Combined HER2 analysis of biopsies and surgical specimens to optimize detection of trastuzumab-eligible patients in eso-gastric adenocarcinoma: a GERCOR study

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Background: HER2 is overexpressed in 10 to 20% of gastro-esophageal adenocarcinoma (GE-ADK), and is a target for trastuzumab in metastatic patients. We conducted a study to compare HER2 expression between diagnostic biopsies (DBs) and surgical specimens (SSs) of GE-ADK, and to determine the influence of non-trastuzumab containing neoadjuvant chemotherapy (NAC) on this expression.

Patients and methods: Pathological specimens from biopsies of 228 patients operated on between 2004 and 2011 were collected. Two cohorts treated (n = 141) or not (n = 87) with a NAC were constituted. Two blind independent pathological HER2 analyses on DB and on SS were carried out using immunohistochemistry (IHC) and colorimetric in situ hybridization (CISH). HER-2 overexpression (HER2+) was defined by a score 3+ in IHC, or 2+ with a positive CISH test, according to the specific HER2 scoring guidelines for GE-ADK.

Results: Paired HER2 status could be determined for 218 out of the 228 patients (95.6%). HER2+ rates were 13.3% on DB (29/218) and 14.7% on SS (32/218). HER2+ tumors were mainly cardial or esophageal adenocarcinomas, with a well-differentiated, intestinal histological type. HER2 status differed between DB and SS in 6% of cases. When DB analyses were added to SS analyses, the relative increase in HER2+ cases was 13.5% (17.1% for patients with NAC and 23.5% for patients with histological response to NAC, versus 7.1% for patients without NAC, P = 0.4, NS). Differences between DB and SS HER2 expression could be explained by intratumoral heterogeneity and by a HER2 expression decrease in SS after NAC in responding patients possibly due to a higher chemosensitivity of HER2-positive clones.

Conclusion: The determination of HER2 status on DB provides results that complete those obtained with SS. Combining the analysis of DB and of SS enables to optimize the selection of trastuzumab-eligible patients in case of metastatic relapse, and particularly in previously NAC-responding patients.

Key words: Her2, gastric cancer, neoadjuvant chemotherapy

introduction

Gastro-esophageal adenocarcinoma (GE-ADK) is the second most frequent digestive cancer [1]. The only curative treatment of GE-ADK consists of surgery for operable tumors. In 2006, the MAGIC trial showed that perioperative chemotherapy increases 5-year overall survival [2]. The benefit of this strategy has been confirmed in several studies since then [3–5], and perioperative 5-FU combined with platinum salt is now the standard of care for localized GE-ADK.

HER2 is overexpressed in 10%–20% of GE-ADK [6] and represents controversial prognostic factors [7, 8]. HER2 status can be determined on pathological samples from both primary GE-ADK and corresponding metastases using immunohistochemistry (IHC) and in situ hybridization, either by fluorescence (FISH) or by colorimetry (CISH). HER2 overexpression is commonly defined as 3+ in IHC or 2+ in IHC with amplification in ISH [9]. In metastatic HER2-overexpressing GE-ADK, ToGA trial showed that trastuzumab combined with platinum and 5-FU significantly improves overall and disease-free survival rates [10] when compared with chemotherapy without trastuzumab, and is now a standard of...
Patients and Methods

Study Population, Therapeutic Strategies and Design

All patients presenting the following inclusion criteria were included:

1. Histological proof of gastric or oeso-gastric (low oesophagus and cardia) adenocarcinoma.
2. Surgery with a curative aim carried out between 1 January 2004 and 31 December 2011 in Institut Mutualiste Montsouris or in Hôpital Saint-Antoine.

We excluded patients for whom diagnostic pre-treatment biopsies could not be collected, or for whom the pathological material from the DBs was depleted or non-interpretable.

Two cohorts were designed; the NAC cohort for patients who received a 5-FU and platinum salt-based NAC before surgery; and the No-NAC cohort for patients who didn’t receive NAC.

All the living patients were informed of the goal of the study by letter and asked whether they agreed to participate. Clinical and pathological data were centralized in a controlled and access-limited database. The protocol was approved by institutional review board according to the national requirements.

Constitution of Pathological Collections

Pathological centers in which diagnostic paraffin-embedded biopsies had been analyzed and stored were asked for tumoral material from each patient. Paraffin-embedded blocks from surgical specimens were stored since the date of surgery in both investigating centers. Paired samples (containing the DBs and one block from the surgical specimen) were anonymised and identifiable by a histology and study number.

Pathological Analyses

IHC was carried out on all available paired samples. HER2 expression was detected using the anti HER2/neu (clone 4B5) antibody on an automated immunostainer (BenchMark XT Ventana) according to manufacturer’s instructions. HER2 status was determined using the validated scoring guidelines for GE-ADK [11].

All tumor pairs that showed a score 2+ on the biopsy and/or the surgical specimen were tested for HER2 amplification in dual CISH (BenchMark XT, Ventana HER2 dual-color ISH assay). Amplification of HER2 was defined as a ratio HER2/CEP17 ≥ 2, as recommended. All IHC and CISH tests were analyzed with two independent blind readings by two specialists in pathology. In case of discordance between both analyses, a third reading was carried out conjointly to reach a consensus. HER2-overexpressing (HER2+) tumors were defined as 3+ in IHC or 2+ in IHC with amplification in CISH.

NAC histological response was established on surgical specimens according to the usual guidelines for GE-ADK [12].

Results

Population

Of the 278 selected patients, 39 were excluded due to impossibility of biopsy collection, and 11 to exhausting of pathological material. Thus, paired samples from 228 patients have been collected. All samples have been tested for HER2 overexpression in IHC and samples with a score 2+ in IHC have been tested in CISH. Among them, 10 samples could not be analyzed due to technical failures or absence or residual tumor cells; thus, 218 paired samples were finally contributive.

The characteristics of the population are summarized in Table 1. No-NAC patients had been mostly operated on before NAC patients, since the benefit of perioperative chemotherapy has been demonstrated in 2006 [2].

Clinical and Histological Characteristics of Tumors

Almost half of the 218 tumors analyzed in the study were located at the eso-gastric junction (cardia and/or low esophagus). HER2-positive (HER2+) tumors were significantly more frequently proximal tumors compared with HER2-negative (HER2−) tumors (P = 0.02) (Table 2).

Table 1. Characteristics of the population at baseline

<table>
<thead>
<tr>
<th></th>
<th>No NAC</th>
<th>NAC</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Number (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Montsouris</td>
<td>38 (96)</td>
<td>32 (85)</td>
<td>70 (98)</td>
</tr>
<tr>
<td>Institute</td>
<td>7 (18)</td>
<td>6 (15)</td>
<td>13 (18)</td>
</tr>
<tr>
<td>Saint-Antoine hospital</td>
<td>7 (18)</td>
<td>2 (5)</td>
<td>9 (13)</td>
</tr>
<tr>
<td>Date of surgery—number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2004–2006</td>
<td>31 (29)</td>
<td>11 (28)</td>
<td>42 (33)</td>
</tr>
<tr>
<td>2007–2011</td>
<td>4 (4)</td>
<td>3 (3)</td>
<td>7 (5)</td>
</tr>
<tr>
<td>Gender—Number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>63 (17)</td>
<td>29 (19)</td>
<td>92 (12)</td>
</tr>
<tr>
<td>Male</td>
<td>55 (12)</td>
<td>112 (14)</td>
<td>167 (21)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (SD)</td>
<td>66 (13.6)</td>
<td>63 (11.5)</td>
<td>64 (12.5)</td>
</tr>
<tr>
<td>Range</td>
<td>37–93</td>
<td>27–80</td>
<td>27–93</td>
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<td>Tumor localization—number (%)</td>
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<td></td>
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<tr>
<td>Esophagus/cardia</td>
<td>34 (15)</td>
<td>79 (36)</td>
<td>113 (49.6)</td>
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<td>Fundus</td>
<td>29 (33)</td>
<td>30 (21.3)</td>
<td>59 (25.9)</td>
</tr>
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<td>Pylorus</td>
<td>24 (27.6)</td>
<td>32 (22.7)</td>
<td>56 (24.5)</td>
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<td>pTNM status—number (%)</td>
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<td></td>
</tr>
<tr>
<td>T0</td>
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<td>2 (1.5)</td>
<td>2 (0.9)</td>
</tr>
<tr>
<td>T1</td>
<td>18 (20.7)</td>
<td>24 (17.0)</td>
<td>42 (18.4)</td>
</tr>
<tr>
<td>T2</td>
<td>34 (39.1)</td>
<td>43 (30.5)</td>
<td>77 (33.8)</td>
</tr>
<tr>
<td>T3</td>
<td>29 (33.3)</td>
<td>58 (41.1)</td>
<td>87 (38.1)</td>
</tr>
<tr>
<td>T4</td>
<td>6 (6.9)</td>
<td>14 (9.9)</td>
<td>20 (8.8)</td>
</tr>
<tr>
<td>N0</td>
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<td>54 (38.3)</td>
<td>78 (34.2)</td>
</tr>
<tr>
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<td>17 (19.5)</td>
<td>34 (24.1)</td>
<td>51 (22.4)</td>
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<tr>
<td>N2</td>
<td>20 (23.0)</td>
<td>29 (20.6)</td>
<td>49 (21.5)</td>
</tr>
<tr>
<td>N3</td>
<td>26 (29.9)</td>
<td>24 (17.0)</td>
<td>50 (21.9)</td>
</tr>
<tr>
<td>M0</td>
<td>84 (96.6)</td>
<td>132 (93.6)</td>
<td>216 (94.7)</td>
</tr>
<tr>
<td>M1*</td>
<td>3 (3.4)</td>
<td>9 (6.4)</td>
<td>12 (5.3)</td>
</tr>
</tbody>
</table>

*pM1: Infraclinic peritoneal or hepatic metastases discovered intraoperatively.
HER2+ tumors, HER2 overexpressing tumors; HER2− tumors, HER2 not overexpression tumors.

Intestinal tumors represented almost half of the general population, and were significantly more frequent in HER2+ tumors compared with HER2− tumors (P = 0.002). In the general population, poorly differentiated tumors were predominant, but HER2+ tumors were mostly well differentiated (P < 10^{-4}).

### analysis of HER2 status on biopsies and surgical specimens

All samples have been analyzed for HER2 overexpression in IHC (supplementary Figure 1A, available at Annals of Oncology online). In biopsies, 163 of 228 samples (71.5%) showed a score of 0/1+, 63 of 228 (27.6%) a score 2+/3+ and 2 of 228 (0.9%) were not contributive. In surgical specimens, 163 of 228 (71.5%) showed a score of 0/1+, 62 of 228 (27.2%) a score of 2+/3+ and 3/228 (1.3%) were not contributive. Despite strong similarities in the rates of 0/1+ and 2+/3+ between biopsies and surgical specimens, 37 (17.0%) paired samples showed discrepancies in HER2 status in IHC between both analyses (score 0/1+ on the biopsy that turned 2+/3+ on the surgical specimen or score 2+/3+ on the biopsy that turned 0/1+ on the surgical specimen).

The 59 paired samples that showed a score 2+ on the biopsy or on the surgical specimen have been analyzed for HER2 amplification in CISH (supplementary Figure 1B, available at Annals of Oncology online). Among them, 38 samples were 2+ on biopsies (the remaining 21 biopsies being 0/1+, while corresponding surgical specimens were 2+), and 39 on surgical specimens (the remaining 20 surgical specimens being 0/1+ while the corresponding biopsies were 2+). HER2 gene amplification was detected in 5 samples (8.5%) of biopsies and in 10 samples (17%) of surgical specimens. In biopsies, on the 38 samples that were 2+ in IHC, only four (10.5%) were HER2 amplified in CISH. In surgical specimens, 9 of 39 (23.1%) that were 2+ in IHC were CISH-positive.

Concordance rates of IHC and CISH results between both pathologists reached 99.1% and 87.3%, respectively (supplementary Table S1, available at Annals of Oncology online).

Finally, 29 of 218 tumors (13.3%) were HER2+ on biopsies, and 32 of 218 (14.7%) on surgical specimens. In no-NAC cohort, HER2 overexpression was found in 10 of 83 tumors (12%) on biopsies and in 13 of 83 tumors (15.7%) on surgical specimens. In the NAC cohort, HER2 overexpression was found in 19 of 135 (14.1%) tumors on biopsies and also in 19 of 135 (14.1%) tumors on surgical specimens, positive tumors on biopsies and surgical specimens being possibly from different patients (Table 3).

### concordance of HER2 status between paired biopsies and surgical specimens

On all 218 paired samples, the overall concordance rate between biopsies and surgical specimens reached 94% (205/218), and this rate was similar in both the cohorts. In no-NAC patients, five tumors showed discordances between HER2 status in the biopsy and in the surgical specimen; among them, four were HER2− on biopsy that turned HER2+ on surgical specimen (positive shift), and only one was HER2+ on biopsy that turned HER2− (negative shift) on surgical specimen.

In NAC patients, eight tumors presented discordant results, with four positive and four negative shifts. Among the four tumors that showed negative shifts, three of them presented major histological response, and one presented minor histological response. The four positive shifts were all detected in patients with no histological response. The four tumors for which HER2 status could not be detected on surgical specimen either due to IHC failure (n = 2) or due to CISH failure (n = 2) were all detected in patients with major histological response.

### contribution of combined analysis of biopsies and surgical specimens

In the general population, combining biopsies with surgical specimens’ analyses enabled to increase the rate of HER2+ tumors from 14.7% (when HER2 status is determined on...
surgical specimens only) to 17% (Figure 1A). This represented a relative increase in HER2+ cases of 13.5% (Figure 1B). In no-NAC patients, combining both analyses enabled to increase only marginally the rate of HER2+ tumors (15.7 to 16.9%; relative increase: 7.1%). Among NAC patients, combining analyses increased the relative rate of HER2+ tumors of 23.5% for patients with histological response to NAC (Figure 1B), but was of no interest in patients with no histological response.

**discussion**

In this study carried out on 228 paired samples of GE-ADK, we evaluated the concordance between HER2 status on coupled biopsies and surgical specimens. Previous studies dealing with HER2 in GE-ADK have mainly focused on the concordance between IHC and ISH techniques [13, 14], or on the concordance between HER2 status on primary tumors and paired metastases [15–17]. Only few studies have evaluated the concordance between paired biopsies and surgical materials, and the impact of a NAC has never been established [8, 18–22].

In our study, the HER2 overexpression rate on surgical specimens reaches 14.7%, and the concordance rate between paired biopsies and surgical specimens is 94%, which is consistent with other studies showing concordance rates ranging from 74.1% to 92.9% (supplementary Table S2, available at Annals of Oncology online).

The concordance rate is similar whether patients have received an NAC or not, but profiles of discrepancies differ, with mostly positive shifts on surgical specimens for no-NAC patients, and a similar frequency of positive and negative shifts for NAC patients. Moreover, combining biopsies with surgical specimen analyses induces a relative 17.4% increase in HER2+ tumors for all NAC patients, and a 23.5% increase for patients with histological response, as opposed to 7.1% for no-NAC patients and to 0% for NAC patients with no histological response.

HER2 intratumoral heterogeneity in GE-ADK is found in 20%–70% of HER2+ tumors [23–25] and is the major cause of discrepancies between biopsies and surgical specimens. We show here that HER2 determination could be modified by NAC on surgical specimens, and that restricting HER2 analysis to surgical specimens after NAC may deprive a notable number of patients of a possibly active treatment. For patients presenting clinical and histological responses to NAC, HER2 analysis on surgical specimens is not reliable, due to a higher frequency of technical failures and negative shifts, and due to the possible
absence of residual tumor cells. HER2+ tumor cells have been shown to have a higher chemosensitivity than HER2− cells [26], which can lead to the impossibility of HER2 detection in tumors with high pathological responses after NAC.

This study suggests that recommendations for HER2 status determination should be adjusted taking into account the prevalence of intratumoral heterogeneity and response to NAC. Gastroenterologists should be encouraged to carry out extensive biopsies during initial gastroscopy. For patients with localized tumors, the analysis of surgical specimens should remain the gold standard in the absence of NAC. However, in NAC patients, HER2 status should be established on initial pre-treatment biopsies, and, if negative, re-assessed on surgical material. This could enable us to optimize the detection of HER2+ tumors, and thus, the number of patients that could benefit from trastuzumab treatment.

title{funding}

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title{disclosure}

The authors have declared no conflicts of interest.

title{references}