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Does microsatellite instability predict the efficacy of adjuvant chemotherapy in colorectal cancer? A systematic review with meta-analysis

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ABSTRACT

Background: Microsatellite instability (MSI) status in predicting the efficacy of adjuvant chemotherapy in colorectal cancer remains controversial.

Materials and methods: Studies were identified through PubMed, Embase and ASCO proceedings with a combination of keywords (colorectal cancer, chemotherapy and MSI).

Results: A MA was performed for treated and non-treated MSI population on seven studies. Statistical calculations were performed on 7 studies representing 3690 patients; mean age: 65.5 years; 810 stage II and 2444 stage III (75%). MSI-high (MSI-H) was found in 454 patients (14% of the global population), and microsatellite stable (MSS) in 2871. A total of 1444 patients received 5-fluorouracil (5FU)-based chemotherapy, whereas 1518 patients did not. For MSI-H patients, there was no statistically significant difference for RFS whether or not they received chemotherapy (5 studies); HR RFS: 0.96 (95% confidence interval (CI): 0.62–1.49); HR OS (6 studies): 0.70 (95% CI: 0.44–1.09; $p = 0.12$). Elsewhere, we found a significant interaction between MSI status (MSI-H or MSS) and therapeutic status suggesting a lesser benefit for MSI-H than for MSS patients (HR interaction RFS: 0.77 (95% CI: 0.67–0.87)).

Conclusion: We found similar RFS for treated and untreated MSI-H patients, showing that MSI-H status, in addition to being a good prognostic factor is also a predictive factor of non response.

1. Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth most frequent cause of cancer death worldwide.[1] In addition to curative surgery, adjuvant chemotherapy is the mainstay of treatment. In adjuvant setting, for high risk stage II and stage III diseases, 5-fluorouracil (5FU) is always part of treatment and now generally used in combination with oxaliplatin (Folfox protocol).[2]

Among genetic abnormalities involved in carcinogenesis, microsatellite instability (MSI) is a major pathway of cancer development.[3] MSI corresponds to a dysfunction of the mismatch repair (MMR) system resulting in a reduction in the length of highly repeated DNA sequences termed

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microsatellites. The MMR system corrects mismatches of DNA nucleotides occurring during replication. A germinal mutation inactivating one of the MMR genes may lead to a hereditary form termed Hereditary Non-Polyposis Colon Cancer (HNPCC).

A previous meta-analysis (MA) found that MSI status was a prognostic factor in CRC.[9] It also concluded to a lack of benefit of adjuvant chemotherapy among MSI-H patients but included only two articles where adjuvant treatment was compared with no treatment.[8,11] In addition, the relative benefit of chemotherapy (meaning predictive value) among patients with high microsatellite instability (MSI-H) and microsatellite stable (MSS) was not detailed. This is a major issue since MSI-H status is not only found in the rare HNPCC forms (Lynch syndrome) but also found in 15% of sporadic forms of CRC.[12]

The main goal of our MA was to assess the predictive value of MSI-H status among patients receiving or not adjuvant chemotherapy for CRC. Although the prognostic value of MSI status has been firmly established, its predictive value is still a matter of debate. The distinction between prognostic and predictive factors has been clearly made.[13]

Many studies assessing the predictive role of MSI status on the efficacy of adjuvant chemotherapy in CRC have been published between 2000 and 2008. These works investigated the relationship between MSI status and relapse-free survival (RFS) or overall survival (OS) among patients with localised disease. Some studies were Randomised Controlled Trials (RCTs) initially designed to evaluate the benefit of adjuvant treatment.[7,8] Until now, no prospective study randomised chemotherapy according to MSI status and compared its efficacy among MSI-H and MSS patients. Also, the relationship between the MSI status and the efficacy of chemotherapy differed among studies. These facts justify the need for a meta-analysis of all publications assessing the predictive value of MSI status on the efficacy of treatments.

2. Methods

2.1. Publication selection

We performed our MA according to a predefined written protocol. To be eligible, studies had to deal with colon or rectum cancer (whatever be the stage at inclusion of patients in the individual studies) and had to assess the relationships between MSI status, chemotherapy and RFS or OS for localised disease. Studies (full articles) were identified by an electronic search using online PubMed, with a set of keywords used simultaneously 'colorectal cancer, chemotherapy, microsatellite instability'. Last query was updated on 29th February 2008. We also performed an electronic search with the same keywords using online EMBASE. Examination of the Cochrane database of systematic reviews did not retrieve additional pertinent references. Our initial selection of articles relied on careful reading of their abstracts. Abstracts were also reviewed from ASCO annual proceedings from 1998 to 2008 available online. We also screened references from the relevant literature, including all the identified studies, but also reviews and editorials for additional information.[9] We included studies written in any language, both as full papers or as abstracts from ASCO proceedings. We carefully tried to avoid duplication of data, by examining the names of all authors and the different medical centres involved for each publication. We excluded studies in which survival data were not available. When needed, we wrote to authors to obtain additional data allowing statistical calculations.

2.2. Methodological assessment

Information was carefully extracted from all publications in duplicate by two readers (Dr. Gaëtan Des Guetz and Dr. Bernard Uzzan), using a standardised data collection form, including the following items: complete reference of the publication, original publication or update of a former publication, mode of making up of the series of cases, prospective study, inclusion of consecutive cases, randomised controlled trials (RCTs), median duration of follow-up, number of patients included in the study, mean or median age, gender, anticancer treatment(s) during follow-up, histological type (adenocarcinoma or mucinous), tumour size, stage of disease, grade (good, moderate or poor differentiation) and nodal status. Assessment of methods used to determine MSI status: analysis of MLH1, MSH2, MSH6 and PMS2 expression by immunohistochemistry or measurement of the size of microsatellite markers by molecular methods (number and type of MSI; i.e. NCI reference or pentaplex panels[14,15]) was done by Dr. Olivier Schischmanoff. To simplify analysis of data, MSI-low and MSS patients were always pooled and will be termed collectively as MSS in the rest of the text.

Disagreements were resolved by consensus between the three readers. We did not set a predefined minimal number of patients for a study to be included in our MA, nor a minimal duration of median follow-up. We did not weigh each study by a quality score, because no such score has received general agreement for use in a MA, especially of observational studies, making the evaluation of its usefulness more difficult.[16] When duplicate studies were retrieved, we included in our systematic review only the one involving the highest number of patients from which data could be extracted (usually the latest). This was done to avoid overlapping between cohorts. A majority of studies were initially designed to assess the benefit of adjuvant chemotherapy and were randomised and controlled. However, determination of MSI status was always performed retrospectively. Some studies compared patients who received or not chemotherapy with or without randomisation. Some studies only included patients receiving chemotherapy. Although their methodological quality and the reliability of their conclusions were variable, their design was almost similar, which is a favourable condition for our MA.

2.3. Statistical methods

In each study, the relationship between MSI status and survival was considered significant when the p-value for the statistical test comparing survival distributions between the MSI-H and MSS groups was below 0.05 in univariate analysis (two-tailed test).

For each trial, Hazard Ratio (HR) was estimated by a method depending on the data provided in the publication. The simplest method consisted in the direct collection of HRs, or odds ratios, and their 95% confidence interval (CI) from the

original article. If not available, we looked at the total numbers of events (deaths, relapses) and at the numbers of patients at risk in each group to estimate the HR. When data were only available as graphical survival plots, the calculations were done only if the number of steps on the curves equalled the number of events given in the publication, assuming that the rate of censored patients was constant during the study follow-up. By convention, a HR lower than 1 meant an improved survival among MSI-H patients compared with MSS, or among MSI-H patients receiving adjuvant chemotherapy compared with no treatment.

We calculated a pooled random HR estimate and its 95% CI by using a fixed-effect model (Mantel Haenszel method) due to the absence of heterogeneity between studies. The statistical calculations for our meta-analyses were performed with EasyMA.net (http://www.spc.univ-lyon1.fr/easyma.net/) application available online (Department of Clinical Pharmacology, Cardiology Hospital, Lyons, France). The statistical analysis was performed by Dr. Patrick Nicolas, who chose the best-fitted statistical tests or decided the exclusion of studies from our MA because their data could not be exploited statistically.

3. Results

Our electronic data search using online PubMed retrieved a total of 159 references including 50 reviews. An EMBASE query did not provide us with any additional reference. After exclusion of the references that were out of the scope of our MA (by reading the abstracts), and of one reference providing only MSH2 gene expression but not MSI status,[17] there remained 17 studies dealing with MSI.[4–8,10,11,18–26] Three studies[5,8,27] were published in duplicate.[20,21,27] We also found 76 abstracts in ASCO proceedings (1998–2008). Additional data corresponding to four recent abstracts were retrieved[28–31] plus one update by Sargent et al. of the study by Ribic et al.[32] We could not include some studies in our MA due to lack of information. We thus wrote (by e-mail) to the authors.[19,23,30,31] Their answers did not allow inclusion of their papers. One article without survival data was excluded.[18] One article was excluded due to inability to obtain HR.[24] Moreover a study by Chang et al.[28] was excluded, since there was no comparison between MSI and MSS patients. Information about the inclusion process is provided in the flow-chart of our MA. Two studies only included treated patients and were not selected.[25,26] And in the study by Popat, HR for treated or untreated patients was not available[29] (Fig. 1).

Seven studies assessed two cohorts, one receiving and the other not receiving an adjuvant chemotherapy[4–8,10,27] and two of these studies included samples from RCTs evaluating the potential benefit of adjuvant chemotherapy.[7,8] Most of the patients were treated with 5FU-based chemotherapy with[5,6] or without folinic acid,[10] or levamisole.[8,11] Chemotherapy was usually administered by systemic route. In two other articles, chemotherapy was based upon 5FU, intra portal Mitomycine[7] or various drugs.[4]

For the analysis of the influence of MSI status for patients treated in adjuvant setting, 7 studies representing 3690 patients were analysed, mean age: 65 years (55–70); 1345 men and 1198 women (1147 missing data); 1777 colon cancers (89%) and 213 rectum (1700 missing data); 810 stage II and 2444 stage III (75%; 436 missing data). MSI-H was found in 454 patients (14% of the global population), and MSS in 2871 (365 missing data).[4–8,10,11]

The number of microsatellite markers analysed differed greatly among the studies (from 1 to 17).[5,6] In some studies, different groups of patients were studied with various microsatellite markers (Table 1). However, Bethesda's markers were often used in 5 studies.[4,5,7,8,10] One study analysed only two markers that included the microsatellite Bat 26 which displays the highest sensitivity and specificity.[11] Two studies included an evaluation of MMR proteins expression by immunohistochemistry (3, MLH1 and MSH2; 2, MLH1, MSH2, MSH6 and PMS2) (Table 1).[5,6]

There was no statistically significant heterogeneity between the studies, pHet = 0.30 for OS and 0.40 for RFS; I^2 = 16% and 4%, respectively. Therefore we used a fixed-effect model. We first examined the HR of events (deaths and relapses) among MSI-H versus MSS patients all receiving chemotherapy. We analysed survival among MSI-H patients receiving or not chemotherapy. We found no benefit of chemotherapy among MSI-H patients. Global HR OS (six studies) was 0.70 (95% CI: 0.44–1.09; $p = 0.12$).[4,6–8,10,11] Global HR RFS (5 studies) was 0.96 (95% CI: 0.62–1.49; $p = 0.86$)[4–8] (see Figs. 2 and 3).

Given the worse prognosis associated with stage III disease compared to stage II, we tried to analyse RFS separately in these two groups. The majority of studies did not analyse stages II and III separately, except the studies by Lanza et al.[5] and Sargent et al.,[32] but the latter did not provide data to calculate HRs. Thus we could not perform a subgroup MA concerning stage III because it assessed only one article.[5] Similarly a MA of stage II could not be performed since it would have included only two articles.[5,32]

We performed a meta-analysis of the interaction as an approach to the issue of whether the benefit of adjuvant treatment differs between MSI-H and MSS patients. In other words, to evaluate if MSI-H and MSS patients benefit similarly from chemotherapy. We found a statistically significant interaction, meaning that chemotherapy had no effect among MSI-H patients compared with a beneficial effect of chemotherapy among MSS patients, HR RFS 0.77 (95% CI: 0.68–0.87; $p < 0.001$).

4. Discussion

Our MA is the first to deal with the predictive value of MSI status to assess the benefit of adjuvant chemotherapy. The issue of the comparative benefits of chemotherapy according to MSI status is crucial since no biological marker has so far been proved effective in predicting the efficacy of adjuvant chemotherapy in CRC.[33] We showed that there was no survival difference among MSI-H patients whether or not they received chemotherapy, whereas MSS patients had a better response to chemotherapy, suggesting that MSI could be considered as a predictive marker of chemoresistance. Moreover, there was a significant interaction on survival between MSI status and chemotherapy status. This means that the effect of adjuvant chemotherapy differs according to MSI status, with no

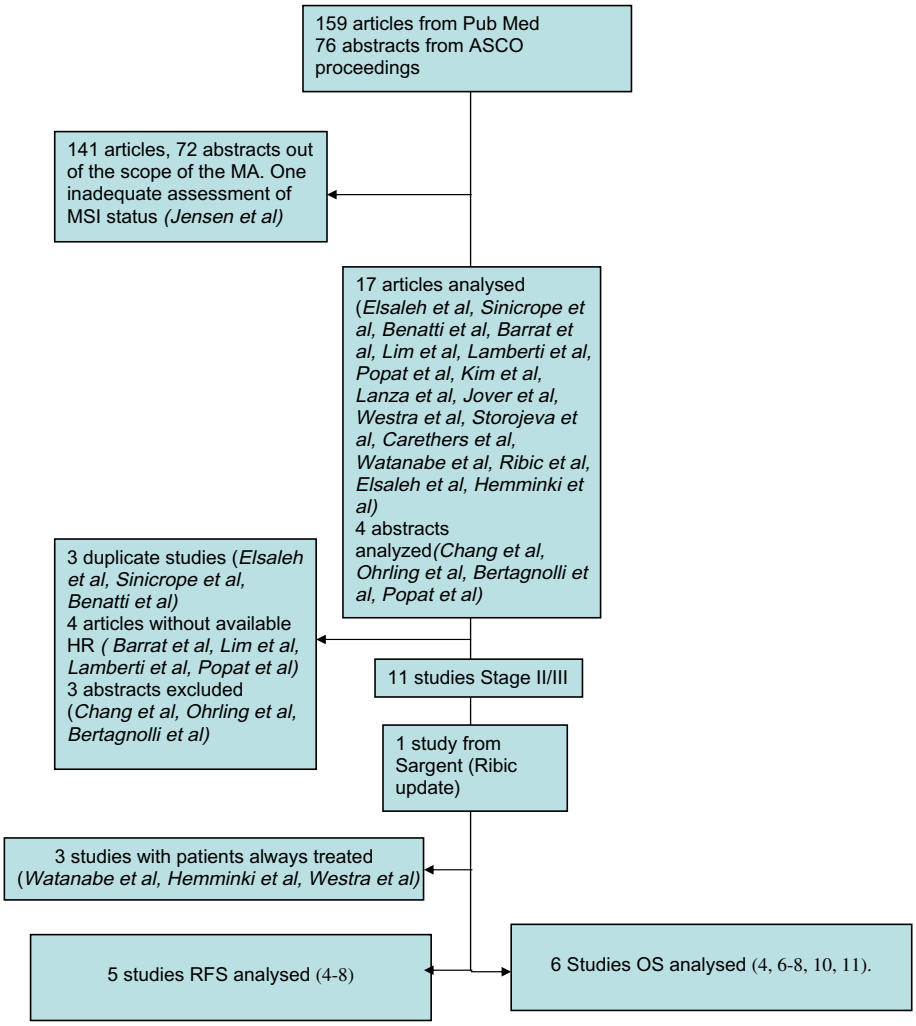

Fig. 1 – Flow-chart of the different steps of the meta-analysis.

difference between treated and untreated MSI-H patients but with a benefit in survival for treated MSS patients.

To determine whether the better survival of MSI-H patients is related to treatment or to the better prognosis of MSI-H, we compared RFS in treated and untreated MSI-H patients. In accordance with the initial MA by Popat et al.,[9] our MA dealing exclusively with MSI-H patients (including the article by Ribic et al.) did not find a significant survival difference between treated and untreated patients. This indicates that MSI-H patients seem less sensitive to adjuvant treatment based on 5FU and is in accordance with the previous data from Ribic (Fig. 4). These results are also in accordance with *in vitro* data finding that 5FU chemosensitivity was due to FU-modified DNA recognition by MMR components (MSH2/MSH6), which are required for the induction of apoptosis.[34] So, a MMR components dysfunction could explain a less efficacy of 5FU-based treatment. We also found a significant interaction between MSI status and chemotherapy status. Taken together, the results suggest a lesser benefit for MSI-H patients. MSI-H patients have a less aggressive disease and therefore would not benefit from chemotherapy.

A specific issue in evaluation of MSI-H chemosensitivity is the potential differences between tumours with HNPCC syndrome where patients are younger and the sporadic MSI-H tumours (due to inactivation of MLH 1 gene by promoter methylation) in older patients. The median age for MSI-H in the studies ranged between 57.9 and 69.2 years.[6,8] For these 2 studies, HRs are similar (see Fig. 2). Thus, this might imply similar relapse rate for HNPCC and sporadic CRC patients.

Although our global MA relied upon all eligible published studies, it should be stressed that almost all these studies were not randomised controlled trials and that each of our subgroup MAs was based upon a few studies. Although these caveats somewhat limit the extent of our findings, they do not put their usefulness into question, since our results reflect the state of the art about this issue.

In adjuvant setting, the treatments are always based upon 5FU administered according to various schedules. Studies differed by their doses and modes of administration of 5FU or by various potentiations (folinic acid or levamisole). However, it is not known whether such differences may influence the survival according to MSI status. Moreover, at present, combined chemotherapy (FOLFOX) has replaced 5FU monotherapy in adjuvant treatment for stage III disease.

For stage II colon cancer, the benefit of chemotherapy is highly controversial. To assess the benefit of chemotherapy

Table 1 – Main characteristics of the studies included in the meta-analysis. C represents studies including consecutive patients, RCT randomised controlled trials. P: prospective study; RR: response rate; RFS: relapse-free survival and OS: overall survival. A positive study showed a statistically significant higher effect of chemotherapy among MSI patients.

First author (reference)	Study from PubMed	Study design	Age	N (M/F)	Colon (n)	Rectum (n)	Stage II/III	Immunohisto chemistry	PCR (markers)	N patients MSI/MSS	HR estimate	Survival analysis	Results RFS (patients treated)
Kim et al.[4]	Yes	P	nd	542 nd	542	0	nd	No	5 (Bethesda markers)	98/444	Reported in text	RFS/OS	Inconclusive
Jover et al.[6]	Yes	C	70	605 nd	nd	nd	II/III	MLH1 MSH2	1	66/688	Data extrapolated	RFS/OS	Inconclusive
Lanza et al.[5]	Yes	C	65	718 (359/359)	548	170	393/325	MLH1 MSH2	6–17 (including Bethesda markers)	75/288	Data extrapolated	OS	Inconclusive
Storojeva et al.[7]	Yes	RCT	63	160 (82/78)	117	43	nd	No	9 (including Bethesda markers)	21/139	Reported in text	RFS/OS	Inconclusive
Carethers et al.[10]	Yes	C	66	204 (131/73)	nd	nd	105/970	No	5 (Bethesda markers)	36/168	Reported in text	OS	Inconclusive
Ribic et al.[8]	Yes	RCT	60	570 (326/244)	570	0	312/258	No	2–11 (including Bethesda markers)	95/475	Reported in text	RFS/OS	Inconclusive
Elsaleh et al.[11]	Yes	C	68	891 (447/444)	nd	nd	0/891	No	1 (Bat 26)	63/669	Reported in text	OS	Inconclusive

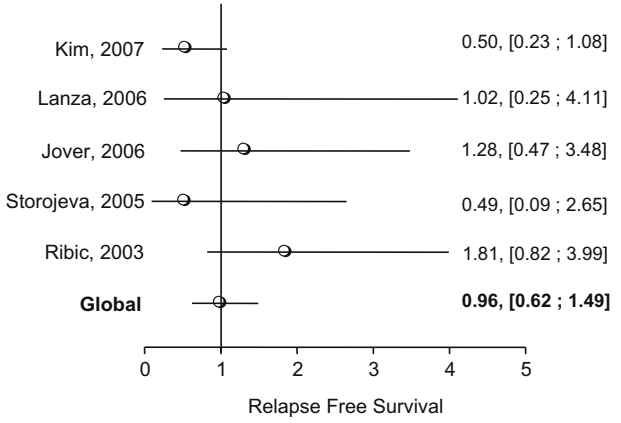

Fig. 2 – Forest plot of the studies assessing relapse-free survival in MSI patients treated compared to MSI patients not treated in adjuvant setting. By convention, a Hazard Ratio (HR) < 1 corresponds to a better survival for treated MSI patients.

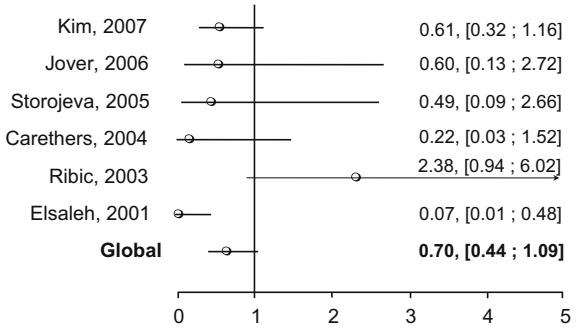

Fig. 3 – Forest plot of the studies assessing overall survival in MSI patients treated compared to MSI patients not treated in adjuvant setting. By convention, a HR < 1 corresponds to a better survival for treated MSI patients.

in stage II CRC, an ongoing RCT (stratifying patients according to two biological markers, MSI status and loss of heterozygosity) has been designed to include more than 3000 patients. In this study, MSS patients (with proven benefit of chemotherapy) are randomised between adjuvant chemotherapy alone and chemotherapy plus bevacizumab, whereas MSI-H patients have no chemotherapy, presuming a better prognosis.[35]

To conclude, our MA showed that among MSI-H patients compared with MSS patients, adjuvant chemotherapy was associated with a significantly better survival. Among the subgroup of MSI-H patients, chemotherapy did not improve survival, compared with no treatment. The favourable effect of MSI-H status on survival might rather be explained by the better prognosis of MSI-H tumours than by the beneficial effect of chemotherapy. Separate analyses of the role of MSI status on treatment efficacy and new specific studies for stage II and stage III patients receiving or not adjuvant chemotherapy are urgently needed since the outcome for patients with MSI-H or MSS tumours is different. Before the results of such studies are available, MSI-H patients should not receive adjuvant treatment except for patients with other factor of poor prognosis such as clinical or pathological signs (occlusion, perforation, T4 tumour, blood or lymphatic vessels invasion).[33,36]

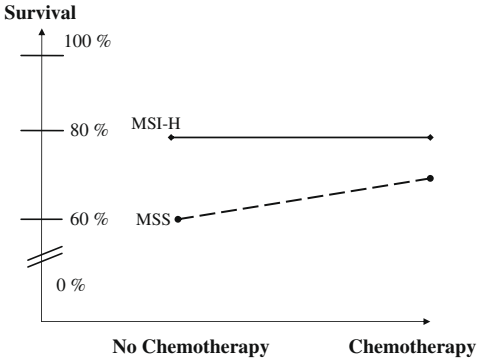

Fig. 4 – Diagram showing the interaction between treatment factors (adjuvant chemotherapy or no chemotherapy), prognostic factor (MSI status) and survival. MSI-H status is a positive prognostic factor (better survival among untreated MSI-H patients than among MSS patients) but not a predictive factor (similar survival among treated and untreated MSI-H patients but better survival among treated MSS patients compared with untreated).

Conflict of interest statement

None declared.

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