Risk factor analysis for involvement of resection margins in gastric and esophagogastric junction cancer: an Italian multicenter study

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Received: 13 July 2015 / Accepted: 21 December 2015 / Published online: 5 January 2016
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Abstract

Background Resection margin (RM) involvement is associated with negative prognosis after gastrectomy. Intraoperative frozen section (IFS) analysis allows radical resection to be achieved in a single operation but is time-consuming and resource-consuming. The aim of this study was to assess risk factors associated with RM involvement to identify patients who would benefit from IFS analysis. **Methods** We retrospectively analyzed patients who underwent gastrectomy with curative intent for gastric or esophagogastric junction (EGJ) cancer from 2000 to 2014 in six Italian hospitals. RM status was assessed by IFS analysis and/or definitive histopathology examination. A set of 21 potential risk factors were compared in a multivariate analysis between patients with positive RMs on IFS analysis or definitive histopathology examination and a control cohort of similar patients with negative RMs, with the samples stratified into three subgroups (T1, T2–T4

Lauren intestinal pattern, T2–T4 Lauren diffuse/mixed pattern).

Results One hundred forty-five patients had positive RMs. Survival was significantly worse in positive RM patients than in negative RM patients (89.5 months vs 28.9 months). Multivariate analysis showed that in T1 cancers a margin distance of less than 2 cm is a risk factor for RM involvement (odds ratio 15.7), in T2–T4 intestinal pattern cancers, serosa invasion (odds ratio 6.0), EGJ location (odds ratio 4.1), and a margin distance of less than 3 cm (odds ratio 4.0) are independent risk factors, and in T2–T4 diffuse/mixed pattern cancers, lymphatic infiltration (odds ratio 4.2), tumor diameter greater than 4 cm (odds ratio 3.5), EGJ location (odds ratio 2.8), and serosa invasion (odds ratio 2.2) are independent risk factors.

Conclusions Survival after gastrectomy is negatively affected by positive RMs. IFS analysis should be routinely used in patients with a high risk of positive RMs, especially in diffuse pattern cancers.

For the Italian Research Group for Gastric Cancer (G.I.R.C.G.).

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Keywords Resection margins · Gastric cancer · Esophagogastric junction · Gastrectomy · Risk factors · Intraoperative frozen section

Introduction

Resection margin (RM) clearance is one of the most debated topics in gastric cancer surgery. Whereas the prognostic impact of RM involvement by the tumor is still to be defined, especially in specific cases such as early gastric cancer (EGC) or low-stage gastric cancer [1–3], it is commonly accepted that positive margins are an independent unfavorable factor associated with worse survival after surgical resection [4, 5]. Therefore, in the field of

gastric cancer surgical oncology, every effort must be made to obtain a complete resection with negative margins and adequate lymph node dissection, which constitutes the only possible curative treatment for nonmetastatic gastric cancer, and reoperation should always be considered in the case of R1 resections [4].

The safe distance between the tumor and resection line that allows one to have a good chance of performing R0 surgery has been investigated in several studies [6–9], and the Japanese Gastric Cancer Association (JGCA) treatment guidelines [10] have established the minimal margin distance that should be observed, depending on the tumor's T category and growth pattern.

However, cases of resection line involvement even with apparently adequate distance are still possible, so the safest way to obtain negative RMs is to perform intraoperative frozen section (IFS) analysis, which has high specificity and sensitivity [11, 12] and allows one to determine the surgical extent of resection in the case of an infiltrated margin so as to achieve single-time radical surgery. Unfortunately, IFS analysis is extremely time-consuming and resource-consuming [13], and despite what some authors suggest [14, 15], its routine use in every case is not feasible.

The aim of this study was therefore to identify specific independent risk factors associated with proximal or distal RM involvement so as to identify those patients who would certainly benefit from IFS analysis.

Materials and methods

We retrospectively analyzed case series from six Italian university hospitals with a high gastric cancer surgery volume (San Raffaele Hospital, Milan; Policlinico A. Gemelli, Rome; Ospedale di Circolo e Fondazione Macchi, Varese; Azienda Ospedaliera Universitaria Senese, Siena; Spedali Civili, Brescia; Ospedale Borgo Trento, Verona). All patients with histologically proven gastric or esophagogastric junction (EGJ) cancer who underwent total or subtotal gastrectomy with curative intent from January 2000 to December 2015 were considered for this study. Proximal or distal RM clearance was assessed by definitive histopathology examinations (DHEs) on a surgical specimen in all cases, and, when available, also by IFS analysis. DHE was conducted with standard hematoxylin–eosin staining after the specimens had been fixed with formalin, whereas IFS analysis was conducted with fast hematoxylin–eosin staining after the samples had been cryofixed at $-70\text{ }^{\circ}\text{C}$ [16].

A negative RM (R0 resection) was defined as the complete absence of both macroscopic and microscopic tumor involvement at the resection line on histopathology

assessments. A microscopically positive RM (R1 resection) was defined as the presence of viable singular tumor cells or cell aggregates at the line of resection on IFS analysis, when available, and on DHE, in the absence of visible tumor involvement of the resection line. A macroscopically positive RM (R2 resection) was defined as the presence of visible tumor involvement of the resection line, confirmed by histopathology examinations.

Only patients with positive R1 proximal or distal RMs assessed either on IFS analysis (regardless of DHE) or, when IFS analysis was not available, on DHE were included in this study (group A). Exclusion criteria were as follows: Siewert type I and type II EGJ cancer, metastatic disease, palliative resections, R2 proximal or distal RM resection, positive (R1 or R2) circumferential margin resections, and previous gastric surgery.

The study group was compared with a homologous control cohort (group B) with the same exclusion criteria who underwent curative total or subtotal gastrectomy during the same time span for gastric or Siewert type III EGJ cancer, with R0 RM resection assessed on DHE. Since the completeness of the data is essential for the multivariate analysis, to correctly identify the risk factors, only patients having no more than two missing values for the variables listed below were included in the control group.

For all patients, the following demographic, clinical, and pathological variables were recorded and inserted in a computerized database: age, sex, BMI, elective/emergent surgery, type of gastrectomy, multivisceral resections, neoadjuvant chemotherapy (NACT), distance between the tumor and the involved margin (or the nearest margin in the case of R0 resection) assessed by the pathologist on a fresh specimen, EGJ location, tumor diameter, tumor grade, T category, N category, Lauren pattern, signet ring cell histological appearance, presence of multifocal disease, presence of tumor invasion within vascular or lymphatic vessels, presence of vascular, lymphatic, and perineural infiltration, human epidermal growth factor receptor 2 (HER2) gene overexpression/amplification [17], Mandard's tumor regression grade (TRG) [18] (only for patients who underwent NACT), and overall survival (OS). A global comparison of actuarial OS was conducted between DHE R0 resection patients and DHE R1 resection patients with the Kaplan–Meier test.

After an overall comparison of the aforementioned variables between group A and group B, patients were stratified into three subgroups according to the JGCA treatment guidelines for a safe RM distance [10]: T1 patients; T2–T4 Lauren intestinal pattern cancer patients; T2–T4 Lauren diffuse/mixed pattern cancer patients.

For each subgroup, a univariate analysis was conducted between group A and group B patients for the following variables:

- T1 patients: age, sex, BMI, elective/emergent surgery, RM distance from tumor (less than 2 cm or 2 cm or greater), EGJ location, tumor diameter, tumor grade (G1–G2 or G3–G4), Lauren pattern, signet ring cell histological appearance, multifocal disease, presence of tumor invasion, vascular infiltration, lymphatic infiltration, perineural infiltration, lymph node status (N0 or N+), HER2 gene overexpression/amplification.
- T2–T4 intestinal pattern patients: age, sex, BMI, elective/emergent surgery, multivisceral resection, NACT, RM distance from tumor (less than 3 cm or 3 cm or greater), EGJ location, tumor diameter, tumor grade (G1–G2 or G3–G4), signet ring cell histological appearance, multifocal disease, presence of tumor invasion, vascular infiltration, lymphatic infiltration, perineural infiltration, lymph node status (N0 or N+), serosa invasion, HER2 gene overexpression/amplification, TRG (1–3 or 4–5).
- T2–T4 diffuse/mixed pattern patients: age, sex, BMI, elective/emergent surgery, multivisceral resection, NACT, RM distance from tumor (less than 5 cm or 5 cm or greater), EGJ location, tumor diameter, tumor grade (G1–G2 or G3–G4), signet ring cell histological appearance, multifocal disease, presence of tumor invasion, vascular infiltration, lymphatic infiltration, perineural infiltration, lymph node status (N0 or N+), serosa invasion, HER2 gene overexpression/amplification, TRG (1–3 or 4–5).

Categorical data are presented as the percent proportion; continuous data are presented as the mean $\pm$ standard deviation. For incomplete data, a dummy variable classified as “missing” was included in the analysis to allow the inclusion of all available cases. To compare differences in categorical data between the two groups, Fisher’s exact test or the χ^2 test was used, as appropriate. The unpaired *t* test was used to compare continuous data between two groups. All variables found to be statistically significant on the univariate analysis were subsequently included in a multivariate binary logistic regression model to determine independent risk factors associated with RM involvement.

For all analyses, a two-tailed *p* value less than 0.05 was considered as significant. Analyses were performed with the SPSS for Microsoft Windows version 22.0 (SPSS, Chicago, IL, USA).

Results

From 2000 to 2014, we identified in our series 191 patients who underwent gastric surgery who had positive RMs on IFS analysis or on DHE. In 92 patients (48 %), the proximal margin was involved, in 78 patients (41 %) the distal

margin was involved, and in 21 patients (11 %) both margins were involved.

Forty-six patients (24 %) were excluded from the initial series: 19 patients (41 %) because of palliative surgery or the presence of metastatic disease, 13 patients (28 %) because of macroscopic evidence of (R2) margin involvement, eight patients (18 %) because of a positive (R1 or R2) circumferential margin, and six patients (13 %) because of previous gastric surgery. The remaining 145 patients were included in group A.

In 45 % of patients the proximal margin was involved, in 45 % the distal margin was involved, and in 10 % both margins were involved. Although there was a higher incidence of proximal margin infiltration in the initial series than in the definitive patient group (48 % vs 45 %), no statistical difference was found (*p* = 0.764). Margin involvement was due to direct infiltration by the tumor in 83 % of cases, whereas in the remaining 17 % of cases it was due to the presence of neoplastic embolization on the resection line.

In 113 patients, because IFS analysis was not performed, RM involvement was assessed directly by DHE; in none of these patients had a second-time radicalization surgical procedure been performed. In the remaining 32 patients, the RM involvement was assessed by IFS analysis, and the subsequent surgical extent (esophageal resection in 20 patients, total gastrectomy in seven patients, and duodenal resection in five patients) allowed R0 surgery to be performed in all patients.

Demographic, clinical, and histopathological characteristics of the 145 patients are reported in Table 1.

The study group was compared with a control group, consisting of 529 patients operated on during the same period, each having no more than two missing values for the variables studied. Overall missing data for the control group amounted to 3 %.

Survival analysis

Postoperative OS of the 113 patients with DHE R1 resection was compared with that of the 561 patients with DHE radical resection (Fig. 1). The mean estimated OS was 89.5 months [95 % confidence interval (CI) 81.9–97.0 months] for R0 resection patients and 28.9 months (95 % CI 18.4–39.4 months) for R1 resection patients (*p* < 0.001). The 5-year OS of R0 and R1 resection patients was 59 and 22 %, respectively (*p* < 0.001).

Overall comparison between group A and group B patients

The overall comparison of analyzed variables between the whole group of positive RM patients and the control group

Table 1 Demographic, clinical, and histopathological characteristics of 145 patients with proximal or distal resection margin (RM) involvement on intraoperative frozen section analysis or definitive histopathology examination

Variable	Value
Age	65.0 ± 14.1 years
Sex	
Male	57 %
Female	43 %
BMI	24.2 ± 3.7 kg/m ²
Type of surgery	
Elective	93 %
Emergent	7 %
Type of gastrectomy	
Subtotal	48 %
Total	52 %
Multivisceral resections	13 %
NACT	10 %
Nearest RM distance from tumor	1.5 ± 1.7 cm
EGJ location	26 %
Tumor diameter	5.6 ± 3.3 cm
Tumor grade	
G1	2 %
G2	19 %
G3	77 %
G4	2 %
T category	
T1	8 %
T2	10 %
T3	25 %
T4	57 %
N category	
N0	21 %
N1	11 %
N2	16 %
N3	52 %
Lauren pattern	
Intestinal	35 %
Diffuse/mixed	65 %
Signet ring cell histological appearance	53 %
Multifocal disease	3 %
Tumor invasion	24 %
Vascular infiltration	24 %
Lymphatic infiltration	25 %
Perineural infiltration	16 %
HER2 gene overexpression/amplification	3 %
TRG (only for NACT)	
1–3	67 %
4–5	33 %

EGJ esophagogastric junction, HER2 human epidermal growth factor receptor 2, NACT neoadjuvant chemotherapy, TRG tumor regression grade

Fig. 1 Five-year overall survival of R0 and R1 resection patients

is reported in Table 2. Patients with RM involvement on IFS analysis or DHE had a higher incidence of EGJ location (26 % vs 12 %; $p < 0.001$) and Lauren diffuse/mixed pattern cancer (65 % vs 36 %; $p < 0.001$). In group A the tumor diameter was greater (5.6 ± 3.3 cm vs 3.9 ± 2.4 cm; $p < 0.001$) and the margin distance was shorter (1.5 ± 1.7 cm vs 2.6 ± 1.7 cm; $p < 0.001$) than in group B. The prevalence of T3–T4 cancer (82 % vs 50 %; $p < 0.001$), N2–N3 cancer (68 % vs 40 %; $p < 0.001$) and grade G3–G4 cancer (79 % vs 59 %; $p < 0.001$) was significantly higher in positive RM patients. Moreover, group A patients had a higher incidence of signet ring cell histological appearance, tumor invasion, and vascular, lymphatic, and perineural infiltration (53 % vs 34 %, 24 % vs 8 %, 24 % vs 12 %, 25 % vs 7 %, and 16 % vs 4 %, respectively; all $p < 0.001$). Finally, in patients with RM involvement, total gastrectomy (52 % vs 27 %; $p < 0.001$) and multivisceral resections (13 % vs 2 %; $p < 0.001$) were more frequently performed.

Risk factor analysis for T1 cancer patients

Among the whole group, 175 patients had T1 cancer: 11 patients had RM involvement on IFS analysis or DHE (group A), whereas 164 had negative RMs (group B). Data regarding the comparison of potential risk factors between these two subgroups are reported in Table 3. Among all analyzed variables, only the RM distance from the tumor was significantly related with RM involvement on histological examination. In particular, there was an RM distance of less than 2 cm in 88 % of group A patients but in

Table 2 Comparison of demographic, clinical, and histopathological characteristics between 145 patients with resection margin (RM) involvement on intraoperative frozen section analysis or definitive histopathology examination (group A) and 529 negative RM patients (group B)

Variable	Group A (145 patients)	Group B (529 patients)	<i>p</i> ^a
Age	65.0 ± 14.1 years	66.9 ± 12.0 years	0.131
Sex			
Male	57 %	57 %	1.000
Female	43 %	43 %	
BMI	24.2 ± 3.7 kg/m ²	24.9 ± 4.3 kg/m ²	0.422
Type of surgery			
Elective	93 %	95 %	0.252
Emergent	7 %	5 %	
Type of gastrectomy			
Subtotal	48 %	73 %	<0.001
Total	52 %	27 %	
Multivisceral resections	13 %	2 %	<0.001
NACT	10 %	14 %	0.303
Nearest RM distance from tumor	1.5 ± 1.7 cm	2.6 ± 1.7 cm	<0.001
EGJ location	26 %	12 %	<0.001
Tumor diameter	5.6 ± 3.3 cm	3.9 ± 2.4 cm	<0.001
Tumor grade			
G1	2 %	12 %	<0.001
G2	19 %	29 %	
G3	77 %	58 %	
G4	2 %	1 %	
T category			
T1	8 %	30 %	<0.001
T2	10 %	20 %	
T3	25 %	33 %	
T4	57 %	17 %	
N category			
N0	21 %	45 %	<0.001
N1	11 %	15 %	
N2	16 %	21 %	
N3	52 %	19 %	
Lauren pattern			
Intestinal	35 %	64 %	<0.001
Diffuse/mixed	65 %	36 %	
Signet ring cell histological appearance	53 %	34 %	<0.001
Multifocal disease	3 %	3 %	1.000
Tumor invasion	24 %	8 %	<0.001
Vascular infiltration	24 %	12 %	<0.001
Lymphatic infiltration	25 %	7 %	<0.001
Perineural infiltration	16 %	4 %	<0.001
HER2 gene overexpression/amplification	3 %	2 %	0.770
TRG (only for NACT)			
1–3	67 %	42 %	0.275
4–5	33 %	58 %	

EGJ esophagogastric junction, HER2 human epidermal growth factor receptor 2, NACT neoadjuvant chemotherapy, TRG tumor regression grade

^a Values in *italic* are significant

Table 3 Univariate and multivariate analysis of potential risk factors for T1 cancers between patients with a positive proximal or distal resection margin (RM) (group A) or a negative proximal or distal RM (group B)

Variable	Group A (11 patients)	Group B (164 patients)	<i>p</i>	OR	95 % CI	<i>p</i>
Age	67.3 ± 8.6 years	65.2 ± 11.7 years	0.569			
Sex						
Male	70 %	63 %	0.747			
Female	30 %	37 %				
BMI	21.8 ± 2.9 kg/m ²	24.9 ± 4.4 kg/m ²	0.126			
Type of surgery						
Elective	100 %	98 %	1.000			
Emergent	0 %	2 %				
RM distance from tumor						
<2 cm	88 %	31 %	0.002	15.7	1.9–131.9	0.002 ^a
≥2 cm	12 %	69 %				
EGJ location						
No	82 %	94 %	0.168			
Yes	18 %	6 %				
Tumor diameter	3.4 ± 2.5 cm	2.6 ± 1.7 cm	0.159			
Tumor grade						
G1–G2	73 %	53 %	0.348			
G3–G4	27 %	47 %				
Lauren type						
Intestinal	54 %	66 %	0.514			
Diffuse/mixed	46 %	34 %				
Signet ring cell histological appearance						
No	70 %	64 %	1.000			
Yes	30 %	36 %				
Multifocal disease						
No	91 %	95 %	0.487			
Yes	9 %	5 %				
Tumor invasion						
No	90 %	96 %	0.353			
Yes	10 %	4 %				
Vascular infiltration						
No	91 %	95 %	0.461			
Yes	9 %	5 %				
Lymphatic infiltration						
No	91 %	98 %	0.237			
Yes	9 %	1 %				
Perineural infiltration						
No	91 %	100 %	0.070			
Yes	9 %	0 %				
Lymph node status						
N0	27 %	22 %	0.711			
N+	73 %	78 %				
HER2 gene overexpression/amplification						
No	91 %	98 %	0.230			
Yes	9 %	2 %				

CI confidence interval, EGJ esophagogastric junction, HER2 human epidermal growth factor receptor 2, OR odds ratio

^a Significant

only 31 % of group B patients ($p = 0.002$). The calculated OR for RM involvement in the case of an RM distance from the tumor of less than 2 cm was 15.7 (95 % CI 1.9–131.9; $p = 0.002$). No other significant differences were found between the two groups concerning all the aforementioned variables.

When the analysis was restricted to only patients with gastric cancer, a free margin distance of less than 2 cm was still identified as the only risk factor (OR 12.3, 95 % CI 1.4–108.8, $p = 0.002$).

Risk factor analysis for T2-T4 Lauren intestinal pattern cancer patients

Among the whole group, 274 patients had T2–T4 Lauren intestinal pattern cancer: 45 had RM involvement on IFS analysis or DHE (group A), whereas 229 had negative RMs (group B). Data regarding the comparison of potential risk factors between these two subgroups are reported in Table 4. In univariate analysis, multivisceral resections were more frequently performed in positive RM patients than in negative RM patients (18 % vs 2 %; $p < 0.001$). The distance between the tumor and the nearest RM was less than 3 cm in 91 % of patients with RM involvement and in 63 % of patients with R0 resection ($p = 0.001$). An ECJ location of the tumor was significantly more frequent in group A patients than in group B patients (36 % vs 20 %; $p = 0.03$). Grade G3 or G4 cancers were present in about 66 % of positive RM patients but in only 49 % of negative RM patients ($p = 0.048$). The presence of tumor invasion, vascular infiltration, and lymphatic infiltration was significantly more frequent in group A patients (24, 33, and 38 %, respectively) than in group B patients (10 %, $p = 0.024$; 16 %, $p = 0.018$; and 10 %, $p = 0.002$, respectively). Finally, patients with RM involvement had a significantly higher incidence of serosa invasion than patients with negative RMs (53 % vs 16 %, $p < 0.001$). No other significant differences in the distribution of the variables considered were found between the two subgroups on univariate analysis.

On multivariate analysis, a distance between the tumor and RM of less than 3 cm (OR 4.0, 95 % CI 1.1–14.5, $p = 0.03$), EGJ location (OR 4.1, 95 % CI 1.7–10.0, $p = 0.002$), and serosa invasion (OR 6.0, 95 % CI 2.5–14.8, $p < 0.001$) were found to be the only independent risk factors for RM involvement by the tumor in patients with T2–T4 Lauren intestinal pattern cancer.

When the analysis was restricted to only patients with gastric cancer, serosa invasion (OR 6.7, 95 % CI 2.1–21.4, $p = 0.001$) and neoplastic embolization (OR 5.6, 95 % CI 1.4–22.5, $p = 0.016$) were the only independent risk factors identified.

Risk factor analysis for T2-T4 Lauren diffuse/mixed pattern cancer patients

Among the whole group, 225 patients had T2–T4 Lauren diffuse/mixed pattern cancer: 89 had RM involvement on IFS analysis or DHE (group A), whereas 136 had negative RMs (group B). Data regarding the comparison of potential risk factors between the two subgroups are reported in Table 5. On univariate analysis, 23 % of RM-infiltrated patients had EGJ-located tumors, whereas only 8 % of negative RM patients had tumors in this location ($p = 0.003$). Tumor diameter was significantly greater in group A patients than in group B patients (6.1 ± 3.7 cm vs 4.1 ± 2.1 cm; $p < 0.001$). Converting this continuous variable in a binary one, we find that 72 % of group A patients had a tumor larger than 4 cm in comparison with 36 % of group B patients ($p < 0.001$). Similarly to the previous analysis, also for this subgroup a significantly higher incidence of tumor invasion, vascular infiltration, and lymphatic infiltration was found in group A patients (26, 22, and 26 %, respectively) in comparison with group B patients (10 %, $p = 0.002$; 11 %, $p = 0.037$; and 9 %, $p = 0.001$, respectively). The presence of lymph node metastases was higher in patients with RM involvement than in patients with negative RMs (87 % vs 74 %, $p = 0.03$). Finally, serosa invasion was significantly more frequent in group A patients (64 %) than in group B patients (37 %; $p < 0.001$). No other significant differences in the distribution of the variables considered were found between the two subgroups on univariate analysis.

On multivariate analysis, EGJ location (OR 2.8, 95 % CI 1.7–7.0, $p = 0.033$), tumor diameter greater than 4 cm (OR 3.5, 95 % CI 1.8–6.7, $p < 0.001$), presence of lymphatic infiltration (OR 4.2, 95 % CI 1.7–10.3, $p = 0.002$), and serosa invasion (OR 2.2, 95 % CI 1.2–4.3, $p = 0.014$) were found to be independent risk factors for RM involvement by the tumor in patients with T2–T4 Lauren diffuse/mixed pattern cancer.

When the analysis was restricted to only patients with gastric cancer, presence of lymphatic infiltration (OR 3.9, 95 % CI 1.6–9.5, $p = 0.002$), tumor diameter greater than 4 cm (OR 3.3, 95 % CI 1.7–6.4, $p < 0.001$), and serosa invasion (OR 2.3, 95 % CI 1.2–4.6, $p = 0.011$) were found to be the only independent risk factors associated with RM involvement.

Discussion

RM clearance is an issue of major importance in gastric cancer surgery. Depending on different case series, the prevalence of R1 resections ranges from 2.8 to 20 % of operated-on patients [2]. The prognostic impact of this RM

Table 4 Univariate and multivariate analysis of potential risk factors for T2–T4 Lauren intestinal pattern cancers between patients with a positive proximal or distal resection margin (RM) (group A) or a negative proximal or distal RM (group B)

Variable	Group A (45 patients).	Group B (229 patients)	p^a	OR	95 % CI	p^a
Age	67.2 ± 13.0 years	69.1 ± 10.8 years	0.306			
Sex						
Male	72 %	61 %	0.215			
Female	28 %	39 %				
BMI	24.9 ± 3.7 kg/m ²	25.6 ± 4.3 kg/m ²	0.610			
Type of surgery						
Elective	97 %	95 %	0.695			
Emergent	3 %	5 %				
Multivisceral resections						
No	82 %	98 %	<0.001	2.5	0.4–16.6	0.372
Yes	18 %	2 %				
NACT						
No	89 %	90 %	0.797			
Yes	11 %	10 %				
RM distance from tumor						
<3 cm	91 %	63 %	0.001	4.0	1.1–14.5	0.03
≥3 cm	9 %	37 %				
EGJ location						
No	64 %	80 %	0.030	4.1	1.7–10.0	0.002
Yes	36 %	20 %				
Tumor diameter	5.2 ± 2.2 cm	4.7 ± 2.5 cm	0.214			
Tumor grade						
G1–G2	34 %	51 %	0.048	1.2	0.5–2.8	0.693
G3–G4	66 %	49 %				
Signet ring cell histological appearance						
No	78 %	87 %	0.127			
Yes	22 %	13 %				
Multifocal disease						
No	95 %	99 %	0.173			
Yes	5 %	1 %				
Tumor invasion						
No	76 %	90 %	0.024	2.3	0.8–6.9	0.129
Yes	24 %	10 %				
Vascular infiltration						
No	67 %	84 %	0.018	0.4	0.1–1.5	0.526
Yes	33 %	16 %				
Lymphatic infiltration						
No	72 %	90 %	0.002	1	0.2–4.9	0.775
Yes	28 %	10 %				
Perineural infiltration						
No	85 %	93 %	0.106			
Yes	15 %	7 %				
Lymph node status						
N0	20 %	32 %	0.113			
N+	80 %	68 %				
Serosa invasion						
No	47 %	84 %	<0.001	6.0	2.5–14.8	<0.001
Yes	53 %	16 %				

Table 4 continued

Variable	Group A (45 patients).	Group B (229 patients)	<i>p</i> ^a	OR	95 % CI	<i>p</i> ^a
HER2 gene overexpression/amplification						
No	96 %	96 %		1.000		
Yes	4 %	4 %				
TRG (only for NACT)						
1–3	60 %	41 %		0.628		
4–5	40 %	59 %				

CI confidence interval, *EGJ* esophagogastric junction, *HER2* human epidermal growth factor receptor 2, *NACT* neoadjuvant chemotherapy, *OR* odds ratio, *TRG* tumor regression grade

^a Values in *italic* are significant

involvement by the tumor has been widely studied by several authors: in a multicenter Italian study, Morgagni et al. [4] showed that RM infiltration is an independent unfavorable factor in patients who underwent gastrectomy (OR 1.5), with a 5-year OS rate of 66 % in R0 resections and 29 % in R1 resections. Eastern authors [3, 5, 14, 15] observed similar results, with an unstratified 5-year survival rate ranging from 53 to 60 % in the case of R0 resections, and from 13 to 26 % in case of R1 resections. In the present study we found that the 5-year survival rate of the whole cohort was 59 % in patients with negative RMs and 22 % in patients with positive RMs; therefore, the data we are presenting perfectly overlap with those reported in the literature.

However, although the effect of R1 resection is undoubtedly negative on the whole series population, the definitive prognostic significance in a specific subgroup of patients is still to be defined. Sun et al. [3] found that R1 resections affect prognosis only in T1–T2 cancer patients, whereas Cho et al. [2] identified a worse survival only in N0–N1 cancer patients. These authors suggest that R1 resections may have a negative impact only in low-stage cancer patients, because the adverse effects of positive RMs might be overwhelmed by the more detrimental impact of the depth of invasion or nodal metastasis. In contrast, Morgagni [1] and Nakamura et al. [19] showed that the prognosis of patients with EGC is not affected by the presence of positive RMs, because of the excellent survival for this type of disease. Finally, in contrast to the above results, Wang et al. [14] found that positive margins affect survival despite the tumor category.

Pattern of recurrence analysis after R1 resection showed conflicting results, and may explain the aforementioned finding. Sun et al. [3] found that locoregional relapse in patients with positive RMs was more frequent than distant or peritoneal relapse, and that it was more frequent than in negative RM patients, as expected. In contrast, Wang et al. [14] and Bickenbach et al. [20] showed that the main recurrence pattern in R1 resection patients was peritoneal rather than locoregional. This finding may indicate that

positive RM patients generally have a more advanced and more aggressive disease [20], whose systemic impact can mask the still present locoregional effects. In addition, in our study we found that patients with positive RMs had clinicopathological features significantly more advanced and more aggressive than those of R0 resection patients, especially concerning T category, N category, grading, and Lauren pattern. Such a pronounced difference between the two groups of patients can then explain the equally pronounced difference in survival as observed in this study as well as in previous ones.

Despite the fact that the real prognostic impact of positive RMs is still a matter of discussion, most authors [1, 2, 4, 21, 22] suggest that, in the case of R1 resection, a surgical retreatment of the patient aiming to achieve radical resection (if possible) should always be considered, in order to ensure optimal prognostic results. Lee et al. [23] showed that the OS rate and the recurrence rate are not changed if R0 resection is achieved by performing additional resection for positive RMs. Other authors, by contrast, found that surgical re-excision of infiltrated margin has a prognostic benefit only when disease extension is limited, as in case of limited nodal involvement [20, 24] or nontransmural disease [20], and should therefore be attempted only in such cases.

Because it was shown that, in the case of R0 resection, the distance of free margin from the tumor does not impact prognosis [25, 26], several authors [6–9] and the Japanese Gastric Cancer Association [10] sought to determine what is the optimal distance of free margin from the tumor in order to reduce the risk of RM infiltration. However, findings have been heterogeneous so far.

IFS analysis has been reported to have excellent sensitivity, specificity, and accuracy [11, 27] in identifying infiltrated margins. Also in the present series, the positive prognostic value and negative prognostic value were 100 %: all cases of positive margins on IFS analysis were confirmed by DHE, whereas no cases of R1 resections with negative RMs on IFS analysis were reported. Therefore, the use of IFS analysis to determine the extent of resection is

Table 5 Univariate and multivariate analysis of potential risk factors for T2–T4 Lauren diffuse/mixed pattern cancers between patients with a positive proximal or distal resection margin (RM) (group A) or a negative proximal or distal RM (group B)

Variable	Group A (89 patients).	Group B (136 patients)	p^a	OR	95 % CI	p^a
Age	63.5 ± 15.1 years	65.3 ± 13.6 years	0.368			
Sex						
Male	47 %	43 %	0.328			
Female	53 %	57 %				
BMI	24.7 ± 3.9 kg/m ²	23.8 ± 4.2 kg/m ²	0.501			
Type of surgery						
Elective	90 %	94 %	0.412			
Emergent	10 %	6 %				
Multivisceral resections						
No	88 %	95 %	0.076			
Yes	12 %	5 %				
NACT						
No	91 %	87 %	0.397			
Yes	9 %	13 %				
RM distance from tumor						
<5 cm	90 %	88 %	0.808			
≥5 cm	10 %	12 %				
EGJ location						
No	77 %	92 %	0.003	2.8	1.1–7.0	0.033
Yes	23 %	8 %				
Tumor diameter	6.1 ± 3.7 cm	4.1 ± 2.1 cm	<0.001			
≤4 cm	28 %	64 %	<0.001	3.5	1.8–6.7	<0.001
>4 cm	72 %	36 %				
Tumor grade						
G1–G2	6 %	7 %	1.000			
G3–G4	94 %	93 %				
Signet ring cell histological appearance						
No	25 %	33 %	0.323			
Yes	75 %	67 %				
Multifocal disease						
No	99 %	98 %	1.000			
Yes	1 %	2 %				
Tumor invasion						
No	74 %	90 %	0.002	1.5	0.6–3.9	0.36
Yes	26 %	10 %				
Vascular infiltration						
No	78 %	89 %	0.037	1	0.4–2.6	0.906
Yes	22 %	11 %				
Lymphatic infiltration						
No	74 %	91 %	0.001	4.2	1.7–10.3	0.002
Yes	26 %	9 %				
Perineural infiltration						
No	88 %	95 %	0.063			
Yes	12 %	5 %				
Lymph node status						
N0	13 %	26 %	0.030	1.5	0.6–3.5	0.671
N+	87 %	74 %				
Serosa invasion						
No	36 %	63 %	<0.001	2.2	1.2–4.3	0.014
Yes	64 %	37 %				

Table 5 continued

Variable	Group A (89 patients)	Group B (136 patients)	<i>p</i> ^a	OR	95 % CI	<i>p</i> ^a
HER2 gene overexpression/amplification						
No	99 %	99 %		1.000		
Yes	1 %	1 %				
TRG (only for NACT)						
1–3	67 %	19 %		0.155		
4–5	33 %	81 %				

CI confidence interval, EGJ esophagogastric junction, HER2 human epidermal growth factor receptor 2, NACT neoadjuvant chemotherapy, OR odds ratio, TRG tumor regression grade

^a Values in *italic* are significant

the safest way to reduce the risk of R1 margins and the subsequent problem of surgical re-excision, but it is not common practice and there is no agreement as to in which patients it should be performed [12].

Firstly, the usefulness of performing IFS analysis should also be related to the site of the margin examined (proximal or distal) and the type of resection (subtotal or total gastrectomy). IFS analysis makes sense if there is the technical possibility to perform an extent of resection in the case of a positive margin. A distal margin, both in total and in subtotal gastrectomy, can be analyzed if the duodenal section is performed just beyond the pylorus. In the case of a positive margin it is generally possible to prepare the first part of the duodenum to further extent the resection. In contrast, if no further duodenal resection is possible, IFS analysis on the distal margin should be performed only on those highly selected cases where pancreaticoduodenectomy can be performed. The proximal margin in subtotal gastrectomy does not present particular issues, because further gastric resection or conversion to total gastrectomy can always be performed. In contrast, in the case of total gastrectomy, it is useful to analyze the proximal margin if an esophageal resection (even by thoracotomy) can be performed.

Moreover, IFS analysis is extremely time-consuming and resource-consuming (the estimated cost for a patient is about \$1000 for both RMs) [13], and its routine use in all gastric resections, as suggested by some authors [14, 15, 23], does not seem reliable.

Therefore, IFS analysis should be selectively performed in those cases where the risk of infiltrated margins is high and there is the possibility to perform an extent of resection. For this reason, several studies have been performed to identify risk factors associated with RM involvement, and a systematic review of this topic has been published recently [28]. According to the different studies, T category [2, 3, 14, 20], N category [2, 3, 14, 20], tumor size [3, 5, 14, 23], proximal tumor location [2, 23, 29], high tumor grading [2, 20], Bormann type IV gastric cancer [2, 23], Lauren diffuse pattern [20, 29], and signet ring cell

histological appearance [23] have been identified as independent risk factors for RM involvement.

All these factors, together with several other variables, many of which have never been previously studied, were selectively analyzed in this study with a clinical-oriented method. To the best of our knowledge, this is the first study in which risk factor analysis has been performed stratifying patients according to T category and Lauren pattern, which have been identified by most authors and by the JGCA as the principal factors associated with RM involvement. This allowed us to perform an analysis aimed at providing the surgeon with a precise assessment of case-specific RM involvement risk, and, consequently, of the usefulness of performing IFS analysis.

Such an outcome was made possible because of our collecting one of the largest cohort of patients with RM involvement, including not only patients with R1 resection on DHE but also patients with positive IFS analysis results, who would actually have had a R1 resection if the IFS analysis had not been performed.

According to the results of the present study, in T1 cancer patients the only identified risk factor for RM involvement is a margin distance of less than 2 cm (OR 15.7). This finding perfectly overlaps with the JGCA guidelines [10], which suggest keeping a distance of at least 2 cm between the tumor and the resection line to avoid the risk of margin infiltration. Thus, IFS analysis is indicated only if the resection cannot respect this safe distance. A limitation of this subgroup analysis is the small number of group A patients, due to the difficulty in finding EGC patients with RM involvement. Nevertheless, because, as previously discussed, the prognostic impact of R1 resection in EGC is still to be defined, the use of IFS analysis in these patients may be a questionable choice.

In T2–T4 Lauren intestinal pattern cancer patients, the 3-cm margin distance suggested by the JGCA was also found to be an independent risk factor for RM involvement (OR 4.0). However, in these patients, other important identified risk factors are EGJ tumor location (OR 4.1) and, above all, serosa invasion (OR 6.0). Because all these

Fig. 2 Discrepancy between the resection margin and the 5-cm cutoff suggested by the Japanese Gastric Cancer Association in patients with T2–T4 Lauren diffuse/mixed pattern cancers with resection margin involvement

factors are known preoperatively and/or intraoperatively, IFS analysis use should therefore be mandatory in these selected cases.

Finally, also in T2–T4 Lauren diffuse/mixed pattern cancer patients, EGJ tumor location (OR 2.8) and serosa invasion (OR 2.2) have been shown to be independent risk factors, together with tumor size greater than 4 cm (OR 3.5) and the presence of lymphatic infiltration on DHE (OR 4.2). Unlike the previous subgroups, however, in this case the margin distance suggested by the JGCA guidelines is not associated with a higher risk of RM infiltration, considering both the entire group of patients and only those affected by gastric cancer. Although the mean distance between the tumor and the RM was only 3.3 cm, we found RM infiltration from 5.1 cm up to even 12 cm away from the tumor (see Fig. 2). In such a scenario, defining a reliable safety margin does not seem to be an easy task. Moreover, the most relevant risk factor (presence of lymphatic infiltration) is a variable that can be known only postoperatively. For these reasons, and since Lauren diffuse/mixed cancer has the higher incidence of RM involvement, the use of IFS analysis should be widely applied in the case of T2–T4 diffuse pattern cancers, especially in advanced forms.

In conclusion, RM involvement has a negative prognostic impact in patients who underwent gastric surgery, and it is associated with more aggressive and more advanced clinicopathological features. The use of IFS analysis can reduce the rate of R1 resection and the need

for surgical re-excision, but it should be selectively applied in patients with higher risk of RM involvement. Margin distance less than 2 cm is an independent risk factor for RM involvement in T1 cancer, serosa invasion, EGJ location, and margin distance less than 3 cm are independent risk factors for RM involvement in T2–T4 Lauren intestinal pattern cancer, and lymphatic infiltration, tumor size larger than 4 cm, EGJ location, and serosa invasion are independent risk factors for RM involvement in T2–T4 Lauren diffuse/mixed pattern cancer. IFS analysis should be routinely performed in those patients with high risk of positive RMs, especially in Lauren diffuse pattern cancers.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human rights statement and informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for their being included in the study.

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