

Research Article

Evaluation of the Expression of the Human Epithelial Receptor 2 (HER2) in Gastric Carcinoma

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Objective. To evaluate the HER2 expression on gastric adenocarcinoma from a Brazilian population and also to analyze the relations between the receptor and clinical characteristics, as well as the survival status. *Materials and Methods.* A retrospective analysis was conducted from January of 2008 to July of 2012, considering only gastrectomies with curative intent. Tumors were tested for HER2 status using immunohistochemistry. The relation between HER2 status and clinical aspects, surgical findings, and survival were also analyzed. *Results.* 222 patients with gastric carcinoma were submitted to surgery during that period, but only 121 (54,5%) were with curative intention. The immunohistochemistry revealed that 4 patients (3,3%) were HER2-positive, 6 patients (4,9%) HER2-undetermined, and 111 patients (91,7%) HER2-negative. There was no statistical concordance between HER2 status and survival or the clinical aspects. *Conclusion.* The HER2 overexpression rate was very low in this Brazilian population sample and cannot be considered as a prognostic factor.

1. Introduction

Gastric carcinoma is one of the most common tumors in the world. An estimate made by the National Cancer Institute of the Brazilian Health Ministry (INCA) for 2016 was 12,920 new cases in men and 7,600 in women, occupying the fourth position for men and fifth for women [1]; in the world setting, it is found with a frequency which varies from second to fifth position, depending on the region under evaluation, with more than 70% of the cases occurring in developing countries [2]. Even though much has been accomplished in the combat of this disease, it still persists as one of the most aggressive tumors; its survival rate of 5 years is approximately 60%, and this drops to 2 to 5% once metastases have been identified. In this manner, the molecular aspects have attracted even more attention on the part of researchers as a promising therapeutic approach, especially in more advanced cases whose surgical limitations reduce the treatment options even further.

The epithelial growth factor receptors (EGFR: HER1, HER2, HER3, and HER4) are cellular membrane structures expressed in several healthy tissues and in various tumors, particularly in those of epithelial origin, and their activation apparently has an important relationship with the genesis of these tumors, due to the stimulation of cellular proliferation stimulus and the inhibition of apoptosis [3]. The superexpression of HER2 has been closely related to breast cancer, colorectal carcinoma, and nonsmall cell lung cancer, in fact suggesting a worse prognosis [4, 5]. In esophagogastric carcinoma, the expression appears to vary between 5 and 25% [6, 7], without reliable data as to its prognosis [8–11].

Over the last few years, diverse medications having an effect on EGFR have been developed. One specific group, which has presented promising results [12–14], is that of the monoclonal antibodies (cetuximab and trastuzumab) that bond with the extracellular domain of the receptor. Another group of agents includes tyrosine-kinase inhibitors

(gefitinib, lapatinib, and erlotinib), acting on the intracellular level, which may present good results when associated with conventional chemotherapeutic treatments [15–17].

The primary objective of this study was to evaluate the positivity of the HER2 expression in gastric adenocarcinomas in the Brazilian population that had undergone surgery. Secondly, we investigated the relationship between this receptor and the patient characteristics and their possible influence on the prognosis.

2. Materials and Methods

2.1. Patients. A retrospective analysis was made based on prospectively acquired data, collected from a specific protocol filled in every gastrectomy due to gastric adenocarcinoma performed with curative intent at the Hospital of Santa Casa of São Paulo Medical School from January 2008 to July 2012. All were submitted to surgery with curative potential (free margins, negative cytology, and adequate lymphadenectomy), independent of the stage, histological type or any other variable. The follow-up time was at least 3 years or until death. Perioperative deaths were excluded.

Right after the resection, the specimens were fixed in 10% neutral buffered formalin for a period not less than 18 h or more than 24 h, as recommended by the Pathology Department of our institution, as well as the reagents' manufacturer. The tissues then were sliced and conserved in paraffin. The paraffin blocks that presented the best tumor representation were analyzed on slides stained with hematoxylin-eosin. Once the block had been chosen, the material was sliced into new sections measuring 3 to 5 μm on a microtome and placed on silanized slides. The whole process of obtaining the immunohistochemical reaction was performed with the *HerceptTest*[®], using the machine *AutoStainer Plus*[®], following the manufacturer's instructions (both *Dako*[®]).

The processes of deparaffinization and hydration were performed at room temperature in xylene and alcohol baths, finishing in a distilled water bath. Antigenic recovery was then performed making use of a solution supplied in the kit and an endogenous peroxidase blocker (incubation in a humid chamber for 5 minutes with 100 μL of the solution on each slide). For all of the processes, 300 μL of the primary antibody anti-HER-2/neu, a polyclonal developed by *Dako* (code A0485) from rabbits, was applied to each slide. The secondary antibody supplied in the kit, called visualizing reagent, is a dextran polymer conjugated from horseradish with anti-rabbit goat immunoglobulins. A quantity of 100 μL of this reagent was used on each slide. Lastly, 100 μL of the 5% chromogen diaminobenzidine (DAB) was placed on each slide and subsequently washed with distilled water, which gave the slides the typical brown color of the reaction. The counter-staining was performed with hematoxylin to identify the nuclei, in order not to have doubts as to the identification and reading of the membrane coloring. The slides were then washed, dehydrated, and assembled. The controls used were obtained from breast adenocarcinoma specimens with a known strong positive reaction (score 3+) to HER2.

All of the slides, both hematoxylin-eosin and immunohistochemical, were evaluated by a very capable pathologist

(Dr. L. Claro). Following well-established protocols [18], the interpretation of the slides was based on the positivity in brown on the cellular membrane, quantified and graded according to the information on the antibody insert.

- (i) Score 0: absence of color or reaction in less than 10% of neoplastic cells; interpretation = negative;
- (ii) Score 1: weak and/or incomplete coloring of the membrane in more than 10% of the neoplastic cells; interpretation = positive 1+;
- (iii) Score 2: moderate and/or incomplete coloring of membrane in more than 10% of the neoplastic cells; interpretation = positive 2+;
- (iv) Score 3: strong and complete and/or incomplete of the membrane in more than 10% of the neoplastic cells; interpretation = positive 3+.

Following what is already widely accepted in the literature, we stratified the results obtained in this manner: score 0 and 1+ defined as absent HER2; score 2+ defined as indeterminate HER2; and score 3+ considered positive HER2.

There was no follow-up loss and the histopathological results were analyzed by both the author and the same pathologist. The adopted classification for the tumor invasion (T), lymph node involvement (N), and final staging was that of the *Japanese Gastric Cancer Association, 3rd English Edition* [19]. There was no sponsorship in the acquisition of the kits.

2.2. Statistical Analysis. In order to determine the association between the presence, indetermination, or absence of the HER2 protein and the studied characteristics, the Chi-square or Fisher's exact tests were used, fixing the rejection level of the null hypothesis at 0.05 or 5%, or in other words, with $p < 0.05$ being considered significant. To analyze the difference in ages (years), survival time (months), and number of positive lymph nodes among the presence, indetermination, and absence of the HER2 protein, the Kruskal-Wallis variance analysis was utilized, also fixing at 0.05, or 5%, the rejection level of the null hypothesis. The software used for the statistical analysis was the Statistical Package for the Social Sciences (SPSS) version 18.0 and the Bioestat version 5.3.

2.3. Ethical Aspects. This study was analyzed and approved by the Ethics Committee of the Education and Research Institute at the Santa Casa of São Paulo Medical School (registry number 44942013.0.00005479). There was no sponsorship whatsoever by any medical companies and the whole of the study followed the most rigid protocols for research using human beings (Helsinki Declaration).

3. Results

During the period of January 2008 to July 2012, 222 patients with gastric adenocarcinoma were treated by the Stomach and Obesity Surgery Department of Santa Casa of São Paulo Medical School. However, only 121 (54.5%) were submitted to surgery with a curative potential and thus being considered eligible for the study. Tables 1 and 2 present the patient

TABLE 1: Characteristics of patients and their relationship with the expression of HER2.

| Characteristic | HER2 | | | Total n (%) | HER2 present % | p |
|-------------------------|-----------------|------------------------|------------------|----------------|----------------------|----------|
| | Absent n (%) | Indeterminate n (%) | Present n (%) | | | |
| Sex | | | | | | |
| Male | 59 (48.7) | 5 (4.1) | 2 (1.6) | 66 (54.5) | 3.1 | 0.3455* |
| Female | 52 (42.9) | 1 (0.8) | 2 (1.6) | 55 (45.5) | 3.6 | |
| Tumor site | | | | | | |
| Proximal | 40 (33.1) | 0 (0.0) | 1 (0.8) | 41 (33.9) | 2.4 | 0.1786** |
| Distal | 71 (58.7) | 6 (4.9) | 3 (2.5) | 80 (66.1) | 3.8 | |
| Surgery | | | | | | |
| Gastrectomy subtotal | 92 (76.1) | 6 (4.9) | 4 (3.3) | 102 (84.3) | 3.9 | 0.3623* |
| Total gastrectomy | 19 (15.7) | 0 (0.0) | 0 (0.0) | 19 (15.7) | 0.0 | |
| Tumor depth | | | | | | |
| T1 | 35 (28.9) | 1 (0.8) | 0 (0.0) | 36 (29.7) | 0.0 | 0.2337* |
| T2 | 12 (9.9) | 1 (0.8) | 2 (1.6) | 15 (12.4) | 13.3 | |
| T3 | 22 (18.2) | 2 (1.6) | 0 (0.0) | 24 (19.8) | 0.0 | |
| T4 | 42 (34.7) | 2 (1.6) | 2 (1.6) | 46 (38.1) | 4.3 | |
| Lymph node involvement | | | | | | |
| N0 | 55 (45.4) | 2 (1.6) | 1 (0.8) | 58 (47.9) | 1.7 | 0.0622* |
| N1 | 12 (9.9) | 0 (0.0) | 0 (0.0) | 12 (10.7) | 0.0 | |
| N2 | 13 (10.7) | 3 (2.5) | 0 (0.0) | 16 (13.2) | 0.0 | |
| N3 | 31 (25.6) | 1 (0.8) | 3 (2.5) | 35 (28.9) | 8.6 | |
| Stage | | | | | | |
| I | 41 (33.9) | 2 (1.6) | 1 (0.8) | 44 (36.4) | 2.2 | 0.4482* |
| II | 20 (16.5) | 0 (0.0) | 0 (0.0) | 20 (16.5) | 0.0 | |
| III | 50 (41.3) | 4 (3.3) | 3 (2.5) | 57 (47.1) | 5.2 | |
| Histological type | | | | | | |
| Well-differentiated | 18 (14.9) | 1 (0.8) | 0 (0.0) | 19 (15.7) | 0.0 | 0.076* |
| Mod. differentiated | 33 (27.3) | 5 (4.1) | 3 (2.5) | 41 (33.9) | 7.3 | |
| Little differentiated | 54 (44.7) | 0 (0.0) | 1 (0.8) | 55 (45.4) | 1.8 | |
| Nondifferentiated | 6 (4.9) | 0 (0.0) | 0 (0.0) | 6 (4.9) | 0.0 | |
| Angiolymphatic invasion | | | | | | |
| Yes | 61 (50.4) | 5 (4.1) | 4 (3.3) | 70 (57.8) | 5.7 | 0.0866* |
| No | 50 (41.3) | 1 (0.8) | 0 (0.0) | 51 (42.1) | 0.0 | |
| Perineural invasion | | | | | | |
| Yes | 50 (41.3) | 2 (1.6) | 1 (0.8) | 53 (43.8) | 1.9 | 0.6340* |
| No | 61 (50.4) | 4 (3.3) | 3 (2.5) | 68 (56.2) | 4.4 | |
| Adjuvant treatment | | | | | | |
| Yes | 50 (41.3) | 2 (1.6) | 3 (2.5) | 55 (45.4) | 5.4 | 0.4124** |
| No | 61 (50.4) | 4 (3.3) | 1 (0.8) | 66 (54.6) | 1.5 | |
| Relapse | | | | | | |
| Yes | 14 (11.6) | 0 (0.0) | 1 (0.8) | 15 (12.4) | 6.6 | 0.4870** |
| No | 97 (80.2) | 6 (4.9) | 3 (2.5) | 106 (87.6) | 2.8 | |
| Death | | | | | | |
| Yes | 45 (37.2) | 3 (2.5) | 2 (1.6) | 50 (41.3) | 4.0 | 0.8443** |
| No | 66 (54.5) | 3 (2.5) | 2 (1.6) | 71 (58.7) | 2.8 | |

*Chi-square test.

**Fisher's exact test.

TABLE 2: Correlation of age, survival time, and lymph node involvement in patients with HER2 expression.

| Characteristic | HER2 | | | <i>p</i> |
|------------------------|----------------|----------------|----------------|----------|
| | Absent | Indeterminate | Present | |
| Age (years) | <i>N</i> = 111 | <i>N</i> = 6 | <i>N</i> = 4 | 0.1195* |
| | Min = 26 | Min = 60 | Min = 48 | |
| | Max = 83 | Max = 89 | Max = 71 | |
| | Median = 61.0 | Median = 89.5 | Median = 56.5 | |
| | Average = 60.1 | Average = 71.0 | Average = 58.0 | |
| | SD = 12.3 | SD = 9.8 | SD = 10.6 | |
| Survival time (months) | <i>N</i> = 111 | <i>N</i> = 6 | <i>N</i> = 4 | 0.6679* |
| | Min = 1 | Min = 1 | Min = 4 | |
| | Max = 82 | Max = 60 | Max = 46 | |
| | Median = 42 | Median = 42.5 | Median = 42.5 | |
| | Average = 39.3 | Average = 34.3 | Average = 33.8 | |
| | SD = 25.0 | SD = 24.7 | SD = 19.9 | |
| Positive lymph nodes | <i>N</i> = 111 | <i>N</i> = 6 | <i>N</i> = 4 | 0.5648* |
| | Min = 0 | Min = 0 | Min = 0 | |
| | Max = 64 | Max = 8 | Max = 11 | |
| | Median = 1.0 | Median = 4.0 | Median = 8.0 | |
| | Average = 5.8 | Average = 3.5 | Average = 6.8 | |
| | SD = 10.2 | SD = 3.1 | SD = 4.8 | |

*Kruskal-Wallis variance analysis.

characteristics according to the HER2 status. Among all the patients studied, 4 (3.3%) were considered positive, 6 (4.9%) indeterminate, and 111 (91.7%) negative. There was no statistical correlation between the presence of HER2 and all studied characteristics.

4. Discussion

The human epithelial growth factor receptors are a family of four structurally similar homologous members (HER1 to HER4), containing an extracellular binder component, a transmembrane hydrophobic segment, and domain with tyrosine-kinase intracellular activity [20]. Its superexpression is generally associated with tumors of epithelial origin, but with much varied frequency; studies focusing on gastric cancer or esophagogastric transition found rates varying from 5 to 25% [6, 9]. We found positivity in 3.3%, a little under the literature data. We can attribute this finding to some situations. One aspect may have been the fact that we did not perform an additional analysis of the group considered "indeterminate" (2+ reactions), through in situ hybridization tests, as some authors suggest [21]. Only for comparative purposes, if we take into account the results found by Van Cutsem et al. [22] when studying the reactions of HER2 obtained both by immunohistochemistry and by in situ hybridization, in which only approximately half of the group with 2+ reactions were in fact positive, we might have obtained a result approaching 6%, which would be in agreement with the current literature. Another situation that seems to increase the incidence of HER2+ is the proximal third tumors [22, 23], but such a site accounts for only one-third of the sample of our patients. Moreover, some studies

[24] suggest an association between greater expression of HER2 and the presence of hepatic metastases. Nevertheless, no patients in this situation were included in our study, as we understand that both the metastatic disease and the residual tumor following palliative resections are situations which clearly compromise the survival time and interfere with the analysis of the results, as one of the objectives of this study was to verify the incidence of HER2 expression in gastric cancer and its possible relationship with survival. Lastly, it is well known that the incidence of HER2 seems to be higher among Japanese patients than Western patients. Even with the very well known Brazilian history of Japanese people migrating to our country over the last decades, only five patients were descendants from Japanese people, with just one positive for HER2 immunohistochemistry reaction. Due to the low number of patients, though, the statistical analysis of this characteristic may not be reliable.

As previously stated, in various published studies the intermediate results (2+ reactions) were submitted to a new analysis using fluorescent in situ hybridization (FISH) or chromogenic in situ hybridization (CISH), thus obtaining only positive or negative results. In a post hoc analysis of the data obtained in the ToGA Trial [12], which studied the effects of trastuzumab associated with chemotherapy in patients with gastric and esophagogastric transition cancers which expressed HER2, Van Cutsem et al. compared the results obtained both by immunohistochemistry and by FISH [22]. The initial analysis showed a general agreement of 87.2%, with 3,280 patients having been tested. For those with 3+ reactions to the immunohistochemistry, the agreement was very high, 94.9%. In the same manner, for reactions 0 and 1+, it was 92.4% for negative FISH. However, the 2+ reactions

presented only 54.6% of agreement for positive FISH. For mathematical test purposes, the authors themselves excluded the 2+ results, thus finding a new overall agreement of 92.8%, which was very satisfactory. Because of some limitations in our Pathology Department, such as absence of the hybridizer instrument for in situ hybridization and lack of financial support for acquiring it and the HER2/CEN-17 probe, we could not perform the test in order to clarify the HER2-undetermined (2+) results. We intend to perform the FISH study in a near future. In this manner we opted not to include the 2+ results in the “positive” group without having the confirmation of their real expression, a factor at times encountered in some publications, which is of questionable methodology.

The real implication of the superexpression of HER2 as a risk factor for a worse prognosis in patients with gastric cancer has not yet been established. In the present study, there was no statistical difference as to the evolution of these patients, even though the low number of positive cases might have influenced such a result. Nonetheless, in a recent revision of meta-analyses, Nagaraja and Eslick analyzed nine large studies [25] and, of these, eight showed some type of worse evolution of HER2-positive patients. Only one revision study (11 publications, 4,569 patients), which followed the same selection methodology as the ToGA Trial, did not show a statistical relationship between the relapse-free survival and the overall survival with the expression of HER2 [11]. If we consider the few studies performed on the Brazilian population, we also found this discrepancy. Following the analysis of the relationship of the four members of the epithelial receptor family, one of the studies showed a worse evolution as for survival for those with superexpression of HER2 and HER3 [26]. This same study, however, presented other results, which were very similar to ours: a low incidence of positive HER2 (only 3% of 3+ reactions) and a greater expression of this receptor in more differentiated histological types (they found a statistically relevant relationship, while our number of cases was close to this, with $p = 0.076$). Conversely, another national study, which also analyzed the expression of the whole epithelial receptor family, showed a worse evolution only in the more advanced stages of the disease, without a relationship with the expression of the same receptors [27]. Finally, in a more recent publication, whose objective was to analyze the clinical-pathological aspects of patients with gastric cancer and the expression of HER2 and IMP3 (cytoplasmic receptor), there was no evidence of a worse evolution upon comparison with the superexpression of HER2 [28].

Even though our study has not shown a statistical difference between the diverse clinical-pathological characteristics and the expression of HER2, the relationship between the lymph node involvement ($p = 0.0622$) and the angiolymphatic invasion ($p = 0.0866$) called our attention. In a recent publication, Jin et al. analyzed the clinical, pathological, and molecular characteristics of 1,104 patients with early gastric cancer submitted to gastrectomy with lymphadenectomy, showing that 104 patients (9.4%) presented with lymph node involvement [29]. After multivariate analysis of risk factors for lymphatic dissemination, one of the characteristics found

was the HER2 expression. Matsumoto et al. analyzed the HER2 expression in patients with gastric cancer and lymph node involvement submitted to neoadjuvant chemotherapy, attempting to define if there was room for trastuzumab or similar drugs in the preoperative treatment [30]. They found 27% HER2 positivity in N2+ or N3+ patients, while in the ToGA Trial, with similar methodology, the overall positivity was 12.2%. This is an interesting piece of data because the lymph node involvement is known to be one of the worst factors for a bad prognosis for the patient with gastric cancer.

In this manner, in light of the similarity with other studies, we found a relatively low HER2 positivity in our gastric carcinoma cohort and we did not find a statistical relationship with the survival or with clinical characteristics. Albeit, when present, HER2 becomes one more treatment option in this disease with so few satisfactory results when in its advanced phase. Studies with new drugs are proving to be more and more promising, especially when combined with already established therapies. It is possible that one might not be able to establish an adequate relationship between prognosis and clinical characteristics specific to the superexpression of HER2, but certainly the research into its expression is very valid. As long as every viable treatment should be offered to the patient, defining if he or she presents with conditions to be submitted to a target treatment seems essential to us.

Competing Interests

The authors declare no competing interests.

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