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Microsatellite instability in rectal cancer: what does it mean? A study of two randomized trials and a systematic review of the literature

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Microsatellite instability in rectal cancer: what does it mean? A study of two randomized trials and a systematic review of the literature

Aim: Currently, compelling evidence illustrates the significance of determining microsatellite instability (MSI) in colorectal cancer (CRC). The association of MSI with proximal CRC is well established, however, its implications in patients with rectal cancer remain undefined. We therefore aimed to determine the role of MSI with respect to incidence and outcome in patients with rectal cancer.

Methods and Results: For this we examined patients from two prospective phase III trials: TME trial and PROCTOR-SCRIPT trial ($n = 1250$). In addition, we performed a literature review to evaluate the overall prevalence, the effect on survival and the response to neo-adjuvant treatment in patients with MSI rectal cancer compared with microsatellite stable (MSS) rectal cancer. Our TME and PROCTOR-SCRIPT cohort showed no differences in terms of overall survival (OS) (hazard ratio [HR] 1.00, 95% confidence interval [CI] 0.69–1.47) and disease-free survival (DFS) (HR 1.00, 95% CI 0.68–1.45) in patients with MSI compared to MSS rectal cancer. The total number of MSI cases in all included studies (including our own) was 1220 (out of 16,526 rectal cancer patients), with an overall prevalence of 6.7% (standard error 1.19%). Both for OS as for DFS there was no impact of MSI status on prognosis (HR 1.00, 95% CI 0.77–1.29 and HR 0.86, 95% CI 0.60–1.22, respectively). The risk ratio (RR) for downstaging and pathological complete response showed also no impact of MSI status (RR 1.15, 95% CI 0.86–1.55 and RR 0.81, 95% CI 0.54–1.22, respectively).

Conclusion: Rectal cancer patients with MSI form a distinct and rare subcategory, however, there is no prognostic effect of MSI in rectal cancer patients.

Keywords: rectal cancer, microsatellite instability, prognosis

Introduction

Microsatellite instability (MSI) is one of the hallmarks of a distinct subtype of colorectal cancer (CRC). Not only is it the diagnostic clue for Lynch syndrome,[1] but in the sporadic setting it is indicative of the serrated pathway.[2,3] Approximately 15% of the sporadic stage II–III CRC have MSI.[4] MSI -CRC have distinct features, such as a more proximal localization, higher grade, a mucinous histology with tumour infiltrating lymphocytes, and the presence of a *BRAF* mutation.[5,6] The relation of MSI with outcome is complex:

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in early-stage CRC it is associated with a prognostic advantage.[7–10] In contrast, in metastatic disease MSI has been associated with a poor clinical outcome.[11,12] Although with conflicting results, accumulating preclinical and clinical evidence reports a resistance to 5-fluorouracil-based chemotherapy in CRC patients with MSI tumours.[8–10,13] Therefore, the advent of immunotherapy for MSI-CRC has totally changed the outcomes in this group of patients.[12]

The role of MSI in patients with rectal cancer is still undefined. Due to the well-documented differences between proximal and distal CRC with respect to prognosis, molecular background, and treatment,[14–16] it is clear that the known implications of MSI (mainly obtained from patients with proximal CRC) cannot be extrapolated to patients with rectal cancer specifically.[17,18] The treatment of rectal cancer patients has shifted towards organ-sparing strategies, where prediction of treatment response has become a key issue. Based on *in vitro* experiments and in a small patient series, an altered radiosensitivity in MSI tumours has been suggested.[19,20] Charara *et al.* suggested that rectal cancer patients with MSI tumours may have increased responses rates,[21] but a recent meta-analysis found no significant difference in pathological complete response (pCR) rate in patients with MSI or microsatellite stable (MSS) tumours.[22]

Compared to colon cancer, the incidence of MSI in rectal cancer is lower, and its prognostic impact is unknown. We therefore aimed to determine the role of MSI with respect to outcome in patients with rectal cancer, by examination of patients from two prospective phase III trials: the TME trial and PROCTOR-SCRIPT trial. In addition, a systematic review of the literature and a meta-analysis was performed.

Materials and methods

PATIENT SELECTION

Data were derived from patients with rectal cancer included in the Dutch TME trial (n = 1530) and the PROCTOR-SCRIPT trial (ISRCTN; 36266738) (n = 470); the results have been published previously.[17,18] Informed consent for participation and retrospective use of samples was obtained from all patients enrolled in both trials. All cases were considered as sporadic rectal cancer, based on the inclusion criteria of both trials, i.e. known hereditary cases were excluded. Formalin-fixed paraffin-embedded (FFPE) tissue of the included Dutch patients was collected. As shown in Figure 1, sufficient FFPE tumour material was available for n = 1061 patients of the TME study. In the PROCTOR-SCRIPT study, n = 324 Dutch patients were included, and tumour tissue could be obtained from n = 268 patients, resulting in a total study cohort of n = 1329 patients with rectal cancer. Histopathological representative tumour regions on haematoxylin and eosin-stained tumour sections were marked by a pathologist (A.v.T.) and punched for the preparation of tumour tissue microarrays (TMA).

MICROSATELLITE ANALYSIS BY IMMUNOHISTOCHEMISTRY

Immunohistochemical staining for mismatch repair (MMR) proteins was performed on 4-μm TMA sections. Briefly, TMA sections underwent deparaffinization and rehydration using xylene and a graded ethanol into water series. Heat-induced antigen retrieval was performed in EDTA for 10 min. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min at room temperature. Sections were incubated in predetermined optimal dilutions (MLH1 1:40, PMS2 1:100, MSH2 1:40, MSH6 1:500) for 60 min at room temperature with anti-MLH1 (clone G168-15, mouse; BD Biosciences, San Jose, CA, USA), anti-PMS2 (clone A16-4, mouse; BD Biosciences), anti-MSH2 (clone GB12, mouse; Calbiochem/Merck, Darmstadt, Germany), and anti-MSH6 (clone EPR3945, rabbit; Abcam, Cambridge, UK). Sections were incubated with Brightvision+poly-HRP-anti Ms/Rb/Rt IgG (Immunologic, Duiven, the Netherlands) for 30 min at room temperature, followed by 7 min incubation with 3,3′-diaminobenzidine (DAB; Immunologic) to visualize antigen expression. Sections were counterstained with haematoxylin, dehydrated, and coverslipped. Tissue stroma served as internal positive control for the staining with anti-MLH1, anti-PMS2, anti-MSH2, and anti-MSH6.

Microscopic analysis of MLH1, PMS2, MSH2, and MSH6 expression was performed by two independent observers (A.v.T. and M.S.) in a blinded manner. When MMR protein expression obtained with IHC on a TMA was inconclusive, additional PCR analysis was performed, as described below.

DNA EXTRACTION AND PENTAPLEX PCR ANALYSIS

DNA was extracted from manual microdissected sections of FFPE tissue focussed on areas with high tumour cell percentage by incubation in 5% Chelex-100 in TET lysis buffer and 10% Proteinase K (20 mg/ml) (Qiagen, Hilden, Germany) for 16 h at

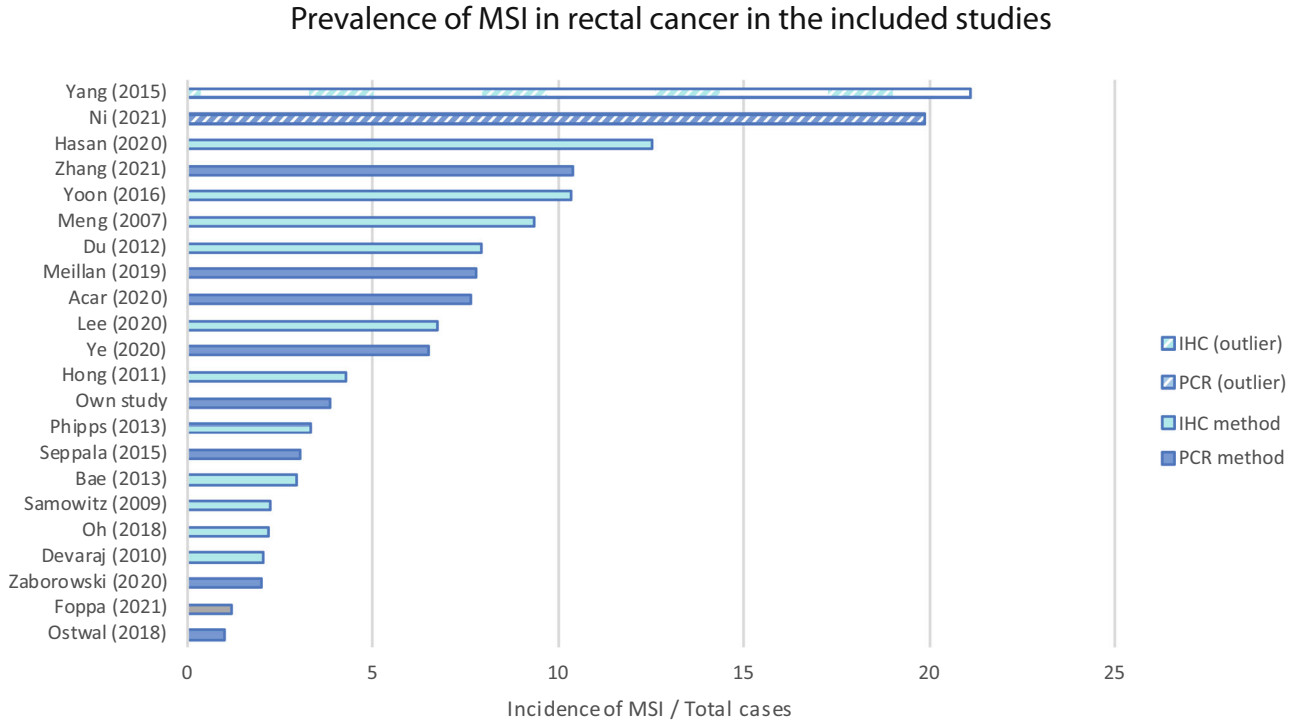

Figure 1. The prevalence of MSI cases per study. In light-blue, all studies that used IHC methods for MSI detection, in dark blue, those that used PCR for MSI detection, and in grey, unknown method for MSI detection. In stripped pattern, studies that did not fall within the IQR limits, as established by the 1.5 × IQR rule. IHC, immunohistochemistry; IQR, interquartile range; MSI, microsatellite instability; PCR, polymerase chain reaction.

56°C. MSI analysis was performed using five mononucleotide repeat markers (NR-21, NR-24, NR-27, BAT-25, and BAT-26) in a single multiplex PCR.[23] The PCR was carried out on a MJ Research PTC-200 Thermal Cycler using 5PRIME HotMaster Taq DNA polymerase (QuantaBio, Beverly, MA, USA) with 1 μl DNA and the following program: initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 20 s, annealing at 55°C for 10 s, and extension at 65°C for 30 s, with a final extension at 65°C for 7 min. DNA fragment analysis was executed on the 3730 DNA Analyser (Applied Biosystems, Foster City, CA, USA). Product sizes for the markers were determined using GeneMarker V.2.6.7 (Applied Biosystems). Normal colon tissues were used as control. A tumour was defined as MSI if at least two of the five markers showed instability.

STATISTICAL ANALYSIS

Statistical analyses were performed using the statistical package SPSS (v 20.0 for Windows; SPSS, Inc., Chicago, IN, USA). Student's *t* test and the Chi-squared test were used for the evaluation of the association between MSI and MSS and clinical-pathological parameters. Overall survival (OS) was defined as time of surgery until death. Disease-free survival (DFS) was defined as time to any recurrence or death, whichever occurred first, or end of follow-up (censored). Distant recurrence (DR) and locoregional recurrence (LR) were defined as time of surgery until DR and LR. Deaths were censored in this analysis. For survival probabilities the Kaplan–Meier method was used and for comparison of survival curves the log-rank test were used. Univariate and multivariate Cox regression analyses were performed to evaluate the differences in OS, DR, and LR. Covariates entered in the multivariate model were age, disease stage, preoperative treatment and adjuvant treatment. For all tests, $P < 0.05$ was considered statistically significant.

SYSTEMATIC REVIEW AND META-ANALYSIS OF PUBLISHED LITERATURE

In cooperation with a trained librarian, we searched for published research comparing patients with MSI rectal cancer and MSS rectal cancer, using MeSH terms

"rectal neoplasms" and "microsatellite instability" in PubMed, including all relevant keyword variations. Titles and abstracts of retrieved articles were screened, followed by full-text review of studies focussing on MSI/MSS status in rectal cancer patients in relation to clinical outcome. Additional eligible articles were manually screened from the reference lists of the retrieved articles. The latest search was performed on January 12th, 2022 (Figure S1).

INCLUSION CRITERIA

Studies in English language with over 100 patients including patients with primary rectal adenocarcinoma with both MSI and/or deficient mismatch repair (dMMR) data were included. Nonhuman studies and case-controls were excluded. For each study the number of patients in both the MSI and the MSS group were retrieved. Data on response rate, 5-year DFS, and 5-year OS for MSI and MSS were extracted from all studies by two independent reviewers. If no HR was reported, it was calculated from Kaplan–Meier curves.[24] Data were entered in Review Manager (RevMan v. 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012). A meta-analysis was performed with all available studies on each endpoint in terms of risk ratios (RR) and hazard ratio (HR) with 95% confidence interval (CI). A random effects model with inverse variance weighting of studies was used. Heterogeneity was assessed using a χ^2 test for heterogeneity with a P-value of <0.10 to show the presence of significant heterogeneity.

Results

STUDY POPULATION

In total, tumour tissue from 1329 patients could be retrieved and was suitable for the preparation of a TMA. Of the total study cohort, 1061 patients participated in the TME trial and 268 patients in the PROCTOR-SCRIPT trial. Patients with ypT0, stage IV or unknown tumour stage were excluded (n = 79). As a result, 1250 patients were included for analysis, with a median follow-up of 7.4 years. Of the included patients 503 patients underwent TME surgery without neoadjuvant treatment, 718 patients received neoadjuvant radiotherapy, 28 patients received neoadjuvant chemoradiation, and one received other neoadjuvant therapy. In the total patient cohort (n = 1250), MSI was observed in 48 (3.8%) and 1202 (96.2%) tumours were considered MSS (distribution of affected MMR proteins can be found in Table S1). The patient and tumour characteristics of the total cohort and stratified by MSS or MSI status are summarized in Table 1. No significant differences were observed between patients with MSI tumours and MSS tumours regarding clinicopathological characteristics.

OUTCOME IN RELATION TO MSI IN OUR STUDY

As shown in Table 2, no differences in terms of OS (HR 1.00, 95% CI 0.69–1.47), DFS (HR 1.00, 95% CI 0.68–1.45), DR (HR 0.94, 95% CI 0.54–1.63), and LR (HR 1.52, 95% CI 0.62–3.74) were observed in patients with MSI or MSS rectal cancer in the whole study cohort in both the univariate and the multivariate analysis. In the multivariate model, treatment was included and there was no difference according to neoadjuvant therapy.

META-ANALYSIS OF PUBLISHED LITERATURE AND THE CURRENT STUDY

The last search was performed on January 12th 2022, resulting in 79 studies. Title and abstract screening were performed and 63 articles were excluded (including nine non-English studies, two studies that did not have full-text, eight studies in which no MSI was performed, 16 reviews and case reports, nine studies not focussed on rectal cancer, and 19 studies in which they included under 100 patients). Based on full-text review, we included 16 original studies, and included an extra five studies through manual inclusion of reference lists of the included articles and our own study. These 22 studies are summarized in Table 3. The total number of MSI cases in these studies (including our own) was 1220 (out of 16,526 rectal cancer patients), with an overall prevalence of 6.7% (interquartile range [IQR] limits −7.44, 18.88, standard error 1.19%). Yang *et al.*[25] and Ni *et al.*[26] were considered outliers after analysis of the 1.5 × IQR rule to find outliers or prevalence of MSI. In Figure 1, the prevalence of MSI cases per study is shown. There was no correlation of MSI rates with nationality, inclusion of stage IV or type of MSI detection test.

OUTCOME IN RELATION TO MSI: META-ANALYSIS

Both for OS (Figure 2A) as for DFS (Figure 2B), there was no impact of MSI status on prognosis (HR 1.04, 95% CI 0.82–1.32 and HR 0.94, 95% CI 0.66–1.34, respectively). There was no heterogeneity between the studies for DFS (I^2 = 43%). One study, in addition to our own study, showed no impact of MSI status on local recurrence rates.[35]

Table 1. Patient characteristics of the total study cohort and stratified for MSI and MSS status

	Total n = 1250		MSI n = 48		MSS n = 1202		
	n	(%)	n	(%)	n	(%)	P-value
Gender							
Male	797	(63.8)	30	(62.5)	767	(63.8)	0.88
Female	453	(36.2)	18	(37.5)	435	(36.2)	
Age median	64.0	(±10.9)	62.0	(±11.5)	64.0	(±10.8)	0.10
Disease stage							
I	325	(26.0)	10	(20.8)	315	(26.2)	0.53
II	337	(27.0)	16	(33.3)	321	(26.7)	
III	588	(47.0)	22	(45.8)	566	(47.1)	
Neoadjuvant treatment							
None	503	(40.2)	18	(37.5)	485	(40.3)	0.98
Radiotherapy	718	(57.4)	29	(60.4)	689	(57.3)	
Chemoradiotherapy	28	(2.2)	1	(2.1)	27	(2.2)	
Other	1	(0.1)	0	(0)	1	(0.1)	
Adjuvant treatment							
Observation	1022	(81.7)	41	(85.4)	980	(81.5)	0.36
Chemotherapy	177	(14.1)	6	(12.5)	171	(14.2)	
Radiotherapy	43	(3.4)	0	(0)	43	(3.6)	
Other	9	(0.7)	1	(2.1)	8	(0.7)	
Circumferential resection margin							
Negative	1066	(85.2)	38	(79.2)	1027	(85.4)	0.40
Positive	180	(14.4)	10	(20.8)	170	(14.1)	
Unknown	5	(0.4)	0	(0)	5	(0.4)	

Data are presented as median ± SD or n (%).
MSI, microsatellite instability; MSS, microsatellite stable; SD, standard deviation.

There are different ways of measuring response: most studies used either downstaging or percentage of complete pathological response. Although individual studies suggest differences, the meta-analysis shows no difference in both downstaging (Figure 2C, RR 1.14 [95% CI 0.89–1.48]) and complete pathological response rate in cases with MSI (Figure 2D, RR 0.81 [95% CI 0.54–1.22]). There was no heterogeneity present (I^2 = 21%, P = 0.32). These results were not influenced by the MSI assay used.

Discussion

In rectal cancer, the incidence of MSI was low, 7% of cases. Due to the relative low incidence of MSI in rectal cancer, limited evidence regarding its prognostic and predictive value existed. We have shown that there is no effect of MSI on OS or DFS, both in our series as well as in the available literature. The lack of association between gender and MSI status was noteworthy, which is entirely different from proximal colon cancers.[45] However, the majority of these were

Table 2. Univariate and multivariate survival analysis for overall survival, disease-free survival, time to distant recurrence, and time to local recurrence according to MSI and MSS status

	Patients n = 1250	Univariate		Multivariate	
		HR (95% CI)	*P*-value	HR[a] (95% CI)	*P*-value
Overall survival					
MSI	48	1.00 (0.69–1.47)	0.99	1.20 (0.82–1.76)	0.35
MSS	1202	1.00		1.00	
Disease-free survival					
MSI	48	1.00 (0.68–1.45)	0.99	1.18 (0.81–1.71)	0.39
MSS	1202	1.00		1.00	
Distant recurrence					
MSI	48	0.94 (0.54–1.63)	0.94	0.98 (0.57–1.71)	0.95
MSS	1202	1.00		1.00	
Local recurrence					
MSI	48	1.52 (0.62–3.74)	0.37	1.53 (0.60–3.86)	0.40
MSS	1202	1.00		1.00	

Covariates entered in the multivariate model were age, neoadjuvant treatment, adjuvant treatment, and disease stage.
CI, confidence interval; HR, hazard ratio; MSI, microsatellite instability; MSS, microsatellite stable; SD, standard deviation.
[a]Adjusted for age, neoadjuvant treatment, adjuvant treatment, disease stage.

derived from hypermethylated sessile serrated lesions, which do not occur in the rectum.[46]

For therapy response, the evidence from our study suggests there is also no difference, although this is less clear-cut. Several different approaches are used to establish therapy response.[47] We did not observe significant differences in downstaging or cases with complete pathological response, but we did see a trend towards more favourable responses in the MSS group. Complete pathological response is a relatively hard criterion, while tumour downstaging is dependent on pretreatment imaging, which is particularly unreliable.[48] Moreover, in one study the pCR cases were excluded,[35] since MSI was determined on the resection specimen. Our data conflicts with results from earlier small studies,[21,49] but are in line with studies of bigger cohorts.[30,50] Testing postradiotherapy material may explain the high MSI rate in some of the studies, as MSH6 loss induced by therapy was not specifically excluded. In our TME cohort, the prevalence of MSH6-loss was comparable, with 5.3% in nontreated patients and 6.4% in RT-treated patients.

The current standard treatment of rectal cancer consists of neoadjuvant chemoradiotherapy followed by surgery. Novel strategies included total neoadjuvant treatment,[51] watch and wait strategies,[52] and immunotherapy.[53] Three recent studies investigated the impact of MSI on neoadjuvant chemotherapy (NCT) treatment[41,54,55] and found conflicting results. Cercek *et al.*[54] found MSI to be an indicator of poor response to NCT. They state that induction chemotherapy is far more efficacious in MSS than in MSI in rectal cancer and that organ preservation strategies such as adjuvant CT or watch-and-wait strategies may be used efficiently in MSS rectal tumours, but may not be so in MSI tumours. Contrary to this, Ye *et al.*[41] found that MSI was associated with improved DFS in patients who received NCT, but associated with worse DFS in those receiving neoadjuvant chemoradiotherapy (NCRT). Moreover, in a recent article de Rosa *et al.*[55] stated that, in their cohort, MSI patients treated with NCRT had the best pCR rates (27%). Thus, while it remains clear that MMR status should be reported in rectal cancer diagnosis, further research with bigger cohorts are warranted to understand the prognostic implications.

In general, limitations in bigger cohorts focus on heterogeneity in diagnostic workup and treatment. While in our cohort diagnostic workup, treatment, and follow-up were strictly standardized, this varies

TABLE 3. Study characteristics

Author (year)	Country	Neoadjuvant treatment	Stage	Total cases	MSI cases	Test type	OS	DFS	LR	Response	IHC + control
Acar *et al.* (2020)[27]	Turkey	NCRT	II–III	341	26	IHC				pCR	Unknown
Bae *et al.* (2013)[28]	Korea	None	I–IV	168	5	PCR	x	x			
Devaraj *et al.* (2010)[29]	USA	Unknown	I–IV	147	3	PCR					
Du *et al.* (2012)[30]	China	NRT	II–III	316	25	PCR		x		pCR, DS	
Foppa *et al.* (2021)[31]	Italy	None, NCRT, NCT, NRT	I–IV	1005	12	Unknown					
Hasan *et al.* (2020)[32]	USA	NCRT	I–III	5086	636	PCR				pCR	
Hong *et al.* (2011)[33]	Korea	None	I–IV	465	20	PCR	x	x			
Lee *et al.* (2020)[34]	Korea	NCRT	I–III	549	37	PCR					
Meillan *et al.* (2019)[35]	France	NRT	I–IV	296	23	PCR/IHC	x	x	x	DS	Yes
Meng *et al.* (2007)[36]	China	None	II–III	128	12	PCR	x				
Ni *et al.* (2021)[26]	China	NCRT, none	I–IV	181	36	IHC	x	x		DS	Yes
Oh *et al.* (2018)[37]	Korea	None	II–III	1103	24	PCR	x	x			
Ostwal *et al.* (2018)[38]	India	NCRT	I–III	296	3	IHC					Yes
Own study	Netherlands	None, NRT, NCRT	I–III	1250	48	IHC	x	x	x		Yes
Phipps *et al.* (2013)[6]	USA, Canada, Australia	Unknown	Unknown	1111	37	PCR/IHC	x				Unknown
Samowitz *et al.* (2009)[39]	USA	Unknown	I–IV	979	22	PCR	x				
Seppala *et al.* (2015)[40]	Finland	Unknown	I–IV	197	6	IHC					Yes
Yang *et al.* (2015)[25]	China	None	II	460	97	PCR		x			
Ye *et al.* (2020)[41]	China	NRT, NCRT	II–III	1015	66	IHC		x		pCR, DS	Yes
Yoon *et al.* (2016)[42]	Korea	NCRT	II–III	145	15	PCR		x			
Zaborowski *et al.* (2020)[43]	Ireland	NCRT	I–III	797	16	IHC		x			Unknown
Zhang *et al.* (2021)[44]	China	Unknown	I–IV	491	51	IHC					Unknown
Total				16 526	1220						

DFS, disease-free survival; DS, downstaging; IHC, immunohistochemistry; IHC + control, immunohistochemistry internal positive control; LR, local recurrence; MSI, microsatellite instability; OS, overall survival; PCR, polymerase chain reaction; pCR, pathological complete response.

in other cohorts. For example, the large population-based study of Hasan *et al.*[32] was subject to criticism on methodology. This register-based exploration grouped together MSI-high, MSI-low and MSI-unspecified in their MSI+ group, leading to a prevalence of 13%, triple that reported in our study and double that reported in our meta-analysis. Although our own study might be underpowered, due to the low prevalence of MSI in rectal carcinomas, we believe the addition of our considerably-sized cohort adds valuable information to the available literature. Immunohistochemistry is a simpler but reproducible

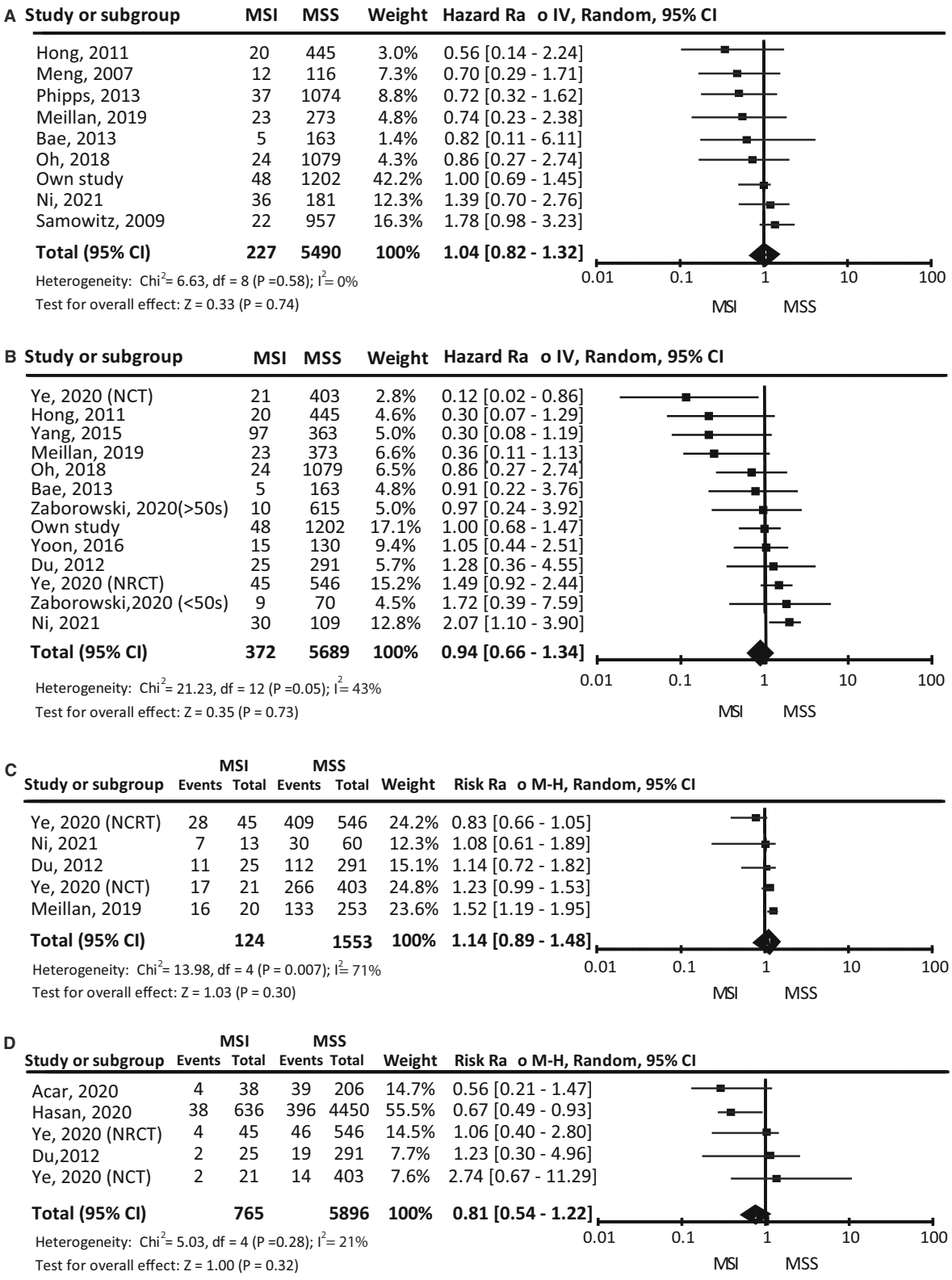

Figure 2. Forest plot of the impact of MSI on outcome. Overall survival (A), disease-free survival (B). Pathological response defined as down-staging (C) and as the presence of complete pathological response (D). CI, confidence interval; HR, hazard ratio; MSI, microsatellite instability; RR, relative risk.

method to dichotomise the population, with the possibilities of adding MSI analysis in cases of doubt. In fact, two recent studies[56,57] aimed to compare both detection methods and found that they were equally proficient tests for establishing microsatellite status. We confirmed this in our dataset, as we did not see a particular cluster of studies with higher standard error of MSI prevalence according to the MSI detection technique (Figure 1), nor did we find that the MSI technique influenced any of the outcomes.

In recent years, promising results have emerged in the use of immunotherapy as treatment, particularly for dMMR CRC. The evidence of effectiveness of immunotherapy as described in case reports or small case series is present for (locally advanced) rectal cancer.[58–61] Whether the positive results of immunotherapy in dMMR colon cancer[62] can be translated into improved treatment of rectal cancer is currently being investigated in several ongoing trials.

In conclusion, although the prevalence of MSI rectal cancer is low and has no prognostic value, the promising results of immunotherapy and the direct link with the detection of Lynch syndrome patients emphasize the need for MSI testing in rectal cancer patients.

Author contributions

The corresponding author ensures that all those designated as authors qualify for authorship, as they have participated sufficiently in the work to take public responsibility for appropriate portions of the content. Marloes Swets: Data Curation, Validation, Writing – Original Draft. Cristina Graham Martínez: Formal Analysis, Data Curation, Writing – Review and Editing, Visualization. Shannon van Vliet: Investigation, Writing – Review and Editing. Arjan van Tilburg: Investigation, Writing – Original Draft. Hans Gelderblom: Writing – Original Draft, Supervision. Corrie A.M. Marijnen: Conceptualization, Methodology, Writing – Original Draft, Writing – Review and Editing, Funding acquisition. Cornelis J.H. van de Velde: Conceptualization, Methodology, Writing – Original Draft. Iris D. Nagtegaal: Conceptualization, Methodology, Writing – Original Draft, Writing – Review and Editing, Supervision, Funding Acquisition.

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Conflict of interest

The authors declare they have no competing interests in the content of this article.

Ethical approval statement

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation at the Radboud UMC and with the Helsinki Declaration of 1964 and later versions. Data were derived from patients included in the PROCTOR-SCRIPT trial (ISRCTN; 36266738), a multicentre randomized phase III trial, that included patients with (y)pTNM stage II-III rectal cancer treated with neoadjuvant (chemo)radiotherapy and TME surgery, randomly assigned to adjuvant chemotherapy or observation. The TME study does not have an ISRCTN number since it was published in 2001. Informed consent for participation and retrospective use of samples was obtained from all patients enrolled in both trials.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, I.D.N.. The data are not publicly available due to privacy restrictions.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow diagram for systematic review of studies via databases and registers.

Table S1. Distribution of MMR proteins affected per patient in each study cohort.