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Original contribution

Approaching heterogeneity of human epidermal growth factor receptor 2 in surgical specimens of gastric cancer

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Summary Gastric cancer shows intratumoral heterogeneity for human epidermal growth factor receptor 2 expression. We evaluated whether the number of tissue blocks analyzed or the antibodies used may influence the immunohistochemical results in gastrectomy specimens. Clinicopathologic data from 148 patients receiving gastric surgery for cancer were collected. One tissue block for each of 88 primary tumors and 60 paired primary tumors and metastases was examined for human epidermal growth factor receptor 2 status by immunohistochemistry using 3 different antibodies (HercepTest, CB11, and 4B5) and by fluorescent in situ hybridization. Two additional tissue blocks of the primary tumor were tested by immunohistochemistry if the results were negative on the first tissue block. The concordance among the 3 antibodies was 94.5% (testing 1 tissue block). Two cases showed a clinically significant discrepancy between primary tumor (score 0) and lymph nodes metastases (score 3+). Additional block analysis increased both the sensitivity (from 63% to 83%) and the accuracy (from 91% to 94%) of immunohistochemistry as compared with fluorescent in situ hybridization. The multiblock approach could potentially identify a greater number of human epidermal growth factor receptor 2–positive gastric cancers, particularly those with higher levels of intratumor heterogeneity. In turn, human

Abbreviations: ToGA, Trastuzumab for Gastric Cancer; ESMO, European Society for Medical Oncology; IHC, immunohistochemistry; SISH, silver-enhanced in situ hybridization result; FISH, fluorescent in situ hybridization result; K, κ of Cohen-Fleiss; WK, weighted κ of Cohen; DSS, disease-specific survival; HR, hazard ratio; CI, confidence interval; CT, chemotherapy; A, amplified; NA, not amplified; TP, true positive; TN, true negative.

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epidermal growth factor receptor 2 positivity correlated with a worse prognosis ($P = .011$) and was an independent variable in multivariate analysis (hazard ratio, 1.57). In conclusion, testing more than 1 tissue block of cancer from specimens of gastric resection provides a more reliable human epidermal growth factor receptor 2 assessment regardless of the antibody used.

1. Introduction

In recent years, several studies have described the negative prognostic role of human epidermal growth factor receptor (HER) 2 overexpression in gastric cancer [1-3]. In 2010, the "Trastuzumab for Gastric Cancer (ToGA)" study [4], a randomized, multicenter, international, phase 3, controlled trial was designed to assess the clinical efficacy and safety of the anti-HER2 agent trastuzumab (Herceptin; Roche, Basel, Switzerland) as an addition to chemotherapy for first-line treatment of advanced or metastatic gastric cancer overexpressing HER2 [4]. The results showed improved survival for patients treated with trastuzumab and chemotherapy compared with patients treated with chemotherapy alone. At the 12th European Society for Medical Oncology/World Congress on Gastrointestinal Cancer, it has been recommended that all patients with metastatic gastric adenocarcinoma who are candidates for first-line chemotherapy should be tested for HER2 status, and patients with a tumor overexpressing HER2, as defined by an immunohistochemical (IHC) score of 3+ or 2+ and a confirmatory silver-enhanced (SISH) or fluorescent (FISH) in situ hybridization result, should be treated with the cisplatin/fluoropyrimidine plus trastuzumab combination [5].

At present, the rate of HER2 positivity in gastric carcinomas varies in the literature from 6.1% to 91% [1,4,6,7]. The HER2 status may be influenced by the histologic type and location of the tumor or the age of the patients. For example, Moelans et al [8] recently reported a 3% rate of HER2 expression in patients with gastric cancer younger than 45 years. On the other hand, the discrepancy could be the consequence of use of anti-HER2 antibodies with different sensitivity [9] and specificity. Another cause of the discrepancy may be the revision of the original definition, as given by Hofmann et al [10], of the membrane location of HER2 in overexpressing gastric cancer cells that was a "moderate to strong complete or basolateral membranous reactivity." This definition was slightly modified in the ToGA trial [4], so that "intense lateral membrane" positivity was considered valid as well. The different quantitative criteria used to define HER2 overexpression in surgical samples and biopsy specimens may be involved as well in the aforementioned discrepancy: although the positivity of 1 cell cluster irrespective of the size in the latter is sufficient, in a surgical specimen, at least 10% of cells have to be present to be scored 3+ and classified as overexpressing HER2 [4]. This percentage value may be influenced by the heterogeneity [11] of HER2 expression in large gastric cancer, so that the assessment of HER2 in just 1 tissue block obtained from surgical specimens of gastrectomy could produce false-negative results. For example, Hsu et al [6] studying HER2 expression in a large case series of gastric resection for cancer showed a rate of positive cases of 6.1%. This low rate of HER2 expression failed to be an independent prognostic factor at multivariate analysis. However, it is worth mentioning that IHC tests were performed on tissue microarrays obtained from a single paraffin block. Recently, an up-to-date guidance on standardizing tissue processing, HER2 testing, and scoring in patients with gastric cancer remarked that HER2 results of tissue microarrays are not suitable for clinical decision due to the heterogeneous nature of HER2 overexpression and amplification in this tumor type [12].

In the present study, taking into account the possible heterogeneity of HER2 expression, we wanted to evaluate whether to examine a single tissue block could impair the IHC results in surgical specimens of large gastric cancers.

2. Materials and methods

2.1. Case series

Formalin-fixed, paraffin-embedded blocks of specimens of surgically resected gastric cancer were retrieved from the files of the Departments of Pathology of Ospedale S. Giovanni Battista of the University of Turin (98 cases) and of Ospedale Policlinico of the University of Bari (50 cases), Italy.

The case series was represented by 88 samples of primary tumor only and 60 samples of paired primary tumors and metastases (58 metastatic lymph nodes and 2 liver metastases). Clinicopathologic and follow-up data (disease persistence, recurrence, and metastases) were collected for all cases.

Institutional review board permission was obtained, and the study was conducted in compliance with the ethical regulatory issues of the participating institutions for the handling of biological specimens from tumor banks, that is, the samples exclusively available for research purposes in retrospective studies. The number of tissue blocks sampled for each primary tumor ranged from 4 to 8. The hematoxylin and eosin slides were reviewed to select the block for HER2 analysis; these blocks had to show at least 50% invasive

cancer cells in the histologic section. The same criterion was used to select additional slides for extra HER2 IHC staining if necessary. Given that the gross mapping of the tumor mass was not reported, we considered the slides that did not show normal mucosa as derived from sampling of the tumor core and slides that showed some normal mucosa as peripheral to the tumor mass. The histologic type, grade of each case, and pathologic staging were reviewed according to the World Health Organization criteria [13].

2.2. IHC tests

Sections were processed for IHC using 3 different antibodies against the HER2 intracellular domain: 4B5 (rabbit monoclonal antibody, prediluted; Ventana Medical Systems, Inc, Tucson, AZ), which was applied using the BenchMark XT automated stainer (Ventana); CB11 (prediluted, Oracle kit; Novocastra Laboratories, Newcastle-upon-Tyne, UK), which was applied on the Bond automated stainer (Menarini Diagnostics, Florence, Italy); and the HercepTest kit (Dako Denmark A/S, Glostrup, Denmark) on the Dako Autostainer.

Three pathologists (S.A., F.M., and G.I.) screened each slide for HER2 expression according to the "magnification rule" [14], so that, for a score of 3+, expression was detectable at low magnification (×2.5-5); a score of 2+, at ×10 to ×20 magnification; and a score of 1+, at ×40 magnification. Definitive IHC scoring was then performed as proposed in the ToGA trial [4]: "score 0/negative," no reactivity or membranous reactivity in less than 10% of tumor cells; "score 1+/negative," faint/barely perceptible membranous reactivity in more than 10% of tumor cells and cells that are reactive only in part of their membranes; "score 2+/equivocal," weak to moderate complete, basolateral, or lateral membranous reactivity in more than 10% of tumor cells; and "score 3+/positive," moderate to strong complete, basolateral, or lateral membranous reactivity in more than 10% of tumor cells. Samples with an IHC staining score of 0 because less than 10% of cells were positive were scanned by the automated image analyzer D-SIGHT (Menarini Diagnostics) to confirm the percentage of positive tumor area. In all cases scored 0/1+, HER2 expression was studied on additional blocks. The "most positive" block was considered for the final evaluation of HER2 results. Discordant cases were reviewed on a multihead microscope to reach a consensus.

2.3. FISH test

FISH was carried out using the same tissue block used for IHC and a dual-probe *HER2* (17q12) and *CEP17* (centromeric probe 17) assay (Vysis, Inc, Downers Grove, IL), according to the manufacturer's instructions as described elsewhere [15]. The whole slide was then screened at ×20 using an epifluorescence microscope (Zeiss, Gottingen, Germany). FISH analysis was performed automatically by the Metafer system through the PathVysion V2 classifier and Isis software by MetaSystems GmbH (Altlussheim, Germany) (Food and Drug Administration approved) and reviewed by at least 2 of the authors (L.V. and A.S.). A *HER2*:CEP17 ratio greater than 2 was defined as positive for *HER2* amplification. In cases with focal overexpression, gene amplification was evaluated using the D-SIGHT system (Menarini Diagnostics), which is able to relocate the cell cluster overexpressing HER2 by IHC on the slide tested by FISH.

2.4. Statistical analysis

Data were analyzed using Stata/SE statistical software (version 17.0; StataCorp LT, College Station, TX). Concordance among the different antibodies and between each antibody and FISH was calculated using the Cohen-Fleiss κ statistic (K) and the weighted κ (WK) statistic.

The cumulative survival rates were calculated by the Kaplan-Meier method, using the date of surgery as the

Table 1 Clinical and pathologic features of 148 cases of gastric cancers

Parameters	Values
Age (y)	
Range	34-89
Mean	69
Sex	
Male	89 (60%)
Female	59 (40%)
Site	
Cardia, fundus, body	71 (48%)
Antrum or pyloric	69 (46.6%)
Anastomosis	8 (5.4%)
Chemotherapy	
CT	34
No CT	47
Histotype	
Intestinal	97 (65%)
Diffuse	36 (24%)
Mixed	5 (3%)
Other	10 (7%)
Stage	
I	15 (10%)
II	48 (32%)
III	72 (49%)
IV	13 (9%)
Grade	
1	10 (7%)
2	58 (39%)
3	80 (54%)
Intestinal metaplasia	
Absent	100 (68%)
Present	48 (32%)
Helicobacter pylori	
Absent	132 (89%)
Present	16 (11%)

Abbreviation: CT, chemotherapy.

Table 2 IHC results of the 3 anti-HER2 antibodies on 1 tissue block of gastric cancer and concordance between each antibody by K and WK

	No. of cases			
	Score 0	Score 1+	Score 2+	Score 3+
Clone 4B5	120 (81%)	10 (6.7%)	2 (1.3%)	16 (11%)
CB11 Oracle	115 (77.7%)	15 (10%)	2 (1.3%)	16 (11%)
HercepTest	114 (77%)	17 (11.5%)	2 (1.5%)	15 (10%)
	K of Cohen-Fleiss 94.5% ($P < .05$)			
Concordance between:	K		WK	
Clone 4B5 and CB11 Oracle	96.2%		96.1%	
Clone 4B5 and HercepTest	94.5%		94.2%	
CB11 Oracle and HercepTest	95.6%		96.0%	

starting point; the follow-up period was assessed at the time of death or at the last clinical investigation of the patient. The disease-specific survival (DSS) was calculated from the date of definitive surgery to the date of death from the disease. Univariate analysis was used to examine which variables had prognostic significance. The survival differences were determined by log-rank analysis, and the variables taken into consideration were age, stage, grade, histotype, site and type of chemotherapy, and IHC results of 1 block and of multiple blocks. The relative hazards and the relative 95% confidence intervals (CIs) were calculated using the Cox proportional

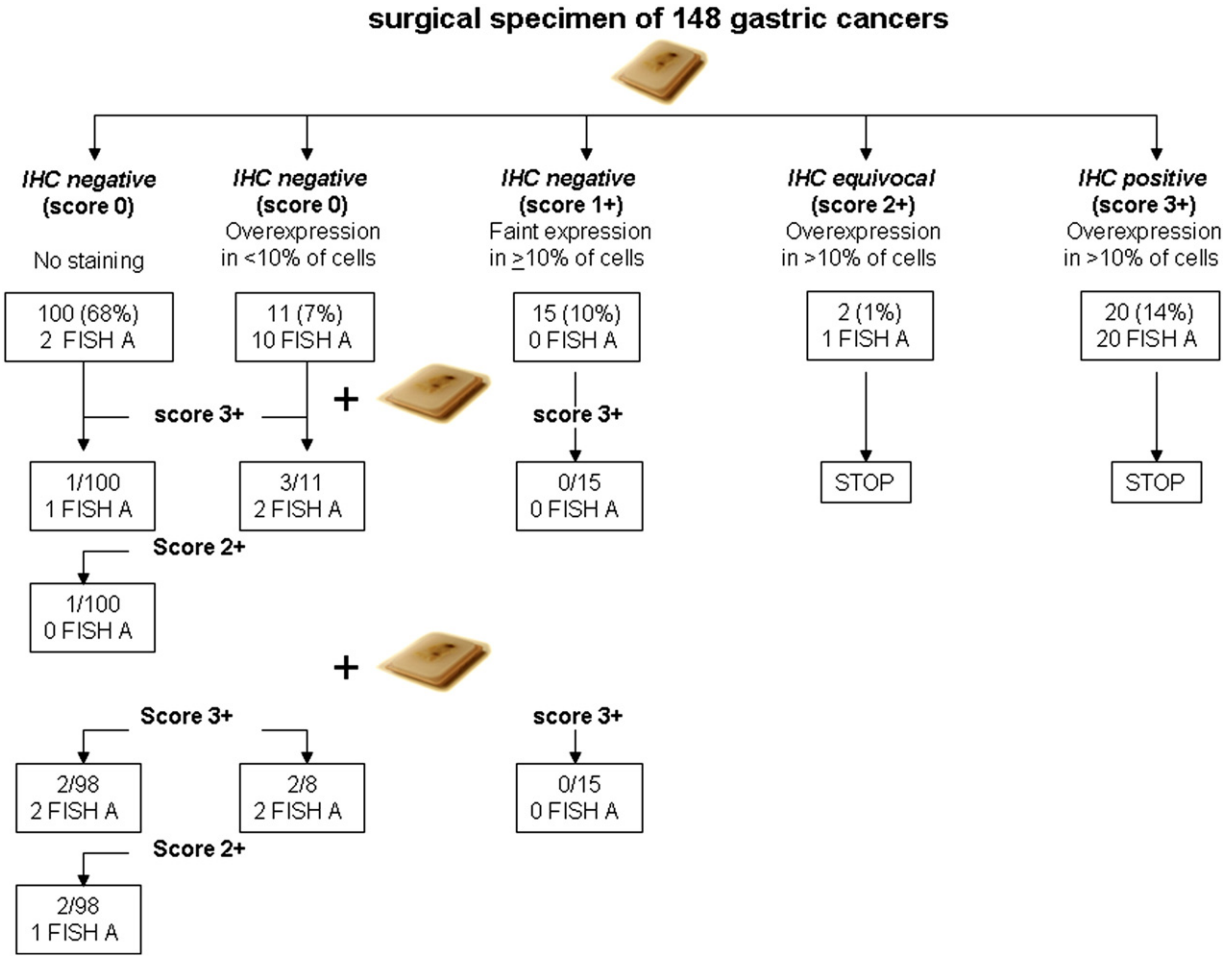

Fig. 1 Decisional workflow for the assessment of HER2 by IHC on 148 surgical specimens of gastric cancer. The decision to analyze 1, 2, or 3 tissue blocks is dependent on the IHC results. Multiblock approach, reserved to HER2-negative cases, enhances the HER2 test sensitivity by identifying cases with heterogeneous expression of HER2.

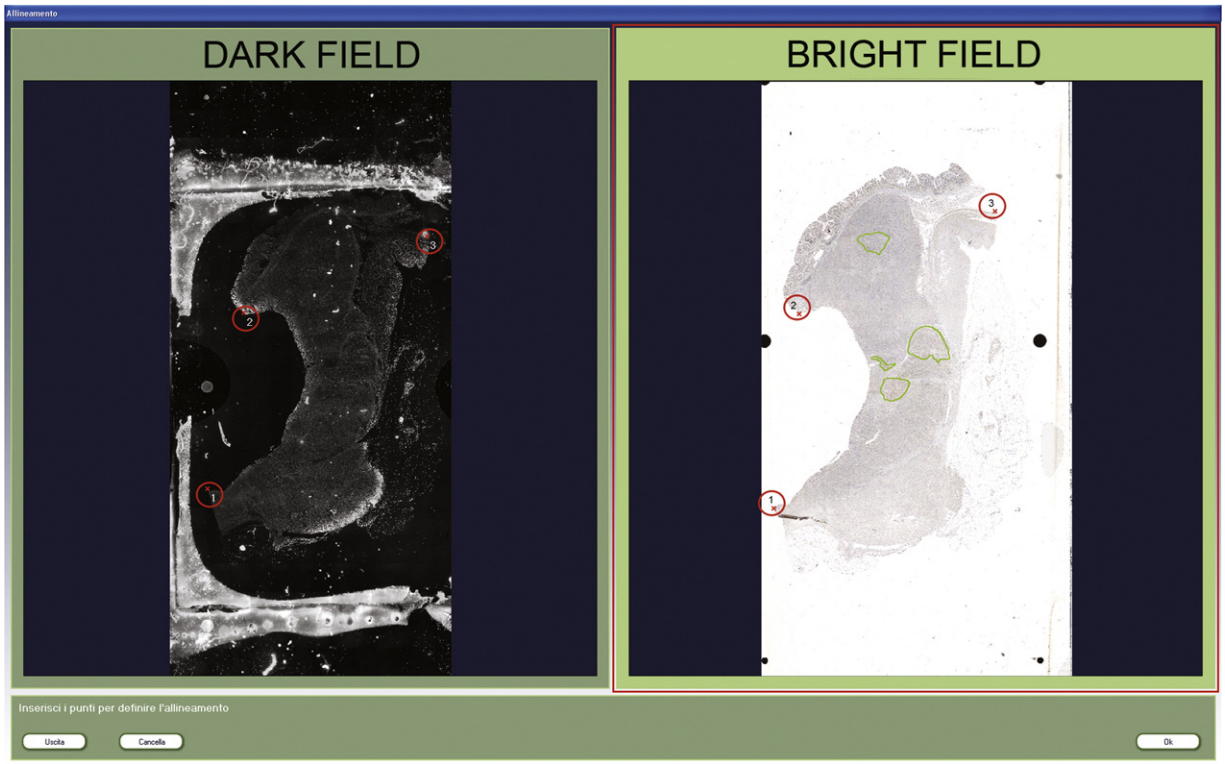

Fig. 2 Example of relocation of image analysis with D-SIGHT software (left, dark-field FISH) (right, bright-field IHC) using the 3-point alignment method (points inside red circles). The software is able to exactly relocate, on the FISH-scanned slide, the same field of interest selected on the IHC-stained slides (green circles).

hazard model. A P value less than .05 was considered statistically significant.

3. Results

The clinicopathologic features of the patients are reported in Table 1. The follow-up period for the patients ranged from 1 to 64 months (mean, 29 months). The therapeutic protocol was known for 81 of 98 cases of the Turin series only. Twenty-eight patients received adjuvant chemotherapy (fluoropyrimidine) after surgery. Three patients received neoadjuvant chemotherapy (platinum-fluoropyrimidine) before surgery. Two of these cases showed HER2 overexpression/amplification both on the preoperative biopsy and on the surgical specimen, whereas 1 case was HER2 negative.

3.1. Analysis of concordances

The comparison of HER2 scores obtained using the different antibodies did not show significant differences (Table 2). The 20 cases scored as 3+ were all amplified regardless of the antibody used, whereas only 1 of the 2 cases scored 2+ was amplified. The K statistic between the IHC results of the primary tumor and the metastases was 79% for 4B5 and CB11 and 71% for HercepTest ($P < .05$). Using 4B5 and CB11, 2 cases showed a clinically relevant discrepancy because the primary tumors were classified as negative (score 0, because HER2

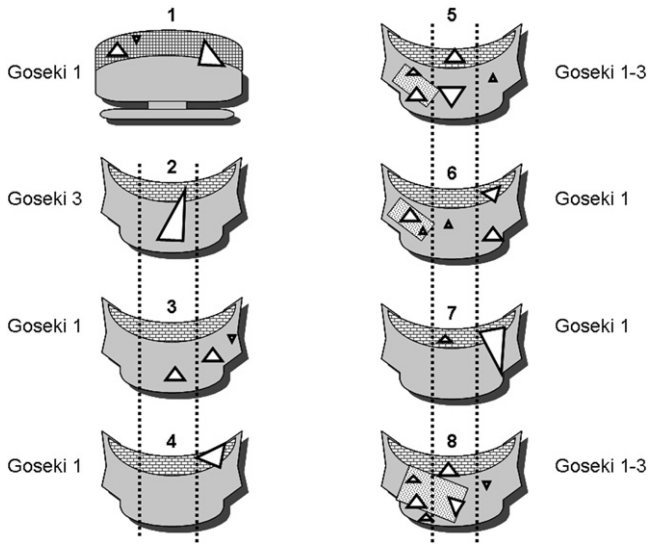

Fig. 3 Graphical correlation of HER2 expression, as detected on multiple blocks with tumor topography in cases originally negative on 1 block. In cases 5, 6, and 8, areas scored as 3+ (white triangle) were randomly distributed within score 2+ areas (dotted rectangle). The variance of HER2 expression was greater in tumors of groups I and III of the classification Goseki et al.

expression was strong but in <10% of cells), whereas the lymph node metastases were diffusely scored 3+. In another 2 cases, there was a minor discrepancy because the HER2 expression was either 3+ or 2+ in the primary or in the metastasis, but both tumor and lymph node metastases were amplified. The concordance between the IHC scoring of the 3 antibodies and the FISH results was slightly better in the metastases (82% for 4B5, 88.6% for CB11/Oracle, and 88% for HercepTest; $P < .05$) than in the primary tumors (80% for 4B5, 84% for CB11/Oracle, and 82% for HercepTest; $P < .05$).

3.2. Analysis of negative cases (score 0/1+)

To evaluate whether the assessment of HER2 using more than 1 block could rescue some of the 126 cases that tested negative (0/1+) with at least 2 of the antibodies, additional tissue blocks were processed for IHC analysis as shown in the workflow (Fig. 1) using the Oracle Kit.

Using 1 block, 100 cases were scored 0 (67.6%) because no immunostaining of tumor cells was observed; 2 of these cases were amplified either focally (1 case) or diffusely (1 case) by FISH. Fifteen cases were scored 1+, and all of them

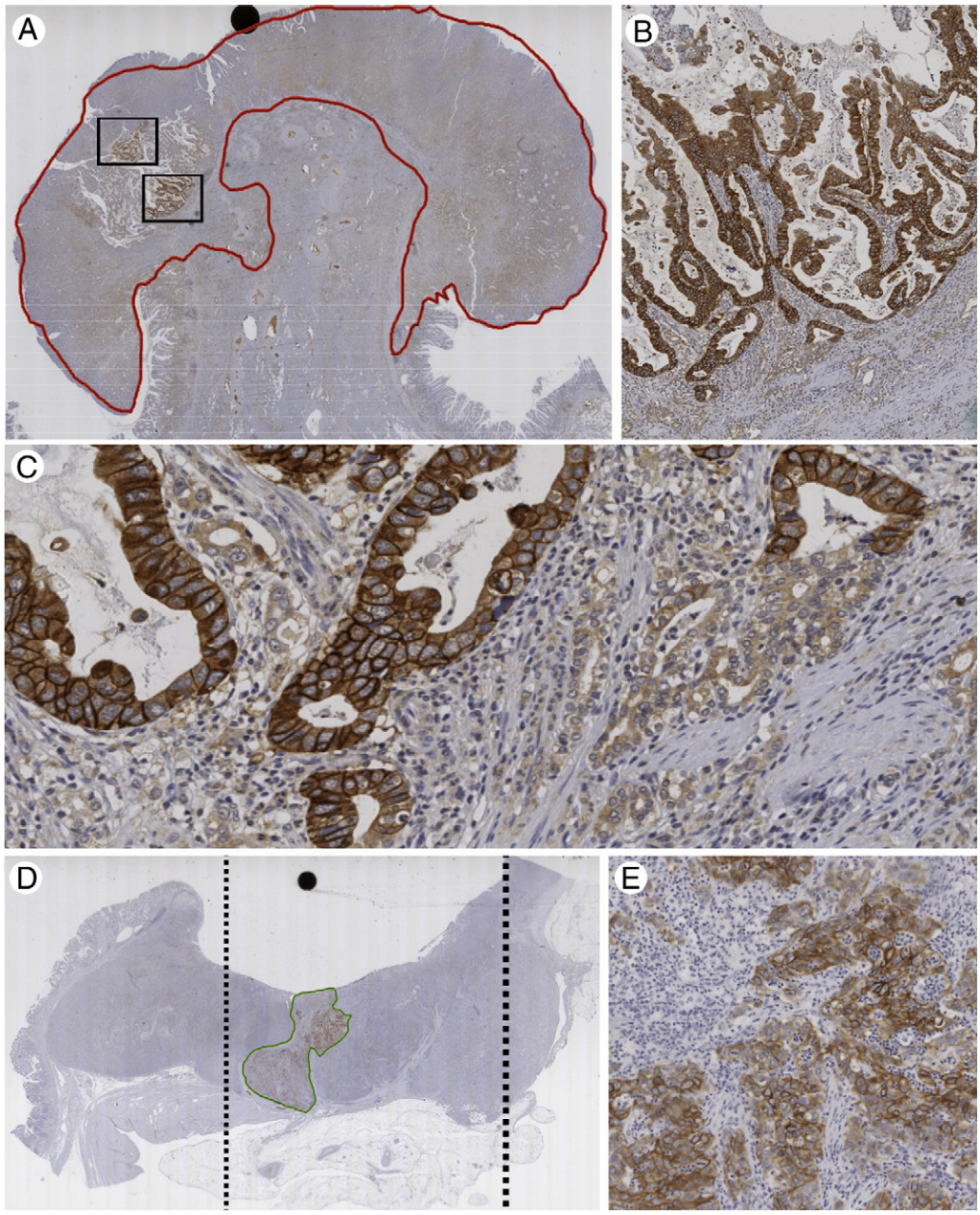

Fig. 4 A, Tumor with a polypoid growth pattern initially classified as negative using a single block evaluated using D-SIGHT. B and C, The area with intense membrane staining represented less than 10% of cancer cells (score 0) and Goseki group I. D-E, An example of tumor with infiltrative growth pattern showing HER2 moderate membrane staining in less than 10% of cancer cells in an area classified as Goseki group III.

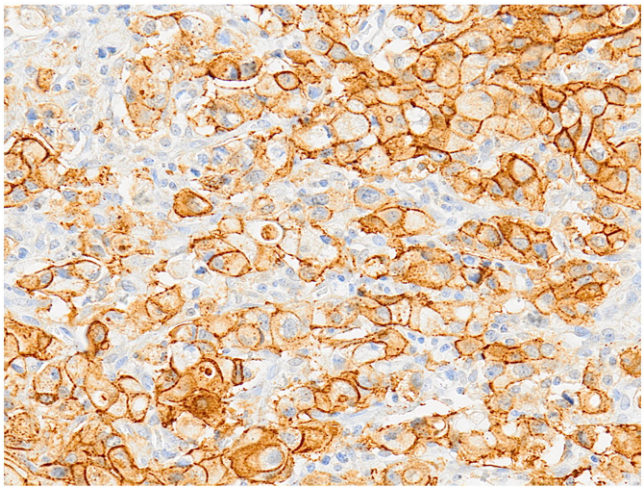

Fig. 5 Gastric carcinoma with signet ring features (group IV of Goseki classification) positive for HER2 (score 3+).

were not amplified by FISH. Eleven cases (7.4%) were scored as negative because the percentage of 2+/3+ tumor cells was greater than 10%. The automated evaluation of the score and percentage of positive cells of these 11 cases confirmed the semiquantitative analysis performed by conventional microscopy. The cell clusters deemed positive by IHC were also amplified by FISH, which was confirmed using the image analyzer D-SIGHT (Fig. 2), which is able to relocate precisely on the dark field (FISH) the area selected on the bright field (IHC).

The IHC score shifted from negative to 2+/3+ in 5 cases using 1 additional block and in 6 further cases using another extra block (Fig. 1). FISH analysis was repeated in the 3 cases that showed equivocal (2+) HER2 results in the additional blocks. One of these was amplified. The single case that was classified as diffusely *HER2* amplified by FISH and IHC negative (score 0) remained IHC negative in the additional block. To evaluate whether technical biases related to processing of gastrectomy specimens (eg, poor fixation) were the cause of this result, we analyzed HER2 in the preoperative biopsy of the same patient, which was amplified by FISH but HER2 negative by IHC as well.

The HER2 variance of the cases that were initially classified as negative using a single block was then correlated with tumor topography and with an at least rough sectorial mapping (eg, tumor center versus advancing edge) as reproduced in Fig. 3. One of these tumors had a polypoid growth (Fig. 4A-C), whereas all the others had an infiltrative growth (Fig. 4D and E). HER2 expression was generally randomly distributed in the tumor with a not-significant prevalence of superficial area as compared with the infiltrative deep edges. In some cases, the heterogeneity was not only related to the topographic distribution but also to the different intensity of the staining, with score 3+ foci being randomly distributed within score 2+ areas (Fig. 3, cases 5, 6, and 8). The correlation using the Goseki classification [16] showed that the variance of HER2 expression was greater in tumor showing tubular differentiation and low mucus accumulation (groups I and III) (Fig. 3). Two cases of the diffuse type with signet ring feature (group IV) were positive on the first block (Fig. 5). No correlation with nuclear pleomorphism or mitoses and HER2 expression was observed.

3.3. Sensitivity, specificity, and accuracy between IHC and FISH

The sensitivity, specificity, and accuracy of the IHC testing of all 148 cases compared with the FISH analysis were then recalculated using the results obtained by testing 2 or 3 tissue blocks of the cases that were negative on the first block. Considering FISH to be the criterion standard, the addition of further blocks to the HER2 evaluation increased both the sensitivity and accuracy of the IHC results, whereas the specificity decreased if cases with 2+ scores were supposed to be amplified (Table 3).

3.4. Clinical correlation

The univariate analysis (Table 4) showed a significant correlation for age, stage, grade, and IHC results performed on multiple blocks with DSS. The Kaplan-Meier analysis did not show any significant correlation ($P = .71$) for the results obtained using a single block to define HER2-negative status and DSS (Fig. 6A); on the contrary (Fig. 6B), HER2 positivity was significantly ($P = .011$) correlated with a worse prognosis when assessed using multiple blocks.

Multivariate Cox regression analysis confirmed that HER2 expression, as defined by the examination of multiple

Table 3 Sensitivity, specificity, and accuracy of immunohistochemistry (compared with FISH) in 148 cases of gastric cancer by adding 1 or 2 tissue blocks in negative cases (score 0/1+)

	Sensitivity		Specificity		Accuracy	
	% (TP/A)	CI (95%)	% (TN/NA)	95% CI	% (TP + TN/total)	95% CI
1 block	63.63 (21/33)	45.14-79.04	99.13 (114/115)	94.54-99.95	91.22 (135/148)	85.15-95.05
2 blocks	73.53 (25/34)	55.35-86.49	98.25 (112/114)	93.18-99.69	92.57 (137/148)	86.77-96.05
3 blocks	83.33 (30/36)	66.53-93.04	97.32 (109/112)	91.79-99.31	93.91 (139/148)	88.43-97

Abbreviations: TP, true positive (IHC score 2+/3+ and FISH amplified); A, amplified cases; TN, true negative (IHC score 0/1+ and FISH not amplified); NA, not amplified cases.

Table 4 Univariate analysis of clinical and pathologic data correlated with DSS

		n	Events	Mean DSS	95% CI	χ^2	*P*
Age	<70 y	67	28	48.52	43.49-53.55	4.279	.039
	≥70 y	81	36	40.27	34.55-46.00		
Stage	I	15	2	54.62	45.09-64.16	11.38	.01
	II	48	12	48.91	41.85-55.98		
	III	72	39	41.72	36.09-47.35		
	IV	13	11	37.03	27.38-46.68		
Grade	I	10	1	63.00	63.00-63.00	6.186	.045
	II	58	21	42.86	36.08-49.27		
	III	80	42	42.51	37.27-47.76		
Histotype	Intestinal	97	34	44.00	38.95-49.06	5.63	.13
	Diffuse	36	25	41.03	33.37-48.69		
	Mixed	5	2	30.62	13.66-47.59		
	Other	10	3	59.66	52.12-62.12		
Site	Cardia, fundus, body	71	28	42.82	36.99-48.65	0.722	.697
	Antrum, pylorus	69	34	44.62	39.05-50.20		
	Anastomosis	8	2	46.00	18.56-73.44		
Chemotherapy	No	47	20	31.19	23.74-38.65	1.959	.162
	Yes	34	12	38.30	30.04-46.57		
IHC with 1 block	IHC score 0-1-2NA	127	54	44.54	40.31-48.77	0.134	.71
	IHC score 2A-3	21	10	42.50	32.13-52.87		
IHC with new protocol	IHC score 0-1-2NA	118	46	46.89	42.66-51.12	6.436	.011
	IHC score 2A-3	30	18	34.67	26.07-43.27		

Abbreviations: NA, not amplified; A, amplified.

blocks, was an independent variable for prognosis (hazard ratio [HR], 1.572; 95% CI, 1.195-2.112; *P* = .001), together with the age of patients (HR, 1.986; 95% CI, 1.187-3.324; *P* = .009), the disease stage (HR, 1.749; 95% CI, 1.2-2.549; *P* = .004), and the grade (HR, 2.797; 95% CI, 1.018-7.682; *P* = .046) of gastric cancer.

4. Discussion

Previous studies have described HER2 expression heterogeneity as a frequent event in gastric cancer [7,10,17,18]. In breast cancer, the intratumor heterogeneity of the *HER2* gene is defined as a tumor showing more than

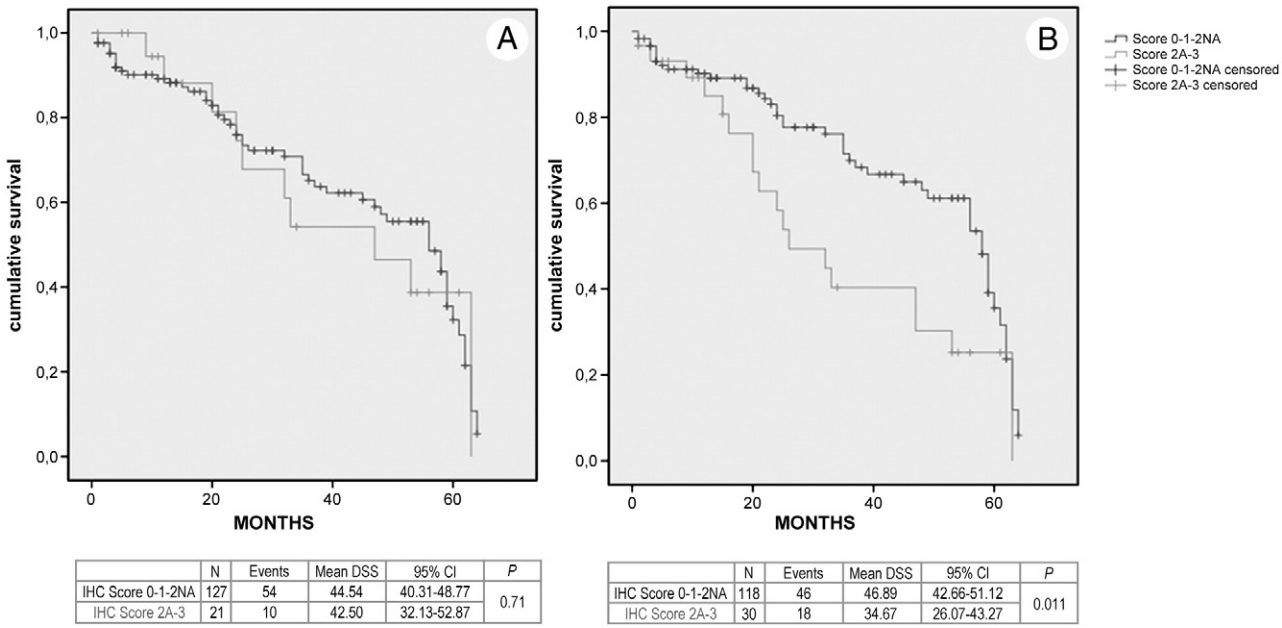

Fig. 6 Kaplan-Meier estimates of DSS (in months) according to HER2 status assessed using 1 block (A) and multiple blocks (B) of surgical specimens of gastric cancer. Abbreviations: NA, not amplified; A, amplified.

5% but less than 50% of infiltrating cells, with a ratio higher than 2.2 by FISH and the incidence ranging from 5% to 30% [19]. In gastric carcinoma, the intratumor heterogeneity has yet to be specifically defined. For example, in a recent work, Lee et al [20] focusing on IHC 3+ or IHC 2+/SISH-positive tumors determined that negative or not uniform weakly stained areas were present in 50% of the surgical and in 42% of biopsy specimens, respectively. Boers et al [9] stated that the number of positive fragments and/or of calculated *H*-score did not predict amplification status in 73% of biopsy specimens due to heterogeneity of HER2 expression of gastric adenocarcinomas.

In the present work, we assessed heterogeneity in a series of 126 gastric cancers, of 148 surgically resected, that were negative at the IHC analyses performed using different antibodies on 1 tissue block. We can exclude a significant influence for the antibody's selection if the IHC procedures are adequately performed [12] and if the scoring follows strict rules [10,14]. On the other hand, in our case series, inadequate sample selection produced 7.1% of false-negative results. This suggests that the HER2 IHC analysis of a single block of the primary tumor could not be sufficiently confident to compensate for the heterogeneity of HER2 expression in a large gastric cancer. For example, in the present series, the mean size of the tumor was around 5.5 cm. Using a single block, 14% of gastric cancers were positive, whereas the addition of further blocks increased the rate to 20%, a value that was slightly higher than that obtained by Hofmann et al [10] using 1 block (17%). However, the analysis of 3 tissue blocks increased the positive rate to approximately 50% of the cases overexpressing HER2 but scored as negative because the percentage of positive cells was less than 10% in a single block. In our series, only 1 (0.7%) of 148 case was diffusely amplified by FISH and constantly negative by IHC, whereas 9 amplified cases were rescued from IHC negative to positive by testing at least 3 blocks. The accuracy of IHC, as compared with FISH, increased from 91% to about 94% using multiple-block analysis. In the recently published guidelines on HER2 in gastric cancer [12], it is suggested that for surgical specimen cases with strong HER2 staining in less than 10% of cells, retesting with ISH may be warranted. If such a sample is FISH-SISH positive, the tumor may be considered to be HER2 positive similar to the scoring on biopsy samples [12].

Heterogeneity may also lead to discrepancies between primary tumors and metastases. For example, in the present work, we showed a clinically relevant difference in 2 cases that were scored 0 (not eligible for trastuzumab therapy) in the primary tumor because of the low percentage of HER2-positive cells and scored 3+ in the metastatic lymph node (eligible for trastuzumab).

The hypothesis that the level of HER2 protein might influence the response to trastuzumab was investigated in a post hoc analysis of the ToGA trial [4]; this analysis revealed a significant increase in the response to trastuzumab in the subgroup with high HER2 expression. However, some benefits were also achieved by treating patients with *HER2* gene amplification by FISH and protein negativity by IHC. The underlying explanations for these results have not been fully evaluated. We demonstrated that multisampling detection of HER2 expression could potentially identify cases with higher levels of intratumor heterogeneity, which may be associated with adverse outcomes but, in turn, could have a therapeutic advantage from trastuzumab treatment. As a matter of fact, HER2 positivity correlated with a worse prognosis and was an independent variable in multivariate analysis only when assessed using multiple blocks. These results seem biologically more reliable than those reported by others [6] on tissue microarrays obtained from a single paraffin block, where a lower HER2 expression failed to be an independent prognostic factor.

Thus, if the HER2 test is performed on surgical samples of gastric carcinoma, the results may be influenced by the tissue block more than by the antibody selection. Gastric tumors at diagnosis are generally larger than breast tumors, and the analysis of a single block may not compensate for the possible intratumor heterogeneity. Particularly, when the HER2 overexpressing cells are focally present, more than 1 tissue block should be tested by IHC.

In conclusion, the workflow we propose (Fig. 1), while producing a relatively higher workload for pathologists, increases the accuracy of the HER2 testing in gastric cancer.

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