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Cancer Investigation

ISSN: 0735-7907 (Print) 1532-4192 (Online) Journal homepage: http://www.tandfonline.com/loi/icnv20

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To cite this article: Karol Polom, Daniele Marrelli, Giandomenico Roviello, Costantino Voglino, Carla Vindigni, Daniele Generali & Franco Roviello (2017): Single Center Experience on Anatomy-and Histopathology-Based Gastric Cancer Molecular Classification, Cancer Investigation, DOI: 10.1080/07357907.2017.1292519

To link to this article: http://dx.doi.org/10.1080/07357907.2017.1292519

CANCER INVESTIGATION
http://dx.doi.org/10.1080/07357907.2017.1292519

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Single Center Experience on Anatomy-and Histopathology-Based Gastric Cancer Molecular Classification

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ABSTRACT

We analyzed the clinical utility of molecular classification based on anatomical and histological background. The study was conducted on 457 patients treated for gastric cancer with additional information about microsatellite instability status. We divided the patients in three groups of molecular classification based on anatomical and histological background: proximal non-diffused, diffused, and distal non-diffused groups. These groups varied in terms of clinical and pathological factors as well as survival rates. The molecular classification based on anatomical and histological data seems to be a useful tool in a simple classification of gastric cancer.

ARTICLE HISTORY
Received 11 September 2016
Accepted 25 January 2017

KEYWORDS
Mismatch repair; personalized medicine; microsatellite instability

Introduction

In recent years, there has been an observable decline in the number of new cases of gastric cancer (GC), yet it remains the third most common cancer-related cause of death in the world, following lung and liver cancer types (1).

Currently, the treatment of gastric cancer depends primarily on the stage of the disease. However, the method of treatment is also related to geography: differences in treatment options are observed, especially between eastern and western countries (2). Moreover, there is no consensus regarding any standard (i.e., universally applicable therapy), which is partially caused by the fact that doctors rely on clinical and pathological information. In addition, there has been an observable improvement in diagnosis, development of new tools used in surgical procedures, and other fields of oncology as well as treatments that help in proposing more tailored therapies (2–9).

Over the past few years many new GC classifications based on different genetic and molecular information have been devised, which are based on anatomical site, histopathology, gene expression, gene amplification, DNA methylation, numerous cancer-relevant aberrations, or oncogenic pathways (8–16). In 2011, Shah et al. proposed a simple division of GC based on tumor position and histopathology, supported by a distinct gene expression profile, which showed more than 85% of accuracy (11). They divided GC into three groups: type-1 proximal non-diffused GC; type-2 diffused GC located anywhere in the stomach and presenting only a diffused pattern of infiltration; and type-3 distal non-diffused GC with tumor located in the middle or distal third part of the stomach with dominant pattern of intestinal histotype (11). Most recently, two molecular GC classifications, independent of each other, have been proposed (8, 9).

One of the new subgroups in molecular GC is microsatellite instability (MSI). Our previous study has already suggested that this division offers a better prognosis, and is associated mostly with females, older age, lower rate of metastatic lymph nodes, and non-cardia position with intestinal histotype (17). In our previous analysis of MSI GC patients we found that the MSI group is not homogenous. Based on clinical information, we found that the difference between

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microsatellite stability (MSS) and MSI in 5-year survival rate is observed in non-cardia intestinal cancer only ($p < .001$) (17). For MSI and MSS, no significant difference in 5-year survival rate was seen in intestinal histotype and cardia location ($p = .899$) and in the diffused-mixed type ($p = .748$) (17).

The primary aim of this study was to compare clinical and pathological information regarding the survival rates of GC patients based on the molecular classification proposed by Shah et al. (11). The secondary aim was to analyze the applicability of this classification in MSI patients.

Material and methods

The analysis was conducted using central database information of GC patients who were treated and followed up in our center. We selected 472 patients with analyzed MSI status whose frozen tissue was stored in our biobank. We excluded 15 patients whom we were not able to classify into the three proposed groups as explained below. Next, we analyzed clinical, pathological, and follow-up survival information.

Details regarding this analysis have been presented in our previous paper (17).

Pentaplex polymerase chain reaction (PCR) and microsatellite analysis

Briefly, we used five quasi-monomorphic mononucleotide repeats for the analysis: BAT-26, BAT-25, NR-24, NR-21, and NR-27. According to the definition of the National Cancer Institute workshop on MSI for cancer, with the additional consensus of 2002 (18), we categorized a tumor as having MSI when two or more markers revealed instability at five loci.

Statistical analysis

Statistical analysis was conducted using commercially available statistical software (SPSS 20.0 for Windows SPSS Inc. Chicago, IL, USA). Statistical association between clinico-pathological characteristics and MSI status was assessed by χ^2 test or Fisher's exact test for categorical variables. The Mann–Whitney U test and the Kruskal–Wallis one-way analysis of variance (ANOVA) were used to compare continuous variables not normally distributed. Cumulative survival was calculated by the Kaplan–Meier life table method, and the log-rank test was used to distinguish significant differences. Survival curves were estimated using the Kaplan–Meier method and were compared using a log-rank test, considering death for cancer as the endpoint (cancer-related survival). A statistical level of $p < .05$ was used for inclusion of prognostic variables.

In addition, we divided the patients into the following three subgroups as proposed by Shah et al. (11):

- Type 1: Proximally located, non-diffused GC with the major part of the tumor (>80%) located in the upper third of the stomach (cardiac area), and presenting non-diffused histopathology according to the Lauren classification (intestinal and mixed).
- Type 2: Diffused GC, located anywhere in the stomach, presenting entirely diffused pattern of infiltration.
- Type 3: Distal non-diffused GC, with the major part of the tumor located in the distal or middle part of the stomach and presenting non-diffused histopathology according to the Lauren classification (intestinal and mixed), with or without components of poorly differentiated carcinoma.

Results

The results of clinical and pathological data of the three subgroups are presented in Table 1. By arranging the data according to this classification, we found that the three subgroups are different in terms of factors such as age, sex, T (tumor) and N (lymph node) status, stage of the disease, WHO histological type, and adjuvant treatments. First, concerning age, the oldest patients tended to be type 3 patients whereas the youngest were type 2 patients. Second, concerning the sex of patients, there were more females as type 2 patients and more males as types 1 and 3 patients. Next, T and N status showed statistical significance with more T3 in types 1 and 3 patients, and more T4 in type 2 patients. Moreover, N0 was observed in 36.6% of type 3 patients, and only 12.1% in type 2 patients. Another statistically significant factor was the stage of the disease, with more stage 1 and 2 cases as types 1 and 3 patients, and less cases of stage 3 and 4 as type 2 patients. The next factor was the WHO histological type: signet ring cell and mucinous were more common in type 2 patients whereas tubular and poorly differentiated as types 1 and 3 patients. Finally, adjuvant treatment was also statistically different according to different types. We found that R (remnant tumor) and M (metastasis) status was statistically insignificant.

Table 1. Clinical and pathological differences according to three types of molecular-based anatomical and pathological divisions.

	Proximal intestinal-mixed		Diffused		Distal-medium intestinal-mixed	*p*
Patient (*n*)	74		107		276	
Age, years (median)	69		63		72	<.001
Sex (male, female)	57:17 (77:23)		53:54 (49.5:50.5)		160:116 (58:42)	.001
pT						<.001
1	4	5.4%	5	4.7%	33 12%	
2	13	17.6%	9	8.4%	52 18.8%	
3	22	29.7%	16	15%	60 21.7%	
4	35	47.3%	77	72%	131 47.5%	
pN						<.001
0	18	24.3%	13	12.1%	101 36.6%	
1	15	20.3%	15	14%	44 15.9%	
2	19	25.7%	23	21.5%	58 21%	
3a	12	16.2%	30	28%	28 10.1%	
3b	10	13.5%	26	24.3%	45 16.3%	
UICC-R						.830
R0	55	74.3%	77	72%	207 75%	
R+	19	25.7%	30	28%	69 25%	
M						.054
M0	58	78.4%	81	75.7%	236 85.5%	
M1	16	21.6%	26	24.3%	40 14.5%	
Stage						<.001
I	9	12.2%	6	5.6%	59 21.4%	
II	22	29.7%	15	14%	74 26.8%	
III	27	36.5%	60	56.1%	103 37.3%	
IV	16	21.6%	26	24.3%	40 14.5%	
WHO histological type[a]						<.001
Papillary	5	6.8%	0	0%	10 3.6%	
Poorly differentiated	32	43.2%	13	12.1%	100 36.2%	
Signet ring cell & mucinous	3	4.1%	93	86.9%	39 14.1%	
Tubular (well/mod. diff.)	34	45.9%	1	0.9%	121 43.8%	
Adjuvant						<.001
No	33	44.6%	26	24.3%	160 58%	
Yes	41	55.4%	81	75.7%	116 42%	

[a]Six cases with unclassified WHO histotype were excluded.

The second analysis was based on the same division into three types but with additional information on MSI status. The patients were divided into MSI and MSS groups. The results for MSI patients are presented in Table 2, and for MSS patients in Table 3. For the MSI group we found that the following factors were statistically significant: N, stage of the disease, WHO histological type, and the applied adjuvant treatment. No differences were observed in sex and T, R, and M status. For MSS patients the difference was observed in sex (more females were seen in type 2 group, and more males in type 1 and 3 groups. Other statistically significant differences were observed in the T and N status, stage of the disease, WHO classification, and adjuvant therapy.

Next, we analyzed cancer-free survival. Figure 1 shows differences in survival rates between all three types of patients ($p = .135$). The 5-year survival rate for type 1 was 31.4%, for type 2 37.6%, and for type 3 it was 49%.

Finally, we analyzed MSS and MSI subgroups for all three types of gastric cancer (Figure 2). We observed a strong statistical correlation in terms of 5-year survival rate according to this division ($p < .001$).

Statistical significance was only observed in type 3 group ($p < .001$). The 5-year survival rate for the type 3 MSI was 76.7%, and for MSS, it was 35.7%. Type 1 and 2 groups did not show statistical difference but, interestingly, the 5-year survival rate in type 1 group for MSI was 33.3% and 31.3% for MSS. The 5-year survival rate in type 2 for MSI was 38.1% and 37.8% for MSS. For better understanding of the above-mentioned results, we analyzed the survival data for stage I and II together versus the survival data for stage III and IV (Figures 3A–C). Statistical significance was found for type 3 for both stages I and II and stages III and IV. No statistically significant differences were found for type 1 and 2 groups no matter the stage of the disease.

Discussion

Gastric cancer is not a homogenous disease, hence for many years different new general classifications, as well as those facilitating differentiation between various subgroups, have been proposed (8–16). A division proposed by Shah et al. is based on two important clinical and pathological factors: tumor position and histotype

Table 2. Clinical and pathological analysis of MSI gastric cancer according to three types of molecular-based anatomical and pathological divisions.

	Proximal intestinal-mixed MSI		Diffused MSI		Distal-medium intestinal-mixed MSI		*p*
Patient (*n*)	3		18		87		
Age, years (median)	72		72		76		.275
Sex (male, female)	0:3 (0:100)		10:8 (55.6:44.4)		33:54 (37.9:62.1)		.137
pT							.073
1	0	0%	0	0%	5	5.7%	
2	0	0%	2	11.1%	19	21.8%	
3	1	33.3%	3	16.7%	35	40.2%	
4	2	66.7%	13	72.2%	28	32.2%	
pN							<.001
0	0	0%	1	5.6%	46	52.9%	
1	1	33.3%	1	5.6%	16	18.4%	
2	2	66.7%	5	27.8%	16	18.4%	
3a	0	0%	8	44.4%	2	2.3%	
3b	0	0%	3	16.7%	7	8%	
UICC-R							.487
R0	2	66.7%	13	72.2%	72	82.8%	
R+	1	33.3%	5	27.8%	15	17.2%	
M							.342
M0	3	100%	15	83.3%	81	93.1%	
M1	0	0%	3	16.7%	6	6.9%	
Stage							<.001
I	0	0%	0	0%	18	20.7%	
II	0	0%	2	11.1%	41	47.1%	
III	3	100%	13	72.2%	22	25.3%	
IV	0	0%	3	16.7%	6	6.9%	
WHO histological type[a]							<.001
Papillary	0	0%	0	0%	1	1.1%	
Poorly differentiated	1	33.3%	3	16.7%	40	46%	
Signet ring cell & mucinous	0	0%	14	77.8%	8	9.2%	
Tubular (well/mod. diff.)	2	66.7%	1	5.6%	35	40.2%	
Adjuvant treatment							.011
No	1	33.3%	9	50%	69	79.3%	
Yes	2	66.7%	9	50%	18	20.7%	

[a]Three cases with unclassified WHO histotype were excluded.

(11). It is well known that intestinal and diffused histotypes differ according to lymph node involvement, type of recurrence, and distal metastases (2, 19, 20). Some authors also consider the clinical outcome where diffused histotype is associated with much worse prognosis compared with intestinal histopathology (2, 19, 20).

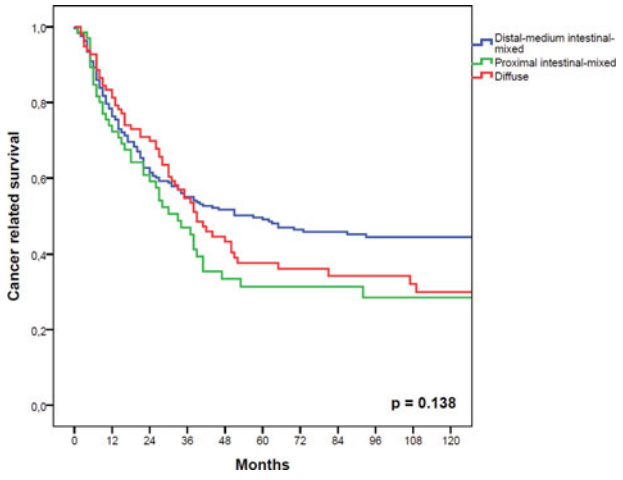

Figure 1. Cancer-related survival of GC patients according to anatomy- and histopathology-based GC molecular classification.

A study by Chen et al. investigated clinical and pathological factors associated with all three Lauren histotypes (21). Clinically, they observed statistically significant differences in overall survival and disease-free survival (both $p < .001$) (21). The survival curves

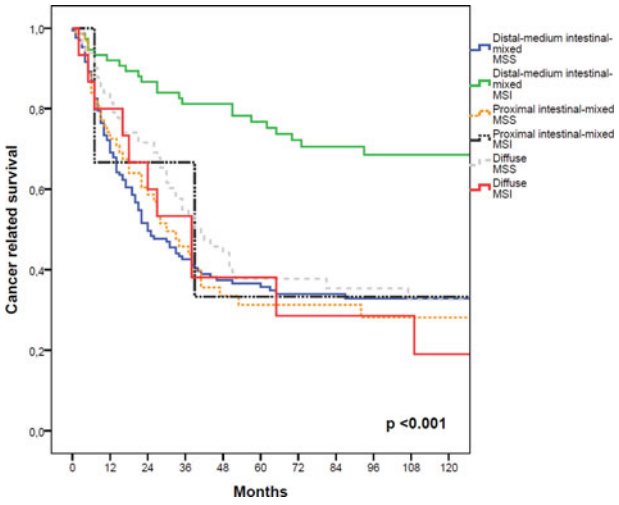

Figure 2. Cancer-related survival of GC patients according to anatomy- and histopathology-based GC molecular classification with MSI and MSS divisions.

Table 3. Clinical and pathological analysis of MSS gastric cancer according to three types of molecular-based anatomical and pathological divisions.

	Proximal intestinal-mixed MSS		Diffused MSS		Distal-medium intestinal-mixed MSS		*p*
Patient (*n*)	71		89		189		
Age, years (median)	69		61		70		<.001
Sex (male, female)	57:14 (80.3:19.7)		43:46 (48.3:51.7)		127:62 (67.2:32.8)		<.001
pT							<.001
1	4	5.6%	5	5.6%	28	14.8%	
2	13	18.3%	7	7.9%	33	17.5%	
3	21	29.6%	13	14.6%	25	13.2%	
4	33	46.5%	64	71.9%	103	54.5%	
pN							.08
0	18	25.4%	12	13.5%	55	29.1%	
1	14	19.7%	14	15.7%	28	14.8%	
2	17	23.9%	18	20.2%	42	22.2%	
3a	12	16.9%	22	24.7%	26	13.8%	
3b	10	14.1%	23	25.8%	38	20.1%	
UICC-R							.873
R0	53	74.6%	64	71.9%	135	71.4%	
R+	18	25.4%	25	28.1%	54	28.6%	
M							.302
M0	55	77.5%	66	74.2%	155	82%	
M1	16	22.5%	23	25.8%	34	18%	
Stage							.002
I	9	12.7%	6	6.7%	41	21.7%	
II	22	31%	13	14.6%	33	17.5%	
III	24	33.8%	47	52.8%	81	42.9%	
IV	16	22.5%	23	25.8%	34	18%	
WHO histological type[a]							<.001
Papillary	5	7%	0	0%	9	4.8%	
Poorly differentiated	31	43.7%	10	11.2%	60	31.7%	
Signet ring cell & mucinous	3	4.2%	79	88.8%	31	16.4%	
Tubular (well/mod. diff.)	32	45.1%	0	0%	86	45.5%	
Adjuvant treatment							<.001
No	32	45.1%	17	19.1%	91	48.1%	
Yes	39	54.9%	72	80.9%	98	51.9%	

[a]Three cases with unclassified WHO histotype were excluded.

of patients with diffused and mixed histotypes were overlapping. Zheng et al. analyzed patients with mixed histotype who had even worse survival rates but without statistical significance ($p > .05$) (22).

The new division proposed by Shah et al., which was based on clinical and pathological factors selected as a result of molecular analysis, seems to be simple and clinically useful (11). They proposed to combine mixed histotype with intestinal histotype in one group versus diffused histotype in the other group. From the clinical point of view, in many studies, mixed histotype is analyzed with diffused histotype rather than with intestinal histotype (2, 19, 23).

Molecular study of methylation status in different GC histotypes revealed a statistically significant result that Lauren mixed group had more methylated genes compared with diffused or intestinal groups (24). Even excluding MSI and Epstein-Barr virus (EBV) patients from the analysis did not affect the results. The authors concluded that mixed histotype showed different CpG island hypermethylation status, and that this process might be involved in the histogenesis of this histotype.

The effectiveness of categorizing intestinal/mixed and diffused histotypes as two separate groups was also confirmed in other molecular studies based on loss of hetrozygosity (LOH) (25, 26). The authors divided GC into two subtypes – high level LOH, which correlates with intestinal or mixed histotype, and low level LOH, which is related to diffused histotype.

In our study, we did not find any statistical difference in survival rates between all three types. Interestingly, there was a difference between groups 1 and 3 representing the same intestinal/mixed histotype. The observation that intestinal and mixed histotypes are not homogenous according to the tumor position seems to be verified in molecular background. A more general analysis of the molecular key is required for understanding this phenomenon.

The location of cardia tumor seems to show different characteristics in comparison with non-cardia tumors. Over the past few decades there has been an increase in the incidence frequency of cardia tumor, which calls for further research into this type of malignancy (2, 23). It has been shown in a recent study by the Italian Research

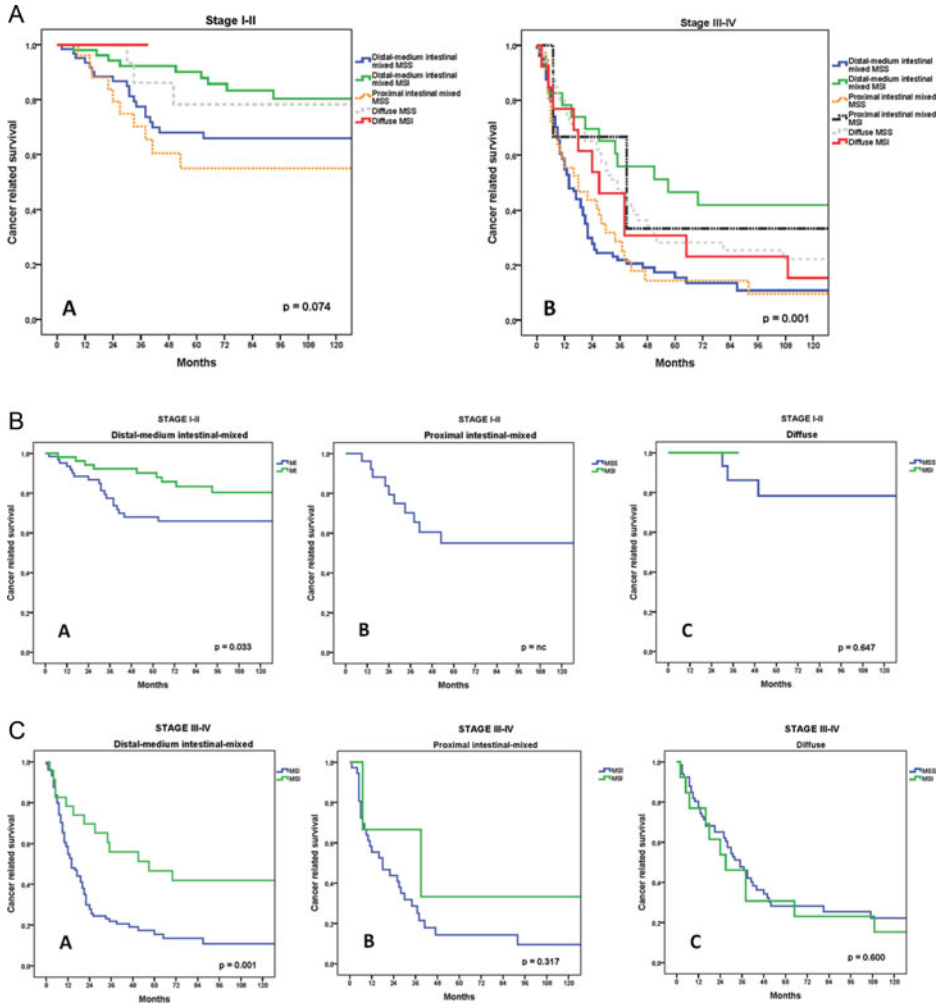

Figure 3. Cancer-related survival of GC patients according to anatomy- and histopathology-based GC molecular classification with division according to MSI status for stage I/II and stage III/IV. (A) All types with MSI and MSS subgroups for stage I/II and III/IV; (B) stage I/II for MSI and MSS patients separately for each type; (C) stage III/IV for MSI and MSS patients separately for each type.

Group for Gastric Cancer (GIRCG) that the incidence of distal stomach intestinal histotype has decreased whereas it has been shown to be stable in proximal location with intestinal histotype and diffuse/mixed histotype GC (2, 27). This has led to relative increase of diffuse/mixed histotype GC over time. Proximal tumors have been found to be clinically more aggressive and to present worse prognosis (2, 28). These conclusions have also been confirmed in our results where type 1 was different from type 3 and the survival rate results were almost the same for types 1 and 2.

Microsatellite instability GC is one of the newly distinguished subgroups of molecular GC classifications (8, 9). Currently, we can learn much more about the clinical and pathological factors as well as the survival rate according to different molecular subgroups. The MSI subtype is characterized as a gastric cancer more often diagnosed in older patients, mostly females, in non-cardia location with intestinal histotype, with lower numbers of lymph node involvement, and presenting better prognosis (2). Kim et al. presented results that intestinal MSI GC was associated with better prognosis in comparison with diffused type MSI GC in stage II, III, and IV ($p < .001$) (29). Furthermore, intestinal MSI showed a better prognosis in comparison with intestinal MSS GC ($p = .004$). The authors distinguished two separate groups: intestinal group, and a group combining diffused with mixed histotypes; however, the mixed histotype was seen only in 6% of MSI GC and in 1% of MSS GC. In our previous study we also found a strong prognostic value of MSI GC in intestinal histotype (17). It should be stressed that this finding was only true for intestinal non-cardia tumors (17). In that study we classified intestinal histotype as one group and diffused/mixed histotype as another separate group.

Research from Korea investigated MSI incidence in gastric cancer analyzing the incidence according

to Lauren histotype (30). The authors found 16 MSI GC types in a group of 116 (13.8%). Out of these 16 patients, 12 had a mixed histotype, mostly of intestinal type (11 cases) and diffused histotype (one case). In our study, mixed histotype was present in nine MSI GC patients (8.1% of all MSI GC patients). In MSS group, mixed histotype was seen in 34 patients (9.4% of all MSS GC patients).

Another interesting factor that can change the choice of oncological treatment in that group of patients is the role of chemotherapy in MSI GC patients. Kim et al. presented in their results that in stage III, in which chemotherapy was applied, the prognosis was worse for MSI tumors with undifferentiated histology and diffused histotype (29). It also confirmed that MSI group is not homogenous, which is also reflected in response to chemotherapy.

Our current results clearly show that this simple classification might be used for MSI GC patients. We have shown that for types 1 and 2 there is no difference in patients' outcome regardless of their microsatellite status. The difference has been observed only in the third group.

Our results showed that this simple division has a clinical potential. First of all, we have found clinical and pathological differences between all three groups. Second, although differences in survival rates have been observed between these subgroups, they did not reach statistical significance. Importantly, we have observed a difference in intestinal/mixed subtype between distal and proximal tumors. It clearly shows that we can apply this classification to better understand the link between molecular and clinical divisions in gastric cancer.

Microsatellite instability as a group of highest importance seems not to be homogenous. In our previous study we analyzed different MSI subgroups based on different numbers of quasi-monomorphic mononucleotide repeats (31). The results revealed that we have to consider a different subgroup of MSI because of differences in mononucleotide repeat numbers (31). From the clinical perspective, we proposed a division of MSI group with different outcomes into four subgroups: intestinal non-cardia; diffused/mixed non-cardia; intestinal cardia; and diffuse/mixed cardia (17). One division that we proposed was based on molecular background, and the second one on clinical experience; the proposition by Shah et al. mixes both these approaches, and is easily applicable clinically, especially for type 3 where the difference in MSI GC is the highest (11, 17, 31).

We have to highlight the fact that in the paper by Shah et al., a leave-one-out cross-validation error was 0.14, which means that 86% of the samples were classified correctly (11). Classifying anatomical and histological factors together with molecular findings is one of the limitations of this simple classification. The authors also stress the fact that gene set analysis with the false discovery rate, which was set at 0.25, allowed to identify several pathways (11). These pathways were differentially regulated when comparing every GC subtype to adjacent healthy gastric tissue.

In conclusion, the new molecular classifications may help in better understanding of GC biology. We have presented clinical and pathological results that might also be used in molecular-based anatomical and pathological GC classification. We have found that this classification might find its place in the subdivision of MSI subgroup of GC, because it is a simple and clinically useful classification. The question of detailed molecular analysis calls for further research.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

Funding

This study was funded by Istituto Toscano Tumori (ITT) grant entitled "Gene expression profiles and therapy of gastric cancer" (grant No. *ITT*-2007) and also by the European Union's Seventh Framework Programme (FP7), Gastric Glyco Explorer under grant agreement No. 316929 (Karol Polom, Franco Roviello).

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