82] However, the efficiency of other DNA repair pathways, such as BER,[83] might become the determinant of the response to this therapy and need to be evaluated when developing treatment strategies.

One additional problem to evaluate the effect of adjuvant chemotherapy on MSI-H cancers is the low number of sporadic MSI-H tumours resulting in a limited statistical power for MSI-H patients. Furthermore conflicting evidence on the benefit of 5-FU chemotherapy for MSI-H CRC patients relies mainly on retrospective case series in which selection bias is a potential confounder.[72] In line with other reports our meta-analysis indicates a significant improved prognosis for MSS patients treated with 5-FU-based chemotherapy. Considering the outcome of our analysis MSS patients with stages II and III colon and rectal cancers will obtain a significant survival improvement after treatment with 5-FU-based chemotherapy. The evaluation of the beneficial effect of 5-FU treatment on MSI-H CRC patients is limited by the heterogeneity present in our data set. Also with the exclusion of non-randomised case series which have the potential of bias due to the reporting of significant positive findings only, heterogeneity was still observed. A clinical trial comparing MSI-H CRC without adjuvant chemotherapy to MSI-H CRC with 5-FU adjuvant chemotherapy is underway to prospectively resolve these conflicting and inconclusive results.[84] However, considering the complexity of the mechanism of 5-FU cytotoxicity would be advisable to further characterise the DNA repair profile of the patients under study.

Single markers, such as MSI, when applied to a complex mechanism have a limited use in predicting therapy response. Another form of epigenomic instability is the CpG island methylation (CIMP) which is a common epigenetic event in colorectal neoplasia. Both MSS and MSI cancers show the CIMP feature which has been proposed as promising molecular CRC marker for predicting the efficacy of 5-FU chemotherapy.[85] Despite the overall improvements in CRC therapy, our understanding of why individual patients respond to therapy and others do not, and why some patients relapse, whereas others do not is limited. Molecular classification in CRC is a rapidly evolving field with a promising importance in understanding the mechanisms of carcinogenesis and in the clinical decision making. It is likely that the inclusion of new molecular markers will provide a better although more complicated understanding of the pathological features of CRC.[71] In conclusion our review indicate that MSI has the potential to be used in the clinical setting as prognostic and predictive marker but with a need to include additional molecular CRC markers to improve the patients' management and the prediction of benefit from adjuvant chemotherapy.

Conflict of interest statement

None declared.

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