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Original Research

Microsatellite instability and sex differences in resectable gastric cancer – A pooled analysis of three European cohorts

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KEYWORDS
Microsatellite instability (MSI-high);

Abstract ***Objective:*** Biological sex differences in cancer are increasingly acknowledged. Here, we examined these differences in clinicopathological characteristics and survival in microsatellite instability (MSI)-high and microsatellite stable (MSS) gastric cancer (GC).

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Gastric carcinoma;
Sex differences;
Prognosis

Design: We analysed MSI status by polymerase chain reaction (PCR) and/or mismatch repair (MMR) status by immunohistochemistry in a pooled analysis of individual patient data from one retrospective cohort from Cologne, and the randomised phase III clinical trials D1/D2 and CRITICS. All patients had resectable adenocarcinoma of the stomach and/or gastro-oesophageal junction. Patients were treated with either surgery only or perioperative chemo(radio) therapy.

Results: MSI and/or MMR analyses on 1307 tumours resulted in 1192 (91.2%) MSS and/or MMR proficient (MMRP) [median age, 65 years; 759 males (63.7%); 619 treated with surgery only (51.9%)], and 115 (8.8%) MSI-high [median age, 69 years; 67 males (58.3%); 76 treated with surgery only (66.1%)] GC cases. Males had shorter overall survival (OS) than female MSI-high GC (5-year OS 34.7% vs. 69.7%; hazard ratio (HR) 2.68, 95%CI 1.60 to 4.49; $p < 0.001$). Females with MSI-high had longer OS than those with MSS/MMRP GC (HR 0.61, 95%CI 0.41 to 0.92; $p = 0.02$). Males with MSI-high did not have longer OS than those with MSS/MMRP GC (HR 1.26, 95%CI 0.94 to 1.69; $p = 0.12$).

Conclusions: MSI-high GC males had a significantly worse prognosis compared to their female counterparts in three independent cohorts. In addition, the favourable prognostic value of MSI was only seen in females and not in males. These observations emphasise the need to consider sex differences in prognosis and treatment effects in oncology.

Clinical trial registration: The CRITICS trial is registered at ClinicalTrials.gov, number NCT00407186; EudraCT, number 2006-004130-32; and CKTO, 2006-02.

1. Introduction

The importance of gender as modulators of health and disease is increasingly recognised in medicine [1] and oncology [2,3]. The concept of a sexual dimorphism in cancer was introduced in 2016 [4] and is supported by rapidly increasing evidence for differences in tumour biology between tumours arising in male and female patients. However, the clinical relevance and implications of these findings are less well understood [5–9].

In primary gastric cancer (GC), a significantly worse outcome for male patients has been observed in different series [10,11]. In contrast, younger women seem to have a worse prognosis than men with advanced stage GC [12]. These studies however did not specify the potential contribution of histological or molecular subtypes to these differences. According to The Cancer Genome Atlas (TCGA) Research Network, four molecular subtypes of GC have been distinguished [13], including the microsatellite instability high (MSI-high) subtype. The MSI-high subtype of GC has both a positive prognostic impact [14,15] and predicts the response to immunotherapy, which has been attributed to its high immunogenicity [16]. Previous data furthermore suggest a prognostic impact of biological sex in patients with MSI-high GCs, with men having a worse prognosis [14]. Interestingly, sex differences have been reported in the immune system [17]. The aim of the present study was to evaluate clinicopathological characteristics and prognostic impact of the patients' biological sex in patients with MSI-high and microsatellite stable (MSS) GC treated with either surgery alone or in combination with perioperative treatment.

2. Methods

2.1. Patients and tumour samples

Formalin-fixed and paraffin-embedded (FFPE) tumour material of a total of 1307 patients from three independent cohorts of resectable GC patients were analysed for its MSI status and/or mismatch repair (MMR) protein status. First, 396 patients treated with surgery alone or perioperative chemotherapy regimens in Cologne, Germany, between 1996 and 2017; second, 449 patients treated with surgery alone between 1989 and 1993 in the Dutch D1/D2 trial [18]; and third, 462 Dutch patients treated with perioperative chemo(radio) therapy between 2007 and 2015 in the CRITICS trial were included in this study [19]. For details see online Supplemental Fig. S1.

2.2. Ethical approval

This study was in accordance with the Declaration of Helsinki. Approval was obtained from the University of Cologne Ethics Committee. The D1/D2 trial was approved by the medical ethical committee of the Leiden University Medical Centre. In addition, the Dutch Code of Conduct for Responsible Use of Human Tissue allows

for the analysis of residual tissue specimens obtained for diagnostic purposes and anonymised publication of the study results (https://www.federa.org/sites/default/files/images/print_version_code_of_conduct_english.pdf). The CRITICS trial was approved by the medical ethical committee of the Netherlands Cancer Institute and the review boards of all participating centres. Patients provided written informed consent for participation into the clinical trial and separately for translational research on residual material.

2.3. MSI and MMR assessment

Tumour areas were marked by an experienced pathologist (AQ or NCTvG) on an H&E-stained slide in all patients. DNA was extracted from corresponding macro-dissected 10 μm thick FFPE tumour tissue sections. MSI status was determined by polymerase chain reaction (PCR). Tumours were considered MSI-high if two or more markers were instable, MSI-low if only one marker was instable, and MSS if none of the markers was instable. MSI-low and MSS tumours were grouped together for this analysis, as previously done in GC studies [20]. Four proteins (MLH1, PMS2, MSH2, and MSH6) were immunohistochemically stained to determine the MMR protein status. For the Cologne cohort, only tumour samples classified as MMR deficient (MMRD) were subjected to PCR to determine MSI status. Tumour samples from Cologne scored as MMR proficient (MMRP) were grouped together with MSS. MSI status of the tumour samples from the D1/D2 and CRITICS trials was published before [21]. A detailed description of the MSI analysis and MMR immunohistochemistry method for each of the three cohorts can be found in online supplementary materials.

2.4. Statistical analysis

Fisher's exact tests were used to correlate clinicopathological variables per biological sex. One-way ANOVA was used to correlate age with biological sex. Log-rank tests were used to compare Kaplan–Meier plots between sexes. Kaplan–Meier plots were truncated the year before <10% of patients per subgroup were at risk. Overall survival (OS), the primary end-point for this analysis, was defined as time from randomisation until death by any cause or time from surgery in case of patients from Cologne. A multivariable frailty model was used to calculate the hazard ratio (HR) and correct for unknown differences in the three cohorts. The three cohorts were put as frailty term in the model. In addition, the multivariable model included the patients' biological sex as variable of interest, as well as variables with $p < 0.10$ from univariate analyses. All analyses were conducted using the program language R (version 3.6.1) and its package survival.

3. Results

3.1. MSI and MMR prevalence

In the present study, MSI and/or MMR status were analysed of 1307 adenocarcinomas from the stomach and/or gastro-oesophageal junction. MSI-high status was observed in 115 of 1307 tumours (8.8%) in the total dataset. Per cohort, MSI-high was observed in 38 of 396 (9.6%, Cologne), 49 of 449 (10.5%, D1/D2 trial) and 28 of 462 (6.1%, CRITICS trial) tumours. All 115 MSI-high cases were confirmed with PCR.

In the Cologne cohort, all 38 MSI-high tumours showed protein loss of MLH1 and PMS2, since only MMRD tumours were analysed for MSI status by PCR in this cohort. In the D1/D2 cohort, 20 of 49 MSI-high tumours showed simultaneous protein loss of MLH1 and PMS2, whereas the 29 remaining cases had insufficient FFPE material left for MMR assessment. In the CRITICS cohort, 23 of 28 MSI-high tumours showed protein loss of at least one of the four MMR proteins, whereas three cases showed no protein loss, and two cases had insufficient FFPE material left for MMR assessment.

3.2. Clinicopathological characteristics of patients with MSI-high and MSS tumours

The clinicopathological characteristics of the 115 MSI-high and 1192 MSS GC cases are shown in Table 1. The median age of patients with MSI-high (69 years; interquartile range (IQR), 64–76 years) was higher than those with MSS GC (65 years; IQR, 56–71 years), regardless of sex ($p < 0.001$). The majority of both female and male patients with MSI-high tumours were treated with surgery only, which was due to the lower percentage of MSI-high in the CRITICS (6.1%) cohort, compared to the Cologne (9.6%) and D1/D2 (10.9%) cohorts. In MSS GC, female patients were more often treated with surgery only, whereas male patients more often underwent additional systemic treatment modalities, which was caused by the higher percentage of female patients in the surgery only D1/D2 (45.2%) cohort, compared to the Cologne (32.3%) and CRITICS cohorts (32.5%; $p = 0.001$). MSI-high tumours were located in the distal part of the stomach in the majority of female cases (66.7%), whereas most male MSI-high tumours were located in the distal (49.3%) or proximal (32.8%) part of the stomach ($p < 0.001$). Distally located tumours were also more often seen in female MSS GC (41.1%), whereas a proximally located tumour was more often seen in their male counterparts (34.3%; $p < 0.001$). The majority of both female and male patients with MSI-high had an intestinal tumour type (56.3% and 68.7%, respectively), whereas MSS GC female patients most often had a

Table 1
Clinicopathological characteristics of female and male patients with MSI-high and MSS tumours.

Characteristic	MSI-high			MSS		
	Female (n = 48)	Male (n = 67)	*P* value	Female (n = 433)	Male (n = 759)	*P* value
Age, median (IQR) [range], y	71 (66–77) [49–84]	69 (62–76) [46–84]	0.15	65 (54–72) [18–87]	64 (56–71) [21–88]	0.51
Treatment						
Surgery only	32 (66.7)	44 (65.7)	>0.99	252 (58.2)	367 (48.4)	0.001
Perioperative chemo(radio)therapy	16 (33.3)	23 (34.3)		181 (41.8)	392 (51.6)	
Tumour localisation						
GE-junction/proximal	2 (4.2)	22 (32.8)	<0.001[a]	85 (19.6)	260 (34.3)	<0.001[a]
Corpus/middle	11 (22.9)	8 (11.9)		141 (32.6)	209 (27.5)	
Distal	32 (66.7)	33 (49.3)		178 (41.1)	258 (34.0)	
>2/3 of stomach	2 (4.2)	4 (6.0)		27 (6.2)	30 (4.0)	
Missing	1 (2.1)	0 (0.0		2 (0.5)	2 (0.3)	
Lauren classification						
Diffuse	5 (10.4)	8 (11.9)	0.22[a]	208 (48.0)	254 (33.5)	<0.001[a]
Intestinal	27 (56.3)	46 (68.7)		130 (30.0)	331 (43.6)	
Mixed	4 (8.3)	3 (4.5)		40 (9.2)	75 (9.9)	
Other	8 (16.7)	4 (6.0)		35 (8.1)	56 (7.4)	
Missing	4 (8.3)	6 (9.0)		20 (4.6)	43 (5.7)	
Characteristic	MSI-high			MSS		
	Female (n = 48)	Male (n = 67)	P value	Female (n = 433)	Male (n = 759)	P value
(y)pT stage[b]						
pT0/Tis	0 (0.0)	0 (0.0)	0.13[a]	2 (0.5)	12 (1.6)	0.001[a]
pT1	7 (14.6)	7 (10.4)		81 (18.7)	107 (14.1)	
pT2	10 (20.8)	6 (9.0)		51 (11.8)	95 (12.5)	
pT3	23 (47.9)	30 (44.8)		140 (32.3)	319 (42.0)	
pT4	8 (16.7)	21 (31.3)		128 (29.6)	177 (23.3)	
Missing	0 (0.0)	3 (4.5)		31 (7.2)	49 (6.5)	
(y)pN stage[b]						
pN0	30 (62.5)	31 (46.3)	0.06[a]	161 (37.2)	273 (36.0)	0.68[a]
pN1	12 (25.0)	11 (16.4)		66 (15.2)	126 (16.6)	
pN2	4 (8.3)	12 (17.9)		72 (16.6)	143 (18.8)	
pN3	2 (4.2)	10 (14.9)		105 (24.2)	171 (22.5)	
Missing	0 (0.0)	4 (6.0)		29 (6.7)	46 (6.1)	
TNM (7th edition)[b]						
Stage 0 (pCR)	0 (0.0)	0 (0.0)	0.08[a]	2 (0.5)	12 (1.6)	0.27[a]
Stage I	14 (29.2)	12 (17.9)		97 (22.4)	148 (19.5)	
Stage II	23 (47.9)	25 (37.3)		115 (26.6)	218 (28.7)	
Stage III	10 (20.8)	23 (34.3)		155 (35.8)	281 (37.0)	
Stage IV	0 (0.0)	4 (6.0)		27 (6.2)	39 (5.1)	
Missing	1 (2.1)	3 (4.5)		37 (8.5)	61 (8.0)	

MSI-high, microsatellite instability; MSS, microsatellite stable; IQR, interquartile range; pCR, pathological complete response.
[a] Excluding those with missing data.
[b] yp denotes T, N, and TNM stage after neoadjuvant chemotherapy and surgery.

diffuse (48.0%) and male an intestinal tumour type (43.6%; $p < 0.001$). In MSI-high GC, there was no difference in T stage between male and female patients. In MSS GC, T4 tumours occurred more often in female than male patients ($p < 0.01$), whereas this was the other way around for T3 tumours ($p < 0.001$). In MSI-high GC, the majority of female patients (62.5%) had lymph node-negative tumours, which was in contrast with male patients who had lymph node-positive tumours in the majority of cases (33/64, 51.6%; $p = 0.18$). Male patients had a more advanced stage of disease (stage III or IV) than female patients in MSI-high ($p = 0.03$), whereas there was no sex difference in stage in MSS GC.

3.3. Survival

Overall, male patients with MSI-high tumours had a significantly shorter 5-year OS of 34.7% (95%CI 24.6%–49.2%) compared to 69.7% (95%CI 57.6%–84.4%) for their female counterparts (HR 2.68, 95%CI 1.60 to 4.49; $p < 0.001$; Fig. 1), both in patients treated with surgery only and when treated perioperatively. In the combined surgery only cohort, 5-year OS in males was 36.0% (95%

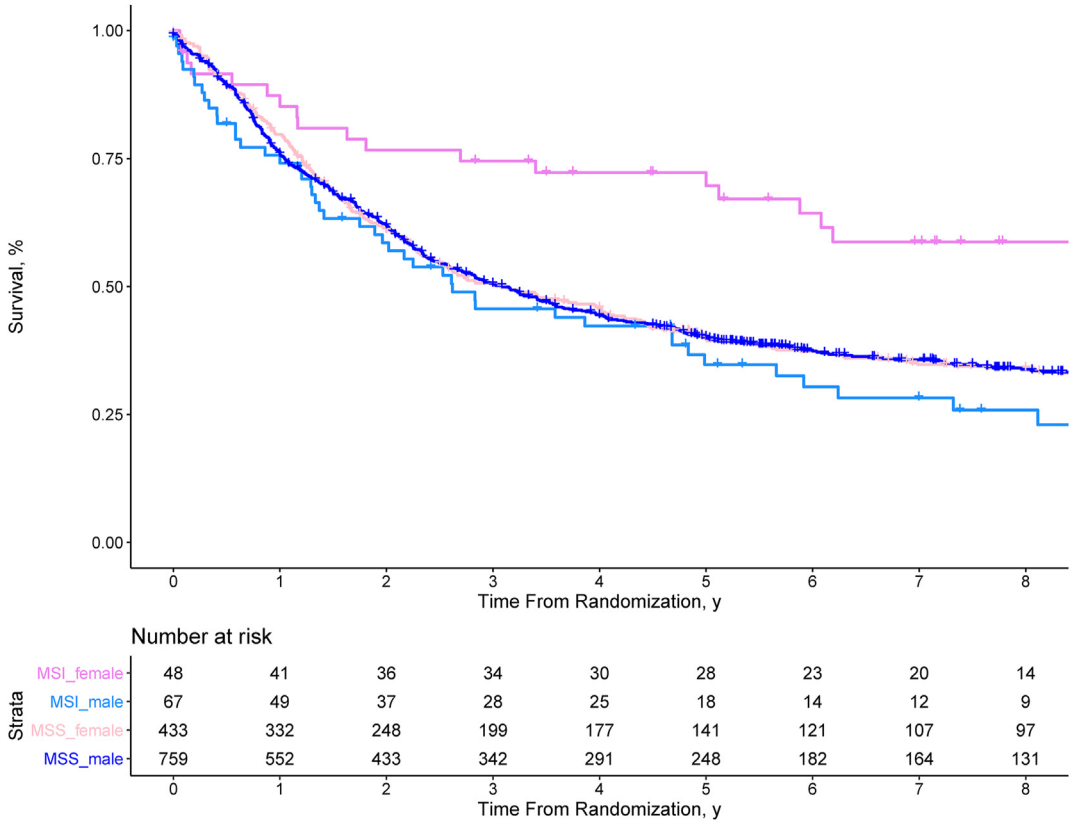

Fig. 1. **Overall survival by sex and MSI status in the 1307 study patients**. MSI, microsatellite instability; MSS, microsatellite stable. Patients were stratified by MSI status and by sex. The hazard ratio was 2.68 (95% CI 1.60 to 4.49; $p < 0.001$) for male vs. female MSI-high, and 1.04 (95% CI 0.90 to 1.20; $p = 0.64$) for male vs. female MSS GC.

CI 23.7%–54.8%) compared to 67.3% (95%CI 52.5%–86.3%) for females (HR 2.53, 95%CI 1.38 to 4.62; $p = 0.003$; Fig. 2). In the perioperatively treated cohort, 5-year OS in males was 32.0% (95%CI 17.1%–59.8%) compared to 75.0% (95%CI 56.5%–99.5%) for females (HR 2.96, 95%CI 1.08 to 8.14; $p = 0.04$; Fig. 3). Overall, female patients with MSI-high had longer OS than those with MSS/MMRP GC (69.7% (95%CI 57.6%–84.4%) and 39.7% (95%CI 35.1%–44.8%), respectively; HR 0.61, 95%CI 0.41 to 0.92; $p = 0.02$). In contrast, male patients with MSI-high did not have longer survival than those with MSS/MMRP GC (34.7% (95%CI 24.6%–49.2%) and 40.1% (95%CI 36.6%–44.0%), respectively; HR 1.26, 95%CI 0.94 to 1.69; $p = 0.12$; Fig. 1). There was no difference in survival between male and female patients with MSS (HR 1.04; 95%CI 0.90 to 1.20; $p = 0.64$), regardless of treatment modality.

The survival curves for male and female patients in the three independent cohorts separately are shown in online Supplemental Figs. S2–4.

3.4. Multivariate analysis of MSI-high tumours

Based on univariate analysis in patients with MSI-high tumours, the frailty model included the variables sex, tumour localisation and pathological N and TNM stage in addition to the three cohorts as frailty. It resulted in a HR of 2.30 (95%CI 1.31 to 4.04, $p = 0.004$) for male compared to female patients with MSI-high GC. Significant variables in this model were sex and TNM stage. In stage I MSI-high GC, 12 males had a 5-year OS of 54.5% (95%CI 31.8%–93.6%) compared to 85.7% (95% CI 69.2%–100.0%) for 14 females (HR 1.81; 95%CI 0.60 to 5.42; $p = 0.29$). In stage II MSI-high GC, 25 males had a 5-year OS of 47.3% (95%CI 30.4%–73.7%) compared to 82.6% (95%CI 68.5%–99.6%) for 23 females (HR 4.44; 95%CI 1.83 to 10.77; $p < 0.001$). In stage III MSI-high GC, 23 males had a 5-year OS of 22.5% (95%CI 10.0%–50.8%) compared to 22.5% (95% CI 6.7%–76.1%) for 10 females (HR 0.73; 95%CI 0.31 to 1.69; $p = 0.46$).

4. Discussion

Differences in tumour characteristics and outcomes of gastro-oesophageal cancers arising in male and female patients are increasingly recognised. In GC, tumours arising in men are more often located proximally, while tumours arising in female patients are typically located in the distal stomach including the gastric corpus. M. Blaser *et al.* have coined the term 'corpus-dominant,

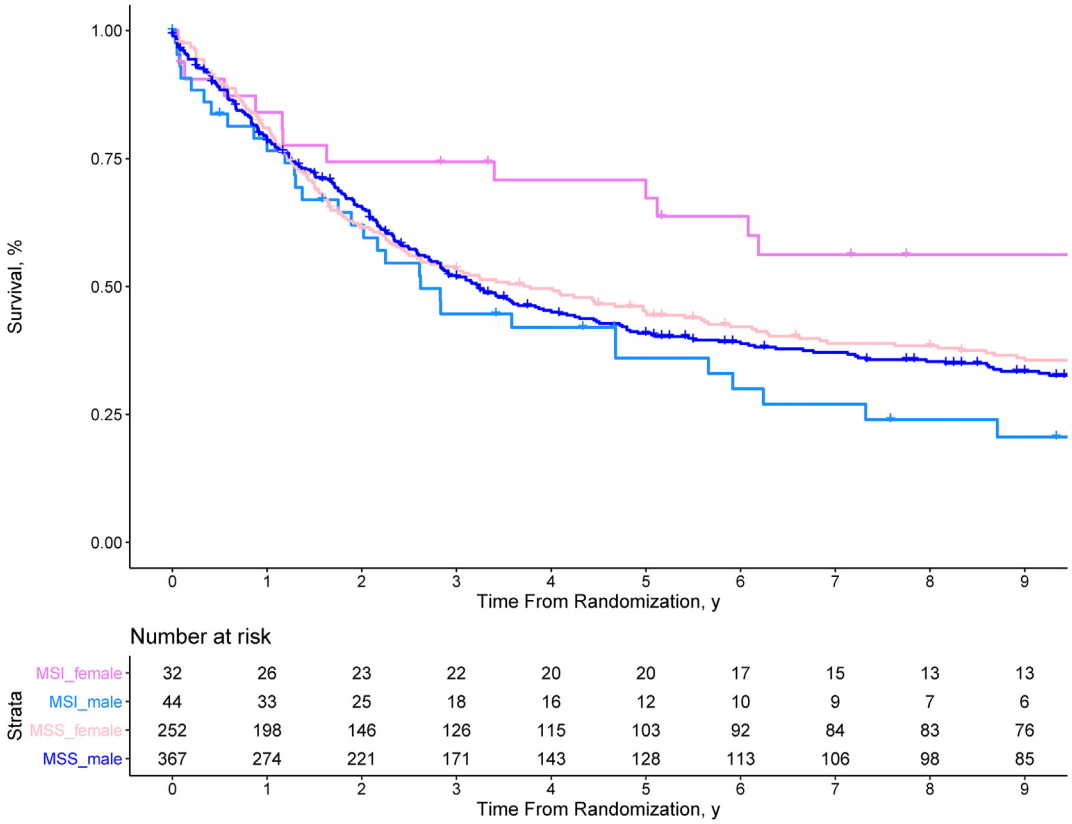

Fig. 2. **Overall survival by sex and MSI status in the 695 study patients treated with surgery only**. MSI, microsatellite instability; MSS, microsatellite stable. Patients were stratified by MSI status and by sex. The hazard ratio was 2.53 (95% CI 1.38 to 4.62; p = 0.003) for male vs. female MSI-high, and 1.09 (95% CI 0.90 to 1.32; p = 0.38) for male vs. female MSS GC.

young age-dominant, female-dominant' (CYF). There is growing evidence that the diffuse subtype of GC is increasingly more prevalent in females [22–25].

As much as location and histology, molecular subtypes of gastro-oesophageal cancers are distributed between the sexes according to a characteristic pattern: according to TCGA, and in line with other series, female patients present more often with MSI-high GC, and less frequently with EBV-associated or chromosomal instable (CIN) GC [25,26]. Although gender differences in the exposure to risk factors, such as the consumption of tobacco and alcohol, exist, behavioural risk factors do not explain the higher incidence of oesophageal adenocarcinoma in men [27], nor the higher frequency of diffuse type and signet cell cancers especially in young women [12,28]. This has been observed in different populations and therefore must reflect sex differences in cancer susceptibility. MSI-high GCs have a high immunogenicity and sex differences in the immune system are well known [17,29,30].

Sex influences the functioning of both the innate and adaptive immune systems, which has implications for the likelihood of the expression of autoimmune diseases and malignant tumours. Sex-related differences in polymorph neutrophil functioning have been described, with diverse implications for immune metabolism [31]. The antigen load is higher in solid tumours in males (e.g. NSCLC) than in females; nevertheless, the inflammatory microenvironment in males is often described as 'cold'.

In this context, our study, for the first time, analysed systematically clinicopathological characteristics and survival of male and female patients with MSI-high GC treated by surgical resection with or without perioperative treatment in multiple independent cohorts. We observed that female patients with MSI-high GC have superior survival compared to their male counterparts. In addition, we showed that MSI-high is only a prognostic factor in females but not in males with resectable GC. Both observations in the Cologne dataset were confirmed in two independent cohorts, the Dutch D1/D2 trial and the CRITICS trial and are thus highly suggestive of a difference in tumour biology between MSI-high GC arising in female and male patients, irrespective of treatment modality.

To the best of our knowledge, our study is the largest to date systematically addressing the question of sex differences in MSI-high GC. Both the relatively large number of 115 patients with MSI-high GC and the confirmation of our findings in different independent cohorts are strengths of this analysis. The result of our multivariate analysis confirms that the prognostic effect of sex occurs independently of stage. It becomes clear that in UICC

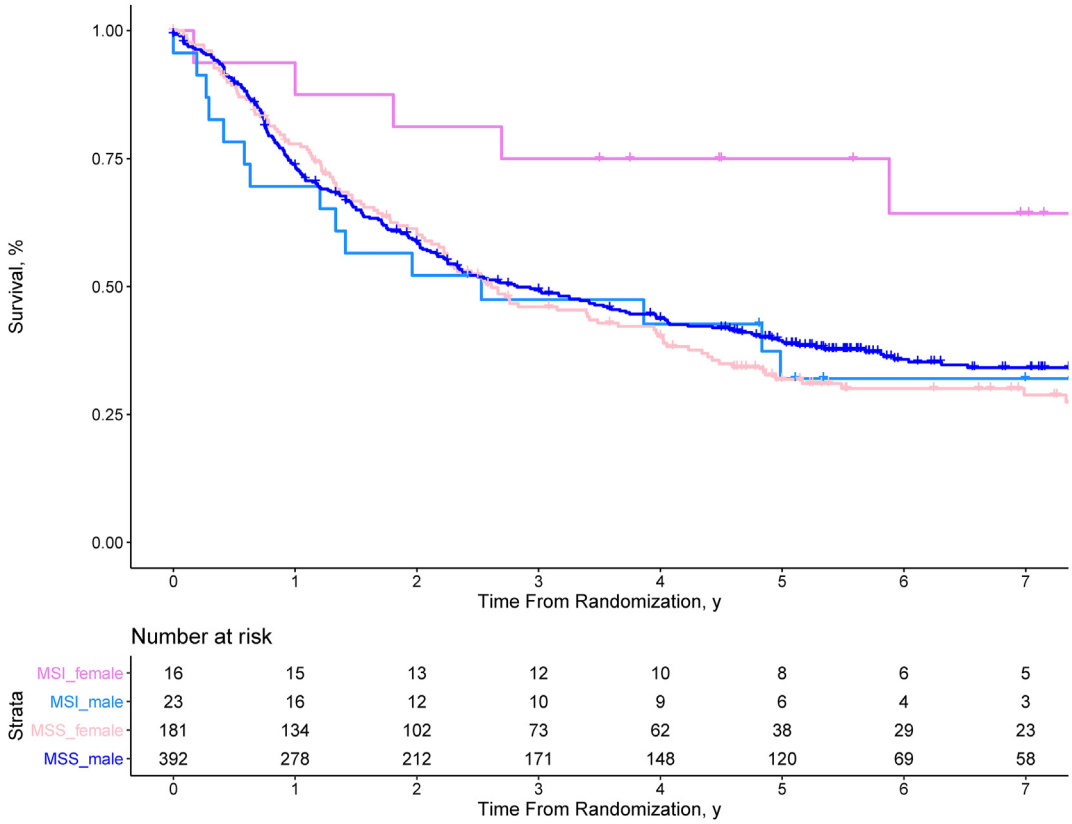

Fig. 3. **Overall survival by sex and MSI status in the 612 study patients treated with perioperative chemotherapy and surgery**. MSI, microsatellite instability; MSS, microsatellite stable. Patients were stratified by MSI status and by sex. The hazard ratio was 2.96 (95% CI 1.08 to 8.14; p = 0.04) for male vs. female MSI-high, and 0.92 (95% CI 0.74 to 1.15; p = 0.48) for male vs. female MSS GC.

stages I and II, women with MSI-high tumour have a significant survival advantage compared to men with MSI-high tumours of the same stage. Before discussing any potential therapeutic implications of this observation, the first step is to consider a potential impact of the patients' sex not only in the overall group of patients with gastro-oesophageal cancer in any trial but as well in subgroups, like those with MSI-high GC, and analyse results in treatment and control groups according to sex. Only after such results are available with sufficient robustness, their therapeutic implications can be discussed. Importantly, male and female patients might react differently to different types of treatments. While prior studies in MSI-high GC noted in fact a detrimental effect of perioperative chemotherapy with epirubicin, cisplatin and fluorouracil (ECF), results were not analysed by sex, and patient numbers were too small to draw any meaningful conclusions regarding sex differences in treatment effects (data from only 19 patients with MSI-high-tumours were included in this report, of which nine patients were treated with perioperative chemotherapy) [20]. However, this detrimental effect of perioperative chemo therapy on survival as well the lack of complete and near complete response in the MSI-high tumours of the MAGIC trial could not be confirmed in other studies [32,33]. Moreover, the recently presented DANTE trial evaluating FLOT with or without atezolizumab as perioperative treatment in GC observed a rate of pathologic complete and near complete responses of 62% among 13 patients with MSI-high tumours treated with FLOT, and 80% among 10 patients with MSI-high tumours treated with FLOT plus atezolizumab [34]. Furthermore, the phase II NEONIPIGA trial in MSI-high GC reported a rate of complete and subtotal regression of 73% in 29 patients after treatment with the combination of ipilimumab and nivolumab [35]. While these results are highly suggestive of a major treatment benefit of these patients from this combination, response rates were not reported according to sex and the magnitude of the benefit of male and female patients from this approach, which might in fact not be the same, is therefore unclear. Given the durable responses in other diseases seen with immunotherapy, the observed high response rates in the DANTE and NEONIPIGA trials furthermore raise the question whether surgery is still necessary in patients with complete response after neoadjuvant treatment. Final publications of this trial and other trials, including a longer follow-up and analyses according to sex, are required to define new treatment standards and decide whether a patients' sex should be considered in future treatment decisions.

Whether the observed differences in prognosis are attributable to tumour-related factors, such as differences

in tumour mutational burden or T-cell infiltration, or systemic factors, such as the sexual dimorphism in the immune system, is currently unclear. It is interesting that the difference in prognosis in our study is particularly evident in the group of lympho-nodal-negative patients although the small proportion of patients in this subgroup must be put into perspective. It is possible that immunological mechanisms in women with MSI-high tumours are able to process the high antigen load of MSI-high tumours in such a way that (lympho-nodal) metastases can be avoided in the long term. In case of metastatic GC, benefit from immune checkpoint inhibitors seems to be higher in male compared to female patients, albeit not statistically significant in a meta-analysis of five clinical trials. In this meta-analysis, MSI-high was the only statistically significant factor for benefit from immune checkpoint inhibitors, but the association between MSI status and sex was not investigated [36].

Recently, sex differences and immunotherapy have been further investigated in GC. Female GC patients with an *ATRX* mutation were associated with MSI-high and favourable clinical benefit to immune checkpoint inhibitors, whereas no association between this mutation and survival was seen in male patients [37].

Male MSI-high GC patients also have a higher mutation prevalence than their female counterparts [38]. Our data add a further important argument for a sexual dimorphism in GC, which – in an age of precision medicine – cannot be longer ignored.

In addition, and most importantly, these differences need consideration in the evaluation of treatment effects: according to the conclusions of the ESMO workshop 'Gender medicine and oncology', especially in diseases or disease subgroups with significant differences in epidemiology or outcomes, men and women with non-sex-related cancers should be considered as biologically distinct groups of patients, for whom specific treatment approaches merit consideration [3]. MSI-high GC is an example of a sexually dimorph disease subgroup.

In summary, we here provide evidence for a significantly longer overall survival in females with MSI-high GC compared to males. In addition, we showed that MSI is only a prognostic factor for female patients. This should be taken into account in upcoming trials of immunotherapy in resectable (MSI-high) gastric cancer. Also, translational studies are warranted to further investigate the biology underlying these sex differences.

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CRediT author statement

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Conflict of interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

MIvBH has served in a consultant or advisory role for Medtronic, Mylan, Alesi Surgical, Johnson and Johnson, BBraun, and received funding for research from Olympus, Stryker, all fees paid to the institution and unrelated to the present study. AC received grants form Dutch Cancer Society, Dutch Colorectal Cancer Group, Hofmann-La Roche, all fees paid to the institute and unrelated to the present study. HWMvL has served in a consultant or advisory role for BMS, Daiichy, Lilly, MSD, Nordic Pharma, Novartis, Servier, and received funding for research or study medication from Bayer, BMS, Celgene, Janssen, Lilly, Merck, Nordic Pharma, Philips, Roche, Servier, and received research funding from the Dutch Cancer Society, Dutch Research Council, European Research Council, MaagLeverDarm Stichting, all fees paid to the institution and unrelated to the present study. NCTvG has served on the advisory boards of BMS and MSD. Topics were unrelated to the present study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2022.06.025.

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